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18TH SYMPOSIUM OF THE INTERNATIONAL SCIENTIFIC CENTRE OF FERTILIZERS

MORE SUSTAINABILITY IN AGRICULTURE: NEW FERTILIZERS AND FERTILIZATION MANAGEMENT

8-12 NOVEMBER 2009
ROME, ITALY

PROCEEDINGS
CIEC 2009

MINISTRY OF AGRICULTURE
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18TH SYMPOSIUM OF THE INTERNATIONAL SCIENTIFIC CENTRE OF FERTILIZER

Organized by

1. Agricultural Research Council
    - Research Centre for the Soil-Plant System, Rome
    - Research Unit for Cropping Systems in Dry Environments, Bari
    - Research Unit for the Study of Cropping Systems, Metaponto (Matera)
2. Institute for Environmental Protection and Research (ISPRA), Rome
3. International Scientific Centre of Fertilizers (CIEC)

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OPENING ADDRESS

to the 18th International Symposium of the International Scientific Centre of Fertilizers (CIEC)

Cristian HERA
President of CIEC

As President of the International Scientific Centre of Fertilizers, known as CIEC, abbreviation coming from the French Language - *Centre International des Engaris Chimiques* - I am delighted and honoured to express heartedly thanks and gratitude to our host, whose efforts enabled us to get together in this marvellous city of ROME.

In the framework of the 18th International Symposium of CIEC, I would like to **recognize and extend my deepest appreciation** to Prof. ***Paolo SEQUI*** from the Agricultural Research Council - Rome Research Centre for Soil and Plant System, to Dr. ***Donato FERRI***, from Bari Research Unit for Cropping Systems in Dry Environments, to Prof. ***Patrizia ZACCHEO***, from the Department of Plant Production, University of Milan, to Dr. ***Francesco MONTEMURRO*** from Metaponto, Matera, to Dr. ***Maurizio DESANTIS*** from Italian Ministry of Agriculture, Food and Forestry Policies as well as to Dr. ***Elvira REA, Francesco FORNARO*** - Symposium Secretary - and to all others who generously devoted their time and efforts in ensuring the success of the 18th International CIEC Symposium. **Please accept L & G my warmest gratitude**.

It is my duty and pleasure to highlight the prolific cooperation between **Italian National Organizing Committee** and ***International Organizing Committee***, represented **primary** by Professors ***Ewald SCHNUG*** and ***Silvia HANEKLAUS*** from Braunschweig, Germany, both strongly involved in CIEC's permanent activities. I would like also to express my appreciations for CIEC Deputy President Prof. Georges Hofman and Prof. Oswald van Cleemput, Laieson Officer for the Environment, for their continuous involvement in CIEC's activity.

It is with great satisfaction that I welcome both the regular members of the International Scientific Centre of Fertilizers (CIEC) as well as the new attendants to the 18th Symposium organized in the spectacular Rome metropolis, **a city world famous for its magnificent history.**

CIEC, like Rome, also has a vibrant history. Not only is Rome the ***birthplace of CIEC*** but it seems to me, that the expression *"all roads lead to Rome"* **certainly holds true for CIEC**, as throughout its existence, ***CIEC keeps coming back to Rome.***

Founded in 1933 as a result of several international conferences held in Rome and Amsterdam, CIEC brought together different players involved in **soil fertility and plant nutrition scientific research**, ***chemical fertilizer technology*** and **their application in agriculture.** Two of the **main founders of CIEC** were Italian Professor ***Francesco ANGELINI*** **-** then Secretary General of International Federation of Agronomists and Professor in the University of Naples - elected at that time as Secretary General of the newly established *Centre International des Engrais Chimiques* (CIEC), and Dr. ***Ernest FEISST***, then Minister of Agriculture of Switzerland, elected at that same time as President. They were both immensely dedicated to CIEC's formation.

The most important event after its founding was the **organization of the 1st World Fertilizer Congress *held again*, in Rome in 1938.**

I would like to take this opportunity to remember and give our heart felt gratitude to our predecessors' hard work and commitment to this organization.

After a nearly 10 years break in its proceedings caused by the 2nd World War, Prof. ***Angelini*** and Dr. ***Feisst*** succeeded in resuming CIEC's activity by **organizing the second World Fertilizer Congress also in Rome, in**

1951, after two previously **preparatory meetings** held in Zurich and Paris, 1949-1950. This Congress infused CIEC with a new momentum in the research of the effects of fertilizers on the soil, on human and animal health, and exploring various methods of fertilizer application and the problems of fertilizer production and manufacturing.

I believe CIEC somehow managed to carve a road of its own to keep its connection to Romania **open ever since its inception**. Romania was **one of the 29 CIEC's founding countries,** hosted the ***XIIth General Assembly in 1971*** and the ***XIIth CIEC Symposium in 2000*.** Thus, **it is with great pleasure and honour that I am now informing that the XVth CIEC World Fertilizer Congress will be held in 2010 in Bucharest, Romania.** I am cordially inviting all of you to participate next year at this Congress (29th August - 02 September 2010).

I must confess that **I am very proud of CIEC's evolution over time**, of its capacity to function as a **valuable bridge between the specialists involved in soil fertility** and **fertilizer research, technical development** and **industry,** from almost all the countries of the world.

The **18th Symposium is a testimony to CIECs solid roots being deeply anchored in the reality of our time,** offering us the opportunity to advance towards **more sustainability in agriculture**, while taking into account **the new challenges we have to confront with the global climate changes, the European and global crises, as well as with the current legislation regulating consumer's rights and environmental demands.**

The Symposium promises to explore and reveal the possibilities to use new fertilizers and make amendments to our conventional and organic agricultural systems, **to inform us about the production, *markets* and economics of these fertilizers and *by-products*, site specific nutrient management**, especially **fertilizers and food quality** under the new fertilizers use.

It is my hope and desire that this Symposium, taking into considerations the efforts made by the Local Organizing Committee leaded by Prof. *Paolo Sequi* and his colleagues - and your efficient contribution *dear participants* - will be crowned with success, and will remain a rewarding and pleasant remembrance for us all.

Thank you very much for your attention and I wish you all great success in the future.

OPENING ADDRESS

to the 18th International Symposium of the International Scientific Centre of Fertilizers (CIEC)

Paolo SEQUI
President of Italian Scientific Centre of Fertilizers

Ladies and Gentlemen, dear Colleagues,

it is a great honour for me to open the programme of this 18th Symposium of the International Scientific Centre of Fertilizers in Rome.

First of all, I want to inform you that the Italian Minister of agriculture, food and forestry policies, **Luca Zaia**, wrote me to express his regret for not being able to participate in the meeting, due to engagements which could not be delayed occurring from the government charge. He wishes full success to our Symposium and gives us his special greetings.

Secondly, I give you greetings and wishes from many authorities of the Ministry. I am speaking you also in the name of **Maurizio Desantis**, Chief of the Fertilizer Office and member of the National Organizing Committee of this Symposium.

The most important matter to remark now, as everybody understands, is to stress the specificity of our meeting, which occurs about one year after the 17th CIEC Symposium in Cairo. The 17th Symposium was devoted to Plant Nutrient Management under Stress Conditions, a topic important of course not only in a Continent like Africa is.

The Programme of the 18th International Symposium is now devoted to New fertilizers and Fertilization Management in order to pursue more Sustainability in Agriculture: such a topic is of primary importance not only in advanced, but also in developing countries. Production of new fertilizers and amendments from industrial by-products and waste materials in general, as well as increasing diffusion of growing media use, specialty fertilizers, as well as the influence of all such fertilizers on food and environmental quality, represent stressing problems in the common policy and life of everyone, so deserving also new problems of characterization and legislation. These topics are included in the seven sessions of the Symposium.

I want to express my personal greetings now to the components of the International Organizing Committee, first of all to the President **Cristian Hera**. Everybody knows Professor Cristian Hera not only for his charge in the CIEC, but also for the high responsibility of Expert during approximately **25** years in the International Atomic Energy Agency of Vienna, guiding specialists from different countries all over the world in the field of stable and radioactive isotopes use in the study of soil fertilization and fertilizer application. For **8** years he was then Head of the Research Section “Isotopes use in the study of plant nutrition, fertilizer applying and water use in vegetal production” of the Joint Division FAO/IAEA of Vienna. I remember him as President of the United Nations Organization for Industry Development (UNIDO) Conference “Chemical fertilization and phytopharmaceutic substances production and utilization and President of the Romanian Academy of Agricultural and Forestry Sciences (AAFS), but, above all, I should like to stress that in 2006 he was elected President of the Union of the European Academies for Science Applied to Agriculture, Food and Nature (UEAA).

UEAA organized three general assemblies with associated scientific conferences or workshops before 2006, the first of them in Italy (Bologna and Florence) under the Presidency of **Franco Scaramuzzi**. No doubt a workshop associated to the 4th Assembly in Bucharest should refer to fertility problems, and in fact its title was "Soil Fertility and the Future of Agriculture in Europe". I was honoured to represent Italy there (the Italian Association of Scientific Agricultural Academies) and I gave a presentation of the topic "Soil Fertility Problems to be faced in Southern Europe", published in a volume edited by Cristian Hera and Cristian Kleps. Nice meeting, nice workshop.

Please accept my apologies for my relatively long dissertations on Professor Hera, but I have some reasons to do that. The first time I met Professor Hera during my life was about forty years ago.

Of course, I should like to represent my high estimation to all the components of the International Organizing Committee. Professors **Ewald Schnug** and **Silvia Haneklaus** came three years ago in Rome and gave very appreciated seminars in a hall where Galileo Galilei spent a productive period of his life some centuries ago; professors **Georges Hofman** and **Tamás Németh** gave a great contribution to CIEC in the last years and I hope that they will come here again soon. My consideration goes also to the Italian National Organizing Committee, from the colleagues of Bari and Metaponto to all the colleagues and friends of Rome or outside Rome who made many efforts to insure a success to this workshop. Particular thanks must be reserved to **Elvira Rea, Francesco Montemurro, Donato Ferri, Francesco Fornaro** and all their collaborators. Additional special thanks are due to the Session Chairman's, some of them cited yet, though some others have not cited before, like **Bill Carlile, Alessandra Trinchera, Massimiliano Valentini, Liviana Leita, Robin L. Walker, Giancarlo Roccuzzo, Francisco Marquez, Anna Benedetti and Claudio Ciavatta**. Most hearty thanks are due also to all the participants and colleagues here.

Thank you, with the hope of a great success!

I SESSION

New fertilizers and amendments from industrial by-products and waste materials

PROPERTIES AND PLANT AVAILABILITY OF PHOSPHORUS FERTILIZERS FROM SOURCE-SEPARATED URINE

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Introduction

In recent years, many efforts were put into developing new alternative sanitation systems (so-called "NoMix" toilets) which, as opposed to the conventional end-of-pipe wastewater treatment systems, focus on closing the nutrient cycle and recycling valuable nutrients back to agricultural land (Larsen *et al.*, 2001, Werner *et al.*, 2003). Indeed, the "Yellow Water" fraction accounts for only 1% of all municipal wastewater, but contains most of the nutrients (more than 75% of the total N load and around 50% of the total P load) (Wilsenach, Van Loosdrecht; 2002). A number of technologies were developed to process source-separated urine and to convert it into a safe fertilizer, with most of these practices focusing on the production of struvite (magnesium ammonium phosphate) (Tilley *et al.*, 2008, Udert *et al.*, 2006, Wilsenach *et al.*, 2007). This practice is also well-known as a source of phosphorus recovery from municipal wastewater treatment works (Booker, 1999, Doyle, Parsons, 2002, Quintana *et al.*, 2004). Much of the research on struvite recovery from urine and wastewater has emphasized on the technological aspects. For that reason, very little is known about the fertilizer efficiency of the materials that are generated by these processes (Johnston, Richards, 2003). The objective of this study was to assess the agronomic effectiveness of five materials produced from human urine and one substance produced from conventional wastewater treatment.

Keywords: agronomic effectiveness, *lolium multiflorum italicum*, nutrient uptake, pot experiment, struvite.

Materials and Methods

Phosphorus Sources

The seven phosphates which were tested in the greenhouse pot experiment are described in table 1. The STR-U sample was produced in CanTho, Vietnam by a pilot-scale process which implies the precipitation of struvite by adding magnesium oxide to source-separated urine.

Samples of SOL, SOL-P and SOL-S were collected from a solar evaporation unit in CanTho, Vietnam after the liquid fraction had evaporated completely. The SOL sample was derived from source-separated urine, whereas the latter was mixed with phosphoric and sulphuric acid in order to produce SOL-P and SOL-S respectively. The sediment which spontaneously precipitated during the storage of source-separated urine (SED) was collected from a storage tank at the University of Bonn, Department of Plant Nutrition. A struvite sample generated from sludge cake of a municipal wastewater treatment plant (STR-W) was collected in order to compare its performance to that of urine-derived material. The phosphates contained in the sludge cake were dissolved by using sulphuric acid. Magnesium oxide or magnesium chloride was added to precipitate struvite from this P-enriched liquor after the pH had been increased to 8.5 by using sodium hydroxide. Single superphosphate (SUP) was purchased commercially from Landgard eG.

Table 1: Description of the phosphorus materials evaluated in the greenhouse pot experiment.

Material	Source of material	Phosphorus content (%)
STR-U	Struvite recovered by a pilot-scale process from source separated urine in Vietnam	13
SED	Sediment recovered from the urine storage tank of a NoMix System at the University of Bonn	11
SOL	Solids recovered from the solar evaporation of human urine in Vietnam	2
SOL-P	Solids recovered from the solar evaporation of human urine mixed with phosphoric acid in Vietnam	18
SOL-S	Solids recovered from the solar evaporation of human urine mixed with sulphuric acid in Vietnam	5
STR-W	Struvite recovered by the pilot-scale “Stuttgart process” from digested sewage sludge in Stuttgart	10
SUP	Superphosphate purchased from Landgard eG	8

The total phosphorus concentration of the materials was determined photometrically after the digestion in nitric acid. The crystal structure was measured by X-ray diffraction spectroscopy. Phosphorus was extracted by mixing 10 g and 5 g of sample with 500 ml of distilled water and citric acid (2%) respectively (VDLUFA, 1995). The solutions were shaken for 30 minutes at 40 rpm, filtered and the phosphorus concentration of the filtrate was measured with an Eppendorf ECOM 6122 photometer (water-soluble P) and MERCK Spectroquant phosphate test (citric acid extracts).

Pot Experiment

A greenhouse pot experiment was carried out using a P-deficient soil ("Meckenheim" subsoil), and ryegrass (*lolium multiflorum italicum*) and corn (*Zea mays* L.) as test crops. All test materials were thoroughly mixed with 6 kg of air-dried, 2-mm-sieved soil and 3 kg of sand in Kick-Brauckmann pots at rates equivalent of 24 mg/kg P, based on the total P content of the material received. Before sowing the seeds, basal nutrients (1530 mg N per pot compensated as ammonium nitrate, micronutrient mixture) were mixed with the soil. A control treatment which received no P was incorporated (BLANK). The total number of treatments was 16, and these were replicated 4 times in a randomised block design leading to a total number of 64 pots. This experimental set-up was conceived to isolate phosphorus as the only growth-limiting nutrient.

Ryegrass was seeded at a rate of 1.8 g per pot, and 4 corn seeds were placed in every pot. The pots were irrigated on a daily basis with deionised water such as to maintain a level of 70% of soil water holding capacity. The ryegrass was harvested at 27, 40, 58 and 82 days after sowing; the corn was harvested at 82 days after sowing. The samples were dried at 65°C, weighed and then milled before chemical analysis for total P concentration by photometry after dry ashing and digestion in nitric acid. Phosphorus uptake was calculated on a pot basis by multiplying the dry matter yield by the phosphorus content of the biomass. Cumulative dry matter yield and nutrient uptake were calculated by combining the data of the 4 harvests.

Results and Discussion

Substrate Characterisation

Analysis by X-ray diffraction spectroscopy has shown that STR-U, STR-W and SED are composed of up to 100% of struvite or magnesium ammonium phosphate (MAP). STR-U contains 15% of dittmarite, which is a dehydration product of struvite (Sarkar, 1991). SOL is almost entirely composed of sodium chloride, whereas SOL-P - through the addition of phosphoric acid – is to 87% composed of ammonium dihydrogen phosphate (biphosammite) (no data available for SOL-S). The mineralogical data is summarised in table 2.

Table 2: Crystal structure of the test materials identified by X-ray diffraction spectroscopy (Struvite: $NH_4MgPO_4 \cdot 6H_2O$, dittmarite: $NH_4MgPO_4 \cdot H_2O$, sodium chloride: NaCl, aphthitalite : $NaK_3(SO_4)_2$, niter: KNO_3, biphosammite: $NH_4H_2PO_4$, sylvite: KCl).

Mineral	STR-U	SED	SOL	SOL-P	STR-W
Struvite (%)	85	98	0	0	100
Dittmarite (%)	15	0	0	0	0
Sodium chloride (%)	0	0	90	13	0
Aphthitalite (%)	0	0	6	0	0
Niter (%)	0	0	4	0	0
Biphosammite (%)	0	0	0	87	0
Sylvite (%)	0	2	0	0	0

Solubility tests (see table 3) show that the phosphorus content of all materials is virtually completely soluble in 2% citric acid. However, only in the case of SOL is it possible for 84% of the phosphates to be dissolved in water. Knowing that SOL is to 90% composed of sodium chloride, it is very likely that large amounts of Na and Cl ions are released at the same time. All other test materials are characterized by water-soluble phosphate values lower than 5% (no data available for SOL-P and SOL-S). Superphosphate, which is a fertilizer known to be completely soluble in water (Müller, 2000), has a relatively low value of 74% only.

Table 3: List of the test materials, their total P content and the relative phosphate solubility in water and in 2% citric acid (in % P, n=2).

Material	% P Total	% P (of total P) water-soluble	% P (of total P) soluble in citric acid
STR-U	13	2	99
SED	11	4	100
SOL	2	84	95
SOL-P	18	No data	No data
SOL-S	5	No data	No data
STR-W	10	1	94
SUP	8	74	90

Yield and Phosphorus Uptake by Ryegrass

For all test substrates but SOL and STR-W, the total dry matter yield and the nutrient uptake by ryegrass were higher than for the reference material SUP (table 4). The poor performance of SOL is probably due to the high content of sodium and chloride ions, which may have had a toxic effect on ryegrass. The SOL-P and SOL-S materials on the other hand have performed much better. The addition of phosphorus and sulphur in the form of sulphuric and phosphoric acid led to an increase of the concentration of these nutrients, with the latter being more plant available due to the lower pH values of the material. SED and STR-U were also characterised by high dry matter yield and nutrient uptake whereas the opposite is true for STR-W. Mineralogical data has shown that all three materials are composed of struvite crystals, hence the yet unexplained nature of the large variation in performance (further crystallographic studies on crystal size and integrity are in progress).

Yield and Phosphorus Uptake by Corn

For corn, only SOL and the reference material SUP led to low values in terms of dry-matter yield and nutrient uptake (table 4). Again, the poor performance of SOL is likely to be related to its high salt content which may have had a toxic effect on corn. The highest P uptake was achieved for STR-W, which did not perform well with ryegrass.

Table 4: List of the test materials and the total dry matter yield (g pot^{-1}) and P uptake (mg pot^{-1}) by ryegrass and corn after a growth period of 82 days (n=4).

Material	Ryegrass		Corn	
	Total dry-matter yield (g pot^{-1})	P uptake (mg pot^{-1})	Total dry-matter yield (g pot^{-1})	P uptake (mg pot^{-1})
STR-U	38.28	152.46	87.93	122.69
SED	38.81	146.35	77.77	100.95
SOL	26.65	63.65	30.89	42.31
SOL-P	34.65	124.46	79.82	104.73
SOL-S	39.70	156.30	92.57	120.62
STR-W	28.30	69.13	88.14	129.57
SUP	28.54	71.56	35.52	44.50
BLANK	17.33	25.67	16.05	20.20

Overall, it appears that the material with the highest portion of water-soluble phosphates (SOL) has led to the lowest dry-matter yield and nutrient uptake in both corn and ryegrass because of its high sodium chloride content. STR-W did not perform well with ryegrass although it is composed of minerals similar to those of STR-U and SED. All other test materials induced much higher dry-matter yields and P uptake values than the reference SUP even though they are characterised by very low water-soluble phosphate contents (<5%). The poor performance and low water solubility of SUP could potentially be related to the long-term storage of the superphosphate batch prior usage in this pot trial.

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ROLE OF BIOFERTILIZERS IN PLANT NUTRITION

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Abstract

The uptake of nutrients by the plants determines the final product, its quantity and quality. One of the main environmental factors determining the effectiveness of crop production is the soil pH. The aim of our studies was to investigate the effects of bicarbonate and the pH of nutrient solution on the growth of test plants in laboratory, as well as to prove the positive role of bio-fertilizers on nutrient uptake, growth and root morphology. The experimental plants (wheat, corn, cucumber) were grown in a climate chamber on nutrient solution. To minimize the input, new technologies were developed, such as the use of bacterium-containing fertilizers. With the use of these bio-fertilizers, the increase of nutrient uptake, and the total organic matter production were observed. It has been proved by our experiments that the growth and development of the treated plants increased when the bio-fertilizer Phylazonit was used. The root development was more intensive, and consequently the green mass production was larger. When the bicarbonate nutrient solution was completed with bio-fertilizer, the dry matter accumulation of shoots increased.

Keywords: bicarbonate, pH, bio-fertilizer, uptake of nutrient.

Introduction

The microelements content of crops growing under micronutrient deficiency is low, which has unfavourable effects on the health of the population living in the given area. Nowadays, the fast-growing investments and the global climate change render agricultural production more difficult. The intensification in food production has emerged in line with the dramatic increase of the use of fertilizers in the past 40 years. The volume of the use of nitrogen has been about 7, and phosphorous 3.5 times larger than in the former period, while agricultural fields has expanded only by 10%. In parallel with the intensive use of macronutrient fertilizers, but the micronutrient fertilization is not so current. Because of the less level of micronutrient fertilization increasing deficiency symptoms (iron and zinc) have been observed in several countries. Around 30% of soils are iron-deficient in the world. These facts have led to the investigation of adaptation mechanisms of various plant species. Marschner *et al.* (1986) reported about two main adaptation mechanisms to iron deficiency. The plants of Strategy I release protons and electrons via their roots, and make the rhizosphera pH lower. The plants belonging to the Strategy I group are the dicots. The grasses belong to the second, so-called Strategy II group. These plants release phytosiderofores (PS) that bind the hardly soluble iron in the soil, and the roots take up the iron together with PS (Kawai *et al.*, 1988; Rengel 2001). Pethő (1992) observed the release of cyclic hydroxamic acids via the roots of corn, and their role in iron uptake was proved. The quantities of organic matters - mainly organic acids - released via the roots can be more than 25% of net photosynthesis (Lynch and Whipps, 1990).

Materials and methods

In this study, we used as experimental plants corn (*Zea mays* L. *cvs. Norma SC*), cucumber (*Cucumis stativum* L. *cv. Rajnai fürtös*) and spring wheat (*Triticum aestivum* L. *var. Paragon*). The seeds were germinated on moistened filter paper at 25^oC. The seedlings were then transferred to a continuously aerated nutrient solution of the following composition: 2.0 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0,5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.1 mM KCl, 1µM H_3BO_3, 1µM $MnSO_4$, 0.25 µM $CuSO_4$, 0.01 µM $(NH_4)_6Mo_7O_{24}$. The nutrient solution of cucumber contains 10µM H_3BO_3. The iron as Fe-EDTA in a concentration of 10^{-4}M alongside with bicarbonate in the form of $NaHCO_3$ and $KHCO_3$, was added to the nutrient solution (uptake by the roots). The concentrations of bicarbonate were 10, 20 and 40 mM, respectively. The bio-fertilizers were added to nutrient solution. We used five commercial bio-fertilizers, such as RhizoVital® (ABiTEP, Berlin, Germany, with *Bacillus amyloliquefaciens* strain FZB42), Proradix® (Sourcon-Padena, Tübingen, Germany, with *Pseudomonas proradix*), Phylazonit MC®, (Corax-Bioner, Hungary, with *Bacillus megatherium* var. *phosphaticum* and *Azotobacter chroococcum*), Phylazonit CE® (Corax-Bioner, Hungary, additional 2 bacterial strains with *Pseudomonas putida*), and a liquid extract from brown algae Kelpak® (Kelp Products, Simon's Town, South Africa).

The experiments were plant cultivation in nutrient solution (hydroponics) enriched with bio-fertilizers (Picture 1).

Picture 1: Cucumber seedlings on nutrient solution (Debrecen/Hungary, 2007).

The seedlings were grown under controlled environmental conditions (light/dark regime 10/14 h at 24/20 ^{o}C, 65–70% relative humidity and a photosynthetic photon flux of 390 $mEm^{-2}s^{-1}$ at plant height). The contents of elements were measured by ICP. The relative chlorophyll contents were measured with SPAD 501 (Minolta), the pH with OPTIMA 200A (USA) pH meter.

Results and Discussion

The soil pH is one of the most important environmental factors determining the productivity of soils, and the success of plant production. Bicarbonate has a significant role in the maintenance of soil pH, therefore we have focused our work on the effects of bicarbonate and bio-fertilizers on the early growth of cucumber, wheat and corn seedlings.

Bicarbonate increases the pH of nutrient solution. As a consequence the uptake of nutrients decreases. The effect of the various treatments on the pH is presented in Table 1 and 2.

Table 1: Effects of the treatments on the pH of the nutrient solutions of corn seedlings (n=10±s.e.).

Treatments	**$NaHCO_3$**		**$KHCO_3$**	
	0th h	**72th h**	**0th h**	**72th h**
Control	5.91±0.08	6.52±0.33	5.91±0.08	6.52±0.33
10 mM	8.14±0.18	7.51±0.46	8.46±0.12	8.14±0.52
20 mM	8.43±0.08	8.36±0.28	8.70±0.04	8.61±0.30
40 mM	8.64±0.04	9.14±0.16	8.87±0.03	8.94±0.42

Table 2: Effects of the treatments on the pH of the nutrient solutions of corn seedlings (n=3±s.e.).

Treatments	Day 0	Day 2	Day 4	Day 6
Control	6.81±1.51	6.72±0.49	6.89±0.38	7.29±0.13
Phylazonit	7.64±2.50	6.87±0.19	6.91±0.04	6.63±0.37
10 mM NaHCO3	8.45±0.25	8.52±0.01	8.49±0.35	8.20±0.28
10 mM NaHCO3+Phyl.	8.37±0.04	7.62±0.04	8.23±0.78	8.19±0.32

The Phylazonit MC in the nutrient solution compensated for growth retardation, and had a stabilization effect on the pH of nutrient solution.

The uptake of nutrients decreases because of pH increasing and in line with this processes the dry matter accumulation of shoots and roots is reduced. The effect of bicarbonate and bio-fertilizer Phylazonit MC on dry matter accumulation is presented in Table 3 and 4.

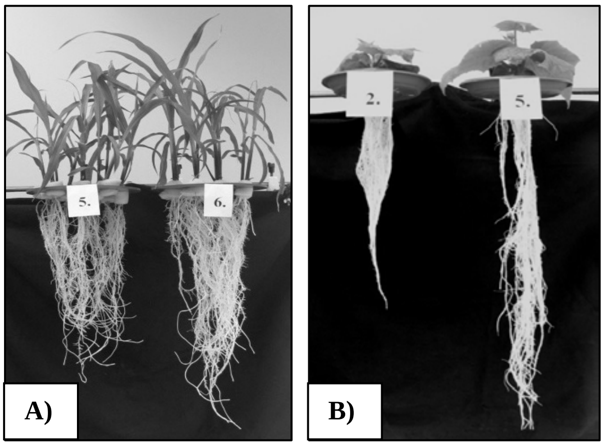
Table 3: Effects of treatments on the dry matter accumulation of 13 days old corn seedlings (n=10±s.e.).

Treatments	$NaHCO_3$		$KHCO_3$	
	shoot (gs^{-1})	root (gr^{-1})	shoot (gs^{-1})	root (gr^{-1})
Control	0.2294	0.0575	0.2294	0.0575
10 mM	0.1362	0.0350	0.2229	0.0769
20 mM	0.1473	0.0655	0.1850	0.0828
40 mM	0.0957	0.0957	0.1079	0.0448

Table 4: Effects of treatments on the dry matter accumulation of 9 days old cucumber seedlings (n=10±s.e.).

Treatments	shoot (gs^{-1})	root (gr^{-1})
Control	0.8581	0.1728
Phylazonit	0.7955	0.1961
10 mM $NaHCO_3$	0.3757	0.0995
10 mM $NaHCO_3$+Phyl	0.4877	0.1283

When the bicarbonate-treated nutrient solution was supplemented with bio-fertilizer, the dry matter accumulation of shoots and root increased. Growth retardation was observed when bicarbonate was added to the nutrient solution. The treatment with bicarbonate decreases the growth of shoots and roots in comparison to the control. When Phylazonit MC was added to the nutrient solution, growth retardation was compensated (Picture 2).



Picture 2: Treated corn (A) and cucumber (B) seedlings 2.3: 10 mM $NaHCO_3$; 5.6: 10 mM $NaHCO_3$ Phylazonit MC.

The bio-fertilizer Phylazonit MC influenced the root morphology, total root length and the number of lateral roots per plant in wheat and cucumber. The roots of treated plants were significant larger in comparison with those of the control plants. The results can be seen on Picture 3 (right). Phylazonit CE enhanced the root development, and the application of bio-fertilizer Phylazonit CE significantly increased the total root length in wheat (Fig. 1.). We observed similar effects on cucumber seedlings, the bio-fertilizer Phylazonit CE improved the root morphology in comparison with the control.

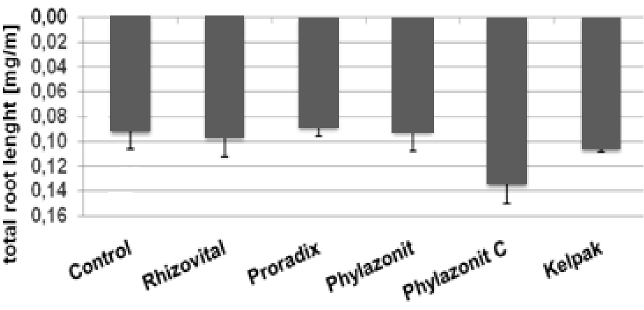
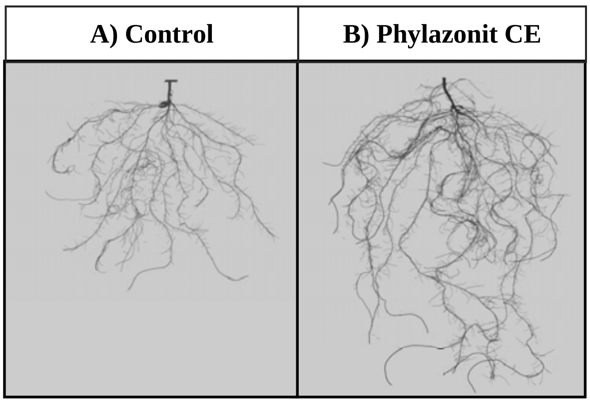


Figure 1: Root length of treated wheat seedlings.



Picture 3: 11-day-old cucumber root mass.

The effects of microorganisms were examined on wheat and cucumber seedlings. With the use of these biofertilizers, the uptake of nutrients and the total organic matter production increased. The effects of different microorganisms on wheat and cucumber seedlings were investigated in our experiments. Our studies have proved that the growth and development of treated plants intensify when the biofertilizer Phylazonit MC is used. The root development was more intensive, and consequently the green mass production was larger.

The treatments with $NaHCO_3$ modified the pH of nutrient solution, and rendered the uptake of nutrients more difficult, and as a consequence the growth was retarded. Decreased iron uptake was observed when the nutrient solution was supplemented with bicarbonate. In the light of the results of our experiments, it is assumed that soil-born bacteria have a significant role in the uptake of various nutrients. The release of organic matters by the bacteria is intensive. These organic acids make the hardly soluble nutrients more available for the plants. Our results also agree with other observations (Nyatsanaga and Pierre, 1973; Bromfield *et al.*, 1983 a, b).

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MODIFIED Zn-LIGNOSULFONATE COMPLEXES AS Zn FERTILIZERS FOR NAVY BEANS (*PHASEOLUS VULGARIS* L.) IN HYDROPONICS

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Abstract

The objective of this study was to compare the efficacy of six lignosulfonates as complexing agents in the formulation of zinc (Zn) fertilizers. Two of the lignosulfonates were obtained through sulfite treatment, from eucalyptus and spruce wood, respectively. Four modified lignosulfonates were obtained from eucalyptus lignosulfonate by oxidation, sulfonation, phenolation and ultrafiltration. Zn complexing capacity (ZnCC) was determined and lignosulfonate efficacy as Zn fertilizer for navy beans (*Phaseolus vulgaris* L.) in hydroponic conditions was assessed in comparison with ZnEDTA, $ZnSO_4$ and a control without Zn.

The ZnCC of the lignosulfonate obtained from spruce was slightly higher than the ZnCC of lignosulfonate obtained from eucalyptus. All the modifications of the original eucalyptus product improved its ZnCC, especially when phenolated groups are included. Biomass, SPAD index and Zn concentration in leaves indicated that the six Zn-lignosulfonate complexes had a similar effect than the ZnEDTA in providing Zn to the plants. Few differences were found between the six lignosulfonates in the correction of the Zn deficiency. Since doses were calculated in function of the complexed Zn, those having a higher Zn complexation capacity were considered more efficient. The six lignosulfonates can be cheaper and environmentally more friendly alternatives to synthetic chelates, although additional studies to relate chemical characteristics with agronomic value of Zn-lignosulfonates are necessary.

Keywords: lignosulfonate; zinc fertilizer; complexing capacity; navy bean; oxidation; sulfonation; phenolation; ultrafiltration.

Introduction

Zinc deficiency is the most widespread micronutrient disorder among different crops (Römheld and Marschner, 1991). Zinc is essential for the normal healthy growth and reproduction of plants, animals and humans. When the supply of plant-available zinc is inadequate, crop yields are reduced and the quality of crop products is frequently impaired (Alloway, 2004). Three different types of compounds are mainly used as zinc fertilisers: Zn inorganic compounds, synthetic chelates and natural organic complexes. Zinc sulfate is the most widely used fertilizer even though zinc chelates, such as ZnEDTA, are regarded as being the most effective sources of plant micronutrients but it is an expensive practice used only in cash crops (Lucena, 2006). Natural organic complexes such as Zn-lignosulfonate (Zn-LS) are characterized by a weak bond and low complex stability which generally implies lower efficacy as Zn fertilizer but also lower mobility and risk of contamination than chelates. Moreover, complexing agents necessary to form natural organic complexes are generally by-products of other industries; consequently, their prices are lower than those of chelates.

Lignosulfonate is a by-product of the pulp and paper industry. Anionic groups included in a highly cross-linked polymer, principally phenolate, sulfonic and carboxylic acid groups are able to bind Zn^{2+}. In this paper modified lignosulfonates with different chemical characteristics are tested to elucidate the efficacy of the increased binding capacity of Zn complexes on their agronomic value.

Materials and methods

Lignosulfonates. Six lignosulfonates, kindly provided by Lignotech Ibérica S.A., were tested in this study. Lignosulfonates (LS) were obtained through sulfite treatment of hardwood (eucalyptus; Euc.LS1) and softwood (SpruceLS) sources. Euc.LS2, Euc.LS3, Euc.LS4 were obtained from Euc.LS1 by industrial modifications, oxidation, sulfonation and phenolation respectively, in order to increase the amount of functional groups of the polymer capable to complex zinc. Through ultrafiltration, Euc.LS5, with a lower molecular weight than Euc.LS1, was obtained.

Zn Complexing Capacity (ZnCC) Analysis. The Zn complexing capacity of six lignosulfonate products was carried out according to the titration method described by Villén *et al.* (2007). In brief, increasing volumes (from 0.2 to 7 mL) of $ZnSO_4 \cdot H_2O$ (100 $g \cdot L^{-1}$) were added to 20 mL of sample solution (100 $g \cdot L^{-1}$). The pH was increased to 9.0 with 0.5 M NaOH and, readjusted after 30 min. The solution was allowed to stand for 1 day in the dark. Thereafter, the pH was readjusted to 9.0 and the samples were diluted to 100 mL. These solutions were centrifuged at 7500 r.p.m. and then filtered through a 0.45 μm Millipore filter.

Zn concentration in the filtrate was determined by atomic absorption spectrometry (AAS) using 0.5% La, 0.2% Cs and 5% HCl as matrix modifier and after the removal of the organic compounds in accordance with method 9.3. (EC 2003/2003 Regulation).

Preparation of Zn-LS complexes. Solutions of Zn-LS complexes (50 μgFe·L^{-1}) were prepared by mixing a $ZnSO_4$ solution and the suitable amount of LS to complex this amount of Zn, calculated on the basis of their maximum complexing capacity. In order to ensure that the entire element is complexed, a 10% amount of lignosulfonate in excess was added.

Biological experiment. Navy beans (*Phaseolous vulgaris* L. c.v. Negra polo) seeds were germinated in the dark at 30 ºC on filter paper moistened with distilled water. After germination, seedlings were transferred to a growth chamber where they were grown until the end of the experiment under controlled climatic conditions: day/night photoperiod, 16/8 h; temperature (day/night) 30/25 ºC; R.H. (day/night) 50/70 %. Seedlings were placed on containers containing 1/5 diluted nutrient solution with a 5 μM Zn. Solution was continuously aerated and the pH was buffered with HEPES $1.0 \cdot 10^{-4}$ M and adjusted at 7.5 with 1.0 M KOH. After five days, the diluted nutrient solution was replaced by a full-strength solution without Zn, with the following composition: (macronutrients in mM) 1.0 $Ca(NO_3)_2$, 0.9 KNO_3, 0.3 $MgSO_4$, 0.1 KH_2PO_4; (cationic micronutrients in μM as buffered micronutrient solution) 2.5 $MnSO_4$, 1.0 $CuSO_4$, 1.0 $NiCl_2$, 1.0 $CoCl_2$, 115.5 $EDTANa_2$, $2.31 \cdot 10^{-4}$ M KOH, 20.0 FeEDDHA; (anionic micronutrients in μM) 35.0 NaCl, 10.0 H_3BO_3, 0.05 Na_2MoO_4. The seedlings were kept in this solution for six days. Then the plants were transferred to polyethylene pots (three pairs of plants per pot) containing 2 L of full-strength nutrient solution with the same composition as the initial one, except for micronutrient content (not buffered micronutrient solution, in μM): 1.0 $MnSO_4$, 0.5 M $CuSO_4$, 0.1 $NiCl_2$, 0.1 $CoCl_2$, 20.0 FeEDDHA. Depending on the treatment, 0.5 μM Zn was added as $ZnSO_4$, ZnEDTA and each Zn-LS tested. A control without added Zn was also used. $CaCO_3$ (0.1 g L^{-1}) was added in order to simulate calcareous conditions. Solution and treatments were renewed every week. Four replicates (four pots) per treatment were done.

SPAD readings were taken with a chlorophyll meter (Minolta SPAD-502) for all the leaf stages every two or three days. Whole plants were sampled 8 days (two pairs of plants) and 22 days (one pair of plants) after the first application of treatments. The sampled roots, stems and leaves were separated and washed as described by Álvarez-Fernández *et al.* (2001), weighed and dried at 65 ºC for three days. Micronutrients were determined in roots and leaves after the dry digestion procedure by Atomic Absorption Spectroscopy.

Results and Discussion

Zn Complexing Capacity. By plotting the measured element, considered as complexed Zn, versus added element the same type of curve is observed for the six tested LS products. As example, the curve obtained for Euc.LS5 (Fig.1) is characterized bya rising segment, which corresponds with the complexing process, followed by a decreasing segment that probably implies the coagulation of the material by the excess of metal (Villén et al, 2007). The Zn complexing capacity (ZnCC) obtained from the intersection point of the two lines is shown in Table 1.

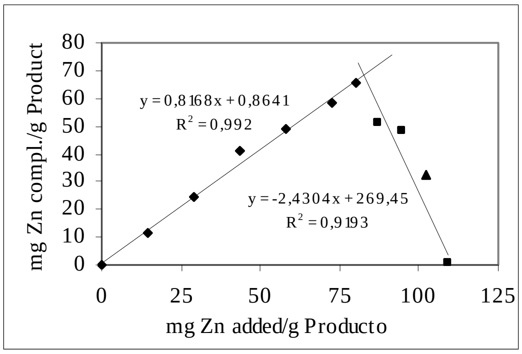

Figure 1: Example of Zn complexing capacity curves.

Table 1: Zn Complexing Capacity of tested products (g Zn /100 g product d.w.).

Spruce LS	Euc.LS1	Euc.LS2	Euc.LS3	Euc.LS4	Euc.LS5
13.9	10.2	15.6	16.4	18.7	14.2

Original LS obtained from eucalyptus wood, Euc.LS1, presents a lower ZnCC that Spruce LS in agreement with the observations by Martín-Ortiz *et al.* (2009). Furthermore modifications from original eucalyptus LS (Euc.LS1) have increased ZnCC over SpruceLS. Pang *et al.* (2008) concluded that the presence of large amount of hydroxyl groups is the main reason for the complexation of calcium by LS and that the amount of complexing Ca^{2+} drops after oxidation because the hydroxyl groups in LS molecule turn mostly into carboxyls. For Zn^{2+}, we have observed that the increment of the electron donor groups (carboxyls and, especially, phenolic hydroxyls) had a clear effect in the ZnCC of the LS.

Efficacy of Zn-LS complexes in recovery of Zn-deficient navy beans (Phaseolous vulgaris L.). Dry weight and Zn concentration in root and leaves of navy beans of each treatment are shown in Table 2. No statistical differences were found in dry weight and Zn concentration in sampling 1 (7 days after treatment), hence data are not shown.

Different Zn sources did not affect the development of root and leaves of navy beans. Dry weight of root and leaves did not present significant differences among treatments which may be due to Zn amount in the seeds being sufficient for the development of the plant in the first growth stages. Zn concentration in roots were significantly greater in navy beans treated with Zn-LS complexes (except Euc.LS5) that in the control without Zn. Whereas Zn concentration in roots of plants treated with Zn EDTA was similar to the control without Zn. Despite that

Table 2: Dry weight and Zn concentration in roots and leaves in sampling 2 (21 days after treatment) and SPAD index.

Treatment	**Dry weight (g)**		**Zn (µg g^{-1} of DW)**		**SPAD**(1)
	Roots	***Leaves***	***Roots***	***Leaves***	
Control–Zn	1.44 ± 0.16 ns	2.49 ± 0.49 ns	18.0 ± 2.0 b	15.9 ± 1.3 b	29.0 f (2)
$ZnSO_4$	1.41 ± 0.12	2.83 ± 0.20	34.8 ± 1.7 a	27.8 ± 0.5 a	32.9 bc(2)
SpruceLS	1.45 ± 0.16	2.74 ± 0.18	33.0 ± 3.1 a	31.6 ± 1.4 a	33.2 abc(2)
Euc.LS1	1.43 ± 0.14	2.52 ± 0.21	43.7 ± 6.1 a	33.4 ± 1.6 a	31.4 de(2)
Euc.LS2	1.60 ± 0.21	2.86 ± 0.11	41.5 ± 6.4 a	32.8 ± 3.0 a	34.5 a(2)
Euc.LS3	1.47 ± 0.11	2.61 ± 0.18	31.8 ± 4.6 a	32.8 ± 1.6 a	32.2 cd(2)
Euc.LS4	1.40 ± 0.14	2.77 ± 0.11	44.5 ± 5.5 a	32.0 ± 1.3 a	30.9 e(2)
Euc.LS5	1.48 ± 0.14	2.86 ± 0.17	31.1 ± 3.0 ab	32.6 ± 3.2 a	33.4 abc(2)
ZnEDTA	1.45 ± 0.14	3.12 ± 0.29	30.9 ± 1.4 ab	30.6 ± 3.3 a	33.9 ab(2)

For each column, different letters denote significant differences among the treatments according to Duncan's test (α = 0.05). ns: not significant

(1) SPAD data are referred to the mean of SPAD measured in leaves of level 3, 4 and 5 during all experiment.

(2) Different letters in the column denote significant differences among the treatments according to Duncan's tri-factorial (treatment, level and day of measured) test (α = 0.05). There was not interaction among factors.

fact, Zn concentration in leaves indicates that translocation of Zn from roots was similar with Zn-LS complexes, $ZnSO_4$ and ZnEDTA and, in all the cases, greater that in controls without Zn.

SPAD index is related to the chlorophyll concentration in leaves (Yavada, 1986). SPAD index was measured in order to determine the grade of chlorosis of leaves of navy beans since interveinal chlorosis is a symptom of Zn deficiency. In Table 2, the average SPAD values of leaves of level 3, 4 and 5 are shown because these levels grew after the first application of treatment and presented the same response about SPAD index. The statistical tri-factorial (treatment, level and day of measured) test indicates that in these three levels, leaves treated with Zn-LS complexes presented a greater SPAD value than the Control without Zn. SPAD values of SpruceLS, Euc.LS2 and Euc.LS5 were similar to those of ZnEDTA.

Few differences among the six Zn-LS tested were observed when added to navy beans plants grown in hydroponic conditions, so we can deduce that a high value of ZnCC did not necessary imply a high Zn supply to plants in hydroponics. But, since doses were calculated in function of the complexed Zn, those having a higher Zn complexation capacity were considered more efficient, because they were used in lower amounts. In general, Zn-LS complexes had a higher effect in the navy beans recovery than control without zinc and similar to $ZnSO_4$ and ZnEDTA. Therefore, new studies are necessary, including the soil and the dosage as factors, to better understand the effect of the binding group modifications on the efficacy of LS.

Acknowledgements

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CHARACTERIZATION AND NITROGEN USE EFFICIENCY OF PIG SLURRY TREATED BY ANAEROBIC FERMENTATION IN COMBINATION WITH ULTRAFILTRATION AND REVERSE OSMOSIS

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Abstract

Intensification of agricultural production has greatly increased fluxes of nitrogen (N) between different compartments of the biosphere and more specifically emissions of N compounds from agroecosystems. Agriculture is one of the main emitters of N compounds (e. g. ammonia, nitrate, nitrous oxide) causing negative impacts to the environment (e. g. emission of greenhouse gases, contamination of surface and ground water). Efficient use of N and reduction of N losses to the environment in order to protect natural resources is still a concern in many industrial countries. New technologies such as anaerobic fermentation (AF) of slurry in combination with ultrafiltration (UF) and reverse osmosis (RO) can be attractive for agriculture as they have potential to optimize nutrient management, reduce transport volumes of slurry and produce renewable energy. In our study anaerobically fermented pig slurry, with or without subsequent mechanical separation and products resulting from additional UF and RO, respectively, were characterized and their apparent N use efficiency was determined in pot and field experiments using the difference method. Treatment of pig slurry with AF, UF and RO increased the ammonium N concentration, which improved plant N availability. But in parallel also pH augmented, increasing the risk for ammonia N losses during storage and application to the field. However, overall such new technologies for slurry treatment in combination with low-emission application techniques (e. g. trail hose) have potential to increase N efficiency of slurry and to reduce N emissions to the environment.

Keywords: anaerobic fermentation, nitrogen use efficiency, pig slurry, reverse osmosis, ultrafiltration.

Introduction

Farmyard manure plays a key role in agriculture. Nutrients contained in farmyard manure are important factors for agricultural production. Nitrogen (N) for example plays a key role because it is the major yield-limiting nutrient and because it provides the basis of dietary N (protein) of animals and humans. Livestock husbandry for milk and meat produces considerable amounts of farmyard manure. Regional N excess due to high livestock density results in increased risk of environmental pollution. Improvement of N use efficiency, the reduction of N losses to the environment and the maintenance of soil N resources are thus of important concern. New technologies such as anaerobic fermentation (AF) of slurry in combination with subsequent ultrafiltration (UF) and reverse osmosis (RO) can be attractive for agriculture as they have potential to i) optimize nutrient management, ii) reduce transport volumes of slurry and iii) produce renewable energy.

In this study different fertilizer products resulting from slurry treatment (AF, UF, RO) have been tested in pot and field experiments to i) investigate the impact of AF, UF and RO on the characteristics of pig slurry and ii) determine the N use efficiency of treated slurry vs. conventional fertilizers (untreated pig slurry, mineral fertilizer).

Materials and Methods

<u>Anaerobic fermentation in combination with ultrafiltration and reverse osmosis</u>

The different procedures by which the fertilizer products have been obtained are shown in Fig. 1. Pig slurry was first anaerobically fermented and subsequently mechanically separated to get the liquid phase of the slurry and the solids. The liquid phase was then subject to the secondary treatments of UF and RO. During UF a liquid - in our case the liquid phase of slurry - is forced against a semipermeable membrane so that solutes of high molecular weight are retained (UF retentate), while water and low molecular weight solutes passes through the membrane (UF permeate). Subsequently UF permeate was treated by RO resulting in a retentate and a permeate (process water). RO works by using pressure in excess of the osmotic pressure to force a liquid from a region of high solute concentration through a semipermeable membrane to a region of low solute concentration. Except for the solids from mechanical separation and process water (RO permeate) from reverse osmosis all intermediate and end products were characterized and their N use efficiency (NUE) was determined in pot and field experiments.

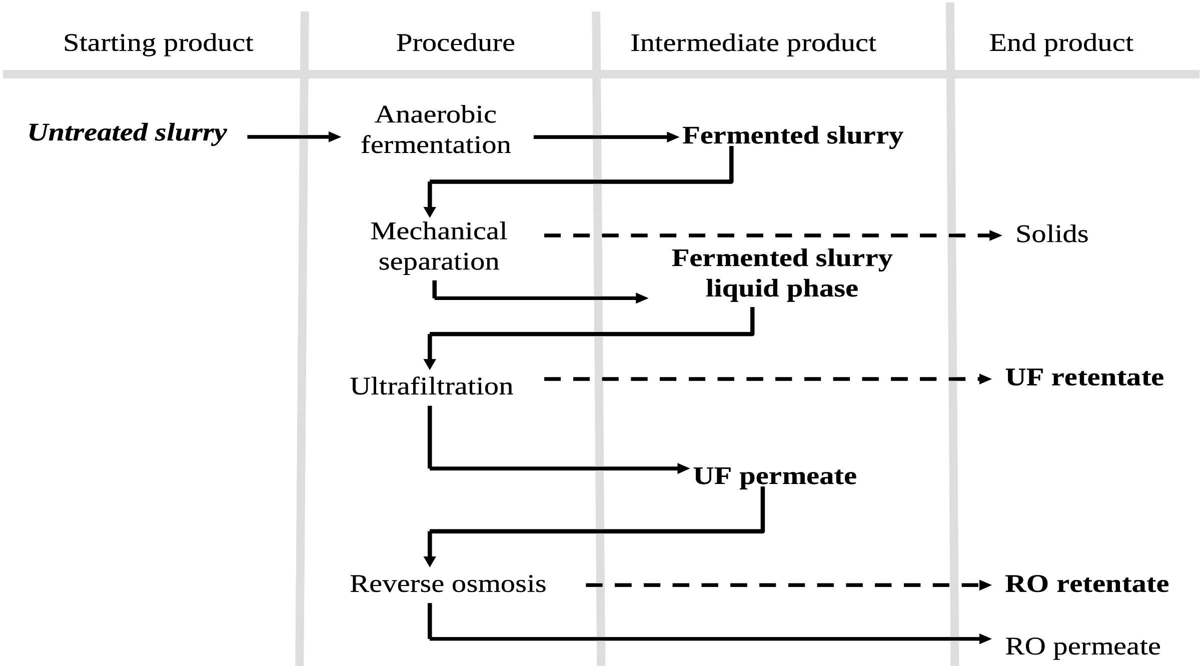

Figure 1: Procedures of slurry treatment to obtain the different fertilizer products. Only fertilizer products written in bold were used for pot and field experiments.

Pot and field experiments

Pot experiments were carried out with summer wheat (*Triticum aestivum* L. var. Fiorina) and maize (*Zea mays* var. Delitop) at Agroscope Reckenholz-Tänikon Research Station ART, Switzerland. Field experiments were conducted with winter wheat (*Triticum aestivum* L. var. Zinal) at two sites (Affoltern and Oensingen, Switzerland). The design of the pot and field experiments was a complete randomized block design with four repetitions for each fertilizer product. The fertilizer products used in the pot and field experiments, respectively, are summarized in Table 1.

Table 1: Fertilizer products used in the pot and field experiments.

Fertilizer product	Pot experiments		Field experiments
	Summer wheat	Maize	Winter wheat
Untreated pig slurry (starting product)	x	x	x
Fermented pig slurry	x	x	x
Liquid phase of fermented pig slurry	x	x	x
UF retentate	x	x	x
UF permeate	x	x	x
RO retentate	x	x	x
Ammonium sulfate[a]	x	x	
Mineral fertilizer[b]	x	x	x
Unfertilized control treatment	x	x	x

[a]Obtained from ammonia-stripping; [b]Ammonium nitrate.

Application of total mineral N was 1 g pot^{-1} (0.038 m^2) for summer wheat, 1.3 g pot^{-1} (0.038 m^2) for maize and 135 kg N ha^{-1} for winter wheat.

Calculations

Apparent N use efficiency was calculated according to Eq. 1 using the difference method (Muñoz *et al.*, 2004):

$$NUE\ (\%) = ((Nuptake_{fert} - Nuptake_{nonfert})/total\ N_{applied}) \times 100 \qquad (1)$$

where $Nuptake_{fert}$ denotes total N taken up by crops from the fertilized treatment, N $uptake_{nonfert}$ total N taken up by crops from the unfertilized control treatment and $N_{applied}$ is total N applied with the fertilizer products.

Statistical analysis

Analysis of variance was performed by using the GLM procedure of the statistical analyses package SYSTAT 11 (Systat Software Inc., USA). The effect of fertilizer product on fertilizer N use efficiency were tested using a complete randomized block design. For analysis of variance percentage data was transformed using arcsin-transformation. In case of significant effects separation of means was conducted using Tukey's HSD (honestly significant difference) test with a significance level of $P \leq 0.05$.

Results and Discussion

Impact of slurry treatment to slurry characteristics

Anaerobic fermentation reduced dry matter (DM) of the pig slurry due to decomposition of organic matter by microorganisms (Tab. 2). The reduction of DM lowers the viscosity of the slurry and thus increases its flowability (Chatigny *et al.*, 2004). Thus, fermented slurry can flow off the crop rapidly and its infiltration into the soil is enhanced compared to untreated slurry. Rapid incorporation of slurry into soil may reduce gaseous N losses. During UF and RO, respectively, DM increases in the retentates (Tab. 2).

Anaerobic fermentation usually increases pH of slurry as a part of organically bound N is converted to ammonium carbonate (Kirchmann and Witter, 1992). In our study we could only detect a very slight increase in pH during AF of the slurry, maybe due to a relatively high pH of the untreated pig slurry. However, UF and RO led to an increased pH of the permeate and the retentates (Tab. 2). As the dissociation equilibrium shifts from ammonium (NH_4) to ammonia (NH_3) at increasing pH there is a higher risk of NH_3 losses during storage or application for these fertilizer products (Pötsch *et al.*, 2004). Thus they have to be incorporated into the soil rapidly after application to keep NH_3 losses low.

Only marginal changes in N_{tot} content of slurry fresh matter should occur during AF as small amounts of N can volatilize to the biogas. The decrease of 15% in total N content of slurry during AF in our study (Tab. 2) could not be explained conclusively. During AF organically bound N is mineralized to plant available N causing an increase in NH_4-N concentration and in parallel a decrease of organic N in the slurry (Gutser *et al.*, 2005; Kirchmann and Lundvall, 1998) (Tab. 2). UF and RO caused a further increase in NH_4-N concentration especially in the RO retentate, whilst NH_4-N of the UF retenate was comparable to the concentration in fermented slurry. This can be explained by the fact that organic N compounds (e. g. proteins) do not pass the semipermeable membrane and thus remained in the UF retentate whilst ions (e. g. NH_4^+) pass the membrane and are recovered in the UF permeate. The conversion of organically bound N into NH_4-N improved plant N availability compared to untreated slurry. N release from the treated fertilizer products thus becomes more predictable allowing a more precise nutrient management. But the concurrent rise of pH with increasing NH_4-N concentration augments the risk of N losses during storage and application.

Table 2: Selected properties (dry matter [DM] content, pH and N content) of the different fertilizer products from slurry treatment.

Fertilizer product	DM	pH (H_2O)	N_{tot}	NH_4-N	Proportion of NH_4-N to total N
	%		….g kg^{-1} FS….		%
Untreated pig slurry	2.8	8.26	4.6	3.1	67.4
Fermented pig slurry	1.9	8.30	3.9	3.4	87.2
Liquid phase of fermented pig slurry	1.9	8.52	4.0	3.4	85.0
UF retentate	4.6	8.53	6.0	3.8	63.3
UF permeate	1.1	8.68	3.4	3.3	97.1
RO retentate	3.7	8.81	7.8	7.6	97.4

Calculation of the mass balance over the full slurry treatment chain (including AF, UF and RO) showed that a considerable amount of water was removed from slurry reducing the volume of RO retentate compared to untreated slurry by approximately 60% (data not shown).

<u>NUE of the different fertilizer products</u>

Pot experiments:

Except for the UF retentate and in part for the RO retentate slurry treated with AF, UF and RO generally showed significantly higher NUE than untreated slurry in the pot experiments with summer wheat and maize (Tab. 3). As mentioned before and as shown in Tab. 2 organic N compounds remained in the UF retentate. With a proportion of directly plant available NH_4-N to total N of about 60% UF retentate was comparable to untreated pig slurry (Tab. 2). In contrast to the UF retentate the proportion of NH_4-N to total N was considerably higher in fermented slurry (85-87%) as well as in the UF permeate (97%) and RO retentate (97%) (Tab. 2), which gave rise to a significantly higher NUE compared to UF retentate or untreated slurry (Tab. 3). Despite a proportion of 97% of direct plant-available N, N from RO retentate was poorly utilized by maize (Tab. 3). As low utilization of RO retentate did not occur in the pot experiment with summer wheat, we assumed that because of a high salt concentration in the RO retentate (data not shown) N utilization by maize might has been restrained due to its susceptibility to high salt concentrations. However, NUE of fermented slurry or fertilizer products resulting from UF and RO was significantly lower than of mineral fertilizer (ammonium nitrate) (Tab. 3). Only ammonium sulfate resulting from ammonia-stripping achieved NUE levels of the mineral fertilizer (Tab. 3).

Field experiments:

No significant differences in NUE between the fertilizer products resulting from AF, UF or RO and the untreated pig slurry and mineral fertilizer, respectively, could be detected in the field experiments; this might be due to higher variability in the field than in the pot experiments (Tab. 3). However, N utilization from treated slurry by winter wheat tended to be higher compared to untreated slurry.

Table 3: Apparent nitrogen use efficiency (NUE) of the different fertilizer products determined in pot and field experiments. Standard deviation is shown in brackets. (n = 4).

Fertilizer product	Pot experiments: Summer wheat	Pot experiments: Maize	Field experiments[a]: Winter wheat
	……………………..NUE (%)……		……………………………
Untreated pig slurry	30.9 (4.3) d	28.0 (3.8) ce	37.1 (8.0) b
Fermented pig slurry	48.3 (4.3) c	52.6 (4.5) b	55.9 (11.3) ab
Liquid phase of fermented pig slurry	50.9 (4.2) bc	46.8 (2.3) b	56.3 (6.9) ab
UF retentate	36.8 (7.3) d	21.7 (1.2) e	42.9 (1.3) b
UF permeate	58.2 (3.3) b	47.7 (2.6) b	53.7 (8.4) ab
RO retentate	50.1 (2.8) bc	36.6 (2.0) c	54.6 (7.3) ab
Ammonium sulfate[b]	77.0 (4.9) a	62.0 (4.7) a	*n.t.*
Mineral fertilizer[c]	67.8 (15.5) a	69.9 (4.7) a	63.3 (9.0) a

[a] Because of no significant differences only values of the experimental site Affoltern are shown.
[b] Obtained from ammonia-stripping.
[c]Ammonium nitrate.
n.t. not tested.
Within columns, means followed by different letters are significantly different ($P \leq 0.05$) by Tukey's HSD test.

Conclusions

Results from the pot and field experiments showed that fertilizer products resulting from UF (UF retenate and UF permeate) and RO (RO retenate) are suitable products for fertilization in agriculture. New technologies (AF in combination with UF and RO) for slurry treatment in combination with accurate storage and low-emission application techniques (e. g. trail hose) have potential to increase N use efficiency of slurry and to reduce N emissions to the environment. Because of the high proportion of direct plant available NH_4-N particularly UF permeate and RO retentate could at least partly substitute mineral fertilizer. The reduction of the transport volume of treated slurry might mitigate the problem of local nutrient excess (transport to regions with undersupply).

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INFLUENCE OF POMACE APPLICATION ON THE EFFECTIVENESS OF A CHEMICAL FERTILIZER

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Abstract

The excessive and exclusive use of chemical inputs without taking into account any appropriate recycling of organic residues has contributed to the reduction of OM contents in many agricultural soils. Consequently, the decrease in these contents propitiates a reduction in the effectiveness of chemical fertilizers.

It is well known, too, that the excessive use of chemical fertilizers triggers the risk of soil and water pollution. Neither are they considered to be improvers of the soil, their effect in this sense can be indirect by means of an increase in biomass production.

However, organic fertilizers can be catalogued as soil improvers as they tend to improve their structure, which facilitates water infiltration and root growth, enabling a better aireation and contributing to erosion control thanks to enhance nutrients availability.

Both types of fertilization have their advantages and drawbacks, so that a complementary effect between both should be sought since neither of these systems in isolation can make agriculture advance towards sustainability.

Keywords: Organic amendment, nutrients availability.

Introduction

The use of organic residues as fertilizers is as old as Agriculture itself. In the past few years, this agricultural practice has been increased due to its fertilizing value and the high financial and environmental costs associated with eliminating this residues. Among these types of residues is pomace, a product resulting from the two-phase extraction process of olive oil, of special interest in an area with a long olive-growing tradition, in which large masses of it concentrated over time are generated, making its management and elimination difficult. Only in Spain, approximately 4,000,000 Mg year^{-1} of this by-product is generated between November and December (López-Piñeiro *et al.* 2008).

One alternative solution to its elimination is using it as an amendment in the agrarian soils. The works of Cabrera (1994, 1995); Tomatti *et al.* (1995 and 1996); Cegarra *et al.* (1996); Cabrera *et al.* (2002), among others, have confirmed the good results observed with the agronomic use of this olive industry's residues. It is one of the organic residues with the highest content in potassium, this having been confirmed in several studies in which the levels of this element in the soil increased notably with the addition of this by-product (González *et al.* 2003), apart from its richness in carbon, a logical result of being an organic residue. The objective of this work was to evaluate the influence of the application of an organic amendment on the main nutrients in the soil, as well as on the amount and quality of harvests.

Material and methods

The assay was carried out on a farm situated in Andalusia, southeastern Spain. On this farm there are some plots on which an average of 10 t/ha of dehydrated pomace has been applied since 1998 in a wheat-sunflower rotation, except in the 2000/01 and 2003/4 growing seasons when its application was stopped. The last amendment was applied in the 2004/05 growing season, and from then on the residual effect of the treatments on the characteristics of the soil and the harvests was estimated. This work gives the data corresponding to the 2004/05, 2005/06 and 2006/07 growing seasons.

As pomace is a residue with a highly unbalanced C/N ratio, this induces an immobilization of the anion nitrate, which can cause, even at the beginning of the treatment, deficits of this element. That is why, in addition to the variable "pomace", that of nitrogenous fertilizer (Urea 40%, P_2O_5 18% and ClK 60%) has been considered in the assay with treatments of 0, 100 and 250 kg/ha in the wheat seasons and of 0, 50 and 125 kg/ha in the sunflower ones. Close to the plots receiving the amendment (A), other witness (T) ones were placed to which only the nitrogenous fertilizer was applied in the same doses as those of the amended plots.

Before beginning the study, the soil in the experiment farm was sampled and analyzed to evaluate their physicochemical characteristics. The result is shown in the following table 1.

Table 1: Chemical characteristics of the study soil.

pH in H_{2O}	8.24	**Carbonate %**	18.4
OM %	2.05	**C.I.C ($mol_c\ kg^{-1}$)**	0.298
ON %	0.11	**Avail.K (ppm)**	611
Textural class	Clayey-silty	**Avail. P (ppm)**	17

It can be seen that it is a soil with a basic pH and a high percentage of carbonates and low level of organic matter.

In the 2004/05 season, the pomace samples used in the trial were collected and analyzed. The result of these analyses appears in the table 2.

Table 2: Pomace characteristics. Total macro and micronutrients. Results referred to dry weight.

pH	6.8	**Mg (%)**	0.96	**N org. (%)**	1.1	**Cu (ppm)**	13.3
EC (dS/m)	1.2	**Na (ppm)**	180.0	**C/N**	32.2	**Mn (ppm)**	66.7
OM (%)	60.3	**K (%)**	0.29	**P (%)**	0.03	**Zn (ppm)**	9.8
OC. (%)	35.5	**Fe (ppm)**	2.6	**Ca (%)**	1.2	**Humidity (%)**	49.6

The experiment was designed as random blocks of plots with four replicates. Each plot measured 6 m*24 m and, in turn, it was divided into subplots of 6 m* 8 m. Each of the plots received all the different nitrogenous fertilizing ie, in the case of the wheat season, one subplot with the dose o kg/ha, one with the dose 100 kg /ha and other with 250 kg /ha. The experiment plots were managed in the same way as was done by the farmer in the rest of the farm and it was characterized by the exclusive use of disk ploughs and scarifiers.

Results and Discussion

Next, the evolution of the contents in the soil of the different nutrients studied for the different treatments considered is shown (A= amended; T= witness).

Nitrogen

The nitric nitrogen content in the topsoil horizon was always higher in the plots treated with amendment regardless of whether they had also been fertilized, these increases were of 5.13 %, 18.2 % and 20.5 % for the doses 0, 100 and 250 kg /ha can be seen in Table 3. This led one to think that the half-term mineralization of the organic residue applied with the amendment could encourage the increase of this anion in the soil, and, with it, its availability to the plant.

These results coincide with those obtained by Hati *et al.* (2006) in a long-lasting trial in which they applied chemical fertilizer treatments at different doses along with an organic amendment. Their results show that, in the case in which the two treatments were applied jointly, the contents in organic matter increased as well as nutrient contents such as nitrogen.

In the deeper soil horizon, the effect of the amendment was observed only in non fertilized soils (A0 vs. T0); while in fertilized plots, the greater concentration of the anion was noted in non amended plots (T), although, as in the previous case, with no significant differences.

Table 3: Content in nutrients in the study soil for the depths of 0-6 and 6-20 cm. Comparison of means on the basis of the analyses of variance and the Tukey test at a level of probability of 0.05. Different letters represent significant differences at a level of probability of $p^{*}\leq 0.05$., $p^{**}\leq 0.01$., $p^{***}\leq 0.001$.

Treatment	$N.NO_3^-$	$N.NO_2^-$	$N.NH_4^+$	P	K	O.M.
	mg/kg					*%*
Depth 0-6 cm						
A 0	11.7 c**	0.29 a	9.5 b**	27.9 a**	862 a**	3.9 a**
A 100	35.1 b**	0.42 a	10.5 b**	28.1 a**	877 a**	3.9 a**
A 250	66.8 a**	0.33 a	17.9 a**	29.1 a**	882 a**	4.1 a**
T 0	11.1 c**	0.25 a	6.8 b**	27.8 a**	600 b**	3.2 b**
T 100	28.7 b**	0.20 a	9.3 b**	26.2ab**	603 b**	3.2 b**
T250	53.1 a**	0.22 a	12.9ab**	22.3b**	576 b**	3.1 b**
Depth 6-20 cm						
A 0	14.6 c***	0.45 a	5.0 a	18.8 ab*	686. bc***	4.3 a*
A 100	32.9 c***	0.38 ab	5.5 a	21.6 ab*	655. bc***	4.0 a*
A 250	69.9 ab***	0.31 ab	5.1 a	25.2 a*	779. a***	4.3 a*
T 0	11.6 c***	0.48 a	5.0 a	20.3 ab*	523. c***	3.6 b*
T 100	40.2 bc***	0.38 ab	6.3 a	21.8 ab*	578. bc***	3.7 b*
T250	74.6 a***	0.23 b	6.1 a	17.0 b*	524. c***	3.8 b*

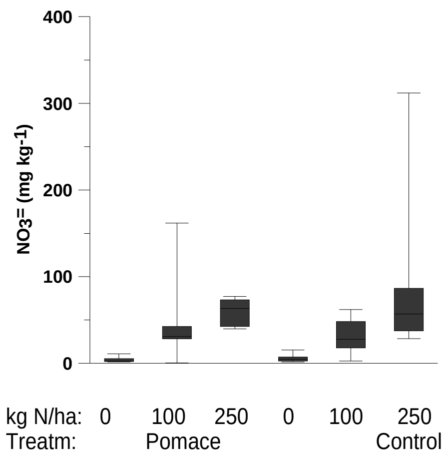

Figure 1: Mean content in NO_3^- for the different treatments in the first 6 cm of the soil profile. Each value represents a total of 348 analyses.

<u>Phosphorus</u>

The same as occurred with nitrogen, the residual effect of the organic amendment applied increased the content in available P in the treated soils in the surface horizon (Table 3) although with no significant differences vs. the only N-fertilized plots, except than in the N2 plots (250) – while in deeper down the P concentration resulted lesser than in only N-fertilized plots, except, one more time, than in N2 plots, with significant differences. The concentration of this element in pomace was not very high, and, also, its presence was associated with organic forms, so that no important increase in the available form of this element in the soil was to be expected.

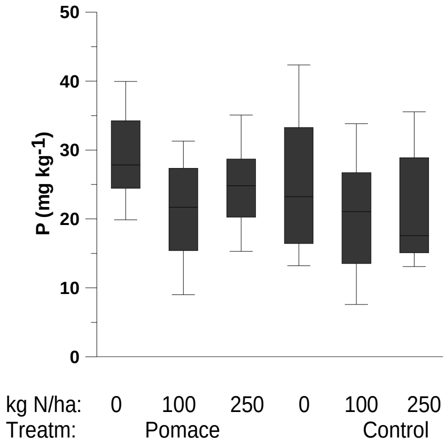

Figure 2: Mean content in P for the different treatments for the first 6 cm in the soil profile. Each value represents a total of 348 analyses.

The degradation of the organic matter accumulated from the application of the successive amendments signified an increase in the content of phosphorus in the A0 treatment soils and in those with the maximum dose of fertilizer, close to 50%. In the case of the soils fertilized with 100 kg N/ha, the available P values of the soils treated with the residue were similar to those of the witness plots to which this fertilizer dose was applied.

<u>Potassium</u>

In all the cases, the potassium concentrations were much higher in the soils treated with pomace regardless of the fertilizer dose received. The differences were significant in both horizons, although they became attenuated as one went deeper down in the profile.

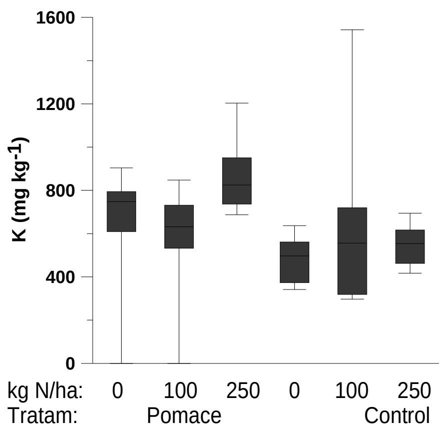

Figure 3: Mean content in K for the different treatments for the first 6 cm of the soil profile. Each value represents a total of 348 analyses.

As can be seen in Figure 3, the most significant increases were produced in the cases of fertilizer doses of 0 and 250. But, above all, one should highlight the data of the treatment with 0 kg/ha since, in this case, there was a notable increase exclusively due to the supply of pomace The gain of this nutrient was of the order of 50% for the non fertilized soils (?) and it dropped with the increase in the dose of fertilizer applied.

<u>Effect of the amendment on harvests</u>

- *Season* 2004/05. Crop grown, wheat.

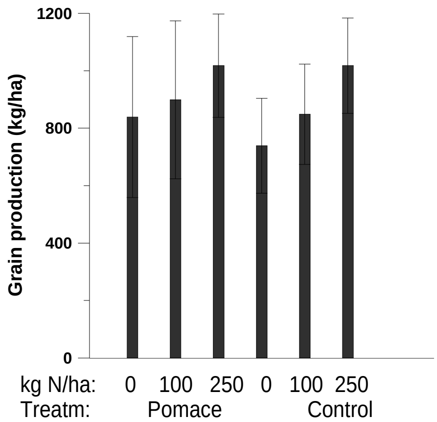

Figure 4: Grain yield for each of the treatments assayed.

The treatments combining both types of fertilization gave the best yields. At same dose of nitrogenous fertilizer, the application of the organic amendment favoured the increase in grain yield. The increment in the plots amendment were 26.1%, 105 and 25 for the doses 0, 100 and 250.

The data recorded indicate that by giving a joint treatment with both fertilizers one can obtain excellent production results as well as a decrease in the amount of fertilizer employed. This implies a saving for the farmer not to mention the great advantage to the environment signified by, on one hand, providing an outlet for a potentially pollutant product like pomace, and, on the other, the diminution in the use of fertilizers which produce so many problems in aquifers and underground water from pollution due to leaching.

- *Season* 2005/06: Crop grown, sunflower

Table 4: Production in kg/ha and fat yield in assay plots.

Treatment*Block	Mean values of the crop parameters	
	Production (Mg/ha)	Fat yield %
A 0	0.84 a	41.9 a
A 50	1.14 a	42.3 a
A 125	0.77 a	41.5 a
T 0	0.66 a	40.2 a
T 50	0.78 a	41.8 a
T 125	0.65 a	39.3 a

As can be deduced from the data given in Table 4, there were no significant differences between the different treatments studied. However, it should be pointed out that when the variable "amendment" was studied, differences were found between the plots treated with the pomace and the witness ones, the former showing a higher production as well as a higher fat yield.

- *Season* 2006/07: Crop grown, wheat.

Table 5: Mean yields found for the different treatments.

N fertilizer dose	Grain	Straw	Weight 100 cc	Weight 100 grains	Nº ears/m²
	Mg/ha		g		
Plots amended with pomace					
A0	1.293 a	1.150 a	83.3 a*	3.4 a	393.2 a
A100	1.232 a	1.279 a	80.6 ab*	3.4 a	426.5 a
A250	1.229 a	1.378 a	82.9 a*	3.5 a	447.2 a
Witness plots					
T0	1.296 a	1.091 a	77.7 b*	3.3 a	404.5 a
T100	1.322 a	1.246 a	79.9 ab*	3.2 a	442.7 a
T250	1.187 a	1.219 a	79.6 ab*	3.3 a	382.0 a

In this season a higher production of grain (+ 35%) and straw was obtained with respect to that of 2004/05, although no significant differences were observed except in the specific weight, which was higher (+ 4%)in the amended wheat.

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THE UTILIZATION OF SEDIMENTS ON AGRICULTURAL FARM LAND IN THE CZECH REPUBLIC

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Abstract

The rate of sediment contamination by inorganic and organic pollutants is a decisive factor for their utilization on agricultural farm land. For manurial effect of sediments, the content of available nutrients is very important, as well as other parameters, such as texture, share of organic matter, and acidity etc.

Present results from monitoring of sediment quality show significant variability of practically all parameters. Texture of sediments varied most and correlated to a great extend with the chemical composition of sediments. In comparison with limits of risk elements and hazardous substances (for sediments and soil), the absolute majority of sediments can be used as fertilisers on agricultural farm land in the Czech Republic.

Keywords : sediments, utilization, farm land, monitoring.

Introduction

Sedimentation is a result of natural erosive and transport processes in a catchment area. Consequently, sedimentation substantially limits and eventually blocks biologic, ecologic and water utilization functions of watercourses and ponds. On the other hand, sediments are valuable materials as fertilisers for agricultural farm land, particularly now that the input of nutrients and organic matter (in form of traditional mineral and organic fertilisers) to the soil in the Czech Republic is very low - for the year 2008 only 85.4 kg N, 13.8 P_2O_5 and 11.4 kg K_2O per hectare of agricultural land.

The estimated total amount of sediments in small ponds and rivers is 197 million m^3 and almost 5 million m^3 of sediments in irrigation channels in the Czech Republic. The Central Institute for Supervising and Testing in Agriculture provides the monitoring of sediments and simultaneously, according to Act No. 156/1998, Coll., on fertilisers, it is entitled to organise inspections of sediment utilization on agricultural farm land in the country. The objective of this paper is the results from monitoring of sediments as an inputs to the soil.

Materials and methods

<u>Monitoring of sediments applied on the agricultural farm land:</u>

Since 1995 to 2008 the Central Institute for Supervising and Testing in Agriculture has sampled and analyzed 319 samples of sediments (174 samples from so-called “field ponds”, 104 samples from so-called “village ponds”, 29 samples from forest ponds and 12 samples from watercourses).

The risk elements and hazardous matters (extracted by aqua regia) are determined in all sediments. The manurial effect of sediments is estimated on the basis of the content of available nutrients according to Mehlich III method.Furthermore, parameters such as texture, content of of organic matter (loss-on-ignition), and the acidity and the ratio C : N are determined.

Results and Discussion

- Considerable variability of sediments is clear from presented results practically in all parameters;
- According to the texture, the sediments contain all categories of soil classification scheme. More than half of sediments have classification “medium-heavy“ sediments;
- For agricultural land, the content of organic matter in sediments is important, but its amount balances very much. In average, the content of organic matter is almost 9% (8.88%);
- The sediment reaction is mostly sub acid and neutral. Acid reaction was determined at 46% of sediments, neutral in 41% and alkaline reaction in 13% of cases. The acidification of sediments is coming up after their exploitation and aeration;
- The available nutrient content is different in the sedimentation processes in comparison with the content of nutrients in the soil. The phosphorus content is lower, the potassium content is roughly parallel and magnesium content is almost doubled in comparison with contents of nutrients in the arable soils;
- The content of risk elements and hazardous matters shows more frequent contamination by cadmium (52 samples, i.e. 16.6%), zinc (30 samples, i.e. 9.6%) and arsenic (12 samples, i.e. 3.9%).

The content of organic pollutants in tested samples of sediments was under limit value.

Table 1: Texture, organic matter content, pH and the concentrations of some nutrients in the sediment samples (monitoring in 1995-2008).

	Texture (% of particles < 0,01 mm)	Organic matter (% of dry matter)	pH/ $CaCl_2$	Available nutrients in $mgkg^{-1}$ (Mehlich III)			
				P	K	Mg	Ca
Total average	29.61	8.88	5.93	41	222	333	4027
Watercourse	18.72	6.00	6.01	62	172	256	2800
Field pond	31.80	8.45	5.95	40	203	353	3729
Village pond	26.72	9.74	6.06	45	274	324	5281
Forest pond	50.37	9.57	5.09	31	189	258	1996

Table 2: Average values of risk elements and risk compounds (monitoring in 1995-2008).

	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn	AOX	PCB*
	$mg.kg^{-1}$ of dry matter (in extraction by aqua regia)									$mg.kg^{-1}$	
Total average	11.06	20.70	12.91	48.28	29.73	0.140	34.33	62.39	159.96	30.23	0.011
Watercourse	11.15	0.57	10.42	45.54	33.32	0.126	23.79	42.94	144.58	46.26	0.0037
Field pond	11.52	37.77	12.58	46.44	26.44	0.117	32.48	36.61	131.83	26.52	0.011
Village pond	10.50	0.70	14.33	53.05	36.31	0.174	40.55	118.29	222.96	28.72	0.013
Forest pond	10.30	0.53	11.32	43.74	24.60	0.161	27.83	27.37	113.77	62.33	0.0019
Limit values	30	1	30	200	100	0.8	80	100	300	no limit	0.2

*PCB = sum of congeners No. 28, 52, 101, 118, 138, 153, 180

Table 3: Number and percent of samples over prescribed limit values (monitoring in 1995-2008).

		As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn	PCB
The sum	**number**	12	52	2	1	3	2	7	11	30	0
	%	3.9	16.6	0.8	0.3	1.0	0.6	2.3	3.6	9.6	0
Watercourse	**number**	0	3	0	0	0	0	0	0	0	0
	%	0	25	0	0	0	0	0	0	0	0
Field pond	**number**	7	25	0	1	1	0	2	5	12	0
	%	4.2	14.8	0	0.6	0.6	0	1.2	3.0	7.0	0
Village pond	**number**	4	21	2	0	2	2	5	6	17	0
	%	4	20.2	2.4	0	2.0	1.9	5.0	6.1	16.8	0
Forest pond	**number**	1	3	0	0	0	0	0	0	1	0
	%	3.6	4.4	1.7	0	0	0	0	0	3.5	0

The results of sediment monitoring were used to determine limit values (for sediments and soil) as well as conditions and rules for their application on agricultural land in Czech legislation - Decree No. 257/2009 Coll., on sediments using on agricultural land (Table 4).

Table 4: Limit value for sediments and soils allowed to be used in agricultural land. The values are based on aqua regia extraction, except total content for Hg.

	The indicator value ($mg.kg^{-1}$ of dry matter)															
	As	Be	Cd	Co	Cr	Cu	Hg	Ni	Pb	V	Zn	PCB	PAU	BTEX	C_{10}-C_{40}	DDT
Sediment	30	5	1	30	200	100	0.8	80	100	180	300	0.2	6	0.4	300	0.1
Common soils	20	2	0.5	30	90	60	0.3	50	60	130	120	0.02	1.0	-	-	-
Light soils	15	1.5	0.4	20	55	45	0.3	45	55	120	105	0.02	1.0	-	-	-

PCB = sum of congeners No. 28, 52, 101, 118, 138, 153, 180
PAU = sum of anthracene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, fenanthrene, fluoranthene, chrysene, indeno (1,2,3-cd)pyrene, naphthalene, pyrene
BTEX – sum of benzene, toluene, ethylbenzene, xylene
DDT including DDD and DDE

Table 5: Maximum application rates (in tons of dry matter of sediments per hectare of agriculture land).

Texture of soil	Texture of sediment			
	sand clay	loamy	clay-loam	Clay
Common soils	600	750	450	300
Light soils (sand, gravel-sand, loamy-sand)	450	600	750	750

Main important conditions and rules for application of sediments on agricultural farmland (according to the Czech legislation – Decree No. 257/2009 Coll.):

- keeping limits of the risk elements and risk matters for sediments and soil,
- the conservation of positive physical, biological and chemical properties of soil by the application of sediments,
- the observance of maximal application rate of sediments to the soil (see. the table above),
- the ratio of applied sediments to plough layer is 1 : 3 (e.g. depth of plough layer is 30 cm - layer of applied sediments is 10 cm),
- the application of sediments on the same field is possible once in every 10 years and it is not allowed to apply sediments and sewage sludge on the same field in the same year.

Extra conditions:

- eco-toxicological tests might be ordered by a supervising body to double-check the quality of sediments.

Acknowledgement

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RELATIVE EFFICIENCY OF DIFFERENT SOURCES OF POTASSIUM IN THE FERTILIZATION OF CROP SYTEM PEAR MILLET AND SOYBEAN

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Abstract

In 2008, the consumption of potassium (K) fertilizer in the Brazilian agriculture was seven million tons, of which 92 % was imported, representing a cost of US$ 5 billion. The objective of this study was to evaluate the efficiency of different sources of potassium in the fertilization of pearl millet (as cover crop) and soybean. Field experiments were conducted at the Embrapa Maize and Sorghum, Sete Lagoas, Minas Gerais state, in the 2006/2007 and 2007/2008 growing seasons, on a Dystroferric Red Latosol (clayey Oxisol), cultivated with pasture of brachiaria and had Mehlich1 extractable K concentration of 40 mg dm^{-3} in the top 20 cm. Pear millet (*Pennisetum glaucum*) was sown in September over the residues of brachiaria, and Soybean (*Glycine max*) was sown in the first week of December, in the two growing seasons. The treatments consisted of three sources of K (Potassium Chloride-KCl, Rock-Biotite schist, and Byproduct-RMS) and four rates (0, 75, 150 and 300 kg K_2O ha^{-1}), applied in the first year, broadcast on the soil surface and incorporated into the soil at 10 cm depth. The rock-Biotite schist occurs naturally in Minas Gerais state and, when crushed present gray color, with 5.0 % of total K insoluble in water. The RMS is a byproduct of the manganese extraction industry, presenting brown color, with 10.0 % of total K soluble in water. The experiment was in random blocks, using a split plot design with three replications. Pearl millet dry matter yield and soybean grain yield were significantly affected by sources and rates of K. Pearl millet and soybean presented greatest response to K fertilization rates of 150 and 300 kg K_2O ha^{-1}, respectively. The byproduct-RMS, when applied at equivalent rates, is almost as effective as KCl and both were superior to Biotite shist for pear millet and soybean production. Our hypothesis that the crop sequence pearl millet/cover crop-soybean could improve the efficiency of the less soluble source of K was not confirmed in this research.

Keywords : sources potassium, natural rock, byproduct, *Pennisetum glaucum, Glycine max.*

Introduction

Potassium (K) requirement of crops is greater than requirements for all other nutrients except nitrogen (N). Whereas biological N fixation provides significant N imputs to terrestrial ecosystems, there are no renewable sources of K in the biogeo-chemical cycle. Crop K uptake is therefore solely derived from existing soil reserves, recycled K in crop residues, and applied K fertilizer (Cassman, 1996). Because the native soil K supply is a fixed quantity, increased food production will require a large proportional increase in K fertilizer use. Application rates to soil which is already deficient must increase in proportion to higher yield levels, and, in addition, K application will be required in many areas where soils do not presently require K inputs to achieve the current yield levels.

The high K demand by crops contrast with the concentrations, in general insufficient, that occur in Brazilian soils (Coelho, 2005). This fact, associated the astounding growth of the Brazilian agricultural production in the past few years, has lead to a great increase in K fertilizers consumption (Nachtigall and Raij, 2005). In 2008, the consumption of potassium fertilizer in the Brazilian agriculture was seven million tons, of which 92 % was imported, representing a cost of US$ 5 billion. These data justify the implementation of governmental policies aiming to explore the canallite ore reserves in Sergipe state as well as the sylvinite deposits in the Amazon state (Lopes, 2005). In addition to that, they must stimulate research concerning the economical feasibility of potassium silicates and mineral byproducts, abundant all over Brazil, as sources of K fertilizer. The objective of this study was to evaluate the efficiency of alternative sources of potassium in the fertilization of pearl millet (as cover crop) and soybean.

Material and Methods

Field experiments were conducted at the Embrapa Maize and Sorghum, Sete Lagoas, Minas Gerais state, during the 2006-2007 and 2007-2008 growing seasons, on a Dystroferric Red Latosol (clayey Oxisol), cultivated with pasture of brachiaria. Soil test indicated a pH 5.8, organic matter content of 34.5 g dm^{-3}, P-Mehlich1 level at 7.3 mg dm^{-3} and K level at 40 mg dm^{-3} in the top 20 cm. The treatments consisted of three sources of potassium (Potassium Chloride-KCl, Rock-Biotite schist, and Byproduct-RMS) and four rates (0, 75, 150 and 300 kg K_2O ha^{-1}), applied in the first year, broadcast on the soil surface and incorporated into the soil at 10 cm depth. The experiment was in random blocks, using a split plot design with three replications. The main plots were sources of K and the split-plot factor consisted of the rates.

The rock-Biotite schist occurs naturally in Minas Gerais State. Sample of this rock was ground to pass a 2-mm screen and chemically characterized. It presents gray color, 5.0 % of total K insoluble in water. The RMS is a byproduct of the manganese extraction industry, presenting brown color, with a degree of fineness less than 2 mm (10 mesh) and a content of 10.0 % of total K soluble in water. Chemical analysis of both materials indicated that they have low concentrations of CaO and MgO, with low effective calcium carbonate (ECC) rating (Biotite shist: 3.20 % and RMS: 29.33 %). The equivalent rates applied to supply the levels of 75, 150 and 300 kg K_2O ha^{-1} were: Biotite schist 1.5, 3.0, and 6.0 t ha^{-1}; RMS 0.75, 1,5 and 3.0 t ha^{-1} and; KCl (60 % K_2O) 125, 250 and 500 kg ha^{-1}.

Pearl millet (*Pennisetum glaucum*), cultivar 'ADR 300', was sown in 3.5- by 6-m plots in 0.35-m rows at a rate of 10 kg ha^{-1} in September, and fertilized with 30 kg N ha^{-1} plus 90 kg P_2O_5 ha^{-1}. In both years, biomass of pearl millet was determined at bloom stage (50 days after sowing). Before harvest, pearl millet was desiccated with glyphosate at rate of 1.0 -l ha^{-1}. The harvested biomass was weighed in the field and sampled for dry matter determinations. The sample was subsequently dried at 65 ºC in a forced draft oven, ground, and then analyzed for total N, P and K.

Fifteen days after pearl millet had been desiccated, soybean (*Glycine max*), cultivar 'Valiosa^{RR}', was sown in the first week of December at 0.35 m row spacing (250 thousand plants per hectare). In the first growing season (2006/07) soybean was not fertilized and the seeds were not inoculated with bacteria for N_2 fixation. In the second growing season (2007/08), soybean was fertilized with 30 -kg N ha^{-1} plus 90 -kg P_2O_5 ha^{-1} and, before planting, the seeds were inoculated with commercial powdered peat based granular *Brady Rhizobium*. Soybean grain yields were determined by hand harvesting six adjacent 5-m long rows (appropriately bordered) and reported based on a moisture content of 130-g kg^{-1}. After each harvest, plant and grain samples were collected and analyzed for total N, P and K. Data were analyzed by conventional analysis of variance procedures for split-plot design, using the PROC GLM of SAS (SAS Inst., 2001). Treatment means were compared by least significant difference ($P \leq 0.05$).

Results and Discussion

Pearl millet production in Brazil has recently been used as a soil improvement, cover crop for on two million ha of no-till production of soybeans (Bonamigo, 1999). In this system, where the pear millet biomass is not removed from the field, and if the soil fertility status is classified as optimum or higher, the application of fertilizers is not recommended (Pereira Filho *et al.*, 2005).

Pear millet dry matter yields responded to alternatives sources (Biotite schist and RMS) and rates of K, compared to KCl (Figure 1). Pear millet dry matter yields ranged from 2.0 to 5.0 t ha^{-1}, with average of 3.48 t ha^{-1}, and large responses to applied K were observed. In the first growing season (2006) the dry matter yield responses showed significant differences ($P \leq$ 0,05) for sources and rates of K application, but the interaction (K sources x rates) was not significant. The maximum dry matter yield was achieved with 75 kg-K_2O ha^{-1} supplied as RMS and KCl, which exhibited statistically similar dry matter, 4.00 and 3.60 t ha^{-1}, respectively. For the Biotite shist a linear response, until 300 kg K_2O ha^{-1}, was observed (Figure 1a). These results agree with the content and solubility of the K previously determined in the sources. For the second year (2007), similar results were observed (Figure 1b). However, for all sources, the maximum dry matter yield was obtained with application of 150 kg K_2O ha^{-1} (Figure 1b) and the production obtained with KCl (5.19 t ha^{-1}) was higher than the one obtained with RMS (3.70 t ha^{-1}) or Biotite shist (3.05 t ha^{-1}).

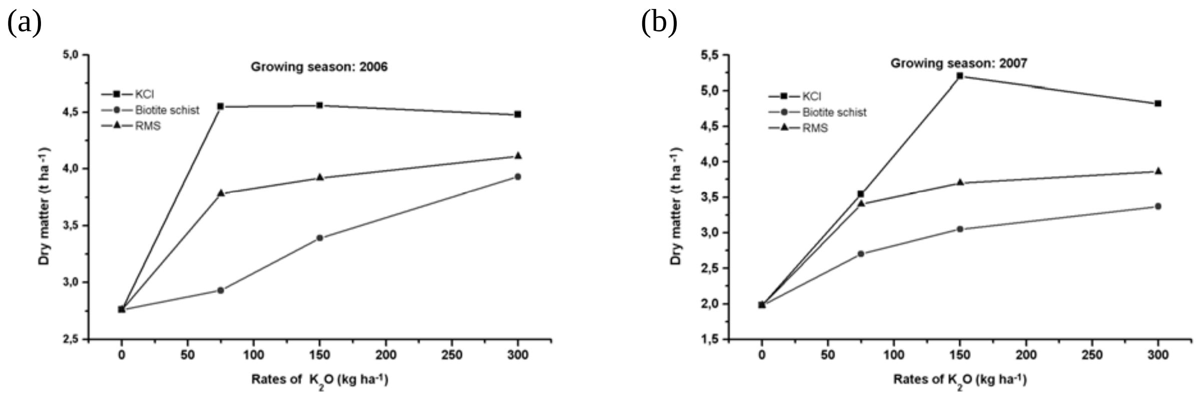

Figure 1: Pearl millet dry matter yields at different rates of K applied in various K sources in 2006 and 2007 growing seasons.

Total K accumulation in dry matter of pearl millet are shown in Table 1. The trends in K uptake in 2006 were similar to those in 2007. Although the sources were applied at equivalent rates, significant ($P \leq 0.05$) differences among rates and sources were observed in the K uptake by pearl millet and they were all greater than the control (Table 1). The K uptake from the Biotite shist treatment was significantly less than from the other sources, which is consistent with the degree of solubility of the K in the sources (Table 1). Upon application of a more soluble source (KCl), a large quantity of K was taken up, which is consistent with luxury uptake of K. Since pearl millet was used as cover crop, a large amount of K (40 - 190 kg ha^{-1}) was cycled from the plant back into the soil (Table 1).

Table 1: Total potassium accumulation in dry matter of pearl millet during the growing seasons.

Source	Rate	K-uptake - 2006		K-uptake - 2007	
	K_2O - kg ha^{-1}	g kg^{-1}	kg ha^{-1}	g kg^{-1}	kg ha^{-1}
KCl	75	20.4	92.74	19.8	70.60
KCl	150	38.2	174.50	21.3	109.71
KCl	300	42.7	189.91	22.6	109.32
Biotite shist	75	14.6	41.33	9.6	25.83
Biotite shist	150	16.4	55.93	9.6	29.23
Biotite shist	300	19.2	73.37	15.7	54.10
RMS	75	24.7	91.30	18.6	61.73
RMS	150	30.6	116.12	22.7	87.42
RMS	300	34.9	148.68	24.7	98.73
Control	0	12.0	32.96	12.3	25.68
Mean		23.1	90.23	16.8	60.31
CV, %		21.0	29.4	38.3	48.7

Soybean grain yields as affected by sources and rates of K application are showing in the Figure 2. Grain yields range from 820 to 2,400 kg ha^{-1} in 2006-07 and from 2,300 to 4,000 kg ha^{-1} in 2007-08 as K was increased from 0 to 300 kg K_2O ha^{-1} (Figure 2). The relative low yields obtained in 2006-07 (Figure 2a) were caused by the fact that the area never had been cultivated with soybean and the seeds were not inoculated with bacteria for N_2 fixation.

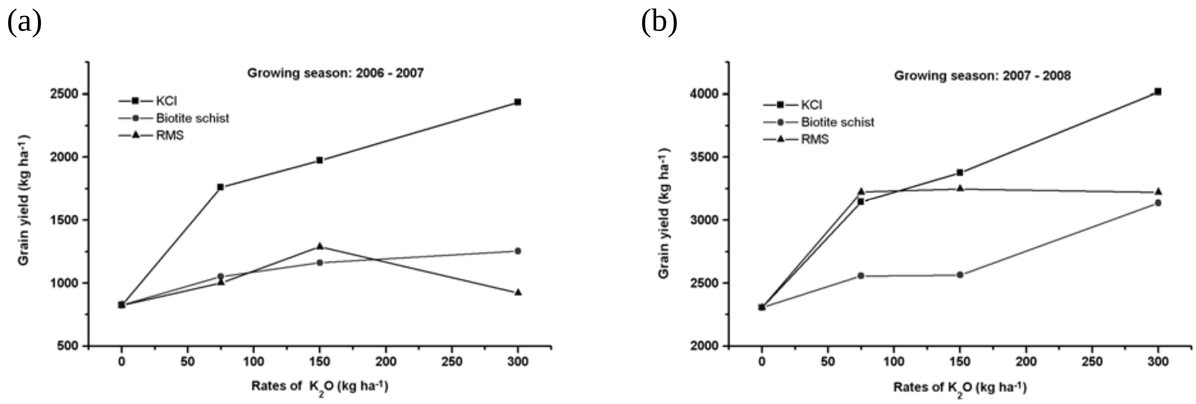

Figure 2: Soybean grain yields at different rates of K applied as various K sources in 2006-07 and 2007-08 growing seasons.

In the first growing season (2006-07), sources, rates and interactions treatments all had a significant effect ($P \leq 0.05$) on soybean grain yield. The treatments with the soluble source (KCl) yielded significantly more than the sources Biotite shist and RMS, with a linear response until the rate the 300-kg K_2O ha^{-1} (Figure 2a). However, in the second growing season (2007-08), while a linear response was obtained to rates applied as KCl and Biotite shist, a linear-plateau response was verified to the RMS with the maximum grain yield (3.200 kg ha^{-1})

obtained with application of 75 kg K_2O ha^{-1} (Figure 2b). These results indicated that the source RMS presented some detrimental effect, limiting soybean grain yield. On the other hand, our hypothesis that the crop sequence pearl millet/cover crop-soybean could improve the efficiency of the less soluble source of K was not confirmed in this research.

Conclusions

The results of this study show that pearl millet dry matter yield and soybean grain yield were significantly affected by sources and rates of K. Pearl millet and soybean presented greatest response to K fertilization rates of 150 and 300 kg K_2O ha^{-1}, respectively. This research also shows that byproduct-RMS, when applied at equivalent rates, is almost as effective as KCl and both were superior to Biotite shist for pear millet and soybean production. Our hypothesis that the crop sequence pearl millet/cover crop-soybean could improve the efficiency of the less soluble source of K was not confirmed in this research.

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ORGANIC AMENDMENTS APPLICATION ON MELON CROPS GROWN IN MEDITERRANEAN CONDITIONS: SOIL CHEMICAL PROPERTIES (SECOND NOTE)

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Abstract

A three-year field experiment (2006-2008) was carried out in a Mediterranean environment to study the effects of organic amendments application (anaerobic digestate and composted municipal solid wastes) on chemical properties of the soil cultivated at melon.

The research was conducted at Metaponto (MT - Southern Italy) on a clay soil (Typic Epiaquerts according to Soil Taxonomy). In a strip-plot experimental design with three replications two irrigations (re-establishment 100 and 50% of the calculated maximum evapotranspiration) and the following four fertilizer treatments were compared: mineral fertilizer (Min); commercial stable manure (Org-min); anaerobic digestate based on wine distillery wastewater (WDD); composted municipal solid organic wastes coming from the separate collection (SUW). Each fertilizer treatment received 150 kg N ha^{-1}.

During the trial period the total organic carbon (TOC) content and the umification fractions (TEC and HA+FA), the macro-nutrient content (total N, exchangeable K, available P) and the heavy metals variation (Cu, Zn, Ni, Pb) were determined.

At the end of trial period, the WDD significantly increased TOC, TEC and HA+FA of the 19, 25 and 14%, respectively in comparison with the Min treatment.

Among the treatments, no significant difference for the content of Zn, Pb and Ni was found, while significant differences where observed for Cu, which reached the highest levels for treatments WDD.

On the whole, the experimental fertilizers seem to increase soil fertility, but their application require careful agronomical practices in order to reduce pollution risks.

Keywords: Organic matter changes, by-products amendment, accumulation of heavy metals.

Introduction

Environmental, social and economical reasons have pointed out that in agriculture is not still possible to pursue a continuous increase of profits by applying conventional agronomical practices that have contributed to the progressive worsening of environmental conditions. Thus, it is necessary to preserve the primary resource, as soil and water, whose availabilities are indispensable for both present and future. Use of chemical fertilizers has been increased worldwide due to availability of inexpensive fertilizers (Graham and Vance 2000), which causes health and environmental hazards (Pimentel 1996). Possible options to reduce chemical fertilizers use may be adoption of recycling of organic wastes. Use of such organic materials in agriculture may contribute to preserve the environmental as well as improve farmland. Application of composts and organic amendments modify soil organic matter and nutrient cycling (Eghball 2002), and increase soil nutrient levels (Chantigny *et al.* 2002). Since organic wastes must be adequately processed in order to obtain an organic fertilizer or an amendment, two main processes are developed to transform organic materials into organic fertilizers for agronomic utilization: aerobic and anaerobic processes. Aerobic transformation of organic wastes takes to a stabilized and well humified material (compost) which is usually characterized by slow mineralization rate in soil. Anaerobic digestion, on the other hand, is based on the degradation of complex organic substances (proteins, lipids, carbohydrates) into less complex compounds which are subsequently transformed in low molecular weight organic acids (acetic acid, propionic acid, butirric acid), alcohols, aldehydes, carbon dioxide, hydrogen, with methane production. Lack of lignocellulosic materials in the digested organic matrices does not allow the production of a fertilizer characterized by high content of humic-like substances. On the light of these considerations, a three-year field research was carried out with the following aims: i) to study the effectiveness of both anaerobic digestate and MSW compost applications on the variation of soil native organic matter and humification process in Mediterranean conditions; ii) to monitor the changes in chemical soil fertility by organic materials compared with mineral fertilizers; iii) to evaluate the accumulation of heavy metals in soil after three years of amendment.

Materials and methods

The research was carried out during three years (2006-2008) at Metaponto (MT) in Southern Italy (lat. 40° 24’ N; long. 16° 48’ E and 8 meters above the sea level) located at the experimental farm of the CRA-Research Unit for the Study of Cropping Systems.

The climate is classified as "accentuated thermomediterranean" according to the UNESCO-FAO classification. The mean annual precipitation at the experimental farm is 490.6 mm, with more than 68% of the rainfall occurring during the winter months. The annual potential evaporation rate is high with a mean annual pan evaporation rate of 1561.5 mm. Evaporation rates are greatest during the months of June, July, and August, with mean monthly rates of 223.1, 268.5 and 232.0 mm, respectively. The total amounts of rainfall, recorded during May-August melon cropping cycles, were 123.5, 63.8 and 60.8 mm for 2006, 2007 and 2008, respectively. Melon plants (Cucumis melo var. Inodorus cv Rugoso di Cosenza), after being grown in the greenhouse to the four-leaf stage, were transplanted by hand on 19, 23 and 14 May for 2006, 2007 and 2008 respectively. Plants spacing were 60 cm between the plants within each row and 150 cm between the rows (density of 1.1 plant m^{-2}). The experimental design was a strip-plot with three replications. The main plots were two irrigation treatments and sub-plots were four fertilizer treatments.

The following treatments were compared: re-establishing 100 (I1) and 50% (I2) of the calculated maximum evapotranspiration (ETm); application of mineral fertilizer (Min), of a commercial stable manure admitted in organic farming (Org-Min) and of two experimental organic fertilizers, anaerobic digestate based on wine distillery wastewater (WDD) and composted municipal solid organic wastes coming from the separate collection (SUW). The fertilizer treatments were applied equally to supply nitrogen amount needs to the melon crop.

N dose was 150 kg ha^{-1} applied in one times, one month before transplanting, for all organic fertilizers and at transplanting for Min treatments as ammonium solphate. In the Table 1 main characteristics of commercial and experimental organic fertilizers are presented. Degree of humification (DH %), was calculated as Sequi *et al.* (1986).

Soil samplings were carried out, at 0-40 cm of depth before the application of the fertilizers and at the end of each cropping cycle; from October 2004 (start time t0) to October 2007 (end time tf). On the soil samples were determined the content of: total organic carbon (TOC), total extracted carbon (TEC), humic and fulvic acids (U+F), humification index (HI), degree of humification (DH), humification rate (HR), total nitrogen (Ntot), available phosphorus (P), exchangeable potassium (K), total copper (Cu), total nikel (Ni), tolal lead (Pb) and total zinc (Zn). The determinations were effected according to the official methods of soil chemical and fertilizers analysis, by the Office of the Agricultural and Forest Politics.

Statistical analysis of variance was made by SAS procedures (SAS Institute 1998). Differences among the means were analyzed at the $P \leq 0.05$ probability level, applying the Duncan Multiple Range Test (DMRT).

Table 1: Mean properties of applied organic fertilizers.

Parameters		Commercial stable manure	Experimental organic fertilizers	
			Wine distillery wastewater	**Municipal solid wastes compost**
Total organic carbon (TOC)	g kg^{-1}	290.9	316.7	220.6
Total extracted carbon (TEC)	g kg^{-1}	151.7	127.0	149.6
Humic and Fulvic Acid (U+F)	g kg^{-1}	82.1	94.4	111.9
Degree Humification (DH)	%	54.1	75.0	74.8
Total N	g kg^{-1}	49.3	42.4	21.1
C/N		5.9	7.5	10.5
Total P	g kg^{-1}	57.2	26.0	13.8
Moisture	%	5.7	63.5	17.5
Cu	mg kg^{-1}	28.7	493.8	98.2
Zn	mg kg^{-1}	156.1	53.0	213.5
Ni	mg kg^{-1}	3.0	3.3	11.0
Pb	mg kg^{-1}	0.0	0.4	92.5

Results and Discussion

At the end of trial period, not conventional treatments WDD and SUW have reached the highest levels of TOC, TEC and U+F content (Table 2), particularly, it shown that both treatments showed statistical significant differences in comparison to “Min”.

Table 2: Chemical soil characteristics**.**

Parameters		L0	Tf			
			Org-Min	**WDD**	**SUW**	**Min**
Total organic carbon (TOC)	g kg^{-1}	11.00	13.47 b	14.23 a	13.80 a	12.32 b
Total extracted carbon (TEC)	g kg^{-1}	5.90	6.89 bc	7.25 a	7.28 ab	5.97 c
Humic and Fulvic Acid (U+F)	g kg^{-1}	4.35	5.05 ab	5.64 a	5.63 a	5.01 b
Humification Index (HI)	%	0.36	0.36	0.28	0.29	0.19
Degree Humification (DH)	%	73.8	73.4	77.8	77.2	83.8
Humification Rate (HR)	%	39.5	37.5	39.7	40.8	40.7
Zn	mg kg^{-1}	73.7	63.3	63.5	65.8	63.5
Cu	mg kg^{-1}	27.0	31.6	37.8	33.6	33.4
Ni	mg kg^{-1}	25.3	32.0	32.4	32.6	32.6
Pb	mg kg^{-1}	6.8	13.5	13.9	15.0	14.1

This aspect underline that the amendment with organic matter characterized by low C/N rate and a good content of humification fraction (Table 1), can improve the soil organic content without generate the effect of triggering mineralization native soil organic matter (Passcual *et al.* 1997, Passcual *et al.* 1999). The humification parameters measured confirm that the treatment SUW and WDD have not disturbed microbiological equilibrium in the soil, in fact the values of HR respect to L0 were unchanged. In the treatment Org-min, the value of DH not shown significant variation respect to L0, while the Min treatment in same period displayed an increase of 14% caused by the increase of U+F content. The treatments SUW and WDD have recorded an increase of DH% generate by increment of both TEC and U+F.

The contents of soil macronutrients was reported in the Figure 1, 2 and 3. These figures shown that at the end of the trial period, the exchangeable K soil content (Fig. 1) was found for all treatments higher than L0 value, this effect was generate by a good soil content of native exchangeable K combined with fertilization. The content of soil available phosphorus at the end of research (Fig. 2) was higher than L0 for all experimental treatments. WDD phosphorus content was increased less than remaining treatments probably due to pH value which allowed to release a larger amount of phosphorus for plant nutrition (first note fig. 1).

Figure 1: K soil content.

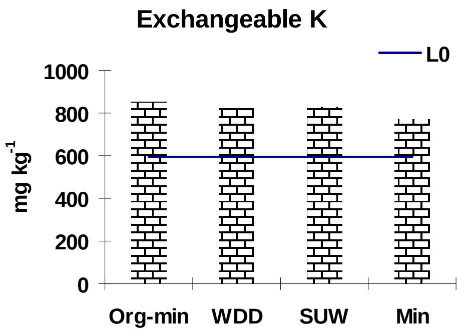


Figure 2: P_2O_5 soil content.

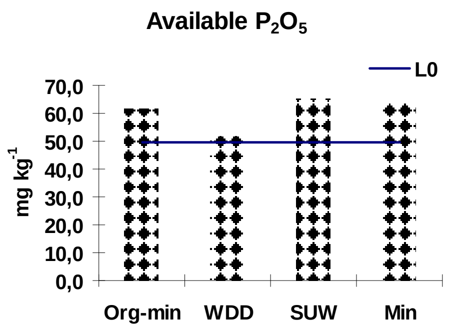


Figure 3: N Total soil content.

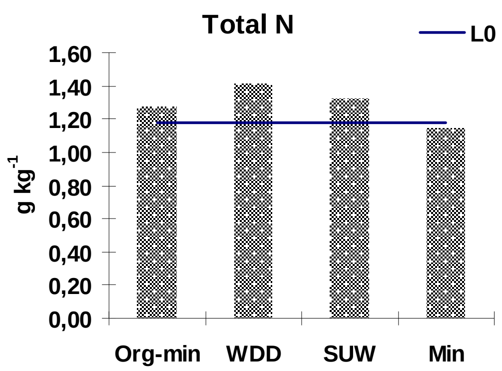
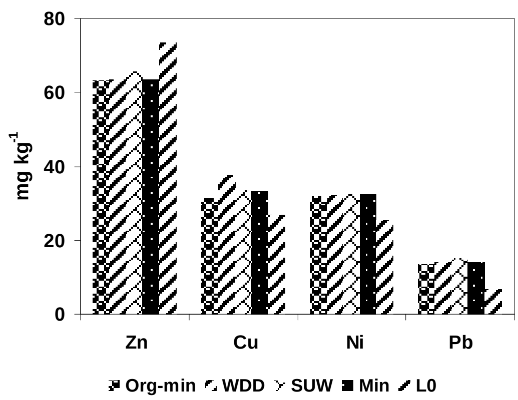


Figure 4: Heavy metals content in soil.



Total N soil content (Fig. 3) in Min treatment was lower than L0, at some time, the organic treatments Org-min, WDD and SUW was recorded an increase of 8%, 19% and 12% respectively.

Organic experimental treatments (SUW and WDD) did not lead to accumulation of heavy metals after three years of application (Fig. 4), however we have recorded in WDD treatment an increase of Cu soil content of the 13% respect Min while the treatment SUW were increase Pb content of the 6.4 %.These variations depended on the content of heavy metals of the same treatments.

On the whole we observed that the quality of the arid soil was improved with organic amendments (Passcual et al, 1999), bat at same time is necessary to control the heavy metals accumulation by agronomical practices.

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POTENTIAL USE OF OLIVE POMACE COMPOST AS AMENDMENT ON A CHICKPEA-EMMER ROTATION IN ORGANIC FARMING*

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Abstract

Olive mill wastes are generated in a large quantity in the Mediterranean area and their incorrect disposal may have a great impact on land and water environments. Olive pomace could be recycled by composting which could avoid potential phytotoxic effects due to its application in the cropland. The objective of this trial was to evaluate the agronomic performance of four kinds of olive pomace compost (OPC), on a chickpea-emmer rotation in an organically managed system.

A field research was carried out in November 2008 in Foggia, Southern Italy. The four types of compost were obtained through an aerobic transformation of two different mixtures of olive pomace blended with pruning wastes and cattle manure, splitted both in a heap led to maturation as a turned windrow and a heap whose composting process was stopped just after the termophilic phase and dried up outside. The following treatments were compared: OPC, from a starting mixture with C/N ratio equal to 45, either not stable (A1) and stabilized (A2); OPC, from a starting mixture with C/N ratio equal to 30, either not stable (B1) and stabilized (B2); a commercial organic-mineral fertilizer and an unfertilized control. Before the beginning of the field trial, a bioassay was performed to assess the phytotoxicity both for the raw pomace and the two typologies of compost with higher risk of phytotoxicity, A1 and B1, by the measurements of seed germination percentage and root elongation of cress.

No phytotoxic effect was detected in OPCs in comparison to raw pomace. The field application of four OPCs showed comparable emmer yield values. On the other hand, only the A2 application revealed good chickpea yield results as high plant residues (19.81 g plant^{-1}),

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seed weight (17.64 g $plant^{-1}$) and pods weight (22.76 g $plant^{-1}$). This outcome showed a presumable crop species specific sensitiveness.

Keywords: Olive pomace compost; Phytotoxicity; Chickpea-emmer rotation; Organic farming.

Introduction

In many Mediterranean countries, the olive oil agro-industrial sector yields, yearly and in a short period of time, great amounts of organic residual materials, both liquid and solid depending on the system used to extract the olive oil (Alburquerque *et al.*, 2009). The modern two-phase system produces, as the only residue, a moist olive pomace with a doughy texture; consisting of lignin and cellulose; rich in polyphenolics, unextracted oil and organic acids (Saviozzi *et al.*, 2001; Niaounakis and Halvadakis, 2006).

A gradual accumulation of olive pomace and its incorrect disposal, may have a damaging impact on the environment, due to phytotoxic and antimicrobial effects of phenolic compounds and lipid fraction (Roig *et al.*, 2006). However, this waste also contains a large proportion of organic matter and nutrients, particularly potassium, which could be recycled by aerobic biodegradation, in accordance with the more basic agro-ecological principles of organic farming. Composting olive pomace, prior to its re-use in agriculture as fertilizer or amendment, should avoid, at the same time, some of its adverse effects owed to toxic compounds (Alfano *et al.*, 2008; Sellami *et al.* 2008). Many complementary residues are usually generated in the Mediterranean area, such as animal manure, olive leaves, cereal straw, almond shells or pruning wastes so that the composting of olive pomace, despite its unsuitable physical characteristics, is feasible (Baeta-Hall *et al.*, 2005; Canet, *et al.*, 2008).

The objective of this field trial was to evaluate the agronomic performance of olive pomace compost (OPC) as amendment on a chickpea-emmer rotation, in organic farming.

Materials and methods

A field research was carried out in November 2008 in the Experimental Farm of the CRA, Research Unit for Cropping Systems in Dry Environments (Foggia, Southern Italy) on a chickpea-emmer rotation under organic farming management. The climate is characterized by winter temperatures which can fall below 0°C, summer temperatures which can rise above 40°C and low rainfall, unevenly distributed during the year. The soil is silty-clay of alluvial origin, classified as a Fine, Mesic, Typic Chromoxerert by Soil Taxonomy-USDA, with total N 1.39 g kg^{-1}; total C 13.5 g kg^{-1}; available P_2O_5 82 mg kg^{-1}, exchangeable K_2O 1108 mg kg^{-1};

pH 8.3. The site was prepared for planting by plowing during late summer and disk harrowing to prepare seedbed. In a randomized-block design with three replications on plots of 40 m^2, the following six treatments, in both crops, were applied, allowing a distribution of 80 kg ha^{-1} of N for emmer and 35 kg ha^{-1} of P_2O_5 for chickpea: OPC, from a starting mixture with high C/N ratio, either not stable (A1) and stabilized (A2); OPC, from a starting mixture with a lower C/N ratio, either not stable (B1) and stabilized (B2); a commercial organic-mineral fertilizer (OM) and an unfertilized control (CT). The four types of compost were uniformly applied in one solution about 1 month before sowing (occurred on 19 December) and buried with a rotary hoe. The OM was applied in two times for the emmer: 1/3 N at sowing and 2/3 N at the fast growth phenological stage, whereas for the chickpea it was applied in one solution at sowing. During the cropping cycles, plants were sampled and tested for LAI, fresh and dry weight and leaves green index (data not reported). Moreover, at harvesting, which occurred at 202 and 216 days after sowing for emmer and chickpea respectively, plants were tested for yields and their components.

The composts used in the field trial were obtained in the experimental plant of CIHEAM-IAMB, through the composting process of two different mixtures of olive pomace collected from a two-step olive oil mill. In particular, one mixture with a high C/N ratio (equal to 45) by 53.8% of pomace, 7.7% of pruning wastes and 38.5% of cattle manure, and another one with a lower C/N ratio (equal to 30) by 9, 45.5 and 45.5%, respectively, of the same three wastes, were prepared and processed in bio-containers. At the end of the active phase, each mixture was removed from the container, put outdoors and splitted in a heap led to maturation as a turned windrow and a heap left immature and dried up outside, in order to obtain the four types of OPCs. The main chemical characteristics of OPCs produced, are presented in Table 1.

Table 1: Chemical characteristics of the composts (means of three values calculated on the dry weight basis).

Parameters	A1	A2	B1	B2
TOC (g kg^{-1})	425.3	416.3	414.4	377.4
Total N (g kg^{-1})	10.5	15.4	14.2	17.9
Total P (g kg^{-1})	1.55	3.21	3.70	6.35
C/N	40	27	29	21
EC (mS cm^{-1})	0.629	0.559	1.373	1.358
Cu (mg kg^{-1})	12.46	58.03	64.36	60.39
Zn (mg kg^{-1})	39.22	288.04	329.10	282.73

To assess the phytotoxicity, both for the raw pomace and the two typologies of compost, the A1 and B1, which were more at risk in terms phytotoxicity, a bioassay was performed.

Therefore, the germination index (GI) for *Lepidium sativum* L. (Zucconi *et al.* 1981) on three dilutions of extract (decreasing from 25% to 50% and 75% in deionized water) used as germination media, as well as on concentrated extract only for raw pomace, was measured.

Statistical analysis was carried out using the SAS software package (Sas Institute, 1990). Differences among treatments were evaluated using Duncan's Multiple Range Test at the $P \leq 0.05$ probability level.

Results and Discussion

The phytotoxicity of raw olive pomace to cress, at decreasing dilutions from 25 to 75%, increased, then the GI decreased from 66.2 to 46.9%. Otherwise, the GI was equal to 88.5 and 97.8% for A1 and B1 respectively, on average of three dilutions. These high GI values confirm a complete absence of phytotoxicity for OPCs, even if not stable, according to Alburquerque *et al.* (2007). In table 2 chickpea and emmer yield and yield components, as affected by fertilization strategies, are presented.

Table 2: Chickpea and emmer yield and yield components (mean values).

Parameter	**Treatments**						**Mean of values**
	A1	**A2**	**B1**	**B2**	**OM**	**CT**	
Chickpea							
Seed yield* (t ha^{-1})	0.58	0.66	0.58	0.75	0.66	0.69	**0.65**
Residues (g plant^{-1})	13.3b	19.8a	12.4b	11.3b	14.2b	10.8b	**13.6**
Seed weight (g plant^{-1})	15.0ab	17.6a	10.9b	11.1b	14.5ab	12.0ab	**13.5**
Pods plant^{-1}	39.0	42.9	28.0	29.4	45.8	30.6	**36.0**
Pods weight (g plant^{-1})	19.2ab	22.7a	13.8b	14.7b	19.8ab	16.4ab	**17.8**
Emmer							
Grain yield** (t ha^{-1})	2.0	2.3	2.4	2.3	1.9	2.1	**2.2**
Straw yield (t ha^{-1})	3.3c	3.5bc	4.4a	4.2ab	4.2ab	3.9ac	**4.0**
Fertile spikes m^{-2}	446.6	338.8	398.2	398.2	431.2	446.6	**409.9**
Infertile spikes m^{-2}	41.8	59.4	57.2	37.4	48.4	79.2	**53.9**
Spike length (cm)	14.1a	12.8ab	12.6ab	12.6ab	11.2b	13.8a	**12.8**

The values in each column followed by a different letter are significantly different according to DMRT at $P \leq 0{,}05$.
*At 14% humidity; ** At 13% humidity.

As regard chickpea, the best agronomic performance (seed weight of 17.6 g plant^{-1}) was found in A2 treatment, despite no significant difference was observed for seed yield, as compared to the other treatments. The maximum seed weight observed for A2, might be attributed to the highest pods weight and high pods plant^{-1}. Otherwise, Hachicha *et al.* (2006) found the best potato yield resulted from compost made of less olive pomace. The mean chickpea yield might have been influenced by local erratic rainfall (ranging up 152.4 to 13.20) during the

experimental trial, high temperature stress during reproductive development (Wang et al, 2006) and by an occurred infection by Ascochyta blight which decreased grain accumulation.
Despite B1 showed a higher grain yield (2.4 t ha^{-1}) value and OM a lower one (1.9 t ha^{-1}), no significant difference in this parameter among all treatments was found for emmer. By contrast, Alburquerque *et al.* (2007) found that ryegrass yield in the first harvest was higher in the compost added treatments than in the control.
Significantly differences were found for the straw yield, B1 showing a value 33 and 12.8% higher than the A1 and CT value, respectively. Nevertheless, the fertile and infertile spikes m^{-2} didn't show any significant difference among treatments. The lowest spike length was observed in OM, reaching a value 20.5% lower than the A1 one, which was the highest.
It seems that the fertility managements able to ensure good emmer yield could be B1 and B2, i.e. the lower C/N compost, either stabilized and not stabilized.
From all the above, it can be concluded that there was a response of emmer even when not stabilized OPCs is applied, showing comparable yield values. On the contrary, there was a species-specific sensitiveness of chickpea because of this crop showed best results only with the stabilized compost derived from the high C/N ratio mixture.
According to several authors (Alburquerque *et al.*, 2006; Montemurro *et al.*, 2006; Montemurro *et al.* 2009), the first findings obtained indicate the possibility to recycle olive pomace, which is so abundant in the Mediterranean area, particularly after composting, to a greater extent on graminaceous crop rather than on leguminous one.

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EFFECTS OF BACTERIA CONTAINING BIOFERTILIZER ON Cd-TOLERANCE OF SOME CROP PLANTS

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Abstract

Biofertilizers promote nutrion uptake, but still there are a lot of questions about their application in polluted soils. Due to the intensive plant growing, considerable amounts of cations are leaching from the soil, and as a consequance the buffer capacity of soils gets weeker, the pH will be lowering, thereby enhancing uptake of heavy metals will be observed. Cadmium (Cd) toxicity is a major problem affecting crop productivity worldwide. The cadmium ion is easily uptakable, and also transportable inside the plants. Thus, the Cd enters the food-chain, causing public health problems. The aim of our work was to investigate the effects of biofertilizers on plant production and nutrient uptake in the case of Cd-polluted nutrient solution. Cadmium accumulated primarily in the roots, while the transport to the shoots was of a rather low level, but there were differences between the two investigated plants. Sunflower took up more Cd (because of the different nutrient-uptake system) and mostly it showed larger stress tolerance to Cd than maize. With the use of the bacterium-containing biofertilizer, the toxic effect of cadmium was moderated.

Keywords: Cd-tolerance, biofertilizer.

Introduction

Within the soil-nutrient-plant system, the mobility and availability of nutrients depend on various factors. During intensive land use, when lots of mineral fertilizers are applied, mainly nitrogen in NH_4 form, the pH of soils will be decreased and as a consequence significant quantity of cations disappears from the soil, which leads to further acidification, due to the decreased buffer capacity thus causing an increase in heavy metal uptake of plants. Therefore the unthinking use of different mineral fertilizers, and other chemicals may cause serious demage of our environment. Contaminated areas pose fundamental environmental problems, the physical, chemical and biological characteristics of the soil take an unfavorable course of change. Soil has the ability to stock toxic heavy metals for many years without even having obvious symptoms caused by their toxic effect. Cadmium is one of the most contaminating toxic elements; it may cause serious human health problems even in small quantities. Cadmium – especially in acidic soils – is easy to take up, and it is transferred very rapidly within plants, as well. Cadmium has a clearly negative influence on the growth and development of intolerant plants (Pinto *et al.*, 2004), and it also blocks the functions of the microorganisms living in the soil (Duxbury, 1985). Therefore, by influencing the accessibility of nutrients it also has a negative effect on nutrient uptake (Moreno *et al.*, 1999). Moreover, since the uptake of cadmium competes with the uptake of other elements because of the use of the same transmembrane carrier (Rivetta *et al.*, 1997), it may act as a substitute for essential zinc without its advantageous physiological effects. The Zn/Cd ratio of the plant may be significantly smaller than the Zn/Cd ratio of the soil, meaning that the plant preferably accumulates cadmium. According to various observations, zinc and cadmium reach cells via different membrane transporters, but these transporters are supervised by the same regulator (Bert *et al.*, 2003). According to Das *et al.* (1997), cadmium hinders the uptake of many elements (Ca, Mg, P, K) and water.

Since one of the advantages of bio-fertilizing is the promotion of nutrient uptake, its application in the case of cadmium contamination may provide interesting and practically utilizable results for the observation of element uptake. In the course of our research, we measured the element content of maize and sunflower sprouts and roots for treatments with different cadmium concentrations and the application of additional bio-fertilizer.

Materials and methods

Corn (*Zea mays, L cv* Norma SC) and sunflower seeds (*Helianthus annus L. cv* Arena PR) were germinated between moistened filter papers at 25°C in dark. The paper was placed in a vertical position in order to provide for the linear growth of seedlings. The seedlings were then transferred to a continuously aerated nutrient solution when the coleoptiles and hypocotyls were 4-5 cm. The composition of the nutrient solution was as follows: 2.0 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.1 mM KCl, 1µM H_3BO_3, (10µM in the case of sunflower), 1µM $MnSO_4$, 0.25 µM $CuSO_4$, 0.01 µM $(NH_4)_6Mo_7O_{24}$. Iron was added to the nutrient solution as the form of Fe-EDTA in a concentration of 10^{-4}M. Phylazonit MC® biofertilizer was added to the nutrient solution in quantities of 1 mlL^{-1}. Phylazonit MC® contains living bacteria, *Bacillus megatherium var. Phosphaticum* acting as phosphorous-mobilizing bacteria in concentration of 1–2 × $10^8 cm^{-3}$, and *Azotobacter chroococcum* as free living N_2 fixing bacteria, in concentration of 1–2 × $10^9 cm^{-3}$. When Cd treatment was applied, the concentration of $CdSO_4$ was 1, 5, 10, 20 mgL^{-1}. The seedlings were grown under controlled environmental conditions (light/dark regime 10/14 h at 24/20°C, 65–70% relative humidity and a photosynthetic photon flux of 390 $mEm^{-2}s^{-1}$ at plant height). For determining the activity of photosynthesis we used chlorophyll fluorescence induction as an indirect method (Schreiber *et al.*, 1994). We carried out the measurement of in vivo chlorophyll fluorescence in dark-adapted leaves – the parameters of chlorophyll fluorescence induction - with a PAM-2001 fluorometer (WALZ Gmbh, Germany). The contents of elements were measured with OPTIMA 3300DV ICP-OA (Perkin-Elmer). The number of repetitions is indicated in the title of figures and tables.

Results and Discussion

In the view of dry matter production, one of the substantial metabolic processes is photosynthesis. The intensity of photosynthesis considerably depends on the operation and quantity of photosynthetic pigments. In our examinations, we measured the changes of relative chlorophyll contents in leaves as a result of cadmium and bio-fertilizer treatments, which is shown as a SPAD-index (*Figure 1)*. As a result of the cadmium treatments, the relative chlorophyll content significantly decreased. According to the results of Stobart *et al.* (1985) and De Filippis and Ziegler (1993), cadmium causes disorders in the chloroplast metabolism by blocking chlorophyll biosynthesis and reducing the activity of enzymes that participate in CO_2 fixation. Our results confirm this observation, because on the sixth day, compared to the control, even the 1 mgL^{-1} Cd-treatment resulted in an approximately 20% reduction in the relative chlorophyll content. The living bacteria in the nutrient solution could compensate the disadvantageous effect of cadmium treatment, supposedly by the release of different organic acids, such as malic, and citric acid. These acids form complexes with cadmium ions, which can not be up taken by the roots.

Figure 2 shows the change of the relative chlorophyll content of the sunflower shoots resulting from the cadmium and bio-fertilizer treatments, as a function of time. While cadmium treatments applied to maize reduced the relative chlorophyll content as a function of concentration, the situation was more complicated in the case of sunflower. 1 mgL^{-1} cadmium concentration significantly reduced the value of SPAD-index even on the first measurement day (day 7). This reduction was apparent in the case of maize as well, but for sunflower we measured a 30% less SPAD-index as a result of the 1 mgL^{-1} cadmium treatment compared to the control. On the other days of the measurements, the difference between the SPAD-indexes of control plants and plants treated with 1 mgL^{-1} cadmium further decreased; in the case of the 13-day-old sunflowers the chlorophyll content of the treated samples was nearly 51% smaller.

Figure 1: Changes in relative chlorophyll contents (Spad units) influenced by treatments of Phylazonit and different cadmium concentrations 7, 8, 10, and 13 days after the treatments. (n=80-100 100 ±s.e) cadmium treatments: $p<0.05$*, $p<0.01$**, $p<0.001$***; biofertilizer (Phyl.) treatments: a<0.05, b<0.01.

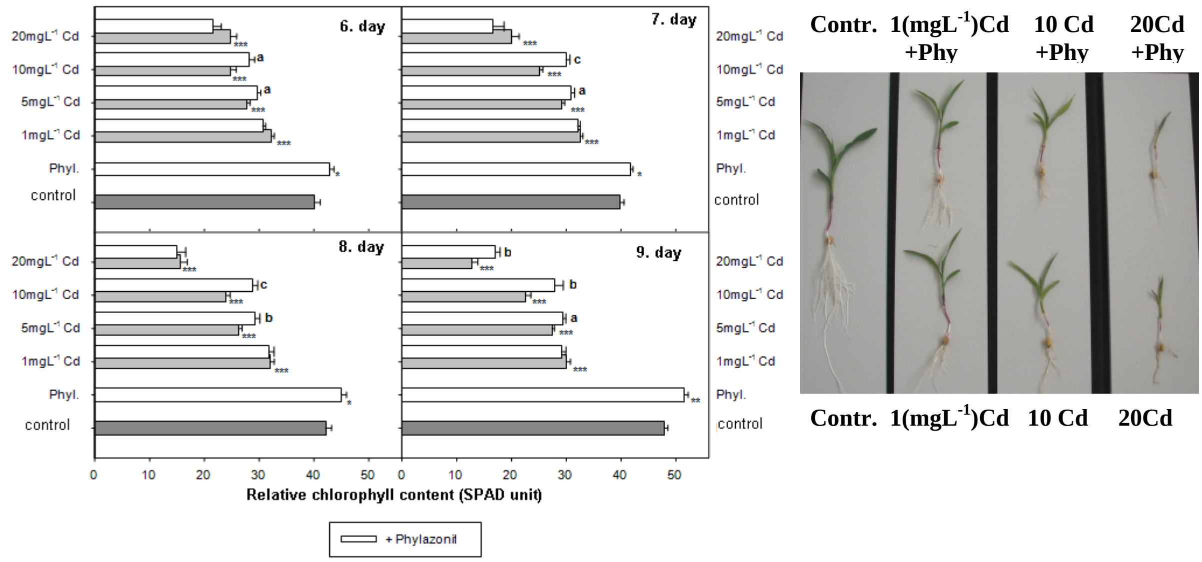
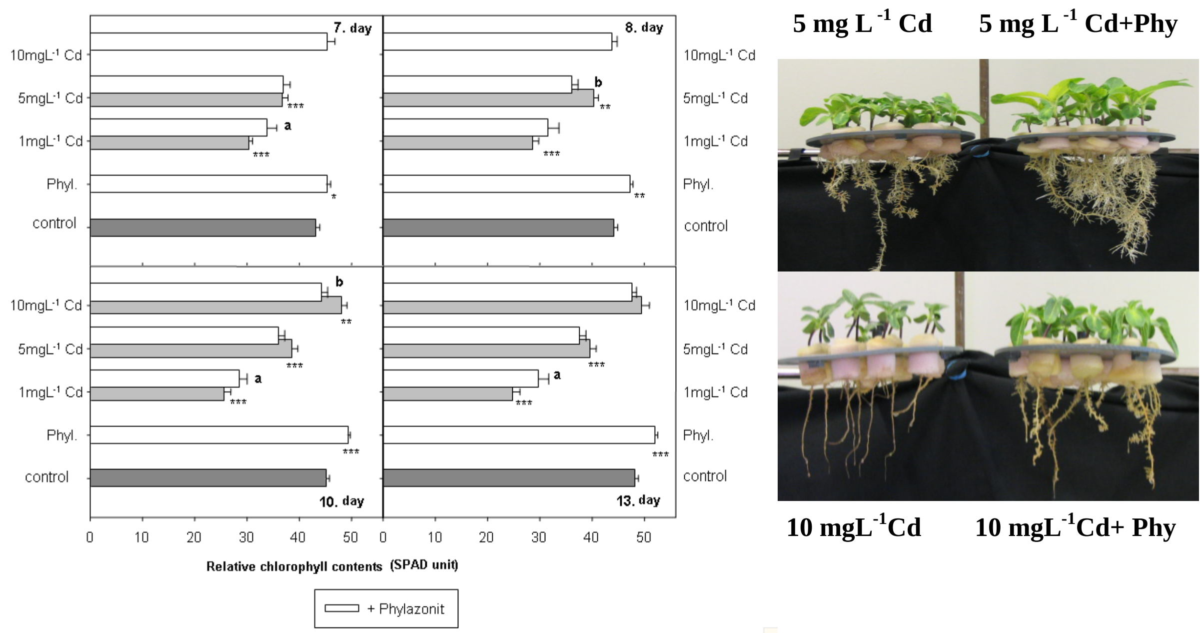


Figure 2: Changes in relative chlorophyll contents (Spad units) influenced by treatments of Phylazonit and different cadmium concentrations 7, 8, 10, and 13 days after the treatments. (n=80-100 100 ±s.e) cadmium treatments: $p<0.05$*, $p<0.01$**, $p<0.001$***; biofertilizer (Phyl.) treatments: a<0.05, b<0.01.



We suppose that the treatment with Cd retarded the dry matter accumulation more intensively than the chlorophyll synthesis, and as a consequence we could measure high chlorophyll contents in a smaller leaf mass. The application of the additional bio-fertilizer treatment at

$1 mgL^{-1}$ cadmium concentration significantly increased the relative chlorophyll content for each measurement; however, it had no effects at higher cadmium concentrations. Intensive photosynthesis is based on the presence of photosynthetic pigments in appropriate quality and quantity. With respect to our results (*Table 1*), the cadmium treatments reduced the amount of chlorophylls and carotenoids (known as protective and aid pigments). The exclusively applied bio-fertilizer (Phyl) increased the quantity of pigments.

Table 1: Effects of cadmium and bio-fertilizer treatments on the total chlorophyll and carotene contents of corn and sunflower shoots (in % of control).

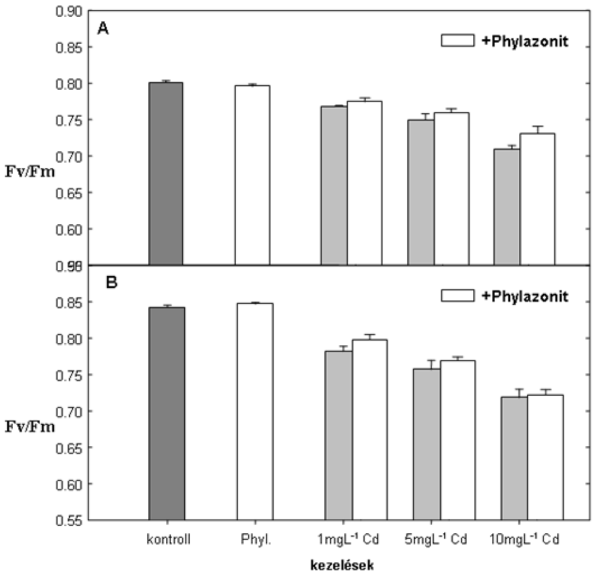
Treatments	Chl. a+b maize	Carotene maize	Chl. a+b sunflower	Carotene sunflower
Control	100	100	100	100
Phylazonit	120	125	138	163
$1 mgL^{-1}$Cd	32	45	65	108
$5 mgL^{-1}$ Cd	28	42	47	70
$10 mgL^{-1}$ Cd	10	30	100	122
1 Cd+Phyl	40	55	74	112
5 Cd+Phyl	26	41	72	105
10 Cd+Phyl	20	38	82	110

While we proved the explicit reduction of photosynthetic pigments as a result of the cadmium treatment in the case of maize shoots, for sunflower the high concentration of cadmium increased the level of photosynthetic pigments in the dry matter. These results conform with the measurement results of the SPAD-index.

Furthermore, the exclusively applied bio-fertilizer increased the quantity of the photosynthetic pigments of sunflower to a larger extent than in the case of maize shoots. The method of chlorophyll fluorescence induction is widely applied for the examination of the physiological condition of plants (Lichtenthaler and Rinderle, 1988; Veres *et al.*, 2000; Tóth *et al.*, 2002).

We examined the change of potential photochemical efficiency as a result of cadmium and bio-fertilizer treatments in maize and sunflower plants. Our results are shown in *Figure 3*. The cadmium contents of the roots is several times higher than of the shoots, which corresponds to the results of Cataldo (1983) wherein the cadmium remained in the root, and only a small amounts were transferred into the shoot. According to our previous results, the application of bio-fertilizers has a positive influence on the uptake of nutrients. The useful microorganisms of the bio-fertilizer compensate for the microbe-destroying effect of cadmium, promote adequate nutritive uptake and the management of nutrients in plants even in contaminated soil.

Figure 3: Changes in potential photosynthetic activity (Fv/Fm) influenced by different Cd and Phylazonit treatments in corn and sunflower plants (n=6 ±s.e).



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ORGANIC FERTILIZERS APPLICATION ON MELON CROPS GROWN IN MEDITERRANEAN CONDITIONS: I. YIELD AND PRODUCTIVE PERFORMANCES

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Abstract

A three-year field experiment (2006-2008) was carried out in a Mediterranean environment to study the effects of organic fertilizers application (anaerobic digestate and composted municipal solid waste) on yield and productive performances of melon. The research was conducted at Metaponto (MT - Southern Italy) on a clay soil. In a strip-plot experimental design with three replications, two irrigations (re-establishment 100 and 50% of the calculated maximum evapotranspiration) and the following four fertilizer treatments were compared: mineral fertilizer; commercial stable manure; anaerobic digestate based on wine distillery wastewater; composted municipal solid organic wastes coming from the separate collection. At the harvest, yield, number of fruits/plant, average weight, were determined. After the three-year field experiment, the quantitative melon performance showed no statistically significant differences among the four fertilizing treatments (commercial and experimental organic and mineral), indicating that mineral fertilization and commercial organic fertilizer could be substituted from those experimental organic ones. Conversely, the highest irrigation treatment showed a significantly higher total yield (25.4%) and fruits number (21%) compared to the lowest irrigation.

Keywords: Winter melon, anaerobic digestate, MSW compost, irrigation.

Introduction

The applying conventional agronomical practices has determined a continuous increase of profits but also has contributed to the progressive loss of soil organic matter, due mainly intensive tillages, and to environmental pollution due to the excessive supplies of nitrogen fertilizer that could cause the contamination of both underground and surface waters. Thus, it is necessary to preserve the primary resources, as soil and water, whose availabilities are indispensable for both present and future. Use of chemical fertilizers has been increased worldwide due to availability of inexpensive fertilizers (Graham and Vance, 2000), which causes health and environmental hazards (Pimentel 1996). Possible options to reduce chemical fertilizers use may be the adoption of recycling of organic wastes. Use of such organic materials in agriculture may contribute to preserve the environment as well as improve farmland. The application of composts and organic amendments modify soil organic matter and nutrient cycling (Eghball 2002), and increase soil nutrient levels (Chantigny *et al.* 2002). Two main processes are developed to transform organic materials into organic fertilizers for agronomic utilization: aerobic and anaerobic processes. Aerobic transformation of organic wastes takes to a stabilized and well humified material (compost) which is usually characterized by slow mineralization rate in soil. Anaerobic digestion, on the other hand, is based on the degradation of complex organic substances (proteins, lipids, carbohydrates) into less complex compounds which are subsequently transformed in low molecular weight organic acids (acetic acid, propionic acid, butirric acid), alcohols, aldehydes, carbon dioxide, hydrogen, with the production of methane (Rechtenbach *et al.*, 2006) and of a fertilizer characterized by less content of humic-like substances. On the light of these considerations, a three-year field research was carried out with the following aims: i) to study the effectiveness of both anaerobic digestate and municipal solid waste (MSW) compost applications on yield in w0inter melon cropped under Mediterranean conditions; ii) to evaluate the agronomic performance of these organic materials compared with mineral fertilizers.

Materials and methods

The research was carried out during three years (2006-2008) at Metaponto (MT) in Southern Italy (40° 24' lat. N; 16° 48' long. E and 8 meters above the sea level) located at the experimental farm of the CRA-Research Unit for the Study of Cropping Systems.

The climate is classified as "accentuated thermomediterranean" according to the UNESCO-FAO classification. The mean annual precipitation at the experimental farm is 490.6 mm, with more than 68% of the rainfall occurring during the winter months. The annual potential evaporation rate is high with a mean annual pan evaporation rate of 1561.5 mm. Evaporation rates are greatest during the months of June, July, and August, with mean monthly rates of 223.1, 268.5 and 232.0 mm, respectively. The total amounts of rainfall, recorded during May-August melon cropping cycles, were 123.5, 63.8 and 60.8 mm for 2006, 2007 and 2008, respectively. Melon plants (*Cucumis melo* var. Inodorus cv Rugoso di Cosenza), after being grown in the greenhouse to the four-leaf stage, were transplanted by hand on 19th, 23 th and 14 th May for 2006, 2007 and 2008 respectively. Plants spacing were 60 cm between the plants within each row and 150 cm between the rows (density of 1.1 plant m^{-2}). The experimental design was a strip-plot with three replications. The main plots were two irrigation treatments and sub-plots were four fertilizer treatments. The following treatments were compared: re-establishing 100 (I1) and 50% (I2) of the calculated maximum evapotranspiration (ETm); application of mineral fertilizer (Min), of a commercial stable manure admitted in organic farming (Org-Min) and of two experimental organic fertilizers, anaerobic digestate based on wine distillery wastewater (WDD) and composted municipal solid organic wastes coming from the separate collection (SUW). The ETm was calculated on the basis of evaporation rate from Class A pan (Doorenbos and Pruitt, 1977) and the crop coefficients was applied according to FAO-56 paper (Allen *et al.*, 1998). The evaporation rate from Class A pan and meteorological data were recorded on hourly basis by an automated data-logger located in the experimental area. Irrigation water was supplied by localized irrigation method. The watering was applied when cumulated crop evapotranspiration value reached 19.44 mm (from transplanting to blooming) and 38.88 mm (from full bloom to fruit ripening). N dose was 150 kg ha^{-1} applied in one solution, one month before transplanting, for all organic fertilizers and at transplanting for Min treatments as ammonium solphate. In the Table 1 main characteristics of commercial and experimental organic fertilizers are presented.

At harvest, total and marketable yield, fruits average weight and number, percentage of pulp,

Table 1: Principals characteristic of the commercial and experimental organic fertilizers.

Parameters		Commercial stable manure	Experimental fertilizer Wine distillery wastewater	Experimental fertilizer Municipal solid wastes compost
Total organic carbon	**g kg^{-1}**	**290.9**	**316.7**	**220.6**
Extracted total organic	**g kg^{-1}**	**151.7**	**127.0**	**149.6**
Humified organic carbon	**g kg^{-1}**	**82.1**	**94.4**	**111.9**
Total N	**g kg^{-1}**	**49.3**	**42.4**	**21.1**
C/N		**5.9**	**7.5**	**10.5**
Total P_2O_5	**g kg^{-1}**	**57.2**	**26.0**	**13.8**
Moisture	**%**	**5.7**	**63.5**	**17.5**
Cu	**mg kg^{-1}**	**28.7**	**493.8**	**98.2**
Zn	**mg kg^{-1}**	**156.1**	**53.0**	**213.5**
Ni	**mg kg^{-1}**	**3.0**	**3.3**	**11.0**
Pb	**mg kg^{-1}**	**0.0**	**0.4**	**92.5**

peel, seeds-placenta and dry matter, in heater to 70 °C for 48 hours, were determined. Data was analyzed using the SAS package (SAS Institute, 1990) considering years as random and replications and fertilizer treatments as fixed factors. The effects of the treatments were assessed through the General Linear Model procedure. The differences amongst treatments irrigation was evaluated using the LSD and the SNK for treatments fertilization and years.

Results and Discussion

The melon cropping cycles were an average 91 days. During growth stages, 9 waterings were effected and water volume applied was an average 2871 and 1436 m^3 ha^{-1} in I1 and I2, respectively. At these amounts the useful rain must be added. In Table 2, the F values from the analysis of variance for the main parameters and their interactions are presented. The yield performance showed significant differences in three years. In particular, the marketable yield (Fig. 1) was the lowest (15.6 t ha^{-1}) in 2006, because of a viral disease verified around 30 days before the harvest, whereas the highest in 2007 (33.3 t ha^{-1}). In 2008, the marketable yield

Table 2: Significance of the F values from the analysis of variance for the main parameters measured.

	Total yield	Marketable yield	Number fruits marketable	Marketable fruits weight
Years (Y)	**	**	*	**
Fertilization (F)	n.s.	n.s.	n.s.	n.s.
Irrigation (I)	***	***	***	n.s.
YxF	n.s.	n.s.	*	*
YxI	*	*	n.s.	n.s.
FxI	n.s.	n.s.	n.s.	n.s.
FxIxY	n.s.	n.s.	n.s.	n.s.

*, **, *** = Significant at the P<0.05, 0.01 and 0.001 levels respectively n.s.= not significant

Table 3: Effects of three years, fertilization and irrigation on yield performance of melon crop.

Treatments	Total yield	Number fruits marketable	Marketable fruits weight
	t ha^{-1}	n m^{-2}	g
Years			
2006	22.9 c	10926 c	1.4 c
2007	34.9 a	17037 a	2.1 a
2008	28.5 b	14164 b	1.9 b
Fertilization			
Min	30.1	13754	1.9
Org	29.3	14444	1.8
WDD	29.0	14761	1.7
SUW	26.7	13209	1.8
Irrigation			
I1	33.0 a	15664 a	1.9
I2	24.6 b	12420 b	1.8

resulted 20% lower than 2007 because of the hail fallen two weeks before harvest (Fig. 1). No statistical difference was recorded on yield and its components among fertilizer treatments (commercial and experimental organic and mineral), indicating that mineral fertilization and commercial organic fertilizer could be substituted from those experimental organic ones (Table 3). Besides, this finding shows that the recycling of differently treated by-products could play the same role of conventional organic fertilizers.

Results of previous researches (Montemurro *et al.*, 2005) concerning municipal solid waste (MSW) compost application on tomato crop, in Mediterranean conditions, indicate the suitability of not conventional organic fertilizers as a N source. However, there is a lack of information regarding a complete substitution of chemical fertilizer by anaerobic digestate and MSW compost. In fact, many studies show that MSW compost can supply essential nutrients (Maynard, 1995; Eriksen *et al.*, 1999), but this material is generally applied to the soil as an amendment (Montemurro *et al.*, 2004; Zaccardelli *et al.*, 2006; Morra *et al.*, 2006). The total and marketable yield presented statistically significant differences between two irrigation treatments (Table 2). In fact, the increase of production obtained in treatment I1, in comparison to I2, was 28% (Fig. 1), in according to Rivelli *et al.* (2003), Kirnak *et al.*, (2005) and in disagreement to some Authors (Cantore and Boari, 2001) that observed winter melon is resistant to the drought. The results showed that fruit weight was not affected by irrigation treatments, while number of fruits was significantly lower in I2 treatment.

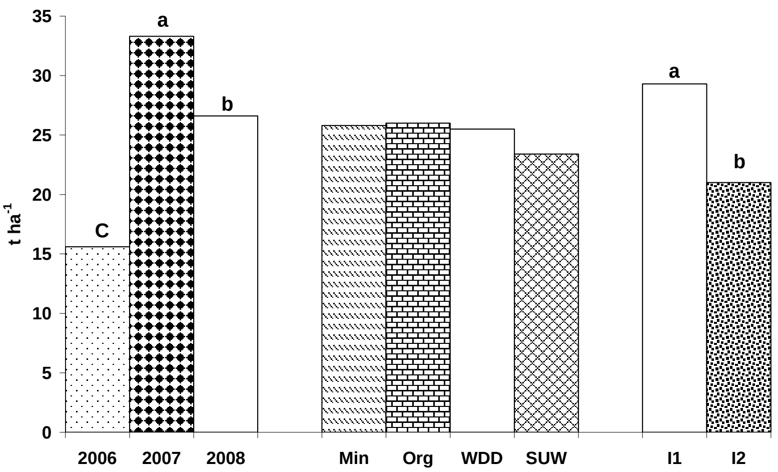

Figure 1: Effects of three years, fertilizer and irrigation treatments on yield marketable.

The values followed by different letters are significantly different at P<0.05 (SNK)

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ANIMAL WASTE FOR PYROLYSIS-DERIVED FERTILIZERS

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Abstract

It has recently been reported that the incorporation into soil of biochar (BC, i.e. the solid fraction of organic matter pyrolysis), exerts a beneficial effect on both soil fertility and carbon sequestration. Up to now the most studied BC source has been represented by woody material with low water content. However, the pyrolysis of solid animal waste for BC production could constitute a potential solution to the problem of animal waste excess disposal. With the general goal of evaluating the potential of pyrolysis as a technique to treat animal waste for energy and fertilizer production, in this preliminary work we considered the influence of process conditions on biochar yield and nutrient content. In a lab experiment, pig manure (PM) at 3 levels of moisture content, and wood chip (WO), were treated at 260, 340, 420 and 500 °C for 20 and 40 min, in anoxic conditions. Residual weight and organic matter, colour and compositional changes of the residual solid fractions were determined. The thermal treatment at 340 °C for 20 min gave a BC yield equal to 48% of the initial dry weight, for the driest PM material, and to 39.6%, for WO. Organic matter and N were partly lost in the combustion process, whereas the phosphorus content did not change remarkably. Further studies are needed to evaluate the fertilizing potential of the pyrolysis-derived solid fractions of animal waste.

Keywords: Pig manure, biochar, carbon recycling technology.

Introduction

Pyrolysis is a thermochemical process, carried out in the absence of air, representing a way for extracting renewable energy from organic material. Some gaseous products of the pyrolysis process, and especially hydrogen, are in fact energy carriers, and may be suitably exploited for energy production. The pyrolysis anoxic environment impedes the full combustion of the organic matter (OM) to be achieved, thus leaving a residual solid product, the charcoal. Recent developments in fertilizer research have pointed out the beneficial effect of charcoal, when incorporated into soil, on soil fertility (Glaser *et al.*, 2002; Steiner *et al.*, 2007) and carbon sequestration (Lehmann *et al.*, 2006). Biochar (BC) is the term used to indicate charcoal, when it is produced specifically for application to soil as part of agronomic or environmental management. Pyrolysis carried out at temperatures < 500 °C, giving higher biochar yields, is more suitable for the obtaining of BC as soil amendment.

As far as the type of OM for use in thermochemical transformations is concerned, up to now the majority of the research work has focussed on organic material with low water content, the cost of its transformation being more sustainable than for materials with high water content. Among animal wastes, chicken litter, which has a low water content, has been the OM source mainly tested (Chan *et al.*, 2008). Animal waste with low water content may also be obtained either by solid-liquid separation, or as co-product of anaerobic digestion, for biogas production. Large amounts of solid manure are or will be available in the near future following the improvement of the manure solid-liquid fraction separation techniques (Vanotti *et al.*, 2007). Despite this situation, information on transformation of solid animal waste other than that for compost production is poor. Very recently, a study was published on thermal decomposition kinetics of swine solids (Ro *et al.*, 2009). However, researches examining the characteristics of the pyrolysis-derived solid fraction from a fertilizing quality point of view are still lacking.

With the general goal of evaluating the potential of pyrolysis as a technique in treating animal waste for energy and fertilizer production, in this preliminary laboratory experiment we considered the influence of process conditions on selected properties of the pig manure solid products. Pig manure (PM) and wood chip (WO) were compared after thermal treatment at low combustion temperatures to i) verify the time-temperature combination giving rise to good BC yields; ii) evaluate the fate of the nutrients in the OM, after thermal treatment, as a prerequisite for evaluation of BC as fertilizing amendment.

Materials and methods

The compared materials were PM at 3 levels of moisture, U (PM_I: U=10%, PM_II: U=14%, and PM_III: U=71%) and WO (U=9%). Five-mm sieved, 5-15 g samples (the amount being established according to moisture content) were thermally treated in a confined environment (porcelain, lid-covered crucibles). Four temperature levels were assessed: 260, 340, 420 and 500 °C, for 2 time lengths: 20 and 40 minutes. Three replicates were performed for PM_I and WO, 2 replicates for PM_III, and one replicate for PM_II. The highest temperature level (500 °C) was tested only for the PM_I and WO materials (one replication). The solid fractions resulting from combustion were characterized in comparison with untreated controls.

Residual weight (RW) of the samples after thermal treatment was reported as percentage of their dry weight (DW) before treatment. The OM content of treated and untreated samples was calculated as the difference between the absolute sample RW (in g) and the ash content in the RW (in g), and reported as percentage of the initial DW content. The DW content of the untreated samples was determined after a night at 105 °C. The ash content of treated and untreated samples was determined after a night at 550 °C.

Biochar quality may be empirically defined by visual inspection: a good BC material is plain black, brilliant, brittle, porous and "clean", that is, lacking of an ashes excess. A more objective evaluation of the BC quality may be obtained by means of its colour determination (Hammes *et al.*, 2008). The BC colour of the treated samples was determined on a tristymulus system basis (CIE xyY colour space), with a Minolta CR300 chroma meter.

The concentration of NH_4-N, total (Kjeldahl) N, and total P (colorimetric determination, after digestion by wet oxidation) was also determined on treated and untreated samples, and it was referred to the initial DW content of the organic material. The organic N concentration was estimated as the difference between total and NH_4-N.

Results and discussion

Residual weight and organic matter content. The RW of the tested material (Fig. 1) decreased exponentially at increasing temperature levels, and tended to become stationary at the highest temperature treatments (420-500 °C), apart from the case of PM_III. As the lower temperature × time treatment combinations (i.e., 260 ° for 20 and 40 min; 340 °C for 20 min) were not sufficient to remove all the water from the PM_III material, in Fig. 1 its RW, when higher than the initial DW, was set equal to 100% of the initial DW.

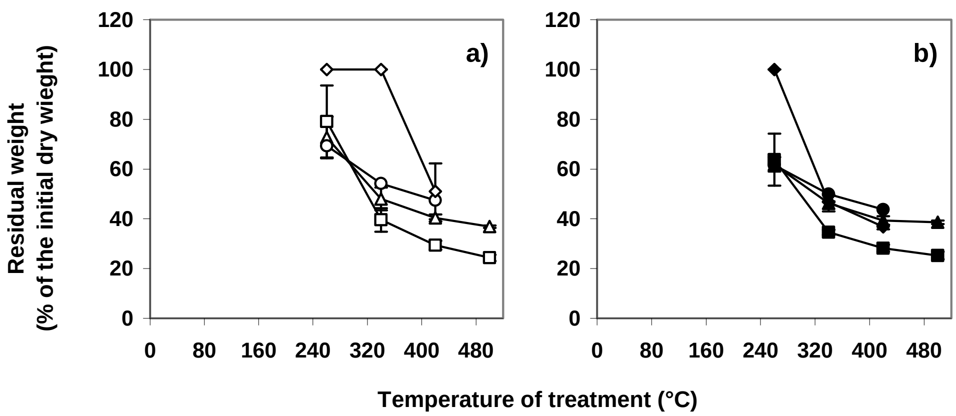

Figure 1: Residual weight of the organic materials after thermal treatment at 260, 340, 420 and 500°C for 20 min (a, open symbols) or 40 min (b, solid symbols). Symbol meaning: squares, WO (U=9%); triangles, PM_I (U=10%); circles, PM_II (U=14%); diamonds, PM_III (U=71%). Bars are the SD of the mean, when available.

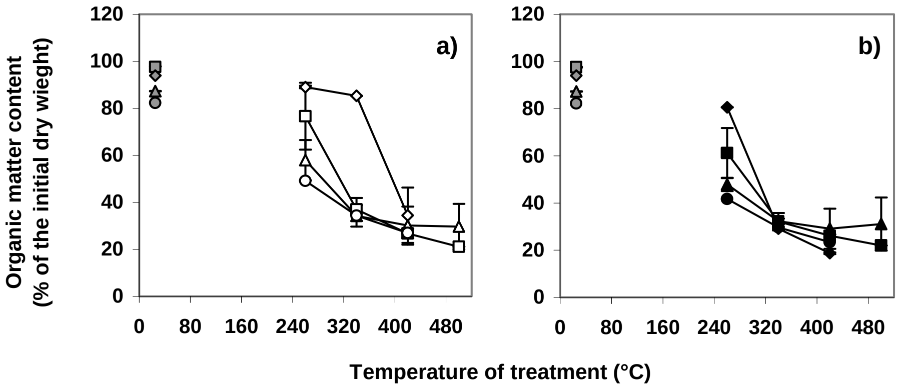

Figure 2: Residual organic matter, as percentage of the initial dry weight, after thermal treatment at 260, 340, 420 and 500 °C for 20 min (a, open symbols) or 40 min (b, solid symbols). Symbol meaning: squares, WO (U=9%); triangles, PM_I (U=10%); circles, PM_II (U=14%); diamonds, PM_III (U=71%). The grey symbols indicate the organic matter content of the untreated material. Bars are the SD of the mean, when available.

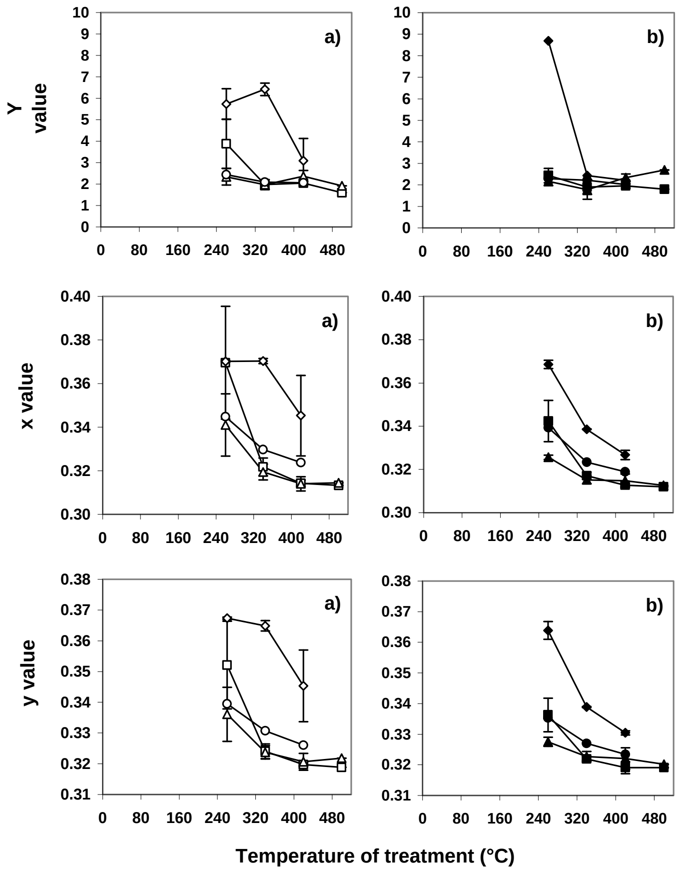

Figure 3: Values of the chromaticity coordinates, Y, x and y, after thermal treatment at 260, 340, 420 and 500 °C for 20 min (a, open symbols) or 40 min (b, solid symbols). Symbol meaning: squares, WO (U=9%); triangles, PM_I (U=10%); circles, PM_II (U=14%); diamonds, PM_III (U=71%). Bars are the SD of the mean, when available.

The curves of the residual OM content (Fig. 2) were similar to those of RW. However, above 260 °C, differences between the WO and the PM materials, both after 20 and 40 min of thermal treatment (apart from the case of PM at U=71%), were lower than those observed for RW. Differences in RW were in fact due to the ash content, higher for PM than for WO.

Colour changes. The colour parameters values (Fig. 3) followed the same pattern as the OM content: the curve shape was that of an exponential decay, the lower asynthote being approached earlier for the materials with a lower initial water content (WO and PM_I).

Composition changes. Despite the

limited differences of OM content between PM and WO (the initial OM concentration range was 82-97%, on a dry weight basis; Fig. 2), the starting PM material was much richer in N and P nutrients than the WO (Fig. 4). The NH_4-N concentration drastically decreased and almost approached 0 following the thermal treatment, due to the well known and expected effect of heating on ammonia volatilization. The total (Kjeldahl) N concentration itself decreased, for increasing treatment times and temperatures, with larger differences between types of material than those observed for the OM content. These differences were due either to differences of initial total N content among the compared materials, or to NH_4-N losses. The N losses by pyrolysis of the organic N fraction increased with time and temperature of treatment on average from 15% (at 260 °C) up to 61% (at 500 °C) of the initial organic N content (data not shown). Differences among materials were greatly reduced when considering the organic-N loss percentage, instead of the total N content. On the whole, the total P concentration did not change remarkably following the thermal treatments.

By visual inspection, the treatment residue had already BC characteristics when treated at 340 °C for 20 min. The OM content of the initially drier materials (PM_I and WO) approached a plateau already after 20 min at 340 °C. In these conditions (340 °C for 20 min) the BC yield (i.e., the RW of the material with visual BC characteristics) of PM_I was

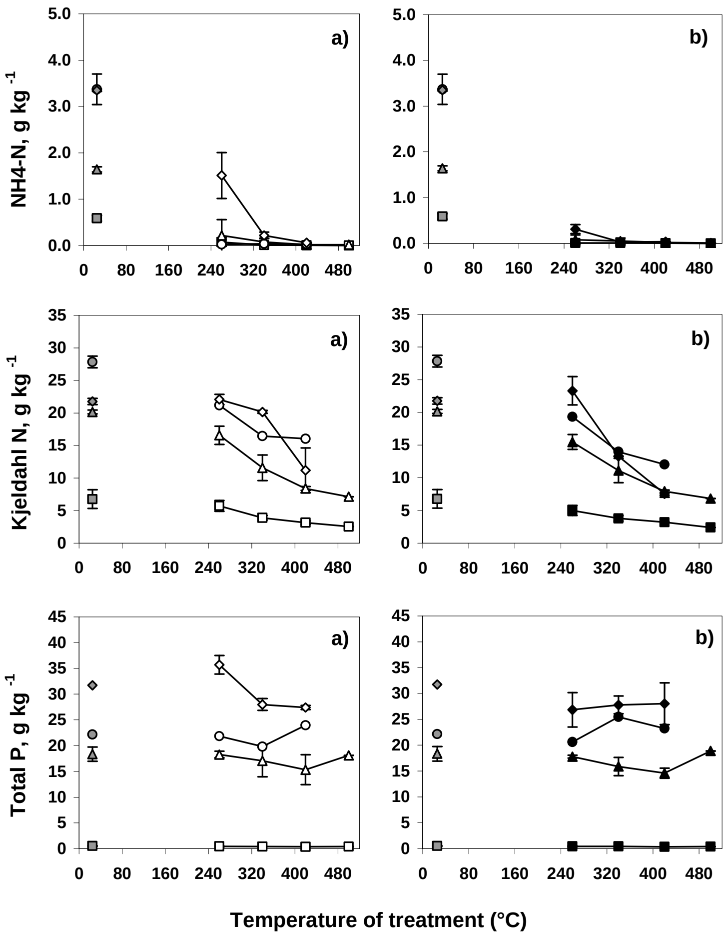

Figure 4: Content of selected components in organic samples treated at 260, 3 40, 420 and 500 °C for 20 min (a, open symbols) or 40 min (b, solid symbols). Their concentration is referred to the initial DW. Symbol meaning: squares, WO (U=9%); triangles, PM I (U=10%); circles, PM II (U=14%); diamonds, PM III (U=71%). The grey symbols indicate the component content of the untreated material. Bars are the SD of the mean, when available.

higher (RW= 48.0%, SD=4.6%, n=4) than the BC yield of WO (RW= 39.6%, SD=4.7%, n=4). These temperature levels, milder than those applied to various matrices in other researches (Shinogi and Kanry, 2003; Chan *et al.*, 2008), seem therefore sufficient for the production of BC, with lower nutrient loss and energy costs than those needed by more drastic thermal treatments. The relationship between visual inspection and colour measurement results suggests the possible usefulness of the colour parameters as indexes of BC quality.

Biochar has several advantages over the original material: it is less voluminous; it is lighter and more stable in time; moreover, the high temperatures of treatment determine a sanitization effect. Whereas, according to our experiment, the phosphorus content of the original material seems preserved, the organic carbon and nitrogen fractions, being combusted during the heating process, move in the gaseous phase of the system and may be lost in the atmosphere, when not suitably entrapped at the system outlet. Their recovery should be pursued for the following reasons: i) the system head space contains gaseous species of interest for the production of energy (i.e., hydrogen); ii) some of them may be of interest for fertilizer production (i.e., ammonia); some of them may have a greenhouse gas effect and/or may contribute to air pollution (i.e., CO_2, CO, CH_4, non-methane hydrocarbons, total suspended particulates). Further studies are therefore needed in order to evaluate the fertilizing value of the pyrolysis-derived solid fractions of animal waste, the possible contribution of the fluid fractions to renewable energy production and the way to minimise the environmental impact of the pyrolysis technology. It should be pointed out if and to what extent the organic forms stabilized in biochar are becoming available to crops when added to soil; the method should be set up for the recovery of the various gaseous-phase components with techniques not so expensive as to compromise the advantage of the rather cheap technology for biochar production at farm level. Positive signals already exist on this way.

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IMPROVING QUALITY AND PRODUCTIVITY OF WHEAT USING DIFFERENT TYPES OF FERTILIZERS IN CONSERVATION AGRICULTURE

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Abstract

The increasing world population requires augmenting crop production. According to current depletion rates of land, water, and energy resources, the challenge is to achieve this objective by applying sustainable techniques, where conservation agriculture plays a key role. This work studies the effect of different types of fertilizers in the amount and quality of wheat production under conservation agriculture (no tillage).The fertilizers used have been not only the conventional ones (urea, ammonium sulphate, etc.), but also some of new generation (with nitrification inhibitor, micronutrient or liquid fertilizers).

The study was carried out during the 2006-07 and 2007-08 seasons, in two different fields situated in typical Andalusian cerealistic areas. The surface of the elemental plot was 50x7.5 m^2, and the experimental design was randomized complete block, with 8 treatments and 4 repetitions.

The results showed that phosphorus micronutrient and the fertilizer with nitrification inhibitor maintained or, in some occasions, enhanced wheat production, without any statistical significant differences. That happened even decreasing the dose (130 UFN compared with 150 UFN with conventional fertilizers). In the grain quality appear some differences, as the nitrification inhibitor fertilizers showed less quality in comparison with the others.

Keywords: conservation agriculture, no till, fertilizers, wheat, production, grain quality.

Introduction

Since the second part of the XX century, the world population has suffered an extraordinary augment, growing from 2.500 million people to 6.100 million (U.S. Census Bureau, 2009). That means an increase of 230 %. Nowadays, 6.700 million people inhabit the Earth, and the ONU estimates that in 2050 the world population will reach 9.000 million.

Many studies alert of the continuous loss of arable land, either by human activity, applying unsustainable practices, or due to the use of land for other purposes. Therefore, agricultural sector should increase food production, taking into account a predictable decrease of the arable land.

Conservation agriculture makes a more rational and efficient use of water, soil and air (Márquez *et al.*, 2009 a). So in these probably conditions of loss of arable land, it would play a significant role. Conservation agriculture, including no-till and cover crops for perennial crops, improves soil fertility and favor nutrient absorption by plant roots, increasing the efficiency of the fertilizers and reducing the nitrogen and phosphorus contamination.

Agricultural technology has developed new products for plants nutrition, such as micronutrient impact starter, incorporating in the line of sowing phosphorus easily assimilated by plants, or the slow release fertilizers with nitrification inhibitors. These fertilizers make a more rational use of the nutrient, reducing leaching and volatilization of the nitrogen, while at the same time improves plants germination and their vigour.

The objective of this work was to study the effect of different types and combinations of fertilizers on the productivity and quality of wheat under conservation agriculture (no till).

Material and methods

The study period covers two seasons, 2006-07 and 2007-08, in two fields located in different cerealistic areas of Andalusia (Spain), which have a typical Mediterranean climate; more specifically in the villages Las Cabezas de San Juan (Sevilla) and Jerez de la Frontera (Cadiz). Their physico-chemical characteristics are described in Márquez *et al.* (2009 b). It is important to remark that in Las Cabezas field the soil is vertic, deep and fertile, while in Jerez exist a calcareous ground, in a relatively mountainous area. No till has been practised in both fields since 8 years; therefore, they present high levels of organic matter.

In the study were used conventional fertilizers (urea, ammonium sulphate, etc.), and new generation fertilizers (slow release, micronutrient impact starter and liquid). The products and their composition are described in the following table:

Fertilizer	Composition (N-P-K)
Urea	46-0-0
Ammonium nitrate	27-0-0
Ammonium sulphate	21-0-0
Nitrogen solution	32-0-0
Diammonium phosphate	18-46-0
Micronutrient starter	11,5-50-0
Slow release	20-10-10

On the first season, the field of Las Cabezas was sown with *Triticum durum* L. (cv. Simeto) and on the second one with *Triticum aestivium* L. (cv. Anapo), in both occasions there was fallow before wheat. In Jerez the situation was opposite, with *Helianthus annus* L. before cereal. The area of the plot was of 50x7.5 m^2, sowed with 360 seeds/m^2 density. The experimental design was a randomized complete block, with 8 treatments and 4 repetitions. Different fertilizer combinations, application rate, nitrogen fertilizer units (NFU) and phosphorus fertilizer units (PFU) appear in Table 1. Notice that the first application was done in January both year, and the second one in March.

Table 1: Type of fertilizer and application rate per hectare in the different treatments.

Treatment	Presowing	Sowing	Incorporated	1st aplication	2nd aplication	NFU	PFU
1	0	0	0	Urea 227 kg	Urea 109 kg	150	0
2	0	0	Diammonium phosphate 125 kg	Urea 168 kg	Urea 109 kg	150	57.5
3	0	0	Diammonium phosphate 125 kg	Ammonium nitrate 287 kg	Ammonium nitrate 185 kg	150	57.5
4	0	0	Diammonium phosphate 125 kg	Ammonium sulphate 369 kg	Ammonium sulphate 238 kg	150	57.5
5	Urea 109 kg	0	Diammonium phosphate 125 kg	Urea 168 kg	0	150	57.5
6	0	0	Starter 43 kg	Urea 200 kg	Nitrogen solution 100 kg	130	21.5
7	0	0	Starter 43 kg	Ammonium sulphate 425 kg	Nitrogen solution 100 kg	130	21.5
8	0	Slow release 310 kg	0	Ammonium sulphate 320 kg	0	130	31

The quality was estimated by the grain protein content, that was calculated with a Leco elemental analyzer of C, N, S (Sparks *et al.*, 1996). The results were subjected to analysis of variance (ANOVA), and mean separation was effected by Tukey's test.

Results and discussion

Table 2 shows the yields , protein content in grain , harvest index (H.I.) obtained in the different treatments , experimental fields and years. Productions are higher in the field of Las Cabezas, as it has a more productive soil than Jerez field. No significant differences appeared related to production, whilst protein content showed significant variation linked to the treatments.

Table 2: Productive summary.

		Year 2006-07			Year 2007-08		
	Treat.	**Yield (kg/ha)**	**Prot. (%)**	**H.C.**	**Yield (kg/ha)**	**Prot. (%)**	**H.C.**
Las Cabezas	**1**	4.523 A	15.1 A	0.9	4.031 A	14.7 A	1.2
	2	4.745 A	14.6 AB	1.0	4.316 A	13.5 ABC	1.2
	3	4.791 A	14.8 A	0.9	4.464 A	13.9 AB	1.1
	4	4.909 A	15.3 A	0.9	4.117 A	13.4 ABC	1.2
	5	4.948 A	14.3 AB	0.9	3.995 A	13.2 ABC	0.9
	6	4.535 A	14.5 AB	1.0	4.103 A	12.6 BC	1.2
	7	5.335 A	14.3 AB	1.1	3.911 A	12.7 BC	1.3
	8	4.888 A	13 B	0.9	4.361 A	11.8 C	1.1
Jerez	**1**	4.197 A	11.9 AB	1.0	3.525 A	15.1 A	0.9
	2	4.304 A	12.5 A	0.9	3.450 A	14.9 A	0.9
	3	4.560 A	12.2 AB	1.0	3.692 A	15 A	1.0
	4	4.057 A	11.5 AB	1.0	3.130 A	14.6 A	0.9
	5	4.356 A	11.1 B	0.9	3.405 A	14.8 A	0.9
	6	4.651 A	11.8 AB	1.0	3.597 A	14.6 A	0.9
	7	4.364 A	11.2 B	1.0	3.837 A	13.7 A	0.9
	8	4.334 A	10.9 B	1.0	3.632 A	13.6 A	1.0

Table 3 illustrate a productive summary expressed as the average of the two years in Las Cabezas and Jerez, and the average of the two fields. Treatment 8, with slow release fertilizer, obtained the worst grain quality results in the climatic conditions and soils where the study was carried out. Referring to grain production, figure 1 shows that treatment 1, with no phosphoric fertilizer, obtained bad results of production, despite the fact that the amount of Olsen P in Las Cabezas was 18.0 mg kg^{-1} and 8.1 mg kg^{-1} in Jerez, and different experiments have shown that plants cultivated in dry-farming areas of the region do not respond to P fertilization when Olsen P>6-7 mg kg^{-1} (Bravo *et al.*, 2006). The average production of this treatment was under the mean, deviation equal to 0, in both experimental fields. Moreover, the grain protein was elevated, due to the negative correlation between amount of production and its quality (López-Bellido *et al.*, 1998).

Table 3: Average production and quality of both campaigns in Las Cabezas and Jerez, and the mean of the 2 fields.

	Las Cabezas		**Jerez**		**Both**	
Trat.	**Production**	**Protein**	**Production**	**Protein**	**Production**	**Protein**
1	4.277 A	14.9 A	3.861 A	13.5 A	4.069 A	14.2 A
2	4.531 A	14.0 AB	3.877 A	13.7 A	4.203 A	13.9 AB
3	4.627 A	14.4 A	4.126 A	13.6 A	4.377 A	14.0 A
4	4.513 A	14.4 A	3.594 A	13.1 A	4.053 A	13.7 AB
5	4.471 A	13.8 AB	3.881 A	13.0 A	4.176 A	13.3 AB
6	4.319 A	13.6 AB	4.124 A	13.2 A	4.222 A	13.4 AB
7	4.623 A	13.5 AB	4.100 A	12.5 A	4.362 A	13.0 AB
8	4.625 A	12.4 B	3.983 A	12.3 A	4.303 A	12.3 B

Between treatments 2 to 5, which had an application of 150 NFU, combination 3, fertilized with diammonium phosphate and ammonium nitrate, which showed the best results of grain yield and good results of protein quality. About treatments 6 and 7, fertilized with 130 NFU and 21.5 PFU, both obtained good productivity results, specially combinations between starter and ammonium sulphate. Treatment 7, because these kinds of fertilizers have to be incorporated in the sowing row, they required an early application to be quickly assimilable by the crops. Treatment 6, also yielded good results, except when the previous crop was sunflower, as this plant extracts a lot of nutrient and soil is too exhausted after its cultivation. As the starter is combined with urea, a fertilizer which needs an elongated period for being available in the soil, the wheat suffered lack of nutrients in its early stages, circumstance that reduced its final production. Variant 8, with slow release fertilizer and an application of 130 NFU and 31 PFU, obtained a production slightly above the field average. However, the grain quality was very low, appearing significant differences with respect to the other treatments, as shown in Figure 1. Treatments with starter fertilizers, did not show significant differences in protein quantity, despite the fact to have 20 PFU less than combinations 1 to 5.

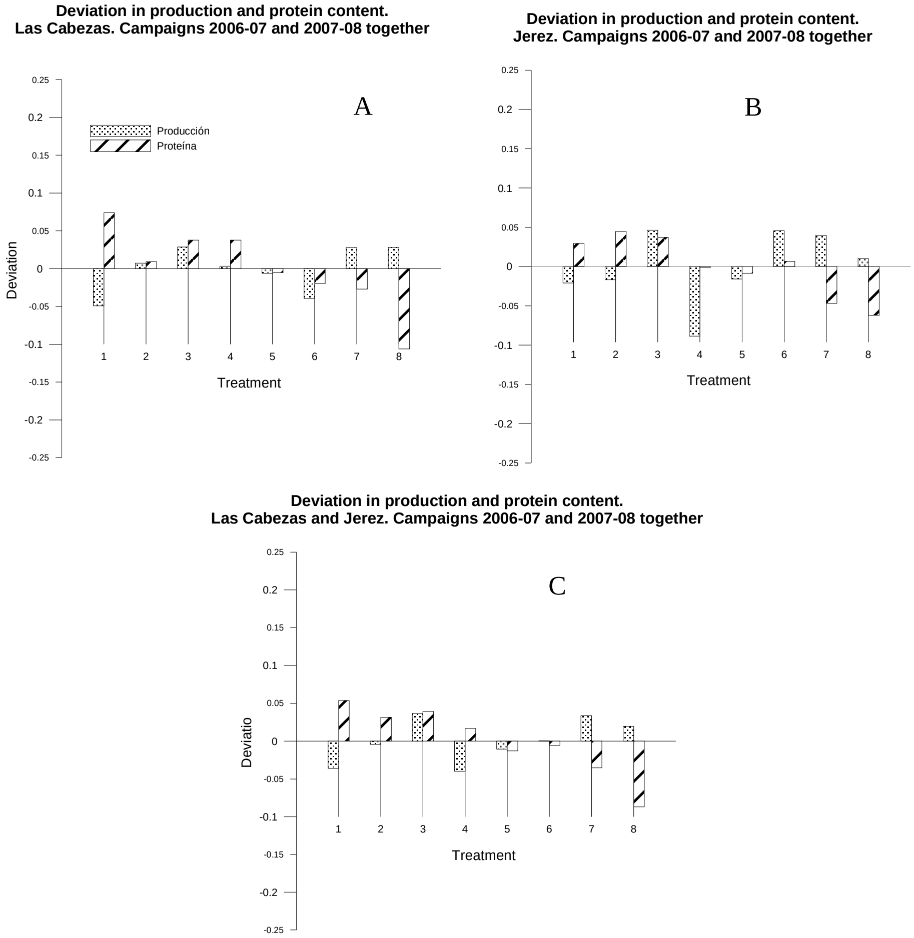

Figure 1. Deviation from the average of production and the amount of protein. A: average of the field of Las Cabezas. B: average of the field of Jerez. C: average of both fields.

Conclusions

In summary, the study demonstrates the importance of the phosphoric fertilization. Also, some new fertilizers, as the slow release with nitrification inhibitors, improve the wheat production. At the same time they help to reduce the amount of nitrogen applied, although also reduce the grain quality. Moreover, micronutrient fertilizers seem to be able to increase grain production maintaining the protein quantity. But this kind of fertilizers need a special management: it is very important to make a first application as soon as possible with fertilizers quickly assimilable by crops. This is particularly important when the previous crop was sunflower; in this situation could be advisable making a ureic fertilization before the sowing.

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COMPOSTING OF OLIVE MILL WASTES WITH UMICA TECHNOLOGY. RAW MATERIALS AND TESTED MIXTURES, PROCESS MONITORING AND QUALITY OF PRODUCED COMPOST

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Abstract

It is reported about trials of composting of olive fluid pomaces by mills with a two-phase system of centrifugation. Trials were carried out in province of Avellino, Southern Italy. It was tested a composting static pile technology named 'UMICA': an in vessel modular insulated container where air supply, air recirculation, wetting and leachate recirculation are enabled and oxygen consumption, temperature and humidity of composting material are continuously monitored. Some cycles of composting were carried out mixing cow manure and straw to olive pomace in different proportions. The first four weeks of process occurred in container where the thermophilic phase (ACT) rapidly started up to 60 °C only with the mixture of olive pomace 63 %, cow manure 30 % and straw 7 % triggered with 10 % of compost from the preceding cycle. The quality of obtained composts was according to tolerance limits fixed by Italian Law excepted for high pH and Salinity. No fitotoxicity was detected after 4-6 months of composting.

Keywords: composting method, biomass recycling, olive mill by-products.

Introduction

The composting of by-products of the agro-food industries is an important goal to reclaim valuable organic biomasses as soil amendants (Rossi and Piccinini, 2003). In countries where is largely diffused the olive oil cultivation, efforts are made to define how to treat the olive mill solid wastes and the wastewaters whose direct distribution are ruled in order to reduce possible soil and water pollution due to phenolic and fat compounds and inhibition of microflora (Pagliai et al., 2001; Hachicha et al., 2006). Composting of olive mill wastewaters or olive pomaces made by solid or fluid materials has been pointed out as an effective way of treatment of these biomasses (Vallini et al., 2001; Alburquerque et al., 2006; Sacchi et al., 2002; Montemurro et al., 2004).

The objective of this research, funded by Campania Region, Assessorato Agricoltura e Attività Produttive, was to evaluate a composting method with static pile in a container named 'UMICA'. In particular, we studied the feasibility to reduce times to trigger the thermophylic phase. Alburquerque et al. (2006), composting 92 % olive pomace and 7,4 % cotton waste , measured a temperature over 50 °C (thermophilic phase) between the eight and the twelfth week. The whole active phase of process lasted 26 weeks. Montemurro et al. (2004), composting 82 % olive pomace, 10 % poultry manure and 8 % straw, reported an active phase of 27 weeks. Hachicha et al. (2006) reported an active composting phase of 16 weeks to compost a mixture of 75 % olive mill cake and 25 % poultry manure with periodic pile turning. The long composting time of piles with olive mills by-products could be related to the low bioavailability of N-compounds, the high proportion of biodegradation-resistant components, such as lignin, the anti-microbial activity of phenolic compounds.

Other objectives of our study were: the optimal mix of raw materials to compost, the manageability of composting with 'UMICA' technology, the quality of the produced compost.

Materials and methods

The research started on November 2008 and was located at Montecalvo Irpino, in Province of Avellino, an internal area of olive-oil production. In order to accelerate the start of the Active Composting Time (ACT) and to achieve maximum control over the composting process parameters, the mixtures to compost were put in a vessel modular insulated container where air supply, air recirculation, wetting and leachate recirculation are enabled and oxygen consumption, temperature and humidity of composting material are continuously monitored. This container had a capacity of 20 m^3 (about 12 t). We composted the more available raw materials in the area: cow manure, straw, olive leaves and olive pomaces very rich in water (75 %) produced by mills with a two-phase centrifugation system. We tested three mixtures in different cycles of composting during winter 2008/09 as shown in Tab. 1.

Table 1: Composition of the mix of raw materials (% on a fresh weight basis) for composting of solid olive mill by-product. Main dates of composting process.

Raw materials	**I Cycle**	**II Cycle**	**III Cycle**
Olive pomace	72	50	63
Olive leaves	9	-	-
Cattle manure	9	40	30
Straw	9	10	7
Start of composting in 'UMICA'	22/12/2008	23/01/2009	25/02/2009
End of composting in 'UMICA'	22/01/2009	23/02/2009	26/03/2009
Maturation phase (months)	6	5	4
Time of compost sampling for analysis	11/05/2009	11/05/2009	11/05/2009

The active phase in container lasted 20-30 days. Then, the container was lifted and emptied out by a mechanical elevator. The slow maturation (curing) phase of compost took place under roof. In the last cycle, a portion (10 %) of the composting mass of the second cycle was added to the mixture as primer. Samplings of compost from the different piles were analyzed for chemical, biological and physical characteristics.

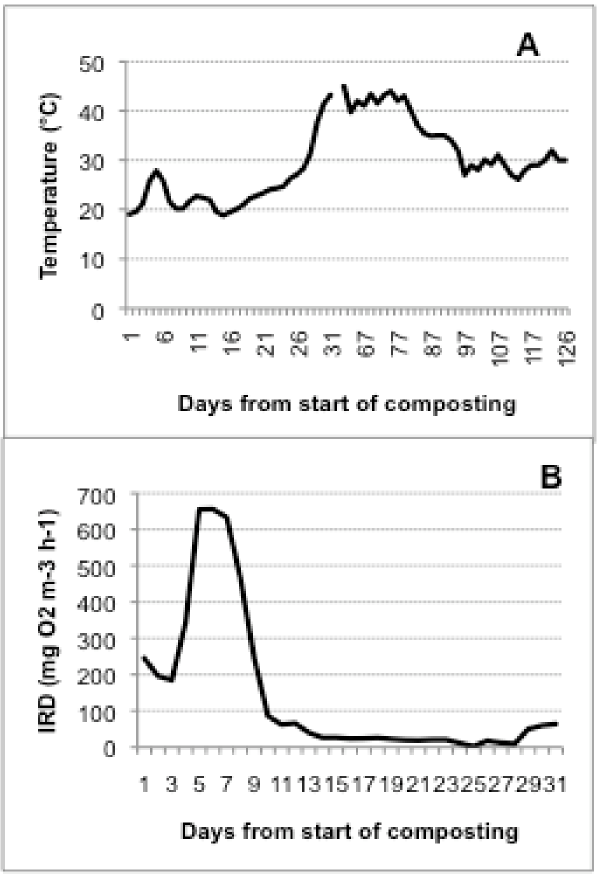

Figure 1: Temperature and instant IRD trends during the first composting cycle.

Results and discussion

In each of the Figures 1-3 is showed the trend of mass temperature recorded during the composting phase in container UMICA and after the unloading (picture A); beside, in each figure is showed trend of the instant Dynamic Respiration Index (IRD) measured during the first part of cycle (picture B).

In the first cycle, temperature of the mixture started to increase only after 30 days, exceeding 40 °C and remaining over this level in the successive two months under roof (Fig. 1). The instant IRD reached a peak only in the first seven days of composting. In the second cycle, during the containerized phase of composting, the ACT did not start as well

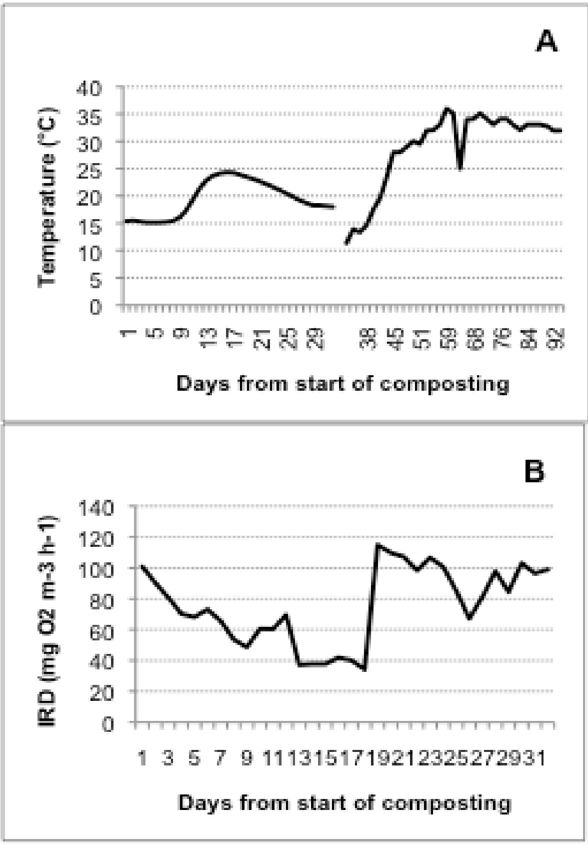

Figure 2: Temperature and instant IRD trends during the second composting cycle.

as the instant IRD was low pointing out a low microbial activity (Fig. 2). After the unloading of UMICA, the temperature slowly rose, but after 90 days a true thermophilic phase did not yet start. The last cycle, showed the best results (Fig. 3). Temperature reached 50 °C after the first week up to 60 °C in the successive weeks in container. Consistently, instant IRD showed two peaks of respiration over 1000 in the thermophilic phase. After the unloading, due to the oxygenation of mixture, the temperature increased again and it was around 45 °C for one month. So, the active phase of this cycle lasted 10-12 weeks. In Table 2 are presented the main chemical and microbiological characteristics of the composts obtained in the three cycles. Samples to analyze were collected about 4,5 months, 3,5 months and 2,5 months from the starting of process of the first, second and third cycle respectively. Composts produced in our experiments showed some characteristics close to that reported in Alburquerque et al. (2006) for a compost with olive mill fluid pomaces after 10 months: high organic carbon content, increase in pH, free of phytotoxicity (data from test of germination of Lepidium sativum not showed), high potassium and organic nitrogen content, low in phosphorus and trace metals.

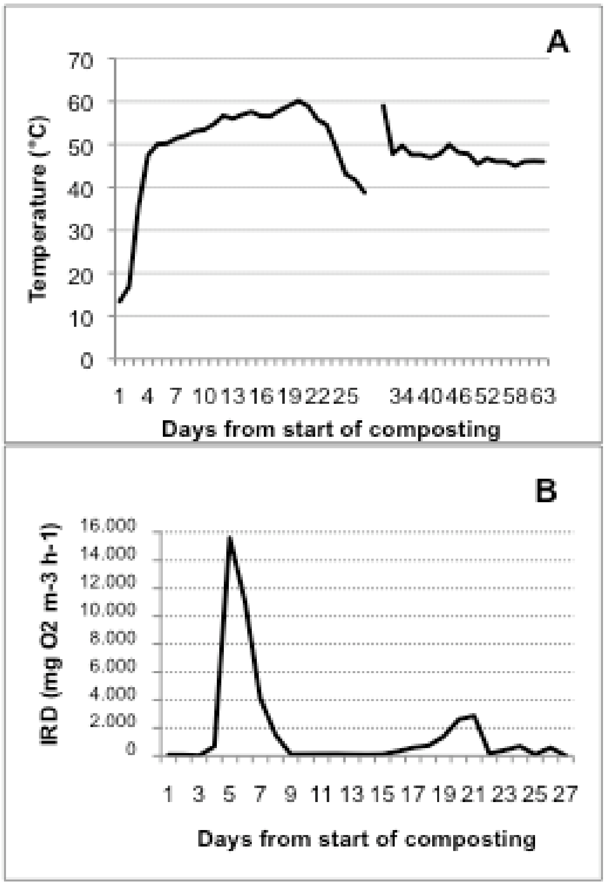

Figure 3: Temperature and instant IRD trends during the third composting cycle.

We found values of Enterobacteriaceae and Streptococci fecal out of tolerance limits.

Our data indicate that the static composting technology UMICA could be represent a flexible approach to match the production of by-products by olive mills in the short season of olive oil extraction (3-4 months).

It is unlikely that a single olive-mill might face alone the costs of a composting plant, but an association of mills could evaluate the rent of a modular technology as UMICA and to compost the olive pomace produced during their activities.

Table 2: Main characteristics of the olive pomace compost obtained in three cycles of composting at Montecalvo Irpino (AV) in 2008/2009. The tolerance limits are according to Italian Decree of Law n. 217/2006.

Parameters	I Cycle	II Cycle	III Cycle	*Tolerance Limits*
Moisture (% f.w.)	37,5*	36,3*	33,7*	*$\leq$ 50*
pH	9.5	9	9.1	*6-8.5*
Electrical Conductivity (dS m-1)	5.3	6.8	4.9	
Total organic Carbon (% d.m.)	39.7	42	40	*$\geq$ 20*
Humic + Fulvic Acids (% d.m.)	12.6	14	10.2	*$\geq$ 7*
Total Nitrogen (% d.m.)	2	2.4	2	*$\geq$ 1*
Organic Nitrogen (% total N)	96	96	95	*$\geq$ 80*
C/N	20	19	29	*$\leq$ 25*
Total P (% d.m. as P_2O_5)	0.76	1.03	0.54	
Total K (% d.m. as K_2O)	3.7	4.8	1.9	
Cd (mg kg^{-1} d.m.)	0.09	0.08	0.04	*$\leq$ 1.5*
Cr VI (mg kg-1 d.m.)	n.d.	n.d.	n.d.	*$\leq$ 0.5*
Hg (mg kg-1 d.m.)	0.12	0.12	0.08	*$\leq$ 1.5*
Ni (mg kg-1 d.m.)	6.8	5.3	2.3	*$\leq$ 100*
Pb (mg kg-1 d.m.)	2.4	3.1	1.4	*$\leq$ 140*
Cu (mg kg-1 d.m.)	20.7	17.8	7.8	*$\leq$ 230*
Zn (mg kg-1 d.m.)	73.5	54.9	21.1	*$\leq$ 500*
Total Enterobacteriaceae (UFC g-1 f.w.)	1.4×10^3	5.8×10^3	1.1×10^4	*$\leq 1.0 \times 10^2$*
Salmonella (presence-absence/50 g f.w.)	absence	absence	absence	*absence*
Streptococci fecal (MPN/g f.w.)	1.7×10^2	2.5×10^4	1.3×10^2	*$\leq 1.0 \times 10^3$*
Nematodes (n/50 g f.w.)	absence	absence	absence	*absence*
Trematodes (n/50 g f.w.)	absence	absence	absence	*absence*
Cestodes (n/50 g f.w.)	absence	absence	absence	*absence*

n.d.: not detected; * values measured on 30 of July 2009

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NUTRIENTS RELEASED IN THE RESIDUE DECOMPOSITION IN DIFFERENT TYPES OF PLANT COVERS IN THE OLIVE GROVE

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Abstract

The characterization of a residue and its decomposition dynamics is the starting-point for the establishment of an appropriate soil management strategy in order to achieve a balance between the farmer's interests and soil and water resource conservation.

The aim of this work was to evaluate the influence of the Mediterranean climatology on the decomposition dynamics of residues from different types of plant covers located in the lanes between trees in an olive grove, and how their evolution over time affects the cover surface, the stubble biomass, and the capacity of the latter to be a source of carbon and of soil nutrients.

The assay was carried out in an olive tree plantation in southern Spain with a random block experiment design of 4 treatments with 4 replications. The cover species tested were: *Brachypodium distachyon*, *Eruca vesicaria*, *Sinapis alba* and spontaneous grass (what kind of grass?). Samplings were done as from the removal of the cover in spring up to the sowing of the following autumn.

The best results of the summer persistence of the cover and of nutrient release were found in the species *Brachypodium*, in which, although it had a bad nascence in its establishment, the spring and early autumn rains triggered regrowth with an important amount of biomass, which ensured the protection of the soil against erosion agents.

Introduction

At present, there are two very important problems not completely solved in olive groves in Andalusia (Spain). One of them is of an environmental type due to erosion, soil compaction and diffuse pollution risks, and the other is the loss of fertility of the soils in which this crop is grown and the replacement of the nutrients extracted by the plant or lost in erosion processes.

To alleviate these problems, since 1980 onwards, some research works have been carried out to facilitate weed control, improve soil management systems, reduce tillage and prevent organic matter mineralization and the loss of structure in the soils. This has been done through no-till techniques and the establishment of plant covers between the olive tree lanes to protect the soil from erosion.

The development of sustainable agriculture production systems requires knowledge of the quality and evolution of plant remains in order to create strategies to manage them, and plant covers are no exception in this sense. The quality of the residue is generally associated with two factors. On one hand, the time it remains protecting the soil, and, on the other, its capacity to supply it with carbon and nutrients at the same time as it decomposes, with the area's climatology and the residue's composition influencing both those aspects (Ernst *et al.*, 2002). Approximately 50% of the weight of crop remains is C, from whence comes its importance as a source of organic carbon to agricultural soils (Crovetto, 2002).

Thus, the permanence of harvest residues on the soil surface promoted by conservative agriculture systems, as well as protecting the soil from erosion agents and improving its water balance, contributes to the long-term maintenance of organic matter and soil fertility (Guerif *et al.*, 2001; Sparrow *et al.*, 2006). The plant cover residues show: a variable water content; a high proportion of organic matter; a different mineral fraction with respect to the total concentration; diverse contents of P and variable neutral detergent fibre; and a generally high C/N ratio, although different between residues (Thorbum*et al.* 2001).

The decomposition process of the harvest remains depends on edaphic-type factors such as temperature, humidity, nutrient availability, soil microflora and fauna; these factors are inherent in the residue like its C/N ratio, content in lignin and soluble carbohydrates, and management factors like the amount and size of the stubble. However, the most important variables are the climatic ones and the susceptibility of the residues to be colonized by microorganisms (Borie, 1994; Soon and Arshad, 2002).

This study aimed to periodically sample the plant remains of different plant cover species in order to evaluate the influence of the Mediterranean climatology on the decomposition dynamics of the residues and to estimate how their evolution over time affects the cover surface, the stubble biomass and the latter's capacity as a source of nitrogen, carbon and soil nutrients.

Material and methods

To conduct the study, different species of plant covers were planted in an olive grove in southern Spain. These were: *Brachypodium distachyon, Sinapis alba,* and *Eruca vesicaria,* and they were compared to the spontaneous grass in each area.

In the olive grove experiment plot, the cover was cleared of weeds around April, and from then on and up to the autumn sowing of the new covers, a periodic sampling of the plant remains was made during the 2008 growing season. For the experiment, the subplots were divided into 4 random blocks, each of them containing the 4 cover species (Brachypodium, Eruca, Sinapis and spontaneous grass). In each species of each block, 3 collecting points of residue were taken, which made a total of 12 samples per cover type and sampling day. In order to assess the spatial variability of the cover, measurements were made of it at 52 points per each species.

The residue mass was estimated from the stubble collected in a metal frame of 0.25 m^2, placed at all the points selected to delimit the sampling areas. The residue collected was sent to the laboratory, where it was rinsed with distilled water to prevent any pollution in its subsequent analysis. It was then placed in a stove at 65ºC until it reached a constant weight, to estimate the amount of dry matter.

The cover percentage was measured following the subjective evaluation per sectors, method described by Agrela *et al.* (2003), which is characterized by using a 1 m^2 frame divided into 100 reticules. This method consists of the valuation of the different percentages of cover estimated in each reticule on a scale of 0 to 5 according to a greater or lesser covering.

Total carbon and nitrogen were analyzed in the residue samples in a LECO elemental analyzer. P and K contents were determined on the ash residue dissolved in 100 ml CH(?) 0.1 N. P was measured by automed (?) colorimetric and K by atomic absorption spectrophotometry. (Sparks *et al.*, 1996).

Results and Discussion

Fig. 1 represents the temporal evolution of the plant residue biomass of the different species of plant cover used in the assay and of the nutrients contained in them.

After weeding, the species with the highest residue mass was *Brachypodium distachyon* with 5180 Kg ha^{-1}, followed by *Sinapis* with 3141 Kg ha^{-1}, *Eruca* with 2960 Kg ha^{-1} , and the spontaneous grass, which at the start of the sampling recorded 2033 Kg ha^{-1}, had the least biomass. Although the trend was towards a loss of mass as the residues evolved, rainfalls of 160 mm recorded in April and of 140 mm between September and October favoured a reshooting in some species such as B*rachypodium* and *Sinapis*, and, to a lesser extent, spontaneous grass, or accelerated the residue's decomposition, as can be noted in the last samplings.

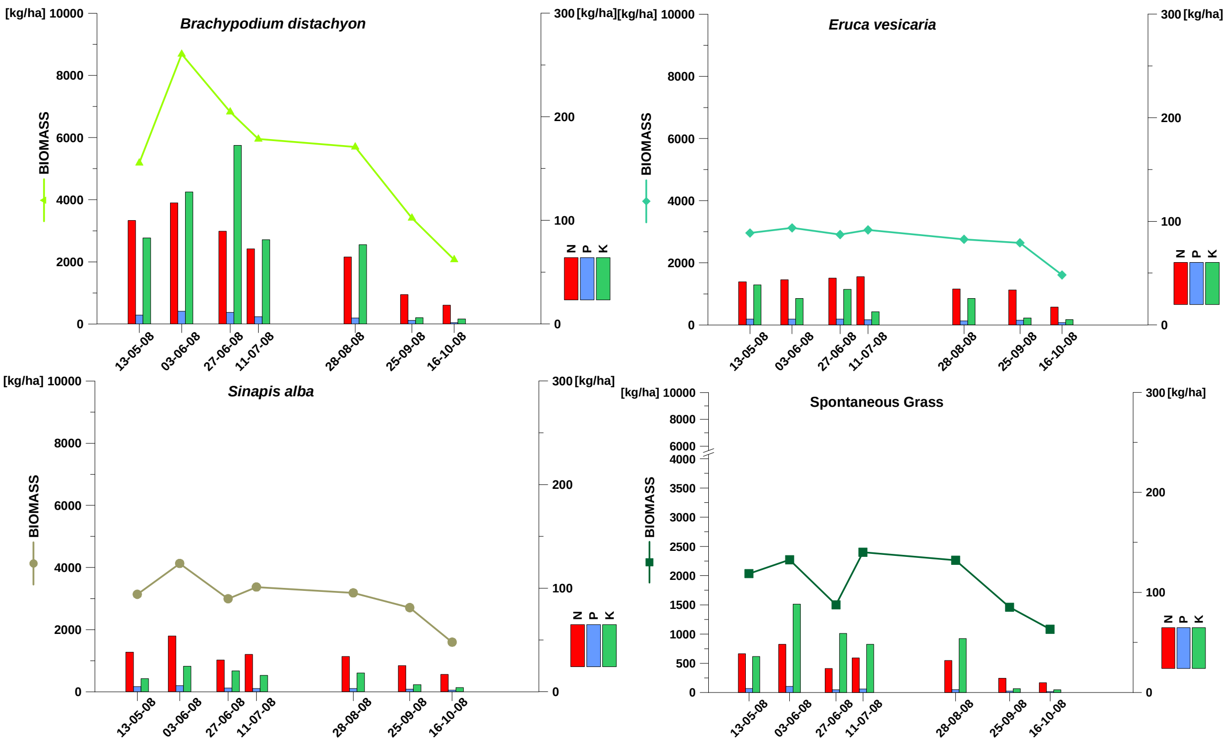

Figure 1: Temporal evolution of the biomass of the different species considered in the study and their content in N, P and K.

The nutrient release followed a similar dynamics to that experienced by the residue mass. A scant diminution in their content in the residue during spring and summer was produced, and a considerable decline in early autumn coinciding with the rainy period was noted.

It was the *Brachypodium distachyon* cover which gave the highest percentage compared to the rest of the species (Fig. 2). While the trend has usually been towards a decrease as the summer period advanced, in this species the degree of soil cover was maintained at above 80%, protecting the soil when the autumn rains began and erosion risks are greater.

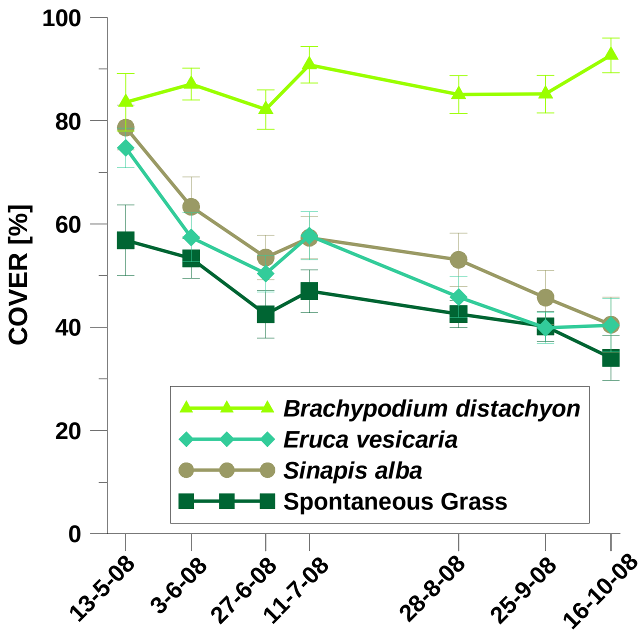

Figure 2: Temporal evolution over time of the cover percentage for the different species considered in the study.

An analysis of variance was made to determine the effect of the species on the dependent variables (biomass, cover), carrying out the subsequent contrast of difference between means using Bonferroni's correction $(\alpha = 0.05)$. In the rest of the cases, the Friedman test was employed, separating the means with Wilcoxon's contrast. The code for the contrasts post-hoc was: 1: Brachypodium, 2: Eruca, 3: Sinapis, 4: Spontaneous cover. The results of this analysis appear in Table 1 and they show significant differences between the different covers since the first sampling, although these differences became attenuated, in the case of the biomass, at the same time as the residues decomposed.

Table 1: Comparative analysis of the behaviour of different species in biomass and cover for the different sampling dates.

Date	Biomass	Cover	Date	Biomass	Cover
13/5/08	$p < 0.0001$ 1a,2b,3b,4c	$p = 0.017$ 1a,2ab,3a,4b	28/8/08	$p = 0.029$ 1a,2bc,3c,4b	$p = 0.001$ 1a,2b,3b,4b
3/6/08	$p = 0.019$ 1a,2bc,3c,4b	$p < 0.0001$ 1a,2b,3b,4b	25/9/08	$p = 0.02$ 1a,2a,3a,4b	$p < 0.001$ 1a,2b,3b,4b
27/6/08	$p = 0.026$ 1a,2b,3bc,4c	$p = 0.001$ 1a,2b,3b,4b	16/10/08	ns ($p = 0.15$)	$p < 0.001$ 1a,2b,3b,4b
11/7/08	$p = 0.001$ 1a,2b,3b,4b	$p = 0.026$ 1a,2bc,3c,4b			

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Table 2 shows the amount of nutrients released and the reduction or increase of the cover and mass of the different species considered in the study after the decomposition period. In general, the loss of cover during the summer period was not very important for any of the covers, with increases in the cover even being noted in *Brachypodium* (Fig. 2). The explanation of this lies in the fact that this species had a very bad nascence and it went on growing as the time passed and some rain fell. This species recorded the highest losses of biomass were recorded, which gives an idea of the ease with which the remains of this cover decomposed.

Table 2: Loss of biomass and cover and release of carbon and nutrients in the different species of plant cover in the farms at Fernán Núñez (187 decomposition days).

FERNÁN NÚÑEZ	Cover loss (%)	Biomass loss (kg ha^{-1})	Organic C (kg ha^{-1})	N total (kg ha^{-1})	P total (kg ha^{-1})	K total (kg ha^{-1})
Brachypodium distachyon	-0.49	3109.5	1279.3	81.7	7.3	78.2
Eruca vesicaria	31.0	1350.3	588.1	24.3	3.4	33.4
Sinapis alba	29.1	1540.3	665.2	21.5	3.5	8.6
Spontaneous Grass	19.9	949.7	413.8	28.8	3.2	33.4

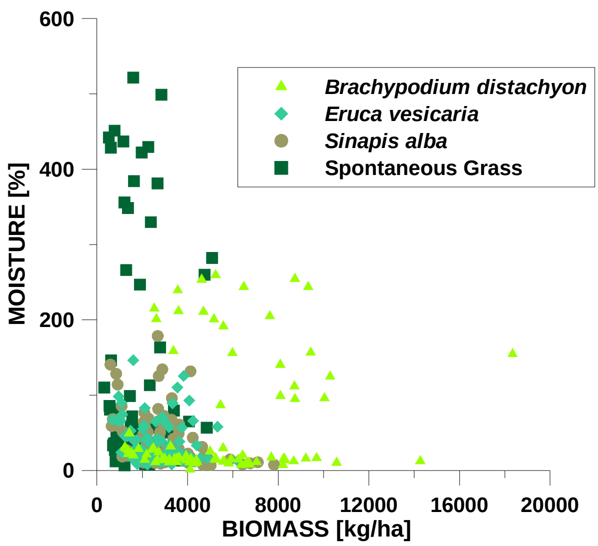

Figure 3: Percentage of moisture in terms of the biomass recorded for the different covers.

With regard to the amount of organic C released, the values were highly disparate and ranged between 1279.3 kg/ha of the *Brachypodium* species and the 413.8 Kg/ha recorded in the spontaneous grass. The edaphoclimatic conditions in the area and the characteristics of the residue, with a higher content of nitrogen in spontaneous grass, played an important role in the evolution of the plant remains. The nutrient release followed the sequence N≈K>P.

The moisture in the different species was also measured and it was seen that the spontaneous grass presented a higher content of water in its composition, which indicated a higher consumption of soil water and a greater competition of this resource with the tree. Also, the lowest values were recorded in Sinapis and Eruca (Fig. 3). The maintenance of water in the soil is a very important aspect as it limits growth and yields in dryland olive groves.

Conclusions

Under the edaphoclimatic conditions in southern Spain, the plant remains persisting in olive grove covers when the latter are removed around April have the double mission of protecting the soil from stormy events during the spring and early autumn rains, and to encourage the maintenance of the soil's fertility with the release of carbon and nutrients as these covers become degraded.

The summer protection of the soil by stubble from the different species has been ensured, since, in the worst of cases (*Sinapis alba*), only 29% of cover was lost until the establishment of the following one.

After 187 days of decomposition, the species releasing the largest amount of nutrients was *Brachypodium distachyon*, with values of 81,6; 7,3 and 78,2 Kg ha^{-1} for N, P and K, respectively. However, in most cases, the differences were not important with respect to the rest of the species.

Acknowledgements

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PHOSPHOGYPSUM-BASED PRODUCTS FOR FARM SCALE PHOSPHORUS TRAPPING

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Abstract

The phosphorus (P) leakage to waters by cultivation is an environmental problem caused by agriculture. This paper addresses two solutions in order to improve P use efficiency and control P discharge on farms. The proposed products can be utilized especially in those areas and farming practises which contribute most to the current P discharge, i.e. erodible fields and intensive manure application. The technologies rely on the use of phosphogypsum from phosphoric acid plant which produces calcium sulphate dihydrate ($CaSO_4 \cdot 2H_2O$) as a by-product. The gypsum is used either for P-sorption in cultivated soils as such, or for P-fractionation in liquid manures as a co-compound. The gypsum originates from Yara's igneous Finnish phosphate rock which does not contain heavy metals or radioactive compounds like do sedimentary phosphate rocks. In the soil treatment, gypsum was spread like lime (2, 4, or 6 ton/ha) after crop harvest and tilled thereafter into the soil where it improved soil particle aggregation and reduced turbidity and erosion by around 50%. Positive growth response and better nutrient uptake was found 2-3 years after the application. In the manure treatment, gypsum was mixed with an additive (5 kg/ton) into a farm pit where it precipitated dissolved slurry P which settled down with manure fibre. P-rich sediment was used in soils of low P-index and upper low-P liquid phase in fields of high soil P-index. Both fractions improved growth and nutrient uptake.

Keywords: crop, erosion, gypsum, phosphate, phosphorus leaching, soil amendment, manure.

Introduction

Phosphorus (P) accumulation into arable soils is a result of manure use or high P fertilization rates in past decades. In Nordic conditions, P leaches from these soils in runoff during wet seasons before and after winter. Particularly water erosion, i.e. transportation of soil particles in runoff, causes significant P load to waters (1-2 kg/ha/a) in spite of considerable decrease in

P fertilization rates from 1990's. More efforts to control erosion and soluble P leakage from cultivated soils are needed especially around the Baltic Sea which is one of the most eutrophicated seas in the world. Vegetated buffer zones (VBZ) along water courses or constructed wetlands are found to be insufficient to prevent phosphorus losses from fields to waterways. New solutions, tools and products to reduce the P load are required.

Soil dissolved P can be precipitated with calcium (Ca), iron (Fe), or aluminium (Al): E.g. Stout *et al.* (1999) reported remarkable decrease (50-60%) in the concentration of soil dissolved P by Ca-, Fe-, and Al-containing soil amendments (10 g/kg), called fluidized-bed combustion FBC fly ash or flue-gas desulfurization FGD gypsum. The neutralizing capacity of FBC increased soil pH to 8.0, where dissolved P was precipitated to non-plant available Ca phosphates. Similarly, FGD gypsum generated Fe- and Al-bound P not readily plant available either. Our goal was to precipitate dissolved P for plant use for slightly acid field conditions, typical in North European soils. Therefore, we focused on Ca-based tools.

Theoretically, $CaSO_4 \cdot 2H_2O$ (gypsum) without Fe or Al additives should bind P without weakening P availability to plants. According to Aura *et al.* (2006), gypsum also improves soil structural properties like aggregate stability of clay soil through elevated Ca and electrical conductivity in soil solution. This is important for erosion control which reduces P transportation within soil particles. It is anticipated that also leakage of dissolved P would decrease by electrical conductivity (EC) in soil solution where elevated EC improves phosphate binding on soil surfaces (Tan 1993; Aura *et al.* 2006). In acid soils this weakly adsorbed P would be easily available for plants because pH is too low for P precipitation.

In order to control P leaching from soils we need to focus also on the controlled use of manure. Liquid manures typically contain more P than the plant needs. A decrease in the P content of liquid manure to an acceptable level without much affecting nitrogen (N) should be provided. There is evidence that by mixing the gypsum with liquid manure, we can separate the liquid part and solids as well as P from N (Heinonen-Tanski *et al.* 2000; Pietola 2008) and in this way we can use the P in an optimal way where it is needed, in the soil for plant use. In contrast to soil conditions, liquid manure treatment aims to Ca-phosphate precipitation prior to soil application. Thus, alkaline conditions are required in a slurry pit to enable also struvite ($MgNH_4PO_4 \cdot 6H_2O$) precipitation (Nelson *et al.* 2003). It is hypothesized that once spread in acid soil conditions, Ca-phosphates or struvite are dissolved and phosphates are plant available.

The paper will present these two innovative tools for P trapping in practical farming, focusing on crop growth response and nutrient use. Quality of pure phosphogypsum is emphasized.

Materials and methods

Raw material originates from fertilizer production where phosphoric acid is used as a P component. Gypsum (phosphogypsum), $CaSO_4 \cdot 2H_2O$ is produced as a byproduct during the production of phosphoric acid by digestion of phosphate ore concentrated with sulphuric acid. The phosphate ore originates from pure igneous phosphate rock of Siilinjärvi in Finland. The gypsum thus not contains radioactive elements or heavy metals like phosphogypsums originating from sedimentary phosphate rocks.

The gypsum was used for P-trapping in soils as such, or for P-fractionation in manures as a main component. The paper presents soil and growth response three field experiments in Yara's experimental station (2 for soil treatment, 1 for manure treatment) and by one farm test (manure treatment) in Southern Finland. The soil gypsum treatments were carried out in 2006 and 2008 on clay soils with low-medium P-index (8-11 mg/l P in acid ammonium acetate AAC) where gypsum was spread 0, 2, 4, and 6 (only 2008) ton/ha after harvest. The fields were then either tilled (by mouldboard plough or cultivator) or left untilled (only 2008). Each treatment had four replicates. Older experiment was sown in May 2007 with spring wheat (*Triticum aestivum L.*), in May 2008 to oil seed rape (*Brassica rapa L.*), and in 2009 again to wheat. Treated manure fractions were tested for cropping by a field experiment and by a farm test. For this purpose, gypsum-based precipitate was mixed (4-6 kg/m^3) into liquid slurry pit (500 m^3) of two farms in April 2009. After settling for 4 weeks, 24 m^3 untreated slurry, low-P liquid fraction and rich-P solid fraction were spread on the field experiment with 70 kg/ha mineral N (CAN), or 130 kg mineral N. Each treatment had four replicates and was sown to wheat in May. Soil texture was clay loam and P-index low-medium (9.5 mg/l P_{AAC}). On farm, soil P-index was high (25 mg/l P_{AAC}, 0-20 cm soil depth) and manure application rate (20 m^3) followed normal farming practises. Despite of high soil P-index, 15 kg/ha P is allowed to spread yearly and farmers can use manure table-index of P 0.8 kg/m^3 despite of manure actual P content. Application rate for the liquid was 25 m^3 after urine P table-index of P 0.5 kg/m^3. Additionally, 100 kg/ha CAN-N was used on these adjacent fields. In all field experiments, soils were sampled to a soil depth of 20 cm each spring and autumn and additional samples were collected from soil surface (0-10 cm) at plant sampling. To estimate erosion sensitivity by gypsum soil amendment, turbidity was measured from 10 g soil aggregates (ø 1-2 mm) suspended to water in 25 ml plastic tubes. After 24 hours settling, turbidity of the liquid was measured by a turbidimeter (Hasch). Additionally, dissolved P leaching from manure amended soils were measured by 0.5 litre soils, moisturized to field capacity in plastic pots with bottom holes. Soils were then watered by 150 ml, and dissolved P was analysed from percolated water. For crop response and plant nutrient uptake, plants were sampled from 0.25 m^2 from different locations per a plot (at 1st sampling 3, at 2nd and 3rd 2 locations). Plant nutrients were analysed from dried material by IPC after wet combustion (perchloric-nitric acid), N by Kjeldahl (Novalab 001.A). Crops were harvested from 15 m^2 area for grain yield measurement.

Results and Discussion

Laboratory tests from field soil samples showed gypsum effect on particle removal into soil solution (Figure 1), in accordance with laboratory incubation tests (Pietola 2008). These data agree also with other larger scale data from the gypsum –treated fields where 4-6 ton/ha of gypsum decreased remarkable turbidity and P load during the first winter after application (Turtola, Ekholm 2009, Personal comm.): These large-scale data base on rain simulation studies and on-line measurements of particle P. Some decrease of dissolved P was shown, though data were not yet completed.

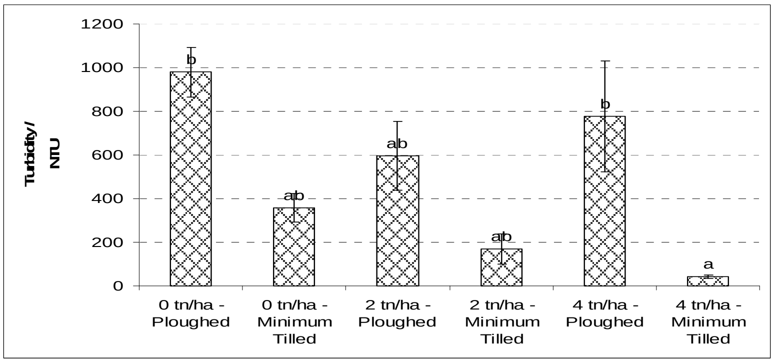

Figure 1: Turbidity of soil solution 6 months after gypsum application, 2 or 4 ton/ha before mouldboard ploughing to a 20 cm soil depth or minimum tillage to a 10 cm soil depth.

The present field studies clearly show that the gypsum did not harm cropping, rather vice versa. Even if the seed yield of oil seed rape was not affected one winter after the soil treatment (2006), the grain yield of spring wheat was improved by 600 kg/ha after the second winter (2008). In ploughed soils the yields were 5410, 5840 and 6140 kg/ha by 0, 2, and 4 ton/ha application rates; in minimum tillage 4940, 5240, and 5360 kg/ha, respectively ($LSD_{0.05}$ 470 kg/ha). By shoot sampling in 2009, the positive response was no more evident although some slight positive effect of increased nutrient uptake was detected especially in mouldboard ploughed soils (Table 1). For the field experiment established in early December 2008, the shoot growth and nutrient uptake of spring wheat was improved (Table 1). These data indicate that the gypsum treatment should be repeated each 3 years, though more soil data is required for the final estimation.

Table 1: Shoot growth response of spring wheat to gypsum applied in late 2006 or 2008: Shoots sampled at the 30th June 2009 for the old experiment, at the 24th June for the new exp.: DW represents dry mass/m^2, and P, N, S nutrient uptake mg/m^2.

Year	Gypsum ton/ha	Moudboard ploughed DW	P	N	S	Minimum till /stubble mulched DW	P	N	S	No till / direct sowing DW	P	N	S
2006													
	0	236	486	3980	442	231	883	6000	613				
	2	239	675	5030	550	241	958	7510	739				
	4	243	567	4240	491	229	883	6330	679				
	$LSD_{0.05}$	60	266	1790	184	73	449	3060	256				
2008													
	0	69	204	2370	228	51	129	1610	157	58	155	1860	197
	2	84	286	3010	305	64	162	2030	199	61	177	2280	225
	4	85	299	3240	320	60	163	2060	217	65	199	2390	252
	6	92	334	3300	357	70	197	2450	277	64	218	2630	293
	$LSD_{0.05}$	14	98	700	75	12	36	530	55	55	96	1050	100

The gypsum-treated manure fractions did not improve remarkably crop growth in the field experiment of low soil P-index (Figure 2), but on farm conditions for high soil P-index the upper liquid fraction was superior. Also nutrient contents and nutrient uptake by shoots before tillering were improved (Figure 3). In the field experiment, soils sampled from 0-10 cm soil depths at early shooting indicated that soil P-index remained similar in treatments of CAN, CAN+manure, or CAN+treated liquid, being 9.5, 10.4 and 10.8 mg/l P_{AAC}, respectively. With CAN+solid fraction the soil P-index increased to 14.5 mg/l P_{AAC} from which shoot P uptake was the highest (800 mg m^{-2}, measured Jun 24). From this treatment, however, dissolved P leaching increased by 50% as compared to the other treatments. Untreated manure was not, unfortunately, of the same origin than the treated one. It was much diluted, containing only total P 0.3 kg/m^3, and thus did not represent normal pig slurry containing P >1 kg/m^3. Thus, P uptake was also lowest, 620 mg m^{-2} (measured June 24, $LSD_{0.05}$ 330).

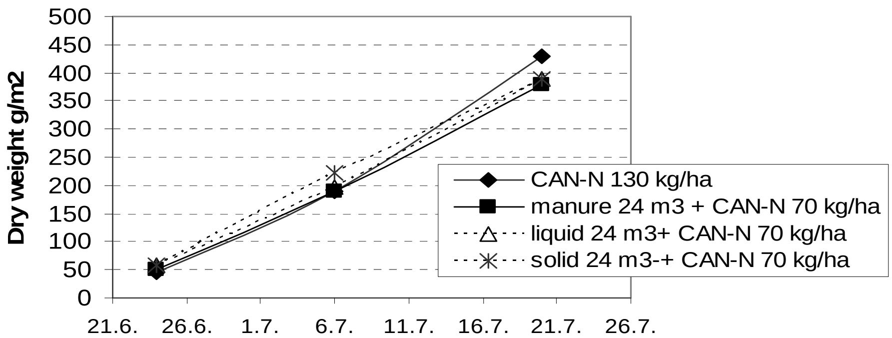

Figure 2: Shoot biomass growth of spring barley (2009) fertilized by CAN only, or with diluted liquid manure, liquid fraction of gypsum treated manure or solid fraction of the treated manure in soils with medium P-index.

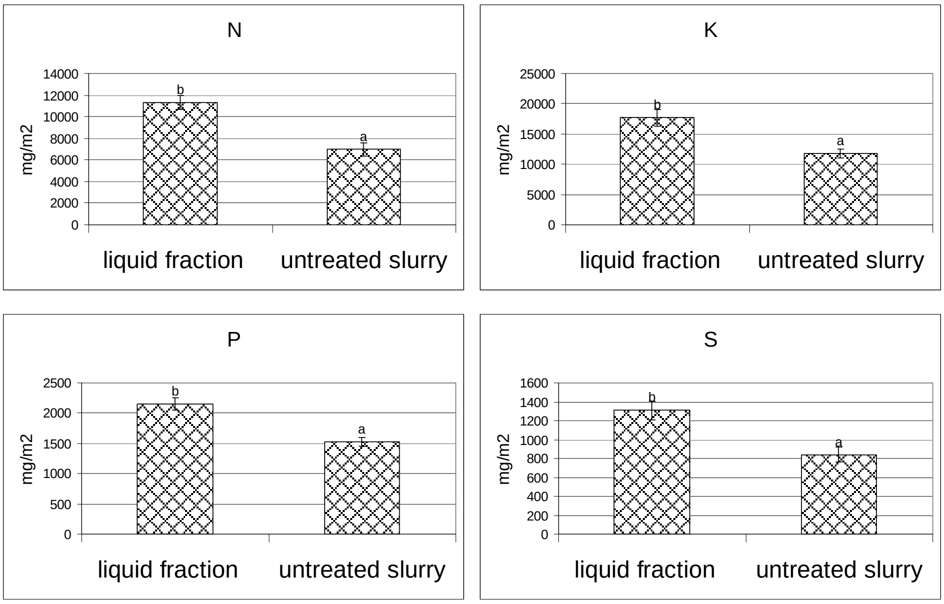

Figure 3: Nutrient uptake of spring wheat shoots before tillering on soil of high P-index, fertilized by pig slurry (P 1.3 kg/m^3, 20 m^3/ha) or by gypsum-treated pig slurry and (P 0.5 kg/m^3, 25 m^3/ha), and CAN for both treatments 100 kg/ha.

Beneficial growth response by the low-P liquid was clearly indicated by the farm test where shoot growth of spring wheat was 570 g/m^2 with the liquid fraction (P rate 12 kg/ha) but only 450 g m^{-2} for the untreated manure (P rate 26 kg/ha). Similarly, nutrient uptake was improved (Figure 3), apparently partly because the treated liquid well infiltrated into soil for emerging spring wheat. At plant sampling, soil P-index to a soil depth 10 cm was 50 mg/l P_{AAC} , typical for a pig farm and risk for P leaching if extra P will be given without manure P fractioning.

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THE “IN FARM” OLIVE MILL RESIDUAL COMPOSTING FOR BY-PRODUCTS SUSTAINABLE REUSE IN THE SOIL ORGANIC FERTILITY RESTORATION

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Abstract

A composting trial of three-phase olive mill and olive orchard by-products has been carried out over a three-year period, to evaluate the efficiency of the “in farm” composting process, and the compost effectiveness on soil characteristics and olive tree productivity. The aim of study will be the valorization of agro industrial by-products through sustainable re-use as amendants in the organic fertility restoration of soils, using a low input technique adoptable in situations where industrial installations are unenvisageables.

In spite of the non optimal trial conditions, has been obtained a “ready” compost typology, and the screening of the micro organisms, done at different stages of the biomass fermentation, confirm the correct way of composting process. These composts, spread and buried in different olive orchard soil at 60 t/ha, sensibly increased the organic fertility, improved the water retention and the nutrients availability, and enhanced the plants productivity.

The results observed in this study, confirm the efficiency of the “in farm” composting process and the agronomic and environmental validity of the olive mill residual compost, as a valid alternative to the “as is” residual spreading, even if obtained under not optimal composting process conditions.

Keywords: Compost; By-products; Renewable biomasses; Sustainable agriculture; Soil fertility.

Introduction

Although the Italian legislation allow the “as is” spreading on soils of olive mill industry by-products (Amirante, 1998), this practice presents management difficulties, environmental risks and agronomical inefficiency, such as: low amendant efficiency of not humified organic matter; inhibition of soil microflora activity; reduction of soil permeability; risk of streaming and water pollution; loss of by-products potential add-value.

The aerobic stabilization of these biomasses, suitably added with olive-orchard and/or other crop by-products, allow to obtain a low-cost stabilized organic amendant, which provides a higher efficiency in the recovery or maintenance of organic soil fertility, with sensitive environmental and cultural advantages, such as: enhancement of permeability and water retention of soil; better endowment and availability of nutrients; fixation of carbon; reduction of desertification processes (Andrich et al, 1992; Centemero, 2002; Lombardo et al, 1995; Paredes, 1998).

The aim of this study is the evaluation of the "in farm" composting process efficiency of olive mill and olive-orchard by-products (Amirante, Montel, 1999; Clodoveo et al, 2002); the evolution of microbiological pools during the process (Galli et al, 1997; Monteoliva-Sanchez et al, 1996); and the amendant and nutritional efficacy of compost on soil characteristics and on olive trees productivity.

Materials and Methods

In a three-year period have been carried out two experimental composting cycles, using a three-phase olive mill and olive orchard by-products, on "in farm" conditions.

The matrix has been composed, depending on by-products availability, by:

CM1 - 200 q pomace; 8 mc waste water; 20 q grinded olive shoots and leaves from pruning;

CM2 - 300 q pomace; 80 q grinded olive shoots and leaves from pruning.

For both matrix, the composting process started in mid of March, weekly remixing the biomass with mechanical shovel, to ensure the oxygenation and to control the heat of fermentation. The monitoring of fermentation process has been weekly effected, checking the temperature and the humidity of the matrix, at the same time of every remixing.

The screening of microbics pools has been effected in different steps of composting process. To evaluate the total bacteria amount, has been used a common and non-selective grown media (Plate Count Agar); for molds and yeast, has been compared the Sabouraud Dextrose Agar media and the Yeast Dextrose Agar (Y.P.D.), added by 150 ppm of chloramphenicol, to inhibit the growth of bacteria. To evaluate the amount of total and faecal coliphorm, has been used the Violet Red Bile Agar (V.R.B.A.) for first group, and the Slanetz – Bartley Medium for the second one. In all substrates, the liquid fraction (distilled water) has been replaced with a sterilized extract of composting matrix. Microbial community has been assessed through plate-counter and expressed as colony-forming units (UCF) per ml.

After approx. 6,5 months, the composts have been spreaded on soil at around 145 kg/tree (60 t/ha), and buried with a disk arrow. At this time, a final sample of compost was collected and analyzed. In respective next year harvest time, a sample of amended and witness soil was analyzed to compare the evolution of their characteristics.

Results and Discussion

The composts parameters at the end of respective composting cycle (Tab. 1), and their respective Microbiological paths (Figg. 1 and 2), show a composition in according to the "ready" compost typology, and a correct way of composting process.

Table 1: Final characteristics of the composts.

	CM1 (2006)	CM2 (2007)
Humidity %	20.0	22.8
pH	6.6	7.1
C/N	21.6	15.2
TOC %	15.6	18.7
N % s.s.	0.72	1.23
P_2O_5 %	0.68	0.57
K_2O %	1.02	0.68

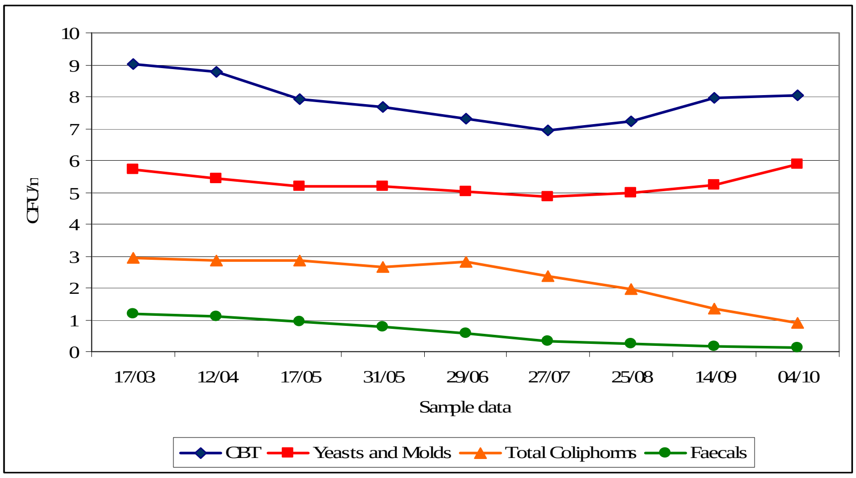

Figure 1: Microbiological path of 2006 composting process (CM1).

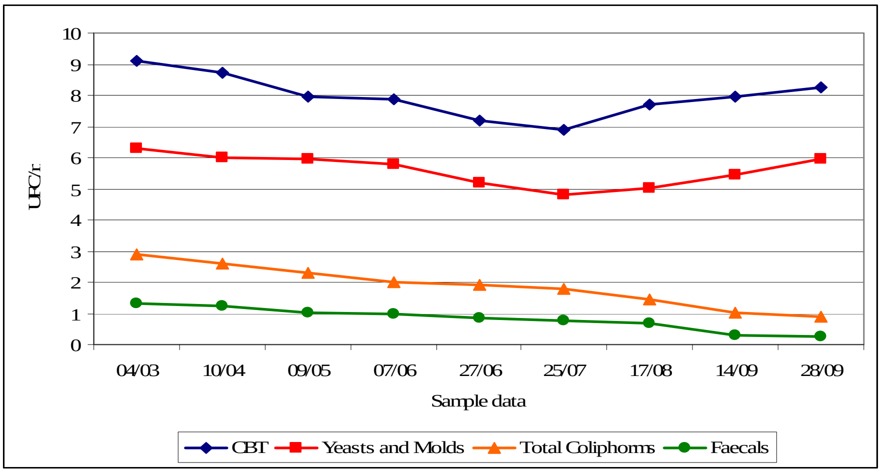

Figure 2: Microbiological path of 2007 composting process (CM2).

The analysis of soils evolution, effected at respective next year harvest time, showed a decrease of pH, and a significant improvement in O.M. (+ 38,5 in CM1; + 33,6 in CM2), (Table 2), with a better water-retention trend (Table 3) vs. respective CT soils.

Table 2: Soil Analyses at harvest time.

	2007		2008	
	CM1	CT1	CM2	CT2
pH	**7.8**	**8.2**	**7.3**	**8.0**
Sand	71.4	70.0	58.1	57.3
Silt	17.9	18.3	26.1	26.6
Clay	10.7	11.7	15.8	16.1
O.M.	**1.48**	**0.91**	**1.52**	**1.01**

Table 3: Soil water retention.

	pF 4.2	**pF 3.7**	**pF 3.0**	**pF 2.5**
CM1	8.8	9.2	10.7	15.3
CT1	7.0	7.8	8.0	12.5
Δ %	**+20.35**	**+15.22**	**+25.01**	**+18.06**
CM2	7.8	8.6	9.4	15.3
CT2	6.9	7.5	8.0	12.9
Δ %	**+11.52**	**+12.79**	**+14.81**	**+15.82**

The amended trees showed noticeable enhancements both in vegetative and productive parameters, vs. untreated trees, confirming the compost effectiveness in nutrient supply and availability, in both cultivar (Table 4).

Table 4: Trees responses.

YEAR	2007 (FS 17)			2008 (Leccino)		
Test	CM1	CT1	**Δ %**	CM2	CT2	**Δ %**
Growth	6.02	8.12	**-34.88**	8.23	10.61	**-28.92**
Flw bud Differ %	75.50	74.55	**+1.26**	70.59	63.16	**+10.52**
Fruit Set %	2.68	2.15	**+20.01**	2.67	1.75	**+34.52**
Fruit Drop %	20.47	28.08	**-37.18**	21.12	32.01	**-51.51**
Yield Kg	13.26	12.34	**+6.94**	18.94	17.40	**+8.13**
Fruit weight	3.87	3.26	**+15.76**	3.11	2.53	**+18.65**
Pulp/pit	5.64	4.98	**+11.70**	5.38	4.91	**+8.74**
Oil %	16.74	15.83	**+5.44**	19.13	18.05	**+5.65**
Oil yield (Kg/tree)	2.22	1.95	**+12.16**	3.62	3.14	**+13.26**

In the trial conditions, the effectiveness of the olive mill residuals compost gotten on “in farm” conditions, has been demonstrated both on soil characteristics and on olive trees productivity, since the first year of application. In a sustainable agriculture and in the valorization of the renewable biomasses, the olive mill residuals composting is a valid alternative to the fresh pomace and waste water spreading on soils, in the restoration and/or maintenance of the organic fertility of farm lands, and to counteract of desertification processes. Moreover, the nourishing effect of these composts, allow to reduce the needs of synthetics agro-chemicals in the fertilization plans. Besides, being a process with low-cost of transformation and management, the composting is in full accordance with the principles of energetic and economic sustainability in the use of renewable resources.

Acknowledgements

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EFFECTS OF CEMENT AND QUICKLIME POWDER ON THE GROWTH OF CORN AND SUNFLOWER SEEDLINGS

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Abstract

The protection of our environment is our common task. All pollution that impacts our soils, plants or the narrowly or broadly conceived environment will appear in the food chain, and sooner or later in human beings at the peak of the food pyramid. The various pollutions justify the heightened focus on the environment. The objective of these studies was to examine the physiological effects of industrial powders originating from the production technologies of factories situated in the vicinity of a city, as well as their potential use in plant nutrition. In our work, we have placed an emphasis on dust pollution entering the environment. The elements of the powders (e.g. Al, Cd, Cr) may modify the life cycle of the living world in the environment. However, we have not found toxic element accumulation in the leaves samples taken at the given sampling sites. It has been confirmed that by-products of cement and quicklime factories can be used for the nutrition of plants.

Keywords: industrial pollution, quicklime powder, plant growth.

Introduction

The main cause of the greenhouse effect is the air pollution, caused by CO_2 and other gaseous pollutants, as well as various solid, small-sized, air-borne materials.
Some of these “solid” pollutant are powders originating from various human activities, such as cement and quicklime production. The different filtration systems are more or less efficient, though there are periods when larger quantities of powder enter the environment.

Materials and methods

Corn (*Zea mays L. cvs. Norma SC*) and sunflower seeds were used in the experiments. The seeds were sterilized with 18% hydrogen peroxide, and then washed in distilled water. The corn seeds were then replaced to 10 mM $CaSO_4$ for 4 hours, but the sunflower seeds were not. The seeds were germinated on moistened filter paper at 25°C. The seedlings were then transferred to continuously aerated nutrient solution of the following content: 2.0 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.1 mM KCl, 1µM H_3BO_3, 1µM $MnSO_4$, 0.25 µM $CuSO_4$, 0.01 µM $(NH_4)_6Mo_7O_{24}$. Iron was added to the nutrient solution as Fe-EDTA at a concentration of 10^{-4}M. The filtrated powders were added to the nutrient solution in a quantity of 0.5g dm^{-3} and 5.0g dm^{-3}. The seedlings were grown under controlled environmental conditions (light/dark regime 10/14 h at 24/20 °C, 65–70% relative humidity and a photosynthetic photon flux of 390 $mEm^{-2}s^{-1}$ at plant height). The repetitions were four; the main differences were marked. The element (Al, Cd, Cu, Cr, Fe, Ni, Zn) contents of samples (sediment powder of quicklime factor, air-borne powder of quicklime factory, air-borne powder of cement factors and air-borne powder of quicklime hydrate factory) were measured with ICP, while relative chlorophyll contents were determined with the use of SPAD 502 (Minolta). The samples were dried at 85°C; the dry matter contents of shoots and roots were measured. To remove the apoplastic-bound ions, the roots were washed in 0.1 nHCl. The powders originated from the quicklime and cement factories in Miskolc-Hejőcsaba (North Hungary). The filtered powder was collected from the filtering systems of factories with the permission of management, as well as from the roofs of houses near the factory.

Results and discussion

The factories are well-equipped with various filtering systems to safeguard the environment, but there are technical difficulties reducing the efficiency of these systems. Lots of powder enters the environment when the filters are replaced, and much smaller quantities when they are in operation. To become aware of the potential threats, the powders originating from the various stages of the filtering process were analyzed.

The contents of elements in different powders can be seen in Table 1.

Table 1: Concentrations of elements taken from different stages of the filtration process (ppm).

Elements	Treatmnets			
	QLF 1	QLF 2	CF	HYD
Al	1,491.00	6,939.00	6,426.00	229.00
Cd	0.80	2.05	3.90	0.31
Cr	3.92	6.26	11.80	2.60
Cu	2.64	3.45	10.70	1.66
Fe	1,067.00	1,980.00	12,941.00	160.00
Ni	6.71	8.40	9.20	2.10
Zn	7.89	15.10	597.00	4.64

QLF1: sediment powder of quicklime factory, QLF2: air-borne powder of quicklime factory, CF: air-borne powder of cement factory HYD: air-borne powder of quicklime hydrate factory.

The analysis shows that there are several metals in the powders. The main interest in aluminium has focused on the ability of some plant species to tolerate high aluminium concentrations in the soil or nutrient solutions. There is no convincing evidence that aluminium is an essential mineral element even for accumulator species. However, there are many reports on the beneficial effects of low aluminium concentrations in the soil or nutrient solutions on plant growth (Bollard, 1983; Foy, 1983). Nevertheless, aluminium and cadmium pollution can be dangerous. Their uptake depends on the prevailing soil conditions.

The physiological effects of any heavy metal depend on its solubility in the soil. The solubility test showed very low solubility in the case of all the collected powder.

Mustard is one of the most sensitive plant usually used for toxicity examination.

The germination percentage is very high the cement dust, but is negligible the quicklime and quicklime hydrate dust. The germination was higher in the cement than the control on the second day of measurements (Table2). It is good, because the early and balanced germination result homogeneous vegetation, which makes plant cultivations work easy.

Table 2: Different filter powder effects on the germination.

measuring days	Treatments				
	control	QLF1	QLF2	CF	HYD
	germination percent (%)				
2.	56.0 ± 5.6	2.0 ±1.4	0.5*± 0.7	77.5*± 10.6	1.0 ± 1.4
3.	24.0 ± 5.6	0.0 ±0.0	0.0 ± 0.0	8.5*± 2.1	0.0 ± 0.0
4.	9.5*± 0.7	1.5*±0.7	0.5*± 0.7	4.0 ± 1.4	0.5*± 0.7
7.	4.5*± 3.5	0.0 ±0.0	0.0 ± 0.0	3.0 ± 1.4	0.0 ± 0.0
summary:	**94.0**	**3.5**	**1.0**	**93.0**	**1.0**

The dusts of quicklime and quicklime hydrate factory hindered plants in growth. They cause average metabolism disorders and reduced growth. When the examination of cement powder brought surprising results, we planted corn germ plants on the powder originating from electrofilter of cement factory. The development of the corn was normal with no symptoms (Picture 1).

Picture 1: Corn on cultivated cement powder.

Differences in dry matter contents were observed during the treatments. The associated results are shown in Table 3.

Table 3: Dry matter of shoots and roots of corn and sunflower seedlings (plant g^{-1}).

Treatments	Corn		Sunflower	
	shoots	roots	shoots	roots
control	0.11	0.17	0.12	0.34
1.25 HYD	0.03	0.10	0.04	0.11
12.5 HYD	0.01	0.09	0.04	0.06
1.25 CF	0.09	0.28	0.07	0.37
12.5 CF	0.08	0.26	0.07	0.41
1.25QLF1	0.07	0.24	0.05	0.10
12.5QLF1	0.01	0.07	0.02	0.07
1.25QLF2	0.04	0.11	0.05	0.11
12.5QLF2	0.05	0.09	0.04	0.07

1.25=0.5g dm^{-3}; 12.5=5.0g dm^{-3}.

In nearly all the cases, the values were below the control ones. The powder from the cement factory decreases the growth of shoots, while the development of root was more intensive than the control. On the other hand, dry matter expansion is below the control with the QLF1 powder collected from the first sediment filter of quicklime factory, the QLF2 powder collected from the second air-borne matter filter of quicklime factory and HDY collected from the air-borne powder of quicklime hydrate factory. In these powders, the growth of shoots is very low. The increase of shoots under more intensive care is favorable, because the plant can take up more water and more nutrients on a larger surface, so the stress tolerance of the plant increases. The results suggest the potential application of cement powder as a microelement fertilizer or as an additive stimulating the growth of roots. The chlorophyll contents affect the photosynthetic activity. The reason underlying the drop of dry matter accumulation is the lower levels of the chlorophyll contents (Table 4).

Table 4: Relative chlorophyll contents of corn and sunflower leaves (Spad units)

Treatments	Corn		Sunflower
	2^{nd} leaves	3^{rd} leaves	1^{st} leaves
control	44.53 ± 2.9	39.38 ± 3.2	43.62 ± 1.1
1.25 HYD	39.90 ± 1.4	27.70 ± 7.7	32.63 ± 1.2
12.5 HYD	35.06 ± 3.9	29.43 ± 3.6	28.55 ± 1.9
1.25 CF	40.90 ± 1.8	35.80 ± 1.8	43.43 ± 2.3
12.5 CF	45.70 ± 4.6	36.30 ± 3.1	44.75 ± 1.1
1.25QLF1	35.43 ± 1.5	29.58 ± 1.3	29.30 ± 3.6
12.5QLF1	28.05 ± 7.6	27.65 ± 7.7	27.67 ± 3.6
1.25QLF2	35.33 ± 0.6	27.10 ± 3.0	31.45 ± 2.0
12.5QLF2	36.50 ± 0.6	32.00 ± 4.1	34.87 ± 2.9

1.25=0.5g dm^{-3}; 12.5=5.0g dm^{-3}.

In the light of our studies, it seems to be obvious that the pollution affects the vegetation. Yet, the related effects are specifically unrevealed because of compensation by the soil and rhizosphere. The laboratory experiment has also evidenced the harmful effects of powders, though some promoting effects on the plant growth were observed when cement factory powder was used (Picture 2–3–4).

Picture 2-3-4: Effects of cement on the corn and sunflower

The root and shoot development were witnessed to be more intensive. Further examinations are needed to clarify the potential use of the cement factory powder in plant production, including in floriculture.

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EFFECTS OF INDUSTRIAL ORANGE WASTE AS ORGANIC FERTILIZER ON GROWTH AND PRODUCTION OF DURUM WHEAT AND SUNFLOWER

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Abstract

In the last ten years increasing attention has been paid towards resource conservation and by-product utilization, making 'waste' a resource to be utilized and not just discarded. In areas where citrus orchards are the prevalent crops, such as north east cost of Sicily, citrus-fruit processing produces a great amount of wastes. Its agricultural use is impeded by limited knowledge on its effect on crop performance. Initial results have proven that the amount of orange waste applied to arable crops needs to be defined according to crop requirements and soil conditions.

A research project was carried out to study the effects of two dried orange waste doses (3 and 9 kg m^{-2}), compared to a control without fertilization, on growth and productivity of durum wheat (*Triticum durum* Desf.) and sunflower (*Helianthus annuus* L.). Our results show that the organic fertilization promoted crop growth, determining in both species a significant increase in leaf area index (LAI) and crop growth capacity (CGR) In wheat, grain yield was significantly and negatively influenced by the excessive vegetative growth induced by organic fertilization; while in sunflower grain yield was promoted.

Keywords: industrial orange waste, durum wheat, sunflower, growth, yield.

Introduction

The agro-industrial waste implies increasing problems due to the high economic cost and the heavy environmental repercussions of its disposal. By contrast, its re-use as organic fertilizer, could represent a sustainable approach to recycle nutrients and to reintegrate organic matter in soil, especially in the Mediterranean environment, where high is the risk of loss of fertility. In areas where citrus orchards are the prevalent crop, one of the most important agro-industrial activities is citrus-fruit processing which produces a great amount of a mixture of pulp (30-35%), peel (60-65%), membrane and seed (0-10%) wastes. In Italy, citrus waste has been estimated from 350,000 to 450,000 t per year (Fisichella, 2004); its re-use is hindered by the complex difficulty of integrating it into agricultural systems, due to economics, logistics and organizational aspects and, last but not least, to the farmer acceptance (Merillot, 1998). Moreover, its agricultural use is certainly impeded by limited knowledge and little research on its composition (Van Heerden, 2002) and its effect on soil characteristics and crop performance (Correia Guerrero *et al.*, 1995; Belligno *et al.*, 2005; Tuttobene *et al.*, 2009). Initial results using orange waste in agriculture as a soil conditioner or organic fertiliser, have proven positive for the physical and chemical characteristics of the soil (Intrigliolo *et al.*, 2005). In orange orchards, the long term application of dry orange waste as an organic fertilizer produced yields similar to mineral fertilizers (Intrigliolo *et al.*, 2005). By contrast, the amount of waste applied to arable soils needs better defining according to crop requirements and soil conditions (Correia Guerrero *et al.*, 1995; Belligno *et al.*, 2005; Abbate *et al.*, 2008). With this in mind, we carried out a research project to study the effects of two dried orange waste doses (3 and 9 kg m^{-2}), compared to a control without fertilization, on growth and productivity of durum wheat (*Triticum durum* Desf.) and sunflower (*Helianthus annuus* L.).

Material and methods

The research was conducted during the winter 2003-04 on durum wheat (cv "Mongibello") and during the spring 2004 on sunflower (cv "Goriasol"), sowed in 0.32 m^3 (0.8 × 0.8 × 0.50) lysimetric tubs, filled with a sandy sub-alkaline soil, total lacking of limestone and organic C. Nine tubs for each species were arranged in a randomized block design with three replications. The following treatments were applied: unconditioned soil (A); 3 kg m^{-2} orange waste (B), and 9 kg m^{-2} orange waste (C). The orange wastes used were previously air-dried for seven months up to 15% of humidity. Orange wastes have an acid reaction (pH 5.8), and they are rich in Total Organic Carbon (40%), nitrogen (21.3‰) and calcium (221 meq 100 g^{-1}). Dried orange wastes have been buried in first 20 cm depth one day before sowing. Sowing was carried out on December 23th 2003 for durum wheat using 400 m^{-2} germinating kernels and for sunflower on April 6th 2004, using 5 plant m^{-2}. Sunflower was watered weekly until R7 stage (the back of the head has started to turn a pale yellow color - Schneiter and Miller, 1981). The leaf area (by area measurement system Delta T Devices Ltd., Burwell Cambridge, England), the biomass dry weight (by drying in forced-air oven at 105°C) and yield were measured. Leaf Area Index (LAI) and Crop Growth Rate (CGR) were also calculated. The Agronomic

Efficiency Index (AE) of dried orange wastes doses were calculated in sunflower with the following formula AE= $(Y_{px} - Y_{p0})/p_x$, where Y represent the grain production, p the dried orange wastes dose distributed to treatment 0 and to treatment x. Air temperature and rainfall were recorded and stored using Campbell Scientific CR21 data logger.

Results and discussion

Growing season, crop growth and production in wheat

The rainfall recorded from December to June (302 mm) showed a regular distribution during the growing season (52% in the winter period, 48% from March to June).

In both treatments with organic fertilization, it was ascertained an increase in the length of the shooting phase (about 18 dd) compared with the control (65 dd), mainly due to an early beginning of the above-mentioned phase; the differences between the organic fertilization and the control were reduced in the following phases and the total cycle ended at 188 days after sowing for all the treatments.

The height of the plants was positively affected by organic fertilization, with increments, on average of two doses, of 2 fold at tillering and 1.6 at heading compared with the control (20.7 cm at tillering and 56 cm at heading).

The organic fertilization determined also a significant increase in Leaf Area Index and Crop Growth Rate, irrespective of the doses in all the samplings (Fig. 1α−β). Leaf Area Index showed, on average of two doses applied, increases of 104% (tillering), 98% (heading) and 87% (milk ripening), when compared with the control (2.5, 2.8 and 3.0 at the three

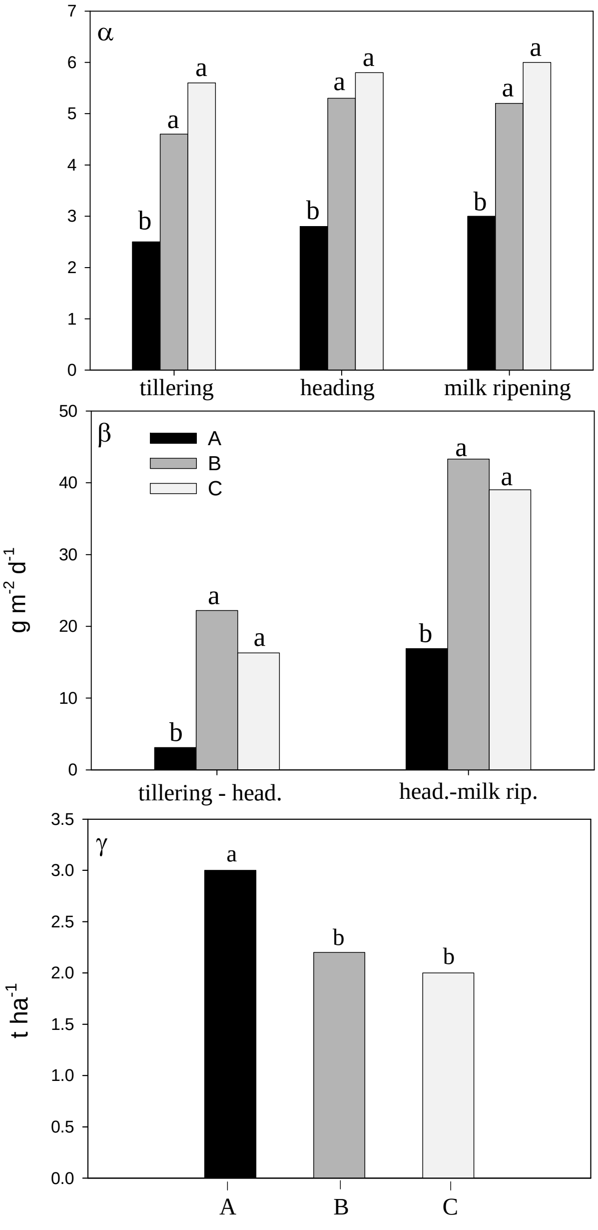

Figure 1: Leaf Area Index (α), Crop Growth Rate (β) and grain yield (γ) in wheat. A: control (untreated soil); B: 3 kg m^{-2} orange waste supply; C: 9 kg m^{-2} orange waste supply. For each sampling different lowercase letters indicate significant differences at $P \leq 0.05$ using the LSD Test.

samplings). On average of the two fertilization treatments, Crop Growth Rate reached values of 19.3 (tillering-heading) and 41.2 g m^{-2} d^{-1} (heading-milk ripening); they were significantly higher than those obtained in the control (2,9 and 16,9 in the tillering-heading and heading-milk ripening phases, respectively)

The grain yield was significantly and negatively influenced by the excessive vegetative growth induced by the organic fertilization (Fig. 1γ). In fact, the thesis treated with wastes produced on average only 2.1 t ha^{-1}, with a decrease of 30% when compared with the control.

Growing season, crop growth and production in sunflower

The rainfall detected during the cycle of the crop was 100 mm, strongly concentrated during the early phases of growing season; during the entire cycle the average of the maximum temperature was 26.1 °C, whereas the average of the minimum temperature was 16.0 °C.

In sunflower, flowering began about 10 days earlier in the organic fertilization treatments as compared to the control (85 dd), but the biological cycle (emergence-maturity) lasted 108 days on average, with no differences as regards the treatments studied. The organic fertilization strongly increased the plant height from 128 cm recorded in the control to 185 cm ascertained in 9 kg m^{-2} orange waste supplied treatment

The organic fertilization determined a significant increase in Leaf Area Index with the increase of citrus waste dose (Fig. 2α); a 5 and 1.6 fold increment was observed between control (0.8) and the minimum citrus dose and this last and the maximum dose, respectively.

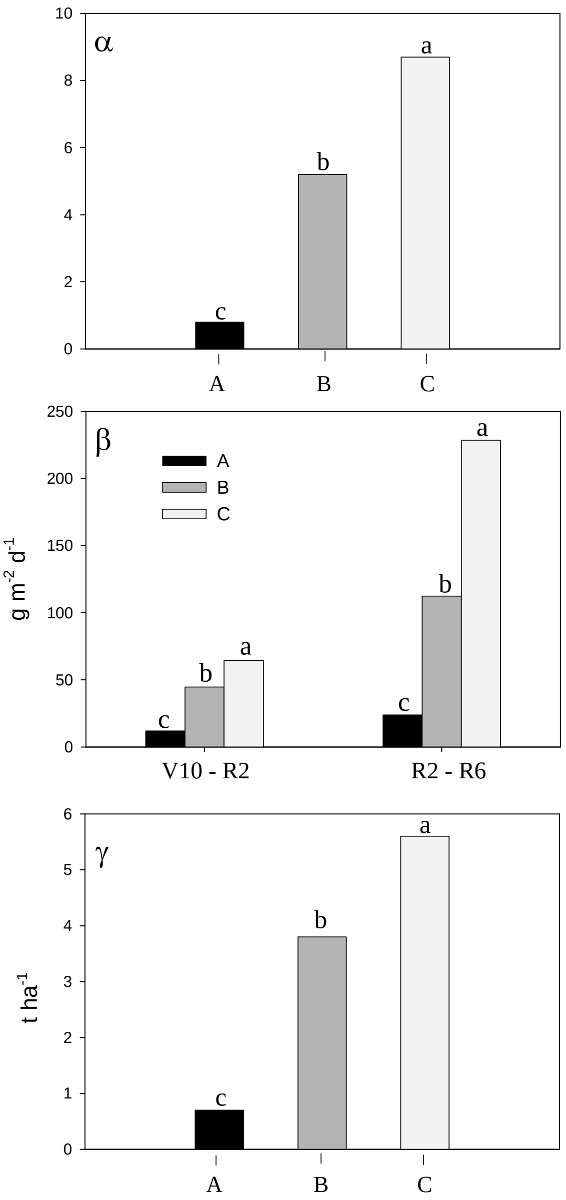

Figure 2: Leaf Area Index (α), Crop Growth Rate (β) and grain yield (γ) in sunflower. A: control (untreated $^{-2}$ orange waste supply. For each sampling different lowercase letters indicate significant differences at $P \leq 0.05$ using the LSD Test.

The Crop Growth Rate index validated that organic fertilization stimulated the crop growth, but with different extent in relation to the doses; in the period R2 - R6 when CGR reached its maximum values, a 4 and a 1 fold increments were observed between control and the minimum citrus dose and between this last and the maximum dose, respectively (Fig. 2β).

The seed yield also increased with successive increases equal to about 5 times between control (0.6 t ha^{-1}) and minimum citrus dose (3.2 t ha^{-1}) and twice between minimum and maximum citrus dose (5.5 t ha^{-1}) (Fig. 2γ). To evaluate the orange waste dose sustainable from an agronomic perspective, we compared the values of the Agronomic Efficiency Index of organic fertilization. The Agronomic Efficiency Index of organic fertilization in sunflower was higher in the treatment with minimum citrus waste dose (88 kg per ton of seed) compared to the maximum citrus waste supplied (54 kg per ton of seed).

Conclusions

The organic fertilization with orange wastes leads to different effects on growth and productivity in durum wheat and sunflower. It stimulated crop growth in both crops but the excessive vegetative growth led to negative effects on grain yield in wheat. On the contrary, positive effects in sunflower were observed. In this last, in fact, the yield increased with the increase of the dose supplied, even if the Agronomic Efficiency Index of organic fertilization was higher with minimum citrus waste dose supplied. In conclusion, the present research indicates that the re-use of dried orange waste as a soil fertilizer in areas in which its disposal has environmental and economic costs, could be a feasible technique low-cost for wheat and sunflower in organic or conventional farming.

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II SESSION

Advances in formulation of growing media

and their components

PLANTED FILTER SYSTEMS IN VIETNAM FOR CLEANING DOMESTIC WASTEWATER AND PRODUCING FERTILIZER SUBSTRATES

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Abstract

The removal of nutrients from domestic wastewater and their concentration in the biomass of plants can provide nutrient-rich amendments for agriculture. A horizontal flow filter system was established on the campus of the University of Cantho in Vietnam and fed with wastewater from the adjacent student dormitory. An unplanted control was compared with filter compartments planted to *Phragmites australis* and *Sesbania rostrata*. Changes in water properties and the dynamics of biomass accumulation and nutrient uptake by the filter plants were evaluated at bi-weekly intervals. The nutrient elimination from the wastewater and its chemical oxygen demand varied by loading rate and filter plant. While *Phragmites* showed the best carbon elimination, its nutrient removal capacity was <25% of the added amounts. On the ther hand, *Sesbania* eliminated >95% of the added N. Eight week-old *Sesbania* accumulated about 1.5 kg m^{-1} of dry matter, irrespective of its position in the filter, and compensated a reduced N supply by enhanced N_2 fixation. *Sesbania* may thus be preferred over *Phragmites* for nutrient recycling in planted filters under tropical climatic conditions.

Keywords : Chemical oxygen demand, COD, Nitrogen fixation, *Phragmites*, *Sesbania.*

Introduction

With local fertilizer shortages and recent price increases it becomes imperative to exploit local resources, i.e., via improved nutrient recycling processes. Domestic wastewater is such a nutrient-rich and underexploited resource. The widely practiced nutrient elimination from wastewater (mainly nitrate-N by denitrification) is not only critical in the face of the ongoing climate debate, but also constitutes a major nutrient loss. Alternatives, such as nutrient precipitation and stripping, are restricted by high requirements for technical infrastructure and relatively large investment costs. In situations where land is available and a market for organic fertilizer sources exists (Vymazal, 2005), the extraction of nutrients from wastewater by plants constitutes a promising avenue for nutrient recycling (Das and Tanaka, 2007). Such planted filter systems or constructed wetlands not only concentrate nutrients in the biomass, but may additionally contribute to wastewater cleaning and potentially to a reduced eutrophization of the environment (Kadlec and Wallace, 2009). So far, biological wastewater treatment in constructed wetlands focused on a reduction of organic C compounds in the rhizosphere of reed grasses (*Phragmites* spp.). Particularly in developing countries it is of additional interest to grow plant species in the filter system that can either use the nutrient elements from the wastewater for providing a marketable harvest good or effectively remove and concentrate these nutrients in the biomass, in view of producing an organic fertilizer substrate to be used in agriculture. Such approaches are seen to also improve the efficiency of wastewater cleaning, thus avoiding the eutrophization of water bodies (Vymazal, 1998). We comparatively evaluated the effect of planted and unplanted horizontal filter systems regarding their wastewater cleaning effects and the accumulation of nutrients in the plant biomass both in space and time.

Material and Methods

A horizontal flow filter system (constructed wetland) was established on the campus of the University of Cantho in Vietnam and fed with wastewater from the adjacent student dormitory with 200 inhabitants. The filters were fed at loading rates of 10 and 20 L of mechanically pre-filtered wastewater per m^2 of filter area and per day. An unplanted control was compared with filter compartments planted to *Phragmites australis* (standard in planted filters) and *Sesbania rostrata,* in three replications. Changes in wastewater properties (pH, EC, COD, N , P) and the dynamics of biomass accumulation and nutrient uptake by the filter plants were evaluated at bi-weekly intervals and at four positions along the filter (2, 4, 6, and 8 m) between the wastewater inlet (0 m) and the filter outflow (10 m). Additionally, the biological N_2 fixation by *Sesbania* was determined by $\delta^{15}N$ using *Phragmites* as reference species.

Results and Discussion

The nutrient elimination from the wastewater and its chemical oxygen demand (COD) varied by loading rate and the type and age of the filter plants. *Phragmites* showed the best carbon elimination with a COD reduction from 37 to about 12 mg l^{-1} (Figure 1). However, its nutrient removal capacity was very low with up to a maximum of 25% of the added nitrate-N (compared to 5% in the unplanted control) and up to 2.8 g N m^{-1} N accumulation in the biomass of 8 week-old plants (Figure 2). This biomass growth and N accumulation increased with plant age and responded to the applied wastewater loading.

On the other hand, *Sesbania rostrata* showed a moderated COD reduction (from 36 to about 18 mg l^{-1}), even if was most effective in nutrient removal and N accumulation (Figure 1).

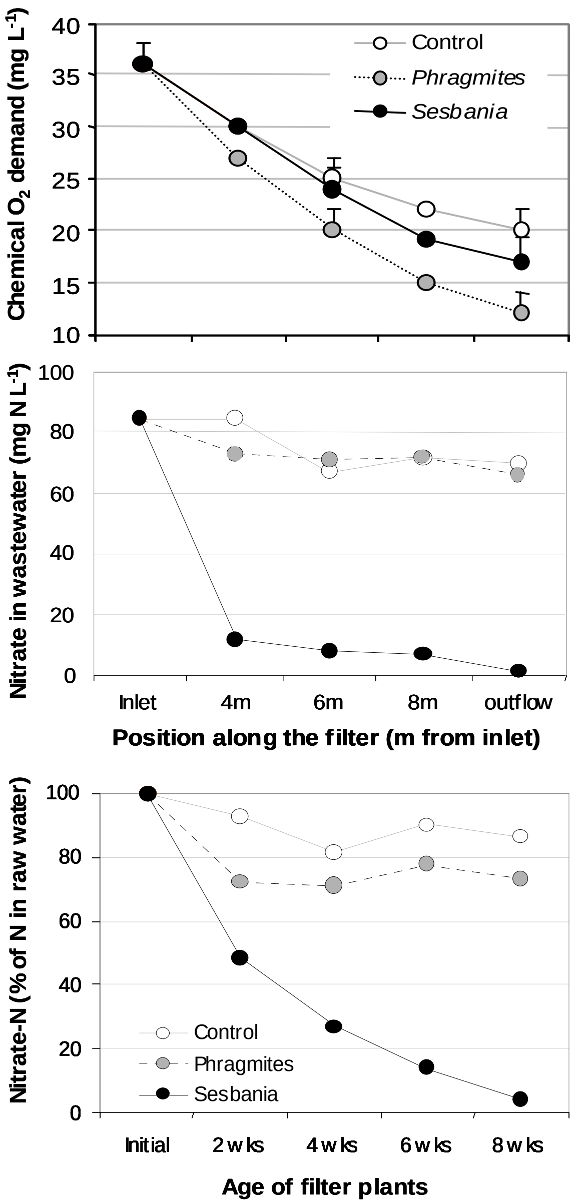

Figure 1: Changes in wastewater properties in space / time as affected by filter species.

Sesbania eliminated >50% of the added N 2-3 weeks after seeding, reaching >95% of the added N at eight weeks. Eight week-old plants accumulated some 1.5 kg m^{-1} dry matter (25-30 g N m^{-2}), irrespective of the wastewater loading or its position in the filter (Figure 3). It compensated a reduced N supply (result of either reduced N load in the wastewater of the gradual N depletion along the filter body) by enhanced N_2 fixation. Thus, the share of N derived from the atmosphere (%Ndfa) increased from 5% in young plants at the full wastewater loading rate and close to the inlet (maximum N concentration in the water) to nearly 82% Ndfa in eight week-old plants at the reduced loading rate close to the outflow (minimum N concentration in the water). Similar substitution effects of soil N supply and biological nitrogen fixation in stem-nodulating legumes have been reported by Becker and George (1995). In the present study, *Sesbania* provided an 8-10 times higher biomass than *Phragmites* and an organic substrate of constant and high quality as expressed by a C:N-ratio of 15-17 and a C:P-ratio of about 200 (Table 1).

Table 1: Biomass accumulation and nutrient content in eight week-old *Phragmites australis* and *Sesbania rostrata* fed at two wastewater loading rates in a horizontal flow filter system in Vietnam.

Species	Loading rate	Biomass (kg m^{-2})	N uptake (g m^{-2})	C:N ratio	P uptake (g m^{-2})	C:P ratio	K uptake (g m^{-2})	C:K ratio
Phragmites	10 L m $^{-2}$	0.10 b	2.0 c	21 ab	0.14 b	297 a	2.63 d	19 a
	20 L m $^{-2}$	0.13 b	2.9 b	22 b	0.20 b	265 a	4.14 c	17 a
Sesbania	10 L m $^{-2}$	1.51 a	17.4 a	15 a	0.94 a	210 ab	13.1 b	20 a
	20 L m $^{-2}$	1.59 a	17.5 a	17 a	1.18 a	190 b	18.3 a	17 a

Values followed by the same letter within a column do not differ significantly by Tuckey Test (0.05)

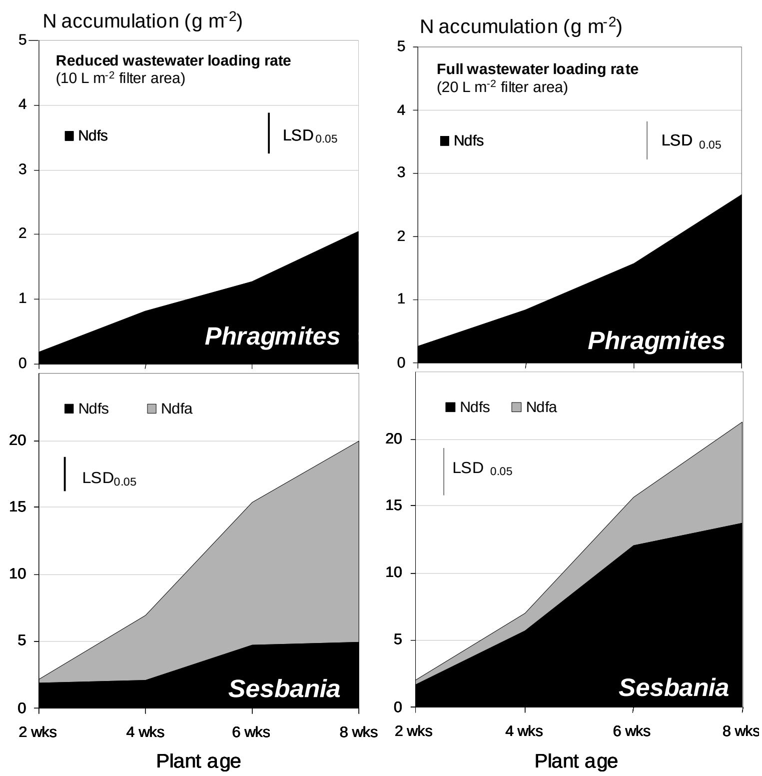

Figure 2: Nitrogen accumulation by filter species in time (plant age up to 8 weeks) with half and full waste-water loading rate in a horizontal filter in Vietnam.

LSD: Least significant difference ($p<0.05$); Ndfs: N derived from the soil; Ndfa: N derived from the atmosphere.

We conclude that *S. rostrata* is largely superior to the standard filter species *Phragmites* and may be preferred for horizontal filter systems under tropical climatic conditions, not only for wastewater cleaning but also for nutrient recycling and provision of organic fertilizer substrates. Field-scale validation trials regarding the potential of *S. rostrata* for wastewater cleaning and the production and of fresh and composted organic amendments in field vegetable production are under way close to the new students' dormitory of the university of Cantho in Hoa An in the Mekong Delta.

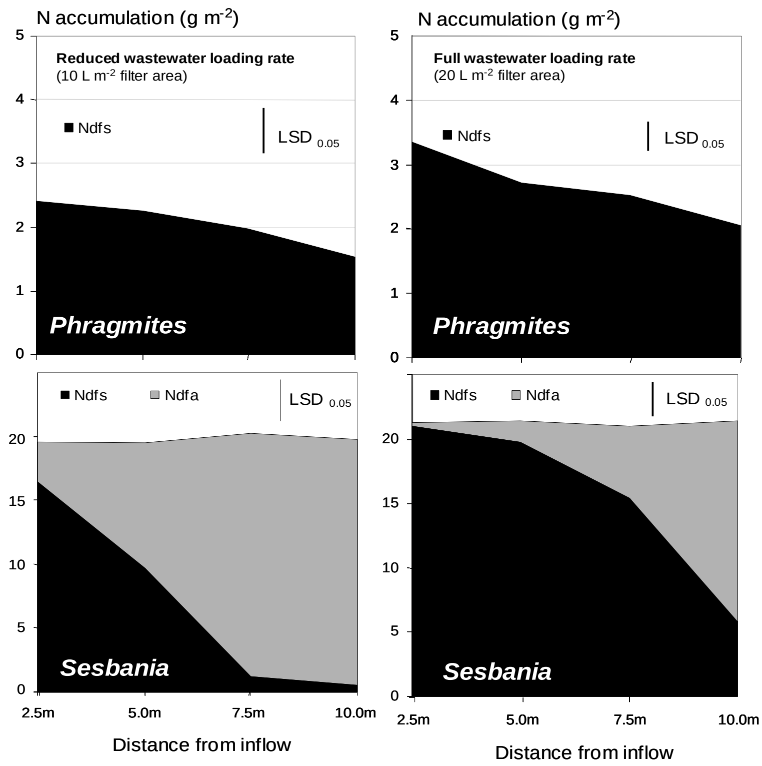

Figure 3: Nitrogen accumulation by eight week-old filter species in space (distance from inflow) with half and full wastewater loading rate in a horizontal filter in Vietnam.

LSD: Least significant difference ($p<0.05$); Ndfs: N derived from the soil; Ndfa: N derived from the atmosphere.

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EVALUATION OF DIFFERENT SUBSTRATE FERTILIZERS IN ORDER TO IMPROVE THE QUALITY OF VEGETABLE SEEDLINGS

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Abstract

Different types of substrate fertilizers have been evaluated on vegetable in order to improve some seedling quality parameters like compactness index, sturdiness index, foliage dry matter, chlorophyll content and rooting.

Results obtained seem to confirm the role played by different nitrogen forms, influencing the analyzed quality parameters for the large part of studied species. Likewise, the use of slow release fertilizers can be a tool to improve the shelf life of young vegetable plants.

Keywords: peat, coated fertilizers, nitrate, ammonium, tomato, plantlets, shelf life.

Introduction

Nowadays, many of the most important vegetables are transplanted. Compared with sowing, transplanting reduces the cropping time, increases the uniformity of yields and the crop timing. Main disadvantages are the seedlings costs and a change in crop management, overall in the first phases. Some growers complain about the poor seedling quality obtained in the nurseries, not assuring satisfactory rooting, foliage characteristics and crop yield. On the other hand, the seedling producers say that without using chemical growth regulators in winter or early spring and/or with high plant density, it is very difficult to produce seedlings with sufficient marketable quality.

In order to avoid the use of chemical growth regulators, potentially harmful for human beings, different physical, mechanical and nutritional tools have been proposed to regulate the growth in the first phases of seedling cultivation.

Physical practices include the regulation of day and night temperatures (DIF method), the reduction of substrate moisture, the variation of natural light in terms of intensity and/or spectral emission with different artificial devices (i.e. lamps or LED). Mechanical methods include brushing, shaking and increased air movements (Latimer,1998). At last, the nutritional approach, include a reduction in total amount of fertilizers applied to the substrate and/or to the plant. Also the variation in the ratio among the three most important macronutrients has been investigated in order to improve the plantlets performance (Whipker *et al.* 2001).

Large part of the 14 billions of vegetable seedlings produced in Italy every year (Trentini, 2002) are cultivated in winter or early spring. In this season, with high levels of $N\text{-}NH_4$ in the fertilizer added to the substrate or with the fertilization applied, low temperature, low pH of the media and low air content into the substrate, it is possible to observe symptoms of $N\text{-}NH_4$ toxicity. The excess of $N\text{-}NH_4$ reduces the growth of the roots that appear often discoloured; whereas on foliage we note leaf epinasty, stem lesions and a general softer and lusher growth (Mills and Benton Jones, 1996; Styer and Koranski, 1997). The final result is a reduction in the marketable value of the plantlets.

Another problem, common for vegetable seedlings produced for the retail market, is the delayed transplant, beyond the right phenological phase. That fact induces nutritional deficiencies, severe transplant crises, and frequently a reduction in the final yield and quality.

The aims of this work were to improve the quality of the seedlings acting on the $N\text{-}NH_4/N\text{-}NO_3$ ratio and to increase the shelf life of seedlings through the use in the substrate of coated fertilizers added to the common readily release fertilizers.

Materials and methods

First experiment

Peat based substrate was prepared using a *Sphagnum* peat with a low degree of decomposition (H3 on the von Post scale) and a very fine texture (0-5 mm). Finally, the substrates were limed with $CaCO_3$ in order to reach a pH of 6,5 and fertilized with 1 kg/m^3 with the following two fertilizer formula:

1) 14 N (2/3 $N\text{-}NH_4$ + 1/3 $N\text{-}NO_3$), 16 P_2O_5, 18 K_2O + trace elements (t.e.);
2) 14 N (1/3 $N\text{-}NH_4$ + 2/3 $N\text{-}NO_3$), 16 P_2O_5, 18 K_2O + trace elements (t.e.).

The polystyrene plug trays (52 x 31 cm) used had a different number of cells depending on the vegetable species: 160 cells for tomato (cv. Perfectpeel), aubergine (cv. Dalia), pepper (cv. Lamuyo), savoy cabbage (cv. Wirosa), lettuce (cv. Canasta), and 40 cells for cucumber (cv. Tasty Green). During the cultivation, starting with the emission of the first true leaf, seedlings were fertilized twice a week (100 ppm) with the following formula 20 N, 20 P_2O_5, 20 K_2O + trace elements were $N\text{-}NH_4$ and $N\text{-}NO_3$ were adjusted in order to obtain the same ratio showed to the point 1 and 2.

<u>Second experiment</u>

Starting with substrates similar to the first experiment, the following three fertilizers were prepared and added to the media.

1) 1,3 kg/m^3 12 N (2/3 N-NH_4 + 1/3 N-NO_3), 11 P_2O_5, 17 K_2O + t.e. (HRRF);
2) 1,3 kg/m^3 (HRRF) +0,5 kg/m^3 of a coated fertilizer 12 N; 11 P_2O_5; 17 K_2O + t.e. (CF);
3) 0,8 kg/m^3 12 N (2/3 N-NH_4 + 1/3 N-NO_3), 11 P_2O_5, 17 K_2O + t.e. + 0,5 kg/m^3 coated fertilizer 12 N; 11 P_2O_5; 17 K_2O+ t.e. (LRRF+CF).

Following the usual practices adopted in nurseries, from the emission to the first true leaf until the optimal vegetative phase for transplanting, seedlings were fertilized twice a week (100 ppm) with the following formula 20 N (2/3 N-NH_4 + 1/3 N-NO_3), 20 P_2O_5, 20 K_2O + trace elements. After that phase, until the end of the experiment (21 days later), the plantlets were irrigated only with tap water.

At the end of the experiments, different quality parameters were measured: compactness index (hypocotyl diameter/height of plantlet); sturdiness index (hypocotyl diameter/lenght of hypocotyl); foliage dry matter; rooting score (based on a judgment of four independent experts) and the chlorophyll content of the first true leaf based on measurements carried out with the Minolta SPAD 502. The experimental design adopted was a randomized block with four replication and all the data collected were submitted to the ANOVA and the Student-Newman-Keuls test.

Results and Discussion

<u>First experiment</u>

As shown in tab. 1, final results seem to indicate significative effects on plantlet growth for almost all the parameters studied except for chlorophyll content. Regarding the vegetables evaluated, there were some species very reactive, like cucumber and others, like aubergine and pepper, few or nothing. The fresh dry matter, a good indicator of the rusticity of the plantlets, seems to be the parameter most sensitive to the nitrogen form applied.

Table 1: effects of the different N-NH_4/N-NO_3 ratio on seedling growth.

Species	Compactness index	Sturdiness index	Fresh dry matter	Root score	Chlorophyll content
Tomato	*	ns	*	ns	ns
Aubergine	ns	ns	*	ns	ns
Pepper	ns	ns	ns	ns	ns
Savoy cabbage	ns	ns	*	ns	ns
Cucumber	**	*	***	***	ns
Lettuce	*	ns	***	ns	ns

ns= not significative; (*) P≤0,05; (**) P≤0,01; (***) P≤0,001.

Analysing parameters showing clear answers, we noted that the compactness index (fig.1) improved clearly tomato (+16,5%), cucumber (+19,4%) and lettuce (+28,4%), whereas for the sturdiness index (fig. 2) we had a positive answer only on cucumber (+12,9%).

Figure 1: Compactness index: high N-NO_3 vs high N-NH_4

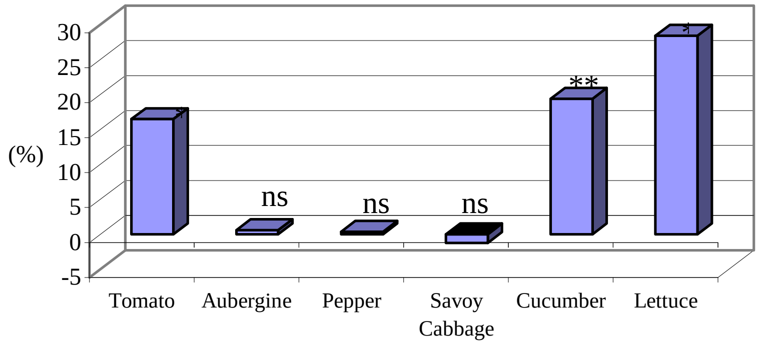
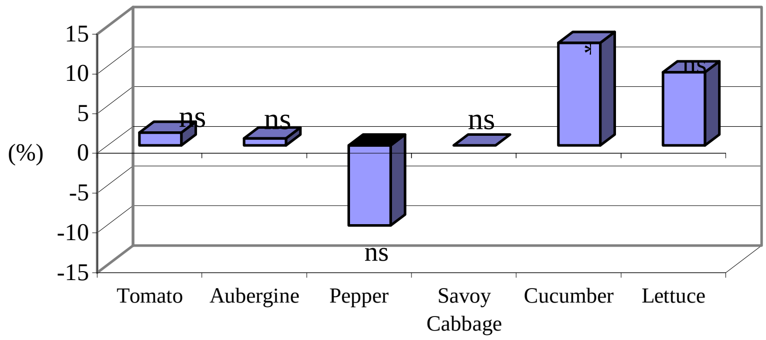


Figure 2:- Sturdiness index: high N-NO_3 vs high N-NH_4



Regarding the foliage dry matter (fig. 3), high N-NO_3 levels promoted a high value on lettuce (+50,8%), cucumber (+19,4%), tomato (+17,1%) and aubergine (+12,8%). Finally, the rooting score (fig. 4) improved only cucumber.

Figure 3: Foliage dry matter: high N-NO_3 vs high N-NH_4

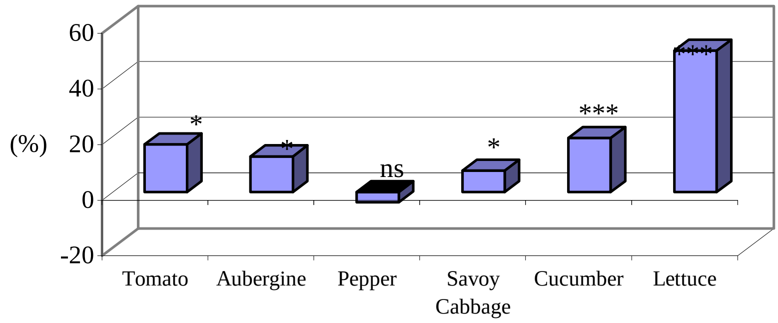
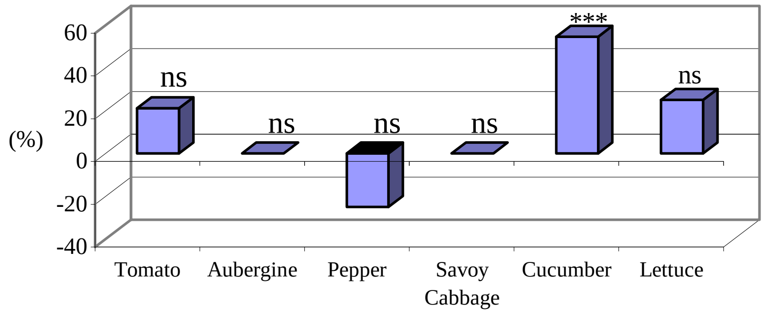
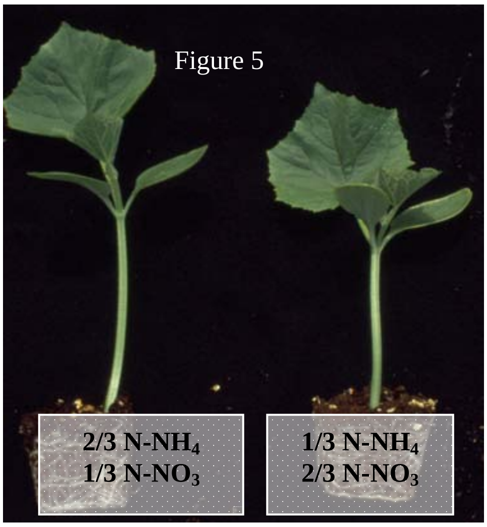


Figure 4 - Rooting score: high $N\text{-}NO_3$ vs high $N\text{-}NH_4$





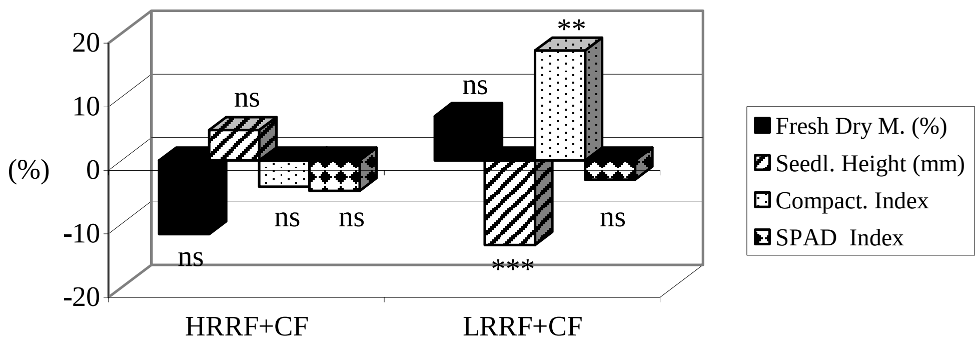
Results obtained on the first experiment seem to assure no detrimental effects on seedling by reversing the ratio $N\text{-}NH_4/N\text{-}NO_3$ from 2/3:1/3 at 1/3:2/3. On the contrary, for the species tested (except pepper), high values of $N\text{-}NO_{3,}$ in comparison with $N\text{-}NH_{4,}$ improved the main quality indicators (fig. 5). The effective height control, due to the different form of nitrogen, seems to indicate that the nitrogen management could be an effectiveness and alternative way to the use of chemical growth regulators.

Second experiment

On plantlets ready to sell (fig. 6) the addition of a coated type fertilizer to the common amount of readily release fertilizer did not change significatively any parameter. Instead, replacing 0,5 kg/m^3 of readily release fertilizer with an equal amount of coated type, affected positively the plantlets in terms of compactness, due to a clear reduction in the seedling height.

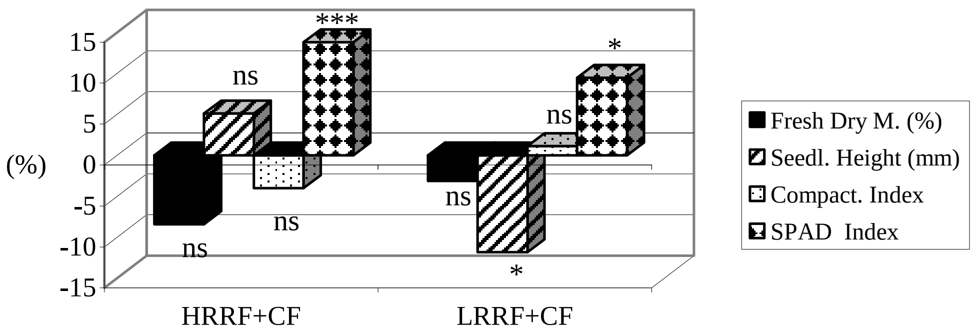
In any case, the chlorophyll content into the first true leaf was not influenced.

Figure 6: Standard vs modified fertilizer on seedling READY to SELL



On aged seedlings (fig. 7), the use of a modified fertilization (HRRF+CF or LRRF+CF) improved the chlorophyll content in the first true leaf. It could be a good indicator of the absence of nutritional deficiencies. Together with a higher chlorophyll content, using a reduced amount of readily release fertilizer, we obtained also a good control in the plantlet height, as previously observed on seedlings ready to sell.

Figure 7: Standard vs modified fertilizer on seedling AGED 21 DAYS



Results obtained indicate that adding a moderate amount of the coated types to the readily release fertilizers the shelf life of plantlets increased. The best result in terms of seedling quality and cost reduction was obtained by replacing part of the readily release fertilizer with the coated types (LRRF+CF). In this way the good height reduction makes chemical growth regulators useless.

To reduce the costs and to evaluate the long lasting effects in terms of transplant crisis and final production other studies are going ahead.

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AGRONOMICAL RESPONSES AND MINERAL COMPOSITION OF TWO *CUCURBITACEAE* SPECIES AS AFFECTED BY ORGANIC AND INORGANIC SUBSTRATES

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Abstract

The expansion of hydroponics in many countries of the world in the last few decades may be ascribed to the ability of soilless growing systems to avoid various problems arising from the use of the soil. Cucumber (*Cucumis sativus* L.) and zucchini (*Cucurbita pepo* L.) plants were grown in closed-soilless culture under unheated-greenhouse conditions typically of the Mediterranean area at the Experimental farm of University of Tuscia, Central Italy, to evaluate the effects of four substrates (rockwool, pumice, perlite, and cocofiber) on growth, yield and plant mineral composition. For both cultures, plants grown in cocofiber, perlite and pumice yielded more than those grown in rockwool. The better temperature regime in cocofiber, perlite and pumice was due to the greater thermal inertia compared to rockwool slabs. The concentration of N in zucchini and cucumber leaves was significantly higher in cocofiber, perlite and pumice in comparison to the rockwool treatment. The concentration of K was significantly affected by the substrate only for the zucchini squash with the highest value recorded on the organic substrate (cocofiber), whereas the Ca concentration was significantly influenced by the growing media only for cucumber with the highest value observed on pumice. Finally, the lowest Mg concentration in leaf tissue was observed on plants grown with the rockwool substrate for both zucchini squash and cucumber.

Keywords : Substrate, hydroponics, cucumber, zucchini, cocofiber.

Introduction

The revolutionary expansion of hydroponics in many countries of the world in the last three decades may be ascribed to the ability of soilless growing systems to be independent of the soil and hence of all problems related to it. Hydroponics has proved to be an excellent alternative to soil sterilization, especially in relation to the use of chemical soil sterilants, such as methyl bromide (Savvas, 2003). Moreover, the cultivation of greenhouse crops and the achievement of high yields and good quality are possible with hydroponics even in saline or sodic soils, or non-arable soils with poor structure, which represent a major proportion of cultivable land throughout the world (Rouphael *et al.*, 2004). A further advantage of hydroponics is the precise control of nutrition. This is particularly true in crops grown either on inert substrates or in pure nutrient solution. However, even in soilless crops grown in chemically active growing media, the nutrition of the plants can be better controlled than in crops cultivated in the soil, due to the limited volume of substrate per plant and its standard, homogeneous constitution, which is well known to the grower (Rouphael and Colla, 2005). The porous materials used as substrates in soilless culture are distinguished as organic or inorganic growing media. The organic materials used in soilless culture originate from plant residuals and are therefore subjected to biological degradation. The decomposed organic materials are more or less chemically active, due to the presence of ion exchange sites, which may adsorb or release nutrients. In contrast, most inorganic materials are chemically inactive (inert). Therefore, many authors use the terms “organic” and “inorganic” growing media as synonyms to “chemically active” and “inert” substrates, respectively (Savvas, 2003). Zucchini squash and cucumber are important crops that have gained popularity for both open field and protected cultivation in the Mediterranean region. However in the last 20 years, growing zucchini squash and cucumber in soilless culture have become increasingly popular among commercial growers. The aim of this study was to determine yield, growth and nutrient accumulation of two cucurbitaceae species (zucchini and cucumber) in relation to the type of substrate (organic vs. inorganic) in unheated greenhouse conditions.

Materials and methods

Two experiments were conducted in two consecutive growing seasons (spring and fall) in a polyethylene 400 m^2 greenhouse situated at the experimental farm of Tuscia University, central Italy. Inside the greenhouse, the high temperature and relative humidity were controlled through ventilation. A randomized complete block design with four replicates (ten plants per experimental unit) was used to compare four substrates: perlite (Perlite Italiana), pumice (Europumice), cocofiber (Cocco Ter), and rockwool (Grodan).

In both experiments, soilless plants were placed in single plastic channel benches (section 26×12 cm; length 5m with a slope of 1.5%) which contained in the bottom a plastic drainage layer (1.5 cm height) covered with geo-textile. Perlite, pumice and cocofiber were added to fill the channels, while rockwool was used as slab (100 cm length x 15 cm width x 7.5 cm depth). In all plots, the surface of substrate was covered with white plastic film.

Seeds of zucchini squash (*Cucurbita pepo* L.) "Afrodite" hybrid (Syngenta, Switzerland) and cucumber (*Cucumis sativus L.*) "Edona" hybrid (Royal Sluis) was germinated in vermiculite on 10 March and 15 August 2001, respectively. Plants remained in the seed pots until the two true leaf stages. The seedlings were transplanted on 23 March and 1 September for zucchini and cucumber, respectively, into cocofiber, pumice, perlite, and rockwool at a plant density of 2.1 plants m^{-2}.

In both experiments, plants were fertilized with the following nutrient solution (mg L^{-1}): N-NO_3 (160), S (26), P (13), Cl (57), K (150), Ca (122), Mg (59), Na (50), Fe (3), Mn (0.8), Cu (0.07), Zn (0.1), B(0.3), Mo (0.05). The EC values were kept within the range of 1.8 to 2.0 dS m^{-1}, while the pH of the solution was maintained between 5.8 and 6.3 by adding an acid mixture. Nutrient solution was pumped from independent tanks through a drip irrigation system with one emitter per plant and an emitter flow rate of $2L \cdot h^{-1}$. The excess of the nutritive solution was recycled for the entire growing cycle. Irrigation scheduling was performed using electronic low-tension tensiometers (LT-Irrometer). During the crop cycle, fruits of zucchini squash and cucumber were harvested when they reached marketable size; fruits that were deformed or badly misshapen were considered unmarketable. Fruits were dried in a forced-air oven at 80°C for 72 hours, then ground in a Wiley mill to pass a 20-mesh screen and analyzed for the following elements: N, P, K, Ca, and Mg.

At final harvest, four plants of zucchini squash and cucumber per plot (experimental unit) were separated into stems and leaves for biomass determination and subsequently ground for major and trace elements determination (N, P, K, Ca, Mg, Na, Cu, Fe, Mn, and, Zn). The nitrogen concentration in plant tissues (leaves and fruits) was determined after mineralization with sulfuric acid by "Regular Kjeldahl method" (Bremner, 1965), P, K, Ca, Mg, Na, Cu, Fe, Mn, and Zn were determined by dry ashing method at 400°C for 24 hours, dissolving the ash in 1:25 HCl, and assaying the obtained solution using an inductively coupled plasma emission spectrophotometer (Karla, 1998).

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 10 for Windows, 2001). Duncan's Multiple Range test was performed at *P*= 0.05 on each of the significant variables measured.

Results and Discussion

The total and marketable yield of zucchini squash and cucumber were significantly affected by the substrates. For both zucchini and cucumber the highest marketable yield was recorded on cocofiber, perlite and pumice, followed by rockwool, with no significant differences observed between the first three treatments. Moreover the lowest marketable zucchini yield observed on rockwool was mainly attributed to a reduction in the fruit number and not to a change in the fruit mean weight, whereas in cucumber, the yield reduction on rockwool was related to a reduction in both fruit mean weight and fruit number.

Table 1: Effects of substrates on total and marketable yield (kg $plant^{-1}$), fruit mean weight (g $fruit^{-1}$), fruit number (n^o $plant^{-1}$), and above-ground dry biomass (g $plant^{-1}$) of zucchini and cucumber plants.

Substrate	Yield		Marketable fruit		Total above ground dry biomass
	Total	Marketable	Mean mass	Number	
			Zucchini		
Cocofiber	2.34 a[1]	2.30 a	113.9 a	20.1 a	375.3 a
Perlite	2.10 ab	2.03 a	115.7 a	17.3 a	364.3 a
Pumice	2.18 ab	2.13 a	118.9 a	17.9 a	341.8 a
Rockwool	1.76 b	1.64 b	116.3 a	14.1 b	305.5 a
			Cucumber		
Cocofiber	3.81 a	3.41 a	183.4 a	18.4 ab	271.1 a
Perlite	3.93 a	3.62 a	177.3 a	20.4 a	287.0 a
Pumice	3.65 ab	2.94 a	169.0 b	17.2 ab	224.7 a
Rockwool	3.31 b	2.40 b	163.2 b	14.7 b	185.3 b

[1]Means within columns separated using Duncan's multiple range test, $p = 0.05$.

The macro- and microelements concentration in zucchini and cucumber leaves as a function of the substrate are displayed in Tables 2 and 3. The concentration of N in zucchini and cucumber leaves was significantly higher in cocofiber, perlite and pumice in comparison to the rockwool treatment (Table 2). Moreover, no significant differences were observed for the P concentration in both crops. The concentration of K was significantly affected by the substrate only for the zucchini squash with the highest value recorded on the organic substrate (cocofiber), whereas the Ca concentration was significantly influenced by the growing media only for cucumber with the highest value observed on pumice. Finally, the lowest Mg concentration in leaf tissue was observed on plants grown with the rockwool substrate for both zucchini squash and cucumber (Table 2).

Table 2: Effects of substrates on major elements concentration of zucchini and cucumber leaves.

Substrates	Major elements (g kg^{-1} of dry weight)				
	N	**P**	**K**	**Ca**	**Mg**
			Zucchini		
Cocofiber	32.6 a[1]	2.1 a	37.8 a	24.4 a	14.8 a
Perlite	38.0 a	2.6 a	26.6 b	23.8 a	13.6 ab
Pumice	36.6 a	2.4 a	28.8 b	23.3 a	12.9 b
Rockwool	29.9 b	2.2 a	24.0 b	26.4 a	14.6 c
			Cucumber		
Cocofiber	29.7 a	5.7 a	36.7 a	32.4 bc	8.7 a
Perlite	31.8 a	4.2 a	36.3 a	36.4 ab	6.9 bc
Pumice	30.3 a	5.6 a	36.7 a	36.8 a	7.1 b
Rockwool	28.2 b	4.4 a	34.8 a	30.2 c	5.7 c

[1]Means within columns separated using Duncan's multiple range test, $p = 0.05$.

The highest Na for both zucchini squash and cucumber was recorded on rockwool, whereas no significant differences were observed for the Cu and Fe concentration (Table 3). Moreover, the highest value of Mn, and Zn were recorded on perlite and rockwool, respectively.

For the zucchini experiment, no significant difference among treatments was observed for the concentration of K, Ca, and Mg in fruit, whereas the lowest value of N, and P was observed on rockwool substrate (Table 4). Finally, in the cucumber experiment no significant effect of substrate was recorded for the N, and P concentration, while the highest K and Mg concentration was observed in fruits harvested from plants grown in the cocofiber substrate (Table 4).

To summarize, we can conclude, that cocofiber, perlite and pumice are suitable for zucchini and cucumber production in closed soilless system, whereas the use of rockwool is more suitable for crops grown under heated greenhouse conditions.

Table 3: Effects of substrates on sodium and trace element concentration of zucchini and cucumber leaves.

Substrates	Trace elements (mg kg^{-1} of dry weight)				
	Na	**Cu**	**Fe**	**Mn**	**Zn**
			Zucchini		
Cocofiber	257.2 b	8.7 a	57.8 a	116.7 b	58.2 c
Perlite	313.7 b	5.7 a	60.7 a	289.1 a	87.0 b
Pumice	260.0 b	6.6 a	50.0 a	142.8 b	70.2 bc
Rockwool	743.7 a	7.2 a	52.1 a	359.7 a	111.6 a
			Cucumber		
Cocofiber	101.2 b	8.6 a	62.0 a	20.5 d	53.0 b
Perlite	96.2 b	9.5 a	61.4 a	49.7 a	70.2 a
Pumice	94.7 b	9.4 a	58.6 a	33.0 c	58.5 b
Rockwool	196.2 a	10.0 a	61.7 a	41.1 b	67.9 a

[1]Means within columns separated using Duncan's multiple range test, $p = 0.05$.

Table 4: Effects of substrates on major elements concentration of zucchini and cucumber fruits.

Substrates	Major elements (g kg^{-1} of dry weight)				
	N	**P**	**K**	**Ca**	**Mg**
			Zucchini		
Cocofiber	47.5 a[1]	5.2 a	46.0 a	4.1 a	5.5 a
Perlite	50.2 a	5.3 a	44.2 a	4.5 a	5.7 a
Pumice	47.8 a	5.1 b	40.9 a	3.9 a	5.4 a
Rockwool	44.2 b	5.1 b	45.7 a	4.3 a	5.7 a
			Cucumber		
Cocofiber	26.3 a	4.9 a	87.9 a	3.8 b	4.1 a
Perlite	25.1 a	4.7 a	79.3 b	4.5 a	3.8 ab
Pumice	26.3 a	4.8 a	75.1 b	4.8 a	3.9 ab
Rockwool	24.1 a	5.0 a	77.7 b	3.9 b	3.6 b

[1]Means within columns separated using Duncan's multiple range test, $p = 0.05$.

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RE.LA.S.CO. PROJECT: ITALIAN PROFICIENCY TEST FOR GROWING MEDIA NATIONAL AND EUROPEAN METHODS

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Abstract

In 2009 the first two types of growing media with specific characteristics were inserted in the Italian legislation on fertilizers (Legislative Decree n. 217/06). Nevertheless no specific analytical methods are until now indicated for labelling declarations. This situation means that laboratories can adopt different methods of analysis (Italian official methods, EN methods, routine labs methods) and that substrate producers and growers can have doubts about reliability and comparability of analytical results. In this context, a voluntary Proficiency Test promoted by AIPSA (Italian association of growing media ad amendments producers) was organized by Di.Pro.Ve on growing media methods. Three different products (peat-based, peat+pumice, peat+compost) were tested for different parameters with different Italian and European methods : pH (3 methods), EC (3 methods), organic matter content, ash, organic carbon, soluble NO_3-N and NH_4-N, total copper and zinc, laboratory compacted bulk density (2 methods), dry bulk density, air volume, water volume, total porosity. For each sample and parameter, data were compiled and processed according to ISO 5725, obtaining: number of outliers, mean value, repeatability and reproducibility standard deviations. Nine laboratories participated to the test and their anonymous results were benchmarked by the Z-score. Results show poor reproducibility for NO_3-N, NH_4-N, and total Zn. Only three labs performed physical determinations.

Keywords: Growing media, proficiency test, EN method, ISO 5725.

Introduction

Before 2006 the growing media were not included in Italian legislation on fertilizers, so they were marketed as soil conditioners, like peat-based amendments or composts, and consequently characterized by the required properties for amendments, mainly carbon level and carbon compounds. In June 2006, the Legislative Decree n. 217 abrogated the previous one and included in the Annex 4 the growing media, but no indications were given on related types or characteristics. Three years later (April, 16th 2009), the Annex 4 was updated, by inserting two types of substrates and the related evaluation criteria The two typologies are the "Basic growing media" and the "Mixed growing media", respectively. The introduced labelling declarations are: pH, electrical conductivity, commercial volume, dry bulk density, porosity and organic carbon, that are parameters more suitable for the characterization of cultivation substrates (Table 1). The stated characteristics required were based on data obtained by EN Methods for Soil improvers and Growing media, nevertheless these methods are not yet acknowledged in the Italian Official Methods of Analysis for fertilizers.

For a lots of Italian laboratories that are experienced in soils, amendments, fertilizers and composts analysis, growing media seem to be new materials. In fact, among 210 laboratories included in the SINAL (Italian Accreditation Body for laboratories) list that usually analyse soils, amendments, fertilizers and composts, only eight laboratories declare to perform analyses on growing media and only a few adopt the EN standards (pH EN13037: four labs; EC EN13038: three labs; dry bulk density EN13041: two labs; quantity EN12580: one lab). The poor knowledge of products appears also in their definitions: effectively, they are called alternatively: *"substrati di coltivazione"*, *"terricci"*, *"substrati di coltura"*.

In this context, problems may arise in comparing analytical results, since labs can adopt different methods (e.g. electrical conductivity, pH, quantity). In this confused situation, substrates producers and growers can have doubts on the reliability and comparability of the analytical data: they need to assess the ability of labs in performing test competently. For this reason, AIPSA (Italian Association of Growing media and Amendment Producers) entrusted to Di.Pro.Ve. (Dept. of Crop Production of Milan) the organization of an inter-laboratory test that took place in 2008, named Re.La.S.Co. project (net of laboratory for growing media).

Table 1: Italian growing media according to revised Annex 4 (16/04/2009).

	Units	**Basic Growing media**	**Mixed Growing Media**
pHw		3.5 - 7.5	4.5 - 8.5
E.C.	dS/m	max 0.7	max 1.0
C org	% d.w.	min 8	min 4
Dry bulk density	kg/m^3	max 450	max 950
Total porosity	% v/v	mandatory declaration	
Commercial volume	L	mandatory declaration	

Materials and methods

About thirty laboratories (Universities, Agricultural Regional Centres, SINAL list, indicated by producers or growers), were invited to participate to the inter-laboratory trial. Only nine labs attended to the Re.La.S.Co. Project: two private labs from SINAL list; five Regional and University labs; two internal company labs. The scheme of the trial consisted of three steps: choice of parameters and methods, draw up of protocol, collection, preparation delivery of samples; analysis of samples; statistical treatments of data and evaluation of lab performances.
Sixteen parameters (Table 2) were selected: for some of them (pH, electrical conductivity and laboratory compacted bulk density) different methods were proposed.

Table 2: Parameters and methods proposed to laboratories attending to Re.La.S.Co. Project.

Parameter	Method	Replicates
pH (3 methods)	A = EN 13037; B = Sonneveld; C = DM 17/06/02	3
Elec. Conductivity (3 methods)	A = EN 13038; B = Sonneveld; C = DM 17/06/02	3
Dry bulk density	EN 13041	4
Total pore space	EN 13041	4
Organic carbon	DM 21/12/00	3
Ash and organic matter	EN 13039	3
Dry matter and moisture	EN 13040	3
Lab. comp. bulk den (2 methods)	A = EN 13040; B = Sonneveld	3
Cu total	DM 17/06/02	3
Zn total	DM 17/06/02	3
NO_3-N water soluble	EN 13652 extraction; determination free	3
NH_4-N water soluble	EN 13652 extraction; determination free	3
Particle density	EN 13041	3
Air volume pF1	EN 13041	4
Water volume pF1	EN 13041	4

In lack of referenced materials and in order to have parameters with a wide range of values, three commercial growing media with different composition were collected, prepared and delivered to labs: S1, white and brown sphagnum peat; S2, sphagnum peat, pumice, clay; S3, white peat and MSW compost.
Statistical analysis followed the UNI ISO 5725-2 (2004) indications: tabulation of data, calculation of means and standard deviations, inspection of outliers, calculation of variances of repeatability (s^2_r), inter-laboratory (s^2_L) and reproducibility ($s^2_R = s^2_r +. s^2_L$).
The final results for each parameter and sample were reported as standard deviations and relative standard deviation (%), as shown in Table 3.
The evaluation of lab performance for each parameter and sample, was made by the Z-score: **Z=(x-X)/s** where lower case **x** is the lab mean, capital **X** is the assigned value of misurand and **s** is the standard deviation value, that are respectively the general mean and the standard deviation of Reproducibility derived from the trial. Scores greater than |3| are considered

unacceptable; between |3| and |2|, questionable; from |2| to |1|, satisfactory; smaller than |1|, perfect.

Table 3: Example of data report.

pH A (EN 13037)	**S1**	**S2**	**S3**
Number of labs retained after eliminating outliers	6	5	5
Number of outliers (laboratories)	0	1	1
Mean value (unit)	6.21	6.47	7.80
Repeatability standard deviation s_r (unit)	0.08	0.10	0.04
Repeatability relative standard deviation (%)	1.27	1.50	0.52
Interlaboratory standard deviation s_L (unit)	0.16	0.04	0.30
Interlaboratory relative standard deviation (%)	2.62	0.54	3.82
Reproducibility standard deviation sR (unit)	0.18	0.10	0.30
Reproducibility relative standard deviation (%)	2.91	1.59	3.86

Results and Discussion

Laboratories were not oblige to perform all the parameters. It is indicative of Italian situation that only three labs performed physical-hydrological analyses (total pore space, dry bulk density, water and air volume at pF1).

Obviously, when different methods are applied, different results are obtained. In particular, it happens for pH and electrical conductivity (Table 4).

Table 4: Data of pH and E.C. (general means) obtained with the three proposed methods.

		S1	**S2**	**S3**
pH A (EN 13037)		6.21	6.47	7.80
pH B (Sonneveld)		6.20	5.80	7.52
pH C (Italian official method)		6.05	6.39	7.82
EC A (EN 13038 mS/m)	µS/cm	298	582	747
EC B (Sonneveld µS/cm)	µS/cm	1061	1868	2391
EC C (Italian official method dS/m)	µS/cm	530	760	810

The results of the collaborative trial are summarized in Table 5 by the reproducibility relative standard deviation percentage: they show that the highest variability occurred for water soluble nitrogen forms and total zinc (values greater than 40%), while the variability of the remaining parameters lies between 1% and 20%.

Table 5: Final results expressed as Reproducibility relative standard deviation (%).

	S1	S2	S3	mean/parameter
NO_3-N water soluble	52.3	31.4	106	63.2
NH_4-N water soluble	42.3	36.3	64.8	47.8
Zn total	72.5	28.2	16.9	39.2
Electrical Conductivity A	29.6	20.3	14.2	21.4
Air volume pF1	18.7	25.8	16.5	20.3
Lab. compacted bulk density B	2.31	27.4	23.9	17.9
Cu total	19.4	14.2	18.4	17.3
Electrical Conductivity C	20.1	13.6	4.17	12.6
pH B	3.89	20.2	6.40	10.2
Organic carbon	12.7	6.37	9.15	9.41
Electrical Conductivity B	12.7	8.02	4.97	8.56
Dry bulk density	5.18	10.7	5.29	7.06
Ashes	10.8	4.47	3.72	6.33
Lab. compacted bulk density A	5.53	3.27	6.98	5.26
Water volume pF1	4.27	6.70	4.71	5.23
Organic matter	1.82	7.18	6.53	5.18
pH C	6.70	2.06	5.06	4.61
Dry matter	3.86	4.12	3.57	3.85
pH A	2.91	1.59	3.86	2.79
Particle density	0.68	1.61	1.04	1.11
Total pore space	0.36	1.77	0.57	0.90
mean/sample	15.6	13.1	15.5	

Comparing the results of Re.La.S.Co. project to the inter-laboratory trial reported on EN standards (Table 6), we are pleased to notice the our variability is similar, and sometimes lower, respect to that of the EN standards one.

Table 6: Comparison of results obtained in Italian and European inter-laboratory trials.

	Re.La.S.Co. Project			**1995 interlaboratory trial by European Committee for Standardization**		
pH A (EN 13037)	S1	S2	S3	Perlite/ peat	Composted bark	Composted straw/sludge
N. of labs retained after eliminating outliers	6	5	5	16	16	16
Number of outliers (laboratories)	0	1	1	0	0	0
Mean value (unit)	6.21	6.47	7.80	5.34	6.43	6.71
Repeatability relative standard dev. (%)	1.27	1.50	0.52	2.24	2.79	1.34
Reproducibility relative standard dev. (%)	2.91	1.59	3.86	9.0	15.34	6.86
EC A (EN 13038)						
N. of labs retained after eliminating outliers	5	6	6	16	16	16
Number of outliers (laboratories)	1	0	0	0	0	0
Mean value (unit)	29.8	58.2	74.7	10.18	31.7	126.9
Repeatability relative standard dev. (%)	2.55	4.51	2.55	11.0	8.61	7.01
Reproducibility relative standard dev. (%)	29.6	20.3	14.2	56.1	47.67	26.39
Dry bulk density (EN 13041)						
N. of labs retained after eliminating outliers	3	3	3	10	11	12
Number of outliers (laboratories)	0	0	0	1	0	0
Mean value (unit)	112	281	267	102.5	190.6	322.3
Repeatability relative standard dev. (%)	2.75	3.08	2.96	4.72	6.15	4.65
Reproducibility relative standard dev. (%)	5.18	10.7	5.29	6.98	12.11	12.4

Except for laboratory 6, labs scores obtained, including outliers, were satisfactory; only in four cases we observed few unacceptable values. The best ones had seventy-eighty percent of results falling in perfect score and the remaining twenty/thirty % in satisfactory score while the less effective ones half and half as perfect or satisfactory (Table 7).

Table 7: Final evaluation of laboratories performances.

Labs		**8**	**4**	**3**	**2**	**5**	**1**	**9**	**7**	**6**
Z-score	n. total analyses	66	66	36	36	36	39	36	30	8
perfect	*n.*	*54*	*55*	*28*	*26*	*25*	*26*	*16*	15	5
< 1	%	81.8	83.8	76.5	72.2	69.4	65.8	44.4	50.0	62.5
satisfactory	*n.*	*12*	*8*	*8*	*9*	*8*	*13*	*18*	*11*	*0*
1 < |z| < 2	%	18.2	12.1	23.5	25.0	22.9	34.2	50.0	36.7	0
questionable	*n.*	*0*	*2*	*0*	*1*	*2*	*0*	*0*	*3*	*2*
2 < |z| < 3	%	0	3.0	0	2.8	5.7	0	0	10.0	25
unacceptable	*n.*	*0*	-	*0*	*0*	*1*	*0*	*2*	*1*	*1*
> 3	%	0	-	0	0	2.9	0	5.6	3.3	12.5

At the end, we noticed from one hand a general low variability within and among the laboratories attending to collaborative trial. The other side of the coin is that, until now, only a small number of Italian laboratories showed to be interested to perform good analysis on growing media. Results confirm the need of clear indication about methods and it is desirable the adoption of EN methods in Italy as it is going on in a lot of European countries.

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EFFECT OF COMPOST BASED SUBSTRATE AND MYCORRHIZAL INOCULUM IN POTTED GERANIUM PLANTS

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Abstract

Two are the critical factors in the commercial production of ornamental plants: the substrate and the fertilization method, since the "sustainable floriculture" discourages the use of synthetic chemical fertilizers and peat-based substrates. The aim of this paper was to test a peat/compost based substrate and a guano + mycorrhizal inoculum fertilizer mixture, in order to obtain a sustainable quality yield of geranium plants. Geranium cuttings (*Pelargonium zonale* cv real polaris and *Pelargonium grandiflorum* cv lotus) were grown for two months in a glasshouse. Peat, in the growing substrate, was replaced with high quality compost (20% and 40% as reduction percentages). The fertilizer mixture was made up of guano (3 g/L) and mycorrhizal inoculum (7,5 L/ m^3). Plants of *Pelargonium* cv Real polaris, grown on a substrate made up of peat and 20% of high quality compost, presented the better trade features, so to satisfy the "sustainable floriculture" need.

Keywords: compost, growing medium, arbuscular fungi, pelargonium.

Introduction

The choices of growth substrate and fertilization method are two critical factors in the commercial production of flowering ornamental plants. Environmental and economical concerns have generated interest in seeking alternatives for peat moss, because of the detrimental effects of peat harvesting on wetland ecosystems (Evans *et al.*, 1996). Composts have proved to be very promising as peat substitute (Verdonck, 1988). However, compost as a nutrient source for plants may require amendments to other organic nutrient sources to meet plant demand. A fertilizer mixture made up of guano and a commercial mycorrhizal inoculum could be solved this problem. Guano is an organic NP fertilizer. Its peculiarities are the fast nitrification and the absence of P insolubilization processes. It is also authorized in organic agriculture. Arbuscular mycorrhizal (AM) fungi are soil microrganisms living in very intimate contact with roots of most plant species and they cannot be found in a compost-peat substrates. These fungi can help the plant to uptake nutrients, such as P, N, Zn, Cu (Rea and Tullio, 2001) and sometimes K (George, 2000). The symbiosis with these fungi may induce earlier flowering and increased flower number (Gaur and Adholeya, 2005). This trait of AM fungi is of particular interest to horticultural production. Researches have tested the interactive effects of nutrient supply and mycorrhizal colonization on flowering. On a peat substrate with organic NPK fertilizer, mycorrhizal pelargonium plants flowered earlier (Nowask, 2004). The effect of compost addition on mycorrhizal plants has been investigated only in a few studies. So, the aim of this work was to the test a peat-compost substrate in association with a fertilizer mixture in potted pelargonium plants, in order to verify if these treatments are complementary in increasing quality of plants in a sustainable management system.

Materials and methods

A high quality compost was used, obtained by the decomposing process of organic waste, such as food and vegetables residues from Rome local markets. It was manufactured by AMA S.p.A. (Italy). In the present study, this compost was mixed with blonde peat (Technostrat srl, Sopram Association), to obtain a compost based substrate with 20% or 40% compost by volume. The fertilizer mixture was made up of guano (Guanito, 3 g/l.; Italpollina S.p.A., Italia) and a commercially available mycorrhizal arbuscular crude inoculum (Aegis granulo; contains *Glomus intraradices*, 100 infective units per g inoculum; administrated dose was 7,5 l/ m^3 ; Italpollina S.p.A., Italia). This mixture was mixed uniformly into the potting substrate before cuttings planting. On these substrates, before the transplanting, the following parameters were detected: pH (CEN, EN 13037 method), electric conductibility (CEN, EN 13037 method) and P and K concentration by incineration and determination at the Inductively Coupled Plasma Atomic Emission Spectrometric (ICP). The Total N concentration was determined by Kjeldhal method, 1883. Cuttings of pelargonium (*Pelargonium x hortorum* cv Real polaris and *Pelargonium grandiflorum* cv Lotus, Vivai Albani & Ruggeri, Italy) were placed, singly, in 200-ml pots filled with the carried out substrates. The control theses had a blonde peat substrate (TP: *P. x hortorum* cv Real polaris cuttings; TL: *P. grandiflorum* cv Lotus cuttings); the theses C20 and C40 had a blonde peat and 20% or 40% vv of compost substrate respectively (C20P and C40P: *P. x hortorum* cv Real polaris cuttings; C20L and C40L: *P. grandiflorum* cv Lotus cutting); the theses CM20 and CM40 had a blonde peat, 20% or 40% vv of compost and the fertilizer mixture substrate (CM20P and CM40P: *P. x hortorum* cv Real polaris cuttings; CM20L and CM40L: *P. grandiflorum* cv Lotus cutting). All pots were watered to maintain favourable water conditions in the substrates. The plants were grown for two months in a “Vivai Albani & Ruggeri”’s automatic greenhouse. A rooting hormone (RIZOPON AA 0,5% - 3-indolyboterzuur- 0,5 Kg) was administrated to the *Pelargonium* *grandiflorum* cuttings, at the cutting surface. The fertilization plan was N:P:K= 1:0,6:1,3 for guanoless theses and N:P:K= 1:0:1,3 for the other ones. At the end of the experiment, shoots were separated from roots, and shoot fresh weight (FW), shoot height (H), leaves number and the branching values were recorded. The mycorrhizal colonization of roots was determined following the method of Grace and Stribley (1991). Percentage of root colonization was recorded by a stereomicroscope (Nikon Instruments S.p.A., SMZ-U, Japan) at 100x using the grid line intersection method (Giovannetti and Mosse, 1980). Pots were arranged in a completely randomized design. Data were analyzed by a two-factorial analysis of variance, with cultivar and treatments as experimental factors. Mean separation was carried out with the test LSD ($P<0.05$). Data were analyzed using Statgraphics Plus 5.1 software (Statpoint techonologies, Inc., Warrenton, Virginia, USA).

Results and Discussion

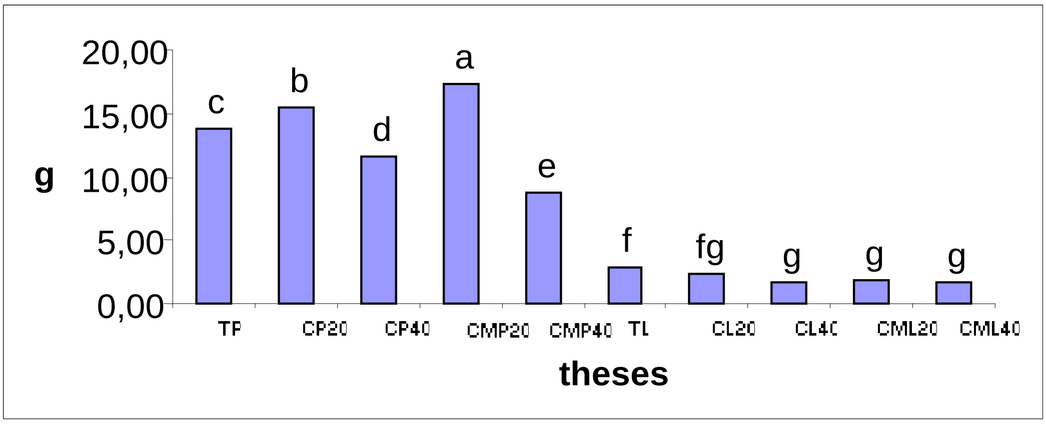
The values of the chemical, physical parameters and the N, P and K concentration of the substrates were in according to their use for pelargonium growing (Tab.1).

Table1: PH and E.C. (mS/cm) values and N, P ,K contents of the substrates.

Potting substrates	pH	E.C. (mS/cm)	N (% ss)	P (% ss)	K (% ss)
control	5.8	0.32	0.37	0.024	0.061
C20	6.9	0.54	0.76	0.097	0.27
C40	7.3	0.63	0.98	0.13	0.41
CM20	7.0	0.86	0.91	0.17	0.33
CM40	7.4	0.90	1.12	0.21	0.52

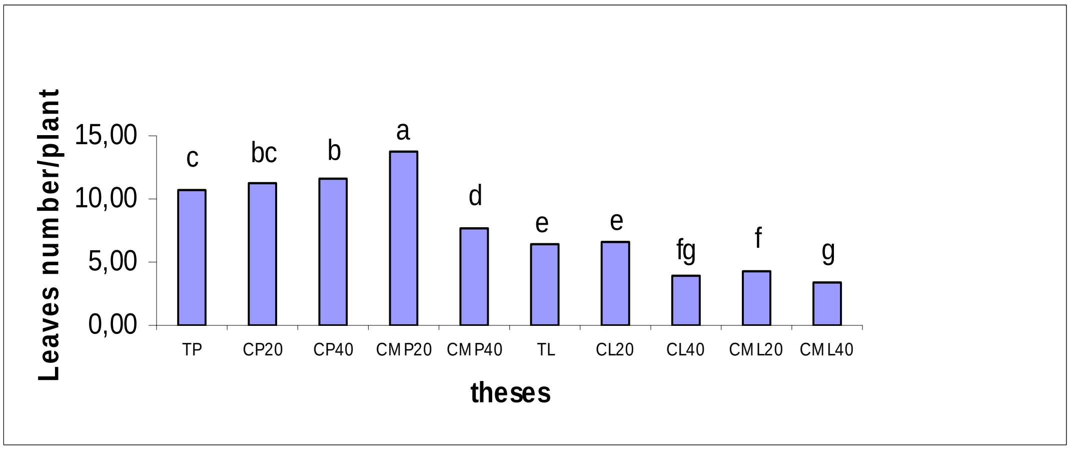
Colonization of *P. x hortorum* cv “Real polaris roots by AM was not significant different between the two compost addition percentages. Average root colonization percentage was 52.33 % (a) for CM20 thesis and 47.33 % (a) for CM40 thesis. Non-inoculated plants remained free of mycorrhizal colonization, although the substrates had not been sterilized before use. No information about *P. grandiflorum* root colonization is available because these plants had a very little root system (1 or 2 little roots, few centimetres long) which didn’t allow any determination. All biometric parameters recorded such as shoot FW, shoot height and leaves number of *Pelargonium x hortorum* plants, grown on 20% compost based substrate with the fertilizer mixture, had the highest values (Fig. 1- 2- 3). *Pelargonium x hortorum* plants, grown on 20% compost based substrate with the fertilizer mixture, had the highest branching values, too (Fig. 4).

Figure 1: Shoot fresh weight values (g) of the two pelargonium species.



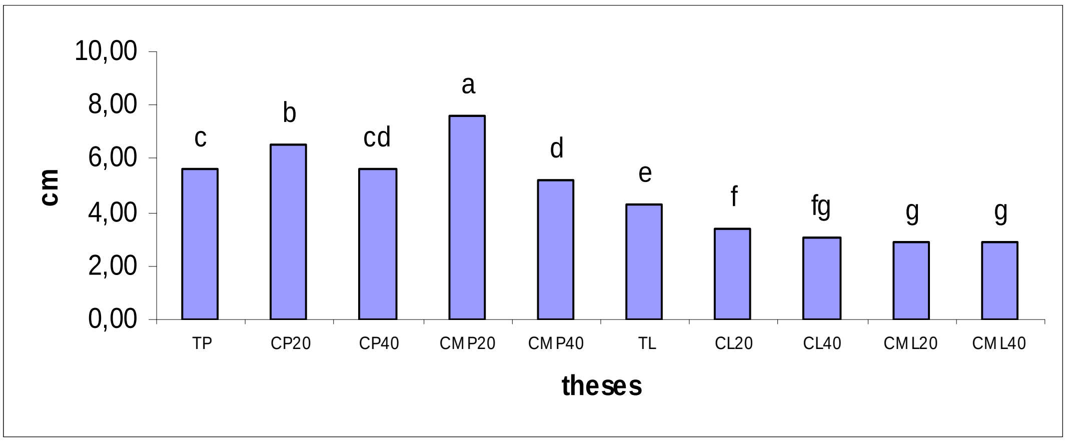
Different letters denote significant differences between means of the treatments determined by Fisher's least significant difference (LSD) procedure ($p \leq 0.05$). P value: P (treatment x cultivar)= 0

Figure 2: Leaves number/plant of the two pelargonium species.



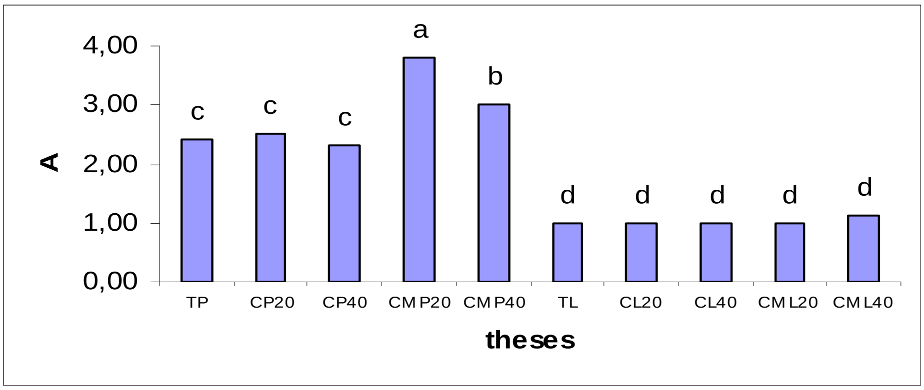
Different letters denote significant differences between means of the treatments determined by Fisher's least significant difference (LSD) procedure ($p \leq 0.05$). P value: P (treatment x cultivar)= 0

Figure 3: Plant high values (cm) of the two pelargonium species.



Different letters denote significant differences between means of the treatments determined by Fisher's least significant difference (LSD) procedure ($p \leq 0.05$). P value: P (treatment x cultivar)= 0

Figure 4: Branching values of the two pelargonium species.



Different letters denote significant differences between means of the treatments determined by Fisher's least significant difference (LSD) procedure ($p \leq 0.05$). P value: P (treatment x cultivar)= 0

Considered parameters for *Pelargonium grandiflorum* plants showed very little differences among them. In particular, the control and CL20 plants had higher values of shoot FW and leaves number with respect to the other theses. The control plants had the highest values of shoot height. All theses had the same values of branching (Fig. 1, 2, 3, 4).

Taking into account that the aim of this research was to check a compost-based substrate with 20 % or 40% compost by volume in association with a fertilizer mixture made up of guano and a commercial mycorrhizal crude inoculum, in potted pelargonium plants, the two compost-based substrates, carried out for the trial, resulted suitable for growing of potted pelargonium plants. Two were the pelargonium species considered: *P. x hortorum* cv Real polaris and *P. grandiflorum* cv Lotus. Roots of inoculated *Pelargonium x hortorum*, after two months from the transplant, were well colonized with mycorrhizal fungi and the root colonization percentage was about 50%. This was true at both compost addition rate (20 and 40%). The quality of *P. x hortorum* cv Real polaris plants, grown on 20% compost-based substrate in association with the fertilizer mixture, was the highest. A significant result of this study for pratical floriculture point of view, was the increased branching of these plants because this parameter is the only economically important one for the plants, in this growth stage. In *P. grandiflorum* cv Lotus, the increasing of the rate of compost in the substrate from 20% to 40% with or without the fertilizer mixture had a very little effect on the growth. Biometric parameters were slightly higher in the control and in the plants grown on 20% compost-based substrate. Lopez *et al.* (1998) showed that compost based substrates utilized as potting media for domestic use, produced an underdevelopment of *Pelargonium zonale* cv Lucky Break F2 plants with respect to the control. This behaviour was related to the inferior physical properties of the compost-based potting media, nitrogen immobilization due to the high C/N ratio of pine bark, and probably lack of available phosphorus originated by high calcium and high pH of the compost-based media. In this trial, the little size of the root system, due to the trouble doing rooting of this specie, has to be taken into consideration such as a possible factor causing the slight plant underdevelopment. We conclude that the AM colonization was well established in pelargonium plants on a horticultural substrate. The compost amount didn't influence the AM capability to colonize the root system and there weren't any differences between the two compost addition percentages. The growing technique of pelargonium plants which uses 20% compost- peat as a potting substrate and a fertilizer mixture made up of guano and arbuscular mycorrhizae inoculum can be used for obtaining high quality horticultural products in a sustainable management system.

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III SESSION

New fertilizers and food quality

NITROGEN FERTILIZATION AND IRRIGATION COMPULSORY EFFECT ON SOYBEAN (*GLYCINE MAX* L. MERR) ISOFLAVONE

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Abstract

Nitrogen fixation and soil residual nitrogen may not supply enough nitrogen for soybeans to maximise yield. Irrigation is an important practice for soybean under different conditions. Isoflavone is primarily limited to leguminous crops. Experiment was conducted during 2006-2007. Factors were distributed in a split-split-plot design; irrigation was applied starting from reproductive stage compared with rain, nitrogen (N)application, in 2006 applied at R1 stage and in 2007 at R3, and two soybean cultivars were chosen to estimate the isoflavones accumulation on seeds formed on main and lateral shoot. The climatic conditions of the two years were different and affected accumulation of isoflavones up to 30%. Significant effect for irrigation was found but drought stress affected cotyledon isoflavone contents. Difference within cultivar was noticed on isoflavones accumulation of germ and cotyledon. Nitrogen application at R1 showed negative effect on germ and cotyledon isoflavone accumulation, whereas at R3 positive effect was observed on soybean cotyledon. Lateral shoot has accumulated more isoflavone than main shoot. The difference in metabolism was noticed between germ and cotyledon. The relation between different molecules varied within cultivars and years. Cotyledon isoflavone accumulation was more linear than its accumulation in germ.

Keywords: Soybean, Isoflavone, Nitrogen, Irrigation, Secondary metabolic, seed quality.

Introduction

Isoflavones are photochemical synthesized in most phenomenon protein family member. The interest in presence of isoflavone has been growing since late 90s, since epidemiological and clinical studies suggested that the consumption of soyfood was associated with health benefits (Sarkar and Li, 2003). N_2 fixing crops present an important option to improve N supply and to maintain soil fertility. Previous studies demonstrated that during drought stress period N_2

fixation is the first process to decrease (Sall and Sinclair, 1991). Although N fertilization is not a common practice there is speculation that the ability of soybean to fix N_2 is not always adequate to reach the maximum yield. In most cases, full-season soybeans can be irrigated only during the reproductive period and one obtains similar yields as those obtained with complete-season irrigation (Ashley and Ethridge 1978). Drought stress decreases yield-related processes and N_2 fixation is more sensitive to drought than many other processes. Therefore, application of nitrogen fertilizer may increase drought tolerance for those plants primarily dependent on N_2 fixation (Purcell and King, 1996). Water content is very important for all plants as effective factor for plant metabolism, isoflavones compound found to increase by irrigation (Bennett *et al.*, 2004) and to decreased by drought (Caldwell *et al.*, 2005; Lozovaya *et al.*, 2005; Seguin *et al.*, 2004). Soil nitrogen enrichment had no effect on total isoflavone concentration, where N application was not correlated with isoflavone concentration (Kim *et al.*, 2005). The aim of this study was to elucidate the irrigation and N application effects on isoflavones accumulation in germ and cotyledon on main and lateral shoot seeds of two soybean cultivars, i.e. Ales and Nikir.

Materials and methods

The experiment was performed in 2006 and 2007 at Padova University Experiment Station, Legnaro - Italy, 45°21'03" N, 11°56'54" E in loam soil. Climate data are reported in Table 1, and water deficit was calculated according to (water deficit= Rain–ETo). A pre-sowing practices were peformed, weed was handly controlled twice during plant development. Factors were arranged in a split-split-plot layout. In both years 2 cultivars (Ales and Nikir) were considered and the following factors taken into account: irrigation, irrigated vs. non irrigated; nitrogen, applied at R1 stage (flower beginning) and R3 stage (pod set beginning). Six rows per plot were cultivated with 40 seed m^{-2} (4m length x 50cm inter-row) in 4 replicates. Irrigation was weekly based on ETm. Pods were collected on main and lateral shoot at harvest time and seeds were collected.

Table 1: Medium temperature, rain, ETo and water deficit of Legnaro in 2006-2007.

	2006				2007			
	med T	Rain	ETo	Water deficit	med T	Rain	ETo	Water deficit
	°C	mm			°C	mm		
May	17.23	92.40	98.07	-5.67	19.15	146.6	116.43	30.17
June	21.74	14.60	123.91	-109.31	22.07	60.8	124.23	-63.43
July	25.19	47.60	131.37	-83.77	23.72	31.2	152.13	-120.93
August	19.95	122.40	94.63	27.77	22.07	48.2	110.71	-62.51
September	20.18	178.20	69.71	108.49	17.39	104.8	74.63	30.17
October	15.92	16.00	40.82	-24.82	13.28	35.8	38.35	-2.55

Data were obtained from Legnaro station belongs to ARPAV (Regional Agency for Prevention and Protection of Environment in Veneto).

For isoflavone analysis, 60 seeds samples were lyophilised and fractionated; germ and cotyledons were isolated, immediately milled, and then frozen to –20 °C. In 7 mL of aqueous methanol solution (80% v/v, 2 h, room temperature) 0.1 g were added, and the solution was than filtered (0.2 µm) and analysed by high performance liquid chromatography (HPLC) with UV sensor (diode array). The mobile phases were a water solution of 0.05% trifluoroacetic acid (v/v) (solvent A) and pure acetonitrile (solvent B). Gradient elution was performed according to the procedure of Murphy *et al.* (1999), with minor modifications by Hubert *et al.* (2005) Data were analyzed by ANOVA using general linear model GLM in both years considered separately because the two years contrasting in complementary N input. Correlation regression analysis used to estimate the relation between different isoflavones group. All statistical analysis were performed using statgraphics centurion XV.

Results and discussion

In our experiments data sets demonstrate that cotyledon had 4 times larger amount of isoflavones than germ, but the relative concentration is smaller due to dilution effect. In soybean germ and cotyledon isoflavone accumulation differ more than 30% within years.

Cultivar: The two cultivars showed large differences in single and total isoflavone of germ and cotyledon (Table 2 and 3). In 2006, Ales germ had larger TISO, TGS and TGY than Nikir, which showed more TDZ accumulation (Table 2). In 2007, Nikir germ showed larger TISO, TDZ and TGS than Ales, which had more TGY accumulation (Table 2). The accumulation of TISO, and other similar forms, in cotyledon generally was greater in Ales than Nikir in both years (Table 3). These differences are due to large genetic differences and such differences are maintained over very diverse environmental conditions.

Irrigation: Average temperature and water deficit were higher during July and August (filling pod period) in 2007. Water supply did not show significant effect neither on germ nor cotyledon for isoflavones accumulation in 2006 (Table 2 and 3); however, in 2007 drought stress increase isoflavones accumulation in soybean germ except TGY, which reflects the relation between isoflavone accumulation and stress. Moreover cotyledon showed significant accumulation of TGS under drought in 2007 plots while TISO and TDZ did not show significant accumulation in irrigated plot.

Table 2: Mean data and Results of Anova for different germ's isoflavone in 2006 and 2007.

		2006				2007			
		TISO	TDZ	TGS	TGY	TISO	TDZ	TGS	TGY
		(mg g^{-1})							
Cultivar (C)	Ales	14.993**	5.951	1.238	7.805**	8.973	3.515	1.786	3.672**
	Nikir	14.174	8.429**	1.212	4.533	9.674	5.504**	1.858	2.312
Irrigation (I)	drought	14.570	7.196	1.240	6.134	9.697*	4.685*	1.922**	3.089
	irrigation	14.598	7.184	1.210	6.204	8.951	4.335	1.722	2.895
Nitrogen (N)	-N	14.861**	7.305**	1.275**	6.281**	9.248	4.487	1.800	2.962
	+N	14.306	7.075	1.174	6.057	9.399	4.533	1.845	3.022
Stem (S)	Principal	14.474	7.173	1.228	6.073	9.064	4.422	1.777	2.864
	Lateral	14.693	7.207	1.222	6.264*	9.584*	4.598*	1.867	3.119*
Anova									
C* I		**	*	ns	**	ns	ns	ns	ns
C*N		ns	ns	ns	ns	ns	ns	ns	ns
C*S		ns	ns	ns	ns	ns	ns	ns	ns
I *N		ns	ns	ns	ns	ns	ns	ns	ns
I *S		ns	ns	ns	ns	ns	ns	ns	ns
N*S		ns	ns	**	ns	ns	ns	ns	ns
C*I*N		ns	*	ns	ns	ns	*	ns	ns
C*I*S		ns	**	ns	ns	ns	ns	ns	ns
C*N*S		ns	ns	ns	ns	ns	ns	ns	ns
I*N*S		*	**	ns	ns	ns	ns	ns	ns
C*I* N*S		ns	ns	*	ns	ns	ns	ns	*

TISO Total isoflavone, TDZ total daidzein, TGS total genistein, TGY total glycitein; *;** at 0.05 P and 0.01 P; ns= not significant.

Nitrogen: Germ isoflavones accumulation were negatively influenced by N supply in 2006, but not in 2007 (Table 2). Large variation between years on the effect of nitrogen supply on cotyledon isoflavone accumulation was found. Nitrogen effect at R1 reduced accumulation of isoflavone in both germ and cotyledon, whereas at R3 increased. Late N application increased plant vigor and delay maturity and elongate seed exposure for low temperature which increased isoflavone (Lozovaya *et al.*, 2005). Our result concerning N application were not in agreement with previously reported data (Kim *et al.*, 2005).

Stem: In both years TGY germ on lateral shoots was 8% larger than main stem (Table 2). Glycitein accumulation came up late in soybean germ after finishing accumulation of genistein and daidzein (Berger *et al.*, 2008). In second year germ on lateral shoot showed significant increment in TISO and TDZ than main stem. In both years cotyledons formed on lateral shoot had 20% more TISO, TDZ and TGS than cotyledons of main stem (Table 3). Lateral shoots form their pods later which may expose them to low temperature during pod filling, increasing consequently isoflavones contents of seed in lateral shoot (Lozovaya *et al.*, 2005).

Table 3: Results of ANOVA for different cotyledon's isoflavone in 2006 and 2007.

		2006			2007		
		TISO	TDZ	TGS	TISO	TDZ	TGS
		(mg g^{-1})					
Cultivar (C)	Ales	1.971**	0.986**	0.871	2.190**	0.801**	1.367**
	Nikir	1.521	0.616	0.819	1.242	0.352	0.874
Irrigation (I)	drought	1.663	0.755	0.801	1.827	0.596	1.208*
	irrigation	1.830	0.848	0.890	1.604	0.556	1.033
Nitrogen (N)	-N	1.770	0.824*	0.872*	1.579	0.534	1.030
	+N	1.723	0.779	0.818	1.853**	0.618**	1.211**
Stem (S)	Principal	1.556	0.703	0.754	1.535	0.516	1.002
	Lateral	1.936**	0.900**	0.936**	1.896**	0.637**	1.239**
Anova							
C* I		ns	ns	ns	**	**	**
C*N		ns	*	ns	**	*	**
C*S		**	**	ns	*	ns	**
I *N		ns	ns	ns	**	**	**
I *S		*	*	*	ns	ns	*
N*S		ns	ns	ns	**	*	**
C*I*N		ns	ns	ns	**	**	**
C*I*S		ns	ns	ns	ns	ns	ns
C*N*S		ns	ns	ns	*	ns	**
I*N*S		**	**	**	ns	ns	ns
C*I* N*S		ns	ns	ns	ns	ns	ns

TISO Total isoflavone, TDZ total daidzein, TGS total genistein, TGY total glycitein; *;** at 0.05 P and 0.01 P; ns= not significant.

Interaction: Germ isoflavone was slightly influenced by interaction between different factors (Table 2), while cotyledon showed great variability under different interactions (Table 3). Nikir in 2006 under drought showed 5% greater accumulation of TDZ and TGS than irrigated plots but Ales was negatively influenced by drought stress for 8%. Moreover drought stress in 2007 showed 50% positive accumulation of TISO, TDZ and TGS in Nikir cotyledon compared to irrigated plots and Ales was negatively influenced by drought stress of 16%. TGS in cotyledon of Ales was greater under non-fertilized plots but Nikir showed a reduction of 40%. TGS accumulation showed difference on lateral shoot cotyledon regarding the stress level, intensity of stress was higher in 2007 and showed greater accumulation in TGS. Level of stress may influence accumulation of isoflavone on different level (Seguin *et al.*, 2004).

Isoflavone metabolism: Both cultivars were varied in their accumulation for three main isoflavones groups. Germ contains all three groups but cotyledon contains only daidzein and genistein forms. However the accumulation of these molecules varied regarding to cultivar and year (Fig. 1 and 2). Great differences were observed between years referring to TDZ, TGS and TGY accumulation (Fig. 1 and 2). It was difficult to estimate both cultivar in one correlation between two molecules due to large genetic differences (Fig. 1-4). In Nikir the correlation between TDZ and TGY germ was linear with R^2 = 90% in 2006 and 71% in 2007 but for Ales did not exceed 55% in both year (Fig. 1).

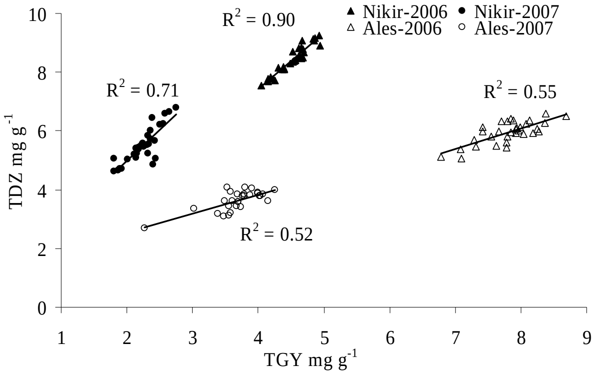

Figure 1: germ total glycitein TGY and total daidzein TDZ correlation for both cultivar in both year.

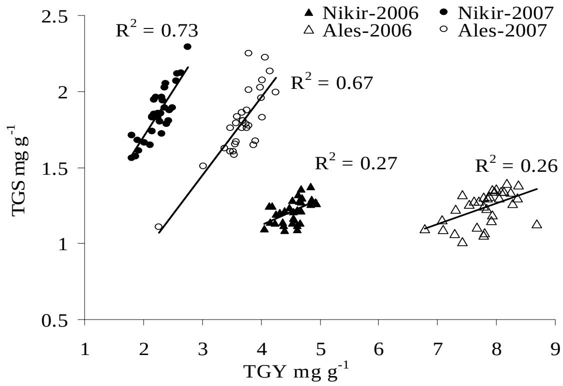

Figure 2: germ total glycitein TGY and total genistein correlationTGS for both cultivar in both year.

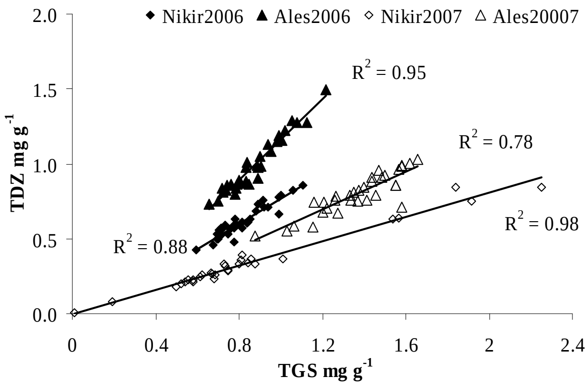

Figure 3: Cotyledon total daidzein TDZ and total genistein TGS relations for both cultivar in different years.

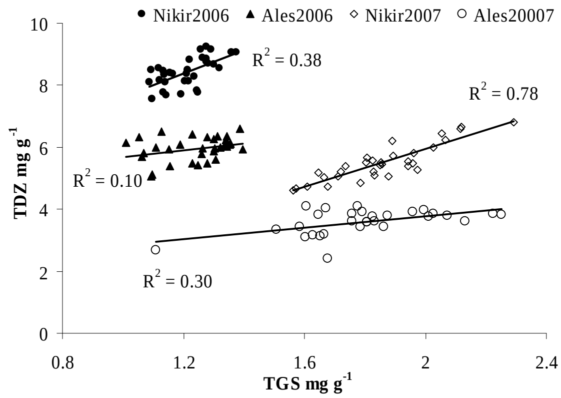

Figure 4: Germ total daidzein TDZ and total genistein TGSrelations for both cultivar in different years.

The correlation between TGS and TGY in 2006 was about 25% for germ of both cultivars whereas in 2007 it was 72% and 67% for Nikir and Ales respectively (Fig. 2). The relation between TDZ and TGS in cotyledon was highly correlated, which reflect that pathway of their synthesis is not contradictory (Fig. 3 and 4), However, germ showed the same trend but the R^2 values were not similar in cotyledon. Both cultivars showed difference in accumulation of TDZ and TGS in cotyledon regarding year, in 2006 they produced higher TDZ and in 2007 more TGS (Fig. 3).

Isoflavones accumulation in both germ and cotyledon was mainly influenced by year and cultivar whereas water and nitrogen supply may affect positively or negatively. Lateral shoot showed higher isoflavone concentration than main shoot. Interaction within factors did not show great stability within year.

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EFFECTS OF ORGANIC AND CONVENTIONAL N-FERTILIZATION ON QUALITY TRAITS IN CORIANDER (*CORIANDRUM SATIVUM* L.)

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Abstract

In organic cropping management of Medicinal and Aromatic Plants, the best quality expression is crucial to gain satisfactory incomes. Coriander (*Coriandrum sativum* L.) is an annual herbaceous plant with a commercial value due to the typical scent of its fruits (commonly termed "seeds"), rich in a pale yellow oil (1-2% in small-sized types, 0.2-0.5% in large-sized ones). Several studies have been done for determining the composition of volatile fraction of Coriander, which was found to vary also as a consequence of cropping techniques, including nitrogen fertilization. In order to gain useful information about the effects, if present at all, of organic N fertilization on Coriander quality in terms of volatiles composition pattern, a three-year trial (2004-2006) was carried out using different types and rates of organic and conventional N fertilizers. Volatile composition of fruits was obtained by means of GC-MS and data were evaluated by multivariate statistical analysis. The most representative compound are linalool, followed by camphor, geranyl acetate and geraniol. The group partition was mainly due to different quantitative ratio of compounds; the differences in volatile composition, however, followed a scheme more resembling the cropping year than the fertilization management.

Keywords: Coriander, N fertilizers, seed composition, volatile compounds.

Introduction

Coriander *(Coriandrum sativum* L.) is an annual herbaceous plant belonging to the *Apiaceae* family, with a good commercial value due to the typical scent of its fruits, rich in a pale yellow oil (1-2% in small-sized types, 0.2-0.5% in large-sized ones) (Catizone *et al.*, 1986).

The diffusion of Coriander into the Mediterranean cropping systems still needs the pointing out of many aspects; one of the most relevant is the fertilization, above all with nitrogen (Carrubba, 2009). N fertilization, as a matter of fact, is claimed to exert on most crop an evident and fast effect, generally promoting growth and increasing plants biomass. Coriander has been targeted by many studies concerning the effects of N fertilization on quantitative aspects of yield; limited information is available, however, as concerns the response of coriander to such practice from the point of view of seed composition (Carrubba and Ascolillo, 2009).

In order to gain useful information about the effects, if present at all, of N fertilization on Coriander quality, the volatiles composition pattern was studied over a three-year trial (2004-2006) by considering different types and rates of N fertilizers, both organic and conventional.

Materials and methods

The trial was carried out from 2004 to 2006 in the experimental farm "Sparacia" (Cammarata - AG 37° 38' N – 13° 46' E; 415 m a.s.l.), in a representative area of the Mediterranean semi-arid environments. The treatments considered were three organic and two organo-mineral NP fertilizers, compared with three rates and application time of a chemical N-fertilizer and one untreated control (table 1).

Treatments were laid out according to randomized complete block design with three replications. Sowing was performed by hand on January 17th 2004, January 19th 2005 and January 14th 2006, distributing seeds of a small-sized Coriander biotype *(Coriandrum sativum* L. subsp. *microcarpum)* on continuous rows 50 cm apart, obtaining a plant population of 40 plants m^{-2}. Climatic parameters (temperatures and rainfall; fig.1) were measured during the period by means of a weather station (CR 10, Campbell Sc., Oregon, USA).

The growth and development of the crop and its phytosanitary state were monitored throughout the trial. Top-dressed N fertilizers were applied as the crop was starting the fast stem elongation phase, i.e. between the middle of March and the first days of April.

The harvest was manually performed as the majority of seeds had reached the full ripening stage, and this occurred in the last ten-days of June. Thereafter, on representative samples of seeds for each treatment and repetition, volatile composition was obtained by means of GC-MS; all data were then submitted to statistical analysis, including ANOVA and MANOVA, by means of the package SAS v. 9.0 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The evaluation of volatile composition of fruits allowed the recognition of 27 different compounds, among which monoterpenes (both hydrocarbons and oxygenated) were the most abundant (98-99%).

Figure 1: Sparacia (Cammarata - AG) - 2004-2006. Coriander (*Coriandrum sativum* L.). 10-day values of climatic parameters measured during the trial.

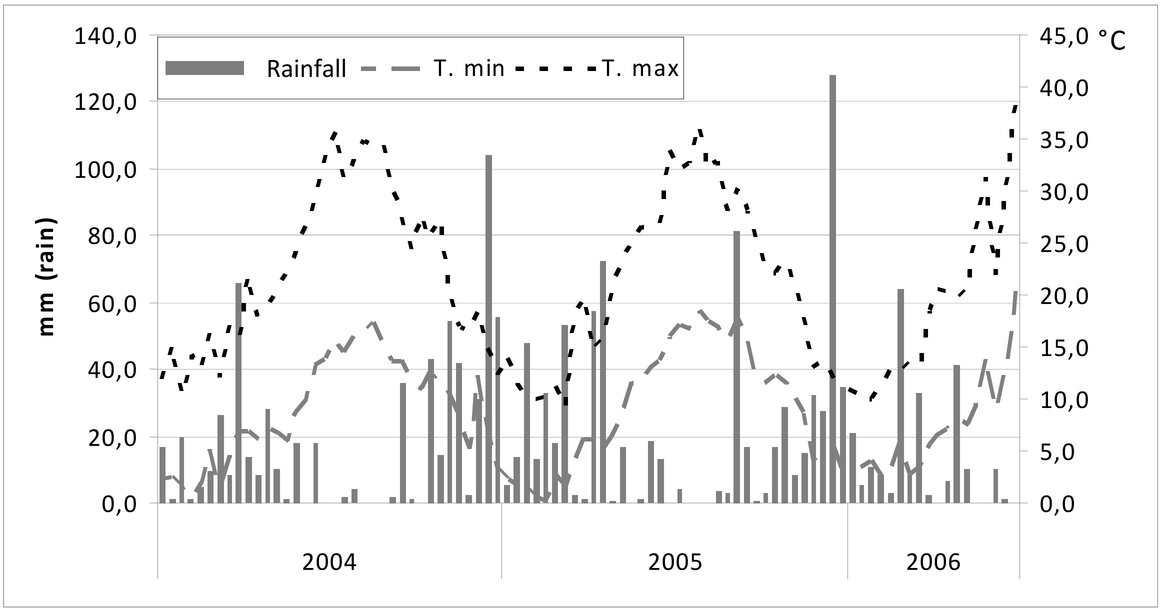

Table 1: Sparacia (Cammarata - AG) 2004-2006. Organic and chemical N-fertilization in Coriander. Treatments tested during the trial.

Treatment /Year	Total N (kg ha $^{-1}$)	N-fertilizer type	Distribution method	Name	Formulation	N content
C1 - 2004 to 2006	80	Inorganic	At sowing	Urea		46%
C2 - 2004 to 2006	80		½ at sowing, ½ top-dressed			
C3 - 2004 to 2006	120		2/3 at sowing, 1/3 top-dressed			
O1 - 2004-2005	80	Organic	At sowing	Natural N8	Pellets	Total N 8.0% (organic 8%)
O2 – 2004- 2005	80			Biagrin	Liquid (solution-sospension)	Total N 5.0% (organic 1%)
O4 - 2006	80			Xena N12	Pellets	Total N 12.0% (organic 12%)
O3 - 2004 to 2006	80	Organo mineral NP		Xena Starter	Pellets	Total N 7.0% (organic 7%)
O5 - 2006	80			Geco Natura	Compost	Total N 5.0% (organic 5%)
T – 2004 to 2006	Non fertilized control					

Oxygenated compounds were the primary group, showing an average content of about 77%, and two of the major compounds, linalool (66.7%) and geranyl acetate (3.4%) belong to this group. The other two dominant compounds, namely γ-terpinene (7.4%) and α-pinene (7.1%), belong to monoterpene hydrocarbons. Other substances, belonging anyway to the monoterpenes, were found in lower quantities: camphor (4%), limonene (2%) and geraniol (2%). A preliminary ANOVA performed on data reported in table 2 allowed us to state that the factor "year", and therefore climatic variability, was the major determinant in assessing the differences of the volatile composition.

Table 2: Sparacia (Cammarata - AG) – 2004-06. Results of ANOVA for some volatiles detected in seeds (SV: source of variability; DF: degrees of freedom; Y: year; T: treatment).

SV	DF	Thujone	α–pinene	Camphor	Limonene	β-pinene	Myrcene	p-cymene	γ–terpinene	Terpinolene	Linalool	Geranyl acetate
Y	2	***	*	**	**	***	*	***	*	***	**	*
T	6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
total	20											

Significance of difference: *: P≤ 0.05; **: P≤ 0.01; ***: P≤ 0.001; n.s.: not significant

Table 3: Sparacia (Cammarata - AG) - 2004-2006 – Mean values (%) of major volatiles detected in Coriander (*Coriandrum sativum* L.) seeds under different N fertilization managements (n=21). Each value is the average of 3 replicates. Data arranged into the clusters obtained through CA. Variables with dark heading were the most significant for clustering.

Cluster	Treatment	Year	α-pinene	Camphor	Limonene	Geraniol	β–pinene	Myrcene	γ–terpinene	Linalool	Geranyl-acetate
Result of F Test			41,71 **	8,11 n.s.	35,34 **	1,67 n.s.	81,15 ***	46,98 **	10,26 *	99,20 ***	6,39 *
1	C1	200304	7,14	4,04	2,29	2,28	0,63	1,08	7,37	65,73	3,67
	C2	200304	7,60	4,06	2,32	2,24	0,63	1,11	7,47	65,74	3,78
	C3	200304	7,03	4,07	2,29	2,39	0,60	1,08	7,32	66,22	3,63
	O1	200304	7,11	4,25	2,29	2,29	0,58	1,08	6,36	66,14	3,61
	T	200304	7,84	4,11	2,33	2,27	0,64	1,11	7,66	64,73	3,56
	O2	200304	7,66	4,15	2,38	2,22	0,61	1,11	8,09	64,76	3,52
	O3	200304	7,27	4,26	2,38	2,36	0,59	1,10	7,70	65,33	3,48
	C1	200405	7,51	4,14	2,37	2,36	0,58	1,09	7,73	66,00	3,34
	C2	200405	7,71	4,14	2,42	2,38	0,59	1,12	8,08	65,59	3,21
	C3	200405	8,26	4,09	2,53	2,20	0,63	1,18	7,91	64,63	3,53
	O1	200405	7,07	4,19	2,38	2,44	0,55	1,09	7,71	66,23	3,29
	O2	200405	7,26	4,11	2,33	2,37	0,57	1,08	7,81	66,43	3,22
	O3	200405	8,09	4,1	2,47	2,30	0,60	1,14	8,04	64,99	3,38
	Average		**7,50**	**4,13**	**2,37**	**2,32**	**0,60**	**1,11**	**7,63**	**65,58**	**3,48**
2	T	200405	6,67	4,19	2,28	2,41	0,53	1,03	7,54	67,29	3,15
	C1	200506	7,00	3,96	2,18	2,27	0,52	1,07	7,55	67,57	3,42
	C2	200506	6,68	3,87	2,09	2,31	0,50	1,01	7,24	67,99	3,39
	C3	200506	7,09	4,02	2,23	2,35	0,53	1,07	7,43	67,04	3,35
	T	200506	6,58	3,54	1,93	1,93	0,52	0,95	7,19	68,10	3,74
	O2	200506	7,03	4,04	2,20	2,31	0,51	1,05	7,33	67,35	3,40
	Average		**6,84**	**3,94**	**2,15**	**2,26**	**0,52**	**1,03**	**7,38**	**67,56**	**3,41**
3	O1	200506	5,25	3,9	1,19	2,48	0,41	0,89	6,34	71,09	3,09
	O3	200506	5,04	3,7	1,74	2,37	0,39	0,83	6,31	71,59	2,89
	Average		**5,15**	**3,80**	**1,47**	**2,43**	**0,40**	**0,86**	**6,33**	**71,34**	**2,99**
			Signif. Cluster analysis								

In order to have a deeper insight on the effects of tested factors on the volatile composition of seeds, a multivariate statistical analysis was performed, including Cluster Analysis (CA) and

Principal Components Analysis (PCA). The tree diagram reported in fig. 2 shows the composition of the groups obtained through CA. Such analysis originated three clusters, whose main characteristics are reported in table 3, where partitioning is mainly linked to a difference in the relative amounts of linalool, γ- terpinene, geranyl acetate and α- pinene. The ANOVA performed on clustered data confirmed such observation.

The scatter diagram plotted against the two main PCAs (fig. 3) confirms that the differences of volatile composition follow a scheme more resembling the cropping year than the fertilization management. In detail, the analysis of volatiles has shown in the first two years similar mean values of the monoterpene content, both for those belonging to the chemical class of hydrocarbons (22% approx.) and for the oxygenated ones (about 76.5 %). The seed samples obtained in the third trial year (2005-06) showed, oppositely, a reduction in monoterpene hydrocarbons (about 20%) and an higher amount of oxygenated ones (about 79%). No definite effect was identified on seeds volatile composition, that in our experiment showed to be rather unaffected by the type and rate of N fertilizer.

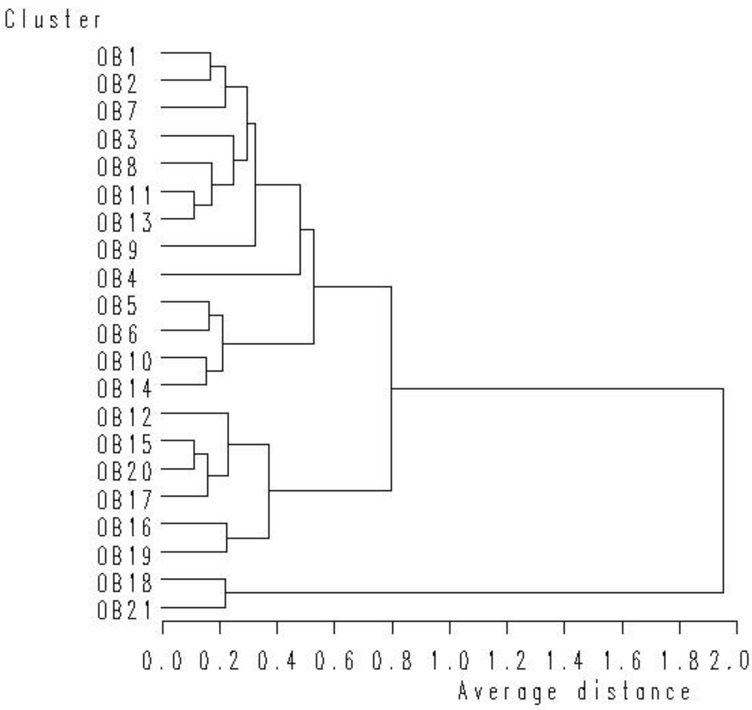

Figure 2: Sparacia (Cammarata-AG) - 2004-2006 - Tree diagram for analytical data of Coriander (*Coriandrum sativum* L.) seeds under different N fertilization managements (n=21).

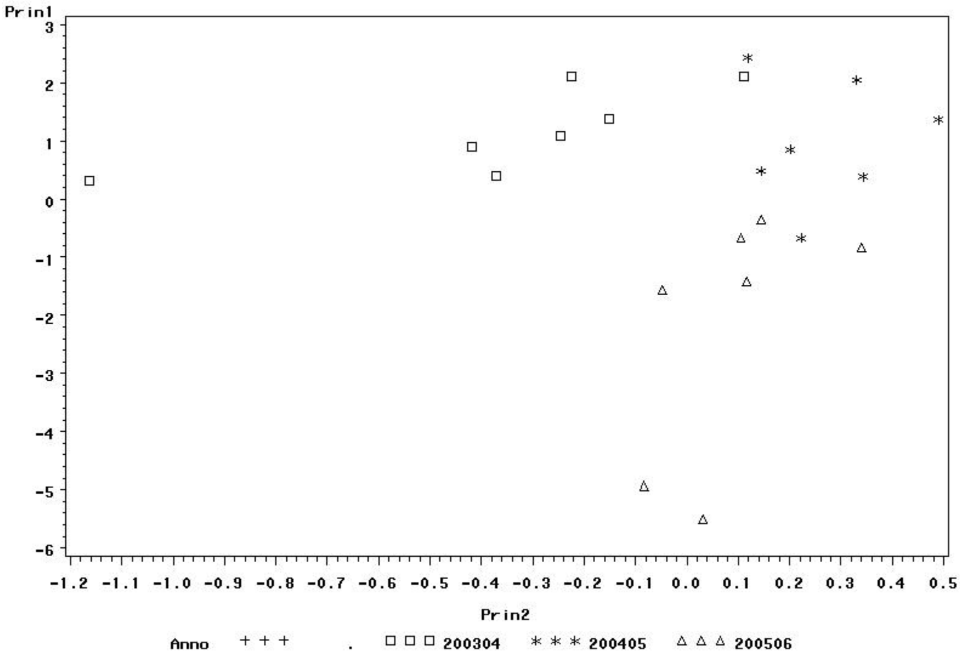

Figure 3: Sparacia (Cammarata - AG) - 2004-2006 - Scatter diagram for analytical data of Coriander *(Coriandrum sativum* L.) seeds under different N fertilization managements (n=21). Each value is the mean of 3 replicates.

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FODDER QUALITY OF MEADOW SWARD IN DEPEND ON THE NITROGEN FERTILIZATION APPLIED IN DIFFERENT DOSES

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Abstract

The experiment was organized on in four replicants in arrangement split – plot with plots having a surface equal to $9m^2$. The basic fertilization applied under first regrowth was the mixture of unary fertilizers (ammonium nitrate, superphosphate, potassic salt) or polifoska. One form of supplementary fertilization was applied under the second and third regrowth. It was the stable form of fertilizer applied to soil. This form of supplemented nitrogen gave respectively: 50 kg N ha^{-1}; 80 kg N ha^{-1}; 110 kg N ha^{-1} per each moving.

During the vegetation season three movings was harvested. From the each movings the samples of green matter were taken for the chemical analysis, i.e. total protein content, soluble carbohydrates and net energy (NEL).

The obtained results showed large differences in fodder quality of the meadow sward fertilized with three doses of nitrogen.

Keywords: total protein, net energy (NEL), nitrogen dose, permanent meado.

Introduction

According to many authors (Winnicka, Bobrecka - Jamro 1996, Wasilewski, Sutkowska 2001), mineral fertilization is one of the basic treatments influencing the botanical composition of meadow sward. Mineral fertilization influences the height and quality of crops. In order to obtain high yields, it is necessary to apply a suitable mineral fertilization regime (Jodełka, Jankowski 2001). High fertilization of grasslands has often negative consequences, such as a worse chemical composition of the fodder, disappearance of some bird or insect species, unfavorable changes in the content of macroelements in soil (Mrkvicka, Vesela 2009; Winnicka, Bobrecka-Jamro 1996). Currently, smaller amounts of fertilizers are being used on meadows or pastures; it can help to maintain the ecological equilibrium of natural grasslands by improving biodiversity (Spatz, Buchgraber 2003, Wasilewski, Sutkowska 2001). However, it is necessary to look for other solutions which would, for example, reduce environmental contamination.

Present intensification of plant production rise a need to search for various solutions, such as new fertilization technologies which reduce environmental pollution. Such technologies allow combination of fertilizing components and improved utilization of nitrogen by plants. It has been empirically confirmed that different doses of fertilization of meadow sward is beneficial (Kolczarek et.al 2008, Jodełka, Jankowski 2001), and it has encouraged us to study the reaction of fodder grasses to fertilization methods in terms of mineral compounds content.

The present study is part of an attempt to formulate guidelines for fertilization of grasslands by testing supplementary nitrogen fertilizations.

Materials and methods

A three-year experiment was established in spring 2003 on permanent meadow in the region of Siedlce. The trial plots were set out on gley proper soil created from light loamy sand on medium silt clay. The soil was slightly alkaline in reaction, both in KC1 solution and in H_2O (pH in l n KC1 7,15); it had a high content of total nitrogen (0.45%), manganese (450 $mg \cdot kg^{-1}$) and iron (1700 $mg \cdot kg^{-1}$), a common magnesium content (0,31 $mg \cdot kg^{-1}$) and a very low phosphorus (0.15 $mg \cdot kg^{-1}$) and potassium (0.25 $mg \cdot kg^{-1}$) amount.

The meteorological measurements (temperature and rainfall) were obtained from the meteorological station in Siedlce, and were quite different across the 3 years experiment. The average air temperature in the growing period (April-September) in the successive years was higher than the long-term average (4.1, 3.9 and 3.8 °C respectively). Also the total precipitation in the growing seasons exceeded the long-term average (about 87.3 mm in 2003, about 74.5 mm in 2004 and about 46 mm in 2005).

Two forms of fertilizer were used for basic fertilization: multiple (Polifoska 15) and a mixture of single-element fertilizers (ammonium nitrate, superphosphate, potassium salt); both added to the soil 60 kg $N \cdot ha^{-1}$, 60 kg $P \cdot ha^{-1}$ and 60 kg $K \cdot ha^{-1}$. Additionally, the second and third cut were fertilized with nitrogen applied in the solid form (ammonium nitrate) to soil. The fertilization treatments introduced respective amounts of nitrogen: N- 50 $kg \cdot ha^{-1}$, N_2- 80 $kg \cdot ha^{-1}$, N_3- 110 $kg \cdot ha^{-1}$.

Three cuts were harvested in the vegetation period; the chemical analysis of the plant material was performed on Infra Lyzer 450 equipment for determination of total protein and soluble carbohydrates.

Mathematical models proposed for this type of experiments by Trętowski and Wójcik (1991) were applied. Significance of differences between means of the experimental factors was determined with Tukey's test at the level of significance $P < 0.05$.

Results and disscusion

The results presented in table 1 shows the significant impact on harvested plant material of fertilization applied in spring on total protein content in dry matter, higher values have been obtained by adding a mixture of fertilizers.

Total protein content in the sward of permanent grassland is one of the most important quality parameters of harvested crop, and its value should be close to 200 g kg^{-1}D.M. Fertilization, especially with nitrogen, has a strong influence on the protein content in plants and higher doses of nitrogen used in fertilization, eliminate legumes plants, which have the biological capacity to collect larger amounts of this component.

Table 1: Content of total protein ($g \cdot kg^{-1}$D.M.) in meadow sward in successive research years in depend on the kind of spring fertilization and nitrogen doses applied in the summer.

Kind of fertilization	Nitrogen dose [$kg \cdot ha^{-1}$]	Years of study			Mean
		2003	2004	2005	
Mulitiple fertilizers (Polifoska)	50	149	157	150	152
	80	160	155	157	157
	110	142	158	168	156
Mixture of fertilizers	50	169	162	165	165
	80	165	157	170	164
	110	170	161	169	167
Mean		159	158	163	160

LSD $P \leq 0.05$ for:
Years of study - 6.0;
Interaction of kind of fertilization and nitrogen dose - 4.9;
Interaction of kind of fertilization and nitrogen dose and study years - 9.8

Analysis of the results indicate the differential impact of applied nitrogen fertilization on the content of total protein in different years. On the objects where the multiple fertilizer was applied in spring and 110 kg N ha^{-1} in the summer, plant material from the first year of the study contained significantly lower amount of total protein (142g kg^{-1}D.M.), but significantly higher amount of this component (168g kg^{-1}D.M.), in comparison with the same objects in the third year of the study. It should be noted that during the same period with the same dose of nitrogen fertilization in the summer, only with a mixture of fertilizers used in the spring, total protein content in the harvested plant material did not differ significantly (170 and 169g kg^{-1} D.M.). These results show that in our system, the evaluation of agricultural production and different ways of nutrients supply depends on the distribution of temperature and precipitation, in agreement with previous work of Jodełka, Jankowski (2000).

An important element of quality of harvested crop is also the content of soluble carbohydrates, which affect the testability of feed and possibility of conservation (Jodełka, Jankowski 2001).

Table 2: Content of soluble carbohydrates ($g \cdot kg^{-1}$D.M.) in meadow sward in successive research years in depend on the kind of spring fertilization and nitrogen doses applied in the summer.

Kind of fertilization	Nitrogen dose [$kg \cdot ha^{-1}$]	Years of study			Mean
		2003	2004	2005	
Mulitiple fertilizers (Polifoska)	50	54.7	60.9	75.2	63.6
	80	59.1	61.9	67.8	62.9
	110	55.7	63.0	69.8	62.8
Mixture of fertilizers	50	51.0	63.0	80.9	65.0
	80	49.2	58.5	70.9	59.5
	110	53.2	58.4	74.9	62.2
Mean		53.8	60.9	73.2	62.6

LSD $P \leq 0.05$ for:
Years of study - 7.4;
Interaction of kind of fertilization and nitrogen dose - 2.2;
Interaction of kind of fertilization and nitrogen dose and study years - 5.9.

Data showed no significant differences in soluble carbohydrate content (table 2). It should be noted that there were significant differences in the content of this component in animal feed harvested in successive years of research. Significantly higher content of soluble carbohydrates was characterized the yield harvested in 2005 (73.2 g kg^{-1}D.M.) On the contrary, the lowest concentration of this nutrient has been found in the plant material from the first year of the study (53.8 g kg^{-1}D.M.). Increasing doses of nitrogen fertilization in summer 2005 was a factor reducing the quantity of soluble carbohydrates in the harvested yield, while in the remaining years of research, such impact wasn’t stated.

Nutrients contained in feed fulfil many important functions in the body of animals must not only cover their living needs, but also placed the higher production requirements. Ruminants for the right conduct of life processes require energy, which must be taken from a given feed.

Synthetic evaluation of nutritive value of feed from different research objects (tab.3) shows higher values of net energy of lactation in the yield from plants fertilized with a mixture of fertilizers, which in terms of composition contain large quantities of mine ballast.

Table 3: Fodder value of 1 kg D.M. of meadow sward in depend on the kind of fertilization and nitrogen dose (mean for three years).

Kind of fertilization	Nitrogen dose $[kg \cdot ha^{-1}]$	Energia netto laktacji (NEL) $[MJ \cdot kg^{-1} D.M.]$	Digestability (%)
Mulitiple fertilizers (Polifoska)	50	5.42	61.76
	80	5.57	63.04
	110	5.56	63.07
Mixture of fertilizers	50	5.71	64.26
	80	5.58	63.58
	110	5.55	63.15
Mean		5.57	63.14

An important indicator of quality assessment of feed, is the digestibility which, according to Pres (1997) in the feed given to cattle should be about 65-67%. Analyzed plant material did not full that standards, that the most similar value (64.26) was obtained on the objects fertilized with a mixture of mineral fertilizer in spring and the lowest dose of nitrogen in the summer. For compare the most alien value (61.76) was recorded at the same dose of nitrogen used in the summer but with the compound fertilizers applied in the spring. Estimation of nutritive value of feed from different research objects indicates, that the highest quality parameters had the plant material from object fertilizer with the mixture of fertilizer used in spring and the lowest dose of nitrogen applied in the summer. While the lowest yield quality parameters of tested yield were stated for using the same dose of nitrogen applied in the summer and multiple fertilizers used in spring.

Acknowledgements

The introduction of multiple fertilizers into model of permanent grassland fertilization significantly worse protein parameters of harvested feed.

Application of higher doses of nitrogen fertilization in the summer not always positively influenced the accumulation of total protein, but decreased levels of soluble carbohydrates.

Increasing doses of nitrogen fertilization during the summer didn’t improved the quality of forage harvested yield on the objects with the using of multiple fertilizers applied in the spring and was the best by using of mixture fertilizers and the lowest dose of nitrogen.

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EFFECT OF DIFFERENT FERTILIZERS ON YIELD AND QUALITY OF NECTARINES IN AN ORCHARD OF BASILICATA REGION (SOUTH OF ITALY)

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Abstract

In a three-year experimental field, which started in the spring of 2006, the efficacy of traditional and controlled release NPK fertilizers has been evaluated improving yield and quality characteristics of nectarines, var. Big Top. The fertilizers were yearly distributed, at 2 different doses (80 and 120 kg ha^{-1}), at start of blossoming. At the end of the experiment the results indicated statistical differences between all treatments and the untreated control for these parameters: spad index, leaf N, solid soluble content, circumference and weight fruit, yield per tree (with increases in confront to the untreated, respectively, of 8.4 - 16.9 - 11.0 - 8.9 - 26.1 and 34.8%). No differences were assessed between traditional and controlled release fertilizers. Moreover fertilizer top dose did not obtain improved results respect to inferior dose, indicating the low dose as the most advantageous.

Keywords: peach, mineral nutrition, controlled release fertilizer.

Introduction

Fertilizer management in orchards should tend to improve the N use efficiency for a sustainable agriculture with advantages for productivity, fruit quality and environment. The use of specific nitrification inhibitors and polymer coated controlled release fertilizers could increase the N fertilizer uptake and decrease the potential groundwater pollution by nitrate leaching. The application of controlled release fertilizers look promising for widespread use in agriculture because they can be designed to release nutrients in a more controlled manner by manipulating properties of polymer coating. However, rather than relying on a standard application rate each year, growers should monitor the nutrient status of trees with a leaf-sampling program (Scott and Uriu, 1989). Mineral nutrition is a pre-harvest factor that affects fruit quality and has to be performed very carefully, since peach quality after harvest cannot be improved, but only maintained (Crisosto *et al.*, 1997). Saenz *et al.* (1997) proposed that N stimulated increases in peach yields extending fruit development period and increasing fruit sink capacity. Arora *et al.* (1999) concluded that tree height, trunk circumference, flowering intensity, fruit set, fruit weight and fruit yield were directly associated with leaf N content, however, there is a lack of these information, especially in Mediterranean condition.

The aim of the present research was to assess the effect, on peach yield and quality parameters, of dose and application frequency of controlled release NPK fertilizers.

Materials and methods

The research was conducted during a period of 3 years (from the 9th to the 11th year of the productive life of trees) in a peach orchard, nectarine cv. Big Top, of a farm located in Metaponto (MT), south of Italy. The soil of the orchard, at 0-30 cm, was characterized by these chemical parameters: clay 30.4 %, silt 37.0 % and sand 32.6 %, pH 8.55, organic substance 1.80 %, N 1.10 g/kg, phosphorus 10.0 mg kg^{-1} and exchangeable potassium 536 mg kg^{-1}. The trees were planted at distances 6 × 4 m and trained as a typical vase shape. The orchard was watered with drip irrigation (200-250 m^3 ha^{-1}) with a weekly timetable. The three NPK fertilizers were: a conventional one, a polymer coated controlled release and a DMPP nitrification inhibitor (respectively Nitrophoska® Perfekt, Nitrophoska® Top15 and Entec® Perfekt). The fertilizers were yearly distributed, at 2 different doses (80 and 120 kg ha^{-1}), at start of blossoming, while 50% of Entec® Perfekt thesis was distributed also at post harvest period. In the discussion of the results it will be considered the total nitrogen fertilization applied in the triennial period as reported in the next table.

Table 1: Nitrogen amounts of fertilizers that were applied to the trees in the triennial period.

Treatments	Product name	Nitrogen fertilization (kg ha^{-1})			
		2006	2007	2008	Total
T1	Untreated	-	-	-	-
T2	Nitrophoska® Perfekt	80	80	80	240
T3	(15-5-20)	120	120	120	360
T4	Entec® Perfekt (14-7-17)	80	80	80	240
T5	with DMPP nitrification inhibitors	120	120	120	360
T6	Nitrophoska® Top15 (15-10-15)	80	80	80	240
T7	with 30% Poligen coated	120	120	120	360
T8	Entec® Perfekt (14-7-17) with DMPP nitrification inhibitors	- 60	60 60	60 -	240

The measurements were performed on plant nutritional status (spad chlorophyll index, N leaf), on fruit quality parameters (soluble solid content, acidity, fruit circumference and weight) and yield (kg and number of fruits per tree). The adopted experimental design was a randomized complete block with 4 replications of 8 treatments.

Differences between means were evaluated using Duncan's multiple range test at P=0.05.

Results and Discussion

In the table 2 are reported the effects of fertilizer applications on plant nutritional status, fruit quality parameters and yield. Results indicated, after a tree trial years, statistical differences between all treatments and the untreated control for several parameters. Particularly the nutritional status was strictly correlated with the N dose. Higher spad index and leaf N contents were assessed with the top fertilizer dose. Leaf N was deficient in the untreated plots, in fact the nitrogen levels should be maintained between 2.6 and 3.0 percent N for peaches and nectarines (Scott and Uriu, 1989).

Considering fruit quality parameters, the weight and dimension, followed by solid soluble content, were principally different between fertilizer and untreated plots. Moreover the number of fruits per tree was higher in the treated plots, respect to the untreated, determining, together with the fruit weight, a considerable higher yield in the fertilizer plots. Moreover no differences were assessed between traditional and controlled release fertilizers.

Table 2: Effect of treatments on plant nutritional status, fruit quality parameters and yield (leaves collected at 9/7/08 and fruits at 16/6/08).

Treatment and total N applied	Untreated	Nitrophoska® perfekt		Entec® perfekt		Nitrophoska® Top15		Entec® perfekt
	0	240	360	240	360	240	360	240
Spad index	38.90 b	41.45 a	42.85 a	41.40 a	42.78 a	41.78 a	42.63 a	42.33 a
Leaf N (%)	2.44 c	2.76 ab	2.92 ab	2.75 ab	2.91 ab	2.70 b	2.98 a	2.94 ab
SSC (°Brix)	8.55c	9.25abc	8.65bc	9.53abc	9.63abc	10.15a	9.55abc	9.70ab
Acidity (meq/100g)	6.38ns	6.46ns	7.36ns	7.19ns	6.71ns	7.34ns	6.74ns	7.26ns
Circumference (cm)	18.45b	20.49a	20.02a	20.00a	19.98a	20.02a	19.96a	20.12a
Fruit weight (g)	110.5b	140.9a	142.7a	138.7a	137.7a	138.9a	134.9a	141.7a
Fruits per tree (n)	203.2b	225.1a	217.8ab	213.3ab	223.5ab	208.9ab	220.9ab	210.3ab
Yield (kg/tree)	22.5b	31.7a	31.1a	29.5a	30.8a	29.0a	29.8a	29.8a

For each line, different letters indicate significant differences for P=0.05 according to Duncan Multiple Range test. n.s.= not significant.

In the next figure is reported the increase effect of N dose respect to the untreated on plant nutritional status, fruit quality parameters and yield. Results indicated that the fertilizer top dose, without considering traditional and controlled release fertilizer types, did not obtain improved results respect to the inferior one, indicating that, in the condition adopted in the trial, about 192 g per tree is the nitrogen yearly dose adequate and satisfactory for the peach orchard tested. In fact spad index, leaf N, solid soluble content, circumference, fruit weight and yield per tree meanly increased, in confront to the untreated, respectively of about 8.4 - 16.9 - 11.0 - 8.9 - 26.1 and 34.8 %.

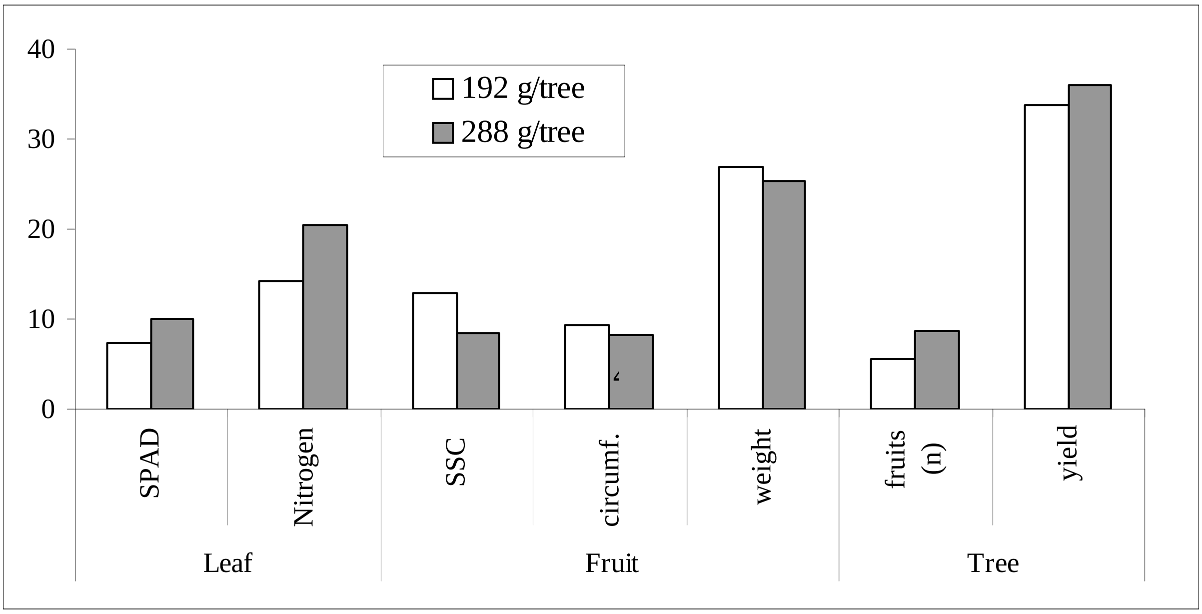

Figure 1: increases effect of N dose respect to the untreated on plant nutritional status, fruit quality parameters and yield.

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ASSESSMENT OF MUNICIPAL SOLID WASTE COMPOST AND SEWAGE SLUDGE APPLICATION USING WHEAT (*TRITICUM DURUM*) ANTIOXIDANT RESPONSE

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Abstract

The efficiency of composted municipal solid waste and sewage sludge to promote wheat (*Triticum durum*) growth was investigated under greenhouse conditions. Plants were cultivated under 0, 40, 100, 200, and 300 t ha^{-1} of MSW compost or S. sludge. Dry weight, heavy metals accumulation, and leaf antioxidant activities (ascorbate peroxidase APX, glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD)) were determined. Plant showed a significant improvement of biomass production at 40 and especially 100 t ha^{-1} of compost amendment (48 and 78% respectively). However under S. sludge treatment the increase did not exceeded 18% at the two doses 40 and 100 t ha^{-1}. At higher amendment doses plants exhibited increasing heavy metal concentrations in shoot and root, correlated with yield decline. In addition, antioxidant activities showed a proportional stimulation with 200 and 300 t ha^{-1} of compost, and with 200 and 300 t ha^{-1} of S. sludge (APX: 26, 24, 24, and 20%; GR: 90, 97, 82 and 100% CAT: 31, 51, 36, and 40%; SOD: 30, 41, 37 and 36% respectively). The study reveals that, in greenhouse experiment, 100 t ha^{-1} of MSW compost seems to be the optimal dose for wheat growth without adverse effects.

Keywords: Antioxidant activities, Compost, Heavy metals, ROS, Sewage sludge, Wheat.

Introduction

In Mediterranean regions, the majority of agricultural soils are poor in organic matter. As a result, it is often necessary to increase the soil humus content, essentially submissive to the intensive cultures, by incorporation of organic amendments (Ben Achiba *et al.*, 2009). Animal manures sewage sludge and composts have been investigated for their effectiveness in soil remediation and plant yield improvement. However, low quality or irrational input of several wastes can cause environmental concern. It can contain excessive concentrations of metals that may be accumulated in the soil almost indefinitely, and may present a long-term problem for plant growth and human health (Lin *et al.* 2007). The continuous input of wastes containing toxic metals on the agricultural land has caused imbalance in ecosystems. Metals such as iron, zinc and manganese are essential as micronutrients for many cellular processes, at higher concentrations their ions act as efficient generators of reactive oxygen species (ROS) (Van Assche and Clijsters, 1990).

ROS are extremely cytotoxic and can seriously disrupt normal metabolism through oxidative damage to lipids, nucleic acids and proteins. In response, the capacity of the antioxidant defence system is increased for protection against ROS playing an important role in detoxification of toxic oxygen species induced by metal ions (Halliwell and Gutteridge, 1993). Efficient destruction of ROS requires the action of several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidise (APX), glutathione reductase (GR) (Gomez-Junior *et al.*, 2006).

Although quite a lot of investigations have been carried out for compost and S. sludge as soil amendments, limited work had been done regarding the beneficial limit of such biosolids. This study aimed at determining the antioxidant response of wheat cultivated in increasing concentrations of MSW compost and S. sludge.

Material and Methods

Wheat seeds (*Triticum durum* L. var. Karim) were sown in soil filled pots (20 cm diameter and 30 cm height) according to the following treatments. **C**: soil, **C1**: soil+MSW compost 40 t ha^{-1}, **S1**: soil+S. sludge 40 t ha^{-1}, **C2**: soil+MSW compost 100 t ha^{-1}, **S2**: soil+S. sludge 100 t ha^{-1}, **C3**: soil+MSW compost 200 t ha^{-1} , **S3**: soil+S. sludge 200 t ha^{-1}**,** **C4**: soil+MSW compost 300 t ha^{-1}, **S4**: soil+S. sludge 300 t ha^{-1}.

Plants were grown in controlled conditions and irrigated twice a week at 2/3 field capacity. In pre-flowering stage (45 day) plant were collected for biochemical analysis.

Table1: Physico-chemical characteristics of MSW compost, S. Sludge, and soil. EC: electric conductivity, N: Nitrogen, C: carbon, Heavy metal (Zn^{2+}, Cu^{2+}, Pb^{2+}, and Ni^{2+}). (Means ± standard deviation calculated on 3 replications basis).

	MSW Compost	**S. sludge**	**Soil**
pH	8.8±0.08	7.07±2.0	7.95±1.0
EC (µS)	400±3	1001±50	263±8
N%	0.11±0.01	3.87±0.1	1.50±0.5
C%	13±2.0	27.24±3.0	1.2±0.5
Zn (µg g^{-1})	290.3±28.34	592.7±21.77	70.0±10.0
Cu (µg g^{-1})	195.2±7.56	284.0±2.35	32.0±5.0
Pb (µg g^{-1})	25.9±1.2	101.7±3.0	-
Ni (µg g^{-1})	192.5±19.27	270.4±10.94	50.0±6.0

Leaves were ground to a fine powder with a mortar and pestle under liquid nitrogen. The proteins were then extracted at 4°C by grinding with a cold 50 mM potassium phosphate (pH 7.0) buffer containing 0.1% (w/v) ascorbic acid, 0.1% (v/v) Triton X-100 and 1% (w/v) polyvinylpolypyrrolidone (PVPP). The homogenate was centrifuged at 4°C for 20 min at 12,000*xg*. The clear supernatant fraction was used for the enzyme assays. Protein content was determined as described by Bradford (1976). All procedures were performed at 4°C as detailed by Iannelli *et al.* (2002). The principle of Catalase (EC1.11.1.6; CAT) measurement method is based on the hydrolysis of H_2O_2 and decreasing absorbance at 240 nm (Chance and Maehly, 1955). Glutathione reductase (EC 1.6.4.2; GR) activity was assayed spectrophotometrically following the formation of thiobenzoic acid (TNB) at 412 nm (Smith *et al.*, 1988). ,Total ascorbate peroxidase (EC 1.11.1.11; APX) activity was determined by measuring the oxidation rate of ascorbate at 290 nm according to Asada (1992). Total superoxide dismutase (EC 1.15.1.1; SOD) activity was assayed according to McCord and Fridovich (1969). The concentration of reduced Glutathione (GSH) was determined with an enzyme-recycling assay spectrophotometrically at 412 nm referring to Griffith (1980).

In addition, shoot and root samples were dried at 60°C until constant weight, ground with a porcelain grinder. Samples were digested in 1/2 (v/v) HNO_3/H_2SO_4 mixture and Ni^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+} concentrations were determined by atomic absorption spectrometry (Perkin-Elmer Analyzer).

The statistical analysis was achieved using the SPSS 13.0 software. Data were subjected to One-Way ANOVA test and means were compared using Duncan's Multiple Range Test at 5% significance level.

Results and Discussion

Compost application improved significantly wheat growth mainly in C1 and C2 treatments (+48% and +78% as compared to the control, respectively). This result is in agreement with several findings, pointing out the positive impact of MSW compost input on plant growth. Data indicate also a significant improvement with S. sludge complements (especially in S1 and S2 treatments). High contents of organic matter and nutrients make S. sludge a good substrate for fertilization and reclamation of degraded soils (Selivanovskaya *et al.*, 2003; Oleszczuk, 2007).

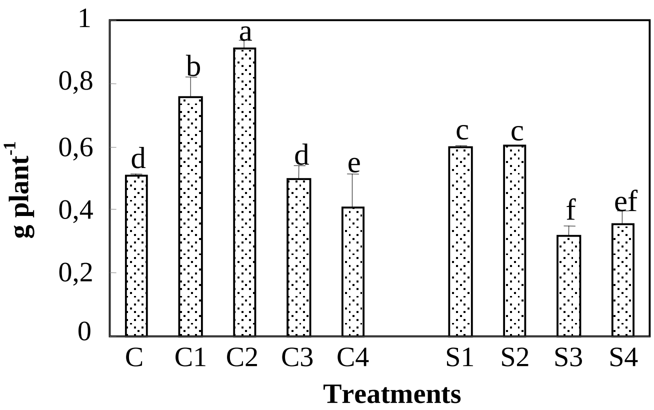

Figure 1. *Triticum durum* dry weight. Data are the mean of 4 replicates ± SE. Means followed by the same letters are not significantly different according to the Duncan's Multiple Range Test at P≤0.05.

In opposite, increasing dose of compost or S. sludge (notably in C4, S3, and S4 treatments) led to a marked reduction of *T. durum* growth activity (-20%, -41%, and -48% of the control). This was concomitant with increasing heavy metal (Ni^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+}) contents in shoots and roots (table 2).

Table 2. Heavy metal shoots and roots concentrations expressed in µg g^{-1} (Sh : shoots, R : roots). Data are the mean of 4 replicates ± SE. Means followed by the same letters are not significantly different according to the Duncan's Multiple Range Test at P≤0.05.

	Ni^{2+}		**Pb^{2+}**		**Cu^{2+}**		**Zn^{2+}**	
	Sh	R	Sh	R	Sh	R	Sh	R
C	127.64 f	142.15 f	22,32 de	22,79 f	13.39 e	12.9 i	66.24 g	89.12 g
C1	148.26 e	165.33e	22,29 cde	22,29 f	41.20 cd	70.04 g	81.93 f	101.15 f
C2	167.15 cd	170.49e	24,51abc	27,95 e	45.72 bcd	90.98 f	87.21e	100.99 f
C3	179.24 bc	180.20d	27,47ab	31,05 cd	54.89 abc	106.31 e	91.58 d	109.82 e
C4	85.11ab	188.34d	30,10 a	32,93 bc	64.77 a	112.85 d	114.16 a	123.54 d
S1	50.17 e	163.82e	20,92 e	23,03 f	39.97 d	49.61h	89.38d e	91.96 g
S2	61.08 d	203.5c	22,02 de	28,90 de	48.72 bcd	76.40 c	91.18 d	148.64 c
S3	73.69 c	231.49b	25,34 bc	35,02 ab	58.88 ab	162.04 b	96.74 c	193.17 b
S4	88.63 a	250.40a	27,15 b	36,48 a	65.13 a	168.79 a	99.50 a	217.88 a

While, metal concentrations were significantly higher in roots compared to shoots, which is in conformity with previous findings (Sinha, 1999; Lakhdar *et al.*, 2008). Such distribution of metals between the plant organs is a common strategy to protect the photosynthesizing tissues from the heavy metal toxicity.

On the other hand, plant antioxidant activities showed no significant variation at low doses of compost (C1 and C2) and S. (S1 and S2), suggesting no oxidative stress was generated by low metal concentrations. In opposite, leaf APX, GR, CAT and SOD activities were significantly enhanced in concomitance with the gradual increase of compost or S. sludge dose in the soil (Table 3).

Table 3. Ascorbate peroxidase APX (µmol AsA min^{-1} mg^{-1} protein), Glutathione reductase GR (µmol mg^{-1} protein min^{-1}), Catalase CAT (µmol H_2O_2 min^{-1} mg^{-1} protein), Superoxide dismutase SOD (U mg^{-1} protein) activities and reduced glutathione content GSH (µmol mg^{-1} protein min^{-1}) in *T. durum* shoots. Data are the mean of 4 replicates ± SE. Means followed by the same letters are not significantly different according to the Duncan's Multiple Range Test at P≤0.05.

	APX	**GR**	**CAT**	**SOD**	**GSH**
C	0.564 bc	2.770 d	0.152 fg	33.805 c	1.698 c
C1	0.615 abc	2.826 cd	0.140 g	38.399 c	1.789 c
C2	0.512 c	3.612 b	0.179 de	39.388 bc	1.821c
C3	0.716 a	5.282 a	0.201 bcd	44.279 ab	1.976 b
C4	0.703 ab	5.479 a	0.231a	47.854 a	2.393 ab
S1	0.524 c	3.434 b	0.172 ef	38.953 bc	1.756 c
S2	0.538 c	3.327 bc	0.186 cde	37.759 c	1.872 c
S3	0.701 ab	5.057 a	0.208 bc	46.397 a	1.954 ab
S4	0.683 ab	5.547 a	0.214 ab	46.031 a	2.832 a

The highest increases were recorded in C3, C4, S3 and S4 treatments. The increased antioxidant activities might be circumstantial evidence to support the hypothesis that heavy metal accumulated (Table 2) at supra-optimal doses of MSW compost and S. sludge could cause the formation of ROS. In fact, many metals cannot participate in biological redox reactions, but there exists evidence that it perform oxidative related disturbances (Sandalio *et al.*, 2001). Hence, ROS production may lead to alterations in the electron transport chain and damages to the thylakoidal membranes. Similarly, Lin *et al.* (2007) observed a strong stimulation of antioxidant activities parallel to the drastic increase of free radicals in wheat exposed to high level of heavy metals. Data showed that SOD activity was mainly stimulated in C3, C4, S3 and S4 (Table 3). This enzyme constitutes the first line of defense against O_2^- by rapidly converting O_2^- to O_2 and H_2O_2 (Alscher *et al.*, 2002). H_2O_2 is a very strong oxidant and requires quick removal, which is achieved by the action of APX in ascorbate-glutathione cycle (Mishra *et al.*, 2006). GR and CAT activities responded more effectively to metal-induced oxidative stress (Table 3). GR and CAT activities were also significantly stimulated (+30% and +17%, respectively) in C2, which represents the optimal dose for wheat growth. In contrast, the beneficial effect of both amendments was suppressed in C3, C4, S3 and S4, despite the strong increase in antioxidant activities (e.g. up to +100% for GR and up to +51% for CAT). This could be due to the failure of antioxidant system to scavenge the ROS generation with increasing heavy metal concentration.

In conclusion, positive effect of compost amendment on wheat growth was more pronounced than S. sludge at moderate doses (40-100 t ha^{-1}). The increasing doses of both biosolids caused an increase of heavy metals concentration in plant shoots and roots. As a response, plant antioxidant system was significantly activated (particularly, SOD, CAT, APX and GR activities) to scavenge ROS generated and mitigate metal stress. However, at higher doses (>100 t ha^{-1}) both MSW compost and S. sludge caused a detrimental impact on plant growth.

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EFFECTS OF DIFFERENT RATES AND FORMS OF N SLOW RELEASE FERTILIZER ON YIELDS OF FENNEL (*PHOENICULUM VULGARE L.*), N BALANCE AND N USE EFFICIENCY

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Abstract

The research was carried out in the experimental farm of Scafati (SA) and started on September 30, 2008 with transplantation of fennel, cv "Aurelio". Two experimental factors were applied: 1. rates of nitrogen: 175 and 122 kg ha^{-1}; 2. forms of N distributed: a) fractioned distribution of ammonium nitrate (N-Split); b) N slow release fertilizer with nitrification inhibitor 3,4 DMPP (Entec); c) N slow release fertilizer with organic complexes (Rhizovit); d) N slow release fertilizer with methilenurea (Record 55). Yield characteristics were recorded at harvest and nitrogen use efficiency indicators and N surplus were calculated. The 122 kg ha^{-1} rate yielded like the higher one (36.6 vs 37.3 t ha^{-1} respectively) and showed a better apparent recovery fraction of N and a lower N surplus. Yields of fennel were not influenced by the forms of N distribution but in N-split treatment plants used N-fertilizer more efficiently than slow release fertilizers and there was the lowest N surplus.

Keywords: Apparent recovery fraction, N absorbed use efficiency, N surplus, fractioned fertilization.

Introduction

N fertilization efficiency depends on N-fertilizer rate and form. Applying low fertilizer amount will not allow a crop to satisfy its needs, whereas applying too much is wasteful, with increased risk for soil, water air (Burns, 2006). Slow release fertilizers can be useful tools in calibrating the availability of N-fertilizer upon plant demand and in reducing the risk of NO_3^- loss by leaching. However, the efficiency of the fertilizer is strongly dependent on its chemical characteristics and on the chemical-physical characteristics of the soil (Gioacchini *et al.*, 2006). The use of slow release N fertilizers could give advantages because of reduced number of fertilizations and, consequently, of labour cost savings. At present, there are not many experiments about the use of these fertilizers for vegetable crops (Minuto *et al.*, 2006;

Chatzoudis and Valkanas, 1995) whose short cycles and growth rates could not be suitably the slow release dynamics. So, an evaluation of the productive, economic and environmental efficiency of these fertilizers is advisable. The network “Centro Orticolo Campano” (constituted by CRA, Eureco S.p.A. and Regione Campania) performed some experiments in three locations with the aim of testing the effects of some N slow release fertilizers on vegetable crops yields, N use efficiency and N surplus in comparison with N fractioned fertilization.

Materials and methods

The experiment was carried out at the experimental farm of CRA–CAT of Scafati. Two experimental factors were tested. The first one was N rate: a) optimal rate of 175 kg ha^{-1} of N according to the Fertilization Manual edited by Regione Campania); b) N rate reduced of 30% compared with the optimal one (122 kg ha^{-1}). The second factor was the form of N fertilizer: a) N split fertilization with ammonium nitrate (N 34%) as control; b) Entec 25-15 (N 25%: 11% nitrate and 14% ammonium), slow release fertilizer with the nitrification inhibitor 3,4 DMPP; c) Rhizovit 2 granulare (N 20%: 12% ammonium and 8% urea), a selected extract of natural organic matters with three specific activator complexes; d) Record 55 (N 35%: 6.7% ammonium, 16.6% urea and 11.7% urea formaldehyde), a N fertilizer with methylenurea. A split plot design with three replications was adopted, laying down the rate of nitrogen to main plots and the form of N to the sub-plots. Every sub-plot measured 25 m^2.The main soil characteristics were: sand 43,8 %, silt 50,8 %, clay 5,4 %, bulk density 1.26 t m^{-3} , total $CaCO_3$ 40 g kg^{-1}, organic matter 19.1 g kg^{-1}, C.S.C. 21.2 meq 100^{-1}, pH (water 1:2 ratio) 8.0, EC 25 °C (1:2) 0.55 dS m^{-1}, total N 1,1 g kg^{-1}, C:N ratio 10.1, P_2O_5 110 mg kg^{-1}, exchangeable K_2O 848 mg kg^{-1}, exchangeable Ca 2405 mg kg^{-1}, exchangeable Mg 312 mg kg^{-1}. Total rainfall during the fennel cultivation cycle was 624 mm; the average monthly temperatures were 18 ^oC, 14 ^oC, 11 ^oC , 10 ^oC, 9 ^oC in October, November, December, January and February, respectively. Fennel, cv Aurelio, was transplanted on 30 September 2008 using a density of 7.1 pt m^{-2} (0.7 x 0.2 m) and the harvest began on 19 February 2009. Slow release fertilizers were distributed in pre-transplant and in top-dressing on 9 December 2008. In N-split fertilization, ammonium nitrate was distributed in pre-transplant and in two top-dressings on 9 December 2008 and 20 January 2009. In pre-transplant 90 kg ha^{-1} of P_2O_5 were distributed in all the fertilized plots. At harvest some parameters were measured on a test area of 8,8 m^2: number and medium weight of marketable heads and marketable yields. Five plants were sampled in every plot and dry matter of leaves and heads were measured; total and mineral N content of dry matter were determined. A soil sample (0-40 cm depth) was collected before the fertilizers distribution and after the harvest in order to analyze mineral N ($N-NO_3$ and $N-NH_4$). In plant samples, total N content was determined by Kjeldhal digestion and distillation method. In plant and soil samples, mineral N was determined by distillation and determination by spectrophotometer (AutoAnalyzer III). N surplus was calculated according to the following formula: (initial soil mineral N content + N fertilizer rate applied + N mineralized from organic matter) – (total above ground N uptake + post-harvest soil mineral N). Nitrogen mineralized from organic matter was calculated in unfertilized plots. To the N uptaken by plants of fennel was subtracted the difference between initial and post-harvest soil mineral N contents (Fagnano *et al.*, 2008). Apparent Recovery Fraction (ARF) and the use efficiency of absorbed N (N_aUE) were calculated as follows:

ARF (%) = $(U_f – U_0)/N_f$ where N_f = fertilizer-N rate (kg ha^{-1}), U_f = N uptake (kg ha^{-1}) when N_f is given and U_0= N uptake (kg ha^{-1}) in unfertilized plots **(**Greenwood *et al.*, 1989);

N_aUE on biomass dry matter= TDM / U_f (Delogu *et al.*, 1998);

N_aUE on marketable yield= Y/ U_f where TDM = total aboveground dry matter, Y= marketable yield (Tei *et al.*, 1999). Data have been analyzed by JMP software (v. 5, SAS, Cary, NC). A two-way Anova for a factorial design was adopted. Means were separated by Tukey HSD Test at P= 0,05.

Results and Discussion

As shown in Table 1, no significant difference between the N fertilizer rates 122 and 175 kg N ha^{-1} was found in number and average weight of heads and in marketable yield. Dry matter and total N uptake did not vary significantly between the two N-fertilizer rates so as N_aUE on total above ground dry matter and N_aUE on fresh marketable yield (tab. 2). However, the low N rate showed a significant higher N apparent recovery fraction than the 175 kg ha^{-1} rate (+ 34%). Consequently, N surplus using the 122 kg ha^{-1} rate was 62% lower than the optimal rate. In agree with Morra *et al.* (1992), an amount of nitrogen below 130 kg ha^{-1} matched fennel nutrient demand in a autumn-winter crop cycle. Further amounts of nitrogen did not increase yields , were not absorbed by plants and were, most likely, lost in groundwaters.

Table 1: Effects of N-rates on fennel yield.

N rate (kg ha^{-1})	Marketable heads (n m^{-2})	Medium weight of head (g)	Marketable yield (t ha^{-1})
122	6.2	595	36.6
175	6.2	605	37.3
Significance[1]	n.s.	n.s.	n.s.

[1]: ** = significant at p< 0.01; *= significant at p<0.05; n.s. = not significant.

Table 2: Effects of N rate on total N uptake, Apparent Recovery of N-fertilizer (ARF), N balance, Use efficiency of absorbed N (NUE) on total above-ground dry matter (DM) and on marketable yield fresh weight (MY FW).

N rate (kg ha^{-1})	Total DW (t ha^{-1})	Total N uptake (kg ha^{-1})	ARF (%)	N_aU E on total DM (t kg^{-1})	N_aU E on MY FW (t kg^{-1})	N balance (kg ha^{-1})
122	42,9	128	55	0,034 b	0,29	50
175	41	132	41	0,032 b	0,29	132
Significance[1]	n.s.	n.s.	*	n.s.	n.s.	n.s.

[1]: ** = significant at p< 0.01; *= significant at p<0.05; n.s. = not significant.

The N split fertilization determined the highest marketable fennel yield (tab. 3). Compared to this form of distribution, the slow release fertilizers appeared to be not significantly different in comparison to Entec and Record 55, while Rhizovit yielded the lowest amount due to the low medium weight of head.

Table 3: Effects of N-fertilizer forms on fennel yield.

Fertilizer	Marketable heads (n m^{-2})	Medium weight of head (g)	Marketable yield (t ha^{-1})
ENTEC	6.1 n.s.	599 n.s.	36.6 ab
RECORD	6.2	608	37.9 ab
RHIZOVIT	6.0	572	34.6 b
N-SPLIT	6.2	621	38.6 a

The values followed by different letters are significant different according to Tukey's test; HSD at P = 0.05; n.s.= not significant

In Table 4 we can observe that fennel crop uptook more nitrogen with N split distribution and Entec while nitrogen absorbed with Rhizovit was significantly lower than N-split. The use efficiency of absorbed nitrogen did not vary among treatments when referred to total above ground dry matter, while if referred to the marketable fresh weight yield, it was significantly higher with Record 55 and Rhizovit. However, apparent recovery fraction of N was 56% and 62 % with Entec and N-split, respectively, 38% and 36 % with Record 55 and Rhizovit. As a consequence, N balance pointed out a significant difference between the value (28 kg ha^{-1}) of N-split on one hand and the values around 100 kg ha^{-1} of the three slow release fertilizers. These data highlighted that during the autumn-winter season the nitrogen distributed with different form of slow release fertilizers was not completely at disposal of crop despite of an unchanged level of productivity. So, the final surplus recorded could represent a pool subjected to leaching to groundwaters. Interactions between the two factors studied were not significant in any of the variables analyzed.

Table 4: Effects of N-fertilizer forms on total N uptake, Apparent Recovery of N-fertilizer (ARF), N balance, Use efficiency of absorbed N (NUE) on total above-ground dry matter (DM) and on marketable yield fresh weight (MY FW).

Fertilizer	Total DW (t ha^{-1})	Total N uptake (kg ha^{-1})	ARF (%)	N_aU E on total DM (t kg^{-1})	N_aU E on MY FW (t kg^{-1})	N balance (kg ha^{-1})
ENTEC	4.4 n.s.	144 ab	56 ab	0.031 n.s.	0.26 b	99 a
RECORD	4.0	116 ab	38 b	0.034	0.33 a	112 a
RHIZOVIT	4.0	111 b	36 b	0.036	0.32 ab	126 a
SPLIT	4.5	149 a	62 a	0.030	0.26 b	28 b

The values followed by different letters are significant different according to Tukey HSD Test at P = 0,05; n.s.= not significant

In conclusion, the results pointed out that rate 122 kg ha^{-1} was better than 175 kg ha^{-1} for the higher N recovery fraction, associated to similar level of yields. N slow release fertilizers determined yields similar to the standard split fertilization but considering the whole plant-soil system, split fertilization left the lowest N surplus and showed the highest apparent recovery fraction. The higher N surplus recorded for slow release fertilizers is probably due to a crop cycle in the autumn-winter season which is characterized by low soil microbial activity and temperature and, consequently, low mineralization rate. In agree to Koivunen and Horwath (2005), on the basis of our data recorded on spring-summer crops (data not published), the use of the slow release fertilizers should be ideal in a Mediterranean climate with a long, warm growing season. The effect of slow release N fertilizers should be valued in long term and with a management system including more than one crop.

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MAGNETIC RESONANCE IMAGING FOR EVALUATING THE EFFECTS OF FERTILIZERS ON FOOD QUALITY

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Abstract

Effects of a new fertilizer containing bio-available silicon, in the form of stable monomeric orthosilicic acid, on fruits and vegetables were investigated by means of Magnetic Resonance Imaging (MRI). Comparison of T_2–weighted and spin density-weighted MRI images has highlighted the variations of the internal morphology, in terms of cellular tissues arrangement and their wateriness. Samples treated with orthosilicic acid led to the formation of tissues with higher consistency, characterized by a longer shelf-life. Studies took into account strawberry, kiwifruit, fennels and tomatoes, and results were for all of them in agreement.

Keywords: MRI, fertilizers, food quality.

Introduction

In the last few years the use of fertilizers to improve fruits and vegetables quality and production has become a common practice. Several analytical techniques are used for foodstuff quality evaluation and for determining the chemical effects of the fertilizers. The most used are those based on chromatography (HPLC or GC), eventually coupled with mass spectrometry (MS) or tandem mass spectrometry (MS/MS)[1, 2, 3]. However, they have the disadvantage of a time demanding sample preparation, and the results assess only the chemical composition aspects, without, in most cases, any hint about the physical condition of the measured sample.

In the last years Magnetic Resonance Imaging (MRI), known mainly for its medical and diagnostic applications, has received general acceptance in food science thanks to its non-invasiveness and non-destructiveness. It produces high resolution spatial images of any internal section or volume of samples, providing information about the internal structures, understanding the importance and function of each tissue and measuring quality-related parameters. The opportunity of studying foodstuff in its wholeness, without any chemical and/or physical preparation of the sample, make MRI a powerful tool for the entire food science research. In the present work we proposed the use of Magnetic Resonance Imaging to investigate the effects of fertilizers on the morphological arrangement and changes occurring during storage period.

Materials and methods

Samples of strawberry, kiwifruit, fennel and tomato were taken into account for the present study. For each foodstuff, control and treated with bio-available silicon samples were considered. MRI images were acquired using a Bruker AVANCE spectrometer (Bruker Biospin, Milan, Italy) operating at a ^{1}H frequency equal to 300.13 MHz and equipped with cylindrical birdcage single-tuned nucleus (^{1}H) coil probehead with an inner diameter of 60.0 mm. Water signal was monitored and used for the image reconstruction. Gradient-Echo (GEFI) and Multi-Slice-Multi-Echo (MSME) experiments, were performed according to standard procedures. In GEFI images the signal intensity is directly proportional only to the water content, thus dark zones are relatively poor in water, while brighter areas contain more. In MSME images the signal intensity is proportional to the transverse relaxation time, i.e. T_2, which in turn is in inverse relation to the correlation time, τ_c. The latter is defined as the average time taken by the molecule to rotate of a radiant about the axis; therefore, MRI images obtained with MSME experiment show dark areas when the water molecule rotate slowly, indicative of intense interaction with the cellular substrate, while in case of weaker interactions with the vegetable matrix MRI images appear brighter in colour.

Parameters were optimized for each sample. Spin-spin relaxation times were calculated by means of Paravision 3.6 software (Bruker Biospin, Milan, Italy), multi-exponential functions with pre-exponential factors were used, according to equation:

$$A(t) = \sum_{i=1}^{n} A_i \exp^{-(t/T_{2i})}$$

where A(t) is the echo amplitude at time t, $T_{2(i)}$ and $A_{(i)}$ are the spin–spin relaxation time and amplitude, respectively, of component *i*. T_2-weighted and spin-density-weighted MRI images were analysed by ImageJ 1.41 software, which allows the greyscale analysis. Images intensities were scaled to an arbitrary scale from 0 to 255; the first limit corresponding to full black and the second one to complete white colours. Therefore parameterised images considered only grey colour intensity and were used to create histograms and pixels' distributions according to intensities.

Results and Discussion

Figure 1 reports the MRI images of the axial section of control (left panel) and a treated with orthosilicic acid (right panel) tomato, obtained with a GEFI experiment, which shows in detail the internal structure of tomatoes, seed size and their distribution within the placental cavities and the septum between the two; also the pericarp and the endocarp are clearly visible. In the control sample a darker zone in the centre of the pericarp can be observed; this is poor in water and is more evident than in the treated sample. From the morphological parameters it's evident that this area is wider in all control tomatoes analysed.

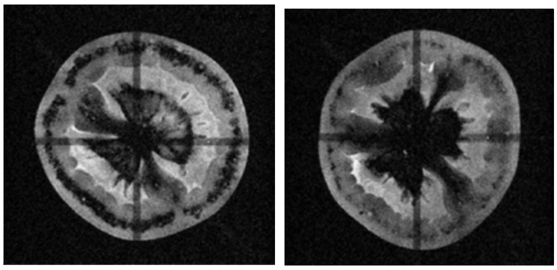

Figure 1: GEFI axial images of a control and a treated with bio-available silicon tomato, left and right respectively.

The same result is evident in the MSME images (data not reported), in which all untreated samples show a concentric darker area appear in the pericarp; this corresponds to a less ripe and fibrous zone, in which water has a strong interactions with cellular tissues and so has a low mobility.

A major concern for fresh strawberries commercialization is storage period, so that we have investigated the effect of bio-available silicon on the shelf-life. In untreated strawberries we found that GEFI images become brighter (data not shown) with increasing storage time., which corresponds to a water release in the different tissues, and became evident after seven day of storage. For strawberries treated with bio-available silicon the same occurs but with four days delay. The same results have been found by analyzing the MSME images, which are reported in Figure 2. Control fruits show damaged tissues and mould appearance after seven days of storage, while in treated strawberries the first hints of deterioration appeared after eleven day.

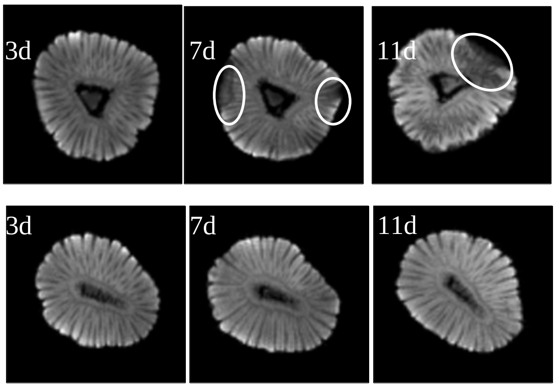

Figure 2: MSME axial images of a control (on the upper side) and a treated with orthosilicic acid (on the lower side) strawberry at different storage times: from the left three, seven and eleven days.

Also T_2 values confirm that the treatment with bio-available silicon slow down the tissues damaging process: in fact, T_2 of untreated strawberries increase with storage time, while for treated fruits, instead, T_2 remains the same until the seventh day of storage and increases after eleven. The treatment with the fertilizer probably slow down the tissues consistency lost process, which is responsible for a longer shelf-life.

To confirm the effect of the fertilizer on the shelf-life, the same measurements performed on strawberry were repeated on fennels. The GEFI images (data not reported) show no significant difference between control and treated samples at the first storage day. Increasing storage period, we observed a consistent change of untreated fennels, which we attributed to the water loss phenomenon. The evapo-transpiration process is evident also from the grayscale analysis of the MSME images reported in Figure 3. In untreated samples the images become brighter (increase in signal intensity) with storage time and the intensity of the pixel is higher than treated ones. This is indicative of a faster evapo-transpiration process in untreated fennels. The difference between the two are due to zones in the sample with low T_2 values (low pixel intensity), in which water is strongly bounded to cellular tissues and so has a low mobility.

Finally we have investigated the effects of the new fertilizer based on bio-available silicon on the shelf-life of kiwifruits. From MRI images obtained with GEFI experiments, Figure 4 left panel, one can see the internal morphology of the fruit: there is a central cavity, poor in water, and various concentric areas with a different water content. One can also easily recognize the seeds and their distribution. Fruits were measured at harvesting and 90 days later.

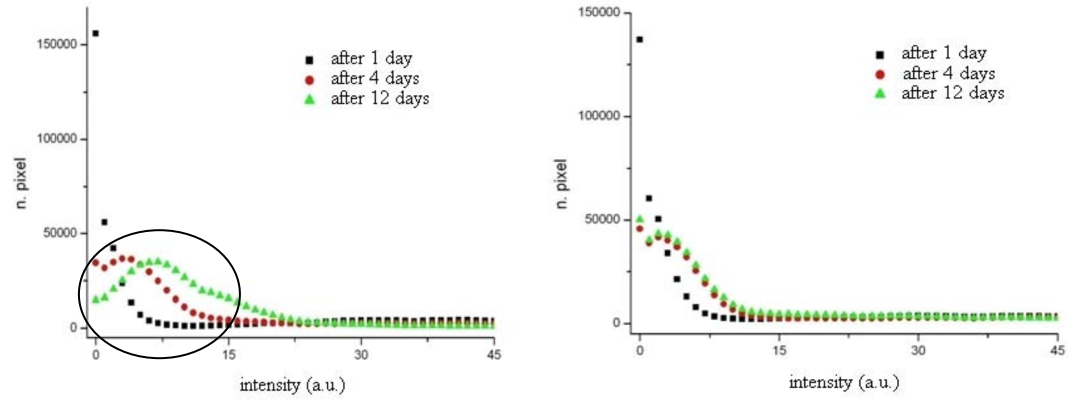

Figure 3: Grayscale analysis of the MSME images of a control (on the right) and a treated with orthosilicic acid (on the left) fennel at different storage times.

At harvesting, both GEFI and MSME images (data not reported) show no significant difference between control and treated fruits. After 90 days storage large variation have been found in the GEFI images, Figure 4 left panel. Treated kiwifruits maintain their internal structure, with the various concentric areas and their different water content still visible. In the control samples, instead, internal tissues structure is progressively lost, with a water release, corresponding to a brighter MRI image.

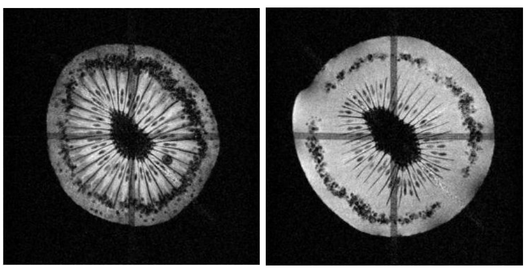

Figure 4, left panel: GEFI images of a treated (on the left) and a control (on the right) fennel 90 days after harvesting.

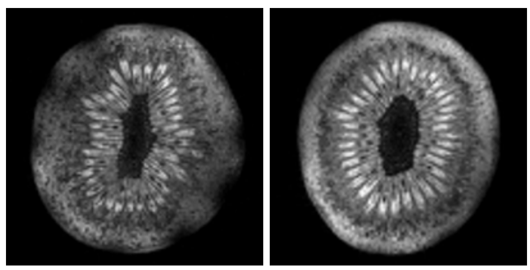

Figure 4, right panel: MSME images of a treated (on the left) and a control (on the right) fennel 90 days after harvesting.

Also MSME images show difference between the two samples at ninety days after harvesting: treated kiwifruits still have the different concentric areas, while the control samples appear with a more homogeneous internal structure, due to the lost of the tissue consistency. This is reflected in a relaxation times values increase: in both cases T_2 increases, but in the treated sample is less marked.

Acknowledgements

We thank Dr. Eugenio Babini for supplying the material for analysis and ILSA S.p.A. for financial support.

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LAYERED SILICATE NANOCOMPOSITES FOR CONTROLLED RELEASE OF NITROGEN FERTILIZER

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Abstract

In this study we considered the development of nanostructured materials capable of reducing the solubilization rate of nitrogen. Polymer composites granules were prepared with four different systems: (1) montmorillonite clay/fertilizer, (2) montmorillonite clay/fertilizer and plasticized starch, (3) montmorillonite clay/fertilizer, plasticized starch and low density polyethylene (LDPE) and (4) montmorillonite clay/fertilizer, plasticized starch and polycaprolactone. The formation of a nanostructured material was assessed by elemental analysis (CHN) and X-ray diffraction (XRD). The compositions were processed in a Haake rheocord mixer and granulated to sizes between 4 and 8 meshes. The kinetics of nitrogen release was measured by colorimetric enzymatic analysis through ultraviolet/visible spectroscopy. The kinetics of fertilizer release was also characterized by using soil columns. The results from enzymatic analysis showed that the nitrogen release from the clay/fertilizer system reached 100% after 60 min. For the montmorillonite clay/fertilizer and plasticized starch systems, 100% release was reached after 3 h. The best systems (LDPE and polycaprolactone) had similar behavior with immediate and prolonged release over 3 h. The results observed in the leachate of soil columns corroborate the nitrogen release trend as the clay/fertilizer system showed a potential for slow release, but much faster than the systems containing polymer. All composites evaluated showed to be potentially useful as controlled release fertilizers.

Keywords: controlled release, nanocomposites, fertilizer, montmorillonite clay.

Introduction

The inadequate control and excessive use of nitrogen and potassium fertilizers in important crops result in large economic and environment losses. The main loss mechanisms are leaching, erosion and, in the case of nitrogen in tropical soils the volatilization and denitrification of NH_3, leading to the formation of nitrous oxides NOx. The evolution of NOx causes serious environmental consequences, as they are active greenhouse gases. Moreover, the leached nutrients can be transported and can potentially contaminate surface and subsurface water sources [1].

The efficient use of nutrients by plants is therefore of extreme importance. The key point concerning efficiency is to have nutrients available the plants during periods of need, so that losses are minimizing. This can be achieved with the use of controlled release fertilizers, the so-called "smart fertilizers", which are capable of releasing the nutrients according to the plant needs during its growth [2,3].

According to Fabunmi et al, the intense search for the development of starch based biodegradable plastics has witnessed the use of different starches under many forms with the aim of achieving profitability and biodegradability [4]. However, most starch based materials form composites with poor performance as far as tensile strength, elasticity, stiffness, elongation at break, humidity resistance and stability are concerned. Nevertheless, for the use in fertilizers production the lack of good mechanical properties, is considered beneficial as degradation may be improved [4].

This work relates the preparation of polymer composites from plasticized starch with glycerin and polycaprolactone (a synthetic biodegradable polymer) and/or low density polyethylene (LDPE) to be used as a new controlled release fertilizer. Urea fertilizer was adsorbed on the montmorillonite clay interlayer, and the kinetics of the fertilizer release was investigated.

Materials and methods

The nitrogen fertilizer was adsorbed in the clay with different adsorption times, by keeping constant the fertilizer concentration for a given volume of solution and clay mass, at 30ºC.

The clay/fertilizer system was dried in an oven for seven days and ground. Clay particles containing the adsorbed fertilizer were evaluated by elemental analysis (CHN), and adsorption isotherms were monitored. From X-ray diffraction (XRD) analysis was used to evaluate the capacity of fertilizer adsorption in inlayered and basal spacing of the clay matrix. Four different systems were prepared, three of them were processed with polymers. The polymer composites were prepared on an intensive Haake mixer (rotor speed: 50 rpm; processing time:

10 min; temperatures of 140 ° C, 140 º C and 90 ° C respectively for the systems (2), (3) and (4). Systems: (1) montmorillonite clay/fertilizer (2), montmorillonite clay/ plasticized starch and fertilizer, (3) montmorillonite clay / fertilizer, plasticized starch and low density polyethylene (LDPE) and (4) montmorillonite clay / fertilizer, plasticized starch and polycaprolactone. The mixtures were then ground and sieved between 4 and 8 meshes. The kinetics of nitrogen release was measured by enzymatic colorimetric analysis and ultraviolet/ visible spectroscope. Kinetics was also evaluated by using soil columns from which the maximum potential release of ammonia to the surrounding liquid was evaluated. The experimental units (soil columns) were built from a 30 cm piece of poly(vinyl chloride) (PVC) tube with a diameter equal to 7.5 cm. A completely randomized experimental design (3 x 4 x 3 + 1 factorial with 3 replications) was carried out, in which the following variables were considered: (a) as nitrogen fertilizer: commercial urea; system clay/nitrogen fertilizer; system clay/nitrogen fertilizer/ plasticized starch/LDPE; (b) the incorporation form: incorporated to 5 cm, and (c) pH soil 6.5. This procedure resulted in 13 treatments with 3 replicates, totaling 38 experimental units. For comparison purposes a control sample was introduced with no fertilizer. After temperature equilibrium has been achieved, 2 mL of urease solution (1 μL) and 110 mL of water were added to each column, the three fertilizing systems (commercial urea, clay/nitrogen fertilizer and clay/nitrogen fertilizer/plasticized starch/LDPE) were introduced in the soil columns in quantities equivalent to 50, 100, 200 and 400 kg/ha. Samples of the leachate solutions were at first collected every 2 days for 12 days, and then every 3 days, for 9 days more. The samples were stored in a freezer (-7 ºC) and analyzed as for nitrate by ion chromatography (DX-120 Ion Cromatograph).

Results and Discussion

Fertilizer adsorption in interlayered clay took place giving rise to nanostructured material; from the carbon content in the clay matrices, determined by CHN, the adsorption curve (Figure 1) was obtained. From the latter one can see that the total nitrogen percentage increased by increasing the concentration of nitrogen fertilizer up to a maximum of ca. 30% - 35%.

Clay/fertilizer was dispersed in the molten polymer on a Haake torque rheometer. From X-ray diffraction analysis, obtained before and after the fertilizer incorporation in the clay, intercalation and formation of nanostructured material occurred. The sodium montmorillonite in the anhydrous form used was characterized by an inter-planar distance of ca. 12 Å, and increased up to 17 Å after adsorption, thus confirming the diffusion of urea in the interlayered clay. The crystalline layers in the network are linked by weak van der Waals interactions, thus allowing water, polar organic compounds, and salts penetrate these structures by expanding them [5].

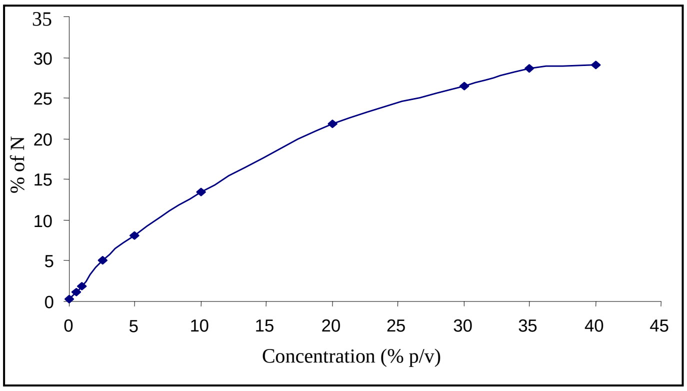
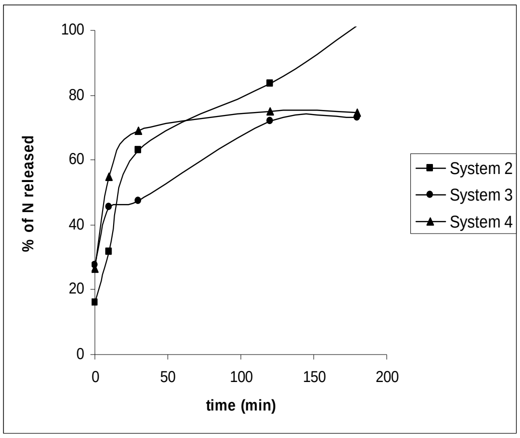


Figure 1: Dependence of Nitrogen fertilizer adsorption with urea concentration.

The results from the enzymatic colorimetric analysis reported in Figure 2 show that the nitrogen release in the clay/fertilizer system reached 100% after one hour of contact with water. Figure 3 reports the results for systems (2), (3) and (4) in long lasting (up to 3 h) release experiments. The results indicate that the hydrophilicity of starch in system (2) nitrogen release was complete after 3 hours. The best results were obtained with the systems (3) and (4), which presented similar behavior, a prolonged release, and after 3 hours N release was still below 70% of total N.



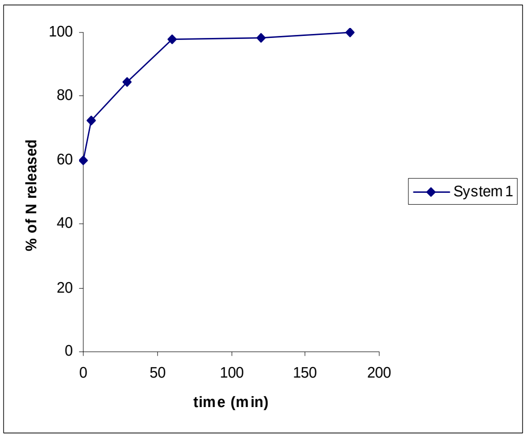


Figure 2: System 1 nitrogen released. **Figure 3:** N released for (2), (3) and (4).

The results shown in Figure 4 (a), cumulative losses of N by nitrate leaching under laboratory conditions, indicate that at 50 kg ha^{-1} dose, which is considered a low fertilizer dosage, there was no significant difference between the control and fertilizers systems containing clay and clay with polymer. However, the commercial urea showed superior results up to the 4th sampling (16th day). As for the urea/clay system, the increase in the N leaching after the 5th sampling (24th day) could be attributed to fertilizer adsorption in the clay inlayer.

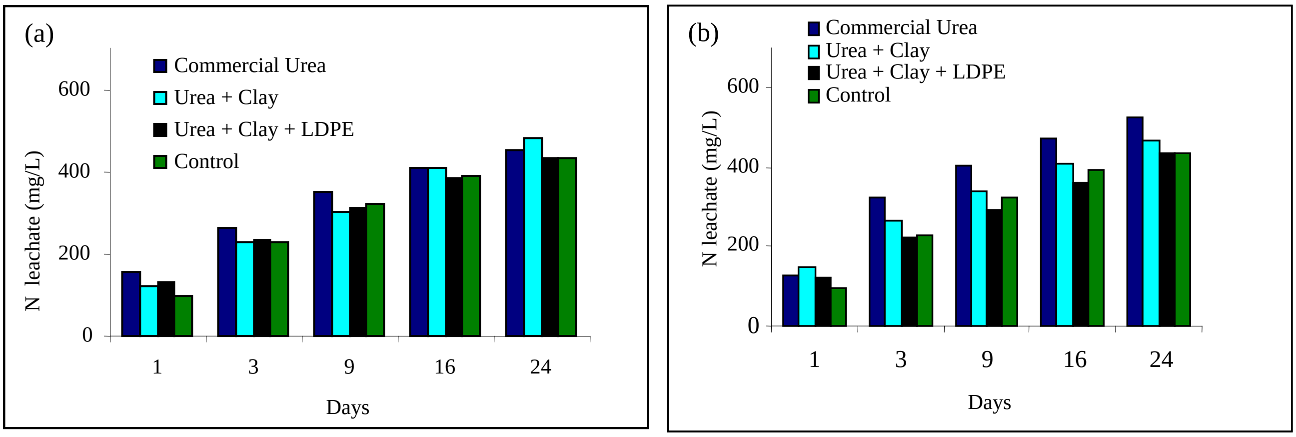


Figure 4: Kinetics of leaching of N in soil columns at doses of (a) 50; (b) 100 kg ha^{-1}.

When dosage is increased to 100 kg ha^{-1}, Figure 4 (b), the fertilizer is adsorbed on the inlayered clay causing a slower release of urea/clay system, as shown in. For 200 kg ha^{-1} dosage, Figure 5, (a) that commercial urea presented the largest N loss.

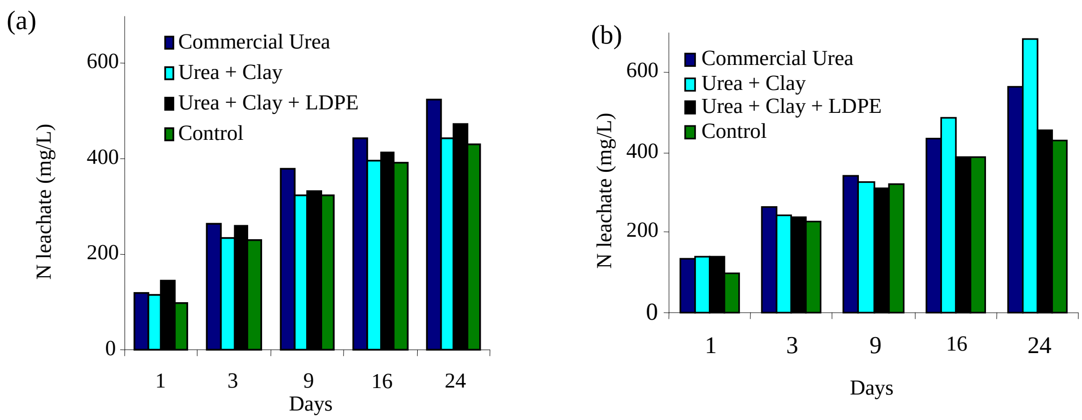


Figure 5: Kinetics of leaching in soil columns at doses (a) 200; and (b) 400 kg ha^{-1}.

At a dose of 400 kg ha^{-1}, Figure 5 (b), the mixture containing polymer had a similar pattern to control sample, while the urea/clay mixture showed a large nitrate loss from the 4th sampling on (16th days). The nitrate losses resulting from the treatments with commercial urea were always significantly higher than those observed for urea adsorbed on the clay or mixed with polymer. The results suggest that the considered materials reduced the nitrogen release rate from urea to the environment. Nanostructured materials based only on clay/fertilizer could be considered a slow release system, since nitrogen release is slower than the conventional system containing only urea. The clay/fertilizer nanocomposites containing either hydrophobic polymers, i.e. LDPE, or hydrophilic ones, i.e. containing plasticized starch or polycaprolactone, led to slower nitrogen releases, even more than 360 min. It can be concluded that all investigated materials are potentially useful for application in the controlled N release from fertilizers.

Acknowledgments

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IV SESSION

Fertilization and environmental quality

INFLUENCE OF SULPHUR FERTILISATION ON THE FLORAL SCENT OF FLOWERING CROPS

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Abstract

Regularly, macroscopic sulphur (S) deficiency can be found in oilseed rape, which causes unique flower symptoms with changes in colour from bright to pale yellow and in shape and size of the petals. In addition, the scent, which is a strong signal for honeybees, is influenced by S deficiency. It is unknown if other flowering crops react to S deficiency with a change in scent, too. It was the aim of the presented study to examine the influence of S nutrition on the release of volatiles during blooming. Crops with (white mustard, oil radish) and without S-containing secondary compounds (chamomile, field beans, and peas) were grown on plots with and without S fertilisation. The S status was monitored and the scent of flowers was characterised 3-times at main flowering by employing an electronic nose. The S content in vegetative plant parts increased with S fertilisation for all crops. Significant differences in scent were determined in relation to S fertilisation at least at one sampling date for all investigated crops except white mustard.

Keywords: chamomile, e-nose, *Faba* beans, floral scent, mustard, oil radish, peas, sulphur.

Introduction

Sulphur (S) deficiency causes a multitude of changes in flowering oilseed rape crops as for instance a change in colour of the flowers, often together with modifications in size and shape of the petals (Schnug and Haneklaus, 1994). Besides these phenological changes S deficiency causes also a change in scent during flowering, which affects the attractiveness of the field for honeybees and other flower-visiting insects (Haneklaus *et al.*, 2007; 2009). Honeybees are attracted by scent, colour and form of the honey-bearing plants, but it is the scent, which is the fastest and strongest signal for pollen and nectar (Menzel *et al.*, 1993). Changes of flowers and scent in relation to S fertilisation have been described for oilseed rape (Schnug and Haneklaus, 1994; Haneklaus *et al.*, 2007). The question remains open, if the scent of other flowering crops is also affected by the S nutritional status.

In glucosinolate containing crops such as *Brassica rapa* nitriles and isothiocyanates, which are degradation products from glucosinolates were found in the floral scent in large quantities (Omura *et al.*, 1999). Previous studies have shown that the S supply increases the glucosinolate content in vegetative plant parts, seeds and petals of oilseed rape (Schnug, 1993). Therefore it is intrinsic that the S nutritional status directly affects the composition of the scent of glucosinolate containing crops such as *Brassica*. Shu and Park (1999) found a positive relationship between the S nutrition and the essential oil content of basil and it was shown that a graded nitrogen supply increased the content of sugars and volatile ingredients in carrots (Schaller, 1999).

Traditionally e-nose systems have been used to distinguish differences in the aroma and the quality of a product like vegetables and fruits (Broda, 2000), wine (Martin *et al.*, 2008), beer, tobacco and coffee. The system is also used in industry for quality purposes like product control. Laothawornkitkul *et al.* (2008) demonstrated the potential use of e-nose technology as a real-time pest and disease monitoring system in agriculture. Therefore e-nose systems can discriminate a wide range of different scents and are suitable for very different research purposes.

It was the aim of the present study to determine the influence of S fertilisation on the floral scent of different crops and to investigate if plants differ in the release of volatiles during flowering.

Materials and Methods

In 2008, a field trial was conducted at the experimental station of the Julius Kühn-Institute (JKI) in Braunschweig (E 10°27´, N 52°18´). The climate is temperate and characterised by frequent changes in temperature, humidity, and winds. The soil type is a Cambisol with a loamy sand soil texture (6.5% clay; 47% sand), characterised by low water retention capacity and high rates of leaching. The soil pH value was 5.5.

Five different crops (white mustard (*Sinapis alba*), oil radish (*Raphanus sativus var. oleiformis*), chamomile (*Matricaria chamomilla*), field beans (*Vicia faba*) and peas (*Pisum sativum*)) were grown in plots each with a size of 288 m^2. Plots were split in half and were fertilised with 100 kg S ha^{-1} as ammonium sulphate nitrate (+ S) which was applied in two equal doses of 50 kg ha^{-1} at sowing and at the start of the main growth. The other half of the plot received no S (- S). N was fertilised at the following rates: white mustard and oil radish: 100 kg ha^{-1}; chamomile, field beans and peas: 30 kg ha^{-1}.

Leaf samples were taken at the beginning of the main vegetative growth in fourfold repitition and leaf samples as well as samples of the flowers were analysed for total S via high temperature combustion method at 1150°C for complete S recovery with subsequent gas analysis for CNS employing the *Elementar Vario Max CNS* equipment. During full blooming in total 4 separate samples (flowers) were taken in each plot on three different days within at minimum 3 days (peas and chamomile) and at maximum 17 days (oil radish) whereby climatic conditions were similar at the sampling dates. Volatiles in the headspace of the flowers were analyzed by means of an e-nose with a sensor system consisting of 32x conductive polymer sensors (CPS; AromaScan® A32/50S). The measurement was conducted with 7 flowers of peas, beans and chamomile, respectively, and 10 flowers of mustard and oil radish, which were directly collected into bottles (22 mL capacity), which were provided by the manufacturer, and which were closed gas-tightly. The measurement was conducted at 40 °C and after each measurement the system was rinsed with synthetic air to clean the sensors. Data analysis was based on the values of maximum change in resistance ($\Delta R)/R_0$ of the sensors, which were interpreted by principal component analysis (PCA), using software supplied by the manufacturer.

For statistical analysis the ANOVA procedure was used to analyse the results and the means were compared by the Tukey test at 5% probability level.

Results and Discussion

S fertilisation increased distinctly the S content of leaves and flowers (Table 1). These differences proved to be statistically significant in leaf tissue of oil radish and chamomile and in the flowers of all crops except pea.

Table 1: Sulphur content in leaves and flowers of different crops in relation to S fertilisation at the beginning of flowering.

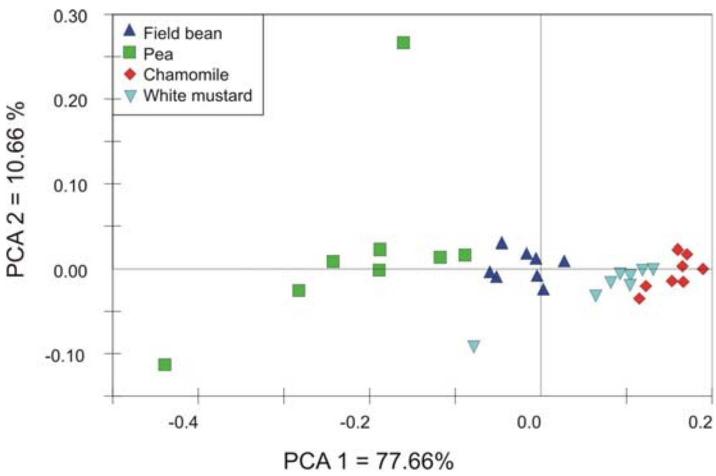
Crop	Leaves [mg S g^{-1} d.w.]			Flowers [mg S g^{-1} d.w.]		
	- S	+ S	*LSD$_{5\%}$*	- S	+ S	*LSD$_{5\%}$*
White mustard (*Sinapis alba*)	6.8	8.6	*1.95*	13.6	15.4	***0.81***
Oil radish (*Raphanus sativus var. oleiformis*)	7.4	9.3	***1.51***	16.8	18.8	***1.45***
Chamomile *(Matricaria chamomilla)*	4.3	6.1	***0.99***	3.8	4.1	***0.33***
Field beans (*Vicia faba*)	4.6	4.6	*0.49*	2.6	2.9	***0.12***
Pea (*Pisum sativum*)	3.7	4.1	*0.70*	4.6	4.6	*0.30*

Bold numbers indicate statistically significant differences

The results of the e-nose measurements revealed significant differences in scent between crops and in relation to the S supply (Fig. 1 and 2). All flowers in figure 1 were sampled and measured on the same day as climatic factors such as air temperature have a strong effect on the results of the e-nose measurement (Paillan-Legue, 1987).

Principal component analysis (PCA) of the sensor data was performed and the calculation of so-called quality factors revealed statistically significant differences. Groups are significantly different from each other when quality factors (QF) higher than 2 were determined.

The data (Fig. 1) reveal that beans and peas as well as beans and chamomile can be discriminated by their scent as well as peas and chamomile and peas and mustard. In comparison it was not possible to discriminate mustard and beans and mustard and chamomile by their scent on that particular day. This result suggests that plants synthesising secondary S-containing metabolites such as mustard do not necessarily show a different pattern in scent, which can be detected by e-nose. Interestingly to note is the fact that it was possible to discriminate the scent of the two legume crops. Similar results were determined for different pairs of crops at the other sampling dates.



2 dimensional PCA map		
Class	Class	QF
Bean	Pea	2.66
Bean	Chamomile	5.25
Bean	Mustard	1.98
Pea	Chamomile	5.30
Pea	Mustard	2.78
Chamomile	Mustard	1.66

Figure 1: Two-dimensional PCA plot for headspace volatiles from flowers of peas, beans, chamomile and mustard sampled on 27th June 2008.

In Fig. 2 the influence of S fertilisation on the release of volatiles is shown for oil radish and chamomile at one sampling date. These crops were selected exemplary because of their significant response to S fertilisation (Tab. 1). The results clearly reveal that S fertilisation and the S nutritional status of the crop significantly influenced the release of volatiles by flowers.

A weak, however, significant linear correlation was found between the total S content in flowers (X) of oil radish and the resistance (dR/R) of all 32 sensors (Y = 0.405X – 0.13;

r^2=66.3 % for the medium resistance of all 32 sensors), which indicates that the intensity of the scent was related to the S status of the plant.

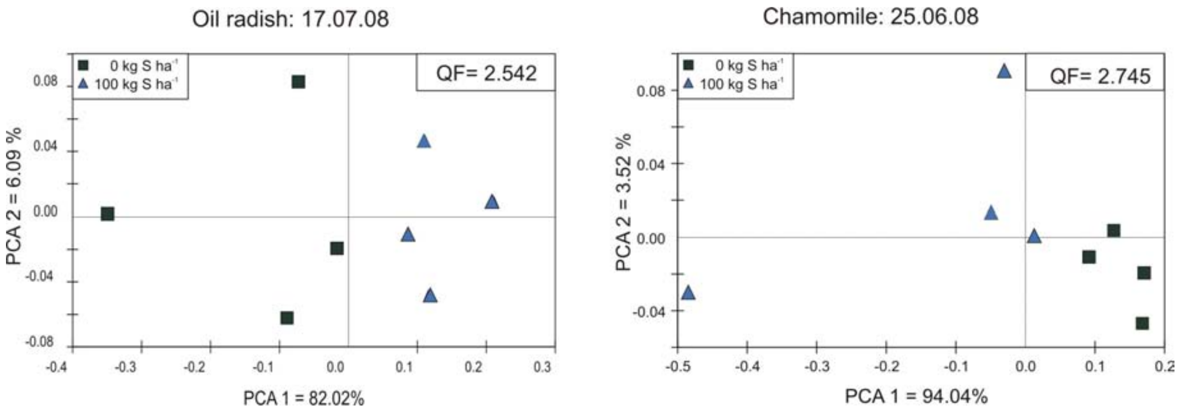


Figure 2: Two-dimensional PCA plot for headspace volatiles from oil radish and chamomile flowers in relation to S application.

Global radiation and temperature are the most important climatic factors influencing the scent of crops. Though sampling dates with similar climatic conditions have been chosen in the experiment the quality factors varied strongly. Thus it can be concluded that single measurements might not reveal basic differences between crops and in relation to S fertilisation.

The significance of changes in the floral scent for instances for pollination by flower visiting, beneficial insects such as honeybees, but also pests remain open. For oilseed rape it was concluded that morphological and physiological changes caused by severe S deficiency resulted in a drastically reduced attractiveness for honeybees (Haneklaus et al, 2007). Haneklaus *et al.* (2009) further demonstrated that S and nitrogen fertilisation influenced the infestation of winter oilseed rape with insects; obviously a higher S supply favoured specialist insects most likely because of increasing glucosinolate contents. In the presented field trial a higher abundance of butterflies of the genus *Pieris* was observed in plots of white mustard and oil radish, which received S fertilisation. Finally it can be speculated if S fertilisation might favour pollination by modifying the floral scent irrespective of the S demand of the crop.

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INFLUENCE OF TILLAGE, CROP ROTATION AND NITROGEN FERTILIZATION ON SOIL FERTILITY

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Abstract

For understanding how production systems can be better managed to sustain long-term soil productivity especially in semiarid climates, a long-term experiment was established at Foggia (Apulia, southern Italy) on a silty loamy soil to evaluate the effects of tillage, crop rotation and nitrogen fertilization on soil fertility over a period of 17 years. Data from this study showed, a longer period may have been required for differences between treatments to be observed. The tillage method did not influence the organic carbon, total nitrogen and available phosphorous contents of the soil, nor did crop rotations. Generally, the nitrogen fertilization at dose of 50 kg N ha^{-1} did not raise the total nitrogen content of the soil. When N was applied at rate 50 kg N ha^{-1}, surface soil (0-20 cm) organic carbon was either equal or slightly greater to that where no nitrogen was applied (0 kg N ha^{-1}). Relative increscent in total soil nitrogen throughout the soil depth (20-40 cm) was evaluated at the high nitrogen rates (100 kg N ha^{-1}) at all locations.

Keywords: Soil fertility. Tillage. Crop rotation. Nitrogen fertilization.

Introduction

Maintaining satisfactory soil organic matter (SOM) level is important to all the three aspects of soil quality, physical, chemical and biological. The levels of SOM can be managed through crop rotation, tillage regime, fertilizer practices and other soil management strategies (Reicosky *et al.*, 1995). Crop rotations usually increase SOM content, when compared with monocultures (Havlin *et al.*, 1990). Nitrogen fertilization has been shown to increase the level of SOC within cropping systems (Bowman and Halvorson, 1998) however; few studies have reported the effects of N rate on SOC accumulation. Halvorson *et al.*, (1999) showed that managing no-tillage cropping systems for optimum yield with adequate N fertility will have positive environmental impacts and that N fertilization will enhance SOC accumulation and soil fertility. Generally, there is a trend towards a stratification of SOC at the surface under no-tillage, without any effect on lower horizons (Mrabet, 2002). Bessam and Mrabet, (2003) reported that no-tillage soil has sequestered 3.5 and 3.4 t ha^{-1} of SOC more than conventional tillage in the 0-200 mm horizon, after 4 and 11 years, respectively. Reduced tillage is also effective in improving aggregate stability and decreased soil mechanical impedance (Lal *et al.*, 1994). The objective of this study was to determine the effect of crop rotation, tillage types and nitrogen fertilization on soil quality and fertility that occurred during 17 years in a long term study in Apulia region, southern Italy.

Materials and methods

Site description

The experiment was established in 1990 on soil has a silty loamy texture at Foggia in Apulia region. Apulia region is located in the south-east of the Italian peninsula, being one of the most important agricultural areas of the country. The climate is mainly of Mediterranean semi-arid type, characterized by hot and dry summer and moderately cold and rainy winter season.

Treatments and experimental design

In this study, tillage treatments included conventional (CT), two layers (TLT), surface (ST) and minimum tillage (MT) were combined with nitrogen fertilizer rates as 0, 50 and 100 kg N ha^{-1}. The four tillage systems were assigned as main plots and different crops (durum wheat, sugar beet, broad been, faba been, triticale, etc.) have been alternated in different two-year rotation as sub plots, and evaluated in a spilt plot design with three replications. Soil samples were taken from each plot (in year 1990 and 2007) from 0-20 and 20-40 cm depth. In the laboratory these soil samples were air dried, crushed, mixed thoroughly and passed through a 2-mm sieve. Analyses were carried out following internationally recommended procedures and the Italian official methods (Italy, 1999). Soil pH was determined by a glass electrode in distilled water (pH_{H2O}) and in 1 M KCl (pH_{KCl}) suspensions at 1:2.5 soil to liquid ratio. Electrical conductivity (E.C.) was measured in a filtrate from 1:2 soil to water ratio using a conductimeter. Texture and particle size distribution were determined by pipette method after dispersion of the soil sample in sodium hexametaphosphate and sodium carbonate solution. Total organic carbon was analyzed by Walkely-Black method and soil organic matter (SOM) content calculated multiplying TOC by 1.72 factor. Available phosphorus (Pava) was determined by UV-Vis spectrophotometry after extraction with sodium bicarbonate/sodium hydroxide, according to Olsen method. Average soil physical and chemical properties of the experimental site before initiation of the study in 1990 are given in Table 1.

Table 1: The average values of the analyzed physical and chemical soil properties before initiation of the study.

Depth	Sand	Silt	Clay	Texture	Bulk density	pH_{H2O}	pH_{KCl}	EC	P_{ava}	SOM	N_{tot}
cm	%				gm cm^{-3}	1:2.5		ds m^{-1}	mg kg^{-1}	g kg^{-1}	
0-20	12.8	55.5	31.8	Silty loamy	1.17	8.8	7.4	0.13	45.2	13.8	2.9
20-40	10.9	50.8	38.2		1.19	8.5	7.1	0.11	38.6	12.5	2.5

Results and discussion

Effects on soil physical properties

The effect of tillage, crop rotation and nitrogen fertilization on soil physical properties seems to be quite small (Table 2). Generally, non-significant effect of used tillage treatments and crop rotation on both texture and soil bulk density had been noted.

Table 2: The average values of the analyzed physical and chemical soil properties in 2007.

N rates	Tillage	Depth	Sand	Silt	Clay	Texture	Bulk density	pH_{H2O}	pH_{KCl}	EC	P_{ava}	SOM	N_{tot}
		cm	%				gm cm^{-3}	1:2.5		ds m^{-1}	mg kg^{-1}	g kg^{-1}	
0 kg N ha^{-1}	CT	0-20	11.6	55.0	33.4	Silty loamy	1.19	9.0	7.4	0.13	34.3	14.8	2.8
		20-40	10.2	54.6	35.2		1.20	8.8	7.1	0.11	31.6	11.5	2.4
	TLT	0-20	14.4	52.0	33.6		1.18	9.2	7.4	0.10	31.7	15.2	3.3
		20-40	13.5	50.6	35.9		1.20	8.8	7.1	0.09	29.8	14.5	2.9
	ST	0-20	15.0	58.3	26.7		1.17	9.1	7.4	0.15	39.5	13.4	2.4
		20-40	13.1	54.3	32.6		1.16	8.8	7.2	0.12	40.6	10.3	2.3
	MT	0-20	18.2	55.2	26.6		1.14	8.7	7.1	0.13	36.2	14.8	2.7
		20-40	15.4	58.7	25.9		1.16	8.6	6.9	0.11	38.5	10.6	2.4
50 kg N ha^{-1}	CT	0-20	10.2	56.8	33.0	Silty loamy	1.18	8.4	6.9	0.14	36.4	15.2	3.0
		20-40	11.7	53.6	34.7		1.18	8.6	6.9	0.12	38.2	11.3	2.6
	TLT	0-20	15.1	55.5	29.4		1.17	9.1	7.4	0.10	29.5	16.2	3.5
		20-40	11.9	55.0	33.1		1.20	8.8	7.2	0.10	32.4	15.4	3.1
	ST	0-20	18.3	55.4	27.3		1.16	8.5	6.9	0.13	38.9	14.1	2.8
		20-40	14.8	54.6	30.7		1.17	8.4	6.9	0.11	39.4	12.6	2.5
	MT	0-20	20.3	54.5	25.2		1.16	8.7	7.1	0.12	38.7	15.5	2.9
		20-40	12.9	59.5	27.7		1.18	8.9	7.2	0.09	38.2	13.8	2.6
100 kg N ha^{-1}	CT	0-20	11.2	52.9	36.0	Silty loamy	1.20	8.6	7.1	0.12	33.8	16.8	4.9
		20-40	11.7	53.5	34.8		1.22	8.5	6.9	0.12	35.2	14.5	5.7
	TLT	0-20	12.1	53.6	34.4		1.16	8.7	7.2	0.11	33.2	18.2	5.8
		20-40	11.7	55.8	32.5		1.18	8.6	7.1	0.09	30.4	17.2	6.2
	ST	0-20	14.4	59.2	26.4		1.16	8.8	7.2	0.12	36.7	16.4	5.9
		20-40	13.7	53.6	32.7		1.18	8.7	7.1	0.10	39.6	14.7	6.5
	MT	0-20	16.5	54.0	29.5		1.19	8.8	7.2	0.11	35.8	17.8	5.2
		20-40	11.9	57.5	30.6		1.20	8.6	7.1	0.10	37.6	15.6	6.8

CT, conventional tillage; TLT, two layers tillage; ST, surface tillage; MT, minimum tillage.

Table 2 showed that silty loamy texture is dominated as the initiation time (Table 1). Results did not show remarkable effects for used systems on the bulk density. The mean soil bulk density was 1.19 and 1.20 gm cm^{-3} at 0-20 cm and 20-40 cm soil depth for (CT), 1.17 and 1.19 gm cm^{-3} for (TLT), 1.16 and 1.17 gm cm^{-3} for (ST) and 1.16 and 1.18 gm cm^{-3} for (MT) tillage, respectively. Several studies (Unger, 1984; Hill, 1990) showed similar findings to the present research, that a non-significant effect of tillage treatments on soil bulk density had been confirmed. According to Blevins *et al.* (1977), different observations indicated that what compaction might occur was rapidly reversed by the mass of roots growing near the soil surface.

<u>Effects on soil chemical properties</u>

While used practices did not show considerable effects on soil physical properties, the effects on soil chemical properties were slightly apparent after the experiment duration. The tillage method did not influence the organic carbon, total nitrogen and available phosphorous contents of the soil, nor did crop rotations.

<u>Total Nitrogen</u>

Table 2 showed there was no significant difference between the used tillage systems. According to the nitrogen addition, the mean total nitrogen under the four tillage systems was 2.8 and 2.5 g kg^{-1} at 0-20 cm and 20-40 cm soil depth under for (0 kg N ha^{-1}), 2.3 and 2.7 g kg-1 for (50 kg N ha^{-1}) and 5.4 and 6.3 g kg-1 for (100 kg N ha^{-1}), respectively. Generally, the nitrogen fertilization at dose of 50 kg N ha^{-1}did not raise the total nitrogen content of the soil. Relative increscent in total soil nitrogen throughout the soil depth (20-40 cm) was evaluated at the high nitrogen rates (100 kg N ha^{-1}) at all locations.

<u>Available Phosphorus</u>

The results showed, mean available phosphorus concentration in the surface layers at the start of the experiment reduced in both used tillage systems. Lal (1997) reported that phosphorus increased with cultivation duration due to application of phosphate fertilizers.

<u>Soil organic matter</u>

Soil organic matter was increased on both tillage systems. The effect of tillage and nitrogen fertilization on soil organic matter in the surface 20 cm of the soil was remarkable. When N was applied at rate 50 kg N ha^{-1}, surface soil (0-20 cm) organic carbon was either equal or slightly greater to that where no nitrogen was applied (0 kg N ha^{-1}). Many authors reported that conservation tillage systems, especially no-till resulted in the accumulation of organic matter in the first few centimeters of the soil profile (Follet and Schimel, 1989; Karlen *et al.*, 1991). Similarly, the level of soil organic matter tends to rise in when the disturbance of the soil is reduced.

Acidity

Values of pH (Table 2) indicated that under the experiment condition, the effect of tillage, crop rotation and nitrogen fertilization on soil acidity appears to be negligible.

Conclusions

Data from this study showed, a longer period may have been required for differences between treatments to be observed. Application of tillage, crop rotation and nitrogen fertilization to silty loamy soil type at Foggia in Apulia region (southern Italy) for seventeen years, slightly improved organic matter content and N concentrations.

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LONG TERM EFFECTS OF DIFFERENT PRACTICES ON SOIL FERTILITY AND SOIL ORGANIC MATTER FRACTIONS IN ALMOND TREE CROPPING IN SOUTHERN ITALY

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Abstract

A long-term field trial was carried out in Bitetto-Bari, Apulia region (Southern Italy) to estimate the effect of soil treatments (herbicides applications, green manure and tillage management) on soil fertility and soil organic matter fractions. Results show that the use of green manure, adequate herbs management and soil tillage systems is crucial to conserve or increase soil fertility in term of organic matter and total nitrogen contents. Practices such as no-tillage with weeds cutting and minimum tillage with green manure caused a considerable increscent in soil organic carbon and total nitrogen comparing with the others treatments. Although there were no significant differences for soil available phosphorus among the used practices. The yields of HAs extracted from the applied treatments were significantly different. Treatment containing persistent organic compound such as broad been green manure increased the humification process in samples and produced higher yield of HA. Spectroscopic analyses revealed that treatments changed the functional groups, alkyl C, and E_4/E_6 of HAs.

Keywords: Soil fertility. Tillage. Weeds control, Green manure, Almond.

Introduction

Maintaining satisfactory soil organic matter **(**SOM) level is important to all the three aspects of soil quality, physical, chemical and biological. The levels of SOM can be managed through crop rotation, tillage regime, fertilizer practices and other soil management strategies (Reicosky *et al.*, 1995). Reduction of tillage intensity with residue management practiced for extended periods of time was shown to increase SOM content near the surface (Bayer *et al.*, 2000). Humic substances (HS) possess a considerable chemical reactivity through which they contribute to the properties and productivity of soils (Galantini and Rosell, 2006). Madari *et al.*, (1998) found higher relative aromaticity in HA from tilled soils than from no-till ones, probably as result of the more oxidative conditions in soils with continuous mechanical disturbance and consequently faster decomposition rate of the SOM, mainly the aliphatic part of the HA. Green manures are known to increase soil N and P availability for the following crop and at the same time, contribute to the conservation of soil organic matter and soil biological, physical and chemical properties (Mc Vay *et al.*, 1989). The objective of this study was to evaluate transitional effects over 30 years on soil quality resulting from different soil treatments included herbicides applications, green manure and tillage management.

Materials and methods

Five systems in a randomized block design was used with five replicates, in 147 m^2 unite plots with three almond plants (cv. Filippo Ceo) of the same age. The five systems and their respective approaches included: (A) no-tillage with chemical control pre-emergence weeds weeding (Bromacile, Propyzamide+Simazine, Dichlorenil+Tiobenzamide, Chlorprofam+ Diuron until 1999; then Glyphosate+Simazine and Oxadiazon+Glyphosate); (B) no-tillage with chemical drying and mulching of weeds (Paraquat); (C) no- tillage with weeds cutting and green mulching; (D) minimum tillage (rotary at 10 cm depth) with green manure of broad bean L. minor Beck); (E) conventional tillage (3-4 five-share plughing at 15 cm depth). The research was initiated in 1976 and has been going on with the same treatments, which are repeated every year so as to test their long-term effects. Soil samples were collected from each plot at the surface horizons (0 to 30cm depth) and analyzed. Analyses were carried out following internationally recommended procedures and the Italian official methods (Italy, 1999).

Results and discussion

There are no significant differences in the pH and EC values and in the total carbonate content of the five plots (Table 1). The content of organic C and total N, and the C/N ratio are higher in soil from Plot C with respect to the other treatments. The lowest organic C content is observed in plot A, possibly because of the limited growth of grass caused by chemical and mechanical treatments. Tables 2 and 3 showed, the HA yield is higher in plots C and D as a consequence of the higher SOM content. The HAs isolated from treatment A and E show higher contents of carboxyl groups, phenolic OH and free radicals, and lower E_4/E_6 spectroscopic ratios. The FTIR spectra of the five HAs (Fig. 1) are substantially similar to each other. In comparison with HA from plot E, the IR spectra of the other HAs show a relatively higher aliphatic character (CH stretching peaks at about 2922 and 2852 cm^{-1}) and polysaccharide-like components (peak at 1040-1041 cm^{-1}). The spectra of the HAs from plots A and E present also a relatively higher content of carbonyl and carboxyl groups (peaks at

Table 1: Relevant parameters measured on dry soils from the five experimental plots.

Parameter measured	Plot A	Plot B	Plot C	Plot D	Plot E
pH (H_2O)	7.8	7.9	7.8	7.7	7.9
pH (1N KCl)	6.8	6.8	6.8	6.9	6.9
EC[a] (µS cm^{-1})	178	183	187	221	187
Sand (g kg^{-1})	281	295	336	314	311
Silt (g kg^{-1})	262	282	319	332	302
Clay (g kg^{-1})	457	423	345	354	387
Total carbonate (g kg^{-1})	2.4	2.5	2.5	3.0	2.3
Organic C (g kg^{-1})	15.5	18.6	23.6	19.9	15.7
Organic matter (g kg^{-1})	26.8	32.0	40.7	34.4	27.1
Total N (g kg^{-1})	1.7	2.0	2.5	2.4	1.7
C/N ratio	9.1	9.3	9.4	8.3	9.3
Available P (g kg^{-1})	57	42	50	59	54

[a] EC: electrical conductivity

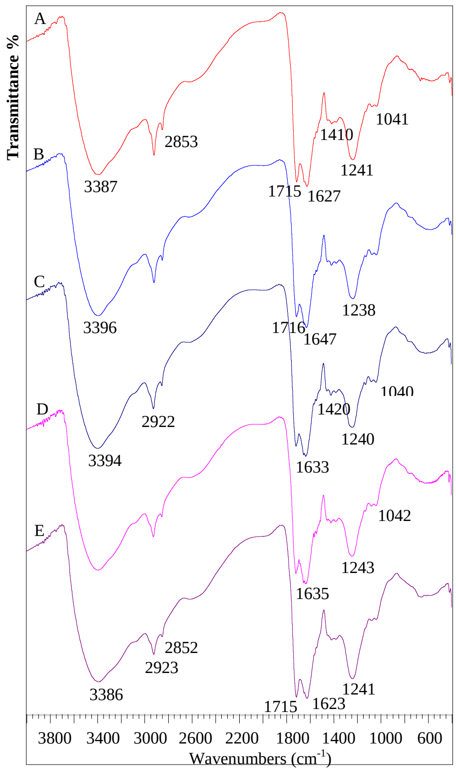

Figure 1: FTIR of the HAs isolated from the soils of the five experimental plots.

Table 2: Ash and acidic functional group contents, E_4/E_6 ratios and organic free radical concentration (FRC) of humic acids isolated from the soils of the five experimental plots.

Origin of humic acids	Ash	COOH	Phenolic OH	Total acidity	E_4/E_6	FRC
	%	mmol g^{-1}			ratio	spin g^{-1} (x 10^{17})
Plot A	1.1	3.9	9.0	12.9	5.0	2.4
Plot B	0.9	3.8	8.0	11.8	5.2	1.8
Plot C	2.4	3.8	8.3	12.1	5.4	2.1
Plot D	1.3	3.8	6.6	10.4	5.2	2.4
Plot E	1.3	4.1	8.6	12.7	5.0	2.7

Table 3: Yield, elemental composition (on moisture-and ash-free basis) and atomic ratios of humic acids isolated from the soils f the five experimental plots.

Origin of humic acids	Yield	C	H	N	S	O	C/N	C/H	O/C
	g kg^{-1}	g kg^{-1}					atomic ratios		
Plot A	0.74	56.4	5.6	4.2	0.3	33.5	15.5	0.8	0.4
Plot B	0.87	55.7	5.7	4.5	0.3	33.8	14.5	0.8	0.5
Plot C	1.07	55.9	5.8	4.4	0.2	33.7	14.9	0.8	0.4
Plot D	0.93	55.6	5.9	4.6	0.3	33.6	14.0	0.8	0.4
Plot E	0.60	57.0	5.6	4.2	0.2	33.0	16.0	0.8	0.4

1722 and at 1219 cm^{-1}) in comparison to the other HAs. These results are in agreement with the carboxyl group content measured by chemical analyses. The total luminescence spectra obtained as excitation-emission matrices (EEMs) (Fig. 2), show a higher number of fluorophores in the HA isolated from plot C in comparison to the other HAs. The fluorophores in this HA present the shortest excitation and emission wavelength, possibly as a consequence of the incorporation of fresh organic matter added. The HAs from the plots A and E present a lower number of fluorophores and fluorescence maxima at longer excitation and emission wavelength.

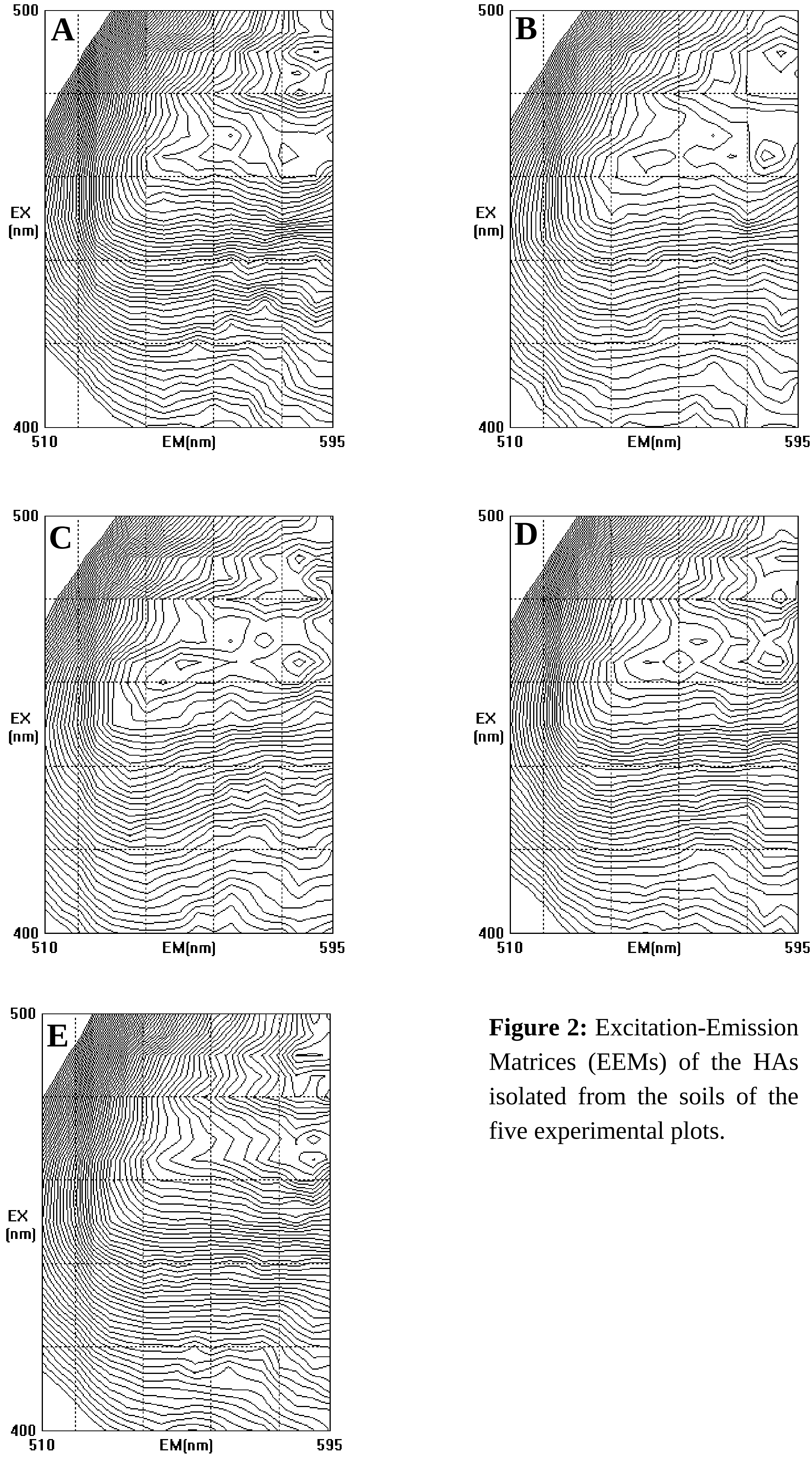

Figure 2: Excitation-Emission Matrices (EEMs) of the HAs isolated from the soils of the five experimental plots.

Conclusions

Results show a higher aliphatic and lower carboxylic content in the HAs from no- tillage with weeds cutting and green mulching and minimum tillage with green manure of broad bean that could be related to the higher content of fresh organic matter in these two plots as a consequence of the different management. In conclusion, management practices of zero tillage with weeds cutting and green mulching (plot C) produced a general increase of soil organic matter with respect to other treatments. However, the humification level of the organic materials results rather poor if compared with traditional tillage practices (plot E).

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NITROGEN AVAILABILITY FROM ORGANIC AND ORGANIC-MINERAL FERTILIZERS

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Abstract

Organic fertilizers represent an important source of nitrogen especially in organic cropping systems; besides organic fertilizers, organic-mineral fertilizers are often used in low-input systems to regenerate soil fertility and to increase the amount of nitrogen immediately available to the plants. However, the nitrogen release characteristics of fertilizers must meet the crop nitrogen requirements during the growing cycle in order to avoid nutrient deficiency or environmental pollution. Soil respiration rate and mineral N dynamic in different soil/organic or organic-mineral fertilizer blends were determined at 48 hours and at 2 weeks intervals of incubation, respectively. Both experiments were conducted at two incubation temperatures: 10 and 25°C. Rapid mineral nitrogen accumulation was observed from all fertilizers especially at 25°C during the initial 2 weeks. Natural organic fertilizers and organic-mineral fertilizers significantly increased soil respiration compared to urea especially at 25°C. Natural organic fertilizers showed a slow release of mineral N in the soil than urea; mineral nitrogen accumulation in soil fertilized with organic-mineral fertilizer containing N as NH_4 was similar to that of soil fertilized with urea while the application of organic-mineral fertilizer containing methyurea significantly delay the release of mineral N.

Introduction

Organic fertilizers represent an important source of nitrogen especially in organic cropping systems; besides organic fertilizers, organic-mineral fertilizers are often used in low-input systems to regenerate soil fertility and to increase the amount of nitrogen immediately available to the plants. The nitrogen release characteristics of fertilizers must meet the crop nitrogen requirements during the growing cycle in order to avoid nutrient deficiency or environmental pollution. The conversion of organic nitrogen into the mineral forms required for plant uptake depends on several factors including the type and nature of organic compounds, soil properties, water availability and soil temperature. Starting from the above considerations, the rate of respiration and mineral nitrogen accumulation in soil amended with different organic and organic-mineral fertilizers were compared in laboratory incubation tests. Moreover, to study the influence of incubation temperature on mineral nitrogen dynamic in different fertilizer/soil blend, the incubation experiment was conducted at 10 and 25°C.

Materials and methods

Soil respiration rate and mineral nitrogen accumulated in soils amended with organic and organic-mineral fertilizers were evaluated in laboratory incubation experiments. A synthetic organic fertilizer (urea) with 46% of N was also included in the experiment. Samples of organic and organic-mineral fertilizers were obtained from Italpollina S.p.A. (Table 1).

Table 1: Selected characteristics of the organic and organic-mineral fertilizers used in the experiments.

Fertilizer	**Components**	**C (%)**	**Total N (%)**	**P (%)**	**K (%)**	**C:N**
Organic-mineral fertilizer						
Ritmo	poultry manure, diammonium phosphate, methylene-urea	34	11	7.4	-	3.1
Radar	guano, poultry manure, borlanda, feather meal, manure, phosphoric acid, chemical fertilizer NP 10-22	27	10	10	-	2.7
Organic fertilizer						
Dix 10 N	guano, poultry manure, borlanda, feather meal	42	10	1.3	2.5	4.2
Phenix	guano, poultry manure, borlanda	29	6	3.5	12.4	4.8
Guanito	guano, poultry manure, borlanda, kieserite	32	6	6.5	2.5	5.3
Italpollina	poultry manure	41	4	1.7	2.5	10.2
Biorex	animal manure	38	2.8	1.3	1.7	13.6

Source: **www.italpollina.com**

These fertilizers were oven-dried, ground to pass 1 mm-screen and analyzed for total N after mineralization with sulfuric acid by "Regular Kjeldahl method" (Bremner, 1965). Mineral N concentrations in 0.5 M K_2SO_4 extracts were determined spectrophotometrically using the method of Cataldo *et al.*, (1975) and the method of Anderson and Ingram (1989) for nitrate and ammonium determination, respectively. A sandy soil from a field grown since long time with vegetables was collected and air-dried; soil was screened trough a 5-mm sieve and brought to 70% of water holding capacity. The soil had 0.6 % organic matter and pH 7.1. The fertilizers were mixed by hand into the soil at variable rate (0.2-3%) in order to keep constant the total N applied (0.84 mg N per g of soil). For respiration rate determination, three replicates of unfertilized soil and of each fertilizer/soil blend were used. The soil and fertilizer/soil blend were placed into 60 ml centrifuge tubes; each tube was placed in a 500 ml flask. In each flask a tube containing 1 N NaOH solution was added. After 48 hours of incubation at 25°C, absorbed CO_2 in the 1 N NaOH solution was precipitated with 0.75 N $BaCl_2$ as insoluble $BaCO_3$ and titrated with 1 M HCl. Soil mineral nitrogen dynamic was measured in three replicate samples of unfertilized soil and of each fertilizer/soil blend after 2, 4, 6, 8 and 12 weeks of incubation in climate-controlled rooms at 10 and 25°C. Soil samples were extracted in 0.5 M K_2SO_4 and analyzed for mineral N concentration as previously described. The mineral N concentration of soil/ fertilizer blends was expressed as percentage of total N in fertilizer.

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 10 for Windows, 2001). Duncan's Multiple Range test was performed at P= 0.05 on each of the significant variables measured.

Results and Discussion

Cumulative soil respiration after 48 hours varied in the different fertilizer/soil blends with lowest values at 10°C in comparison to 25°C (Fig. 1). At 25°C, soil respiration rate was highest in soil amended with natural organic fertilizers while the lowest values were observed in unfertilized soil and soil fertilized with urea. Organic-mineral fertilizers gave intermediate values. At 10°C, soil respiration rate was significantly higher in all fertilizer/soil blends than in unfertilized soil.

At 25°C, the highest soil respiration rate observed after organic fertilizer application can be expected due to the high fertilizer rate and the presence of natural microorganisms in the fertilizers.

At both temperatures, mineral N increased during the incubation period especially in the initial 2 weeks (Table 2). Soil fertilized with urea showed the highest mineral N accumulation while the lowest values were observed in soil amended with animal manure fertilizer (Biorex).

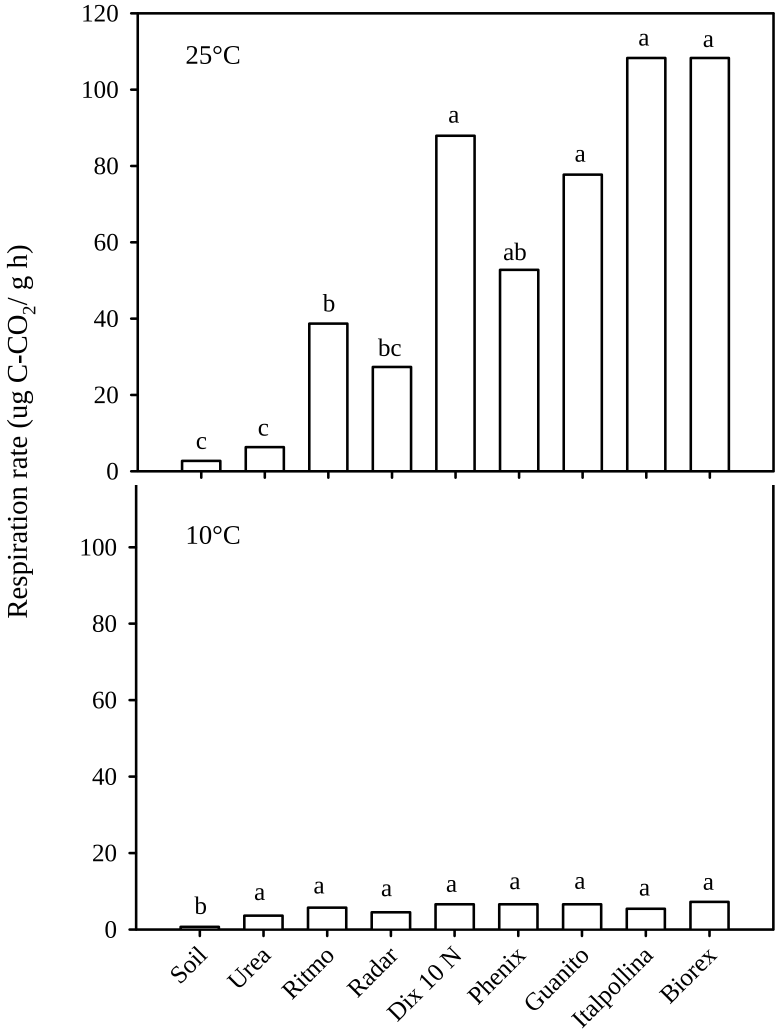

Figure 1: Soil respiration response to added fertilizers determined at 10 and 25 °C.

Intermediate soil mineral N values were recorded in the mix of soil and the others organic and organic-mineral fertilizers. Organic-mineral fertilizer containing methylurea showed a significant delay of mineral N accumulation compared to urea. The mineral N concentration in the soil/fertilizer blends decreased when the incubation temperature drop from 25°C to 10°C. In all fertilizers the majority of mineral N at 10°C in the first weeks of incubation remained in NH_4 form while at the highest temperature the NO_3 was the predominant (data not shown).

N mineralization dynamics in guano and feather meal-based fertilizers (Dix 10N, Phenix, Guanito) observed in this study were similar to those observed by Hartz and Johnstone (2006) who reported in a 8 week soil incubation at 10 and 25°C a net N mineralization of 55 and 63% for feather meal and 54 and 67 % for seabird guano, respectively. Moreover, Yadvinder-Singh *et al.* (2009) reported a N release of about 46% from poultry litter incubated for 60 days at 30°C while Qafoku *et al.* (2001) observed a net N mineralization ranged from 24 to 74% for poultry litter incubated for 112 days. In our experiment we determined a net N mineralization of 67.7 and 72.6 % from poultry manure (Italpollina) incubated at 25°C for 56 and 84 days, respectively.

Table 2: Cumulative mineral nitrogen (N) from organic and organic-mineral fertilizers as influenced by temperature and time of incubation.

Fertilizer	N (% of total N)				
	2 wk	**4 wk**	**6 wk**	**8 wk**	**12 wk**
	Incubation temperature 25 °C				
Urea	80.1 a[1]	92.4 a	94.0 a	96.2 a	97.4 a
Ritmo	51.3 b	57.4 bc	68.3 b	75.6 b	85.1 bc
Radar	67.5 ab	70.0 b	81.1 ab	83.4 ab	87.7 b
Dix 10 N	53.9 b	57.8 bc	67.2 b	72.8 b	78.3 bc
Phenix	53.7 b	61.7 bc	72.3 b	76.3 b	79.8 bc
Guanito	42.2 bc	52.2 c	63.2 b	67.3 b	75.3 bc
Italpollina	38.0 bc	53.0 c	62.3 b	67.7 b	72.6 c
Biorex	27.5 c	36.1 d	41.3 c	44.5 c	50.4 d
	Incubation temperature 10 °C				
Urea	55.0 a	62.4 a	70.9 a	74.6 a	82.0 a
Ritmo	41.7 b	47.0 b	52.9 ab	58.8 ab	68.1 b
Radar	54.3 a	58.0 a	61.4 a	63.7 a	68.0 b
Dix 10 N	35.0 b	44.6 b	48.2 b	52.6 b	59.7 b
Phenix	42.4 b	46.6 b	50.3 b	53.4 b	60.1 b
Guanito	33.0 b	38.0 bc	44.5 bc	49.4 bc	58.7 b
Italpollina	24.4 c	31.3 c	39.8 c	45.3 c	55.3 b
Biorex	16.8 c	19.6 c	23.3 d	26.6 d	32.7 c

[1]Means within columns separated using Duncan's multiple range test, $P = 0.05$.

The heterogeneity (chemical composition) of the poultry manure may be responsible for the different N-mineralization rates. Total N availability was highest in urea fertilizer due to the fast mineralization rate. Among the organic and organic-mineral fertilizers, Radar had the higher total N availability (mineralized organic N + initial inorganic N) due to its higher initial inorganic N. The high N mineralization rate in the first 2 weeks of the incubation test is in agreement with the findings of Hartz and Johnstone (2006) and come primarily from enzymatic hydrolysis of urea and simple proteins. The much slow rate of nitrogen release from the organic fertilizers after the initial 2 weeks suggested a microbial mediated degradation of more complex organic N forms. Moreover, the results indicated that substantial N availability can be obtained from organic fertilizers even at low temperature but due to the slow rate of nitrification at low temperature, the mineralized N tends to remain in NH_4 form for a significant period.

To summarize, we can conclude, that natural organic fertilizers and organic-mineral fertilizers significantly increased soil respiration compared to urea especially at 25°C. Natural organic fertilizers showed a slow release of mineral N in the soil than urea; mineral nitrogen accumulation in soil fertilized with Radar was similar to that of soil fertilized with urea while the application of organic-mineral fertilizer containing methyurea (Ritmo) significantly delay the release of mineral N.

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DELINEATION OF MANAGEMENT ZONE USING MULTIVARIATE GEOSTATISTICS AND EMI DATA AS AUXILIARY VARIABLE

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Abstract

Crop yields often vary widely across the field. Both financial and environmental gains can be achieved if fertilisers could be dosed more accurately according to site and season specific potential yield. Delineation of management zones to represent clustering of soil properties relevant to crop productivity allows increased precision in fertiliser management. An EMI survey provides data on the apparent soil electrical conductivity of the soil profile and this is often locally correlated with soil properties such as texture and pH. We carried out an EMI survey of a 200 ha cropping field in Western Australia to delineate management zones. We applied a multivariate approach called factor cokriging using pH measurement at four depths and an EMI sensor in both types of polarization. The first factor based on a linear combination of the six variables explained about 92% of the structural variance. Factor cokriging resulted in partitioning of the field into three areas using the interquantile values as breakdown points after filtering outliers. The resulting spatial classification did not show clear spatial long-range structures and this is thought to be also due to the scarce number of soil pH points (38) compared with EMI (11,500) data points used in the analysis. The EMI data actually dominated the structure of the homogenous areas whereas the pH data contributed to noise within these areas. This information together with additional data on soil and / or plant may be very useful for the implementation of VRT fertilization.

Keywords: EC_a, pH, factor cokriging.

Introduction

Crop yields often vary by a factor of ~3-5 across the field due to variation in soil and landscape properties. Variations in crop growth and yield result in corresponding variation in nutrient requirement. When fertilisers are applied uniformly to cater for the field-average crop yield, excess nutrient remains in parts of the field at harvest while other parts may suffer from nutrient depletion and deficiency. Both financial and environmental gains can be achieved if fertilisers could be dosed more accurately according to site and season specific potential yield (Wong *et al.*, 2001). Delineation of management zones to represent clustering of soil properties relevant to crop productivity allows increased precision in fertiliser management. When available, yield maps are often used in conjunction with other spatial data for this purpose (Wong *et al.*, 2008). An EMI survey provides data on the apparent soil electrical conductivity of the soil profile and this is often locally correlated with soil properties such as texture, plant available soil water storage capacity and pH which are often yield-determining in dryland cropping (Wong and Asseng, 2006) to improve fertilization. However, determination of management zones is difficult due to the interactions among several biotic, abiotic and climate factors that affect crop yield. Different approaches have been developed mostly based on clustering analysis. In the traditional theory of clustering classes are supposed to be crisply or fuzzy delineated in both attribute and geographic spaces. It may be erroneous, whenever within-class variation is greater than between- class variation, as in the case of tilled and cultivated fields, where soil properties vary gradually rather than abruptly. On the contrary geostatistics can treat multivariate indices of soil variation as continua in a joint attribute and geographical space (Castrignanò *et al.*, 2008).

The objective of this work was to apply a multivariate geostatistical approach for delineating management zones based on EMI survey and soil pH values to improve fertilization.

Materials and methods

The 200 ha cropping field is situated 350 km north of Perth, at Buntine in Western Australia. The average annual rainfall for Buntine is 335 mm, of which 240 mm falls in May to October growing season. The main crops grown were wheat in rotation with lupin and canola. The soil types across the field consisted of what the grower termed as good sand (Eutric Regosol), medium sand (Eutric Regosol), poor sand (Ferralic Arenosol), gravels (Xanthic Ferralsol) and red clay (Ferralic Cambisol). We measured apparent soil electrical conductivity (ECa) across the field on 30 m line spacing with an electromagnetic induction equipment (EM38, GEONICS, Ltd, Ontario-Canada) set in both horizontal and vertical polarization (EM38h and EM38v) simultaneously with differential corrected GPS location. ECa and GPS location were recorded at 1 second intervals with a data logger. Details of the ECa measurements are given in Wong and Asseng (2006). Soil pH in calcium chloride were measured across the field to represent soil profiles in 0.10 m intervals to 0.40 m (pH0, pH10, pH20 and pH30).

Geostatistical Procedures

In Geostatistics each measured value at a set of locations is interpreted as a particular realization or outcome of a random variable. For a detailed presentation of the theory of geostatistics, interested readers should refer to textbooks such as Chilès and Delfiner (1999), Wackernagel (2003), among others.

MultiGaussian approach

Even if ordinary cokriging does not require the data to follow a normal distribution, variogram modelling is sensitive to strong departures from normality, because a few exceptionally large values may contribute to many very large squared differences. In this scope, to produce the map of the variables we used multiGaussian cokriging. It is based on a multiGaussian model and requires a prior Gaussian transformation of the initial attribute into a Gaussian-shaped variable with zero mean and unit variance. Such a procedure is known as Gaussian anamorphosis (Chilès and Delfiner, 1999; Wackernagel, 2003) and consists in determining a mathematical function which transforms a variable with a Gaussian distribution into a new variable with any distribution.

The Gaussian anamorphosis can be achieved using an expansion into Hermite polynomials Hi(Y) (Wackernagel, 2003) restricted to a finite number of terms.

Multivariate geostatistical approach

The multivariate spatial data were analysed by cokriging and Factor Cokriging Analysis (FCKA,) which is a geostatistical method developed by Matheron. The theory underlying FCKA has been described in many papers (Castrignanò *et al.*, 2000; Wackernagel, 2003). The approach consists of decomposing the set of original second-order random stationary variables into a set of reciprocally orthogonal regionalized factors, related to NS spatial scales.

The three basic steps of FCKA are the following:

1) modelling the coregionalization of the set of variables, using the so called Linear Model of Coregionalization (LMC);

2) analysing the correlation structure between the variables, by applying Principal Component Analysis (PCA) at each spatial scale;

3) cokriging specific factors at each characteristic scale and mapping them.

1) Linear Model of Coregionalization (LMC)

The LMC, developed by Journel and Huijbregts (1978), considers all the studied variables as the result of the same independent physical processes, acting at different spatial scales. The simple and cross variograms of the variables are modelled by a linear combination of N_s standardized variograms to unit sill. Fitting of LMC is performed by weighted least-squares approximation under the constraint of positive semi-definiteness of the matrix of sills (coregionalization matrix) at each spatial scale, using an iterative procedure.

The transformed data are estimated at all unsampled locations using ordinary cokriging. Finally, the estimates are back transformed to the raw data through the mathematical model calculated in Gaussian Anamorphosis.

2) Regionalized Principal components Analysis

It consists in decomposing each coregionalization matrix into eigenvalues and eigenvectors matrices (Wackernagel, 2003).

The transformation coefficients correspond to the covariances between the original variables and the principal components, called regionalized factors, at a given spatial scale.

3) Cokriging and mapping of Regionalized Factors

The behaviour and relationships among variables at different spatial scales can be illustrated by cokriging and then mapping the regionalized factors (Castrignanò *et al.*, 2000). The cokriging systems for regionalized factors have been widely described by Wackernagel (2003).

All statistical and geostatistical analyses were done by using the software package ISATIS®, release 9.03 (Geovariances, 2009).

Results and Discussion

Table 1 lists the correlation coefficients of the soil properties. The two types of polarization were strongly correlated between them, whereas their relationship with pH data kept high up to 0.20-m depth.

Table 1: Correlation matrix of the soil properties. The correlation coefficient > 0.32 is significant at 0.05 probability level.

VARIABLE	EM38h	EM38v	pH0	pH10	pH20	pH30
EM38h	1	0.99	0.58	0.67	0.47	0.45
EM38v	0.99	1	0.61	0.67	0.43	0.42
pH0	0.58	0.61	1	0.76	0.54	0.5
pH10	0.67	0.67	0.76	1	0.83	0.77
pH20	0.47	0.43	0.54	0.83	1	0.95
pH30	0.45	0.42	0.5	0.77	0.95	1

Distributions of soil parameters were found to be strongly positively skewed (skewness varying between 1.07 and 2.61). Moreover, as the variables are characterised by different sizes and measurement units, before performing the multivariate approach, they were normalised and standardised to mean 0 and variance 1, by using Hermite polynomial expansions restricted to the first 30 elements. All the successive procedures of variography and estimation were performed on the calculated Gaussian variables. An isotropic LMC was fitted including two basic structures: 1) a nugget effect and 2) a spherical model with range = 450 m.

The spatial maps of the six raw variables, obtained by cokriging on a 2.5 m x 2.5 m square grid and then applying back transformation (Figure 1), display some distinct spatial patterns and also reveal some degree of spatial association among the different soil attributes.

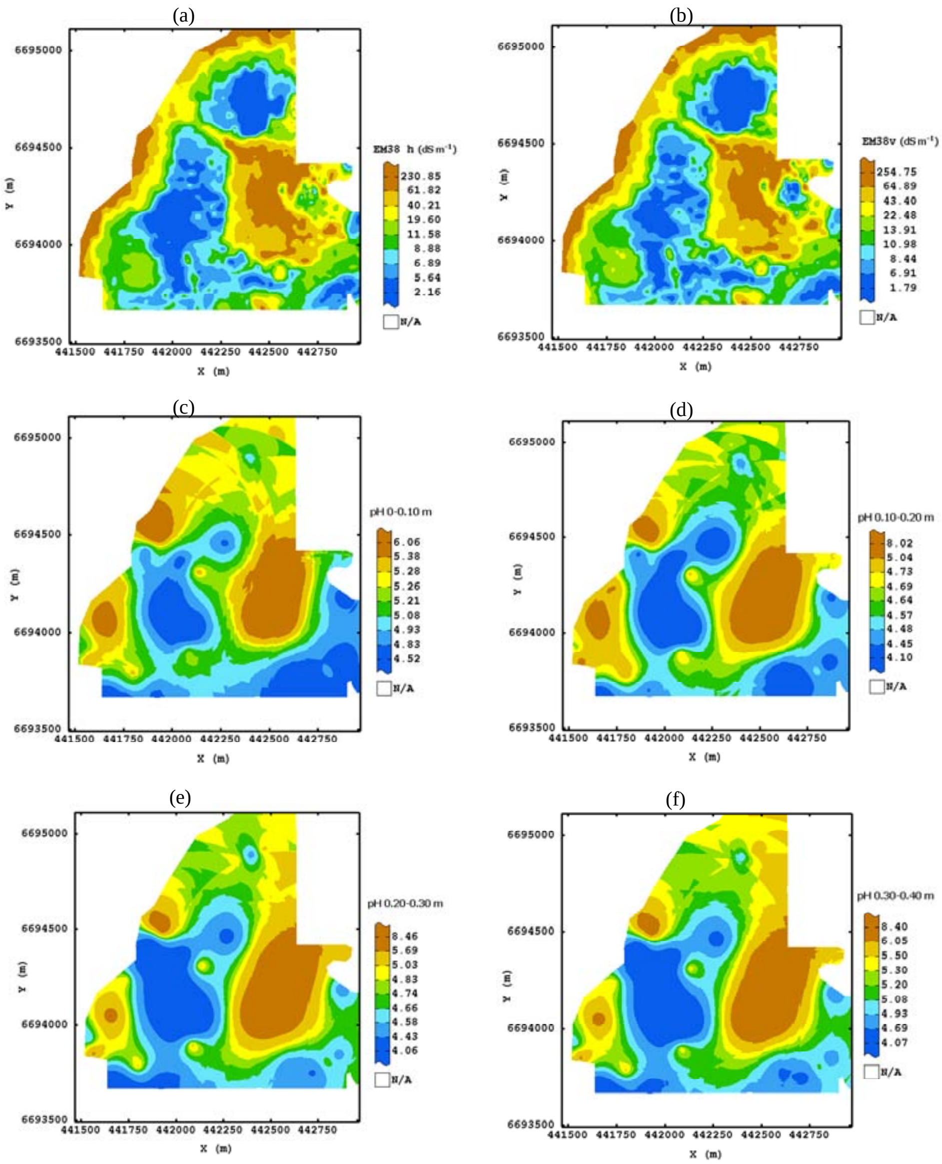

Figure 1: Spatial estimates of EC_a (dS/m) in horizontal (a) and vertical polarization (b) and pH at different depths (0-0.10 m (c); 0.10-0.20 m (d); 0.20-0.30 m (e); 0.30-0.40 m(f)).

Low values of EC_a <10 mS/m occur approximately along the axis 442000 east where field observations show occurrence of deep sandy soils. Another roughly circular area of low EC_a values occurs on shallow gravelly soils at ~ 6694500-6695000 North (Figure 1). Deep sandy and shallow gravelly soils occurring elsewhere in the field are likewise characterised by low EC_a values. Areas of high EC_a >45 mS/m occur along the western boundary of the field due to occurrence of a salty creek and to the central east part of the field due to a patch of clayey soil.

The cokriged maps of soil pH at the four depths look quite similar among them, which means that soil profile should not show clear discontinuities at least up to 0.40-m depth. This is also confirmed by the high correlations between the pH values at the different depths (Tab. 1). Compared with the EM38 maps, they appear more “spotty” spatially in the northern part of the field due to the quite sparse number of data points. The deep sandy soils are mostly acidic with pH < 4.5 whereas the clayey soil is alkaline (pH > 7). From a visual comparison between the EM38 maps and the pH ones there can be observed a great coherence in the central and southern parts of the field, whereas in the northern part the relationship looks not so clearly defined owing to the larger uncertainty in pH estimation.

To synthesise the multivariate variation of the field in a restricted number of zones, to be submitted to the same fertilisation, the factor cokriging analysis was applied to the data set of the six Gaussian transformed variables (Tab. 2). Since the sum of the eigenvalues at each spatial scale gives an estimation of variance at that scale, it is evident from the inspection of table 2, that the main component of variation (about 85% of the total variance) occurs within a range of 450 m, corresponding approximately to the size of the main spatial structures shown in the EM38 maps. In contrast, the contribution of the spatially uncorrelated component (nugget effect) to the total variance is quite low (15%). In the analysis we retained the eigenvectors producing eigenvalues greater than one and omitted the ones corresponding to nugget effect, because the latter are mostly affected by measurement errors. Therefore, we focused only on the first factor at 450-m range, which accounts for about 92% of the variation at that spatial scale. The loading values indicate that all the variables affect positively the first factor and the pH values between 0.10 m and 0.40-m depth intervals and EM38 data in horizontal polarization are the most influencing ones.

Figure 2 shows the map of the first factor whose scores, proportional to pH and ECa values, were split into three zones by using interquantile values as breakdown points after filtering outliers. The main patterns observed in the EM38 maps are still evident, but the factor map looks much more variable, characterised by many spots of size smaller than 450 m, probably related to local variability of pH.

These results show VRT Fertilisation might be more efficiently implemented in this field using a near infrared spectrophotometer, integrated with a fertilization control system, rather than delineating "homogeneous" zones of such size to be differentially managed by the farmer.

Table 2: Decomposition into regionalized factors at the different spatial scales. Only Factors corresponding to eigenvalues > 0 are reported.

Nugget effect

	pH0	pH10	pH20	pH30	EM38h	EM38v	Eigen Value	% Variance
Factor 1	-0.0472	-0.2425	-0.5678	-0.485	0.4566	0.4158	0.5469	47.49
Factor 2	0.9053	0.2541	-0.1669	-0.2404	-0.1216	-0.1239	0.4474	38.86
Factor 3	-0.3646	0.8517	-0.103	-0.3544	-0.0706	-0.0212	0.1439	12.5
Factor 4	0.0203	0.0293	-0.1707	0.1856	-0.6497	0.7163	0.0132	1.15

Spherical model - Range = 450.00m

	pH0	pH10	pH20	pH30	EM38h	EM38v	Eigen Value	% Variance
Factor 1	0.363	0.4465	0.4169	0.4292	0.4125	0.3751	5.7654	91.68
Factor 2	0.0157	-0.1864	-0.3669	-0.4456	0.3949	0.6899	0.5045	8.02
Factor 3	-0.2826	-0.6517	0.6138	0.1436	-0.0886	0.3002	0.0186	0.30

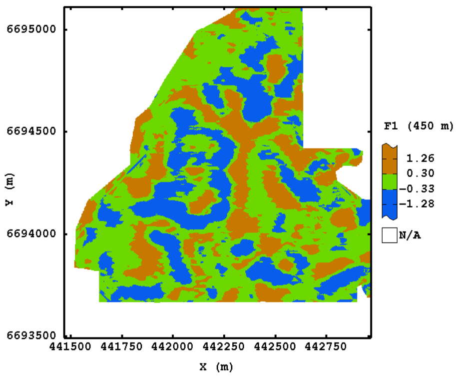

Figure 2: Spatial estimates of the regionalized factor.

Conclusions

Research in Precision Agriculture has focused on dividing a field into few relatively uniform management zones as a practical and cost-effective approach for variable application of fertilisers. In this study soil pH values at four depths and EMI data were used to delineate agricultural management zones by adopting a multivariate geostatistical approach. The resulting partitioning has not shown clearly distinct homogenous areas which could be differentially fertilized. This was thought to be due to local variation in pH and large uncertainty in pH estimation owing the scarce number of soil samples. The EM38 data actually dominated the structure of the homogenous areas whereas the pH data contributed to noise around these areas.

Like ECa this approach did not differentiate between deep sandy and shallow gravelly soils and between salt-affected and clayey soils. This clustering information if supplemented with soil texture may be very useful for yield and fertiliser requirement prediction.

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EVALUATION OF SPATIAL AND TEMPORAL VARIABILITY OF SOIL AGROCHEMICAL PROPERTIES

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Abstract

There are many different sources of heterogeneity of soil agrochemical properties. Combinations of all possible sources lead into different levels of spatial and temporal variation across the field. The investigation is focused on the illustration of the temporal changes and spatial variability of the available nutrients (Mehlich 3 available phosphorus, potassium and magnesium) and pH level in the field of an 21 ha area of the University Farm, located near Lány (Czech Republic). Soil samples were taken in 2004 – 2008 every year from the topsoil (0–30 cm) using the point sampling method with a regular grid square pattern 50 × 50 m. For the description of field variability of selected soil parameters coefficient of variation (CV), experimental variograms and relative nugget effect parameters have been used. A geostatistical analysis indicated that among all determined parameters soil pH showed the lowest variability. Higher variability was found in the nutrient status (P, K, Mg). Results shows, that available P, K, Mg and pH level are too little temporally variable on the field. The content of available nutrients was from low to good level.

Keywords: precision agriculture, nutrient, pH, spatial variability, temporal variability.

Introduction

Spatial variability of soil has been studied for many years. For the most part, this variability has been measured using samples collected in the field and analyzed in a laboratory. Understanding the spatial variability in the samples has increased with the availability of global positioning system (GPS) measurements of the sample locations, and geographic information systems (GIS) for the spatial mapping and analysis of these data. Development of GPS and GIS and their utilization in commercial field were not first tools for description of spatial differences in soils. Burrough (1993) has reviewed many of these studies, and stated that if the spatial variation of soil fertility over a field can be mapped, one has enough information to adjust the amount of fertilizer spread at any spot to that which will be needed for the crop. These studies and new results show that the spatial variability of soil properties vary from sampled field. Plant nutrition optimizing based on site-specific application of fertilizers can not go without accomplished mapping of spatial variability of soil agrochemical properties. The agrochemical properties of interest include pH, nutrients (particularly nitrogen, available phosphorus, potassium and magnesium) and organic matter content. Soil agrochemical properties are affected from large amount of soil processes, which are working together but their activity and interaction vary both in time and space. The higher spatial variability of parameters mentioned above, the higher opportunity of variable rate application of P, K and Mg fertilizers. Štípek (2003) relate soil available P, K and Mg to the properties with medium spatial variability. Term and technique of soil sampling are very important factors for site-specific management activities. If the spatial structure of nutrients is to be used in the control of fertilizer application, it would be necessary to know its stability in time (Delcourt *et al.*, 1996). Wollenhaupt *et al.* (1997) presented a review of soil sampling and recommended the use of unaligned systematic sampling for site specific management as it reduces the chance that important patterns slip through unnoticed.

In this study the objective was to describe the spatial and temporal variability of nutrients and pH.

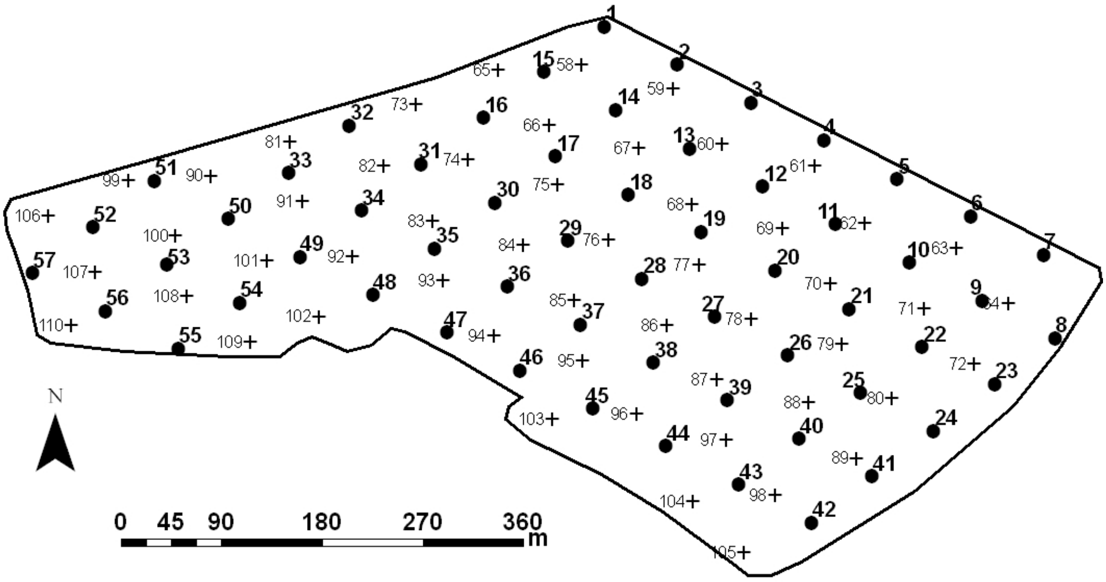
Material and Methods

The study of spatial heterogeneity and temporal variability of soil properties was examined at Ruda field of area 21 ha located near Lány, Czech Republic (Loc: 50°8'45.416''N, 13°52'0.08''E). Soil samples were collected from topsoil (0 to 30 cm) using the point sampling method with regular grid square pattern of 50×50 m across the whole field and for comparsion was used unaligned systematic sampling grid. Seven individual core samples were taken from each point (sampling area) from a circle area with a radius of 3 m from the centre point. The total number of soil samples collected from field was 57 in regular grid and 53 in unaligned systematic sampling (Figure 1).

Soil samples were collected three years on spring and two years on autumn in both grids and then every year on spring were collected samples from exclude points. A set of soil samples was homogenized, air-dried and sieved on the 2 mm mesh. Fine earth fraction was analysed for available P, K and Mg in Mehlich 3 extraction (an official method for soil testing in the Czech Republic). The Skalar San System segmented continuous flow analysis with photometric detector was used for the detection of P. The atomic absorption spectrometer (Varian AA-300) was used for the detection of K and Mg. Soil pH was determined in 0.01 M

$CaCl_2$ extract. Summary statistics data were processed using Statistica. Geostatistics, using GS+, was used to explore spatial variation of soil in the field. The coordinate system is in UTM projection, WGS-84 datum To express the level of spatial dependence was used the relative nugget effect [Co/(Co+C)] described in Cambardella and Karlen (1999).

Figure 1: Point sampling design (● regular grid square pattern; + unaligned systematic sampling).



Results and discussion

The soil agrochemical properties data sets, obtained from soil analysis, were initially evaluated using basic statistics parameters. Results summarized in Table 1 show available P average from 46 to 57 ppm. Average values of available K ranged between 153 and 228 ppm and available Mg from 54 to 76 ppm. Value of pH ranged between 6.4-6.6

The highest spatially variable was soil available K content with coefficient of variation (CV) from 27 % to 40 % in regular grid and from 19 % to 49 % in unaligned systematic grid. To evaluate the spatial dependence of the soil available K data sets experimental variograms with fitted models were constructed. Relative nugget effect presented as [Co/(Co+C)] ranged from 40 to 45 % which means a medium spatial dependence of soil available K. The spatial dependence of most properties can be classified as moderate according to the classification proposed by Cambardella *et al.* (1994). Most of an experimental field has good soil available K content. Differences in soil available K in samples, taken in diverse sampling time could be influenced by various soil moisture conditions. Peck and Melsted (1973) suggest that existing of seasonal variability of soil test results are going to change because of factors affecting the nutrient uptake by plants and nutrient replenishment of soil solution (sorption, desorption, water transport, soil microbial activity, soil pH, CEC). However, correlation coefficients between data collection show the week change in soil spatial variability of the soil available K content.

Table 1: Summary statistics of soil available nutrients (P, K, Mg) and pH in the experimental field over the period 2004-2006

	regular grid					unaligned systematic grid				
Sampling	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**
					P (ppm)					
average	51	47	57	57	54	52	46	55	49	46
min	24	18	31	32	31	29	23	44	28	39
max	110	89	123	94	80	104	130	64	95	55
SD	16	15	19	15	13	15	18	10	14	6
median	49	44	54	57	55	47	41	60	45	46
CV (%)	30.4	32.1	33.0	26.5	23.4	29.4	39.4	17.3	28.5	13.9
					K (ppm)					
average	179	228	197	158	167	153	225	171	194	180
min	104	109	105	79	84	71	99	127	96	79
max	312	604	359	368	332	337	578	205	567	508
SD	49	92	60	61	56	59	109	32	95	86
median	166	212	180	141	162	143	192	167	149	150
CV (%)	27.2	40.3	30.3	38.8	33.6	38.6	48.6	18.9	49.1	47.8
					Mg (ppm)					
average	74	59	76	72	66	67	56	71	60	54
min	36	29	32	35	30	33	22	53	7	26
max	167	159	189	183	173	130	116	89	134	106
SD	29	23	32	29	31	22	21	15	30	17
median	67	53	65	66	58	63	52	69	56	52
CV (%)	39.4	39.9	41.6	40.1	47.0	31.9	37.3	21.7	50.1	32.1
					pH					
average	6.5	6.5	6.4	6.4	6.4	6.5	6.6	6.5	6.6	6.6
min	5.2	5.2	5.2	4.9	5.2	5.6	5.9	5.6	5.7	5.9
max	7.2	7.3	7.1	7.2	7.1	7.2	7.1	7.2	7.2	7.1
SD	0.4	0.4	0.4	0.5	0.4	0.4	0.3	0.4	0.3	0.3
median	6.6	6.6	6.5	6.6	6.5	6.6	6.7	6.6	6.6	6.7
CV (%)	6.0	6.0	6.1	7.4	6.1	6.1	4.7	6.1	5.2	4.7

Soil available P variability derived from coefficient of variation shows relatively low levels (CV = 23 - 33 % in regular grid and from 14 % to 39 % in unaligned systematic grid). Relative nugget effect varied from 40 % to 50 % shows medium spatial dependence of soil available P. Similarly to available K content, the temporal variation in P content did not influence site-specific application of P fertilizers, derived from soil analysis data. But most of an experimental field has a low level of soil available P content (Figure 2). The spatial variability of soil available Mg content characterized by coefficient of variation fluctuated in range 39 – 47 % in regular grid and from 22 % to 50 % in unaligned systematic grid. Relative nugget effect ranged between 14 and 23% of the total semivariance, which means moderate to a strong spatial dependence. Generally low soil available Mg content in most of this field, allow to say that neither spatial variability of soil Mg nor site-specific application of Mg fertilizers, derived from soil analysis results was affected by the different sampling times.

Figure 2: Map of phosphorus spatial variability.

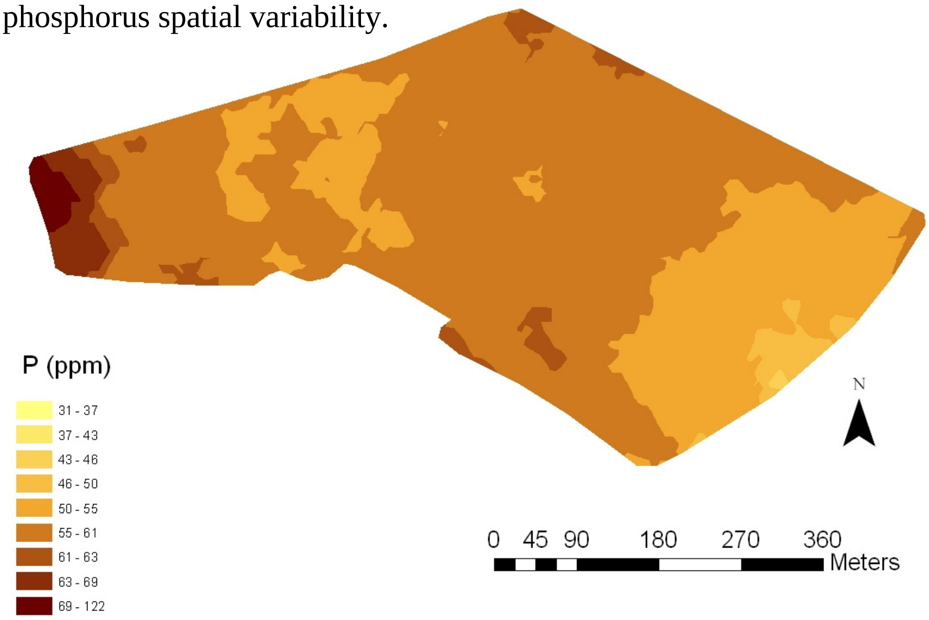


Table 2: Correlation coefficients between sampling data sets

	regular grid				unaligned systematic grid			
Sampling	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
				P				
II	0.77				0.33			
III	0.83	0.76			0.63	0.67		
IV	0.81	0.69	0.93		0.73	0.89	0.69	
V	0.80	0.72	0.85	0.86	0.69	0.56	0.77	0.81
				K				
II	0.73				0.74			
III	0.79	0.69			0.76	0.64		
IV	0.81	0.90	0.78		0.71	0.83	0.75	
V	0.88	0.64	0.74	0.67	0.53	0.74	0.43	0.83
				Mg				
II	0.79				0.69			
III	0.94	0.79			0.70	0.77		
IV	0.82	0.96	0.82		0.76	0.87	0.75	
V	0.95	0.74	0.91	0.76	0.67	0.72	0.76	0.69
				pH				
II	0.70				0.80			
III	0.78	0.83			0.83	0.79		
IV	0.88	0.43	0.78		0.93	0.85	0.76	
V	0.82	0.79	0.80	0.56	0.90	0.82	0.75	0.80

The lowest spatially variable was pH value with coefficient of variation from 6 % to 7 % in regular grid and from 5 % to 6 % in unaligned systematic grid. Relative nugget effect ranged between 3 and 23% of the total semivariance, which means a strong spatial dependence.

Similar results described Delcourt *et al.* (1996) with relative nugget effect was small for pH (5 %), medium for P (20 - 30 %) and Large for K and Mg (40 - 55%)

The correlation between all sampling data sets of the different topsoil nutrients was quite strong (Table 2). This indicates that temporal variability did not change much over the evaluate years. High spatial variability together with low temporal change in soil available nutrient content, determined using appropriate soil test gives an assumption for the cost benefit of mapping soil agrochemical properties and variable-rate application of fertilizers (Štípek 2004)

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DURUM WHEAT AND COMMON VETCH PERFORMANCES UNDER CONVENTIONAL AND CONSERVATIVE CROPPING SYSTEMS

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Abstract

Cropping systems in the Southern Italy were characterized by continuous cereal cropping system, leading to environmental risks, and soil fertility deterioration. To reduce those problems, a growing interest in conservative cropping systems has occurred. Under rainfed conditions and over the period 2004-2007, a field study was carried out in a Mediterranean environment (Foggia, Southern Italy), on a cereal-livestock farm to assess the effects of conservative agronomical practices on grain yield and quality of durum wheat (*Triticum durum* Desf.) cropped as continuous crop (CC) and in rotation (ROT) with common vetch (*Vicia sativa* L.). On elementary plots, laid out in a randomized complete block, two levels of crop management were evaluated: i) conservative (CONS) with soil tillage 15-20 cm deep combined with organic-mineral fertilization and ii) conventional (CONV) with deep soil tillage at 40-50 cm and mineral N fertilization.

The possible effect of common vetch in rotation with durum wheat on soil fertility was also evaluated, by comparing yields of durum wheat in rotation and under continuous cropping. The results obtained showed either the effects of weather conditions, or the effectiveness of conservative practices on crops productions. In fact, for durum wheat cropped in CC and ROT, similar grain yields were obtained with the two levels of crop management (2.08 *vs*. 2.39 t ha^{-1} for CC, and 2.31 t ha^{-1} *vs*. 2.54 t for ROT, respectively for CONS and CONV treatments). While, the responses of common vetch showed that the CONV treatment allowed the significantly highest dry matter production (5.64 *vs*. 4.80 t ha^{-1} of conservative treatment). Thus, these results pointed out the possibility to apply agronomical interventions with low environmental impact, almost always without compromise the crops performance.

Keywords: conventional practices, conservative practices, durum wheat, common vetch, agronomic performances, quality

Introduction

One of the most important problems in agriculture is the decrease of soil productivity as a consequence of erosion, salinization, compaction and losses of organic matter associated with extensive tillage. In particular, the Mediterranean area is characterized by a high seasonal rainfall variability and also by a low soil organic matter content. Such factors were considered as important constraints limiting crops growth and production specially under rainfed conditions in the Mediterranean region.

In addition, the application of both intensive agronomical interventions and cropping systems can cause agronomic and environmental negative effects. Particularly, traditional (conventional) soil tillage system, continuous cropping systems and the use of high rates of inorganic N fertilizers are the most practices that must be monitored to avoid soil resources depletion and crop yield reduction.

Therefore, there is a need for sustainable management practices in cropping systems (no-till or minimum tillage, rotation and supply of organic fertilizers) able to conserve and increase soil resources, to improve environmental safety and to increase crops productivity and quality (Al-Issa and Samarah, 2006., Bazzoffi et al. 1998., Campbell et al., 1995).

In this regard, Cambardella and Elliott (1993) indicated that traditional tillage can reduce soil organic C and N and disrupt soil aggregates, while no-tillage or minimum tillage are more efficient in conserving moisture, controlling soil erosion and improving C and N storage in the soil (Franzluebbers *et al.*, 1995; Lal, 1989). In the other hand, Wiatrak et al (2006) reported that wheat (*Triticum aestivum* L.) production is influenced by management of the previous crop but is highly dependent on current year management. Moreover, the addition of organic source of N could be a good way for developing soil organic belt as well as sustaining crop growth and productivity.

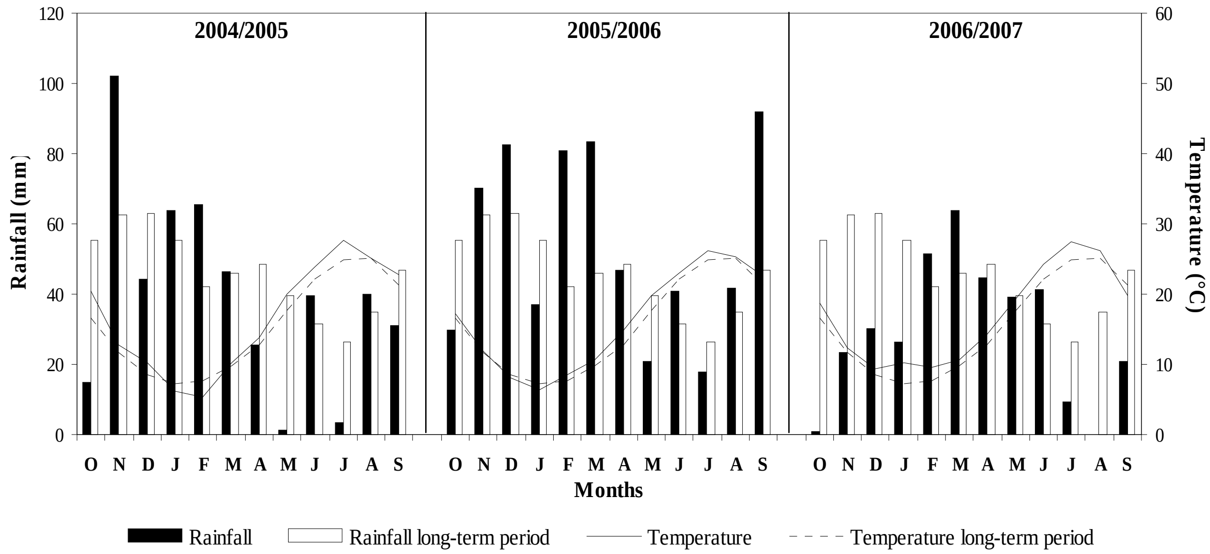
In the light of these considerations, the CRA - Research Unit for Cropping System in Dry Environments has achieved a study for evaluating the possibility to reduce soil tillage depth and to supply organic fertilizers in durum wheat and common vetch cultivation. Therefore, the objective of this research, carried out in Southern Italy, was to assess the responses of some typical Mediterranean crops to the application of conservative management techniques (shallow soil tillage and organic-mineral fertilizer), for developing some recommendations concerning environmental and economical point of view.

Materials and methods

The trials were carried out during the 2004-2005, 2005-2006 and 2006-2007 growing seasons at Foggia (Southern Italy, 41° 27' lat. N, 3° 04' long. E, 90 m above sea level) in a cereal-livestock farm, by the CRA - Research Unit for Cropping Systems in Dry Environments, Bari, Italy. The trial location is a typical area of Mediterranean environment (Apulian Tavoliere).

The soil was a silty-clay Vertisol of alluvial origin (silt 41.09%, clay 37.80%, sand 21.11%, total N 1.66 g kg^{-1}, available P 12.62 ppm, exchangeable K 554.10 ppm, organic matter 20.6 g kg^{-1}, pH 7.59), classified as Typic Chromoxerert, Fine, Mesic, by Soil Taxonomy USDA.

The climate is "accentuated thermomediterranean" (UNESCO-FAO classification), with rains concentrated mainly in the winter months. The monthly mean temperatures and the rainfall are shown in the figure 1. In particular, in the second trial year plentiful rains fell in September (35 mm), October (95 mm) and November (76 mm). In these conditions, the soil was tilled in the last days of December 2005 and sowed in January 2006, with consequent negative effects on crops production, while these practices are usually carried out in August and October-November, respectively.



Durum wheat (*Triticum durum* Desf.) was cropped as continuous crop (CC) and in rotation (ROT) with common vetch (*Vicia sativa* L.) in rain-fed conditions to evaluate the effects of the following two levels of crop management: i) Conservative (CONS), with shallow tillage at 15-20 of depth, combined with organic-mineral nitrogen (N) fertilizer, and ii) Conventional (CONV), with traditional soil tillage at 40-45 cm deep and mineral N fertilizer. The N fertilizer (70 kg N ha^{-1}) was broadcasted, as mineral or organic-mineral, always as a top dressing, in February-March only on durum wheat.

Furthermore, for evaluating the possible effects of leguminous crops on soil fertility and productions of following crops, the yields of wheat in rotation with common vetch were compared with those of wheat in continuous cropping.

On elementary plots of 350 m^2 each, the experimental design was a randomized complete block, with three replicates. In accordance with normal practice, all the trial fields received the phosphorus fertilizer (100 kg P_2O_5 ha^{-1}), as mineral or organic-mineral, in pre-sowing, at the time of main soil ploughing (August). In addition, the cultivations in rotation were sown contemporaneously every year to avoid the climatic pattern effect and to have contemporarily both the crops in rotation every year.

At harvesting time, the most important quanti-qualitative parameters of durum wheat (plant height, grain and straw yields, hectolitre weight and protein content) and common vetch (plant height, green forage, protein content and dry matter productions obtained after oven-drying at 70 °C till a constant weight) were determined.

Statistical analysis was carried out using the SAS procedures (SAS Institute, 1998). The effect of the treatments was evaluated considering the years as a random effect and the crop management levels as a fixed one. Differences among means were analyzed at the $P \leq 0.05$ probability level, by applying the Duncan Multiple Range Test (DMRT) for more then two means comparison.

Results and discussion

The Table 1 shows the mean effects of years, experimental treatments and cropping systems on yield, yield components and quality of durum wheat. In particular, during the trial period, grain and straw yields and also hectolitre weight were significantly highest in the first year (2005), followed by the third one (2007), trial years in which the rains were lower than 2006, but evenly distributed during the wheat cropping cycles and not concentrated in the winter period as in 2006. These results confirm the important role played by climatic conditions in Southern Italy. According to Lòpez-Bellido (1992), climatic restrictions of the Mediterranean region provide an unfavourable scenario for agriculture.

Concerning the experimental practices, the significantly highest grain, straw yields and plant were produced under the CONV treatement (2.47 t ha^{-1}, 3.13 t ha^{-1} and 61.33 cm, respectively). These findings are in consistence with the results of Al-Issa and Samarah (2006), who reported that conservative tillage system gave the lowest grain and straw yields in comparison with the traditional system. Furthermore, similar results were found only for grain yield by Lòpez-Bellido et al. (2001) in Mediterranean environments.

Same trend of CONV treatment was also recorded for hectolitre weight (81.19 and 81.16 kg, for CONV and CONS, respectively) and protein content (11.9 and 11.55% for the same treatments, respectively) but without significant differences (Table 1). Lòpez-Bellido et al. (1996) reported similar results, but with significant differences, for wheat grain protein content, while Campbell et al. (1998) report that the tillage system has no effect on grain protein content.

Table 1. Effect of years, experimental treatments and cropping systems on quanti-qualitative parameters of durum wheat.

	Grain yield (t ha^{-1})*	Straw yield (t ha^{-1})	hectolitre weight (kg)	Plant height (cm)	Protein content (%)
Years					
2005	3.11 a	4.66 a	83.90 a	63.88 b	11.08 b
2006	1.10 c	2.46 b	79.93 b	48.96 c	10.20 b
2007	2.80 b	1.32 c	79.68 b	67.68 a	14.01 a
Treatments					
CONS	2.19 b	2.49 b	81.16	59.02 b	11.55
CONV	2.47 a	3.13 a	81.19	61.33 a	11.97
Cropping systems					
CC	2.24	2.64 b	81.21	59.68	11.64
ROT	2.43	3.00 a	81.14	60.67	11.89

* At 13% of moisture
Values with different letters in the columns are significantly different at P≤0.05.

Finally, the durum wheat in rotation with common vetch showed the highest both grain yield, plant height and protein level, but without significant differences (Table 1). This finding was probably due to the capability of the leguminous crops to increase soil fertility.

In Table 2 the mean effects of years and experimental treatments on yield, yield components and quality of common vetch are presented.

Particularly, the common vetch shows as the durum wheat, similar productive trends were found in common vetch, for which the best forage and dry matter yields and plant height were always obtained in 2005 (Tables 2).

With reference to the experimental treatment, for common vetch was recorded the significantly highest dry matter productions with the CONV treatment, with 5.64 *vs.* 4.80 t ha^{-1} of CONS treatment. However, some findings, in other crops and in similar trials carried out in Mediterranean environments, have reported that the CONS treatment has reached, generally, good agronomical responses (Lòpez-Bellido *et al*., 2000; Montemurro and De Giorgio, 2005).

While, regarding the protein content, the present study demonstrated the superiority of the CONS treatment over the CONV one (19.58 and 18.39% , respectively), in confirmation of the inverse relation between the quantitative and qualitative characteristics of the production.

Table 2. Effect of years and experimental treatments on yield, yield components and quality of common vetch.

	Forage yield ($t\ ha^{-1}$)	**Dry matter yield ($t\ ha^{-1}$)**	**Plant height (cm)**	**Protein content (%)**
Years				
2005	20.23	5.54 a	100.27 a	18.03
2006	18.43	4.27 b	84.12 b	19.94
2007	19.95	5.84 a	91.20 ab	18.90
Treatments				
CONS	18.46	4.80 b	91.58	19.58
CONV	20.62	5.64 a	92.14	18.39

Values with different letters in the columns are significantly different at $P \leq 0.05$ (Duncan's multiple range test)

Conclusions

The study has pointed out the possibility to apply agronomical interventions with low environmental impact level of agro-techniques (minimum soil tillage and mineral fertilizer application), almost always without compromise the crops performance and improving the durum wheat productivity in rotation with common vetch respect to the wheat in continuous cropping systems.

Furthermore, it is be noted, that our results appeared very satisfactory from both the economical and the environmental aspects, considering that the slightly lowest grain and dry matter yields of durum wheat and common vetch, respectively (reduction about 10 and 15% respectively) could be balanced by the lowest production costs and pollution risks.

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SITE SPECIFIC SOIL FERTILITY MANAGEMENT OF AN OXISOL CULTIVATED WITH CORN FOR APPLICATION OF LIME AND GYPSUM

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Abstract

Due to the necessity to improve soil fertility diagnostic, the researchers have been searched for more efficient technologies on agronomic, economic and environmental aspects. One of these technologies is the use of the concept of site-specific for soil fertility management. This research was conducted in a farm field (100 ha) located in Corinto, Minas Gerais state, starting in the 2007-2008 growing season. The soil is classified as Clay Oxisol, cropped with corn (*Zea mays* L.) and irrigated with a center-pivot sprinkler irrigation system. Grid cell of 1 ha was used for collecting soil samples from 0 to 20 cm and 20 to 40 cm depths. Data of pH, Al^{3+}, Ca^{2+}, Mg^{2+}, CEC, base saturation (BS), Al^{3+} saturation in the CEC at soil pH (effective CEC) and organic matter were submitted to geostatistical analysis and interpolated by point-kriging using the modeled semi-variograms. Based on the maps of BS and Al^{3+} saturation, it was possible to define zones of management for application of lime and gypsum. The threshold used to the definition of the rates of lime was 60 % of BS in the top of 20 cm. The criteria based on values of Ca (<0.5 $cmol_c\ dm^{-3}$) and Al^{3+} saturation (>25 %) in the subsoil (20 to 40 cm) were used for gypsum application. With these informations, maps of application of lime and gypsum at variable rate were generated. The rates of lime range from 0 to 3 t ha^{-1} and for gypsum of 0 and 1 t ha^{-1}. The costs of soil sampling with GPS, soil chemical analyses, field mapping with GIS and application of lime and gypsum, were evaluated. Allocating the cost of site-specific soil acidity management, over a useful lifetime of four years, the cost of the technological package, US$ 18.40 per hectare, become economically feasible.

Keywords: soil acidity, spatial variability, variable rate, economic aspects, *Zea mays* L.

Introduction

Soil analysis should be the basis for all programs of soil fertility evaluation and the building of a nutrient management plan. It can be complemented by other techniques, but it is the only one that efficiently, on a routine basis, makes it possible to anticipate the existing soil constraint for the growth and development of plants. In general, the current soil tests performed in the laboratories in Brazil, are both precise and accurate in determining the nutrient supplying power of the soil that is being analyzed. Most states also have certification programs that ensure that the laboratories are performing the correct test and are proficient in the exact procedure (Cantarella, 1999). However, the greatest difficulty for soil fertility evaluation is collecting the right soil samples to test and that represents the area sampled. Also, soil sampling has often been described as the weak link in soil testing. Thus, there is the necessity to improve soil fertility diagnostic, and the researchers have been searched for more efficient technologies on agronomic, economic and environmental aspects.

One of these technologies is the use of the concept of site-specific for soil fertility management. It is based on information about spatial variability of soil attributes and involve customize management of fertilizer and soil amendments (lime and gypsum), in sub-regions of a field, in order to reduce soil variability, optimizing productivity and profitability (Mulla and Hernández, 2006; Coelho, 2008a). The aim of precision farming research is to help farmers optimize the management of their fields so that gross returns are maximized for each management area. To do this, the farmer needs to know how the conditions for growing crops vary over the area of interest. The objective of this research was to identify the spatial variability of soil fertility, with emphasis in the soil acidity, to define management zones for application of lime and gypsum at variable rate, in an Oxisol cropped with corn. Also, the profitability was evaluated to obtain the balance cost of soil sampling with the value of information.

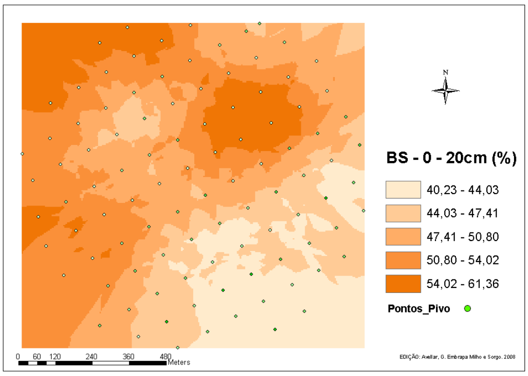
Materials and Methods

This research was conducted in a farm field (100 ha) located in Corinto (18º 13'S, 44º 36'W, 550 m above sea level), Minas Gerais state, starting in the 2007-2008 growing season. The soil is classified as Clay Oxisol (47 % of clay), cropped with corn and irrigated with a center-pivot sprinkler irrigation system. A georeferenced grid cell of 1 ha, was used for collecting soil samples from 0 to 20 cm and 20 to 40 cm depths. Five soil cores were randomly collected within a 5-m radius of the grid-line intersection (node) and composited as one soil sample. Soil test analyses were carried out according to methodology described by Embrapa (1997). Data of pH, Al^{3+}, Ca^{2+}, Mg^{2+}, CEC, percent base saturation (BS), percent Al^{3+} saturation in the CEC at soil pH (effective CEC) and organic matter were submitted to geostatistical analysis, as described by Isaaks and Srivastava (1989), and interpolated by point-kriging using the modeled semi-variograms. Based on the maps of BS and Al^{3+} saturation, it was possible to define zones of management for application of lime and gypsum. The threshold used to the definition of the rates of lime was 60 % of BS in the top of 20 cm. The criteria based on values of Ca (<0.5 $cmol_c$ dm^{-3}) and Al^{3+} saturation (>25 %) in the subsoil (20 to 40 cm) were used for gypsum application. The calculation of the rates of lime to bring soil BS to 60 %, was based on the following equation: RL_{100} = [(60 – BS)*CEC/100]. Where, RL_{100} is the rate of lime with the effective calcium carbonate rating of 100 %. The rates of gypsum were based on clay content, according to Alvarez V, et al (1999). With these informations, maps of application of lime and gypsum at variable rate were generated. Based on these maps, lime and gypsum were applied at variable rates, on the soil surface, using a commercial spreader applicator. The costs of soil sampling with GPS, soil chemical analyses, field mapping with GIS and application of lime and gypsum at variable rate, are evaluated.

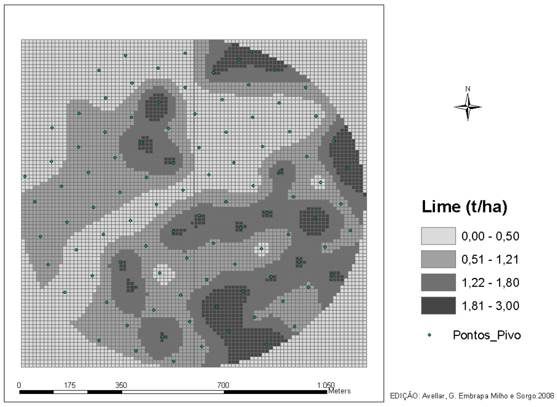
Results and Discussion

Since this study is ongoing, a complete analysis of site-specific soil fertility management is not yet possible. However, the observed spatial variability in the corn crop yield and nutrient levels has important implications for both variable and constant rate fertilizer and soil amendment (lime and gypsum) applications. The site in this study show large spatial variability in the soil attributes used as indicators of the soil acidity (Figure 1ac). In the topsoil (20 cm), spatial patterns in kriged BS (Figure 1a) show values ranging from about 40-60 % in a 100-ha field. The BS is approaching the deficiency in part southwest of the field (< 50 %), yet is plentiful (BS > 50 %) in parts northwest and southeast of the field. This variability (Figure 1a) afford the opportunity to differential application of limestone and the modern technology of precision farming. The spatial scale makes it feasible technologically. According to National Research Council (1997), soil variation is of interest when conditions vary over patches manageable by agricultural machinery.

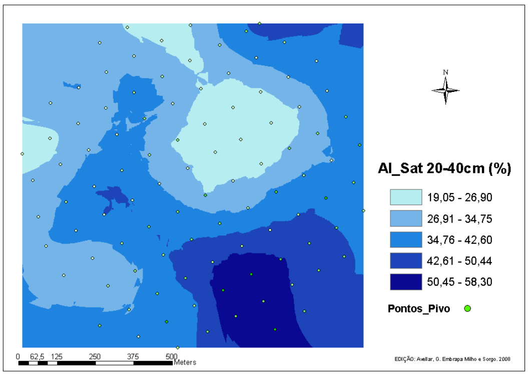
Based on map of BS (Figure 1a), four management zones were established for limestone application (Figure 1b): zone 1 - 0.0 t ha^{-1}, representing 27 ha; zone 2 - 0.3 to <1.0 t ha^{-1}, representing 21 ha; zone 3 - 1.0 to <2.0 t ha^{-1}, representing 38 ha and; zone 4 - 2.0 to 3.0 t ha^{-1}, representing 13 ha. Field-averaged soil test for BS was 49 ± 10 %. For corn crop, the values of BS of 50 to 60 % are recommended (Coelho, 2008b). If the field is to be uniformly liming at a single rate to bring BS to 60 %, according to recommendation for corn, there is a necessity of 1.0 t ha^{-1} of limestone. Although the total comsuption of the limestone (99 t), is similar for both, uniform and variable rate application, the large variability in the soil acidity, justify the use of variable rate technology.



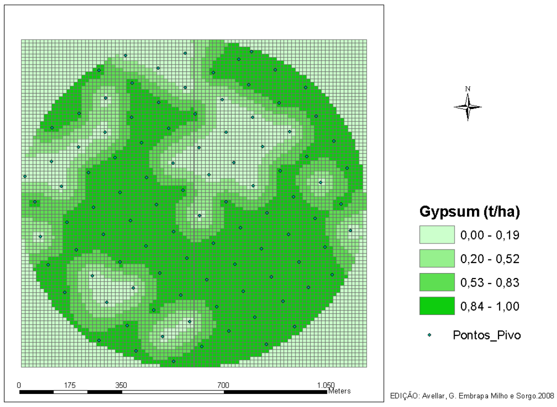
a) Map of base saturation 0-20 cm depth



b) Map of lime application



c) Map of Al^{3+} saturation 20-40 cm depth



d) Map of gypsum application

Figure 1: Interpolated maps of base saturation (a); lime application (b); aluminum saturation (c) and, gypsum application (d).

For Al^{3+} saturation in the CEC at soil pH, the values in the soil surface range from 0 to 35 % (data not showed). The subsoil (20 - 40 cm) presented high acidity, with values of Al^{3+} saturation ranging from 20 to 58 % in almost all field (Figure 1c). Thus, according to criteria for gypsum recommendation, in 70 ha of the field, there is the opportunity for gypsum application at maximum rate of the 1.0 t ha^{-1} (Figure 1d). Research reported by Prado (2001) showed for some corn hybrids, that grain yield was reduced by 47 % in an oxisol with Al^{3+} saturation of 23 % in the topsoil (0-20 cm). The results presented and discussed here, show that the conventional approache to soil testing, based on average, was inadequate for characterizing spatial variation of soil acidity. However, only the economic conspire against it, because of the need to analysis the soil for nutrients at least 99 points at two depths, and the cost of that in any one year could be more than the farmer can expect to gain in greater efficiency. In the Table 1, are showing the annual costs for application of lime and gypsum at variable rate for a 100-hectare field.

Table 1: Annual information costs for application of lime and gypsum at variable rate for a 100 hectare field, with a 4-year soil sampling cycle.

Activities	Price (US$/ha)	Amount (US$)	%
Soil sampling labor 0-20 cm depth(1ha grid)	5.00	500.00	8.42
Soil sampling labor 20-40 cm depth (1ha grid)	5.00	500.00	8.42
Soil test lab analyses (198 samples)[1/]	10.00	1,980.00	33.35
Constructing soil test results maps with GIS	4.00	400.00	6.74
Lime application (72 ha)	18.00	1,296.00	21.83
Gypsum application (70 ha)	18.00	1,260.00	21.23
Total variable cost		5,936.00	100.00
Cost of capital (discount rate 6 % a.a.)		356.16	
Depreciation (4 years)		1,484.00	
Annualized cost for 100-hectare field		1,840.16	
Annualized cost per hectare		18.40	

[1/]Assumes a composite sample for each hectare. Cost of soil test lab analyses US$10,00 per sample for analyses of pH, Al, H+Al, Ca, Mg, P, K and organic matter.

The total variable cost (Table 1), for grid soil sampling, laboratory analyses, building soil maps and, application of lime and gypsum for 100 ha field was US$ 5,936.00, which represent a cost of the US$ 59.36 ha^{-1}. However, according to National Research Council (1997), the information gained from sampling and mapping soil phosphorus, potassium and, soil acidity, is often used for three to five years, allowing sampling and analysis cost to be amortized over several growing seasons. Thus, allocating the cost of soil acidity management over a useful lifetime of four years, the cost of this technological package become relatively reasonable, US$ 18.40 ha^{-1} (Table 1). It is important to remember that this cost was obtained for an intensive grid soil sampling of 1 ha and, collecting samples from two depths. According to Hennessy *et al.* (1996), optimal sampling depends on trade-offs between potential savings in input expenditures, potential gains from increased yields due to improved management, and sampling costs.

Conclusions

The conventional approache to soil testing, based on averages, was inadequate for characterizing spatial variation of soil acidity. Intensive grid sampling conducted for precision farming studies showed that the indicators of the soil acidity levels within field are variable and can be mapped into management units suitable for variable rate application technology. The annualized cost (US$ 18.40 per hectare) of intensive grid sampling was economically feasible. Since this study is ongoing, little information is available to describe the long-term effects on soil productivity. Innovative soil fertility manager should consider field heterogeneity as a potential alternative to conventional strategies and a way to improve fertilizer profitability, particularly if appropriate yield goals and fertilizer recommendation can be developed.

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DYNAMIC OF NH_3 VOLATILIZATION FOLLOWING DIFFERENT FERTILIZERS SPREADING

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Abstract

Ammonia (NH_3) losses by volatilization following application of both organic and mineral fertilisers have to be investigated in order to both optimize N fertilizer usage and to reduce the environmental issues, such as acidification of soil and eutrophication of aquatic ecosystems. The monitoring of biosphere-atmosphere NH_3 fluxes have to be performed by means of non intrusive methodologies since NH_3 volatilization is a process strongly affected by microclimate. The micrometeorological approach has the right requirements for NH_3 studies. The results of two experimental campaigns carried out using the aerodynamic gradient method and the eddy covariance technique are presented here. Considering the dynamic of NH_3 volatilization, the purpose was to compare what happens after the spread of slurry on bare soil and after the application of urea on a cropland. Following slurry spreading NH_3 volatilization was very strong but last only a few hours, until incorporation of the slurry into the soil. Following urea application NH_3 volatilized only when the soil moisture was sufficient for the hydrolysis to start.

Keywords: micrometeorological methods, slurry, urea, semi-arid climate, humid-temperate climate.

Introduction

World organic and inorganic fertilizer application has greatly increased agriculture production, inducing, on the other hand, environmental issues due to an excess of nitrogen (N) input with respect to system requirements (Galloway *et al.*, 2003). In this context, ammonia (NH_3)

volatilization is one of the major pathways of N losses following N fertilizer applications, representing an economic loss too. Furthermore, NH_3 is implicate in a wide range of environmental stresses such as acidifying deposition, eutrophication in aquatic ecosystems and change in biodiversity (i.a. Galloway *et al.*, 2003). Then, a quantification of NH_3 losses is needed in order to face both environmental and economic issues linked to this N compound.

Animal manure and synthetic fertilizer are the primary sources of NH_3 emissions, accounting for about 23% and 14% of the global NH_3 emissions, respectively (Bouwman *et al.* 2002).

Studies on NH_3 volatilization after slurry/manure report variable NH_3 emission varying from 0 to 60% of the applied ammoniacal N (Sommer *et al.*, 2003) in few hours following the spreading. On the other hand, NH_3 losses by synthetic fertilizers such as urea are characterized by a slow initial phase needed to start urea hydrolysis under optimal temperature and soil moisture conditions. The volatilization in this case can have a long phase of increasing NH_3 fluxes before the extinction of the phenomenon occurs (Pacholski *et al.*, 2008). However, to carry out NH_3 measurements in actual field conditions is a challenge due to the sticky nature of this N compound and the strong dependence of the volatilization phenomenon by microclimate. Nowadays, scientific community is improving methodologies to investigate NH_3 exchanges over a wide range of ecosystems and climatic regions (see for example the European project www.nitroeurope.eu). In particular, micrometeorological approaches are advisable in order to investigate on large space scale (few hectares) without altering the processes involved in NH_3 transfer in the continuum soil-plant-atmosphere.

In this study, the NH_3 dynamic after slurry spreading on bare soil in a humid-temperate region and urea application on an irrigated cropland submitted to a semi-arid climate have been investigated by means of innovative devices employed in micrometeorological methodologies.

Materials and methods

Experimental sites

The first trial **(T-I)** was carried out during the spread of cattle slurry on bare soil in a 19 ha field located in Grignon (48°51' N, 1°58' E) near Paris (France) during spring 2008. The soil type is classified as luvisol (loamy clay: 25% Clay, 70% Silt, 5% Sand). The climate is humid-temperate with mean annual temperature around 11.5 °C and mean annual precipitation of 700 mm. The surface spreading has been made by means of a tanker fitted with a splash-plate, followed by the slurry incorporation into the soil by conventional tillage implementation two days after. The total N supply was around 76 kg ha^{-1}. During the slurry application the weather was mostly fine.

The second experimental campaign **(T-II)** was carried out during and after urea application on a 2 ha field located in Rutigliano (41°N, 17°54' E, 122 m a.s.l.) near Bari in Southern Italy during summer 2008. The soil type is classified as loamy clay (Clay 41%; Silt 44%; Sand 15%). The climate is semi-arid Mediterranean, with mean annual temperature is 15.7 °C and mean annual precipitation of 600 mm. The field was cultivated with *Sorghum vulgare* (cv. *Hay Day*) sowed on 10th June 2008 and irrigated by sprinkler irrigation. Commercial granular urea was broadcast at a rate of 240 kg N ha^{-1} divided into three applications. The amount of urea was artificially increased as compared to usual agronomic practices to increase the probability to detect NH_3 volatilization by innovative devices. During the application the weather was fine and the sky clear.

NH3 measurements

Aerodynamic gradient method (AGM)

The aerodynamic gradient method was used during **T-I** to measure NH_3 fluxes following the approaches developed by Sutton *et al.* (1993). The flux (F_χ) is calculated by:

$$F_x = -u_* \chi_* \tag{1}$$

where u_* is the friction velocity and χ_* the concentration scaling parameter given by:

$$\chi_* = k \frac{\partial \chi}{\partial [\ln(z-d) - \Psi_H]} \tag{2}$$

Here k is von Karman's constant (= 0.41), z is height above the surface, d is zero plane displacement, Ψ_H is the integrated stability correction functions for scalar properties, calculated from the Monin-Obukhov length (L) according to the description of Sutton *et al.* (1993). The NH_3 concentration χ was obtained at three levels by means of an innovative device, ROSAA, developed at INRA (Loubet *et al.*, 2008). It relies on the coupling of NH_3 capturing by means of an acid stripping solution (wet-effluent denuders) to convert NH_3 in NH_4^+, and the analysis of the NH_4^+ concentration by means of a detector based on a semi-permeable membrane and a unit to perform temperature controlled conductivity measurements (ECN, Netherlands). In the ROSAA system the three denuders were sampling at three heights above ground and the averaged concentration was measure every 30 minutes.

Eddy covariance method (EC)

The eddy covariance method (i.a. Kaimal & Finnigan, 1994) was used during **T-II** to directly measure the NH_3 flux, correlating the instantaneous deviations of vertical wind speed (w') and NH_3 concentration (χ'):

$$F_\chi = \overline{w'\chi'} = \overline{w\,\chi} - \overline{w}\;\overline{\chi} \tag{3}$$

The overbar represents the mean of the product over the sampling interval (30 minutes). The application of the EC method requires to sample at a high enough frequency (10 Hz): ultrasonic anemometer are used to monitor wind speed, while fast instruments for real time concentration monitoring of trace gases such NH_3 are based on tunable diode laser (TDLs) technology applied to infrared spectroscopy. The compact QC-TILDAS-76 SN002-U developed by Aerodyne Research Inc. (ARI, USA) (Zahniser *et al.*, 2005) was used during this study.

Results and Discussion

The difference between the slurry and urea dynamic of NH_3 volatilization can be highlighted by comparing the cumulated NH_3 losses (Figure 1) During the T-I, the fast decrease of volatilization, which ended with the slurry incorporation into the soil two days after spreading, is shown by the sharp rise of the normalised cumulative loss of NH_3 immediately after spreading. About 98% of NH_3 emission occurred during the first 24 h in accordance with results of other authors such as Klarenbeek *et al.* (1993) and Wulf *et al.* (2002), which reported about 80% and 88% of the total losses were observed during the first day after slurry spreading. The slow NH_3 release by urea hydrolysis in T-II is confirmed by the trend of the relative normalised cumulative flux pattern (see Figure 1). First of all, the trend showed a slow initial phase (lag of about 7 days) during which the dry soil moisture conditions inhibited urea hydrolysis and consequent NH_3 emissions. Only after irrigation, the second phase of increasing emissions started, after which a third phase of decreasing fluxes (Pacholski *et al.*, 2008) was not observed before the end of the experiment. The slower dynamic of NH_3 volatilization detected during T-II confirms observations carried out by other authors under semi-arid conditions. Sanz-Cobena *et al.* (2008) found that NH_3 losses reached a maximum 4 days after urea application and the phenomenon was monitored for a long period of 36 days. The time evolution of NH_3 losses following both organic and synthetic fertilizers was investigated by means of non intrusive micrometeorological approaches, obtaining realistic cumulative flux patterns in accordance with results reported in literature. The need to perform long term measurements in field after synthetic fertilizer employment was highlighted by the absence of the plateau in the normalized cumulative curve relative to urea trial (T-II), which prevents from estimating the total N losses during this campaign. Then, sufficiently robust devices have to be employed to carry out these kind of experimental campaigns and the equipments employed during these trials seems to be suitable, even if they have to be adequately monitored.

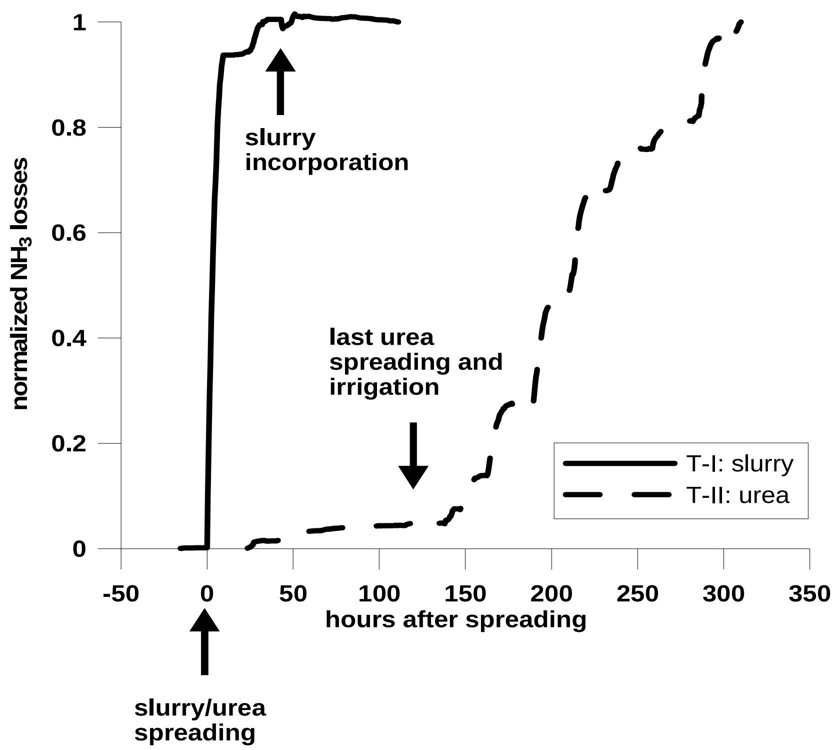

Figure 1: Cumulated NH_3 volatilization normalized to the total NH_3 loss recorded during each trial: T-I, slurry spreading; T-II, urea application.

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WHOLE PLANT REGULATION OF SULFATE UPTAKE AND DISTRIBUTION IN *BRASSICA* SPECIES

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Abstract

Uptake and distribution of sulfate in Chinese cabbage and curly kale were modulated by the sulfate supply to the root and were coordinated with the sulfur requirement for growth. The uptake of sulfate by the root and the expression of the sulfate transporters in root and shoot were up-regulated when the sulfate supply to the root was limited. Prolonged sulfate deprivation led to depleted pools of sulfur metabolites and the development of sulfur deficiency symptoms, e.g. a decreased plant growth and shoot to root ratio. There was poor shoot to root signaling for regulation of expression and activity of the sulfate transporters in the root, whereas the presumed significance of sulfate and thiols as signal compounds in the regulation of sulfate transporters was ambiguous.

Keywords: *Brassica*, sulfate uptake, sulfate transporters, sulfur assimilation, sulfur nutrition.

Introduction

Sulfate is the primary sulfur source for plants, and uptake by the root is under strict metabolic control and is driven by the plant's sulfur demand for growth (Hawkesford and De Kok 2006; Zhao *et al.* 2008). Different sulfate transporter proteins are involved in the uptake, transport and distribution of sulfate in the plant. Plants may contain 12-14 different sulfate transporters, which have been classified in up to 5 different groups according to their cellular and subcellular expression and possible functioning (Hawkesford and De Kok 2006).

Brassica species are characterized by their high sulfur demand and seedlings of Chinese cabbage and curly kale required an overall sulfate uptake of approximately 40 μmol g^{-1} fresh weight root day^{-1} to maintain plant growth (Koralewska *et al.* 2007, 2008, 2009a,b; Shahbaz *et* *al.* 2009). In this paper the current knowledge of the regulation of uptake and distribution of sulfate in *Brassica* will be briefly reviewed.

Materials and methods

Two *Brassica* species: curly kale (*Brassica oleracea* L. cv. Arsis; Royal Sluis, Enkhuizen, The Netherlands) and Chinese cabbage (*Brassica pekinensis* (Lour.) Rupr. cv. Kasumi F1; Nickerson-Zwaan, Made, The Netherlands) were used in the studies. Seeds were germinated in vermiculite and 10-day-old seedlings were transferred to a 25 % Hoagland nutrient solution (pH 6.0) at various sulfate concentrations (0 to 500 µM sulfate; Koralewska *et al.* 2007). For sulfate uptake plants were transferred to a 25 % Hoagland nutrient solution labeled with [^{35}S]sulfate (2 MBq l^{-1}) and incubated for 30 min. Thereafter, roots were rinsed, roots and shoots separated and digested in 1 N HCl for 5 or 7 days and subsequently the radioactivity was measured by using a liquid scintillation counter (Koralewska *et al.* 2007). For sulfate content, plant material was extracted in de-mineralized water and the anions were separated by HPLC on anion exchange column and their contents determined refractometrically according to Maas *et al.* (1986). Total RNA isolation from root and shoot and Northern hybridization of the sulfate transporter transcripts was carried out as described by Koralewska *et al.* (2007).

Results and Discussion

<u>Regulation of sulfate uptake in *Brassica*:</u>

Under field conditions plants face a constant variability of nutrient supply and for example the sulfate concentration in soil may vary from 0.2 to 365 µM (Haneklaus *et al.* 2007a,b). Plants need to adapt their overall sulfate uptake efficiency in order to maintain growth and to avoid sulfur deficiency (Hawkesford and De Kok 2006; Haneklaus *et al.* 2007a,b; Zhao *et al.* 2008). The high affinity Group 1 sulfate transporters, Sultr1;1 and Sultr1;2, are involved in the primary uptake of sulfate by the root (Hawkesford 2003, 2007, 2008).

In *Brassica* species Sultr1;2 was the sole sulfate transporter highly expressed in the roots at ample sulfate supply and was therefore responsible for the sulfate uptake (Table 1; Buchner *et al.* 2004; Koralewska *et al.* 2007, 2008, 2009a,b; Parmar *et al.* 2007). The sulfate contents of both root and shoot were hardly affected by the sulfate concentration in the root environment. Even at 5 µM, a concentration lower or close to the K_m values of the high affinity sulfate transporters, the sulfate content of root and shoot was only slightly lower than that at the higher sulfate concentrations, whereas the total sulfur was hardly affected by the external sulfate concentration (Koralewska *et al.* 2007). At sulfate concentrations ≤ 25 µM, the level of constitutively expressed Sultr1;2 increased, moreover the expression of the second high affinity sulfate transporter Sultr1;1 was slightly induced (Koralewska *et al.* 2007; Table 1). The increased level of mRNAs transcripts for sulfate transporters was accompanied by an up to 2-fold increased sulfate uptake capacity (Koralewska *et al.* 2007; Table 1).

Table 1: Regulation of the expression and activity of the sulfate transporters, and the shoot/root ratio of *Brassica* as affected by the sulfate concentration in the root environment. The data are presented as relative values of that measured at an ample sulfate supply (data derived from Koralewska *et al.* 2007, 2008, 2009a).

Sulfur supply	Sulfate concentration	Sulfate uptake capacity	Transcript abundance		Shoot/root ratio
			Sultr1;1	Sultr1;2	
Ample (>V_{max})	100 - 500 µM	1	0	1	1
Sufficient (≈K_m)	5 - 10 µM	2	4	2	1
Deficient (<K_m)	0 µM	4	35	4	0.5

Sulfate deprivation resulted in a rapid decrease in sulfate and thiol contents in both roots and shoots of *Brassica*, and in an increased sulfate uptake capacity and expression of sulfate transporters in the roots (Buchner *et al.* 2004; Koralewska *et al.* 2007, 2008, 2009a,b; Parmar *et al.* 2007, Stuiver *et al.* 2009; Table 1). Upon a more prolonged sulfate deprivation (longer than 4 to 6 days), the shoot dry matter content started to increase and sulfur deficiency symptoms (yellowing of the young developing leaves) started to develop (Buchner *et al.* 2004; Koralewska *et al.* 2007, 2008, 2009a,b, Table 1). Plant growth started to be adversely affected, although the growth of the shoot was more rapidly affected than root growth, resulting in a decreased shoot to root ratio (Stuiver *et al.* 1997; Buchner *et al.* 2004; Koralewska *et al.* 2007, 2008, 2009a,b). On a whole plant level, the combination of an up-regulated sulfate uptake and a decrease in shoot to root biomass partitioning might facilitate a more efficient overall sulfate uptake. Upon sulfate deprivation the level of constitutively expressed Sultr1;2 was increased and the expression of high affinity sulfate transporter Sultr1;1 was highly induced (Koralewska *et al.* 2007, 2008, 2009a,b; Stuiver *et al.* 2009; Table 1), however, the overall affinity of the sulfate transporters was not substantially affected (Koralewska *et al.* 2007). The substantial increase of expression of sulfate transporters was accompanied with an only up to 4-fold increased sulfate uptake capacity, which demonstrated that there was no direct relation between the level of expression and activity of the sulfate transporters. Upon prolonged sulfate deprivation the Sultr1;2 sulfate transporter was also induced in the shoot of both curly kale and Chinese cabbage and that of Sultr1;1 in curly kale. Furthermore, also the Sultr1;3 (phloem transporter) and the Group 2 transporters (Sultr2;1, and Sultr2;2; low affinity sulfate transporters involved in vascular transport), and Group 4 transporters (Sultr4;1 and Sultr4;2; involved in vacuolar efflux) were highly up-regulated in the root and that of both Group 4 transporters also in the shoot (Koralewska *et al.* 2007, 2008, 2009a; Parmar *et al.* 2007; Stuiver *et al.* 2009). The up-regulation of the Group 2 and 4 sulfate transporters indicated that upon the occurrence of sulfur deficiency, plants try to remobilize and redistribute all possible available sulfate from, for instance, vacuoles and other resources.

Signal transduction pathway involved in the regulation of uptake and distribution of sulfate:

Glutathione is the pre-dominant water-soluble non-protein thiol compound present in plant tissues and may be involved as an inter-organ signaling molecule in the shoot to root regulation between the reduction and assimilation of sulfate in the shoot and the uptake of sulfate by the root, as well as in the cellular regulation of the expression and activity of sulfate transporters (Hawkesford and De Kok 2006). The expression and activity of the sulfate transporters would be modulated by the *in situ* glutathione concentration and would be down-regulated at high and up-regulated at a low levels. Additionally, high levels of sulfate, sulfide and cysteine would down-regulate sulfate transporters (Hawkesford and De Kok 2006).

There was no direct relation between the thiol levels in the root and the activity and expression of the sulfate transporters in *Brassica* (Koralewska *et al.* 2007; Stuiver *et al.* 2009). It is likely that the external and internal sulfate concentration itself was involved in the control of the expression and activity of sulfate transporters. A decrease of the internal sulfate content was accompanied by an increased expression and activity of sulfate transporters in curly kale at $\leq$ 25 μM sulfate, which was probably due to a more rapidly decreasing sulfate content in the cytoplasm.

Foliarly absorbed H_2S may be utilized as a sulfur source for curly kale and Chinese cabbage and result in a down-regulation of the uptake of sulfate by the roots (Westerman *et al.* 2001, 2002; De Kok *et al.* 2002, 2007; Koralewska *et al.* 2008). A simultaneous exposure of sulfate-deprived Chinese cabbage to atmospheric H_2S, at levels which were sufficient for plants to maintain growth at normal level (plant yields were similar to those of sulfate-sufficient plants), had very little effect on the expression and activity of sulfate transporters (Koralewska *et al.* 2008). Sulfate uptake capacity and expression of Sultr1;1 and Sultr1;2 in sulfate-deprived H_2S-exposed plants was quite similar to that of sulfate-deprived non-exposed plants (Koralewska *et al.* 2008). In addition, the decrease in shoot to root biomass partitioning remained largely unaffected. The latter showed a poor regulation of the shoot to root biomass partitioning in the absence of sulfate in the root environment but at an ample sulfur supply from absorbed sulfide.

This may indicate that changes in shoot to root biomass partitioning are rather determined by the sulfate concentration in the root environment than by the sulfur status of the plant itself.

From cut off shoot experiments with curly kale, it was apparent that the sulfate uptake capacity (*viz.* the activity of Sultr1;2) was strongly determined by the sink capacity of the shoot (Koralewska *et al.* 2009b). The shoot sink capacity had only an effect on the sulfate uptake rate itself and not on the expression of sulfate transporters in the root. The discrepancy between the level of gene expression and activity of sulfate transporters in sulfate-deprived plants which shoot had been cut off, also indicated the occurrence of a strong translational and/or post-translational controls (Koralewska *et al.* 2009b).

Further evaluation is required to determine to what extent the observed changes in gene expression and concentrations of metabolites provide insight for the dissection of the signal transduction pathway, as determined at the whole organ level, or at a more localized level, for example in specific root cells or cell layers. Furthermore, cells of both shoot and root have the capacity to reduce and assimilate sulfate in their plastids, which makes it hard to distinguish local signaling at a cellular level from that at an integrated tissue level, *viz.* shoot to root interactions.

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CHANGES OF MINERAL SULFUR CONTENT IN SOILS AFTER $CaSO_4$ FERTILIZER APPLICATION TO OILSEED RAPE

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Abstract

Precise field experiments were established on 3 sites with oilseed rape under different soil-climatic conditions in the Czech Republic. For this experiment, four fertilizing treatments were evaluated. Nitrogen rate was 200 kg $N.ha^{-1}$ for all the treatments. Treatment 1 was fertilized only with N, while treatment 2, 3 and 4 received 12.5 kg $S.ha^{-1}$, 25 kg $S.ha^{-1}$ and 50 kg $S.ha^{-1}$, respectively. Each treatment was conducted in 4 replicates. Sulfur was applied as $CaSO_4$ (NS fertilizer). Fertilizers were applied in two split doses: i) at the beginning of spring vegetation, ii) at the vegetation period BBCH 30-32. Soil samples (0-30, 30-60 cm) and above ground biomass were taken after reaching the periods BBCH 18-20, 30-32 and 50-52 and straw and grain were taken after the harvest. The mineral S content was measured using water extraction. Water soluble S content before regeneration reached about 2.9 mg $S.kg^{-1}$. The biggest increase was obtained in the S fertilized treatments in vegetation periods BBCH 20-28 and BBCH 30-32. Then, the decreasing trend followed. Similar trends were reached measuring S content in plants. The differences between soil samples from 30-60 cm depth were not statistically significant. All obtained values related to soil were compared with S uptake by oilseed rape. Spearman correlation coefficient shoved close relation between S content in soil and plant except samples during harvest. The r^2 reached 0.57 at the p-value 0.01.

Keyvords: Sulfur, water extractable soil S, total S in plant.

Introduction

Sulfur is an important macroelement in plant nutrition. It is part of amino acids, e.g. cystein and methionin, it appears in plant oils (mustard, garlic) and in vitamins (thiamin) (Fecenko 2002).

The request of sulfur content on optimal plants growth ranges between 0.1 and 0.5 % of plant dry mass. The requests on sulfur increase in the row *Poaceae* < *Fabaceae* < *Brassicaceae*. From thus, *Brassicaceae* needs in average 1.1-1.7% (Deloch 1960).

Plants receive the sulfur especially in the form of sulfur anion SO_4^{2-}. This uptake is relatively low influenced with other ions in soil solution and with soil conditions (Balík and Tlustoš 2000).

The total content of S in soil usually ranges between 0.01 and 0.1% (Balík et al. 2007). The main portion of total S in soils is bounded in soil organic matter (Eriksen et al. 1998, Kertesz and Mirleau 2004, Yang et al. 2007). Plant available S consist of: i) soluble inorganic SO_4^{2-}, ii) sorbed inorganic SO_4^{2-}, iii) the portion of organic S in soil that is mobilized during vegetation periods.

Sulfur deficiency in plant nutrition is an actual problem in the Czech Republic (Matula and Pechová 2005, Vaněk et al. 2008) and in other European states (Scherer 2001, Lehmann et al. 2008). It can be attributed to the decrease of atmospheric emissions, less intensive application of mineral fertilizers, restriction of manure and cropping of plants with high S uptake in crop rotation (e.g., rape) on the bigger area. Because of that is important to test the efficiency of new fertilizers.

Material and methods

Precise field experiments were established on 3 sites with oilseed rape under different soil-climatic conditions in the Czech Republic (Humpolec, Hněvčeves and Uhříněves). The sites are characterized in table 1.

Table 1: Characteristic of experimental fields.

Site	**Altitude (m)**	**Mean yearly**		**soil type**	**soil sort**	**pH ($CaCl_2$)**
		rainfall (mm)	**temp. (°C)**			
Uhříněves	295	575	8.3	luvisol	silty loam	6.5
Hněvčeves	265	573	8.2	luvisol	silty loam	6.3
Humpolec	525	665	7.0	cambisol	sandy loam	5.1

For this experiment, four fertilizing treatments were evaluated. Addition of nitrogen at all treatments was 200 kg $N.ha^{-1}$. Treatment A was fertilized only with nitrogen, treatment B with 12.5 kg $S.ha^{-1}$, treatment C with 25 kg $S.ha^{-1}$ and treatment D with 50 kg $S.ha^{-1}$ (table 2). Each treatment was conducted in 4 replicates. Sulfur was applied in the form $CaSO_4$ (NS fertilizer). Fertilizers were applied in three split doses: i) at the beginning of spring vegetation, ii) at the vegetation period BBCH 30-32 and iii) at the vegetation period BBCH 50-52 (only nitrogen).

Table 2: Fertilizing of the experiment.

Fertilizing treatment	1.rate (regeneration)	2.rate (BBCH 30-32)	3.rate (BBCH 50-52)	Total	Total
	kg N ha^{-1} + kg S ha^{-1}			kg N ha^{-1}	kg S ha^{-1}
A	80 + 0	80 + 0	40 + 0	200	0
B	80 + 12.5	80 + 0	40 + 0	200	12.5
C	80 + 25	80 + 0	40 + 0	200	25
D	80 + 25	80 + 25	40 + 0	200	50

Soil (0-30, 30-60 cm) and plant samples were taken at the beginning of vegetation and in the periods BBCH 18-20, BBCH 30-32 and BBCH 50-52 and straw and grain after the harvest. The mineral S content was measured using water extraction 1:10 (w/v). The total sulfur contents in above ground biomass, seed and straw were analyzed using microwave digestion method. Extracts were measured with ICP-OES.

Results and discussion

In the initial uptakes, similar contents of water extractable sulfur (S_w) in topsoil (0-30 cm) and in 30-60 cm depth were found. In dependence on the site, they ranged between 1.8 and 4.2 mg S kg^{-1}. Higher values were always observed in the 30-60 cm depth.

The influence of fertilizing was observable in samples taken in the vegetation period BBCH 18-20. Average content of S_w in the non-fertilized treatment was 4.40 mg S kg^{-1}. After the addition 12.5 kg S ha^{-1} (B), the S_w value increased to 7.2 mg S kg^{-1} and in both treatments fertilized with 25 kg S ha^{-1} (C and D), the contents increased to12.9 mg S kg^{-1} and 17.5 mg S kg^{-1}, respectively. Measured data corresponds with results published by Shan et al. (1997), who estimated the ratio of S_w between 8.0-34.2 mg S kg^{-1} at soils with different fertilizing systems.

A similar trend appeared after reaching the vegetation period 30-32. In the topsoil from the non-amended treatment, an average 4.5 mg S kg^{-1} was estimated. The content of S_w at the treatment B was 7.4 mg S kg^{-1} and at the treatments C and D (25 kg S.ha^{-1}) it reached about 8.5 mg S kg^{-1}. Also in the vegetation period BBCH 50-52, the differences between treatments were evident. The lowest content of S_w was estimated again at non-fertilized treatment A (3.1 mg S kg^{-1}). The content at the treatment B was 4.2 mg S kg^{-1} and at treatment C 5.4 mg S kg^{-1}. From the soil samples after harvest it is evident, that the content of S_w decreased and was almost equal for all treatments. The average value from all treatments reached 1.4 mg S kg^{-1}

with a standard deviation of only ±0.2 mg S kg^{-1}. The changes in the S_w contents in the topsoil during the whole experiment are depicted in Figure 1.

Significant differences were not found between the S_w contents in the 30-60 cm depth. The measured values reached about 4.0 mg S kg^{-1} at all treatments during the experiment.

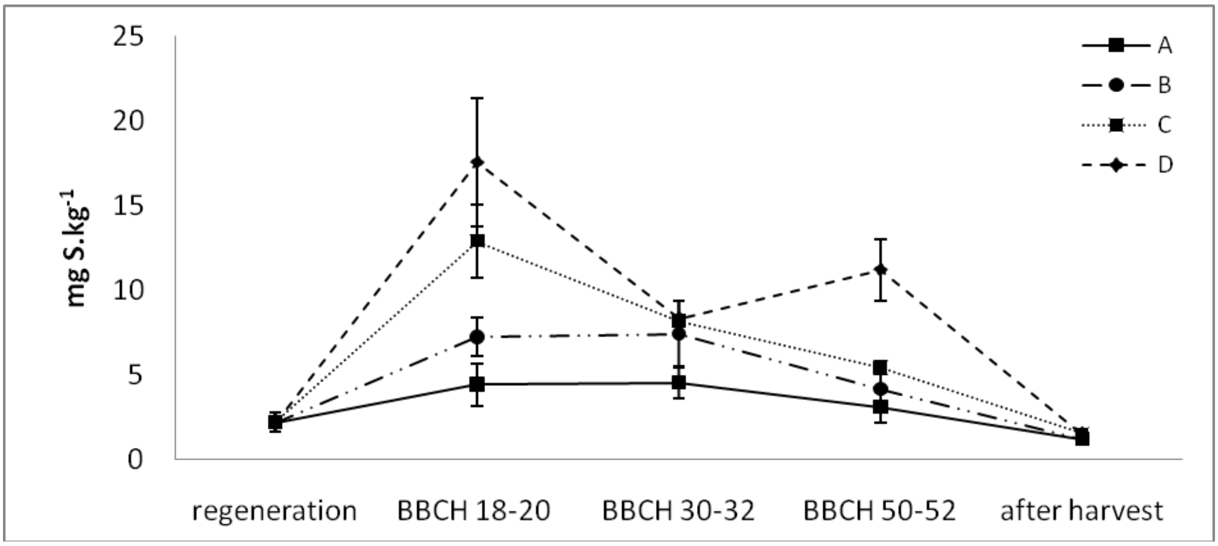

Figure 1: Average contents of S_w in topsoil during vegetation.

The samples of above ground biomass were taken at the beginning of regeneration, during the vegetation periods BBCH 18-20, BBCH 30-32 and BBCH 50-52 and the samples of straw and seeds were taken during the harvest. From Figure 2 it is clear that the total contents of sulfur in plants taken before the regeneration did not significantly changed. The average value from all sites reached 0.42% S (±0.01 % S).

After the first addition of fertilizers (samples from the vegetation period 18-20), the content of total sulfur increased at all treatments included the non-amended one. The highest increase was observed at treatments C and D, where 25 kg S ha^{-1} was added. Here 0.55% S and 0.54% S, respectively, was estimated.

The highest increase of sulfur content in above ground biomass was observed in the vegetation period BBCH 30-32. The amount of total sulfur reached 0.51% S at the control treatment, 0.57% S at treatment B (12.5 kg S ha^{-1}) and at treatments C and D (25 kg S ha^{-1}) it was estimated to be 0.62% S and 0.64% S, respectively.

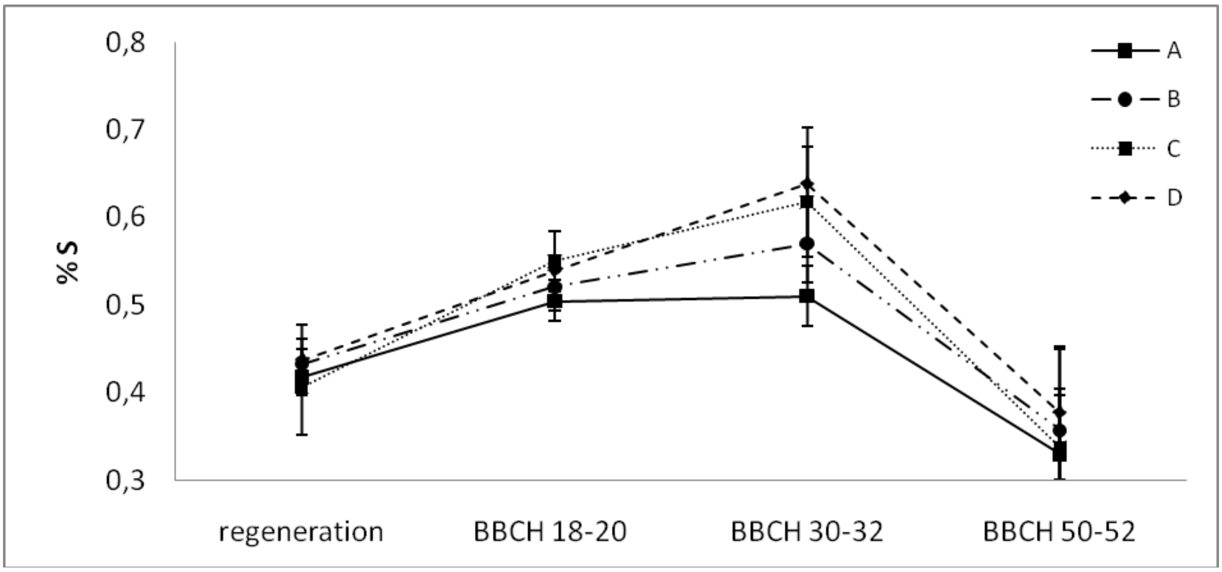

Figure 2: The total content of sulfur in plants during vegetation.

In the vegetation period BBCH 50-52 a significant decrease of total S content in above ground biomass was observed at all treatments. Significant differences were not found between different treatments. The lowest average content was found at the non-fertilized treatment (0.32 % S) and the highest at treatment D (50 kg S ha^{-1}), where it was estimated to be 0.37 % S.

The results of seeds and straw analysis are reported in Table 3. No significant differences were found between fertilizing types.

Table 3: Average contents of total sulfur in seed and straw of oilseed rape.

Treatment	% S in straw	std. deviation	% S in seeds	std. Deviation
A	0.35	0.14	0.12	0.02
B	0.37	0.12	0.12	0.02
C	0.38	0.12	0.13	0.02
D	0.37	0.12	0.12	0.02

The highest content of sulfur in straw was found at the Uhříněves site, where the values reached about 0.52 % S. It resulted in relatively high standard deviations, because the values at the Humpolec and Hněvčeves sites reached only about 0.30 % S. The S contents in seed were almost equally at all sites and they ranged about 0.12 % S with minimal standard deviations.

The contents of S_w were compared with total S contents in upper biomass using correlation analysis. Because of non-normal distribution of the evaluated data, the Spearman correlation coefficient was used. Close relations were found: correlation coefficient r^2 reached 0.57 at the p-value of ≤ 0.01.

Acknowledgements

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RISK ELEMENTS IN THE SOIL IN RELATION TO THE ENVIRONMENT

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Abstract

Based on the results of project, which was applied in the frame of the Community Initiative program INTERREG IIIA and focused on environment protection, critical loads for agricultural and forests soils were determined - a quantitative estimate of an exposure to one or more pollutants which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge.

For the critical loads calculation, it was necessary to determine risk elements (heavy metal) and risk matters (organic pollutants) in the soil - in the several soil horizons.

Keywords: EU project, monitoring, risk elements, risk matters, soil acidity.

Introduction

Continuous mountain range along Bohemia–Bavarian border represents a huge, homogenously composed and exploited, natural environment. This geographical unit acts like a barrier to prevailing western air flow and related long-range transport of various substances. Due to that, deposition of ever-present pollutants like heavy metals, polychlorinated hydrocarbons, dioxins, furans and acidifying substances, produced by human activity is much higher there. Forrest damage often present in this region is strongly connected with this stressing situation, mainly atmospheric (acid) deposition.

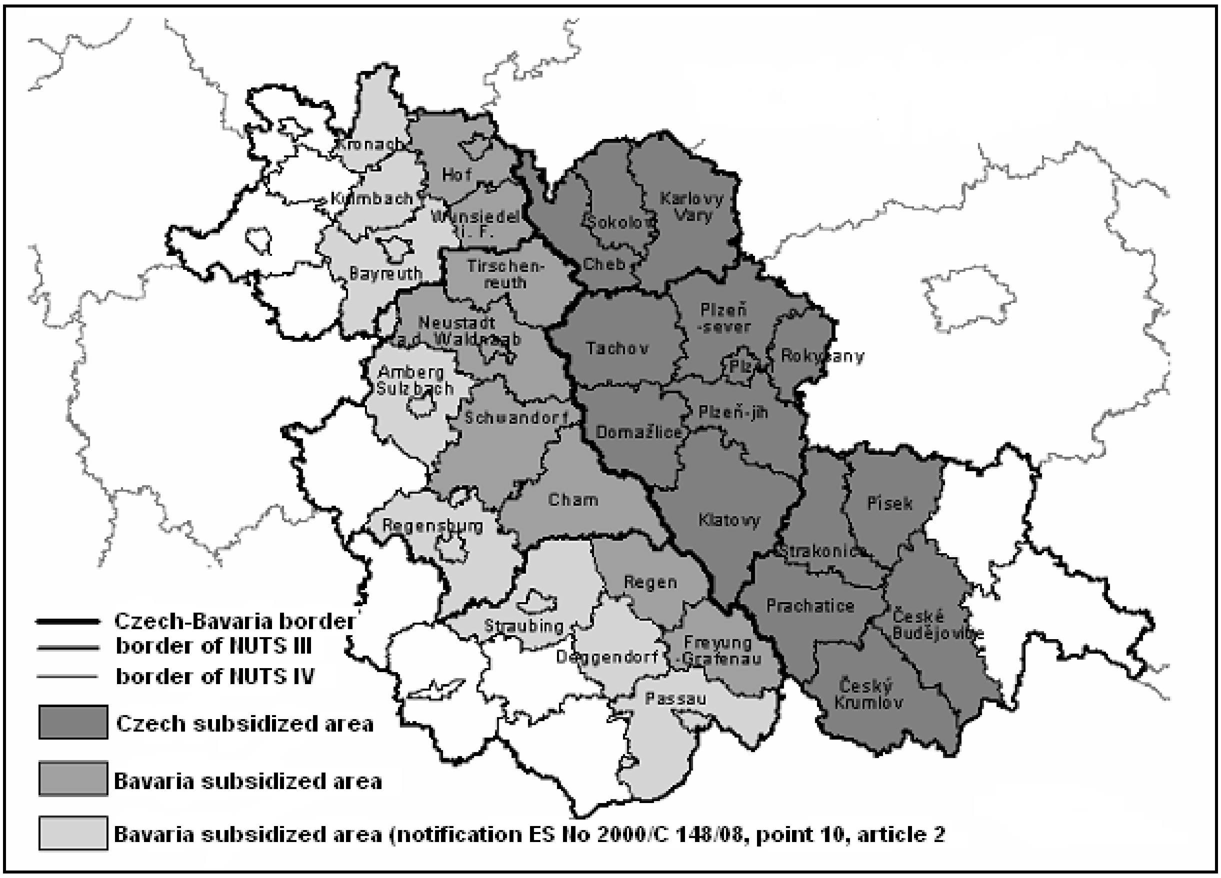
The results of the project are being compiled into well-arranged database giving freely accessible information about basic soil properties as well as risk elements in the soil. These results are important for next work – critical loads calculation.

The critical loads calculation (mainly in forest soils) is the main task of the next project No 75 "Effects of acidification on the soil and water sources", supported and co-financed by structural EU funds in frame initiative European community "Aim 3" (Figure 1).

Acidification causes of forest soils:

- natural acidification
- biomass extraction from the forest
- human industrial activities – SO_2 emission and NO_x traffic emission

Figure 1: The map of area of interest - initiative European community "Aim 3".



Materials and methods

The international project on monitoring of agricultural and forest soils started in 2006 under the EU subsidy. A special monitoring grid 8 x 8 km of sampling pit-points was designed along the Czech-Bavaria border area. There are 278 monitoring points along the Czech-Bavaria border (Figure 2). On monitoring points, the soil pits were made and the description of soil profiles and soil sampling followed. Soil pit was dug manually; no machinery was allowed in order to avoid possible contamination. No smoking, cosmetics or repellents were allowed.

Figure 2: The grid of monitoring point on the Czech territory (yellow points = arable land, green points = grassland, grey points = forests).

Aims of the project

- determination of soil physical properties,
- determination of organic pollutants in the soil: PCB, PAH, DDT, HCH,
- determination of sellected elements in Aqua Regia mineralisate and in water and 1M NH_4NO_3 leach,
- determination of available nutrients and sulphur in the soil in Mehlich III leach,
- determination of exchangable pH,
- determination of C_{tot}, N_{tot},
- determination of cation exchangable capacity (CEC),
- determination of amount of released and possible leaching of e.g. Al, Fe and heavy metals from (acidified) soil into groundwater,

- determination of real buffering capacity of soils in relation to acid deposition,
- prognoses of possible inputs of risk matter into groundwater,
- presentation of results in form of cross-border thematic maps.

Results and Discussion

Risk elements (heavy metals)

Within the frame of the project 14 elements were analysed (As, Be, Cd, Co, Cr, Cu, Mo, Ni, Pb, Sb, Sn, Tl, V, Zn). The most ecology important five elements (Cd, Co, Ni, Pb, Zn) were included into evaluation of the fraction available in 1M ammonium nitrate solution.

Table 1: Median of risk element content (ppb; 1M NH_4NO_3).

		Cd	**Co**	**Ni**	**Pb**	**Zn**
organic horizon	forest	118	322	1 040	2 440	11 200
organomineral horizon	forest	38.5	234	327	2 635	2 180
	grassland	17.6	50.3	67.1	10.0	285
	arable land	5.4	20.2	20.5	4.5	103
mineral horizon	forest	16.6	396	169	504	878
	grassland	7.0	77.1	111	4.5	185
	arable land	4.7	89.0	95.5	4.5	127

Elements contents (available in 1M ammonium nitrate solution) in organic horizons of forest soils are several times higher than in other horizons. The content of element in this fraction consists in many factors, especially on:

- pH value,
- organic carbon content,
- cation exchange capacity,
- total content of element.

Carbon content in the forest soil organic horizon (C_{ox}) is 27% (median), a range of values is 12 - 40%. Carbon content in other soil horizons (C_{tot}) is 1,58% (median; arable land) and 2,08% (median; grassland).

Chosen elements are pH-negatively dependent. Forest soils have the lowest pH and on the other side arable lands have the highest pH. The highest element contents are related to pH about 3.4 (forest - figure 3), a decline of element contents follow up to pH about 5.8 (arable land).

Figure 3: pH value in forest soil - horizon A.

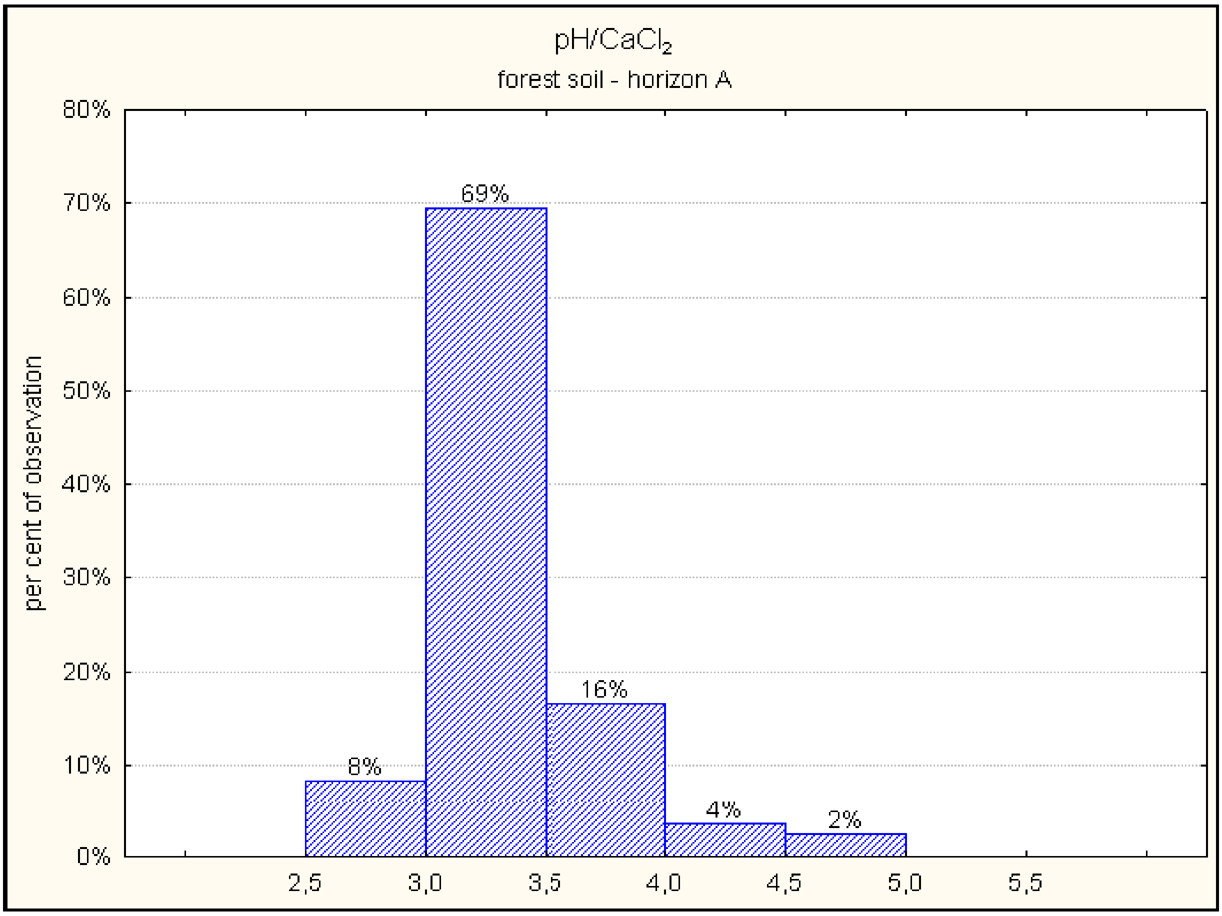


Figure 4: Example - cadmium content (1M NH_4NO_3, ppb), pH, C_{tot} (%) in soil samples from organomineral horizon.

Persistent organic pollutants (POPs):

Within the frame of this project PCB (7 congeners), OCP (HCH, HCB, DDT) and PAH (16 individual hydrocarbons) were determined.

Table 2: Example - PCB (polychlorinated biphenyls) in soil.

	organic horizon	organomineral horizon			mineral horizon		
	forest	forest	grassland	arable land	forest	forest	grassland
Average	21.10	2.31	1.94	2.27	1.77	1.76	1.78
Median	23.35	1.75	1.75	1.75	1.75	1.75	1.75
Minimum	4.15	1.75	1.75	1.75	1.75	1.75	1.75
Maximum	62.0	11.1	4.8	12.9	3.9	2.0	2.5
Number of samples	104.0	104.0	88.0	56.0	104.0	88.0	56.0

- the highest contents of substances are in organic horizon of forest soils
- PCB -the major part of samples are below a limit of quantification
- HCB, lindane –contents decrease in following sequence: forest >grassland >arable land
- Lindane -contents aresignificantlydifferent between plantations(A, B horizons)
- DDT –values distribution is different from PCB, HCB, HCH
- DDT –the highest contents are in altitude below 500m
- DDT –metabolic transformation to DDE in SW part of interest area is well-marked

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HBED/FE^{3+}, A NEW SOLUTION OF IRON CHLOROSIS IN DICOT PLANTS

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Abstract

Fe fertilizers applied to the soil are widely used, and constitute the most common Fe chlorosis remediation technique. Among them, only the most stable chelates, especially chelates derived from polyamine-carboxylic acids, are able to maintain Fe in calcareous soil solution and to transport it to the plant root. Nowadays, *o,o*-EDDHA (ethylenediamine-N,N´-bis(*o*-hydroxy-phenylacetic) acid) and its analogues are the most efficient. HBED (N,N'-bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid) presents a similar structure to *o,o*-EDDHA and forms a very stable Fe chelate. This chelating agent has been traditionally used in oral drugs to remove excessive Fe from humans but had never been used in agriculture due its elevate Fe stability constant and price. Now Fe-HBED is available as a pure and affordable product. In this work, different doses of *o,o*-EDDHA/$^{57}Fe^{3+}$ and HBED/$^{57}Fe^{3+}$ were studied in soybean plants grown in a calcareous soil. Chelates prepared with the isotope ^{57}Fe were used to differentiate the Fe uptake from the chelate and from other sources. Several parameters related to the plant nutritional status, such as plant growth, SPAD index, ^{57}Fe and total Fe concentration in leaf and root, and Fe/Mn ratio were determined. We conclude that *o,o*-EDDHA/Fe^{3+} presents a faster action than HBED/Fe^{3+} and HBED/Fe^{3+} presents more long lasting effect than *o,o*-EDDHA/Fe^{3+}. The results obtained show that HBED/Fe^{3+} can be a good chelate to correct Fe chlorosis.

Keywords: iron, HBED, fertilizer, ^{57}Fe.

Introduction

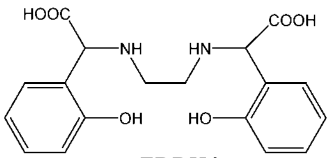
HBED (N,N'-bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid) is a strong Fe^{3+} chelating agent which has traditionally been used as an oral drug to remove excessive Fe from humans. Its structure (Figure 1) is similar to that of *o,o*-EDDHA (ethylenediamine-N,N´-bis(*o*-hydroxy-phenylacetic); however HBED has never been used in agriculture to solve Fe chlorosis because of the high stability constant of the iron chelate (~10^4 times greater than that of *o,o*-EDDHA (Ma *et al.*, 1994, Yunta *et al.*, 2003)) and price. Recently, Nawrocki *et al.*, (2009) propose a new process for the preparation of HBED in order to reduce production costs and make this chelate more affordable to be used as iron fertilizer.

Chaney (1988) showed that HBED offered better selectivity for Fe^{3+} compared to EDDHA in computer-modelled nutrient solutions, and that adequate Fe was supplied for growth of non-graminaceae species at pH 7.5. However, the use of HBED instead of EDDHA was suggested only for plant research. In line with our research work towards the development of new chelating agents for the treatment of Fe chlorosis, we have taken up again the study of the suitability of HBED to provide Fe to plants. López-Rayo *et al.* (2009) have proved that HBED is able to maintain available Fe in solution under different agronomic conditions (nutrient solutions and soil conditions), even when competing cations, such as Cu^{2+} or Ca^{2+}, are present. Nadal *et al.* (2009), in agreement with Chaney (1988), have concluded that HBED/$^{57}Fe^{3+}$ supplied sufficient Fe for the growth of Strategy I species (soybean plants) at pH 7.5 in hydroponic cultures. Besides, we have shown that plants treated with HBED/$^{57}Fe^{3+}$ had a lower Fe uptake rate than plants treated with *o,o*-EDDHA/$^{57}Fe^{3+}$ in hydroponic conditions, with both behaving similarly when used as substrates of the ferric-chelate reductase (FC-R). In agreement with Lucena and Chaney (2007), this raises a question about the effect of the chelate, not only on the reduction mechanism, but also on the transport across the root plasma membrane (IRT1).

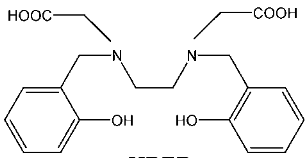
In this work, the efficacy of HBED/Fe^{3+} to supply Fe to plants in calcareous soil conditions, compared to that of *o,o*-EDDHA/Fe^{3+}, was evaluated. The Fe nutrition was studied considering the Fe uptake from the chelates prepared with the isotope ^{57}Fe and measured by ICP-MS (Rodríguez-Castrillón et al, 2009) in order to differentiate the Fe uptake from the chelate and from other sources.

Materials and methods

For the experiment, 6 days old, middle chlorotic soybean seeds (*Glycine max* L. cv 'Klaxon') were transplanted into 1 L pots (three plants per pot) filled with a sandy clay soil (pH in H_2O 7.70, O.M. 9.2 g·kg^{-1}, total $CaCO_3$ 380 g·kg^{-1}, active lime 89 g·kg^{-1}) from Picassent (Valencia, Spain) and with sand (975 g·kg^{-1} $CaCO_3$, 1-3 mm size) in 2:1 v:v mixture. The experiment was carried out in a research growth chamber model CCKF 0/16985. The growth daily cycle was day: 16 hours, 30ºC 50% humidity; and night: 8 hours, 25ºC, 70% humidity. Pots were initially irrigated till 80% field capacity and then daily with the amount of solution necessary, determined by weight loss, to achieve again 80% field capacity. Irrigation was made with a macronutrient nutrient solution (2 times concentrate) with 0.1 $g \cdot L^{-1}$ lime and 0.1 $g \cdot L^{-1}$ sodium bicarbonate. Trays were put under the pots to avoid leaching. The experiment consisted on two treatments with six different doses and one control without Fe (three pots). Treatments were applied 7 days after transplanting. The concentrations of ^{57}Fe in the treatments were 0, 1.7, 3.4, 8.4, 16.8, 25.1 and 41.9 µmol $^{57}Fe \cdot kg^{-1}$ of soil in the form of *o,o*-EDDHA (Promochem, Spain) or HBED (Strem Chemicals, England) (see figure 1). Two and one plants were sampled at 7 and 21 days after the treatment, respectively.



o,o-EDDHA



HBED

Figure 1: Chelating agents used.

SPAD index was determined in all the leaf levels every two days. At sampling times, leaves and stems were separated and washed, weighed and dried. Total Fe and ^{57}Fe were determined in leaves after dry digestion by AAS and ICP-MS, respectively.

After the plant experiment was finished the soluble and available ^{57}Fe in the soils were determined. The complete pot content (including roots) was submerged in 1 L distilled water and shaked until complete dispersion of the substrate. Roots were taken apart to be analyzed and 40 ml of the substrate-water mix were centrifuged and the supernatant filtrated. For the analytical determination HNO_3 suprapur (1%) was added. ^{57}Fe was determined in these extracts by ICP-MS. The remaining solid in the centrifuge tube was extracted for 20 minutes with 25 ml of Soltanpour and Schwab (1977) extractant (DTPA + Ammonium bicarbonate) and then filtrated. The extraction was repeated for three times. The extracts were joined in a 100 mL volumetric flask and made up to volume. HNO_3 was added to eliminate excess bicarbonate and to allow an acid media for the analytical determinations of ^{57}Fe by ICP-MS and total Fe by AA.

Means were compared using Duncan's test at $p<0.05$ in order to find significant differences among treatments. Statistical analysis was done using SPSS 16.0.

Results and Discussion

SPAD readings in the 3rd leaf stage (the youngest fully developed leaf at the start of the treatment period, t = 0) has been presented in Figure 2. No significant differences between treatments could be observed and the doses that provide a higher SPAD index were 8.4, 25.1 and 41.9 µmoles ^{57}Fe/kg of soil.

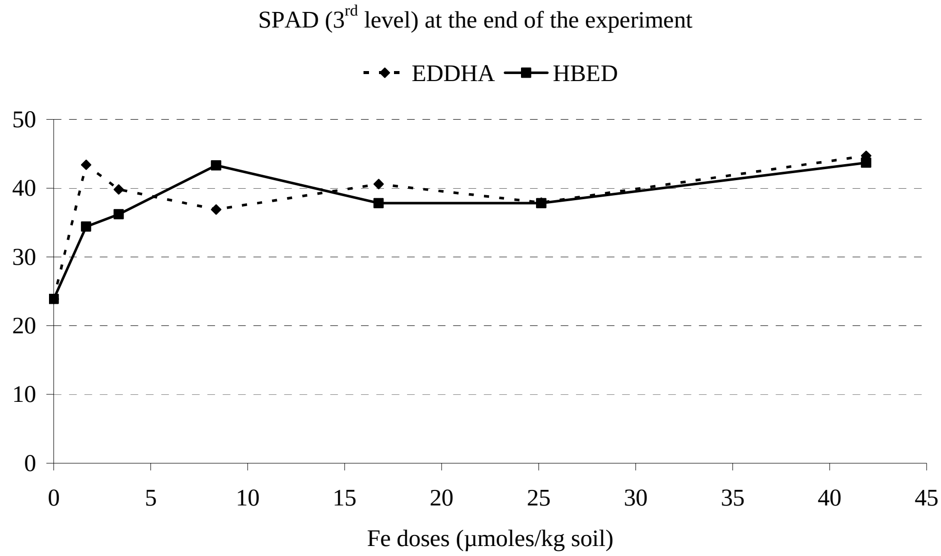


Figure 2: Evolution of SPAD index for the 3rd leaf stages along time.

In figure 3 the ^{57}Fe concentration in leaves with the different Fe doses is presented for the two treatments in the second sampling time. The results obtained have been adjusted to an exponential equation ($^{57}Fe = K_d * Dose^{1/n}$). K_d is the constant that determinate the chelate affinity. The higher the K_d the higher the affinity. *n* represent the order. As *n* increase, the saturation is reached sooner. In this case, HBED/$^{57}Fe^{3+}$ presented a better affinity in the second sampling. With low doses no differences could be observed between treatments, however these different are more significant when higher doses are applied. These results show that to obtain the same ^{57}Fe absorption by the plant it is necessary a higher dose of HBED^{57}/Fe^{3+} than of *o,o*-EDDHA/$^{57}Fe^{3+}$. This difference between doses is lower in the second sampling time than in the first sampling (data not shown). These results indicate that *o,o*-EDDHA/$^{57}Fe^{3+}$ has a faster effect than HBED^{57}/Fe^{3+}, however HBED^{57}/Fe^{3+} has a longer lasting effect. This is in good agreement with the higher stability of HBED^{57}/Fe^{3+} (log Kº = 39.01 (Ma *et al.* 1994)) than *o,o*-EDDHA/$^{57}Fe^{3+}$ (log Kº = 35.09 ± 0.28 (Yunta *et al.* 2003)) and with previous findings that show that lower stable chelates (e.g *o,p*-EDDHA/Fe^{3+}) have a faster effect, but during less time, than chelates of high stability (e.g *o,o*-EDDHA/Fe^{3+}) (García-Marco et al, 2006).

At the end of the experiment (21 days after treatment application) the solubility and availability of ^{57}Fe in soil were measured. In Table 1 the percentage of the applied isotope measured in the soluble and available forms in the soils and in leaves + roots is presented. According to known absorption patterns, the higher the doses applied, the lower the percentage of Fe that is taken by the plant and the higher the amounts remaining in the soils. Between chelates the differences are low.

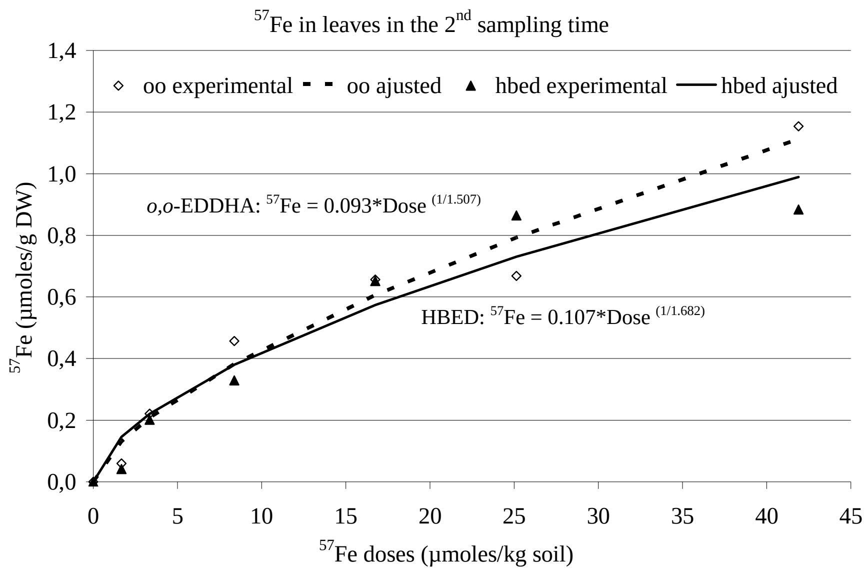


Figure 3: ^{57}Fe concentration (µmol/g DW) in leaves in the second sampling time.

The results obtained are in agreement with previous work of our research group that showed that plants treated with HBED/$^{57}Fe^{3+}$ had a slight lower Fe uptake rate than plants treated with *o,o*-EDDHA/$^{57}Fe^{3+}$ in short-terms hydroponic culture, both behaving similarly when used as substrate of the FC-R (Nadal et al, 2009).

Table 1: Percentage of applied ^{57}Fe measured in the soluble and available forms in the soils and in leaves + roots.

Treatment	Doses (µmoles ^{57}Fe/kg)	% ^{57}Fe		
		Soluble in soil	Available in soil	Roots+Leaves
***o,o*-EDDHA/$^{57}Fe^{3+}$**	3.35	0	0	48
	8.38	11	0	25
	16.75	15	27	20
	25.13	18	37	17
	41.88	22	31	15
HBED/$^{57}Fe^{3+}$	3.35	0	0	37
	8.38	9	0	25
	16.75	21	21	20
	25.13	29	23	18
	41.88	31	38	12

We can conclude that HBED/Fe^{3+} presents a slower action and more long lasting effect than *o,o*-EDDHA/Fe^{3+}. However, the experiment here presented is a short experiment of three weeks, so it is expected that at longer periods the efficacy of HBED^{57}/Fe^{3+} would increase with respect to *o,o*-EDDHA/$^{57}Fe^{3+}$. Anyway, in agreement with other authors as Chaney (1988) and with other assayed of our group (López-Rayo *et al.*, 2009, Nadal et al, 2009) the results obtained show that HBED/Fe^{3+} can be a good chelate to correct Fe chlorosis.

Acknowledgements

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THE WHOLE-OF-BLOCK EXPERIMENTAL APPROACH FOR MEASURING SPATIALLY VARIABLE RESPONSES TO TREATMENTS

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Abstract

Precision agriculture offers opportunities for the development of new approaches to on-farm experimentation to assist farmers with site-specific management decisions. Farmers faced with variable conditions need to optimize their management to variation over space and time on their farm, which is not solved by conventional approaches to experimentation. New designs for on-farm experiments were developed in the 1990s for cereal production in which the whole field was used for the experiment rather than small plots. We explore the extension of this type of experiment to a vineyard in the Clare Valley of South Australia in order to evaluate options for increasing grape yield and vine vigour. Manually sampled indices of vine performance measured on georeferenced 'target' grapevines were analysed applying geostatistical procedures. The major advantage of such an approach is that the spatial variation in response to experimental treatments can be examined. The results indicate that both treatment responses and the significance of differences between them are spatially variable. Thus, we conclude that whole-of-block on-farm trials are a useful tool to provide valuable spatial information about treatment responses and will therefore aid farmers in improved decision making.

Keywords: On-farm trials, Viticulture, Geostatistical analysis, Whole-of-block experimentation.

Introduction

Appropriate and targeted fertilisation is one of the most important management practices for farmers to achieve economically acceptable yields and to minimise negative impacts such as phosphorus and nitrogen pollution of ground and surface waters. The development of precision agriculture (PA) technologies involving GPS, GIS, a variety of sensors and geostatistical analysis to detect and manage in field variability, also enables new opportunities for field experimentation. For more robust decision-making, especially when PA management strategies are used, farmers, advisors and scientists need to know the likely crop response to a certain management practice across the range of soil and climate conditions of the farm or field. New designs for on-farm trials were developed in the 1990s (Adams and Cook, 1997, 2000, Cook *et al.*, 1999, Doerge and Gardner, 1999 and Pringle *et al.*, 1999) for cereal production in which the whole field was used for the experiment rather than small plots. This approach, which we refer to as 'whole-of-block experimentation', has also recently been applied in viticultural experiments (Bramley *et al.*, 2005, Panten *et al.*, 2009). It involves the establishment of experiments over an entire management unit, such as a cereal field, vineyard block or orchard and therefore covers the whole range of spatially heterogeneities within the unit. The highly replicated designs used, together with high density data obtained from systems like yield sensors, allow the analysis of the results using geostatistical methods. However, analysis of the results of the early experiments relied predominantly on simple map algebra for determining treatment response and was not supported by rigorous statistical analysis. Recently, Bishop and Lark (2006) developed a geostatistical process for analysing whole-of-block experiments. Here, we illustrate the benefits of this approach with a trial which was carried out in an organically managed vineyard (4.8 ha) planted to Merlot in the Clare Valley, South Australia.

Materials and methods

The experiment was established in response to concerns that the organic management system may be placing a constraint on vine nutrition resulting in low yield and vine vigour. The vineyard manager was also concerned that the permanent ryegrass (*Lolium perenne*) cover crop in the mid rows of the vineyard was competing with the vines for water and nutrients. The experiment was therefore established with the objective of evaluating the merits of three mid-row management strategies aimed at enhancing vine vigour and yield through promotion of nutrient cycling. These were: ryegrass combined with either undervine compost (RGC; control) or mulch (RGM), or a cereal (*Triticum triticosecale*) cover crop sown in alternating rows with a legume (*Vicia faba*) in the intervening rows (CL) (Fig. 1a). The treatments were implemented in winter 2004 with one buffer row between treatments.

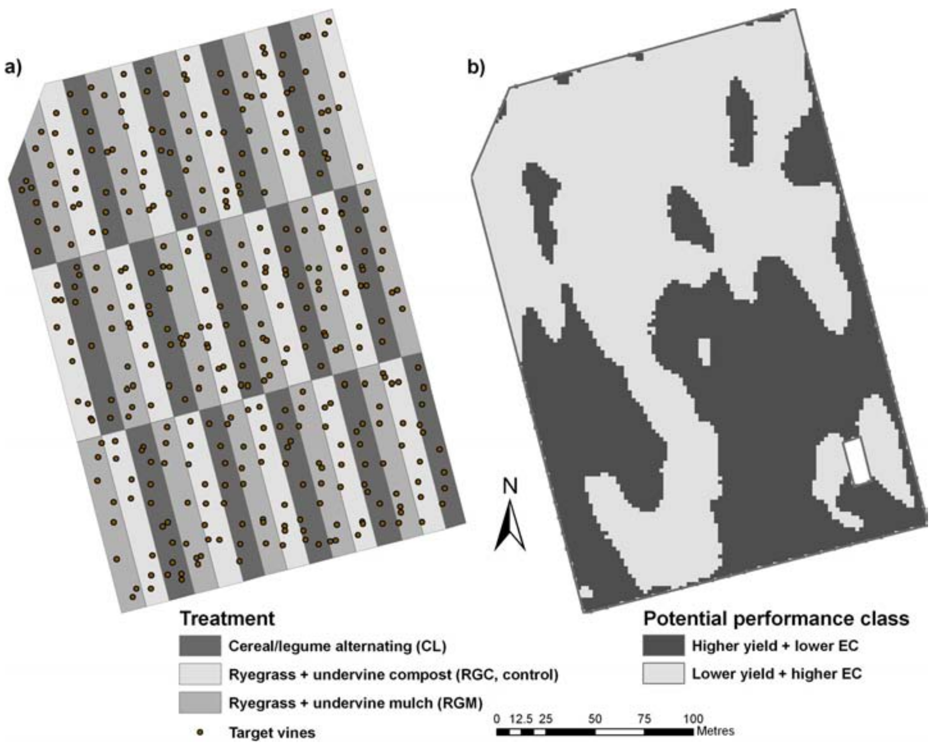

Figure 1: (a) Experimental layout and (b) potential performance classes generated from yield and EC_a data in 2004.

Treatment responses at vintage 2006 were assessed through a combination of data collected at high spatial resolution (airborne remote sensing, yield mapping) and through a program of intensive hand sampling of target vines (126/treatment). The latter permits the evaluation of vine vigour parameters such as bunch numbers, bunch weight, berry weight and berries per bunch for which sensors are not available.

The use of the *k*-means cluster technique on bulk soil conductivity (EM38) data and the pre-experiment yield map from 2004 resulted in the delineation of two potential vine performance classes (Fig. 1b). Treatment response data was evaluated firstly by analysis of variance (ANOVA) conducted with R (R Development Core Team, 2007), modelling the spatial dependency of the error term using the 'gls' function to fit the extended linear model with residual maximum likelihood (REML). The suitability of the spatial correlation models was evaluated using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC).

Secondly, treatment responses were spatially analysed using the approach developed by Bishop and Lark (2006). In this approach, treatment responses are seen as random variables and therefore can be estimated for any part of the experimental site. This also allows the calculation of contrasts between different treatments. Bishop and Lark (2006) recommend to use cokriging after modelling the treatment responses as coregionalized variables with a linear model of coregionalization (LMCR; Journel and Huijbregts, 1978). Because the treatment response for different treatments can only be observed at different locations, pseudo cross-semivariograms (Papritz *et al.*, 1993) are used to model the cross-covariance of the treatment responses. Bishop and Lark (2006) found that standardized ordinary cokriging (SOCK) was the best predictor of treatment responses and contrasts and we therefore used SOCK to estimate these for each cell in a 2 m grid derived from the vineyard block boundary. Additional details of this methods of data analysis are given in Panten *et al.* (2009).The statistical significance of treatment differences was tested for each grid cell by evaluation of the standardized normal variable z: if $|z| > 1.96$ the difference is regarded significant at the 5 % level and if $|z| > 1.654$ and < 1.96 the difference is regarded significant at the 10 % level.

Results and discussion

Comparisons of the average treatment responses using ANOVA (Tab. 1) reveal that the CL treatment has the strongest effect in terms of increasing bunch number, bunch weight and the number of berries per bunch.

Table 1: ANOVA results of vine performance variables recorded during vintage 2006 on 378 target vines by treatment and by treatment and potential performance class.

Variable	Treatment			ANOVA - *p*
	RGC	RGM	CL	
Bunch numbers	45[b]	43[b]	56[a]	*** < *0.0001*
Bunch weight [g^{-1}]	57[b]	63[a,b]	68[a]	*** < *0.0001*
Berry weight [g^{-1}]	0.77[a]	0.82[a]	0.81[a]	* *0.0409*
Berries/Bunch	71[b]	76[b]	84[a]	*** < *0.0001*
n	*126*	*126*	*126*	

	Treatment & potential performance class						
	RGC HY	RGC LY	RGM HY	RGM LY	CL HY	CL LY	
Bunch numbers	53[a]	35[b]	49[a]	37[b]	59[a]	54[a]	*** < *0.0001*
Bunch weight [g^{-1}]	76[a]	35[d]	77[a]	48[c]	87[a]	60[b]	*** < *0.0001*
Berry weight [g^{-1}]	0.87a	0.66[c]	0.92[a]	0.71[b,c]	0.92[a]	0.77[b]	*** < *0.0001*
Berries/Bunch	86[a,b]	53[d]	84[b]	68[c]	96a	79[b]	*** < *0.0001*
n	*67*	*59*	*65*	*61*	*38*	*88*	

RGC = Ryegrass and compost under the vine; RGM = Ryegrass and mulch under the vine; CL = Row alternating cereal and legume; HY = Higher Yield and lower EC_a; LY = Lower yield and higher EC_a.
[1] Levels not connected by the same letter are significant different at the 5 % level (Tukey-Kramer *t*-test).

These three variables also show significant differences between the treatments. By separation of the treatments into a higher and lower potential performance class (Fig. 1b) a lot more information is gained. For example, whereas bunch numbers in the CL treatment are generally higher than in the RGC and RGM treatments, it can also be seen that the increase was much bigger in the lower yielding areas than in the higher yielding parts of the block. These results can be taken as a first indicator that the treatment effects indeed vary spatially.

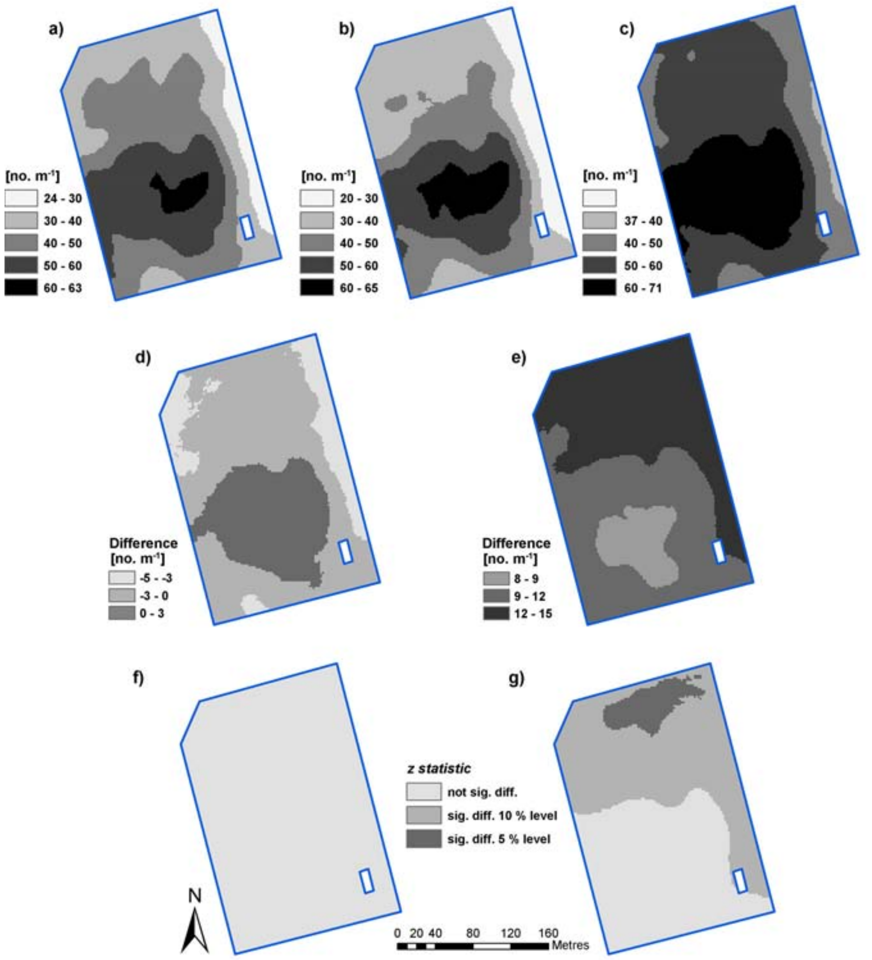

Figure 2: Treatment effects estimated in terms of bunch numbers per metre for the: (a) RGC, (b) RGM and (c) CL treatments. Also shown are maps of the difference between the RGM and RGC treatments (d) and between CL and RGC (e). Maps of the related z-statistics are also shown for point-wise comparisons between: (f) RGM and RGC and (g) CL and RGC.

Using these experimental results for decision making requires knowledge of where in the vineyard each treatment was beneficial. Unlike researchers, most vineyard managers will not base their decisions on a significance test but will be more interested in the absolute magnitude of the difference. Nevertheless, it would help to have the ability to delineate areas of the vineyard where significant treatment benefits can be expected. The method developed by

Bishop and Lark (2006) enables spatial exploration of treatment differences and for conclusions to be drawn about the significance of treatment responses. Using bunch number as an example, Figure 2 illustrates the efficacy of this method.

Clearly, the CL treatment (Fig. 2c) outperforms the other two treatments. The difference maps gained by subtracting the control treatment (RGC) from the RGM treatment (Fig. 2d) and the CL treatment (Fig. 2e) exhibit the absolute difference between the treatments. The *z*-statistic reveals where in the vineyard significant differences are accomplished (Fig. 2f and 2g). No significant differences can be detected between the RGC and RGM treatment (Fig. 2f). Most of the significant differences between the RGC and CL treatment are achieved in the potentially poorer performing areas of the vineyard (Fig. 2g). Table 2 lists the percentage area with significant treatment differences for all four yield components. It can be seen that the CL treatment delivered benefits compared to the control in at least some parts of the block in terms of bunch number, bunch weight and the number of berries per bunch.

Table 2: Percentage experimental area with significant treatment differences of grape yield parameters in 2006 calculated with the method developed by Bishop and Lark (2006).

	Vineyard area with significant treatment difference (%)					
	RGM > RGC			**RGM < RGC**		
Level of significance	**1 %**	**5 %**	**10 %**	**10 %**	**5 %**	**1 %**
Bunch number	0	0	0	0	0	0
Bunch weight	0	0	0	0	0	0
Berry weight	1	3	6	1	0	0
Berries / bunch	0	0	0	0	0	0
	CL > RGC			**CL < RGC**		
Bunch number	0	7	53	0	0	0
Bunch weight	0	2	13	0	0	0
Berry weight	0	0	1	0	0	0
Berries / bunch	0	20	100	0	0	0
	CL > RGM			**CL < RGM**		
Bunch number	0	41	57	0	0	0
Bunch weight	0	0	1	0	0	0
Berry weight	0	1	1	2	1	0
Berries / bunch	0	0	26	0	0	0

RGC = Ryegrass and compost under the vine; RGM = Ryegrass and mulch under the vine; CL = Row alternating cereal and legume.

With this information, along with consideration of economic factors and other yield influencing attributes, the vineyard manager is able to make a decision as to if and where to target mid row management for a specific outcome in this vineyard. Correlation of treatment response with an indicator variable (eg EC_a) promotes extrapolation to other vineyards.

Conclusions

This case study demonstrated the potential of whole-of-block experiments for measuring spatially variable responses to experimental treatments in vineyards. The technique could equally be applied in other production systems. It can be concluded that on-farm trials established using the whole-of-block design provide valuable spatial information about treatment responses and will therefore aid farmers in improved decision making. With more high spatial resolution sensors available, the data recording will become easier and more economically viable which will improve the practicality for farmers to set up their own whole-of-block experiments.

Acknowledgements

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SOIL FERTILITY MONITORING PROJECT: A TOOL FOR IMPROVING THE FERTILIZATION PLAN

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Abstract

With the objective to improve the fertilization management, Pioneer Hi-Bred Italy started in 2001 the "Soil Fertility Monitoring Project".
First step of the project was the development of a new analytical system, based on NIR spectroscopy that allowed performing quick and reliable soil analyses for the determination of physical and chemical parameters useful to compiling fertilization plans. Capability of first NIR calibrations was enlarged in the following years, updating the databases with the reference data of the selected outliers. In 2009 the Standard Error of Validation and R2 of the calibrations were respectively: 26.1g/kg and 0.90 for Clay, 87.0g/kg and 0.72 for Sand, 0.31 and 0.90 for pH, 1.96% and 0.98 for Total Lime, 3.60g/kg and 0.95 for Organic Carbon, 0.31g/kg and 0.97 for Total Nitrogen.
Fertilization plans have been prepared according to the guidelines provided by Emilia Romagna region. Nutrients uptake is defined taking into account also the specific Pioneer hybrids yield potentials, based on Pioneer field trial results across all Italian corn areas.
The most considerable impact of this project was the capability to set fertilization plans on a very large scale. More than 39,000 soil samples have been analyzed, involving about 17,000 corn growers, covering more than 200,000 corn hectares.

Keywords: fertilization, soil analysis, NIR.

Introduction

Nitrogen is a critical element for corn, because of its strong uptake during growing season, and its extremely complex dynamic in the soil profile. Organic Carbon (OC), Total Nitrogen (TN), Clay (CL), Sand (SA), pH and Total Lime (TL) are main parameters commonly utilized to characterize soils and to estimate nitrogen changes in their profile. Having an analytical tool, able to measure these soil parameters in a rapid and accurate way, is a critical step to implement fertilization plans based on the element balance with a wide number of farmers. NIR spectroscopy, already known as rapid and easy in food and forages analysis, showed excellent capability in predicting soil Organic Carbon [1, 2, 3, 4], Total Nitrogen [1, 3], particle size [4, 5], Total Lime [6] and pH [3].

Materials and Methods

Soil samples collected and analyzed within the project came from all the main Italian corn areas. The first calibration set was built in 2001, using the Italian pedological maps as a guide to select from a larger set 420 samples, characterized by as much variability as possible. All the samples were collected at a depth of 0.3 m, according to the official soil sample protocol [7].

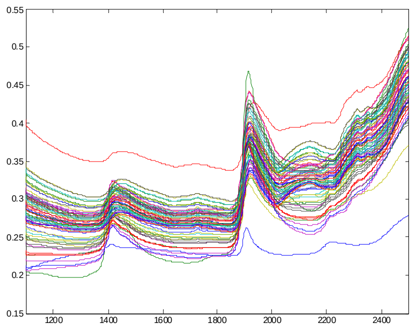


Figure 1: NIR spectra of the first data set.

After drying at 40 °C, the samples were sieved at 2 mm and a quota of sieved sample was ground at 0.5 mm. Analysis of OC, TN and TL were performed on ground sample, while CL, SA and pH were performed on sieved sample. All the analysis were done at least in two replications, according to the reference methods [7]; when the coefficient of variation between replications was higher than 7.5 or the standard deviation was higher than the limit set for each single parameter, a third replication was performed.

NIR spectra of ground samples were collected using a FOSS NIRSystems 5000, equipped with a sample transport device. For each sample two spectra, coming from two filling of the cell, were collected in the range between 1100 and 2500 nm with a resolution of 2 nm.

NIR data processing and calibration development were performed using WinIsi Version 1.5.

Macronutrient balances and fertilization plans were set according to the guidelines provided by Emilia Romagna region. Nitrogen uptake was calculated considering yield potential of each Pioneer hybrid in every single district, whose performances were based on data coming from Pioneer field trials in all the Italian corn areas.

Results and Discussion

The first analytical dataset showed a very high variability in Italian soils composition, both for organic and mineral compounds. Range, average and standard deviation values, expressed in g/kg, were respectively: 4.0-114, 15.3 and 9.1 for OC; 0.50-8.50, 1.62 and 0.76 for TN; 0-818, 111 and 147 for TL; 31-680, 220 and 106 for CL; 62-930, 502 and 167 for SA. A very high variability was collected also with NIR spectra, in terms of offsets, baselines slopes and scattering effects.

The *Principal Component Analysis* identified 15 samples as outliers that have been excluded from the dataset. The remaining population was randomly split in two sub-set, two thirds used as calibration set and one third used as validation set. The best results were obtained using a Local calibration approach (Shenk patented), based on the selection from the global library of the sub-set of samples those best describing the unknown; a specific calibration was calculated for each unknown. Standard Error of Validation and the R^2 were respectively: 3.20 g/kg and 0.88 for OC, 0.30 g/kg and 0.84 for TN, 3.8 % and 0.95 for TL, 32 g/kg and 0.92 for CL, 75 g/kg and 0.79 for SA.

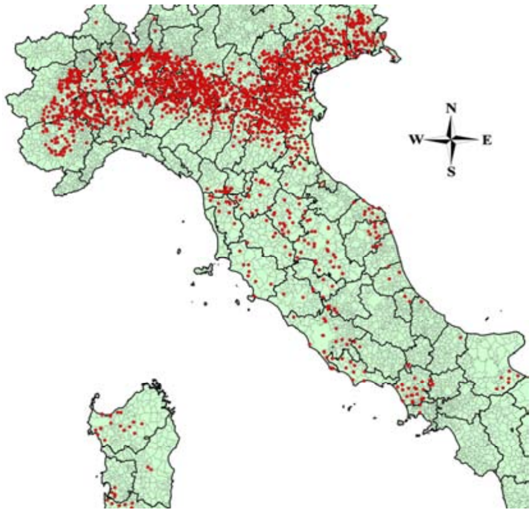


Figure 2: Locations included in the calibration set since 2001 to 2009.

Calibrations created in 2001 were the starting point for developing a very effective analytical instrument and represent the core of the stronger calibrations obtained later, updating the database year by year.

In 2002 Pioneer Hi-Bred Italia started using the calibrated NIRSystems to analyze soil samples and to improve corn fertilization management. In the period 2002-2008 an average of about 5500 soil samples/year were collected and predicted. Samples that showed a Mahalanobis distance in PLS space higher than 3 were analyzed by reference methods. Number of outliers decreased from about 12 % of collected samples in 2002 to about 2% in the next years.

Since 2001 till 2008, an important enlargement of the data range took place for OC (2.07-288.1 g/kg) and TN (0.35-23.91 g/kg). In 2003 a new dataset was built for texture, classifying soils according to USDA instead of ISSS and in 2004 a new data set was also created for pH.

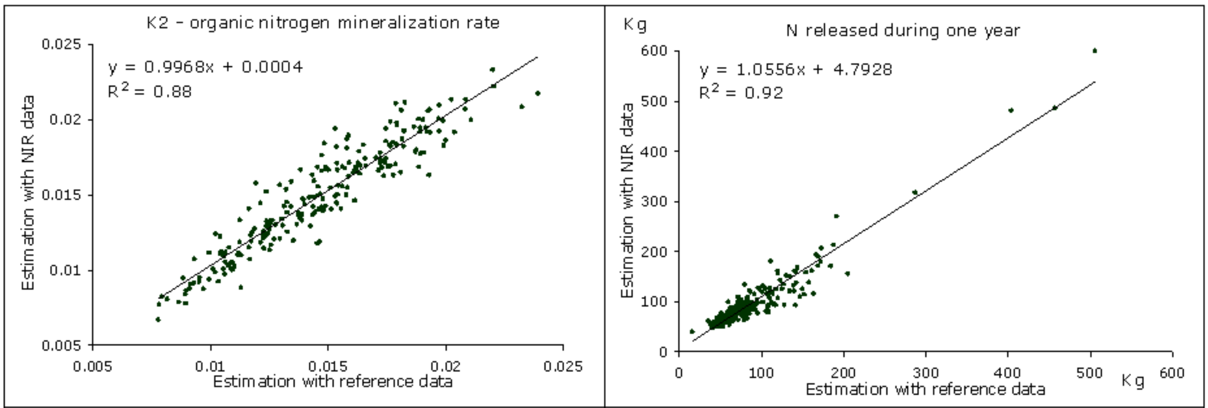
In 2008 the predictive performances (Standard Error of Validation and R^2) of the calibrations were the following: 3.60 g/kg and 0.95 for OC and 0.31 g/kg and 0.97 for TN, 1.96 % and 0.98 for TL, 26.1 g/kg and 0.90 for CL, 87.0 g/kg and 0.72 for SA, 0.31 and 0.90 for pH.

Table 1: Calibration performances on an external validation set.

Soil variable	U.M.	Selected samples for calibration	n° of PLS fact. used in regression	Math Treatment	R^2 val	SEV	RPD	RER
OC < 30.0	g/kg	120	21	SNV, der.1				
OC > 30.0	g/kg	180	21	der.1				
OC	g/kg				0.95	3.60	3.8	31.1
TN < 3.0	g/kg	120	21	SNV, der.1				
TN > 3.0	g/kg	150	21	der.1				
TN	g/kg				0.97	0.31	5.4	40.3
CL	g/kg	110	23	SNV, der.1	0.90	26.1	2.9	15.7
SA	g/kg	180	32	SNV, der.1	0.72	87.0	1.9	7.6
TL	%	150	10	SNV, der.1	0.98	1.96	5.6	32.3
pH		250	31	SNV, der.1	0.90	0.31	3.1	14.0

SEV: standard error of validaton
RPD: SD of validation set/SEP
RER: Range in the validation set/SEP

Results obtained since 2001 up to today demonstrate the capability of NIR spectroscopy to predict main parameters in heterogeneous soils. Performances of the calibrations were also tested calculating K2 and N released during one year using data NIR predicted and obtained by wet chemistry. Fig.3 shows a very high correlation between N released estimated using NIR predicted data and using reference data on a validation set randomly selected in 2008.



K2 = 1200/(Cl+200)(TL+200)
where: CL = clay (g/kg)
TL = lime (g/kg)

Boiffin et al., 1986 and
Bockstaller e Girardin, 2000

N released during one year = TN*K2*SV*BD
where: K2 = organic nitrogen min. rate
TN = total nitrogen (g/kg)
SV = soil volume (3000 m3)
BD = bulk density (1.2)
Emilia Romagna region, 2004

Figure 3: Correlation between N mineralized estimated with NIR predicted data or with reference data.

The most considerable impact of this project was the capability to set fertilization plans based on soil nutrient balances on a very wide scale. More than 39 000 soil samples have been analyzed, involving about 18 000 corn growers, accounting for more than 200 000 corn hectares.

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ABATEMENT OF NH_3 EMISSIONS FOLLOWING APPLICATION OF UREA TO GRASSLAND BY MEANS OF THE NEW UREASE INHIBITOR 2-NPT

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Abstract

Following the application of granulated urea to grassland, volatilization of up to 30% of the contained nitrogen as ammonia (NH_3) are reported in literature. These emissions are partly deposited into semi-natural ecosystems, where they not only cause eutrophication, but also entail an increase in N_2O losses. As an immediate incorporation of the fertilizer is not possible on grassland, the addition of a urease inhibitor (UI) is the most promising alternative for mitigating NH_3 emissions. Two experiments including a total of seven measurement periods were conducted on intensive grassland. Granulated urea with and without the new urease inhibitor 2-NPT (N-(2-nitrophenyl) phosphoric triamide) was surface applied and NH_3 emissions were measured using a dynamic chamber method for 10 days following fertilization. Without the UI, on average 4.0% of the fertilized N were emitted as NH_3, ranging from 1.5% to 7.4%. The addition of 2-NPT strongly reduced these losses by up to 100%, allowing a safe top application of urea onto grassland.

Keywords: grassland, granulated urea, ammonia losses, urease inhibitor, 2-NPT.

Introduction

Urea is the most important fertilizer worldwide with a market share of 53% in mineral nitrogen fertilizer (IFA, 2009). Though, it is known that high emissions of ammonia (NH_3) may occur following its surface application. On grassland, these nitrogen losses can amount to 30% within the first three days following application (van der Weerden and Jarvis, 1997). Emitted into the atmosphere, NH_3 causes an over-fertilization of semi-natural ecosystems entailing their acidification and eutrophication, which results in losses of certain plant communities. But, it also indirectly enhances N_2O losses from these sites, which gives NH_3 an indirect greenhouse potential of 2.98 CO_2 equivalents (IPCC, 1996). As an incorporation of the fertilizer is not possible on grassland, the addition of a urease inhibitor (UI) is the most promising alternative for abating NH_3 losses. Throughout the loss phase, the UI should reversibly block the active center of the ubiquitous enzyme urease, which is responsible for hydrolysis of urea to CO_2 and NH_3. The new urease inhibitor 2-NPT (N-(2-nitrophenyl) phosphoric triamide) has already been successfully tested on arable land, mitigating NH_3 losses from granulated urea by up to 60%. The aim of the present work is to evaluate the potential of 2-NPT for reducing NH_3 losses following the application of granulated urea to grassland.

Material and Methods

In the years 2007 and 2008, two field experiments were conducted on two intensive, perennial grassland sites in Southern Germany (Veitshof [48°24'09.80'' N; 11°41'24.09'' E] and Dürnast [48°23'35.90'' N; 11°43'51.76'' E], Technische Universität München, Freising, Germany). Both sites consisted of established swards of perennial ryegrass (*Lolium perenne* L.), which were cut four to five times a year. The mean annual temperature and rainfall were similar for both experimental sites adding up to 7.8°C and 786 mm. Further soil characteristics are listed in Table 1.

Table 2: Soil characteristics of the experimental sites.

location	year	pH	soil texture			urease activity	CEC	C_{org}
			clay [%]	silt [%]	sand [%]	[µg NH_4^+-N (g soil)$^{-1}$ 2 h^{-1}]	[cmol (kg soil)$^{-1}$]	[% of d.m.]
Veitshof	2007	7.2	26.8	45.7	27.5	557	26.85	7.12
Dürnast	2008	5.7	28.1	46.6	25.3	210	14.18	10.65

In both years, experiments were carried out between the end of May to the end of July. Expected warm and dry climatic conditions following a period of low precipitation were chosen for fertilizer applications to detect enhanced ammonia losses. The day before fertilization, swards on the experimental plots were cut down to a stubble size of 5 cm and fertilized with 80 kg N ha^{-1}. Treatments consisted of surface applied granulated urea with and without the urease inhibitor (UI) 2-NPT at different concentrations of 0.075%, 0.10% and 0.15% related to the N content, and a control treatment which received no N. The measurement system was installed and started immediately after fertilization, and continuously detected NH_3 emissions over a period of 10 days.

For measurements a dynamic chamber system was selected. The "open top"-chambers (area 0.125 m^2, height 0.40 m) consist of acrylic glass combined with a stainless steel ring at the bottom, which is pressed into the soil (Figure 1). Chambers were daily relocated within the plot to avoid a "greenhouse" effect on NH_3 emission. Within this system, samples are analyzed by a device for detection of the nitric oxide (NO) content in the air (CLD 700 AL, EcoPhysics, Gürnten, Switzerland) based on the chemiluminescent gas phase reaction of NO with ozone (O_3). For this purpose, NH_3 has first to be converted into NO, which is achieved in a stainless steel converter by addition of O_3 at temperatures of 600°C. The ammonia content is then determined as the difference: $[NH_3] = [NOx_{amines}] - [NOx]$. Concentration of NH_3 in ambient air is also detected and deducted from the NH_3 content in the treatment sample. Throughout the day, samples are collected and analyzed every 1.5 hours, which allows to establish the progression of NH_3 emissions for every measured plot. Daily emission averages were calculated for each chamber and used for statistical evaluation.

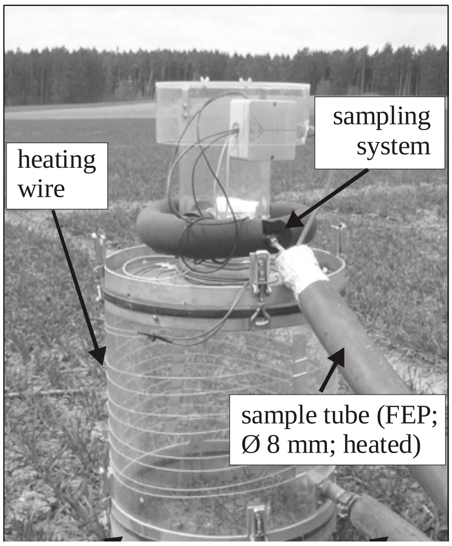

Figure 2: "Open top" chamber for detection of NH_3 emissions.

Climatic data were collected at a nearby weather station and provided by the Deutscher Wetterdienst (DWD).

Statistical analysis was performed using SAS (SAS 9.1 TS Level 1M3, SAS Institute Inc., Cary, NC, USA). Differences in means of daily NH_3 emissions as well as of yield parameters were evaluated using ANOVA combined with the Tukey's HSD procedure ($p = 0.05$).

Results and Discussion

In all measurement periods, ammonia (NH_3) losses occurred within the first 2 to 5 days after the application of granulated urea, which is congruent with observations made by Watson *et al.* (1990) and van der Weerden and Jarvis (1997). The maximum rate of NH_3 emission was emitted between the first and second day. Losses varied between 1.5% and 7.4% of the fertilizer N (Table 2). On average 4.0% got lost into the atmosphere as NH_3.

Except for the first two applications in 2007, NH_3 losses from urea alone were significantly higher than from urea with the urease inhibitor (UI) 2-NPT (Table 2). The addition of 2-NPT abated NH_3 emissions by 49% to 100% compared to urea without the UI, onto a level not significantly different from unfertilized plots. All concentrations of 2-NPT similarly and highly reduced NH_3 losses by 73% to 76% for 0.075% of 2-NPT, 49% to 75% for 0.10% of 2-NPT, and 69% to 100% for 0.15% of 2-NPT. In 2008, a doubling of 2-NPT concentration from 0.075% to 0.15% did not result in significant lower NH_3 losses.

Table 3: Sum of NH_3 emissions [kg NH_3-N ha^{-1}] occurring within 10 days following fertilization of grassland with 80 kg N ha^{-1} as granulated urea with or without the urease inhibitor 2-NPT at different concentrations.

fertilizer	10/06/2007 [kg NH_3-N ha^{-1}]	19/06/2007 [kg NH_3-N ha^{-1}]	13/07/2007 [kg NH_3-N ha^{-1}]	26/07/2007 [kg NH_3-N ha^{-1}]	26/05/2008 [kg NH_3-N ha^{-1}]	27/06/2008 [kg NH_3-N ha^{-1}]	10/07/2008 [kg NH_3-N ha^{-1}]
urea	1.68a ± 0.62	2.86a ± 2.03	3.73a ± 1.07	3.37a ± 0.67	3.72a ± 1.83	6.44a ± 1.95	3.38a ± 0.60
urea + 2-NPT 0.075%	---	---	---	---	0.91b ± 0.34	1.65b ± 0.16	0.92b ± 1.08
urea + 2-NPT 0.10%	0.86ab ± 0.52	1.18a ± 1.05	1.61b ± 0.38	0.83b ± 0.50	---	---	---
urea + 2-NPT 0.15%	---	---	---	---	0.30b ± 0.29	2.00b ± 0.94	0.48b ± 0.46
control (nil fertilizer)	0.47b ± 0.35	0.60a ± 0.45	0.56b ± 0.31	0.19b ± 0.10	0.29b ± 0.29	0.49b ± 0.24	0.34b ± 0.39

Compared to literature reports, NH_3 losses following the application of granulated urea were low on both sites. However, in nearly all experiments, humid top soil conditions at the time of fertilization entailed a quick dissolution of fertilizer granules and hydrolysis of urea, which caused higher concentrations of NH_3 in the soil solution (Black *et al.*, 1987). The only exception was the first period in 2007, characterized by an initially low soil water content in the top soil at time of fertilization. This condition resulted in a slow dissolution of fertilizer granules and a consequently slow urea hydrolysis (Fenn and Miyamoto, 1981; Black *et al.*, 1987). Thus, at this time gaseous nitrogen losses as NH_3 were kept on an even lower level of only 1.5% of the fertilized N. Independent of the actual conditions for conversion of urea granules, the addition of 2-NPT at all concentrations reliably inhibited urea hydrolysis from the very start and kept NH_3 losses on a constantly low level.

A quick drying-out of the soil may generally account for the continuously low level of emissions following the early peak on day 1. Both experiments were located on relatively wind-exposed sites and the dry conditions were even intensified, due to the fact that the grassland is cut down to a stubble size of 5 cm prior to fertilization of urea. Thus, congruent with observations made by Bouwmeester *et al.* (1985), wind could have caused a rapid drying-out of the top soil mitigating and finally stopping NH_3 losses.

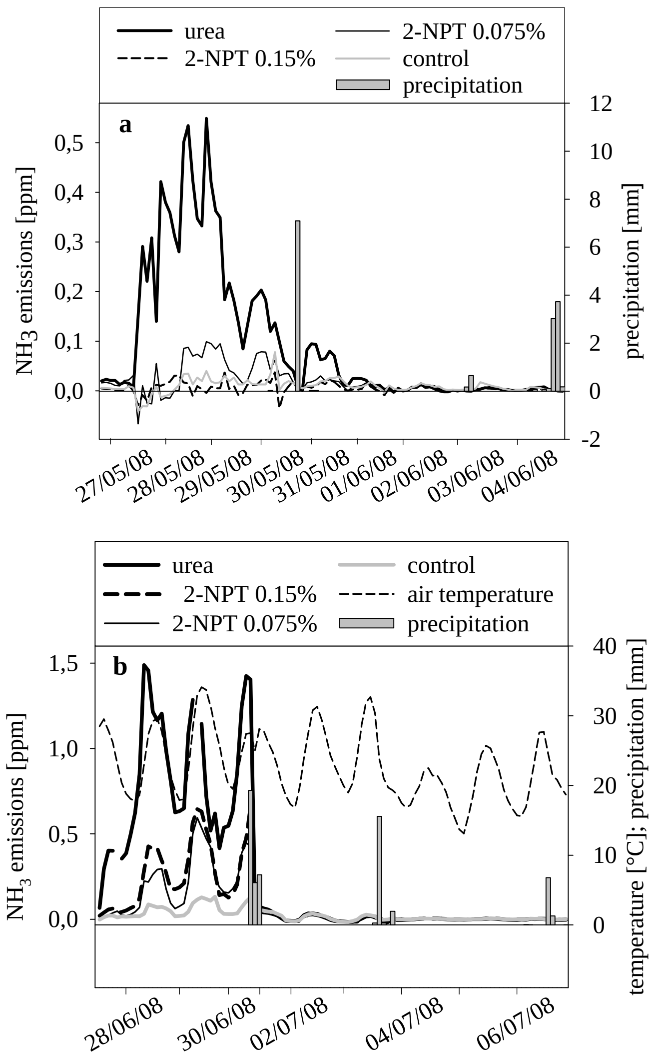

Figure 3: Courses of NH_3 emissions [ppm] following fertilization of grassland swards with 80 kg N ha^{-1} as granulated urea with or without the urease inhibitor 2-NPT at different concentrations, influenced by a) precipitation [mm] and b) temperature [°C].

Besides, some phases of NH_3 losses were precociously terminated due to precipitation. The higher the precipitation were and the closer this event occurred to the date of fertilization, the stronger was its effect on reducing emissions for both, urea and urea + 2-NPT. However, while low precipitation enhanced NH_3 losses from urea alone, no increase in NH_3 emissions was detected for urea + 2-NPT, suggesting, that no spatial separation of urea and 2-NPT took place (Figure 2a).

The enhancing effect of higher temperatures on NH_3 losses could be supported by our continuously measuring system, which documents how strongly NH_3 emissions correlate with the course of daytime temperature (Figure 2b). The addition of 2-NPT dramatically mitigated the extent of emitted NH_3, but did not influence the temporal occurrence of losses. Temperature related losses from urea + 2-NPT followed the same diurnal course as from urea alone.

Acknowledgments

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IMPACT OF COPPER EXPOSURE ON PHYSIOLOGICAL FUNCTIONING OF CHINESE CABBAGE (*BRASSICA PEKINENSIS*)

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Abstract

Arable soils may be contaminated with copper as the consequence of scarce attention on soils and fertilizers management. Exposure of Chinese cabbage (*Brassica pekinensis*) to elevated Cu^{2+} levels resulted in leaf chlorosis, a loss of photosynthetic capacity and lower biomass production at ≥ 5 µM. The decrease in pigment content was probably not the consequence of its degradation, but more likely due to a hindered chloroplast development at high Cu levels. The toxic effects of copper were substantially reduced when the UV-B was filtered out. Elevated Cu^{2+} levels (≥ 5 µM) strongly substantially affected, the uptake, distribution and metabolism of sulfur and the overall mineral nutrient composition of both root and shoot.

Keywords: Brassicacea, copper, sulfur metabolism, mineral composition, toxic metals, UV-B.

Introduction

Soils may be contaminated with high Cu levels as the result of mining, smelting and other industrial activities, but also by the application of manure and other copper-rich organic and inorganic fertilizers or copper-containing fungicides (Brun *et al.* 2001; Dach and Starmans 2005; Yruela 2005, 2009). Cu is rather immobile in soil, since it strongly binds with organic soil material. The application of manure and fertilizers, which are generally rich in Cu (and other trace metals) results in a cumulative accumulation of copper in soils, since its influx is often exceeding the crop removal rate (Sauve *et al.* 1997; Dach and Starmans 2005). Cu is an essential plant nutrient and it functions as a redox-active transition metal in enzymes in many physiological processes, e.g. photosynthesis, respiration and the oxidative stress response. However at elevated levels, Cu may become phytotoxic and the general symptoms are chlorosis and stunted growth (Yruela 2005, 2009; Kopsell and Kopsell 2007).

The present paper briefly reviews the impact of enhanced Cu levels on physiological functioning and represents data on the interaction between Cu and UV-irradiation and the impact of Cu on mineral content in Chinese cabbage (Brassica pekinensis).

Materials and methods

Chinese cabbage (*Brassica pekinensis* (Lour.) Rupr. cv. Kasumi F1 (Nickerson-Zwaan, Made, The Netherlands) was germinated in vermiculite and 10 to 14-day-old seedlings were grown on a 25 % Hoagland nutrient solution, pH 5.9-6.0, containing supplemental concentrations of $CuCl_2$ (1 - 10 μM) in 13 l containers (10 sets of plants per container, 3 plants per set) in a climate-controlled room. Day and night temperatures were 25 and 20 °C, respectively, and the relative humidity was 60-70 %. The photoperiod was 14 h at a photon fluence rate of 340 ± 20 μmol m^{-2} s^{-1} (within the 400-700 nm range) at plant height, supplied by Philips HPI-T (400 W) lamps. In some experiments UV-B was filtered out by polyester foil (Mylar type D, transmittance > 313 nm; Du Pont de Nemours, Luxembourg). Plants were harvested 3 h after start of the light period and roots were separated from the shoots and weighed. For pigment analysis shoots were frozen immediately in liquid N_2 and stored at –80 °C and for mineral analysis, plant tissue was dried at 80 °C for 48 h. For the determination of the pigment content, shoots were homogenized in 100 % acetone using an Ultra Turrax (10 ml per g fresh weight) and centrifuged at 800 g for 20 minutes. The chlorophyll *a*, *b* and total carotenoid contents were determined according to Lichtenthaler (1987). For the determination of the mineral content, dried plant material was pulverized by a Retsch Mixer-Mill (type MM2; Haan, Germany) and content of the different minerals were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES). 5 ml of a 85:15 (v/v) mixture of nitric acid (Primar, Aristar s.g 1.42, 70 %) and perchloric acid (Aristar/Primar, 70 %) was added to 250 mg of oven dried plant material and allowed to stand at room temperature for at least 2 h. Samples were digested in a heating block (Carbolite, London, UK) for 3 h at 60 °C, 1 h at 100 °C, 1 h at 120 °C and 2.5 h at 200 °C. Temperatures were ramped at either 1 or 2 °C min^{-1} for the first and last or 2nd and 3rd periods. Subsequently 5 ml of 9.25 % (v/v) HCl (AR grade) was added to the dried samples and heated for 1 h at 80 °C. After a further addition of 20 ml of water, the samples were heated for 30 min at 80 °C. Finally extractions were made up to 25 ml with water and subjected to ICP-OES (Applied Research Laboratories, Vallaire, Ecublens, Switzerland).

Results and discussion

Exposure of Chinese cabbage (*Brassica pekinensis*) to 1 - 10 µM Cu^{2+} resulted in shoot chlorosis and lowered plant biomass production at ≥ 5 µM (Fig. 1; Shahbaz *et al.* 2009). The decrease in pigment content was accompanied by a loss of photosynthetic capacity, however, expressed on a chlorophyll basis, the rate of photosynthesis was hardly affected at levels up to 10 µM Cu^{2+} (Shahbaz *et al.* 2009). The decrease in pigment content, which first was visible in young developing leaves, was probably not the consequence of pigment degradation, but due to a hindered chloroplast development at high Cu levels (Shahbaz *et al.* 2009). The impact of Cu on pigment content and biomass production in Chinese cabbage appeared to be strongly by the level of abundant UV-B (Fig. 1). If the UV-B irradiation was filtered out, the development of the toxic effects of elevated Cu levels was strongly reduced and both Cu-induced chlorosis and loss of biomass production was decreased (Fig. 1). UV-B (280 - 320 nm) may interfere with several physiological processes and high levels may impair protective mechanisms (Kakani *et al.* 2003; McKenzie *et al.* 2007). The nature of the interference between relative low levels UV-B and enhanced Cu in plants needs to be evaluated further.

Elevated Cu levels affected the uptake, distribution and metabolism of sulfur in Chinese cabbage. Cu exposure resulted in an increase in water-soluble non-protein thiol content of the root and to a lesser extent of the shoot at ≥ 1 µM, which was ascribed partially to a Cu-induced enhancement of the phytochelatins content (Shahbaz *et al.* 2009). These compounds, which are derived from glutathione, may complex with Cu and may buffer the Cu concentration in the cytosol, however, their significance in actual Cu detoxification appears to be restricted (Verkleij *et al.* 2003; Ernst *et al.* 2008; Yruela 2005, 2009). The total sulfur of the shoot was increased at ≥ 2 µM Cu^{2+} (Table 1), which might be attributed mainly to an increase in sulfate content, as a consequence of a Cu-induced enhanced sulfate uptake by the root (Shahbaz *et al.* 2009).

The Cu content of roots of Chinese cabbage increased with the increased external Cu concentration from 0.4 (control) to 14 µmol g^{-1} DW at 10 µM Cu^{2+} (Shahbaz *et al.* 2009). However, within the plant the Cu was relatively immobile and only a minor proportion (maximum of 4 %) was transferred to the shoot, although a more than 3-fold increase in Cu content of the shoot of Chinese cabbage initiated toxic effects (Shahbaz *et al.* 2009).

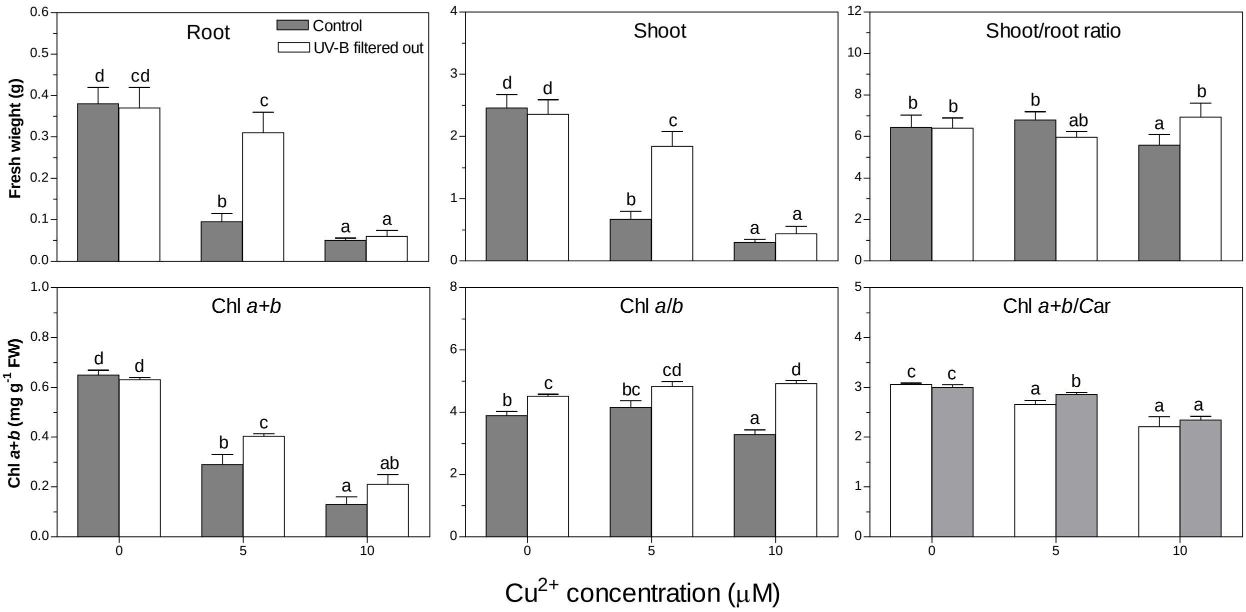

Figure 1: Impact of Cu^{2+} and UV irradiation on biomass production and pigment content of Chinese cabbage. 10 day-old seedlings were grown on a 25 % Hoagland solution containing 0, 5 and 10 µM $CuCl_2$ for 10 days. Control (grey bars; 1.5 mW cm^{-2} UV-A+B) and UV-B filtered (white bars; 1.0 mW cm^{-2} UV-A+B). The initial shoot and root fresh weights were 0.036 ± 0.002 and 0.009 ± 0.002 g, respectively. All data represent the mean of 3 measurements with 3 plants in each (± SD). Different letters indicate significant differences between treatments ($p < 0.01$, Student's t-test).

The overall mineral nutrient compositions of root and shoot were substantially affected at ≥ 5 µM Cu^{2+} (Table 1). Exposure of Chinese cabbage to Cu^{2+} resulted in a decrease in the content of Ca and Mg in root and that of Fe in the shoot, and a decrease in the content of Mn, P, K in root and shoot, whereas that of Zn increased. Similar alterations in mineral composition at excessive Cu levels have also been observed in other plant species (Ouzounidou *et al.* 1998; Patsikka *et al.* 1998, 2002; Fageria 2002; Sheldon and Menzies 2005; Alaoui-Sosse *et al.* 2004). To what extent the changes in mineral composition are the consequence of an altered competition in the uptake and transport of the minerals or altered root architecture at the occurrence of root injury at toxic levels of Cu levels needs to be further evaluated (Sheldon and Menzies 2005). It is not yet established to what extent a change in mineral composition is involved in the onset of the phytotoxity of Cu.

Table 1: Impact of Cu^{2+} levels on mineral content of Chinese cabbage. 14 day-old seedlings were grown on a 25 % Hoagland solution containing 0, 1, 2, 5 and 10 µM $CuCl_2$ for 7 days. Data are expressed as µmol g^{-1} dry weight and represent the mean of 3 measurements with 9 plants in each (± SD). Different letters indicate significant differences between treatments ($p < 0.01$, Student's t-test).

	µM Cu^{2+}				
	0	1	2	5	10
Shoot					
Calcium	776 ± 20b	735 ± 5a	776 ± 16b	841 ± 18c	807 ± 44bc
Iron	1.33 ± 0.09c	1.20 ± 0.11bc	1.05 ± 0.07b	0.76 ± 0.00a	0.76 ± 0.03a
Magnesium	194 ± 7a	190 ± 13a	210 ± 4b	236 ± 16c	216 ± 26abc
Manganese	2.10 ± 0.22b	2.09 ± 0.05b	2.12 ± 0.18b	2.42 ± 0.09c	1.71 ± 0.10a
Phosphorus	225 ± 3b	204 ± 6a	199 ± 7a	234 ± 7b	234 ± 12b
Potassium	1781 ± 47d	1713 ± 10c	1651 ± 65bc	1562 ± 88ab	1417 ± 105a
Sodium	18 ± 4a	18 ± 1a	20 ± 2a	26 ± 3b	35 ± 9c
Sulfur	268 ± 7a	282 ± 20ab	375 ± 29c	443 ± 23d	314 ± 27b
Zinc	0.44 ± 0.04a	0.45 ± 0.06a	0.50 ± 0.11a	0.67 ± 0.03b	0.65 ± 0.04b
Root					
Calcium	1029 ± 159c	1139 ± 247c	729 ± 300bc	646 ± 114b	250 ± 8a
Iron	62 ± 10a	84 ± 26ab	74 ± 30ab	75 ± 30ab	109 ± 20b
Magnesium	194 ± 23bc	200 ± 10c	163 ± 14b	108 ± 5a	111 ± 3a
Manganese	19.5 ± 5.8b	18.1 ± 2.2b	19.3 ± 2.4b	28.4 ± 3.7c	7.2 ± 1.8a
Phosphorus	334 ± 17a	335 ± 34a	339 ± 19a	349 ± 24a	383 ± 20b
Potassium	1475 ± 56c	1439 ± 80c	1440 ± 77bc	1341 ± 57ab	1275 ± 61a
Sodium	8.4 ± 1.7a	9.3 ± 1a	9.2 ± 1.2a	7.7 ± 1.0a	9.4 ± 1.4a
Sulfur	317 ± 5a	315 ± 10a	345 ± 6b	389 ± 12c	402 ± 13d
Zinc	0.67 ± 0.10ab	0.59 ± 0.08a	0.70 ± 0.02b	0.92 ± 0.09c	1.37 ± 0.13d

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PREDICTION OF NITROGEN MINERALIZATION FROM SOIL ORGANIC MATTER USING A COMBINED PHYSICAL AND CHEMICAL FRACTIONATION TECHNIQUE

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Abstract

N mineralization is a crucial parameter in optimizing N use efficiency but difficult to predict on the basis of chemical fractionations. This paper reports on an attempt to use a combined physical (ultrasonication and wet sieving) and chemical (oxidation with NaOCl, treatment with hydrofluoric acid) fractionation technique to try to predict N mineralization rates from soil organic matter. Absolute N mineralization rates found here were surprisingly low, and there was no good correlation with chemical fractions. The best correlation of relative N mineralization (i.e. as % of total soil N) was with sand size particulate organic N (r = 0.519*). Incubations at higher temperatures may provide larger differences in N mineralization of these light textured soils, while further physical separation of soils through particle size separation, aggregate fractionation and density fractionation could yield fractions that are better correlated to N mineralization.

Keywords: N mineralization, fractionation, ultrasonication, NaOCl extraction, HF extraction.

Introduction

N mineralization from soil organic matter is an important contributor to overall N supply. Therefore, it is a crucial parameter to optimize N fertilization and N use efficiency. Considering that the determination of N mineralization by laboratory incubations or field measurements is very expensive and labor intensive, many attempts have been made during the past decennia to try to predict N mineralization on the basis of chemical fractionation techniques. However, none of these attempts has allowed to successfully predict N mineralization for a wide range of soils. In this paper, we present results of a combined physical and chemical fractionation technique and its potential to predict N mineralization from soil organic matter.

Materials and methods

We selected 20 soils from an intensive agricultural area in West and East Flanders, divided in three subsets: arable, alternating arable-vegetable and intensive vegetable rotations (Table 1). Samples from the upper 30 cm soil layer were incubated aerobically at an average temperature of 13.7°C for 4 months for measuring N mineralization rates. Samples were also incubated aerobically for measurements of net C mineralization during a 6-weeks incubation at an average temperature of 19.7°C. We used a combined fractionation consisting of an ultrasonication-wet sieving physical part subsequently followed by chemical fractionation, with determination of NaOCl-oxidation labile and resistant OC and N, mineral bound [(10% HF extractable) OC and N (MOC and MN)] and recalcitrant (10% HF resistant) OC and N fractions. At the end of the 6^{th} and 14^{th} week of incubation, we also measured enzyme activities of β-glucosidase and dehydrogenase, and microbial biomass C (data not presented here).

Table 1: Soil total C, total N, textural class, N and C mineralization for different cropping groups.

Sample Location	Total C g C kg^{-1}	Total N g N kg^{-1}	C:N	Texture (USDA)	Mineralization (kg N $ha^{-1}yr^{-1}$)	% C Mineralization 6 $weeks^{-1}$	Cropping group
Staden	9.39	0.93	10.1	Loamy sand	111	2.28	Alternating vegetable
Poelkapelle	11.7	1.06	11.1	Sandy loam	132	2.35	Alternating vegetable
Nevele	11.5	0.92	12.5	Loam	109	1.99	Alternating vegetable
Alter	10.4	0.97	10.8	Silt loam	114	2.64	Alternating vegetable
Lendelede 1	7.3	0.63	11.6	Sandy loam	71	2.27	Arable vegetable
Lendelede 2	6.9	0.55	12.6	Sandy loam	70	3.25	Arable vegetable
Otegem 1	9.7	0.97	9.9	Silt loam	97	2.60	Arable vegetable
Otegem 4	10,0	0.99	10.1	Silt loam	80	2.64	Arable vegetable
Otegem 2	7.8	0.70	11.2	Silt loam	52	1.75	Arable vegetable
Otegem 3	16.4	1.62	10.2	Silt loam	53	2.06	Arable vegetable
Deurle	17.5	1.24	14.1	Loamy sand	105	1.75	Pure vegetable
Kruishoutem	10.9	0.81	13.6	Loamy sand	62	1.70	Pure vegetable
Aarsele	11.5	0.97	11.8	Sandy loam	60	1.21	Pure vegetable
Vosselare	15.8	1.14	13.8	Loam	98	1.34	Pure vegetable
Merendree	8.9	0.79	11.2	Loamy sand	93	2.47	Pure vegetable
Torhout	14.9	1.20	12.4	Loamy sand	101	1.40	Pure vegetable
Handzame	10.2	0.96	10.6	Sandy loam	95	1.76	Pure vegetable
Reningelst	11.3	1.15	9.8	Silt loam	127	2.85	Pure vegetable
Heule	6.4	0.55	11.7	Loam	95	9.25	Pure vegetable
Waregem	7.95	0.69	11.5	Loamy sand	108	2.75	Pure vegetable

Results and Discussion

Significant differences in annual N mineralization (kg N ha^{-1} yr^{-1} for 30 cm depth) (Table 1) were found among the different cropping groups in the order, alternating (116.5 ± 10.3) > pure vegetable (94.5 ± 20.1) > arable (70.5 ± 17.1). The zero order N mineralization rates measured here, however, were in general much smaller than expected and than observed in similar studies in this region. Likewise, alternating arable vegetable production surprisingly showed larger N mineralization than pure vegetable rotations.

The C:N ratio of the sand fraction (average 20.9 ± 9) was almost twice as high compared to the silt+clay (average 9.0 ± 1.5). The relative proportion of sand sized OC and N were found to be higher in pure vegetable soils compared to alternating arable and arable groups (Table 2). This might be due to the fact that pure vegetable fields receive a larger input of OM from fresh crop residue and animal manures compared to the alternating and arable soils.

The relative proportion of N was found to be higher in the silt+clay sized fractions as compared to relative proportion of OC in that fraction (Table 2). This is in line with often reported enrichment of N in the finest soil particles (Schulten and Leinweber, 1995). Indeed N containing compounds such as peptides and recalcitrant N-heterocyclics have been found to be enriched with decreasing particle size. This can furthermore be explained by the selective accumulation of microbial N-containing metabolites in the finer soil fractions (Schulten and Leinweber, 1995).

Table 2: The size fractions (g 100 g^{-1} soil) obtained by physical fractionation (ultrasonication and wet sieving) (avg. with stdev.) and C, N and C:N ratio of sand, silt+clay fraction.

Field	Sand (>50µm)	Silt +Clay (<50 µm)	Sand (>50µm)			Silt +Clay (<50 µm)		
	g 100 g^{-1}	g 100 g^{-1}	N g N kg^{-1}	C g C kg^{-1}	C:N	N g N kg^{-1}	C g C kg^{-1}	C:N
Alternating arable vegetable								
Staden	53.8 ± 0.25	47.2 ± 0.32	0.11 ± 0.01	1.7 ± 0.06	16.6 ± 2.2	0.82 ±0.01	6.5 ± 0.47	7.9 ± 0.48
Poelkapelle	52.8 ± 0.32	46.2 ± 0.25	0.09 ± 0.01	2.1 ± 0.08	22.9 ± 3.7	0.97 ±0.01	7.8 ± 0.04	8.1 ± 0.14
Nevele	42.1 ± 0.52	57.9 ± 0.52	0.08 ± 0.01	2.1 ± 0.1	26.2 ± 2.8	0.84 ±0.05	6.6 ± 0.42	7.8 ± 0.55
Aalter	35.7 ± 1.26	64.3 ± 1.26	0.09 ± 0.01	1.7 ± 0.02	19.1 ± 2.1	0.88 ±0.01	6.7 ± 0.16	7.6 ± 0.11
Arable vegetable								
Lendelede 1	48.9 ± 0.67	51.1 ± 0.67	0.04 ± 0.01	1.2 ± 0.04	27.6 ± 0.6	0.59 ±0.01	4.9 ± 0.36	8.3 ± 0.64
Lendelede 2	50.6 ± 1.1	49.4 ± 1.1	0.09± 0.02	1.1 ± 0.1	12.6 ± 0.7	0.46 ±0.02	4.3 ± 1.94	9.2 ± 3.12
Otegem 1	27.2 ± 0.41	72.8 ± 0.41	0.1 ± 0.01	1.7 ± 0.03	16.7 ± ns	0.87 ±0.01	6.8 ± 0.24	7.8 ± 0.26
Otegem 2	24.9 ± 0.07	75.1 ± 0.07	0.07 ± 0.01	1.5 ± 0.07	22.0 ± 2.7	0.61 ±0.03	5.8 ± 0.36	9.6 ± 1.07
Otegem 3	22.0 ± 1.42	78.0 ± 1.42	0.11 ± 0.08	2.0 ± 0.99	20.8 ± 6.4	1.51 ±0.08	11.7 ±0.12	7.7 ± 0.49
Otegem 4	25.1 ± 0.05	74.9 ± 0.05	0.07 ± 0.01	2.0 ± 0.26	29.6 ± 1.1	0.92 ±0.01	6.7 ± 0.56	7.2 ± 0.52
Pure vegetable								
Deurle	77.4 ± 0.21	22.6 ± 0.21	0.16 ± 0.07	1.5 ± 0.34	9.9 ± 2.1	0.65 ±0.07	7.2 ± 1.23	11.0 ± 0.75
Kruishoutem	79.5 ± 0.58	20.5 ± 0.58	0.12± 0.0	3.0 ± 0.37	26.0 ± 3.2	0.81 ±0.06	7.6 ± 0.68	9.3 ± 0.19
Aarsele	49.3 ± 0.72	50.7 ± 0.72	0.14 ± 0.13	3.6 ± 0.07	43.7 ±40.3	1.10 ±0.13	12.8± 2.06	11.8 ± 3.25
Vosselare	47.0 ± 0.96	52.9 ± 0.96	0.13 ± 0.01	4.0 ± 0.22	30.2 ± 3.0	0.95 ±0.08	10.9 ±0.68	11.5 ± 1.58
Merendree	74.4 ± 0.64	25.6 ± 0.64	0.12 ± 0.01	1.4 ± 0.07	11.6 ± 6.2	0.67 ±0.01	6.2± 0.13	9.2 ± 0.12
Torhout	78.3 ± 1.38	21.7 ± 1.38	0.17 ± 0.04	2.2 ± 0.54	13.5 ± 0.2	1.04 ±0.04	12 ± 0.12	11.6 ± 0.36
Handzame	67.5 ± 0.44	32.5 ± 0.44	0.13 ± 0.08	1.8 ± 0.13	16.9 ± 9.2	0.83 ±0.08	7.1 ± 0.02	8.6 ± 0.78
Reningelst	35.6 ± 0.36	64.4 ± 0.36	0.08 ± 0.00	2.6 ± 0.17	31.9 ± 1.9	1.07 ±0.01	7.6 ± 0.09	7.1 ± 0.08
Heule	52.7 ± 0.13	47.4 ± 0.13	0.12 ± 0.05	1.0 ± 0.12	9.7 ± 4.8	0.43 ±0.05	4.5 ± 0.04	10.3 ± 1.18
Waregem	82.1 ± 0.22	17.9 ± 0.22	0.14 ± 0.05	1.3 ± 0.19	9.6 ± 1.9	0.55 ±0.05	5.9 ± 1.08	10.7 ± 1.05

From Fig. 1- 3 it becomes clear that the major part of N was oxidized by NaOCl. The proportions of NaOCl-resistant N were relatively lower than the NaOCl-resistant OC in all cropping groups in following order vegetable production > alternating > arable. Differences in the (relatively low) % of NaOCl-resistant N may be due to differences in management (rates of animal slurry and mineral N application, as well as amounts and C/N ratios of crop residues). Indeed, the pure vegetable fields have since long received frequent and high manure N inputs, high N crop residues and high mineral fertilizer N. The NaOCl resistant fractions of the "vegetable production" cropping group had the the highest C:N ratios, whereas the C:N ratios of the bulk soil were the lowest.

The proportion of mineral protected N (MN) was higher than the recalcitrant N (i.e. HF resistant N), but the MOC (i.e. mineral bound OC) was lower than ROC (i.e. HF resistant OC/ recalcitrant OC) for all different cropping groups (Fig. 1 - 3). This finding suggests that binding of organic N to the mineral phase would be involved as relatively important mechanism in these soils. The differences in RN (i.e. recalcitrant N or HF resistant N) between the cropping groups were small. Sleutel et al. (2009) found that the ROC was unaffected by land-use history in sandy croplands in Flanders, and in this study ROC equally showed no significant differences among cropping groups.

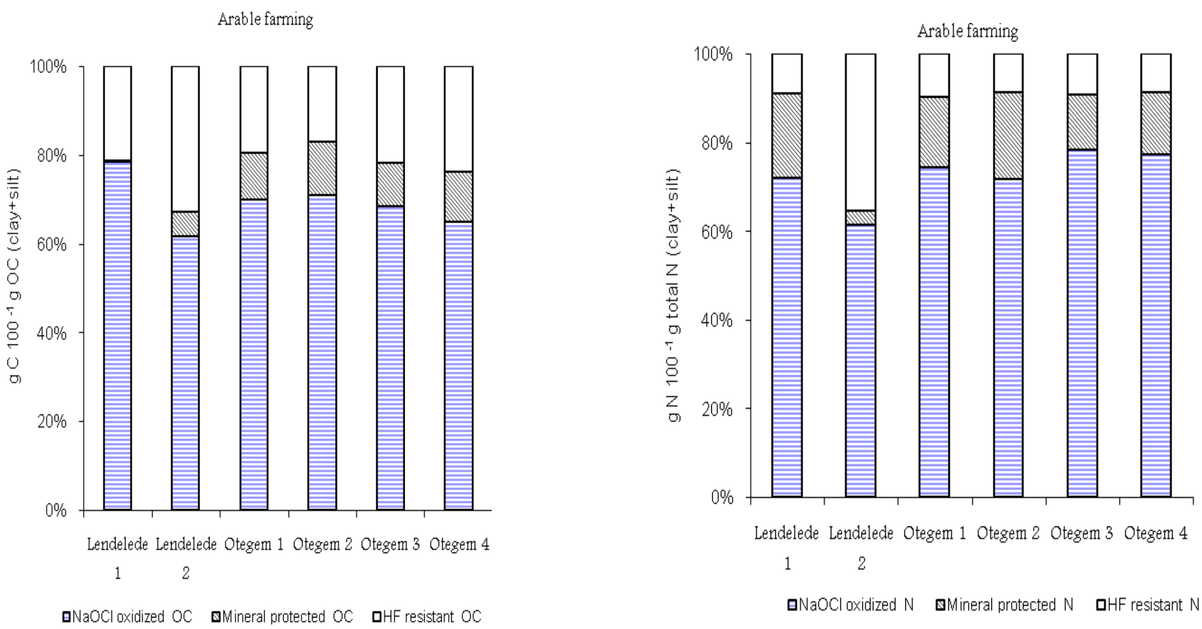

Figure 1: Relative distribution of NaOCl oxidizable (labile), mineral protected and recalcitrant OC and N of the arable group.

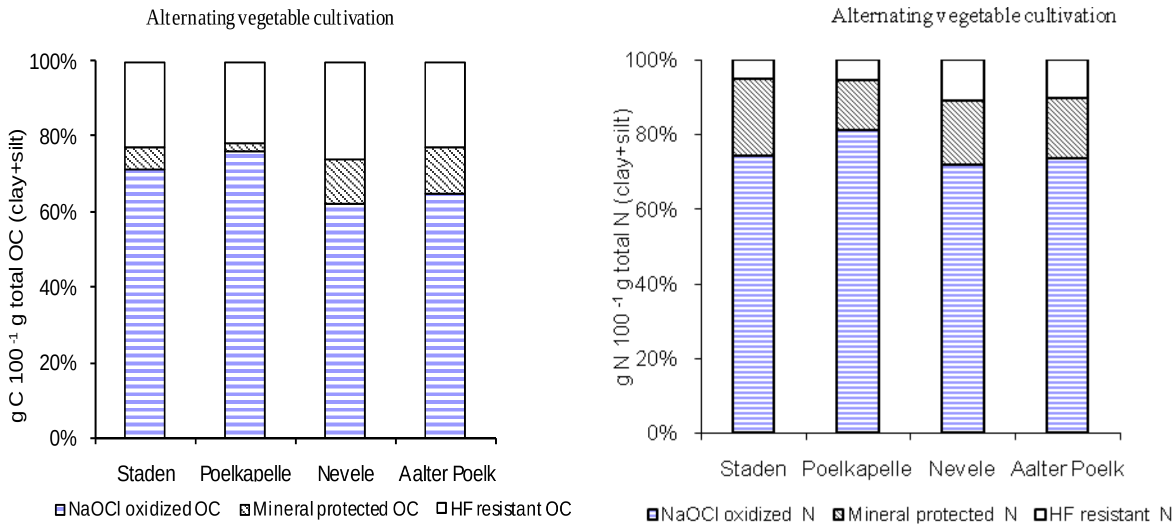

Figure 2: Relative distribution of NaOCl oxidizable (labile), mineral protected and recalcitrant OC and N of the alternating arable vegetable group.

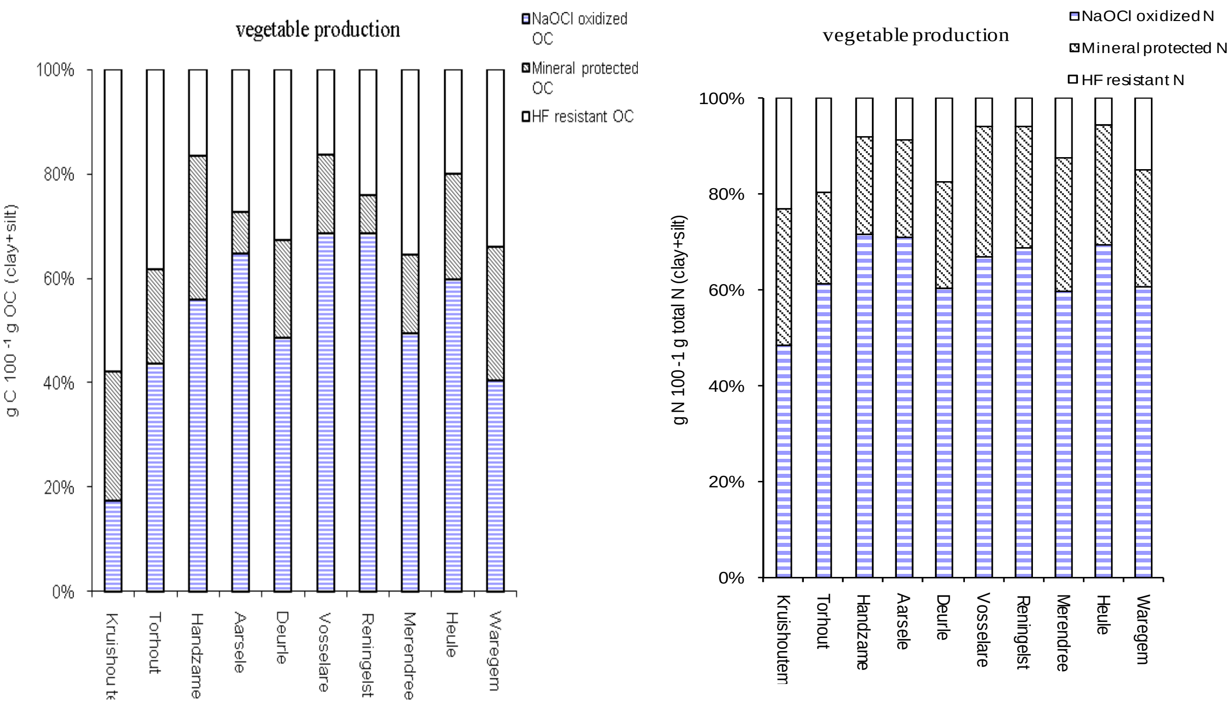

Figure 3: Relative distribution of NaOCl oxidizable (labile), mineral protected and recalcitrant OC and N of the pure vegetable group.

Surprisingly there was no correlation between absolute amounts of N mineralization and measured physical and chemical fractions as well as no correlation for % N mineralization and relative % of different chemically treated silt+clay fractions (Table 3). The % N mineralization had a strong positive correlation ($p<0.05$) with sand size particulate organic N ($r = 0.519^*$).

Table 3: Pearson correlation coefficient of absolute and % N mineralization with different parameters

Parameters	Absolute N mineralization	Relative % N mineralization
Initial N	-0.099	-0.672**
Initial C	-0.134	-0.648**
Silt	-0.231	-0.262
Clay	-0.107	-0.314
Sand	0.218	0.274
MBC 6th week	-0.037	0.27
MBC14th week	-0.175	0.267
Silt+clay N	-0.117	-0.428
Silt+clay C	-0.161	0.016
Sand N	0.142	0.519*
Sand C	-0.091	-0.295

Parameters	Absolute N mineralization	Relative % N mineralization
NaOClRes N	-0.059	0.285
NaOClLia N	-0.119	-0.285
MON	0.186	0.2
RN	-0.293	0.14
NaOClRes C	-0.071	0.2
NaOCl Lia C	-0.169	-0.2
MOC	-0.056	0.156
RC	-0.071	0.174

We conclude that N mineralization experiments at higher temperatures will probably yield clearer differences between soil, hence increasing the possibility of finding correlations with physically-chemically isolated fractions. A future combined physical-chemical fractionation should have a more detailed physical part, with more detailed particle size separation and aggregate and density fractionation.

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IMPROVED MANAGEMENT OF NITROGEN TO RAISE PRODUCTIVITY OF FOOD CROPS

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Abstract

Increased nitrogen use efficiency, raising yield potential and closing existing yield gaps to avoid yield stagnation are pivotal components of a sustainable agriculture that meets human needs and protects natural resources. The better the yield determination is understood the more likely the breeding or management strategies designed to raise productivity will efficiently apply. Improved efficiency of nutrient use at a field and farm scale, both aiming at increasing crop yield and reducing losses, is dependent upon the magnitude of matching nutrient supply and demand of the crop. Matching nitrogen and water supply to the demand of the crop requires knowledge of crop growth processes and critical phenological stages in crop development.

Two cases are presented: one on N use in irrigated lowland rice and another on nitrogen use efficiency in wheat under abiotic stress. Irrigated lowland rice cropping systems show low ANR-values of about 0.30. The agronomic nitrogen use efficiency, derived from apparent nitrogen recovery (ANR) and physiological nitrogen use efficiency (PNUE), amounts to 0.50 on average for crops under temperate conditions. Water availability strongly affects N uptake and recovery. A quantitative systems approach is needed to identify the prospects for improving the agronomic N-use efficiency.

Keywords: wheat, rice, N uptake, N use efficiency (NUE), apparent N recovery (ANR).

Introduction

It was found that at the global level the industrial N fertilizer input exceeded the total crop N uptake around 1980 (Goudriaan *et al.*, 2001). In the developed countries the consumption of Nfertilizers declined from about 1985 onwards; however the N-consumption in developing countries continues to increase (Eickhout *et al.*, 2006). More than 60 % of the food supply for the human population currently depends on chemical N fertilizers. With the change to a more protein-rich diet in countries with emerging economies and a growing population, the dependence on N-fertilizers will increase strongly. Sylvester-Bradley & Kindred (2009) reported that yields of winter wheat in England increased strongly, but NUE only slightly from 20 to 24 kg DM. kg^{-1} N over the last three decades. They concluded that NUE improved more through better resource capture than physiological conversion. Thus, improvements were more depending on agronomic measures than breeding.

A comprehensive overview of opportunities to improve fertilizer N management is presented by Zebarth *et al.* (2009). They listed several research areas providing opportunities to improve fertilizer N use in cropping systems, such as: breeding for improved NUE in association with heat and drought tolerance, development of gene expression profiling to identify crop N stress, practical soil N mineralization tests, development of decision support systems and refinement of the decision-making process for variable rate fertilizer N application, use of controlled-release fertilizer N, capturing and recycling nutrients from drainage water. Successfully addressing the complex problem of the low agronomic NUE requires a more integrated approach than is currently used in most on-going monodisciplinary and sometimes scattered research activities (Neeteson *et al.*, 2002). Various stakeholders should be involved in this development process: researchers, farmers, consumers, policymakers, etc.

An integrated and interactive approach will be the most cost-effective approach for improving resource-use efficiency and profitability of sustainable agricultural systems (Spiertz, 2009).

Increased N use efficiency, raising the yield potential and closing existing yield gaps to avoid yield stagnation are pivotal components of a sustainable agriculture that meets human needs and protects natural resources (Cassman *et al.*, 2003).

<u>Crop growth and nitrogen utilization in wheat</u>

Strategic research on growth, development and yield formation of cereals has contributed to research-based crop management with a time- and dose-specific approach for crop protection and N fertilization, which increases both yield and yield stability in Northwest Europe (Stockdale *et al.*, 1997). Combining the genetic potential of modern cultivars with best practices in N management and pest and disease control almost doubled the yield of winter wheat in the better endowed regions of Northwestern Europe in a time-span of 30 years. In the Netherlands, average wheat yields increased from 4,820 to 8,200 kg ha-1 (Spiertz, 2004). The progress in raising yields has even been more successful in winter wheat compared to spring wheat and barley. Commercial grain yields (ca. 15% moisture) of winter wheat in regions with long days and a mild climate, e.g. Northern Germany and Scotland, currently average about 9,000 kg ha^{-1} with top yields up to 11,000 kg ha^{-1} under conditions with optimal fertilization and chemical control of pests and diseases.

Given the change in market demands, N management should become more directed to quality traits. A shift to split-dressing with an additional late N application around flowering has proven to increase the protein content of the grain in both rainfed (Ellen and Spiertz, 1980) and irrigated wheat (Wuest and Cassman, 1992). Decision support systems based on the N status of the leaves may be an option to improve the synchrony between N supply and crop demand. Wang *et al.* (2004) reported a good relationship between plant pigment ratio (R550 - R450)/(R550 + R450) measured at anthesis and grain protein content in winter wheat. This ratio is obtained from the reflectance's (R) measured at wavelengths of 450 and 550 nm.

<u>Nitrogen utilization in aerobic and flooded rice production</u>

In quantifying N response and NUE in *rice-wheat* (RW) cropping systems Jing *et al.* (2009) found lower apparent N recoveries (ANR) in wheat (0.27-0.34) than in rice (0.32-0.49). Lower ANRs may have been caused by higher N losses by denitrification and ammonia volatilization due to the change from anaerobic to aerobic soil conditions in RW systems. Aiming at saving water and N use, the *aerobic rice* concept was developed to produce high-yielding rice grown in non-puddled soils just like upland crops. Bouman *et al.* (2005) reported that the highest yields under aerobic conditions were realized with an improved upland cultivar (5.7 t ha^{-1}) and a lowland hybrid rice cultivar (6.0 t ha^{-1}). Total water input (irrigation and rainfall) was 1,240-1880 mm per season in flooded fields and 790-1,430 mm in aerobic fields. However, the consequences of transforming a flooded soils into an aerobic soil for N-use on the long term are still poorly understood. In flooded rice with saturated soils, ammonium is the dominant form of available N. Since nitrate is barely present in flooded rice soils, very little nitrate-N is leached to the groundwater. The intermittent application of irrigation water will create soil moisture conditions close to saturation during short spells. These alternating wet-dry soil conditions may stimulate denitrification/nitrification processes, resulting in gaseous losses of N through N_2 and N_2O. In addition, nitrate is prone to leaching. The differences in soil N dynamics and magnitude and pathway of N losses between flooded and aerobic systems may result in different fertilizer-N recoveries. Thus, water availability strongly affects N-uptake and Nrecovery (Belder *et al.*, 2005). Agronomically, farmers should aim at the minimum input of each production resource required to allow maximum utilisation of all other resources (de Wit, 1992).

<u>A system approach in nutrient management</u>

In N management, the goal is to make predictions of crop N demand based on the expected growth and yield while taking into account the soil N reserves and net N-mineralization during the growing season. However, there are many uncertainties in predicting the yield and mineralization due to weather extremes and the incidence of pests and diseases. In reality, the N-demand of a crop can be explored by measuring on site the dynamics in soil N availability and N-concentrations in the leaves. A more advanced method is using validated crop growth models and actual weather records to support Decision Support Systems. Crop diagnostics, like a leaf colour chart or imaging methods, can be used to increase the specific nature of recommendations during the growing season. The progress made by site-specific nutrient management (SSNM) in irrigated rice is relatively small compared to split-dressings of N taking into account crop N demand (Wang *et al.*, 2001). A greater use of SSNM in dynamic optimization of N management would be possible when phenology of the crop is taken into account.

Mathematical modeling has begun to integrate our understanding of the soil-plant N cycle and the soil, plant, environmental factors that govern it. Various models of crop growth, the soil N cycle and plant - soil models have been developed. Good examples are ORYZA2000 (Bouman *et al.*, 2001) and APSIM-Nwheat (Asseng *et al.*, 2000). However the complexity of the cycle and the large number of interactive factors that control it implies that the models do not closely approach reality.

Environmental concerns are focused on N losses from soils that may pollute the environment. Leaching is the major route by which nitrate enters the ground and surface waters, while nitrification-denitrification results in significant sources of N_2O, an important greenhouse gas. In irrigated rice-based cropping systems, N-losses from nitrate leaching are very low; however, large N losses occur from volatilization of ammonium sources. Improved efficiency of N-use at the field and farm scale to increase crop yield and quality and reduce N losses depends on dynamic optimization to match supply of N and the N requirements of the crop at a field scale. This optimization requires measurement and prediction of soil N supply, crop uptake and their variability (Cassman *et al.*, 2002).

Differences between fields are in part due to historical differences in management. But the major cause of varying fertilizer use efficiency, particularly for N, is that the supply of nutrients from soil reserves and fertilizers is not well synchronized with the demands of the crops (Raun *et al.*, 2002). This mismatch will be greater when crops depend mainly on organic N-sources because the mineralization rate is governed by temperature and soil moisture and may not be closely matched to crop demand.

Conclusions

Improving N use efficiency depends on securing attainable yields and matching nutrient supply and demand. Attainable yields should be secured by applying best farming practices.

The optimisation of N management requires measurement and prediction of soil N supply, and alleviation of factors that limit N uptake and utilization. Knowledge of temporal and spatial variation in N demand and supply is needed to improve N use efficiency. Integrated system analyses of the economic, ecological and social performance of conventional and alternative agro-production systems are also needed to guide a sustainable development and to meet societal concerns. A multi-scale approach to evaluate the efficiency of nutrient management in cropping and farming systems is recommended.

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PEAK PHOSPHORUS: A NEW DIMENSION FOR FOOD SECURITY AND WATER QUALITY IN THE LAKE WINNIPEG BASIN

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Abstract

Evidence is emerging that global supplies of rock phosphate used for production of phosphorus (P) fertilizers are declining. The term *peak phosphorus* has been applied to the situation that phosphorus, the first non-renewable, non-substitutable life-supporting element, will become scarce in the foreseeable future. As a result, management of phosphorus on the land and the prevention of nutrient pollution in aquatic ecosystems are aligned by the common goal of keeping phosphorus on the land for crops. The objective of the study was to investigate the confluence of intensive management of phosphorus in agriculture with the goals of securing food productivity and water pollution prevention as symbolized by Lake Winnipeg, visibly the most eutrophic Great Lake in the world. The method was an action research approach designed to disseminate and gather information on existing and future challenges related to P management and social process of change. Based upon review of science, analysis of the basin and dialogue with 40 stakeholders, action recommendations for best management practices, P recycling and stewardship were formulated. The results demonstrate the urgency and importance of establishing an integrated watershed management approach including all aspects of Lake Winnipeg-related water security, phosphorus recycling, traditional knowledge and governance.

Keywords: peak phosphorus; food security; water quality; social change; eutrophication; Lake Winnipeg.

Introduction

The prospect of depleting the world's easily minable mineral rock phosphate reserves, predicted to be as early as 20 years (Cordell, Drangert & White, 2009) is of great concern and is being increasingly compared to 'peak oil' (Déry & Anderson, 2007). Unlike irreversible impacts such as the consumption of fossil fuels, phosphorus can neither be created nor destroyed, but is endlessly recycled through the natural processes of the Earth. While we can never 'run out' of phosphorus, when washed from the land to the oceans it is not returned to mineral forms that can be mined for tens of millions of years. Yet, phosphorus stores within agricultural soils, crops, food, composts, biomasses, and wastewater can be controlled, managed, and recycled rather than being lost to the oceans.

The objective of our study was to investigate the confluence of intensive management of phosphorus in agriculture with the goals of securing food productivity and water pollution prevention as symbolized by Lake Winnipeg, visibly the most eutrophic great lake in the world (Fig. 1).

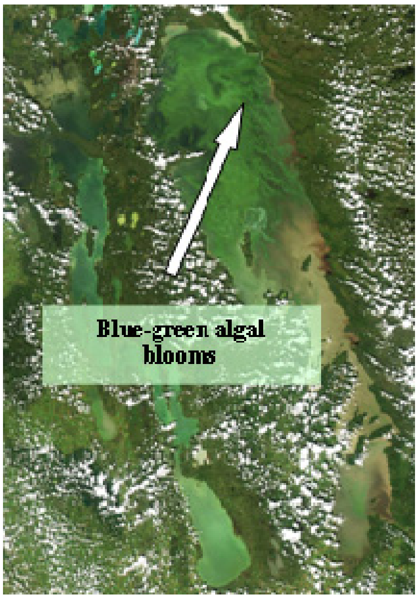

Figure 1: Satellite view of Lake Winnipeg in 2009 showing the large expanse of surface blooms in the North Basin, covering 13.000km^2 in 2005 (Supplied by Greg McCullough).

Home to 6 million people, 17 million livestock, the basin (Fig. 2) covers nearly 1 million km^2, including 55 million hectares of agricultural land. Lake Winnipeg serves multiple purposes, including recreation, commercial fisheries, and is the world's largest hydro-electric power generation reservoir. These functions affect or are being affected by high phosphorus levels.

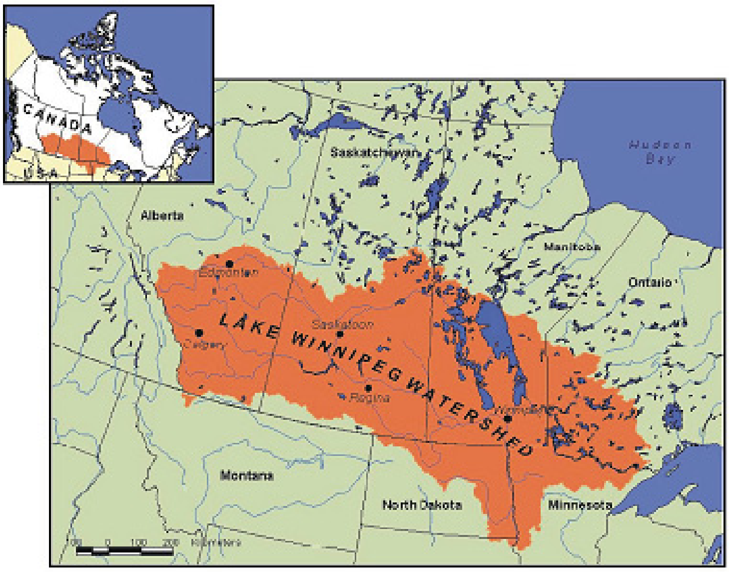

Figure 2: The Lake Winnipeg basin (in orange) is the second largest watershed in North America. It is nearly 40 times greater in area than Lake Winnipeg, the largest ration for any large lake in the world (Source: Manitoba Water Stewardship,

Fig.3 shows the phosphorus cycle consisting of natural processes and human influences. In this interdisciplinary work, while we consider all pools as important, we see agricultural operations (Fig. 4), consumption, social change, life-cycle assessments as well as wastewater treatment as central.

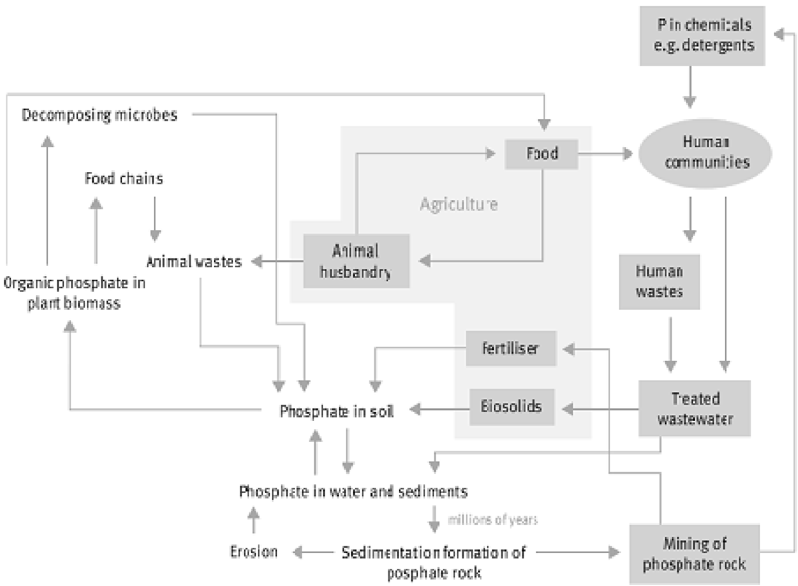

Figure 3: The natural phosphorus cycle and its human influences (shaded) (Source: Green Alliance, 2007).

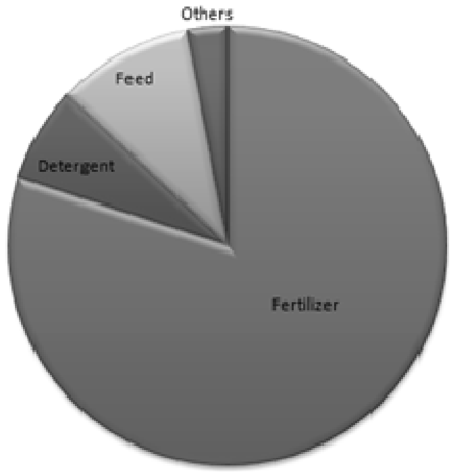

Figure 4: The most important commercial use of phosphorus is the production of fertilizers for food production (Source: International Fertilizer Industry Association, 2007).

The realization that there is a finite limit to easily accessible phosphorus must lead to a paradigm shift in its management: No longer can phosphorus be seen as pollutant, managed to protect the environment. Instead, it must be managed as a finite, life-supporting resource to secure regional and global food production.

Method

The method was an action research approach designed to disseminate and gather information on existing and future challenges related to declining phosphorus reserves, water and phosphorus resources management, governance and changing the level of awareness, attitudes and practices with regard to planning, wastage and disposal. Based upon review of science, literature, and websites of more than 40 organizations and agencies, analysis of the basin and dialogue with 40 stakeholders, action recommendations for best management practices, phosphorus recycling and stewardship were formulated.

Results

In general, the problem of declining phosphorus reserves is neither well-known nor widely acknowledged within the discourse of food security and nutrient management in respect to the prevention of eutrophication. Nevertheless, dialogue is emerging and opportunities to better use, recover, and recycle phosphorus are at hand. A successful process requires an integrated, systematic perspective and includes relevant ecological, geopolitical, social and technological factors affecting supply and demand. Such a multi-level, long-term strategy must aim at protecting, sustaining and improving water quality and phosphorus conservation. This agenda must be advocated and advanced basin-wide.

The following activities are recommended to pave the way to a food and water secure Lake Winnipeg Basin:

- municipalities, householders and the agricultural sector take immediate steps to promote the stewardship and recycling of P by
  - reducing and composting food waste
  - recovering P from wastewater (i.e. in form of struvite)
  - improving knowledge of dynamics of P in soil and on landscapes, including availability of P to plants
- establish an integrated watershed management approach to include all aspects of Lake Winnipeg-related water security, indigenous perspectives, and sustainable governance
- develop a comprehensive watershed communication plan among all stakeholders, commissions, boards and agencies
- First Nations traditional knowledge be integrated in the development of a healthy Lake Winnipeg basin vision
- establish funds for research and development in monitoring P flows in and between commodities, wastes, land, and water
- a comprehensive review of policies on phosphorus is initiated with all governance partners to design new multi-jurisdictional mandates for sustainability
- enhance science, social science, education and information with the goal of identifying roles for students, families and other individuals in a sustainable approach to peak P and food security related issues

Discussion

While declining supplies of rock phosphate are triggering concern about future limitation in the supply of phosphate fertilizers manufactured from rock phosphate for food production, it is more important to focus on that fact that phosphorus is indestructible. It is abundant on the earth. The urgency is to begin and maintain monitoring of the global flows of phosphorus in foods, feeds, and other products. It is crucial to put in place effective, global recycling activities to keep the phosphorus in these flows cycling via human and domestic animal waste management back to the land. Land and soils should become a greater focus of attention for better understanding of nutrient dynamics and availability to promote efficient reuse of the phosphorus by plants. As well, soils and agricultural lands can be a point of integration of nutrient dynamics, climate change, water management, and biodiversity for the provision of healthy land-based foods and fiber. In this way, peak phosphorus is transformed from a discussion of scarcity to a vision of sustainable abundance.

Conclusion

The Lake Winnipeg Basin is highly complex and its phosphorus monitoring, management and conservation strategies could provide models for peak phosphorus responses in other parts of the world. Engaging urban and rural communities, national, state and provincial jurisdictions and First Nations communities to endorse research, technological developments, and social change is a crucial endeavor for improving the health of Lake Winnipeg and maintaining the phosphorus supply to agricultural lands in the breadbasket of Canada.

Acknowledgements

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V SESSION

New fertilizers and fertilization management

in Organic Farming

N USE AND PARTITIONING IN CORIANDER (*CORIANDRUM SATIVUM* L.) AFTER ORGANIC AND CONVENTIONAL N FERTILIZATION

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Abstract

In coriander, a well known spice herb, many studies concerning the effect of N fertilization have been conducted in special areas where the cultivation of such plant has a major importance, such as India. Limited information is available as concerns the response of coriander to N fertilization under Mediterranean climatic conditions, above all when organic N fertilizers (mandatory when organic cropping management is chosen) are used. This work refers about some observations realised from 2004 to 2006 by an experiment on organic and mineral N fertilization techniques in coriander, carried on in the experimental farm “Sparacia” (Cammarata, AG, Sicily). Similarly to what suggested for other species, each year at harvest time, for each fertilizer treatment, seeds yield and plant biomass were weighed and the respective N content was determined in order to compare N plant uptake with total and mineral N measured in the soil before and after cropping cycle. From such data a few indices were calculated in order to get information about the efficiency of use by plants of the tested N forms. Some differences in N partitioning and use efficiency according to treatment were noticed, suggesting an overall higher efficiency of N chemical fertilizers.

Keywords: Coriander, N fertilizers, organic fertilization, Nitrogen use efficiency.

Introduction

The widespread utilization of N fertilization worldwide is probably a consequence of the generally evident and quick effect that the addition of this element exerts on the overall performance of vegetation in most crops. As a matter of fact, such an effect may in some cases push farmers to an excess in distribution, in the belief that a more luxury habit of vegetation also means a higher healthiness condition for the plant. In this way, supplying crops with excessive N doses, that plants are unable to adsorb and soil cannot retain, may generate losses due to volatilization or to solubilization and leaching, with many environmental risks. The growing concern related to N excesses and misuses, therefore, calls attention to the necessity of a fine tuning of N management in field, keeping into account the exact requirement from crops and the real advantage of N supply (Sangwan *et al.*, 2001). In coriander, a well known spice herb, many studies concerning the effect of N fertilization have been conducted in special areas where the cultivation of such plants has a major importance, e.g. India or Pakistan. A review of world data about N fertilization in coriander (Carrubba, 2009) has shown that, in most examined cases, N fertilization allowed a 10 to 70% increase in seed yields in comparison with untreated control; notwithstanding, a large variability according to the site fertility could be ascertained, with the maximum yield advantages under high fertility conditions, whereas in less favorable environments the advantages of additional N supply were significantly lower. Limited information is available as concerns the response of coriander to N fertilization under Mediterranean climatic conditions, above all when organic N fertilizers are taken into consideration, as it is mandatory under organic cropping management (Carrubba and Ascolillo, 2009). The high costs of organic fertilizers for each single N unit push towards the necessity to a deep insight of the efficiency of use of the element by crops. A few considerations about N partitioning among the different parts of some crops have been exposed by Sinclair (1998), who suggested some interesting algorithms in order to quantify the relationships between HI and N use by plants, since in cereals seeds and straw have not the same N content, and therefore a shift in the relative fractions of both components (i.e. a modification in harvest index) involves a great modification in N accumulation and allocation by plants. Coriander is, by far, a not improved crop, and its plant architecture and metabolism have not been improved to increase N use efficiency; its HI often takes values lower than 10%, a value that may possibly decrease after N fertilization.

With the aim to give some detailed information about the bio-agronomical behavior of Coriander after organic and conventional N fertilization, a field trial was performed from 2003-04 to 2005-06.

Materials and methods

The work was carried on in the experimental farm "Sparacia" (Cammarata, AG, Sicily), comparing the types and rates of N fertilizers that are listed in table 1. Each year, prior to sowing, on representative soil samples (0-30 cm depth), total N content (both in organic and mineral form) was determined; at harvest time, both biomass and grain yields were weighed and converted to kg ha^{-1} of dry matter. Thereafter, the following soil and plant data inputs (all expressed in kg ha^{-1}) were determined for further analyses:

Nt: N total, i.e. N amount stored in aerial plant biomass at harvest time;

Table 1: Sparacia (Cammarata – AG) 2004 – 2006 – Organic and chemical N-fertilization in Coriander. Treatments tested during the trial.

Treatment/year	Total N (kg ha^{-1})	Distribution method	N-fertilizer type, name, formulation	Producer, provenience	N content	Technical details
C1 - 2004 to 2006	80	At sowing	Inorganic (urea)		46 %	
C2 - 2004 to 2006	80	½ at sowing, ½ top-dressed.				
C3 - 2004 to 2006	120	2/3 at sowing, 1/3 top-dressed.				
O1 – 2004-2005	80	At sowing	Organic (Natural N8) Pellets	SCAM (Modena, Italy)	Total N 8,0 %, of which organic 8 %	C of biological origin (TOC) 37,0 % Organic matter of biological origin 63,0 % C/N ratio 4,6
O2 – 2004-2005	80	At sowing	Organic (Biagrin) Liquid (solution-sospension)	PFB (S. Giuseppe Jato, PA, Italy)	Total N 5,0 %, of which organic 1%	Humic extract obtained from residual olive waters. C of biological origin (TOC) 30,0 % Humic acids 15% C/N ratio 6,0
O3 – 2004 to 2006	80	At sowing	Organo mineral NP (Geco Natura) Compost	Gecos Fertilizzanti (Scordia, CT, Italy)	Total N 5,0 %, of which organic 5 %.	C of biological origin (TOC) 12 % C/N ratio 2,4 Total P_2O_5 7 % Water soluble K_2O 1 %
O4 – 2006	80	At sowing	Organic (Xena N12) Pellets	Nuova Geovis spa (Ozzano dell'Emilia, BO, Italy)	Total N 12,0 %, of which organic 12 %	C of biological origin (TOC) 40,0 % C/N ratio 3,3
O5 – 2006	80	At sowing	Organo mineral NP (Xena Starter) Pellets	Nuova Geovis spa (Ozzano dell'Emilia, BO, Italy)	Total N 7,0 %, of which organic 7%.	C of biological origin (TOC) 38 % C/N ratio 5,4 P 10 %, of which soluble in 2% formic acid 6 % (upon 10)
T: non fertilized control - 2004 to 2006						

Nf: N supplied by means of fertilization;

Nh: N in inorganic form measured in soil at harvest time;

Ns: N supply, i.e. all N potentially available in the soil, given by Nt0 + Nh0 + Nf, where Nt0 was total N amount stored in plant biomass; Nh0 was inorganic N originally in soil (both directly measured in the unfertilized plots), and Nf was, as written above, N supplied by means of fertilization;

Nav: N available, i.e. soil N effectively available for plants, given by Nt + Nh, determined for each fertilization treatment.

Similarly to what suggested for other species such as corn (Moll *et al.*, 1982), in order to get information about the efficiency of use of different N forms by plants, from such measured data a few indices were calculated, among which:

NUE = Gw/Ns, Nitrogen use efficiency, i.e. the ratio between grain yield and N supply;

NAE = Nt/Ns, Nitrogen absorption efficiency, i.e. the ratio between N uptaken by plants and N supply.

Results and discussion

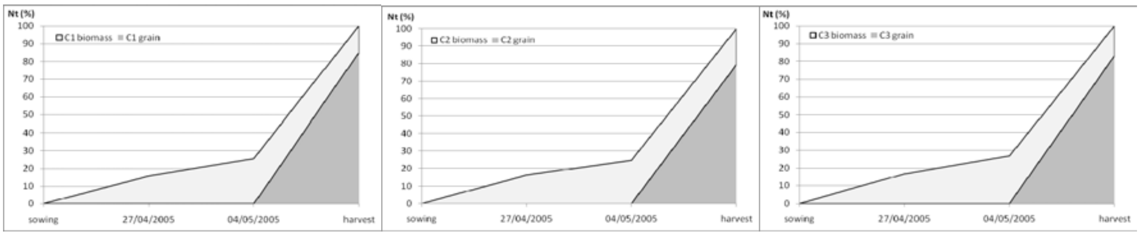

Figure 1: Trend of N storage (% of Nt at harvest) in coriander aerial biomass and grain in conventional N fertilization treatments.

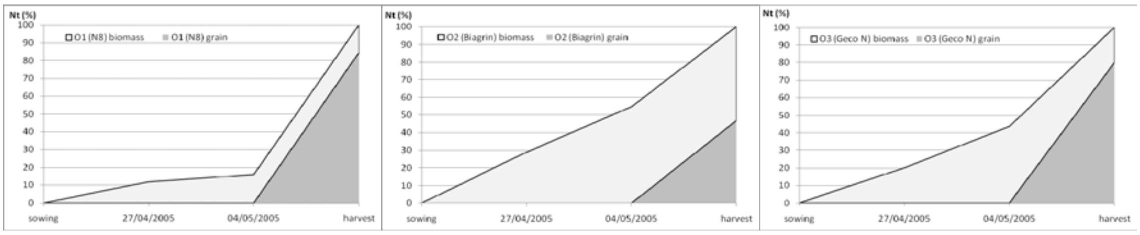

Figure 2: Trend of N storage (% of Nt at harvest) in coriander aerial biomass and grain in organic N fertilization treatments.

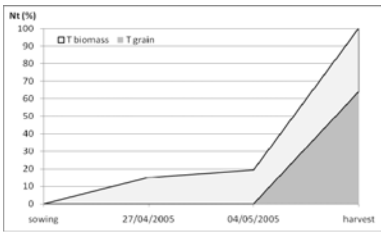

Figure 3: Trend of N storage (% of Nt at harvest) in coriander aerial biomass and grain in unfertilized plots (T).

Figs 1 to 3 show the trend of N accumulation in plants biomass recorded in 2004-05 in all groups of treatments, expressed in percentage of total N measured at harvest time. In the conventional treatments (C1 to C3) a substantially homologous trend is shown, apparently independent upon tested distribution rates and timing. Total N measured in seeds represented more than 80% of total N detected in plant biomass at harvest time (70-90 kg ha^{-1}). Among organic N fertilizers a higher variability was observed: Nt values ranged from 46 (O2) to 108 (O1) kg ha^{-1}, with a proportionally higher N storage in plants biomass than in seeds.

Additional remarks are reported in fig. 4, that summarizes the values calculated for some index related to N uptake and use in coriander plants. First, it is worth to notice the high variability among the three trial years, as a consequence of the effect exerted on N uptake and storage by the variations in rainfall and temperature. The high instability of the climatic pattern caused a strong variability in some data, such as grain yield, in relation to the cropping year. This variability explains, probably, a large part of the differences in some of the calculated indexes, such as Gw/Ns. The trend of the Gw/Ns ratio may be interpreted as an evaluation of plant's ability to convert all potentially available N into marketable biomass. In our experiment, it took rather low values, ranging from less than 5 to a maximum of 30, in this assessing the scarce ability of coriander to use efficiently N supply in soil. Generally speaking, the conventional treatments showed values higher than the organic ones, and among them no advantage was found for N fertilization at the highest rate (C3, 120 kg ha^{-1}). However, although the effect exerted by variability among years is still evident, the overall efficiency of organic treatments rises to levels closer to the conventional treatments when the Gw/Nav values are taken into consideration. As a matter of fact, since Gw/Nav= Gw/Nt x Nt/Nav, it comes out that such efficiency value must be attributed to the concurrence of the efficiency of use of stored N (Gw/Nt, kg of grain for each kg of N uptaken by plants), generally unmodified across years for the conventional treatments, and the ability of plants to uptake N from soil and to convert it into plant biomass (Nt/Nav), which appeared to be greatly dependent upon climatic constraints. Similarly, being Gw/Ns = Gw/Nav x Nav/Ns, the variations detected in nitrogen use efficiency of coriander plants may be due to the highly variable reactivity of crop to N uptake from soil and in its allocation into plant's tissues.

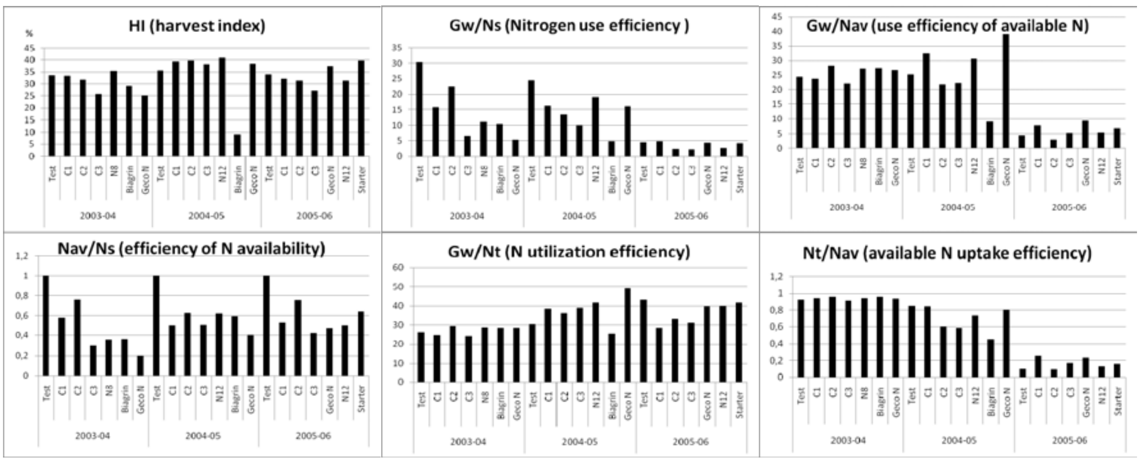

Figure 4: Sparacia (Cammarata – AG) 2003-04 to 2005-06. Indexes related to some aspects of N use efficiency in coriander under different N fertilization managements.

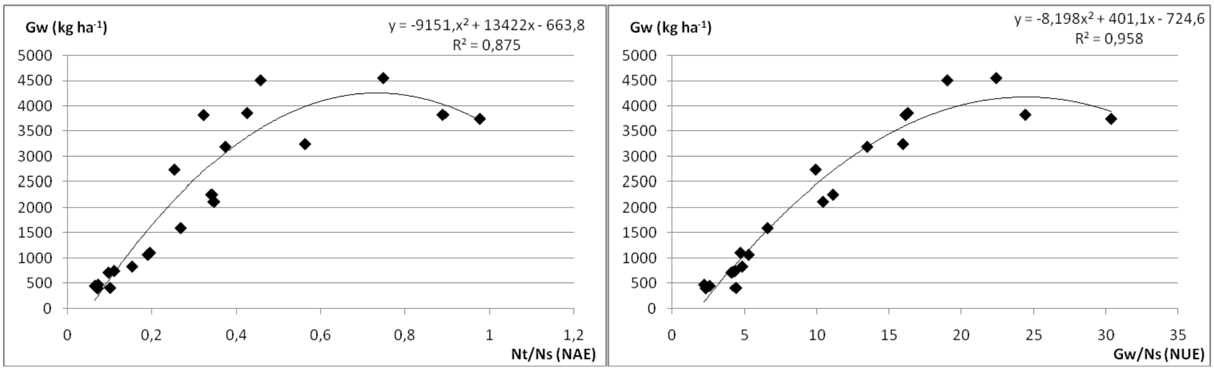

Figure 5: Sparacia (Cammarata - AG) 2003-04 to 2005-06. Relationship between coriander grain yield, nitrogen adsorption efficiency (NAE, on the left) and nitrogen use efficiency (NUE, on the right).

The importance to deepen the mechanisms that underlay N adsorption and use in coriander may be driven by the observation of the graphs in fig. 5, that stresses the tight relationship between grain yields and both examined aspects of efficiency, namely NAE (adsorption efficiency) and NUE (use efficiency).

As a matter of fact, organic fertilization is often considered as one of the recommended choices for growing medicinal and aromatic plants (Biffi, 2005). In our trial, however, organic fertilization did not allow advantages on yields, and its utilization gave erratic results. Expecially under Mediterranean climatic conditions, in which climatic patterns are often responsible of aleatory yield results, the lack of knowledge about behaviour of organic fertilizers could add a negative effect on the expected yields, with serious economical risks for farmers.

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NEW FERTILIZATION STRATEGIES ON OLIVE-GROVES CROPPED AS TRADITIONAL AND ORGANIC AGRONOMICAL INTERVENTIONS IN SOUTHERN ITALY

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Abstract

The aim of this research was to study the alternative strategies of fertilization of olive trees cropped according to the principles of intensive and organic agriculture.

The trial was carried out in two experimental fields of olive trees in Apulia and Basilicata Regions (Southern Italy). The following four fertilization strategies were compared in the first research (Apulia Region): nitrogen and phosphorus mineral fertilizers, shallow applied; mineral fertilizers incorporated into the soil at 10 cm depth; organic fertilizer as olive pomace compost incorporated into the soil at 10 cm depth and the same organic fertilizer shallow applied.

In the second trial (Basilicata Region), four fertilizer treatments were compared: organic fertilization with olive waste water; organic-mineral fertilizer; organic fertilizer with olive pomace compost and green manure amendment.

The results show that the application of olive pomace compost positively affects olive trees performances in the intensively cropped grove with yields very similar to those obtained through mineral fertilization. Furthermore, in the trial carried out as organic farming, it appears that olive pomace compost and olive mill wastes can be useful organic fertilizers in substitution of green manuring, which is normally applied in the area. The application of olive mill by-products (compost, wastes) increased the level of main soil nutrients and improved the soil organic matter composition, as showed by the values of total extraction carbon (TEC), humification carbon (HU+FU), humification rate (HR) and humification degree (DH).

No significant accumulation was observed for heavy metals content in soil.

Keywords: olive-grove, organic fertilizer, chemical composition soil.

Introduction

The Italian olive oil industry produces about the 20% of olive oil in the world, while the estimated production of olive mill wastes is nearly 2 millions tons of olive solid pomace and 1.7 millions tons of olive waste water (COI, International Olive Concil). Of these about 80 %, of these wastes, are produced in the southern Italy.

At the same time, in the last years, in Southern Italy, the conventional mineral fertilizations have contributed to a progressive worsening of soil fertility. The agriculture plays an important role for a sustainable development of the environment. The applications of not conventional organic fertilizers can offer many advantages, as the improvement of physical soil fertility parameters (Pagliai *et al.* 2001) by an increase of soil organic matter content (Montemurro *et al.* 2006). West and Post (2002) points out that "a change in the agronomic management, of the agrarian lands, it can increase in the soil the content of organic carbon, contributing to seize carbonic anhydride of the atmosphere".

Soil organic matter, and especially its humified fractions, constitute an important source of nutrients, and are also a key factor in maintaining or improving soil structure (Lòpez-Piñeiro *et al.*, 2007). Changes in total soil organic matter content induced by agricultural practice are important, changes in organic matter quality (the organic matter fractions) are also of considerable significance (Graham *et al.*, 2002). For this reason, waste processing of the oil industry (olive waste water and olive solid pomace) can be used as fertilizers. Several studies have investigated the use of three-phase olive-mill waste water as a soil amendment (Parades *et al.*, 2005). The use of olive mill wastes as fertilizers could be an appropriate strategy to reduce the progressive depletion of soil organic matter and recycling these organic materials.

The purpose of our research was to study different strategies of organic fertilizing compared to the traditional plans of fertilization in same Southern Italy areas (Puglia and Basilicata Regions).

The applications of oil waste are evaluated in olive-groves in full production.

Materials and methods

The study, conduced from 2005 to 2008, was carried out in two experimental fields (EF):

1) EF A (sandy soil - Monopoli, Apulia Region), situated near the coastal zone in an olive-grove, at few meters on the sea level.

The planting space is 5 x 5 m and the olive cultivar is *Nociara*, very diffused in the research area.

2) EF B (clay soil - Matera, Basilicata Region), layed in an olive-grove at 250 m a.s.l., in hilly position. The investigated cultivars were *Raccioppa* and *Ghiannara*, with a planting space of 10 x 10 m, typical of the secular olive-groves of the zone.

Both the experimental areas are situated in Mediterranean environment. The climate is "accentuated thermomediterranean" (UNESCO-FAO classification), with total rainfall ranging from 400 to 550 mm, with rains mainly concentrated in the winter months.

EF A

The experimental design was a randomized block with three replications and elementary plots of 100 m^2. Different fertilizing strategies have been compared (Table 1): traditional mineral fertilization with ammonium sulphate and simple superphosphate, without ploughing in (Min-sh); traditional mineral with ammonium sulphate and simple superphosphate, with ploughing in 10 cm depth (Min-inc); organic fertilizer with olive pomace compost and ploughing in 10 cm depth (Comp-inc); organic fertilizer with olive pomace compost, without ploughing in (Comp-sh).

Table 1: Amount of both fertlizers applied and nitrogen and phosphorus supplied to plant. EF A.

Treatments	Fertilizers amount for plant (kg)	N amount for plant (g)	P amount for plant (g)
Mineral fertilizer shallow applied (Min-sh)	4 (*)	420	320
Mineral fertilizer incorporated (Min-inc)	4 (*)	420	320
Olive pomace compost incorporated (Comp-inc)	50	420	260
Olive pomace compost shallow applied (Comp-sh)	50	420	260

(*) 2 kg of sulphate ammonium + 2 kg of superphosphate.

The organic fertilizer (olive pomace compost) used was produced mixing residues of pressed olives without core, wheat straw, chicken-dung and urea, in the following proportions, respectively: 90.6%, 3.6%, 5.3% and 0.5%. The mixture obtained was submitted to a process of composting for about 80 days. During this period moisture and temperature was constantly monitored. The table 2 show the chemical characteristic of olive pomace compost applied in EF A in the three-years of trial.

Table 2: Chemical characteristics of olive pomace compost applied. EF A.

Year	Moisture %	pH	N % s..s.	P % s..s.	C % s.s.	C/N	Zn mg kg^{-1}	Ni mg kg^{-1}	Cu mg kg^{-1}	Pb mg kg^{-1}
2006	29.4	8.03	1.85	0.55	24.00	12.97	91.32	6.04	41.13	5.49
2007	38.2	7.91	1.05	0.10	17.23	16.41	46.00	25.00	17.00	36.0
2008	25.0	7.97	1.23	0.67	18.70	15.20	22.74	3.02	6.54	4.07

Limiti di concentrazione (mg kg^{-1}) D. Lgs 217/06: Pb 140; Zn 500; Cu 150; Ni 100.

The sampling of both plants and soil were collected during the productive cycle. On plants, at the main phenological stages (resumption vegetative, flowering and fruit setting, drupe formation, hardening of the core, to dark-coloured turn, ripeness), the nutritional state was evalued through the leaves analysis of four sprigs for plant (one year old), one for every cardinal point (Lacertosa *et al.*,2001; Tittarelli *et al.*, 2002). On the leaves sample were determined: green index (SPAD); content of nitrates in the leaf stem; contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg). The green index was determined with a chlorophyll reader (Spad-502 Minolta) and was measured on ten fresh leaves (three determinations were effected in the middle each leaf). The nitrates content was determined on stem juice of the same leaves through Nitrachek (Merck), while the chemical characteristics were also determined on the ten leaves.

Two soil samplings were carried out at the 0-25 cm depth (maximum depth allowed because of the presence of rock) before the application of the fertilizers (t0, 29 December 2005) and at the end of the crop cycle (tf, 15 December 2008). On the soil samples were determined the content of: total organic carbon (TOC), total extraction carbon (TEC), humic and fulvic acid (H+F), total N, available P, exchangeable K, heavy metals Cu, Zn, Ni and Pb. The determinations were effected according to the official protocols of soil chemical analysis, vegetables and fertilizers, by the Office of the Agricultural and Forest Politics. Statistical analysis of variance was made by SAS procedures (Sas Institute, 1998). Differences among the means were analyzed at the $P \leq 0.05$ probability level, applying the Duncan Multiple Range Test (DMRT).

EF B

The experimental design was a randomized block with three replications and elementary plots of 200 m^2 each. Different fertilizing were compared (tab. 3): olive waste water, distributed at the rate of 80 m^3 ha^{-1}, (OWW), organic-mineral fertilizer admitted in biological agriculture (Org-min), organic fertilizer with olive pomace compost (Comp), organic fertilizer with green manure amendment of broad bean, *Vicia faba* L. minor Beck, (GMA).

Table 3: Amount of both fertilizers applied and nitrogen and phosphorus supplied to plant. EF B.

Treatments	Fertilizers amount for plant (kg)	N amount for plant (g)	P amount for plant (g)
Olive waste water (AWW)	800*	640	240
Organic mineral fertilizer (Org-min)	15	900	314
Olive pomace compost (Comp)	60	900	371
Green manure amendments (GMA)	150	645	113

* Litre.

The table 4 shows the physical and chemical characteristics of compost olive waste water and broad crop bean. The samplings and the experimental determinations were carried out following the same methodologies described in the EF A.

Table 4: Chemical characteristics of biomass applied. EF B.

	Moisture %	**pH**	**N % s.s.**	**P % s.s.**	**C % s.s.**	**C/N**	**Zn mg kg^{-1}**	**Ni mg kg^{-1}**	**Cu mg kg^{-1}**	**Pb mg kg^{-1}**
Compost										
2006	29.4	8.03	1.85	0.55	24.00	12.97	91.32	6.04	41.13	5.49
2007	38.2	7.91	1.05	0.10	17.23	16.41	46.00	25.00	17.00	36.0
2008	25.0	7.97	1.23	0.67	18.70	15.20	22.74	3.02	6.54	4.07
*Broad bean**	83	//	2.90	0.053	29.8	10.3	< 0.1	< 0.1	< 0.1	< 0.1
*O.W.W.**	//	4.91	0.08^{+}	0.032^{+}	2.50^{+}	31.2	3.80^{+}	6.54^{+}	2.02^{+}	1.53^{+}

* Average value of the three year trial; + mg l^{-1}.

Results and Discussions

<u>EF A</u>

After tree years of trial period the 4 different treatments, on the average nutritional state of the leaves, showed a substantial equilibrium of P, K and Mg contents (Figure 1). The content of nitrogen in the olive leaves was higher in the mineral treatments, particularly, the N total content increased of 11.8 and 16.0% in the treatment Min-sh and Min-inc, respectively, compared to the treatments Comp-sh and Comp-inc. The results can be attributed to the different chemical form of N in fertilizer treatments. The Ca content reached the highest value in Min-sh, while in the remaining treatments the values were almost identical.

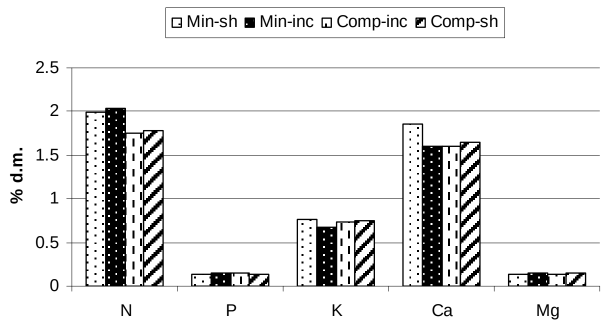

Figure 1: Chemical composition leaves. EF A (average of tree years).

The table 5 shows the average values of the three year trial of SPAD and nitrates. These values did not pointed out any significant difference within the experimental treatments according to the chemical characteristics of leaves. This results underline that in long-term organic and mineral fertilization can to reach the same potential fertilizer.

In the tf time (table 6), even if not statistical differences among the treatments were recorded, for soil contents of total organic carbon (TOC), total extraction carbon (TEC), humic and fulvic acid (H+F), the mean content of same parameters is greater in the organic treatments than the mineral one, respectively of the +8, +7.7, +6.4%.

Table 5: SPAD and nitrates. EF A (average value of the three year trial).

Treatments	SPAD	NO_3 mg kg^{-1}
Min-sh	77.73	114.11
Min-int	77.54	103.77
Comp-int	73.46	107.91
Comp-sh	75.08	101.15

Values with different letters in column are significantly different at P≤0.05 (DMRT)

Tabella 6: Chemical soil composition at tf. EF A.

Chemical characteristics	t0	tf							
		Min-sh		Min-inc		Comp-inc		Comp-sh	
TOC (g kg^{-1})	18.73	22.66		21.79		26.15		22.04	
TEC (g kg^{-1})	11.60	15.19		15.04		17.18		15.36	
U+F (g kg^{-1})	9.04	11.12		10.87		12.98		10.42	
DH %	79.12	73.18		72.17		74.23		67.77	
HR %	48.47	49.13		49.84		49.18		47.24	
N tot. (g kg^{-1})	1.77	1.73		1.92		2.06		1.95	
$NaHCO_3$-P (mg kg^{-1})	5.55	9.93	b	15.81	a	15.23	a	9.01	b
NH_4Ac-K (mg kg^{-1})	455	535	ab	391	b	671	a	776	a
Zn (mg kg^{-1})	54.47	55.26		55.66		57.14		56.10	
Cu (mg kg^{-1})	24.16	28.17		30.56		32.38		28.68	
Ni (mg kg^{-1})	34.61	34.80		34.32		34.37		33.53	
Pb (mg kg^{-1})	29.67	29.30		30.31		28.15		28.84	

Values with different letters in row are significantly different at P≤0.05 (DMRT)

Within the treatments there were not significant differences in total nitrogen contents, which reached the highest levels with the treatment Comp-inc and the lowest one with the treatment Min-sh. Conversely significant differences were showed in the content of available phosphorus and exchangeable potassium. About this aspect it appear that the variation of P in soil content depend more of the application technology than fertilizer chemical form, while the opposite is true for the content of potassium. The heavy metals content showed similar values among the fertilizer strategies.

EF B

In general, chemical leaves composition did not point out any substantial difference among the treatments (Figure 2), even if the treatment Org-min (organ-mineral fertilizer) has shown a N, K and Ca content higher than the other treatments. In both research the diagnostic leaves confirmed that the organic fertilization initially determines a partial immobilization of mineral nitrogen, that is furtherly available with the following organic matter mineralization processes (Elliot *et al.* 1981).

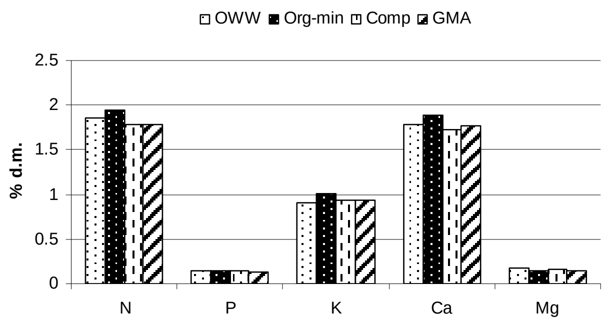

Figure 2: Chemical composition leaves. EF B (average of tree years).

In the table 7 are reported the mean values of SPAD and nitrates, which are similar among the treatments, according to the chemical characteristics of leaves.

Table 7: SPAD and nitrates. EF B (average of tree years).

Treatments	SPAD	NO_3 mg kg^{-1}
OWW	76.42	102.87
Org-min	77.83	100.30
Comp	75.96	97.99
GMA	77.51	100.38

Values with different letters in column are significantly different at P≤0.05 (DMRT)

The soil fertility evolution (Table 8) has shown that compared treatments to the end of trial period does not affect significantly TOC and TEC contents. The content of organic humificatied carbon rate was significantly lower in the treatment GMA, while in same parameter values not particularly different was observed from Org-min, OWW and Comp. The content in $NaHCO_3$-P was always higher with the application of organic alternatives treatments compared to Org-min and was statistically different with the treatment OWW. About the organic matter content and its humification fractions was observed, at the end of the trial (tf), that in all treatments the values of TOC remain unchanged versus start period (t0), while the values of TEC and U + F in same time were increased. This has led to an increase of DH and HR compared to t0, particularly the values are higher with the treatment Comp.

Not significant differences there are in the soil total N content and NH_4Ac-K. (Table 8), which reached the highest value (1.16 g kg^{-1} and 1109 mg kg^{-1}, respectively) with the treatment OWW (Olive Waste Water). The content of heavy metals in soil after three years of application does not show large accumulations, even if with Comp treatment were recorded the highest level in Zn and Pb content.

Therefore, the nutritional status of the crop and the evolution of soil fertility in both experimental fields show that organic fertilizers, when applied systematically and repeatedly at least one month before the resumption vegetative, are able to meet the nutritional requirements of the olive trees and are comparable to mineral fertilizers, as soil chemical fertility; finally organic fertilizers seem to improve the physical and biological soil fertility.

Tabella 8: Chemical soil composition at tf. EF B.

Chemical characteristics	t0	tf							
		OWW		Org-min		Comp		GMA	
TOC (g kg^{-1})	11.3	11.2		11.7		10.6		11.1	
TEC (g kg^{-1})	5.89	7.91		7.76		8.31		7.57	
U+F (g kg^{-1})	3.29	6.00	ab	6.06	a	6.62	a	5.35	b
DH %	55.4	76.0	ab	78.1	a	79.7	a	70.5	b
HR %	28.9	53.8		52.0		66.0		48.4	
N tot. (g kg^{-1})	1.23	1.16		1.14		1.15		1.12	
$NaHCO_3$-P (mg kg^{-1})	9.87	29.5	a	22.4	b	24.2	ab	25.2	ab
NH_4Ac-K (mg kg^{-1})	834	1109		963		934		978	
Zn (mg kg^{-1})	34.0	39.8	bc	42.6	ab	45.5	a	38.0	c
Cu (mg kg^{-1})	13.4	15.0		13.7		14.5		12.9	
Ni (mg kg^{-1})	21.0	22.8		21.6		22.9		22.4	
Pb (mg kg^{-1})	7.90	7.75	c	9.33	ab	10.3	a	8.70	bc

Values with different letters in row are significantly different at $P \leq 0.05$ (DMRT)

In order to widen the knowledge on the tree crops nutritional implications and to study the quantity and the quality productions, it is necessary to deepen the evolutionary aspects of the organic fertilization and then it is planned to continue the research over the years.

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INOCULATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA AS INFLUENCED BY ORGANIC FERTILIZATION: EFFECTS ON PLANT AND SOIL P CHARACTERISTICS

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Abstract

Rhizobacteria are able to promote the phosphorus (P) supply of plants essentially, but their efficiency in interaction with organic fertilization is scarcely known. In a pot experiment the effects of two plant growth promoting rhizobacteria (PGPR) *Pseudomonas fluorescens* strain DR 54 and *Enterobacter radicincitans* sp. nov. strain DSM 16656 on plant growth and plant P uptake of *Phacelia tanacetifolia* cv. Boratus as well as soil P characteristics were assessed depending on organic fertilization (without and with cattle manure or biowaste compost). The application of cattle manure significantly enhanced the P uptake of *Phacelia tanacetifolia* cv. Boratus. Applications of both bacterial strains increased activities of phosphatases under *Phacelia tanacetifolia* cv. Boratus. The magnitude of this effect varied between the different organic fertilization treatments and between the two bacterial strains. Our results indicate that the application of PGPR can response in significantly changed soil P turnover, and that organic fertilization modifies the efficiency of applied rhizobacteria.

Introduction

Soil microorganisms are considered to mould soil fertility and plant health (Nannipieri *et al.* 2003, Johansson *et al.* 2004). In this context the use of bacterial inoculants in crop production is regarded to be a non hazardous biological approach for plant establishment especially under nutrient deficiency conditions (Egamberdiyeva and Höflich 2004). Hence, such applications might serve to ease the demand of agrochemicals and contribute to the alignment of agricultural ecosystems with the principle aims of sustainable development (Zahir *et al.* 2004, Kohler *et al.* 2007).

Beside nitrogen (N), phosphorus (P) often represents the plant growth limiting element (Rodriguez and Fraga 1999), but might also cause eutrophication of surface water bodies after improper fertilization (Oehl *et al.* 2002). A huge amount of P that is applied to the soil due to fertilization rapidly gets plant unavailable, conglomerating in either organic P fractions that are immobilized in soil organic matter or in inorganic P fractions which are fixed by chemical adsorption and precipitation (Richardson *et al.* 2001). It is known that soil organic P forms between 20 and 80 % of total P in the surface layer of soils and needs to undergo mineralization in order to become plant available (Oehl *et al.* 2004, Chen *et al.* 2004). Several plant growth-promoting rhizobacteria (PGPR) are especially able to mineralize organic phosphorus compounds by phosphatases converting them into plant available forms (Vessey 2003). However, information on the performance of PGPR in combination with organic fertilization is rare.

Therefore our study assessed the complementary effects of organic fertilization in combination with application of two PGPR strains (a *Pseudomonas fluorescens* strain as well as an *Enterobacter radicincitans* strain) on growth and P uptake of *Phacelia tanacetifolia* cv. Boratus as well as on selected soil properties of the P cycle.

Materials and methods

A pot experiment was carried out to test the complementary effects of inoculation with PGPR strains × organic fertilization on growth and P uptake of *Phacelia tanacetifolia* cv. Boratus as well as soil P characteristics. The trial included three treatments with PGPR (B-control without bacteria application, BE+ application of *Enterobacter radicincitans* DSM 16656, BP+ application of *Pseudomonas fluorescens* DR54, 10^9 bacteria per m^2) and three fertilization treatments (nonfertilized control, treatment with application of cattle manure, treatment with biowaste compost). Initial soil characteristics indicated a suboptimal plant available P supply comprising all fertilization treatments. After growth of the plants for three weeks, strains of either *E. radicincitans* or *P. fluorescens* and no PGPR for control were applied, respectively. Harvest was done after eight weeks of plant growth. Each treatment comprised four replicates. Shoot biomass P concentrations of phacelia were determined after dry ashing using the vanadad-molybdate method (Page *et al.*, 1982). Shoot biomass P uptake per pot was calculated by multiplying shoot biomass and P concentrations of the shoot biomass. Acid and alkaline phosphatase activities in the soil were measured according to Tabatabai and Bremner (1969). Analysis of variance (ANOVA) was realized by means of Duncan's multiple range tests at the 95 % level of probability in order to evaluate significant effects of fertilization, PGPR and their interacion on soil and plant parameters.

Results and Discussion

The acid phosphatase activities in the rhizosphere were always increased after the application of the *P. fluorescens* strain (Fig. 1). The strongest effect of this strain (+36 % compared to control) was obtained in the control treatment without fertilization (Fig. 2).

The alkaline phosphatase activities showed an even higher response (+123 % compared to control) indicating the strongest effect without fertilization after the application of the *P. fluorescens* strain (Fig. 2).

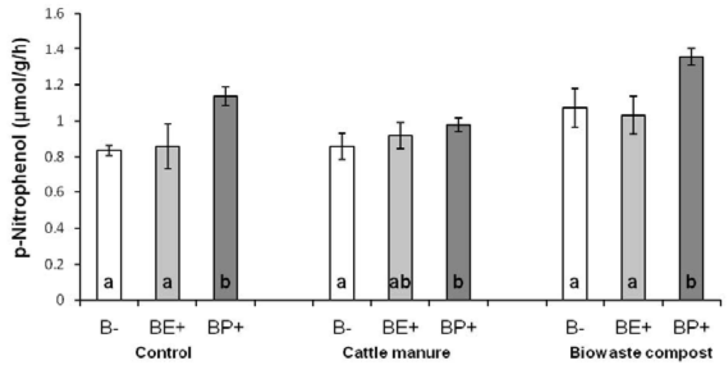

Figure 1: Acid phosphatase activities (mean ± SD) after fertilization (control without fertilization, cattle manure application, biowaste compost application) and application of PGPR (B- non-treated control, BE+ application of *Enterobacter radicincitans* DSM 16656, BP+ application of *Pseudomonas fluorescens* DR54) under *Phacelia tanacetifolia* cv. Boratus after two months of growth in an outdoor pot experiment. Mean values with different letters indicate significant differences (p ≤ 0,05) using Duncan's multiple range test after one way analysis of variance.

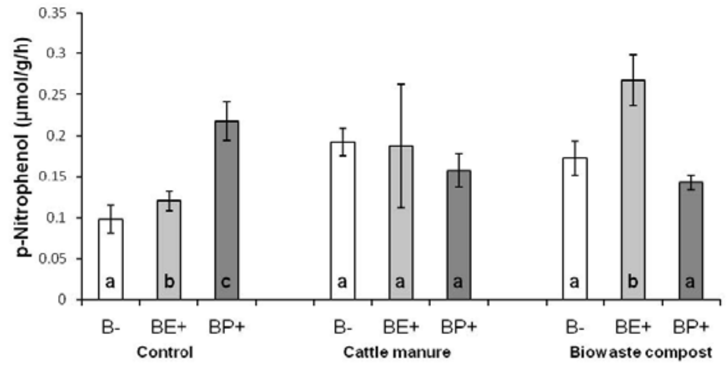

Figure 2: Alkaline phosphatase activities (mean ± SD) after organic fertilization (control without fertilization, cattle manure application, biowaste compost application) and application of PGPR (B- non-treated control, BE+ application of *Enterobacter radicincitans* DSM 16656, BP+ application of *Pseudomonas fluorescens* DR54) under *Phacelia tanacetifolia* cv. Boratus after two months of growth in an outdoor pot experiment. Mean values with different letters indicate significant differences (p ≤ 0,05) using Duncan's multiple range test after one way analysis of variance.

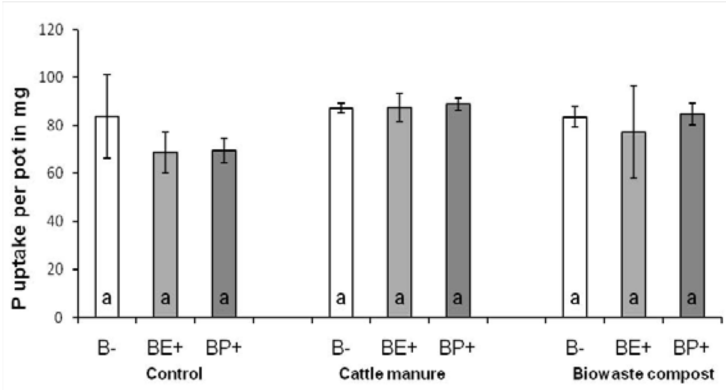

Figure 3: Shoot biomass P uptake per pot in mg (mean ± SD) after organic fertilization (control without fertilization, cattle manure application, biowaste compost application) and application of PGPR (B-nontreated control, BE+ application of *Enterobacter radicincitans* DSM 16656, BP+ application of *Pseudomonas fluorescens* DR54) under *Phacelia tanacetifolia* cv. Boratus after two months of growth in an outdoor pot experiment. Mean values with different letters indicate significant differences ($p \leq 0,05$) using Duncan's multiple range test after one way analysis of variance.

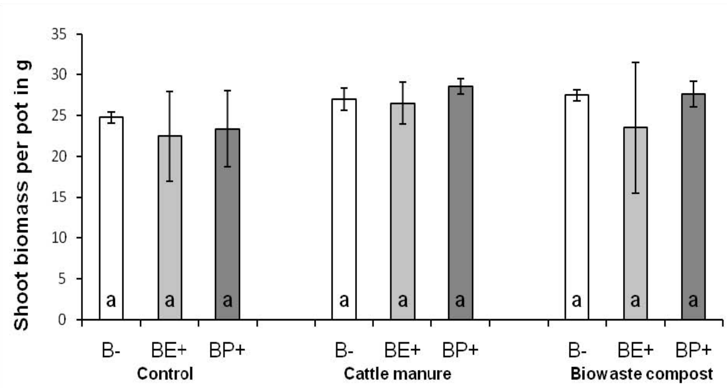

Figure 4: Shoot biomass per pot in g (mean ± SD) after organic fertilization (control without fertilization, cattle manure application, biowaste compost application) and application of PGPR (B-non-treated control, BE+ application of *Enterobacter radicincitans* DSM 16656, BP+ application of *Pseudomonas fluorescens* DR54) under *Phacelia tanacetifolia* cv. Boratus after two months of growth in an outdoor pot experiment. Mean values with different letters indicate significant differences ($p \leq 0,05$) using Duncan's multiple range test after one way analysis of variance.

However, the application of the PGPR strains did not show significant effects on plant growth and P uptake (Fig. 3 and 4). According to Egamberdiyeva (2007), stimulatory effects on plant growth derived by bacterial inoculation are more likely to occur in nutrient deficient soils. Therefore we suggest that plant growth promotion might appear after a longer growth period. The results demonstrated that an application of PGPR can influence soil P dynamics before plant growth stimulation might be observed and that the interactive effect with organic fertilization depends on bacterial strain and organic matter origin.

Acknowledgements

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YIELD, NUTRITIONAL STATUS AND SOIL CHEMICAL PROPERTIES AS RESPONSE TO CATTLE MANURE, REACTIVE NATURAL ROCK PHOSPHATE AND BIOTITE SCHIST IN MASSAI GRASS

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Abstract

In animal production, grasses planted in the pasture lands have especial value to improve aggregate value of products. This paper evaluates the effects of applying cattle manure, reactive natural phosphate and biotite schist on soil fertility, yield and nutritional content of *Megathyrsus* spp. cv. Massai. The experiment was conducted under field conditions, in an Oxisol. The experimental design was randomized blocks with the treatments confounding. The treatments consisted of three rates of natural reactive rock phosphate from Algeria - Djebel-Onk (0, 100 and 200 kg ha^{-1} of P_2O_5), three biotite schist rates (0, 150 and 300 kg ha^{-1} of K_2O) and three cattle manure rates (0, 20 and 40 Mg ha^{-1}). The application of reactive natural rock phosphate increased dry matter yield (DMY), however, this effect was not observed for cattle manure and biotite schist. The foliar contents of N, K and Mg (cattle manure), P and B (natural rock phosphate) and K (biotite schist) were significantly influenced by the treatments. The same effect was found for P levels in soil, dry matter of the aerial part, Mg and B content in the dry matter.

Keywords: *Megathyrsus* spp., mineral nutrition, soil fertility, alternatives sources.

Introduction

In intensive cattle dairy system, grazing capacity of the pasture needs to be improved by adopting adequate management practices. Adequate rates of fertilizers including limestone, N, P and K and micronutrients are necessary to improve growth of pasture grasses. In the dairy industry, constant fluctuations in the price of milk make it advisable to introduce new products with higher aggregate value. Although organic milk is a promising product, with demand growing by 30% a year, it is still restricted to a small niche in relation to conventional milk (Castro *et al.*, 2008). The same observation holds for organic meat and other animal products.

But in organic systems, the sources of potassium are restricted to ashes, potassium sulfate, vinasse (from sugarcane processing) and mulch (AAO, 2000), making it difficult to add nutrients to the system. Studies are thus necessary to find new alternative sources of K. The objective of this study was to evaluate the effect of applying cattle manure, reactive natural rock phosphate (RNRP) and biotite schist on the production, nutritional value and soil fertility of a hybrid of *Megathyrsus* spp., Massai cultivar.

Materials and methods

The experiment was conducted in an Oxisol, with medium texture (483 g kg^{-1}). After correction with dolomitic limestone, the top layer (0-20 cm) presented the following chemical characteristics: pH in $CaCl_2$ = 6.0; P = 16.1 mg dm^{-3}; K = 0.8 $mmol_c$ dm^{-3}; Ca+Mg = 66.0 $mmol_c$ dm^{-3}; and H+Al = 22.0 $mmol_c$ dm^{-3}. The treatments consisted of three rates (0, 100 and 200 kg ha^{-1} of P_2O_5) of reactive natural rock phosphate (RNRP) from Algeria, with 29% P_2O_5 (9% being soluble in citric acid) and 12% of Ca, three rates (0, 150 and 300 kg ha^{-1} of K_2O) of biotite schist: pH_{H2O} = 8.5, K_2O = 4.3%, CaO = 2.8%, MgO = 15.1%, P_2O_5 = 0.01%, PN = 0% and SiO_2 = 54.6%, and three rates (0, 20 and 40 Mg ha^{-1}) of cattle manure: N = 5.0 g kg^{-1}, P = 2.6 g kg^{-1}, K = 6.0 g kg^{-1}, S = 1.0 g kg^{-1} and Ca = 2.0 g kg^{-1}. The full rates of natural rock phosphate, $^2/_3$ of the cattle manure and $^2/_3$ of the potassic rock were incorporated 20 days before planting. After the third cutting, the remaining $^1/_3$ of the potassic rock and $^1/_3$ of the cattle manure were applied as top dressing to complete the treatments.

The experimental design was a randomized block with the treatment confounding, and an additional treatment with cattle manure, natural rock phosphate and potassium chloride, in proportions of 40 Mg ha^{-1}, 100 kg ha^{-1} of P_2O_5 and 150 kg ha^{-1} of K_2O. The first cuttings occurred 60 days after planting the Massai cultivar, a spontaneous hybrid between *Megathyrsus maximum* and *Megathyrsus infestum*. Six cuttings were made. After collection, the vegetable material was weighed and dried at 65^oC to determine the dry matter yield (DMY). Before planting and after each cutting, samples were taken from the topsoil at a depth of 0-20 cm to determine the pH ($CaCl_2$), available P and exchangeable K, Ca, Mg and H+Al, soil organic matter (SOM) and S-SO_4 (Raij *et al.*, 2001). After each cut, cattle were allowed to graze on the parts of the plots not sampled. After being dried, the samples from the aerial part were ground and submitted to chemical analyses. The total N P, K, Ca, Mg, S, Cu, Fe, Mn and Zn were extracted according to the method described by Malavolta *et al.* (1997). The data were compared by Tukey test and regression analysis at 5% and 10% probability and finally, Pearson correlation coefficients between the dry matter yield and the levels of available and exchangeable elements in the soil and plants were also calculated.

Results and discussion

The variance analysis showed a significant effect of applying natural rock phosphate on the dry matter yield (DMY) of the Massai grass, while the use of up to 40 Mg ha^{-1} of cattle manure and 300 kg ha^{-1} of K_2O in the form of biotite schist and their interactions showed no influence of the treatments (Table 1).

Table 1: Least square means of dry matter yield (DMY) of Massai grass obtained under different treatments.

Cattle manure	Dry matter yield	Natural rock phosphate	Dry matter yield	Biotite schist	Dry matter yield
Mg ha^{-1}	Mg ha^{-1}	P_2O_5 - kg ha^{-1}	Mg ha^{-1}	K_2O - kg ha^{-1}	Mg ha^{-1}
0	10446.28	0	8929.17b	0	9943.84
20	10075.74	100	10278.86ab	150	9714.94
40	9880.21	200	11194.20a	300	10743.45
Cattle manure		Natural rock phosphate		Potassium chloride	
40	9800.28	100	9800.28	150	9800.28
Analysis of variance				F test	
Cattle manure (a)				NS	
Natural rock phosphate - RNRP (b)				*	
Biotite schist (c)				NS	
CV%				16.63	

*,NS Significant at the 5% probability and non significant, respectively. [1]Means followed by the same letter in the each column are not significantly different at the 5% probability level by Tukey test. Σ of six cuttings.

In the case of RNRP, there was a 25% increase in forage volume, which is equivalent to an estimated enhance of 2.3 Mg ha^{-1} $year^{-1}$. These gains in yield are due to the function of phosphorus in plant metabolism. It plays an important role in the energy storage and transfer, acting mainly in the form of ADP and ATP on plants' respiration, photosynthesis, synthesis of nucleic acids and transport of ions through cell membranes (Fageria, 2009).

The lack of a significant increase in dry matter yield with the addition of cattle manure (Table 1) is possibly due to the predominantly organic form of the N present after incorporation of the manure into the soil (Kiehl, 1999). For N to be assimilated from fresh manure, mineralization or ammonification of the soil organic matter (SOM) must occur first, but in this period the N becomes immobilized by the decomposing microorganisms, which is intimately related to the metabolism of carbon (Aita & Giacomini, 2007).

The effects manure application has also been analyzed by Oliveira *et al.* (2007). In these studies, was obtained a quadratic effect, showing a significant increase in productivity, except at the highest rates applied, which caused a reduction. In the case of biotite schist, although other authors have found significant enhancement of dry matter yield of sunflowers and soybeans, in other crops such as corn (Resende *et al.*, 2006) there was no significant effect, irrespective of the rate applied. According to Straaten (2007), in biotite schist's structure, the K is present in the phyllosilicates between the tetrahedron layers of Al and octahedron layers of Si or Mg, hindering the nutrient's release and availability to the plants. In this study, the DMY with application of biotite was similar to that with application of KCl (Table 1). The analyses of the DMY of the aerial part showed that the addition of cattle manure raised the concentration of N e K and reduced the Mg (Table 2).

Table 2: Least square means of N, P, K, Ca, Mg and S concentration in aerial dry matter of forage (Massai grass) under different treatments.

Variable	N	P	K	Ca	Mg	S
	------------------	------------------	------------------ g kg^{-1}	------------------	------------------	------------------
Manure, Mg ha^{-1}						
F test	*	NS	*	NS	*	NS
Phosphate, kg ha^{-1}						
F test	NS	*	NS	NS	NS	NS
Biotite, kg ha^{-1}						
F test	NS	NS	**	NS	NS	NS
CV%	5.56	22.91	21.00	5.89	21.91	15.80
CPK(2)	20.37	6.97	27.26	5.65	11.27	2.67

*, **, NS Significant at the 5 and 10% probability; and non significant, respectively. Natural rock phosphate – quantity in P_2O_5, biotite schist – quantity in K_2O. [1]Means followed by the same letter in the each column are not significantly different at the 5% probability level by Tukey test. [2]CPK = Cattle manure (40 Mg ha^{-1}), Natural rock phosphate (100 kg ha^{-1} of P_2O_5) and potassium chloride (150 kg ha^{-1} of KCl).

The application of natural rock phosphate caused an increase in the P and a reduction in the B, while the addition of biotite schist significantly raised only the K concentration (Table 3). The three types of fertilizers did not influence the concentrations of Ca, S, Cu, Fe, Mn and Zn in the DMY (Tables 2 and 3).

The smaller B concentration with the addition of phosphate is possibly due to the limitation of the nutrient itself in the soil, because of the positive interaction of the P and B, enhancing the absorption of the latter (Fageria, 2009). In the absence of foliar concentration indicated as standards on the evaluation of the nutritional state of Massai grass in Brazilian conditions, the average concentrations in the plant tissue (aerial dry matter) presented the following order of macronutrients uptake: K>N>Mg>P>Ca>S (Table 2), and in the micronutrients themselves the sequence was: Fe>Mn>Zn>B>Cu (Table 3).

Table 3: Least square means of B, Cu, Fe, Mn and Zn concentration in aerial dry matter of forage (Massai grass) under different treatments.

Variable	B	Cu	Fe	Mn	Zn
	------------------	------------------	mg kg^{-1}	------------------	------------------
Manure, Mg ha^{-1}					
F test	NS	NS	NS	NS	NS
Phosphate, kg ha^{-1}					
F test	*	NS	NS	NS	NS
Biotite, kg ha^{-1}					
F test	NS	NS	NS	NS	NS
CV%	11.98	7.27	20.11	13.61	7.97
CPK(2)	6.01	5.88	307.77	88.19	21.81

*, **, NS Significant at the 5 and 10% probability levels and non significant, respectively. Natural rock phosphate – quantity in P_2O_5, biotite schist – quantity in K_2O. [1]Means followed by the same letter in the same column are not significantly different at the 5% probability level by Tukey test. [2]CPK = Cattle manure (40 Mg ha^{-1}), Natural rock phosphate (100 kg ha^{-1} of P_2O_5) and potassium chloride (150 kg ha^{-1} of KCl).

Assuming as adequate the nutrient concentration in *Megathyrsus* spp., regardless of the cultivar, were found that the average of N, P, K, Mg, S, Fe and Zn were above those found by Gallo *et al.* (1974), while those of Ca, B and Mn were considered low (Tables 2 and 3). The application of cattle manure boosted the content of SOM and available S in the soil, while the pH, available P, K, Ca, Mg and exchangeable H+Al were not influenced by the treatments (Table 4). The addition of 20 and 40 t ha^{-1} of cattle manure (treatments) increased the organic matter content by approximately 2 and 4 t ha^{-1} of C, respectively, without considering the mulch and manure left by the grazing animals after each cutting. In the case of the enhanced content of $S\text{-}SO_4$ in the soil verified, it is estimated that over 95% of the S in soils is contained in organic compounds, with plant residues and animal droppings constituting the major source. The levels of available P, exchangeable H+Al and SOM increased with the higher rates of natural rock phosphate (Table 4). This enhance of available P to the plants was directly related to the natural rock phosphate rates. In the case of exchangeable H+Al, considering which ammonia is one of the first products formed, the application of P must have been accelerated the initial decomposition of the soil organic matter, increasing the soil acidity. When the ammonia is converted into nitrate, H^+ ions are released, temporarily raising the exchangeable and non-exchangeable acidity of the soil (Sousa *et al.*, 2007).

The application of biotite schist raised the concentration of exchangeable K in the soil from 0.8 $mmol_c\ dm^{-3}$ to 1.1 $mmol_c\ dm^{-3}$ (Table 4). Although this rock contains high quantities of Ca (2.8%), Mg (15.1%) and SiO_2 (54.6%) and has a high pH_{H2O} index (8.5), these variables were not influenced ($p > 0.05$) by the treatments. The DMY was positively relationship with the available P and concentration of P and Mg in the dry matter, and negatively with the B concentration in the plants (Table 5).

Table 4: Least square means of pH_{CaCl2}, P, K, Ca, Mg, H+Al, MOS and $S\text{-}SO_4$.

	Cattle manure, Mg ha^{-1}			F test	CPK(2)	CV
	0	20	40			%
pH ($CaCl_2$)	5.92	5.72	5.73	NS	5.77	6.03
P (mg dm^{-3})	21.00	17.22	21.78	NS	23.00	23.86
K ($mmol_c$ dm^{-3})	0.77	0.94	1.19	NS	3.07	19.27
Ca ($mmol_c$ dm^{-3})	41.22	36.33	35.67	NS	37.00	20.32
Mg ($mmol_c$ dm^{-3})	20.22	17.11	17.00	NS	19.33	20.73
H+Al ($mmol_c$ dm^{-3})	20.78	23.11	23.00	NS	21.33	19.83
$S\text{-}SO_4$ (mg kg^{-1})	3.00c	5.33b	8.11a	*	6.00	20.04
MOS (g kg^{-1})	32.78b	34.33ab	35.89a	**	37.00	9.77
	RNRP, kg ha^{-1} - P_2O_5					
	0	100	200			
pH ($CaCl_2$)	5.82	5.78	5.78	NS		
P (mg dm^{-3})	14.56b	19.11b	26.33a	*		
K ($mmol_c$ dm^{-3})	1.24	0.82	0.83	NS		
Ca ($mmol_c$ dm^{-3})	38.00	35.78	39.44	NS		
Mg ($mmol_c$ dm^{-3})	17.78	18.33	18.22	NS		
H+Al ($mmol_c$ dm^{-3})	22.67a	35.22b	35.78b	*		
$S\text{-}SO_4$ (mg kg^{-1})	5.89	5.44	5.11	NS		
MOS (g kg^{-1})	32.00b	35.22a	35.78a	*		
	Biotite schist, kg ha^{-1} - K_2O					
	0	150	300			
pH ($CaCl_2$)	5.76	5.83	5.76	NS		
P (mg dm^{-3})	19.00	22.89	18.11	NS		
K ($mmol_c$ dm^{-3})	0.80b	1.00ab	1.10a	**		
Ca ($mmol_c$ dm^{-3})	37.56	39.56	36.11	NS		
Mg ($mmol_c$ dm^{-3})	18.11	18.22	18.00	NS		
H+Al ($mmol_c$ dm^{-3})	23.44	22.22	21.22	NS		
$S\text{-}SO_4$ (mg kg^{-1})	4.11	6.44	5.89	NS		
MOS (g kg^{-1})	34.56	34.11	34.33	NS		

*, **, NS Significant at the 5 and 10% probability levels and non significant, respectively. [1]Means followed by the same letter in the each line are not significantly different at the 5% probability level by Tukey test. [2]CPK = Cattle manure (40 Mg ha^{-1}), Natural rock phosphate - RNRP (100 kg ha^{-1} of P_2O_5) and potassium chloride (150 kg ha^{-1} of KCl).

The maximum estimated yield was obtained when the concentration of available P in the soil and plants and of Mg in the plants were 29.5 mg dm^{-3}, 18.3 g kg^{-1} and 14.3 g kg^{-1}, respectively. For the foliar B, the lowest production of DMY estimated was obtained with a foliar content estimated at 8.5 mg kg^{-1}. In the absence of data, these values can be used a reference to define the suitable levels of these elements in the soil and plants.

Table 5: Significant relationship between principal soil and plant chemical properties (x) with forage aerial dry matter yield (ŷ) of Massai grass[1].

Variables	Regression equation	r
P (mg dm^{-3})	$\hat{y} = 8.422.04 + 132.90x - 2.25x^2$	0.45**
P – DMY (g kg^{-1})	$\hat{y} = 1243.57 + 1463.51x - 39.98x^2$	0.66*
Mg – DMY (g kg^{-1})	$\hat{y} = 18639.13 - 1274.17x + 44.50x^2$	0.61*
B – DMY (mg kg^{-1})	$\hat{y} = 10794.49 - 1127.73x$	-0.47*

[1]The values are average of six soils and plant collections. DMY – Dry matter yield.
*, **, NS Significant at the 5 and 10% probability levels and non significant, respectively.

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ORGANIC PLANT PRODUCTION - LIMITED BY NUTRIENT SUPPLY? AN OVERVIEW

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Abstract

In organic plant production the number of approved mineral fertilisers is strictly limited. Additionally, various by-products and residues from industrial processes, mainly from the food industry can be used for fertilisation. The early organic farming movement in the 20th century used household-composts and sewage sludge, but the awareness of the existence of organic and inorganic xenobiotics in those products excludes nowadays categorically nutrient recycling. The vitality of organic plant production is mainly based on farmyard manure and legume growth. Organic farming aims at closed nutrient cycles despite the risk of negative nutrient balances and nutrient mining. Nevertheless the productivity level in organic farming is constant since many years. Residues from local bio-energy production on the basis of organically grown crops could be the key to break up mental reservations against fertilisation with externally recycled materials as the concept is in agreement with legitimate organic directives. Biogas residues from fallow-legumes offer for instance a new tool to optimise N-fertilisation on organic farms and could increase productivity substantially. Other options to improve nutrient management on organic farms comprise nutrient mobilisation in the soil by roots and/or mycorrhiza, improved nutrient uptake and utilisation efficiency of plants by breeding and the recycling of environmentally sound waste-materials.

Keywords: biological nutrient mobilisation, organic farming, macronutrients, micronutrients, farm residues, biogas slurry, waste recycling.

Introduction

Plant nutrition plays a key role in organic farming as a harmonic nutrition of the crop is essential not only for producing high quality feedstuff and food, but also for promoting natural plant resistance mechanisms against pests and diseases. The major difference between organic and conventional farming is summarized by Schmidt and Haccius (1998): "The primary concern of organic agriculture is not the substitution of depleted nutrients and not that of feeding plants, but rather feeding soil life. Consequently an organic farmer does not aim at defined crop yields by providing for a certain input of fertilizers and nutrients." Organic plant production relies on internal nutrient cycling and on the application of nutrients with organic materials from organic production. With the introduction of mineral fertilisation mid of the last century crop yields increased rapidly in conventional agriculture. In comparison, crop productivity in organic farming relies on a high soil organic matter and soil N content and the conservation of soil water resources so that organic farming may have special advantages under drought conditions (Pimentel *et al.*, 2005) and may contribute to food security in regions, which rely on local farming systems (Badgley *et al.*, 2007; Scialabba, 2007; Scialabba and Hattam, 2002). In contrast, in industrial countries with favourable cropping conditions, high input of resources and crop rotations that are limited by strong market interactions an improved nutrient management is the key to raise yields in organic farming and to secure further yield benefit from future technical-biological developments. For the application of external fertilizers the regulations of organic farming laws on fertiliser use in different countries and special guidelines of different growers associations have to be taken into account. In Europe the EC-Council Regulation No EU 834/2007 is the minimum basis for organic production. Accredited fertilisers are generally listed in the Commission Regulation No EU 889/2008. Trade names and products are published in special lists like the German 'Betriebsmittelliste' (www.FiBL.org) or the US 'National List of Allowed and Prohibited Substances' (www.ams.usda.gov). In this paper current knowledge on nutrient limitations and possibilities to maintain and improve plant nutrient supply in organic production are discussed.

Results and Discussion

<u>Macronutrients</u>

Nitrogen (N) is regularly the strongest limiting growth factor in organic farming systems. The use of mineral N fertilisers is prohibited in organic farming. The N supply is mainly covered by rhizobial N from legume cultivation (Peoples *et al.*, 2009). By-products of animal or vegetable origin (horn meal, feather meal, extracted oilseed rape meal, legume seed meal, molasses, vinasses) are frequently used in organic vegetable production to compensate an insufficient N supply (Laber, 2009; Mueller *et al.*, 2007). Cropping systems relying on organic N import, which is a common problem in arable vegetable production (Koller and Lichtenhahn, 2004) run contrary to the directives of organic farming, which unambiguously demand closed nutrient cycles. In this context Haneklaus *et al.* (2005) described particular methods of soil analysis for organic farming, which comprise structural and biological soil parameters to evaluate the soil fertility status. The necessity of humus and nutrient recycling from by-products of the food chain was already accentuated in the early days of the organic movement (Rusch, 1964; Sir Albert Howard, 1943). Nowadays the use of household wastes and sludge on agricultural land is excluded for basic reasons of hygiene and contamination with organic (such as pharmaceutical residues) and inorganic (heavy metals) xenobiotics. The development of a complete extraction of all xenobiotics would be necessary before restarting a discussion on their use in organic farming (Rahmann *et al.*, 2009; Gethke *et al.*, 2008). The use of materials produced on an organic farm, particularly its by-products was emphasized by Sir Albert Howard (Sir Albert Howard and Wad, 1941) and an increased use of biomass for bio-energy production might deliver residual products which provide the minimum key nutrient N (Moller *et al.*, 2008, Stinner at al. 2008). Thus organic farmers may intensify productivity of cash crops without compromising the primary ideal of organic farming.

In contrast to N, for all other essential plant nutrients the off-take by harvest products is higher than the input. Nutrient balances are required for keeping a balanced nutrient input (Watson *et al.*, 2002). But threshold values available for crops produced on conventional farms (Barker and Pilbeam, 2006) have not been validated for organic farming. Static critical nutrient values are not applicable in organic farming systems because the target is not a targeted crop yield. Interactions between growth factors need to be taken into account site-specifically. An appropriate approach to interpret results and derive critical nutrient values for soils and plants is provided for instance by the BOundary LIne DEvelopment System BOLIDES (Schnug and Haneklaus, 2008). So far, lower soil nutrient levels are supposed to be sufficient because of the lower nutrient demand of crops for a reduced yield level (Kolbe, 2001). Such approach, however, does not live up to the basic ideas of organic farming. It will be necessary to compare separately critical nutrient values for different organic faming systems and on arable and livestock farms.

The K supply on organic farms may be marginal or insufficient on coarse structured soils (Oborn *et al.*, 2005; Askegaard *et al.*, 2003). Here, deficiencies can easily be compensated with K salts. In case of S (Paulsen, 2005) and Mg deficiencies natural gypsum, kieserite and Mg or S containing limestone can be used.

Looking at the limited world phosphate (P) reserves an efficient recycling of residual P from industries, households and incineration of biomass is inevitable (Schnug *et al.*, 2003). There are consistent results that organic farming takes advantage of soil P levels from former (conventional) fertilisation (Van Den Bossche *et al.*, 2005; Oehl *et al.*, 2002; Loes and Ogaard, 2001). The application of raw phosphates is not adequate to satisfy the P demand of crops because of their insufficient solubility. A sustainable use of P in organic farming requires a substantial improvement of the solubility of rock phosphates and bone meals as otherwise this non-renewable resource is deposited in a non-accessible form for plants in soils. The result: plant available soil P levels will decrease and soil fertility will diminish. An *in situ* digestion with a combination product of elemental S and rock phosphate enhanced significantly the solubility of non water-soluble P sources (Schnug and Haneklaus, 2006). Other options to improve P uptake is mobilisation by mycorrhiza (Kahiluoto and Vestberg, 1998) and P-efficient plants, which are able to explore P reserves by high root density or root exudates (Eichler-Lobermann *et al.*, 2008).

<u>Micronutrients</u>

Usually an insufficient supply with micronutrients is a problem of limited plant availability and not a limited reserve. In general, adjustment of the soil pH by liming will ensure a sufficient supply with micronutrients.

The application of organic manures or composts increases the micronutrient supply (Mn, Zn, Cu) (Herencia *et al.*, 2008). Studies on microelement concentrations in herbage indicated that Cu, Mo, Co, Zn, Fe or Mn were not limiting plant growth in organic herbage production (Govasmark *et al.*, 2005a; Govasmark *et al.*, 2005b). A higher mycorrhizal colonization of roots because of a lower P supply in organic systems may yield higher Zn concentrations when compared to conventional wheat grain (Ryan *et al.*, 2004). Another external source of micronutrients are feed additives. Mineral feed additives may charge soils with micronutrient loads (Cu), which needs to be evaluated critically (Gustafson *et al.*, 2007; Linden *et al.*, 2001; Olsson *et al.*, 2001). Heavy metal contents (Cd, Pb, Hg, As) and other undesirable substances in animal feed are limited in the Directive 2002/32/EC. Particularly phosphates may contain considerable amounts of undesired elements (Sullivan *et al.*, 1994). The Cd content in fertilisers is regulated to a maximum of 90 mg kg^{-1} P_2O_5 (EC 889/2008), but lower values are discussed and part of the fertiliser directives of some EU Members states (e. g. Germany 60, Finland 50 mg Cd kg^{-1} P_2O_5). So far no thresholds exists for U in fertilisers and feed additives (Knolle *et al.*, 2008; Uberschar, 2006). U is a toxic heavy metal and anthropogenic U contamination of agricultural soils is closely related to nationwide fertilization practices. The U content in rock phosphates may be as high as 220 mg kg^{-1} U. Particularly P fertilizers are known to add significant U loads to soils (Schnug and De Kok, 2008).

The results clearly reveal research deficits in the field of development of methods and criteria for evaluating nutrient availability and nutrient supply, and fertiliser demand in organic farming. In addition organic farming requires not only innovative approaches to improve nutrient availability as it was shown exemplary for P, but also regulatory updates of fertiliser regulations concerning environmentally relevant elements such as U.

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THE CONTROLLED GRASS-COVER SOIL MANAGEMENT IN SOUTHERN ITALY OLIVE ORCHARD ENVIRONMENTS

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Abstract

The influence of controlled grass-cover on soil characteristics and olive tree growing parameters has been tested in two different environments of dry-farmed olive orchards of Southern Italy (Calabria). In the first trial, carried out on hilly and slope soil, the permanent grass-cover management improved both soil characteristics, with significant reduction of erosive phenomena, and olive trees yield, also reducing the alternate bearing, in comparison with the tilled soil management.

On flat coastal environment has been instead evaluated the effects of different soil grassing management on soil characteristics and olive trees responses. On the contrary to hilly environment, in flat soil the permanent turf showed worse results than temporary grassing, in most of considered parameters. In this environment, without soil erosion events but with lower yearly rain amount, the olive trees yet needs of irrigation to express their vegetative and productive potentiality, apart from soil management system.

These results confirms that the growing management techniques must be selected and applied in order to the specific environmental parameters, to optimize their effects.

Keywords: Soil grassing; Soil fertility; Soil defence; Desertification; Sustainable management.

Introduction

About two-thirds of Italian olive-groves are located in hilly areas with varying degrees of slope. In these environments, it’s often difficult to apply adequate soil and cultivation management, in order to avoid nutrient depletion and soil deterioration or loss by erosion.

In flat olive groves, where soil erosion events do not occur, cultivation management is normally realised by mechanical soil tillage techniques, to control the temporary flora, bury the fertilizers, and store the rain water. To protect natural resources and restore olive orchards profitability, it is necessary to apply a low environmental impact cultivation techniques, that are also less expensive than mechanical tillage methods. Among the possible options, the grass-cover soil management offers significant advantages in terms of reduced management costs, improvement of soil characteristics, and defence against soil erosion. However, soil grassing can lead to competition for water and nutrients between the olive trees and the surrounding vegetation, if cultural techniques are not carefully handled.

To evaluate the influence of different grass-cover managements on the evolution of soil characteristics, and their effects on cultivation parameters of olive trees, a study was conducted in two representative olive orchard environments of Southern Italy (Calabria).

Materials and methods

The first trial was carried out for five years in a young hilly and dry-farmed olive grove of Carolea cv 6 x 4 scaled, on a stock-clay-sandy texture soil with an 23% average slope. On a l0 trees row and the two adjacent inter-row areas per treatment, were compared the Permanent Spontaneous Grassing (PG), controlled by grass-chopper, vs. Tilled Soil (TS) using rotary hoeing machine and disk harrow to keep the soil without flora. Both soil plots were yearly fertilized with approx. 1 kg/tree of 20-10-10 NPK at bud break, and 0.5 kg/tree of Urea at fruit set. The influence of meadow cover on rain downflow and soil erosion has been evaluated collecting and analyzing the turbid water streamed from each soil plot after every rainfall.

The second trial was carried out for four years in a young flat coastal and dry-farmed olive grove of Carolea cv 6 x 4 scaled, on a stock-sandy texture soil. In the same plot test as in the previous trial, were compared the Permanent Spontaneous Grassing (PG) controlled by grass-chopper, vs. Temporary Spontaneous Grassing (TG) using grass-chopper and disk harrow to bury the turf at end of spring. The annual fertilization was applied partly on soil at bud break, and partially to the leaves at fruit set, using various fertilizers to supply a total amount of 340 gr. N, 60 gr. P_2O_5, and 180 gr. K_2O per tree.

On olive trees, were yearly collected the vegetative parameters data, and, at the harvest time, the yield and carpometric parameters. At end of both study period, soil samples were taken at depth range of 2-30 cm from all plot areas, and analyzed to evaluate the evolution of their characteristics.

Results and Conclusions

In slope soil the meadow cover (PG) significantly increase all considered plant parameters vs. the tilled test (TS), in the average of yearly responses. The higher productivity of PG trees did not ***affect the pulp/pit ratio, nor*** inolition processes (Table 1), moreover significantly reducing alternate bearing vs. the TS trees (Figure 1).

Table 1: Plants parameters (Avg values of trial period).

	PG	TS	Δ %
Growth	5.91	6.44	-8.97
Flw bud Differ %	60.97	58.66	+3.79
Fruit Set %	2.00	1.66	+17.00
Fruit Drop %	45.79	52.07	-13.71
Yield Kg	13.05	10.73	+17.79
Fruit weight	5.84	5.49	+5.99
Pulp/pit	7.56	7.30	+3.44
Oil %	22.14	22.12	+0.09
Oil yield (Kg/tree)	2.89	2.37	+17.85

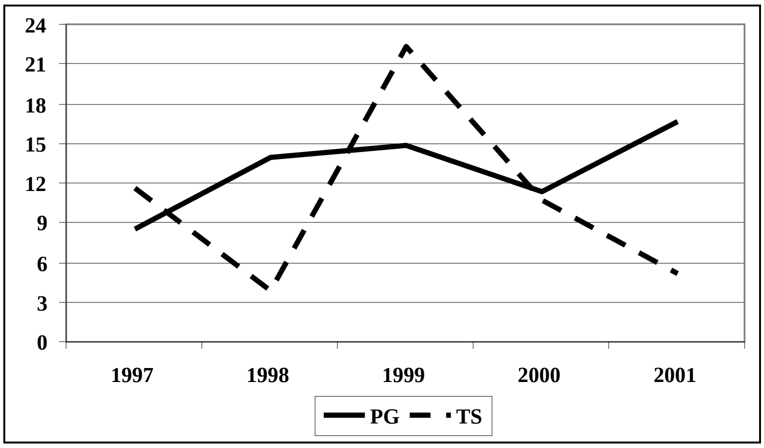

Figure 1: Average trees yield.

The analysis of streaming data show that most rainfalls did not cause a downflow in PG soil, whereas in TS a remarkable volume of rain water was lost through streaming, thus ***displacing*** a great quantity of soil (Table 2). The turf protection against rainfall downflow, avoiding the soil erosion, can be an useful mean to contrast desertification processes which are overextending in agrarian lands.

Table 2: Effects of annual rainfalls on downflow and soil erosion.

Years	Rainfall mm	Test	Downflow mc/ha	Downflow %	Soil Erosion (t/ha)
1997	789.4	PG	99.87	4.40	0.27
		TS	416.17	13.82	60.11
1998	914.1	PG	126.62	4.61	1.24
		TS	843.50	18.29	70.94
1999	1059.6	PG	115.77	4.99	0.26
		TS	1573.80	22.72	94.99
2000	691.8	PG	140.35	21.76	0.24
		TS	541.61	30.94	41.39

The consequent evolution of soil texture, show higher Skeleton and Sand percentage, as well as a lower percentage of Silt, Clay, and O.M. in TS vs. PG soil (Table 3).

Table 3: Soil analyses (average values of 0-30 cm depth).

	Skeleton %	Sand %	Silt %	Clay %	O.M. %	pH
Start	----	52.40	22.60	24.95	0.40	6.12
PG	19.00	51.40	22.80	25.80	0.60	6.40
TS	37.40	57.85	21.85	20.25	0.29	6.22

In flat coastal orchard with lower yearly rainfall, the olive trees showed to suffer the environment dryness (Table 4), that reduced the efficacy of fertilizers, and worsened trees performances in both plot test, also enhancing yield alternance (Figure 2).

Table 4: Water balance.

Year	Rain mm	ET mm	Balance
2000	592.3	717.1	-124.8
2001	465.4	731.3	-265.9
2002	896.8	730.5	+166.3
2003	585.8	824.2	-238.4

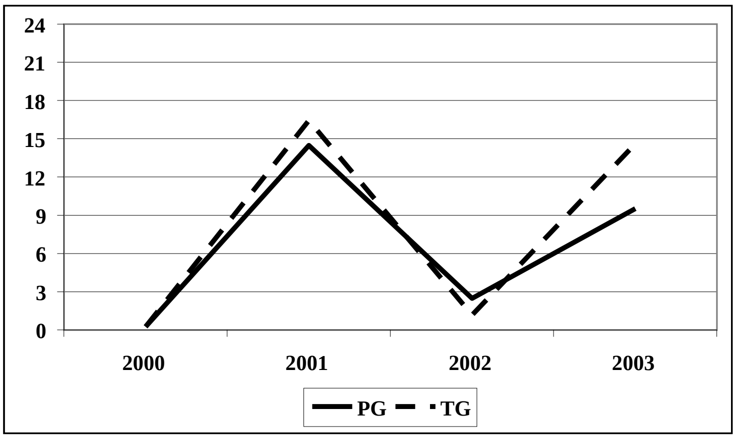

Figure 2: Average trees yield.

In this study case, the permanence of meadow cover caused a sensible summer water competition, further reducing fruiting and inolition processes in PG vs. TG trees (Table 5).

Table 5: Plants parameters (Avg values of trial period).

	PG	TG	Δ %
Growth	10.63	10.56	+0.66
Flw bud Differ %	49.84	64.87	-30.15
Fruit Set %	2.21	1.77	+19.61
Fruit Drop %	38.76	35.15	+9.32
Yield Kg	6.67	8.09	-21.28
Fruit weight	5.09	5.15	-1.18
Pulp/pit	8.23	7.65	+7.11
Oil %	18.63	20.40	-9.54
Oil yield (Kg/tree)	1.21	1.64	-35.81

In this environment, olive trees needs irrigation to express their vegetative and productive potentiality, apart from soil management system.

In absence of downflow erosion, no differences due to different soil management technique were found on soils textures at end of trial, while organic matter levels were slightly higher in TG soil (Table 6).

Table 6: Soil analyses (average values of 0-30 cm depth).

	Sand %	Silt %	Clay %	O.M. %	pH
Start	63.66	24.92	11.42	1.63	7.91
PG	74.53	17.11	8.36	2.15	8.37
TG	78.31	13.20	8.49	2.28	8.26

Results obtained in grassing experiences on different olive orchard environments, showed that both trees responses and soil characteristics evolution can be modified by the different soil management approaches.

The controlled soil grassing management, when properly applied, increases yield and oil production and improves soil characteristics at lower costs, representing one of the most sustainable cultivation technique, for Southern olive orchards without irrigation systems, too.

The differences in results of grassing management trials, confirms that the implementation of general principles of cultural techniques must be fitted to the specific environment peculiarities to obtain the best results.

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BUCKWHEAT: POTENTIAL TO IMPROVE P USE EFFICIENCY IN ORGANIC CROPPING SYSTEMS

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Abstract

Farmers who manage systems organically have limited options available to optimise soil P status, particularly if not linked to the readily available sources of P from livestock enterprises. In such cases, phosphate rock (PR) can be applied under the discretion of the organic certification body. However, the solubility of P from this source is unpredictable and site specific. Plants vary in their inherent ability to acquire soil-P, with the literature suggesting buckwheat possesses attributes favourable to solubilising P. This paper quantifies the ability of buckwheat to solubilise PR applied under field conditions and a temperate climate. An organically managed farm located in SE Scotland, had soils of low P status, and was used to investigate treatments with and without PR and the specific effects of crop species. The experiment tested buckwheat's suitability as either a green manure crop, or a cash crop, which could be included in a rotation to improve P efficiency of the system, particularly to the benefit of following crops. Under the test conditions, buckwheat proved extremely efficient at mobilising and accessing P relative to the other test species. It thus has potential in the roles described.

Keywords: Buckwheat, field beans, red-clover, wheat, phosphate rock, P.

Introduction

Phosphorus (P) inputs to organically managed farms in the EU is limited to either various recycled organic materials (e.g. compost or FYM) or new inputs such as phosphate rock (PR)

(EC, 1991). FYM and compost are bulky to transport and are not always available locally, particularly in predominantly arable areas, so if soil P levels are low, PR application is often the only option to improve P fertility. However, solubility of P from most PR sources is extremely low and it can take several years for appreciable levels of P to be made available to the crops. A number of crop species can reportedly increase solubility of P from either soil reserves or PR applications (e.g. Horst *et al.*, 2001; Imas *et al.*, 1997; Hinsinger, 2001). Modifying rotations to use these crops as either a cash crop or as a green manure, offers an opportunity to increase the availability of P and the efficiency of its use in cropping systems. Appropriate cash crops can potentially solubilise and use P directly from these poorly available P sources, as well as make P available indirectly to following crops through breakdown of the resulting residues. The green manure approach would be used to optimise P availability to following crops primarily from this latter scenario. The experiment described in this paper investigated buckwheat as a candidate crop in these roles and compared its performance as a P solubiliser with several more commonly grown crop species.

Materials and methods

An experiment to investigate crops with potential to improve rotational P use efficiency was undertaken using spring crops at Windshiel farm, near Duns in the Scottish Borders (latitude 55^o59'N; longitude 20^o14'W). This farm is predominantly grassland that is grazed by sheep and cattle, with a small area of arable crops produced for on-farm feed use. The P level of the soil where the trial was located was measured at 0.5mg/L (Morgan extraction), which is very low. The field was previously under long term pasture and was ploughed in March 2008. PR was surface applied to sub plots in mid April 2008 and crops were sown using a drill in the first week of May 2008, which was later than ideal. The crops sown were buckwheat, spring field beans, red clover and spring wheat at seed rates of 60, 250, 8 and 220 kg/ha respectively. They were sown in split plots with sub plots having either 0 or 600 kg/ha PR (Gafsa) applied (-PR and +PR respectively). The treatments were arranged in a randomised block design with four replicates. Crops emerged well, although vertebrate pest attack (birds and hares) required the beans to be re-sown two weeks later. No additional inputs or weeding were used on the trial plots.

Plant herbage and soil samples were taken from all plots eight weeks after sowing (mid July 2008) and prior to harvest (early September 2008). Plant samples were oven dried at 70^oC. Dried samples were ground to <1mm then a 0.5-1.0 gram sample was placed in a silica crucible and ashed in a muffle oven at 600^oC over night (~16 hours). Once cool, 10ml of 2M

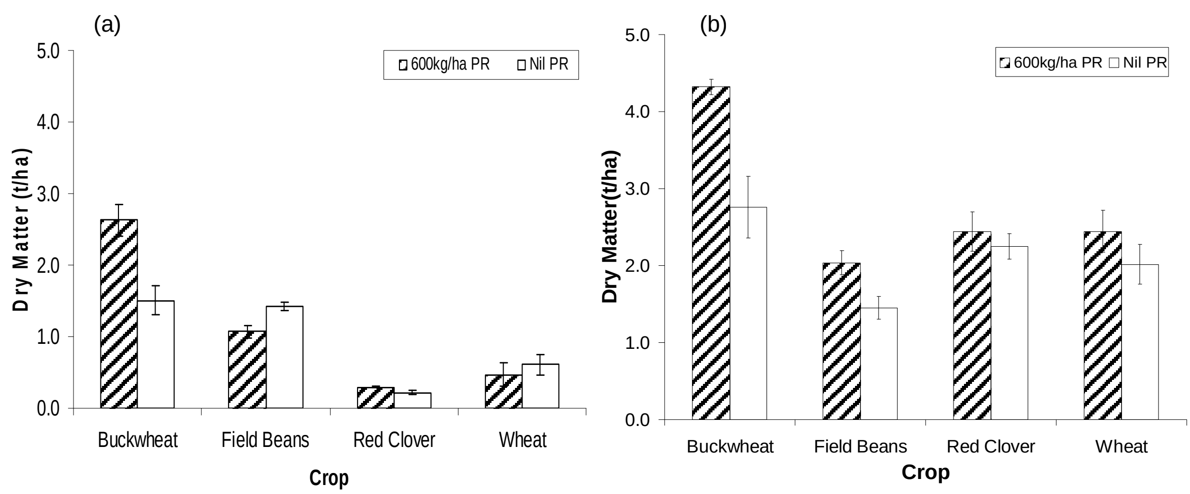
HCl was added to the ash and transferred to glass tubes. 5ml deionised water was added to the crucible to remove any residues, with this also being added to the tubes which were then made up to 50ml in total with deionised water. Samples were analysed colorimetrically using ammonium molybdate-metavanadate determination at 400nm wavelength.

Soil samples were dried in an oven at 60^0C. These samples were then crushed using a pestle and mortar and sieved (maximum diameter 2mm). Soil anion exchange P (AEM-P) was measured by shaking 10g of the sieved soil in 100ml of deionised water with 4 anion exchange membranes, each of approximately $3cm^2$, for 16 hours. The P was then removed from the membranes in 40ml of 0.5M HCl shaken for 1 hour. An aliquot of this extracted P was then analysed colorimetrically using a modified Murphy-Riley ammonium molybdate procedure.

Results and discussion

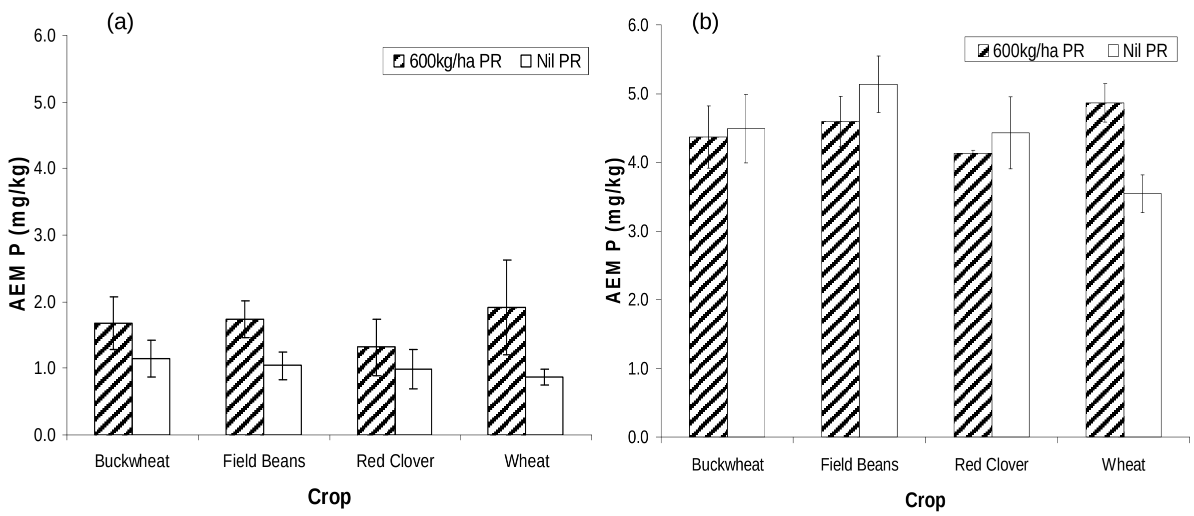
Early season growth was rapid for buckwheat with field beans not far behind, although red clover and wheat were much slower to establish and produce biomass (Figure 1a). Buckwheat continued to produce biomass consistently well throughout the season, although the biomass accumulation by the beans slowed down after its initial flourish. Red clover and wheat grew better from mid season onwards and actually performed better than the beans in terms of biomass accumulation by harvest time (Figure 1b). The ability of buckwheat to produce large amounts of biomass in a relatively short space of time, in combination with its relatively broad leaves, 'bushy' growth habit and allelopathic capabilities, provided good evidence of its weed suppressing qualities compared to the other crops tested (Bond & Grundy, 2001), although this data is not presented here.

Figure 1: Biomass yield at (a) mid-season (July 2008) and (b) harvest (September 2008). Error bars show SE of means (n=4).



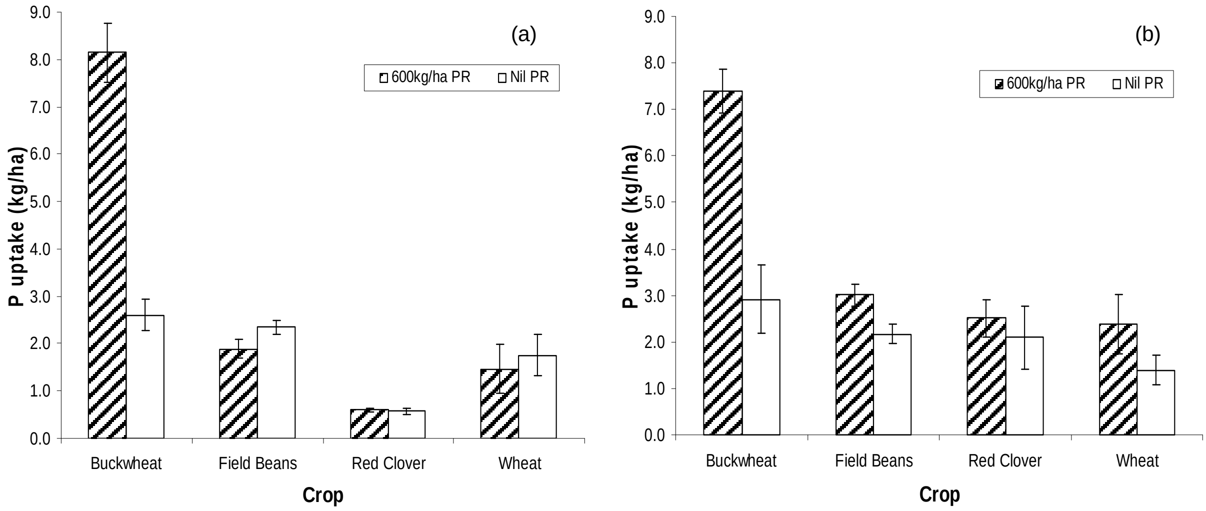
There were no obvious differences between the AEM-P availability in the soils for each of the crop treatments in the early season (July), although the +PR treatments appeared to have consistently higher levels of AEM-P for all crops (Figure 2a). AEM-P levels in the soil were similar for all crops late season (Figure 2b), with only the wheat continuing to show higher AEM-P levels in soil for the +PR treatments. These results suggest that P solubilisation was occurring across all treatments, particularly from mid-season onwards. It is likely that some combination of plant induced solubilisation and, other naturally occurring P-solubilising soil processes was involved.

Figure 2: AEM P concentration in the soils of each treatment at (a) mid-season (July 2008) and (b) harvest (September 2008). Error bars show SE of means (n=4).



Buckwheat showed rapid uptake of P in the early season, especially for the +PR treatment (Figure 3a). Red clover showed very slow uptake of P in the early season, but improved towards the end of the season when its P content became comparable to that of field beans and wheat (Figure 3b). There was no further uptake by buckwheat after mid-season for both PR treatments although all other crops showed some increase in P content, especially for the +PR treatments (Figures 3a & 3b). The rapid uptake of P in the early season suggests that it was actively able to solubilise P from either the soil reserves, PR, or both, probably by exuding organic acids from its roots into the rhizosphere. This solubilised P was subsequently taken up by the plant. The efficiency with which buckwheat was capable of extracting P from the soil would appear to be evidenced by the fact that there was almost four times the amount of P in its tissues compared to the other crops on test (Figures 3a & 3b), but little difference in soil AEM-P levels between crop treatments. If buckwheat was only improving P availability, and not extracting it as well, then it would be logical to conclude that the AEM-P levels in the buckwheat plots would be greater than those of the other crops. However, limitations in the AEM-P methodology, as well as the possibility that solubilised P may have subsequently been made immobile through other soil processes (physical or chemical), are all factors making interpretation of the results problematic (Edwards *et al.*, 2009).

Figure 3: P uptake by each crop at (a) mid-season (July 2008) and (b) harvest (September 2008). Error bars show SE of means (n=4).



Buckwheat proved to be a crop suited to low soil fertility situations. In this Scottish trial where P in particular was known to be at low levels in the soil, the Buckwheat performed well compared to the other crops tested in terms of biomass production and P uptake. This was particularly true when comparing the buckwheat with the field beans and red clover, both of which have been reported in the literature as being good P solubilisers, although under the experimental conditions found here, their performance did not warrant such claims. In most instances, these two crops were only marginally better than the spring wheat, which was included in the experiment as a control crop, for the biomass and P measurements described. The rapid uptake of P by the buckwheat in the first two months after sowing suggest that it may be a good option for green manuring if not grown to full maturity in soils with low P status.

Acknowledgements

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EFFECTS OF CEREAL / LEGUME INTERCROPS WITHIN A ROTATION

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Abstract

Intercropping systems with legumes as a component can simultaneously provide symbiotically fixed nitrogen (N) and increase yield through improved resource use efficiency. Key objectives investigated in this project were: a) to determine if there are yield or environmental benefits from the intercropping phase in a rotation compared to the associated monocrops and b) to determine if there are cumulative benefits of the cereal-legume intercrop over more than one growing season. Experiments were established at two sites in 2006 in Scotland, UK. Treatments include spring barley (*Hordeum vulgare* cv. Westminster), oat (*Avena sativa* cv. Firth) and pea (*Pisum sativum* cv. Zero 4 or cv. Nitouche) monocultures and intercrops of the cereals with white clover (*Trifolium repens* cv. *Alice*) or peas with all treatments followed by an oat crop in spring 2007. No fertilisers, herbicides or pesticides were used. This paper presents grain yield data, land equivalent ratio (LER) estimates as well as estimates of N losses from the system at various points of the 2006 and 2007 growing seasons. The choice of legume cultivar or species is a key factor influencing the amount of N available to the system in the year of use and / or the following year, with this impacting significantly on the final grain yield in both years.

Keywords: Intercrop, barley, oats, peas, clover, legumes, nitrogen, GHG, nitrate.

Introduction

Intercropping can be defined as a system that uses multiple synchronous cropping, i.e the simultaneous cultivation of two or more crops on the same area of land. Sowing can take place at the same or different times, but the crops are grown together for most of their growth period. There is evidence that intercropping can increase yields and their stability compared with monocrops (Hauggaard-Nielsen *et al.*, 2006) by improving crop resource use efficiency (e.g. light, nutrients, water). If the intercrop has a legume component, nitrogen transfer between legume and non-legume component species is possible (Hauggaard-Nielsen and Jensen, 2001; Corre-Hellou *et al.*, 2007).

There is a particular interest in intercropping in low input and organic systems. High N fertiliser costs and environmental legislation are driving farmers to consider alternative sources and approaches to N management such as intercropping.

In developing more sustainable cropping systems it is important to take stability of crop yield and N dynamics over time (whole rotations or periods of several years as well as within growing seasons). To date, intercrop studies have tended to concentrate on benefits to final yield (Connolly *et al.*, 2001) and data from only one growing season (Hauggaard-Nielsen and Jensen, 2001; Andersen *et al.*, 2005). However, some studies, including the present one, consider the system N dynamics over a longer period and the economic and environmental implications of this (Hauggaard-Nielsen *et al.*, 2003).

The main objectives of the current work were: a) to determine if there are yield or environmental benefits from the intercropping phase in a rotation compared to the associated monocrops and b) to determine if there are cumulative benefits of the cereal-legume intercrop over more than one growing season.

Materials and methods

An intercrop experiment was established near Edinburgh (55.9°N, 3.2°W), on twelve hydrologically-isolated plots that had been fallow for three years. The soil was a sandy loam (Eutric Cambisol, Macmerry Series). In Aberdeen (57.2°N, 2.2°W) an experiment was established on a sandy loam soil (Leptic Podzol, Countesswells Series) in a field which had been in grass/clover and grazed for three years. The treatments consisted of either component monocrops (barley, oats, peas or clover), or cereal-legume intercrops. At the Edinburgh site, barley was the only cereal used, but two pea varieties (either cv. Zero4 or cv. Nitouche) were used for comparison. At the Aberdeen site, the cereal component consisted of either barley or oats for comparison, but only one pea variety (cv. Zero4) was used. In the intercrops the seed rates for the cereals and peas followed a 50:50 replacement design. Thus, the target intercrop density was 50% of the monoculture density of each crop. Full monocrop seed rates were 250 kg ha^{-1} for pea, 200 kg ha^{-1} for the cereals (barley or oats) and 5 kg ha^{-1} of white clover. In both experiments, plots were replicated three times in a randomised block design. No manure, fertiliser, herbicide or other agrochemicals were applied to the plots. N_2O fluxes at both sites were measured at intervals of between one and four weeks by the static chamber technique (Clayton & Smith, 1994) and samples were analysed by gas chromatography. Drainage water from the plots at the Edinburgh site was monitored for nitrate and ammonium concentrations, determined by continuous flow analysis. Grain yields from each plot were determined off the combine (except for clover), and the land equivalent ratio (LER) was calculated from the available data as follows:

$$LER = \frac{\text{Yield Intercrop A}}{\text{Yield Monocrop A}} + \frac{\text{Yield Intercrop B}}{\text{Yield Monocrop B}}$$

An LER > 1 implies the intercrop is more efficient than the component monocrops per unit land area. During the 2006-07 winter the plots remained fallow and in spring 2007, oats were grown in all plots.

Results and discussion

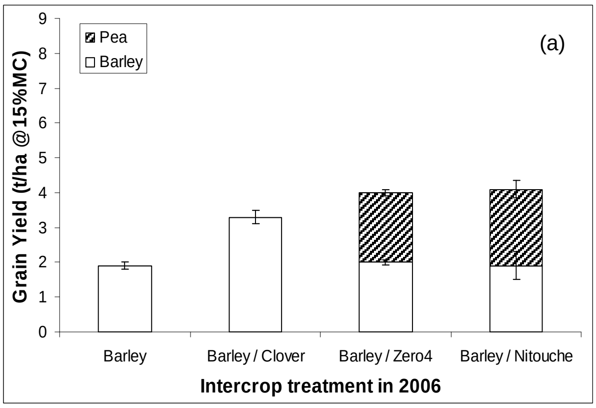
All the treatments at the Edinburgh site that had a legume component produced significantly more grain than the monocrop barley in 2006 (Figure 1a). There was no difference between the barley or pea yields for the two intercrops comparing pea varieties at this site. Monocrop pea yields were approximately 4.6 t/ha at this site, and performed very well. The LERs (Table 1) highlight the particularly high values associated with the pea/barley intercrop. In the following year (2007), the oat crop grown on the plots that had previously grown the barley-clover intercrop was significantly greater than all other treatments, with the oats grown on the pea-barley intercrop plots having comparable yields to the oats grown on the previous years barley monocrop plots (Figure 1b). This highlights the suitability of oats as a crop for lower fertility soils, particularly as the site was known to have low fertility prior to 2006, and was part of the reason for its use here.

Table 1: Calculated LERs and their SE of means based on grain yields for the intercrop treatments compared to the monocrop components at both sites in 2006.

	LER	SEM
Edinburgh		
Barley / Clover	1.27	0.10
Barley / Pea cv. Zero4	1.46	0.11
Barley / Pea cv. Nitouche	1.46	0.12
Aberdeen		
Barley Clover	1.12	0.16
Barley / Pea cv. Zero4	1.46	0.18
Oat / Clover	1.15	0.09
Oat / Pea cv. Zero4	1.35	0.14

There was a similar, but less pronounced, relationship shown by the treatments at the Aberdeen site (Figures 2a & b), particularly in the intercrop year (2006). This site was higher in fertility and probably accounts for the smaller yet still significant, differences shown in this year compared to the Edinburgh site. Monocrop pea yields (Zero4) were just over 1.5 t/ha at this site, which were low, but not uncommon for commercially grown peas in northern Scotland. However, the LERs shown in Table 1 continue to highlight the advantages that cereal-legume intercrops can have over their monocrop components. The real advantage is shown in the following year's oat crops, where again, plots which had previously had a cereal-legume intercrop growing in them performed significantly better in virtually all cases than when grown after a cereal monocrop (Figure 2b). Based on the data from these two Scottish trial sites, the combined yield data over the two years suggests that economic benefits are obtainable for the intercrop year, with these benefits carrying over into the following year.

Figure 1: Grain yield of (a) barley and peas for selected intercrop treatments in the year the intercrops were grown (2006) and (b) grain yield of oats grown on the same treatment plots the following year (2007) for the Edinburgh site. Error bars show SE of means (n=3).



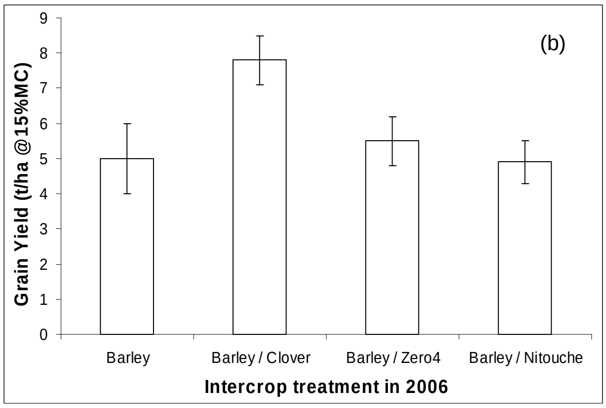
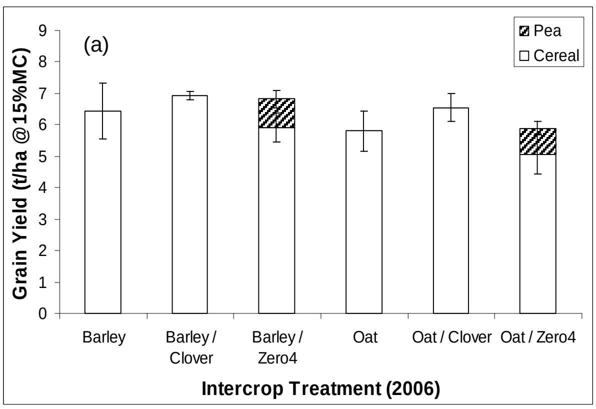


Figure 2: Grain yield of (a) cereal and peas for selected intercrop treatments in the year the intercrops were grown (2006) and (b) grain yield of oats grown on the same treatment plots the following year (2007) for the Aberdeen site. Error bars are SE of means (n=3).



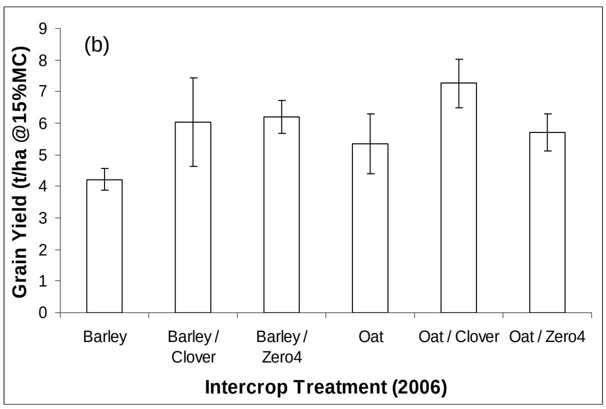


Table 2: Comparison of cumulative N_2O emissions (kg N ha^{-1}) from plots sown in spring 2006 with barley / legume intercrops at both sites. Periods are "summer" (June 2006 - September 2006) and "winter" (October 2006 - March 2007). Similar letters within a column indicate treatments not significantly different from each other (P≤0.05).

Treatment 2006	Aberdeen		Edinburgh	
	Summer	Winter	Summer	Winter
Barley	0.63^a	0.61^a	0.19^a	0.21^a
Barley/ Clover	0.53^a	0.85^{ab}	1.23^c	0.51^c
Barley/ Pea Zero4	0.75^b	0.92^b	0.19^a	0.19^a
Barley/ Pea Nitouche	-	-	0.92^b	0.33^b

Table 3: Cumulative nitrate-N leaching values (g NO_3^-:N ha^{-1}) from plots sown in spring 2006 with barley/ legume intercrops at the Edinburgh site. Similar letters across columns indicate treatments not significantly different from each other (P≤0.05).

Month (2006)	**Clover / Barley**	**Pea 04 / Barley**	**Pea Nit / Barley**	**Barley**
May	1193	129	198	455
June	503	1	21	1
July	14	0	0	0
August	5	0	0	0
September	188	9	169	175
Total	**1903^d**	**139^a**	**388^b**	**631^c**

Emissions of N_2O (a potent GHG and ozone depleter) for the intercrop season and following winter are shown in Table 2. Differences in emissions between the two sites were probably due to a combination of climatic and soil factors (e.g. base fertility was originally higher at Aberdeen, with more rainfall at this site). In general, treatments with a legume component produced more N_2O over this period. At the Edinburgh site, of the two pea varieties, Zero4 produced significantly less N_2O although, as highlighted in Figure 1a, there was no difference in yield between the two varieties. The oat yield was slightly higher after barley-Zero4 than after barley-Nitouche in 2007 (Figure 1b). Both pea treatments showed low levels of NO_3^- leaching compared to the other treatments (Table 3), with the cereal-clover intercrop being particularly poor in this respect, although it produced consistently high grain yields in both years. This, combined with the reduced levels of NO_3^- leaching from the Zero4 compared to

Nitouche treatments, suggests Zero4 may be an appropriate pea variety to reduce N losses compared to the other treatments in this study.

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NITROGEN DYNAMICS IN LEGUME BASED ROTATIONS

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Abstract

There is increasing interest in legume based cropping systems as an alternative to systems based on nitrogen fertiliser inputs. Systems comprising a grazed, legume based ley followed by a period of arable cropping are common. However, as grass-legume leys provide no economic return from a system without livestock, there is increasing interest in stockless systems which rely on grain legumes and alternative management of forage legumes such as undersowing or companion cropping. Using data from a long-term organic rotation trial in the North-East of Scotland, nitrogen flows in stocked and stockless rotations have been assessed using the NDICEA model. The initial predictions suggest that the stockless and the stocked systems are likely to lead to a reduction in the soil organic matter. The stocked systems have higher estimated leaching and dentrifications losses. However, the assumptions made regarding the amount of green manure that is returned to the system and the nitrogen fixation rates for the legumes are crucial in determining the overall sustainability of the system. In addition, the actual losses from the system will also be affected by the actual weather conditions experienced by each crop.

Keywords: Legumes, organic, stocked rotation, stockless rotation, N use.

Introduction

The inclusion of legumes within crop rotations provides an alternative to nitrogen fertiliser inputs and the high energy costs and greenhouse gas emissions (GHG) associated with their manufacture. In some circumstances, legumes may also contribute to lower nitrogen losses and GHG emissions in the field, although further research is required to quantify the magnitude of these benefits. However, in arable cropping systems that are reliant on legumes for the supply of nitrogen, the management of the soil fertility and the nitrogen supply is vitally important (Stockdale *et al.*, 2001). This is because the nitrogen mineralised can exceed the needs of the following crop (e.g. Eriksen *et al.*, 1999) or be released out of synchrony with crop demand (Berry *et al.*, 2003), which can result in losses to the environment (Ball *et al.*, 2007). In North-West Europe crop rotations comprising a grazed legume based ley followed by a period of arable cropping are common. Nevertheless, stockless rotations based on legumes are of increasing interest given the rising price of fertiliser nitrogen. However, as grass-legume leys provide no economic return in a system without livestock, stockless systems will necessarily rely more on grain legumes and alternative management of forage legumes such as undersowing or companion cropping. In this paper we compare nitrogen flows in stocked and stockless organic rotations using the NDICEA (Nitrogen Dynamics in Crop rotations in Ecological Agriculture) model developed for ecological agriculture (van der Burgt *et al.,* 2006).

Materials and methods

The data used is from the long-term organic crop rotations trials at SAC at Aberdeen (57° 10.5' N, 2° 15.7' W; 125 m asl) in North-East Scotland. The average rainfall is 820mm and the soil type is a sandy loam of Countesswells association, Dess series (Glentworth and Muir, 1963). This trial originally compared two contrasting stocked ley-arable rotations (Taylor *et al.*, 2006). However, in 2007 one of the rotations was converted to a stockless system. The six-course stocked rotation consists of three years of a grass-white clover sward which is followed by spring oats, swedes and spring oats which are undersown with grass-white clover. The manure applications are based on a stocking density of 1.7 livestock units ha^{-1}. The six-course stockless rotation consists of spring beans then spring barley, which are undersown with white clover, followed by spring oats which is undersown with a grass red clover mixture. This green manure (GM) is cut and mulched up to six times during the subsequent season. The GM is followed by either spring wheat, which is undersown with white clover, and then potatoes, or potatoes followed by the undersown spring wheat. There were two replicates of each rotation, and all phases of the rotation occurred in each year. Plots are approximately 30m x 15m in area. Sowing took place using calibrated seed drills / planting equipment at sowing densities and timing typical for each crop in the region.

The nitrogen flows of the two stockless rotations and the stocked rotation have been assessed using NDICEA model version 5.5.1. The model simulates the dynamics of water, nitrogen in the organic matter and inorganic nitrogen, and it has been used extensively in Europe to assess organic fertilization strategies of organic systems (Koopmans and Bokhorst, 2002; van der Burgt, 2004; van der Burgt *et al.,* 2006). The inputs to the model are average weekly temperature, total weekly rainfall and total weekly evapotranspiration, which is calculated according to Makking (1957). Requested crop management data are restricted to planting and harvesting dates, and to the quantities, timings and mineral content of the manure and fertilizer applications. In addition, the yields of the crops are also entered and thus the model is target-orientated. There is no feedback between the resource requirements and the yield. As a consequence of this, the user must ensure there is an adequate nitrogen supply. The nitrogen budget has been calculated six times for each rotation, using each phase of the rotation as a starting point. In this scenario, the weather data for 2007 has been used to represent the climate.

Results and discussion

The stockless rotations have appreciably less nitrogen input than the stocked rotation, Table 1. This was because the model estimated that levels of nitrogen fixation were less for the stockless than stocked systems. In addition, the stocked rotation also had additions of farm yard manure (FYM). However, the nitrogen actually removed by the crop is also slightly higher for the stockless system than the stocked system and therefore the predicted leaching and denitrification losses are also much lower. The model predictions suggest that there will be a decline of nitrogen in the organic matter in the soil over the course of the rotation for the stockless and stocked.

The denitrifcation and leaching losses are lower for the stockless rotations than the stocked rotation, Table 1 and as illustrated for one plot of the stockless rotation in Figures 1 and 2. With the exception of oats following the grass crop for the stocked rotation, the denitrification losses tend to be less than 25 kg N ha^{-1}, Figure 2. The leaching losses tend to be higher across all phases of the stocked rotation. The greatest losses for the stocked are predicted when the ground is bare over the winter period (Figure 1). In contrast, the greatest losses fro the stockless are predicted during the spring wheat crop following the grass-clover crop. This is probably because the spring wheat is not fully utilising the nitrogen released from the mulching of the grass-red clover crop. However, for the oat crop following the grass crop for the stocked rotation, the denitrification and leaching losses may be an overestimate as there is a tendency weed growth, which will utilise some of the nitrogen in the soil, during the period from the oats being harvested and the swedes being planted. In addition, the actual leaching and denitrification losses that occur will be highly dependent on the weather conditions during the growing season and the fallow period.

Table 1. The average annual nitrogen budget for the stocked and stockless rotations.

	Stocked	Stockless	
		Cereal[1]	Potatoes[2]
	Kg N ha^{-1} yr^{-1}	Kg N ha^{-1} yr^{-1}	Kg N ha^{-1} yr^{-1}
Supply with FYM	39.00	0.00	0.00
Nitrogen fixation	96.83	77.83	80.83
Deposition	12.00	12.00	12.00
Total supply	148.17	89.83	92.83
Removal with produce	65.33	73.00	73.00
Calculated remains	82.83	17.17	20.00
Denitrification	15.83	7.50	7.33
Leaching	89.50	41.67	44.83
Accumulation org. matter	-26.38	-31.45	-31.50

[1] The spring wheat follows the grass - red clover crop; [2] The potatoes follows the grass - red clover crop

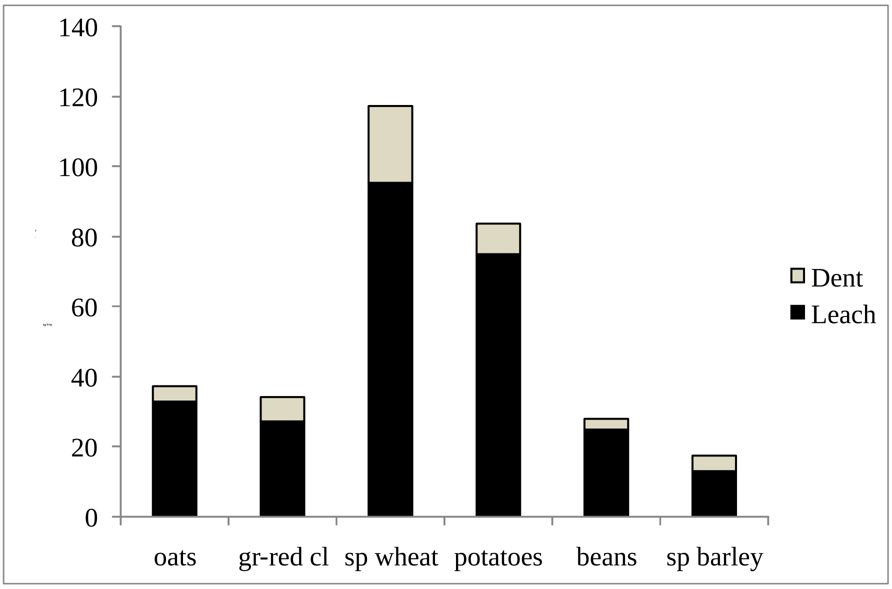

Figure 1. The nitrogen leaching and denitrification losses for each phase of the stockless rotation.

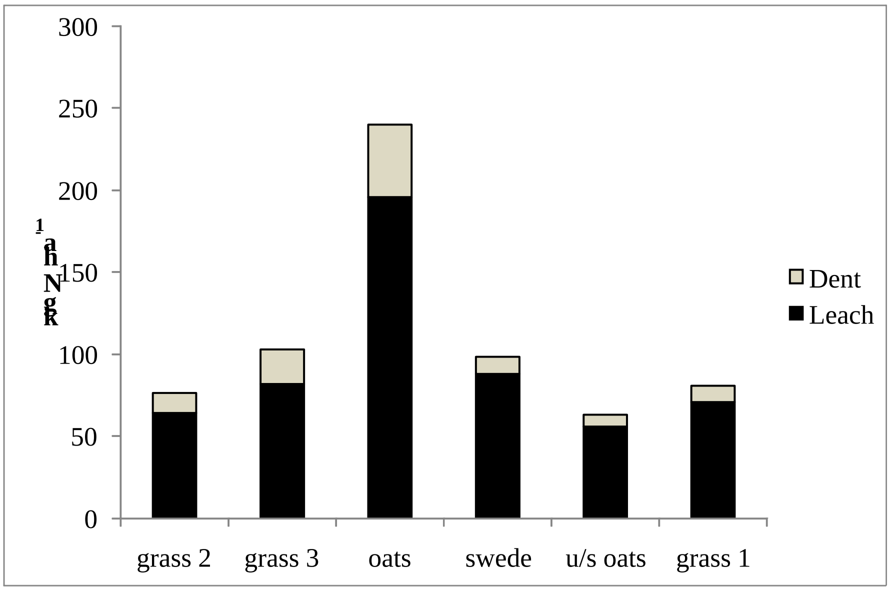

Figure 2. The nitrogen leaching and denitrification losses for each phase of the stockless rotation. (u/s oats = undersown oats).

The model predictions suggest that there is little impact on the sustainability of the rotation as a whole between spring wheat or potatoes following the grass-red clover crop. However, depending on which crop is used as the starting phase of the rotation, there are marginal differences in the differences in the nitrogen fixation, and hence the supply of nitrogen, and the changes in the nitrogen in the organic matter. However, the annual variability in weather conditions will clearly have an impact on the overall sustainability of the stockless rotations as a wet autumn following the potato crop will results in increased leaching compared to planting an undersown spring wheat white clover crop.

However, the results and hence the sustainability of the stockless systems is largely dependent on the assumptions that are made in terms of the amount of and the availability of the nitrogen in the green manure that is added back during the rotation. In addition, the ability of the undersown white clover crops to fix nitrogen throughout the rotation will also impact on the sustainability of the system. With regards to the stocked system, the factors that have the largest impact on the sustainability of the system are the nitrogen fixation, and the actual yield during the periods that the sward is grazed by sheep.

Future work will explore the impact of varying the assumptions in terms of green manure, nitrogen fixation, and the management of the cereal straw. The impact of the variability in climatic conditions on the systems will also be assessed.

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THE EFFECT OF NATURAL FERTILISATION OF GRASSLANDS ON SILAGE QUALITY IN ORGANIC FARMING SYSTEM

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Abstract

The aim of the study conducted in 2008 was to evaluate the effect of fertilisation with different natural manures on the quality and nutritive value of meadow sward and grass silage. Treatments were: i) mineral NPK fertilisation (N - 60 kg·ha-^{1}, P_2O_5 - 30 kg·ha^{-1}, K_2O - 60 kg·ha^{-1}) as a control; ii) cattle manure (22 t·ha^{-1}) and iii) liquid manure (25 m^3·ha^{-1} + 30 kg P_2O_5·ha^{-1}). Meadow sward was cut in May 2008 and after pre-wilting (to 40% dry mass) it was ensilaged in big bales. The content of nutritive components in meadow sward and silage was evaluated. Products of fermentation, the number of selected bacteria, yeasts and moulds and mycotoxin level in silage samples were also analysed. Nutritive value of silage made of meadow sward fertilised with natural fertilisers was higher than that made of sward fertilised with mineral fertilisers. No negative effect of liquid manure fertilisation on fermentation quality was noticed. Only silages made of sward fertilised with cattle manure had higher ammonium and volatile acids concentration and lower content of lactic acid. It was also found that natural fertilisation favoured yeast and moulds development in silage and resulted in the higher concentration of mycotoxins (aflatoxin B1).

Keywords: fertilisation management, organic fertilisers, organic farming.

Introduction

Organic fertilisers of animal origin i.e. manure, liquid manure and slurry are important for plant production in many farms, particularly in organic farms. Meadow sward from grasslands in these farms is commonly preserved by making silage (Jankowska-Huflejt, Domański, 2008). From a few recent studies it appears that grassland fertilisation with natural fertilisers may affect the process of ensilaging and consequently - the quality of obtained silage and indirectly - the quality of produced milk. Particularly negative effect on ensilaging and the quality of silage is exerted by non-fermented manure applied in large doses and on improper terms (Davies, Merry, Bakewell, 1996; Johansen, Todnem, 2002; Pauly, Rodhe, 2002; Rammer, Lingvall, 1997; Rammer *et al*, 1994).

The aim of this study was to evaluate the effect of various forms of natural fertilisation on the nutritive value of meadow sward and the quality of silages produced in big bales from this sward.

Materials and methods

Studies were carried out in 2008 in a plot experiment on permanent grassland situated in mineral soil of the Experimental Farm, Institute for Land Reclamation and Grassland Farming in Falenty. Three plots of an area of 0.3 ha each were fertilised as follows: plot 1 –mineral NPK fertiliser (control) at a dose of N - 60 $kg \cdot ha^{-1}$ (20 kg in spring and after the I and II cut), P_2O_5 - 20 $kg \cdot ha^{-1}$ (once in spring), K_2O - 60 $kg \cdot ha^{-1}$ (30 kg as in N fertilisation); plot 2 – manure (after 6 months long storage on manure slab, 20% dry mass) at a dose of 22 $t \cdot ha^{-1}$ in autumn; plot 3 –liquid manure (4% DM, to soil at a depth of 1.5 cm with applicators spanned by 15 cm) – at a dose of 25 $m^3 \cdot ha^{-1}$ (12.5 m^3 in spring and after the I cut) + 30 kg $P_2O_5 \cdot ha^{-1}$ (once in spring). The doses of manure and liquid manure were equivalent to c. 60 kg N applied in the control.

Meadow sward was first cut in May. Three representative sward samples for chemical analyses were taken in the time of mowing. Mown grass after preliminary drying on meadow surface was collected with the rolling press and ensilaged in big bales without any additives. Three big bales of silage wrapped up with four foil layers were made from each fertilisation object (plot) after transporting to the place of storage. A half year later (in November) 5 silage samples were taken from every bale for chemical analyses. Dry mass (after drying at 105°C) and the content of nutritive components (NIRS method using NIRFlex N-500 spectrofotometer and ready to use calibrations of the firm INGOT^R^) were determined in samples of green grass and silage. Moreover, in silage samples the following parameters were analysed: pH of fresh mass (potentiometric method), content of lactic acid, volatile fatty acids and ammonia (NIRS method), and total number of aerobic bacteria, enterobacteria, number of yeasts and moulds (cultures on Petrifilm™ 3M plates), the level of aflatoxin B1 (with the method of Eliss using RIDASCREEN® Aflatoxin B1 tests in the Stat Fax apparatus).

Results and discussion

Botanical composition of sward in studied objects was similar; grasses were the dominating plants with 92% contribution in the object fertilised with liquid manure and 86% contribution to the sward in the two other objects. *Alopecurus pratensis* L., *Poa pratensis* L., *Dactylis glomerata* L., and *Festuca pratensis* Huds. dominated among grasses. The share of herbs and weeds ranged from 6% (liquid manure) to 10% (manure) and that of legumes – from 2 to 7%.

All experimental plots were mown 3 times and obtained annual yields were: 5.9 t dry mass/ha after manure fertilisation, 7.5 t/ha in the control and 8.3 t/ha after fertilisation with liquid manure.

Chemical composition of meadow sward was affected by the type of fertilisation (Table 1). The samples of green grass fertilised with manure had higher content of total protein, crude fibre and its NDF and ADL fractions, lower content of carbohydrates (WSC) and worse digestibility of organic mass than those from the two other objects. The carbohydrate to protein ratio was very low (only 0.94) which might indicate worse usefulness of this material for making silage (Table 1). The content of nutritive components, carbohydrates and the ratio of carbohydrates to protein in sward fertilised with liquid manure was similar to the respective values in sward from control object (NPK). Dry matter content in ensilaged plant material was low – c. 350 g kg^{-1} – due mainly to atmospheric conditions (frequent showers and relatively low temperature) during harvest and silage preparation. In similar studies on the effect of different fertilisation on the quality of sward and silage made by Rammer *et al.* (1994) the differences in chemical composition of the sward were ambiguous.

Table 1: The content of nutritive components (g kg^{-1} DM) in meadow sward intended for ensilage.

Analysed parameters	Fertilisation			Significance
	NPK (control)	Manure	Liquid manure	
Dry mass	354.7	371.6	327.5	ns
Total protein	100.6a	119.9b	105.1a	**
Crude fibre	286.3a	297.8b	293.2ab	*
NDF	496.4	495.9	505.4	ns
ADF	326.9a	342.9b	336.7ab	*
ADL	40.8a	45.4b	40.5a	**
Crude ash	86.2	88.2	87.7	ns
WSC (carbohydrates)	152.9b	112.4a	144.2ab	**
Digestibility of organic matter (%)	54.10b	52.11a	53.23ab	*
The ratio of WSC to protein	1.52b	0.94a	1.37b	**

ns - not significant ; *,** - differences significant at P<0.05 and P<0.01, respectively

The quality of obtained silage assessed in the sensory analysis was good, irrespective of the type of fertilisation. Results of chemical analyses were more diversified. The content of dry matter in silages was lower than in the original plant material (270-300 g kg^{-1}) and did not depend on the type of fertilisation, as observed for pH (Table 2). Fertilisation had, however, a significant effect on percentage share of ammonia in the total nitrogen and on the content of lactic acid and volatile fatty acids in silage. Silage from the sward fertilised with liquid manure had the highest quality. It showed significantly (P<0.01) lower share of ammonia in the total nitrogen content than silages from objects fertilised with manure and NPK. The former had also a significantly lower content of volatile fatty acids (acetic, butyric, propionic acid) with a significantly higher content of lactic acid in the dry mass of silage and in the sum of all acids. Fertilisation with manure exerted most unfavourable effect on the quality of silage. The highest content of ammonia and volatile fatty acids and the least of lactic acid were found in this treatment (Table 2). Similar results: worse quality of silage from the sward fertilised with manure (high pH >4.5), high contents of ammonia (>150 g kg^{-1} N) and butyric acid and low content of lactic acid with better and comparable results obtained in the control and in objects fertilised with liquid manure were found by Rammer *et al.* (1994). They explained the differences by the presence of fragments of not decomposed manure applied in spring.

Table 2: An assessment of the quality of silage made of meadow sward.

Analysed parameters	Fertilisation object			Significance
	Control (mineral NPK)	Manure	Liquid manure	
Dry mass (g/kg)	298.8	305.6	275.8	ns
pH	4.87	4.84	4.75	ns
Ammonia (% of total N)	12.74b	13.82b	9.62a	**
Lactic acid (g kg^{-1} DM)	43.05ab	41.51a	56.49b	*
Volatile fatty acids (g kg^{-1} DM)	28.91ab	34.03b	24.56a	*
The sum of fermentation products (g kg^{-1} DM)	71.96a	75.54ab	81.04b	*
The share of lactic acid in the sum of fermentation products (%)	59.70ab	54.59a	69.29b	*

ns - not significant ; *,** - differences significant at P<0.05 and P<0.01, respectively

The content of most basic nutritive components in analysed silages depended on the type of applied fertilisation. The exceptions were ash and digestible dry matter (tab. 3). Silages from the object fertilised with manure, similarly as green grass from that object, showed the highest content of total protein. Silages from the control variant (NPK) showed the lowest content of total protein (significantly lower than those from sward fertilised with manure) and crude fat (significantly lower than those from sward fertilised with liquid manure) and the highest content of NDF and ADF fibre fractions – higher than in silages from objects fertilised with manure (significantly higher) and liquid manure. All this affected the index of relative feed value (RFV). The highest value of the index (the best nutritive value) had silages from grasses fertilised with manure and liquid manure. According to the scale elaborated by Linn and Martina [1989] they might be qualified to the II quality class i.e. the fodder intended for good cows, young heifers pre-selected for reproduction.

Table 3: An assessment of the nutritive quality of silages.

Analysed parameters	Fertilisation object			Significance
	Control (mineral NPK)	Manure	Liquid manure	
Total protein (g kg^{-1} DM)	117.3a	134.7b	124.9a	**
NDF (g kg^{-1} DM)	462.9b	434.5a	446.9ab	**
ADF (g kg^{-1} DM)	279.5b	261.6a	263.1ab	*
ADL (g kg^{-1} DM)	68.9b	69.2b	66.0a	**
Crude fat (g kg^{-1} DM)	29.7a	31.2ab	32.0b	**
Crude ash (g kg^{-1} DM)	85.2	91.9	92.5	ns
DDM, (%)	67.1	68.5	68.4	ns
DMI, (% body weight)	2.59a	2.76b	2.69ab	**
RFV	135a	147b	142ab	*

ns - not significant ; *,** - differences significant at P<0.05 and P<0.01, respectively
ADF - acid detergent fibre
NDF - neutral detergent fibre
DMI - dry matter intake (% body weight), DMI=120/NDF
DDM - digestible dry matter, DDM=88.9-(0.779 x ADF)
RFV - relative feed value, RFV= (DDM x DMI)/1.29

Applied fertilisation exerted a significant effect on selected parameters of microbiological evaluation of silages (Table 4). The number of aerobic bacteria and yeasts in fresh weight of silage from sward fertilised with manure was the highest and significantly higher than in that ferilised with liquid manure. Swedish studies on silages (Rammer *et al.*, 1994) also showed more bacteria (in that case *Bacillus* spores and *Clostridium* spores) after fertilisation with manure.

The number of mould colonies also depended on applied fertilisation – significantly fewer moulds were found in silages from sward fertilised with manure. In silages deriving from plots fertilised with liquid manure and in the control silage the number of moulds was significantly higher. It was also found that the application of natural fertilisers enhanced the occurrence of larger amount of aflatoxin. The lower value of aflatoxin B1 was determined in silages from the control (mineral NPK) and the higher value in silages from sward fertilised with liquid manure. However, amounts of aflatoxin B1 did not exceed the concentration of 20 ppb allowed in fodder.

Table 4: Microbiological assessment of silages.

Analysed parameters	Fertilisation object			Significance
	Control (NPK)	Manure	Liquid manure	
Total number of aerobic bacteria (log_{10} cfu g^{-1} fresh wt.)	5.52ab	5.85b	5.23a	*
Enterobacteria (log_{10} cfu g^{-1} fresh wt.)	1.63	1.57	2.59	ns
Yeasts (log_{10} cfu g^{-1} fresh wt.)	3.03b	3.15b	2.15a	**
Moulds (log_{10} cfu g^{-1} fresh wt.)	3.20b	1.13a	3.55b	*
Aflatoxin B1 (ppb)	4.42	5.06	5.90	ns

ns - not significant ; *,** - differences significant at P<0.05 and P<0.01, respectively

Nutritive value of silages from meadow sward fertilised with natural fertilisers was higher than those from sward fertilised with mineral NPK. Application of natural fertilisers, especially manure, significantly increased the content of total protein and decreased the content of NDF and ADF fractions in fodder.

No negative effect of liquid manure fertilisation on the quality of silages was found but fertilisation with manure increased the share of ammonia in the total N and the content of volatile fatty acids at a low content of lactic acid.

Application of natural fertilisers may also favour the greater number of yeasts and moulds in silages and the higher concentration of mycotoxins (aflatoxin B1).

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VI SESSION

Specialty Fertilizers

FERTILIZING EFFECT OF BIOGAS SLURRIES

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Abstract

The substance and nutrient cycling is one criterion of sustainability in bioenergy production. In particular, the reuse of the phosphorus (P) from bioenergy residues is important, since P is a limited resource and the prices of P fertilizers are going to increase. The effects of the residues of biogas production (biogas slurries) from cattle slurry on P uptake of different crops, soil available P and K content and dehydrogenase activity were investigated in green house experiments in comparison with non-digested cattle slurry and high soluble Triple-Super-P. Our results showed that biogas slurry has a similar P fertilizing effect like non-digested cattle slurry and high soluble Triple-Super-P. However, in comparison to non-digested cattle slurry, microbial activities in soil (measured in activity of dehydrogenase) were found to be lower when biogas slurry was applied. Our results underlined the high P fertilizing potential of biogas slurries. But they also showed the degradation of organic compounds during the digestion process may result in lower enhancement of microbial activity in soil.

Keywords: biogas, phosphorus, crops, microbial activity.

Introduction

Phosphorus resources are limited and not substitutable (Schnug et al. 2003). According to estimation of the FAO, at constant consumption, the P resources will be exhausted in the next 100 to 130 years (Niehörster 2005). Therefore, an efficient P use is one of the most important tasks in agriculture worldwide. The recycling of P with residues is one essential measure to meet these needs.

The strong promotion of biogas - as a renewable energy source - during the last years in Germany resulted in increased amounts of biogas slurry as a residual product. Usually, this product is applied on agricultural areas. Whereas there are already results available regarding the effect of biogas slurries on nitrogen (N) and organic matter cycles in soil, the effect of biogas slurries on soil P cycle is much less investigated.

Biogas slurries contain P between 0.4 and 0.8 kg/m³. Regarding the solubility of the P in these slurries different opinions exist. Generally, a high P availability is expected which is due to the mineralization of the nutrients during the digestion process (Gerardi 2003), whereas investigations of Umetsu et al. (2001) and Loria und Sawyer (2005) showed only delayed P release from biogas slurries.

Furthermore, the biogas slurries also contain N and C compounds which have an important effect on the soil micro flora. During the digestion process the readily decomposable organic compounds are degraded and compounds like lignin remain (El-Shinnawi et al. 1989). However, lignin is not an adequate C and energy source for micro-organisms. Therefore, a general effect of digested slurries on microbial activity can also be expected.

In our study we investigated the effect of biogas slurries on P uptake of different crops as well as on soil double lactate soluble P (Pdl) and dehydrogenase activity.

Materials and Methods

A pot experiment was established in 2008 in order to investigate the effect of different P sources (TripelSuperP (TSP), dairy slurry (slurry) and digested dairy slurry (biogas slurry)) on soil available P and plant nutrient uptake. The composition of the slurry and biogas slurry are presented in table 1.

Table 1: Composition of the substrates used in the pot experiment (% of fresh weight).

	DM	**OM**	**N_{total}**	**NH_4-N**	**P_{total}**	**K_{total}**	**Mg_{total}**	**pH**
slurry	9.3	7.5	0.46	0.23	0.08	0.32	0.07	6.4
biogas slurry	8.1	6.2	0.5	0.25	0.08	0.33	0.07	8.9

DM = dry matter; OM = organic matter

Furthermore, a control without any P supply was established (without P). Mitscherlich pots were filled with 6 kg of an air dried loamy clay soil. The initial Pdl content of about 33 mg kg^{-1} soil indicated a suboptimal P supply.

The P amount added with the slurries and TSP was 200 mg per pot (not for the control). The K (as KCl) and N (as NH_3NO_4) supply was 800 and 1100 mg, respectively, for all the pots (control included) .

Two different crops were cultivated; maize (Zea mays) and amaranth (Amaranthus hypochondracus).

All treatments were replicated 4 times. The pots were placed outside in a cage, under natural weather conditions. Distilled water was used for irrigation according to crop demands. Pots could freely drain to field capacity and percolated water was replenished. The plants were harvested after 8 weeks. Plant and soil samples were taken from each pot for subsequent analyses at the end of experiment. Harvested shoots were dried in an oven at 60 °C, weighed and afterwards ground with a plant mill. The P content in plant tissue was measured after dry ashing using the vanadate-molybdate method (Page et al. 1982).

Microbial activity was evaluated by measuring dehydrogenase activity (DHA) on moist soil samples (Schinner et al. 1996). For the other parameters measured, soil samples were air-dried and sieved (0.2 mm) before analysis. Content of double lactate soluble P (Pdl) and K (Kdl) as well as pH ($CaCl_2$) were quantified according to Blume et al. (2000).

Soil and plant data corresponding to 4 replications were subjected to the analysis of variance (General linear model, GLM). The means of soil and plant parameters were compared by the Duncan multiple range test. Significance was determined at $p < 0.05$, and significantly different means were indicated by different letters.

Results and Discussion

The investigations showed a significant influence of the fertilization on crop yield, Pdl content, and dehydrogenase activity in soil (Table 2).

Table 2: Effect of fertilization and crop species on crop and soil parameters. Results of the two-factorial analysis of variance.

factor	**yield**	**P uptake**	**Pdl**	**DHA**
fertilization	***	n.s.	***	***
crop	***	***	***	***
fertilization*crop	***	n.s.	**	n.s.

*** P≤0,001, **P≤0,01, *P≤0,05, n.s.=not significant

Regarding the P uptake, only amaranth was affected by fertilization (Table 3). The highest values of P uptake were found after application of biogas slurry and TSP (without significant differences between the two fertilizers). The same applied regarding the crop yields. Similar high yields were found after application of TSP and biogas slurry, whereas non digested slurry resulted in slightly lower yields (data not shown).

The effect of the treatments on soil Pdl was more pronounced. The Pdl increased after P supply, independently of whether P was applied as TSP or with the slurries. However, after maize cultivation highest Pdl values were found in the TSP treatment. For amaranth no significant differences between the P fertilization treatments were found. Generally, amaranth cultivation resulted in lower Pdl values, which is in agreement with the higher P uptake of amaranth in comparison to maize.

The K content was also investigated, although it was not a proofed factor in this experiment. The differences in dependence of fertilization were rather low. Highest K values after amaranth cultivation were found in the slurry treatment. For maize no significant differences were obtained.

The pH increased significantly when slurry or biogas slurry were applied in comparison to the control or TSP treatment.

Table 3: P uptake (mg/pot), soil Pdl and Kdl content (mg/kg), pH and dehydrogenase activity (DHA, µg TPF/g 24 h) in dependence of cultivated crop and fertilization in a 8 week pot experiment.

Parameter	Fertilization	Maize	Amaranth
P uptake	without P	90.3 a	103 a
	TSP	97.7 a	161 b
	slurry	108 a	137 ab
	biogas slurry	109 a	167 b
Pdl	without P	26.6 a	25.6 a
	TSP	36.4 c	30.3 b
	slurry	33.0 b	31.0 b
	biogas slurry	34.0 b	28.9 b
Kdl	without P	49.7 a	55.2 ab
	TSP	52.0 a	52.0 a
	slurry	50.7 a	66.5 c
	biogas slurry	49.6 a	58.4 b
pH	without P	5.63 a	5.43 a
	TSP	5.72 a	5.44 a
	slurry	5.87 b	6.03 b
	biogas slurry	6.01 b	6.01 b
DHA	without P	189 a	114 a
	TSP	239 b	131 ab
	slurry	372 c	234 c
	biogas slurry	318 bc	173 b

Mean values followed by different letters in the same column indicate significant differences (Duncan, $p < 0.05$).

Since the slurry application also provides the soil with N and C, usually it also affects soil biology parameters. As expected, higher values of dehydrogenase activity were found when slurry or biogas slurry were applied. However, the application of digested slurry resulted in lower values than the non digested slurry. Since the nutrient supply with the slurries was similar, the different results can only be explained by the different quality of organic compounds in both substrates. Slurries contain higher amounts of readily available substrates that are known to stimulate microbial activity.

Further investigations of soil biochemical parameters are necessary for a better evaluation of the effect of biogas slurries on the soil P cycle, mainly on the organic P pool.

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MARINE ALGAE FILTRATES: PHYSIO-ACTIVATORS® THAT STIMULATE PLANT NUTRITION AND GROWTH

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Abstract

Biological effects of seaweed on plant growth and development have been investigated for more than 30 years: the studies showed that *Ascophyllum nodosum* (Fucales, *Phaeophyceae*) extracts stimulate plant growth and nutrition, which translates into nutrient enrichment and increased plant weight and yield (Klarzynski *et al.*, 2005; Klarzynski *et al.*, 2006).

It was demonstrated that these extracts (Physio-activators®), obtained by a patented industrial process, activate specific plant enzymes involved in absorption and assimilation of some important nutrients, thus acting as physiological activators. For instance, they stimulate the nitrate reductase and root phosphatase enzymes, involved in nitrogen and phosphorus nutrition, respectively (Klarzynski *et al.*, 2005; Klarzynski *et al.*, 2006). Furthermore, recent studies showed that *Ascophyllum nodosum* extracts implement the iron assimilation and translocation mechanisms, by stimulating both the Fe-chelate reductase activity - which reduces Fe^{3+} into Fe^{2+}, more easily assimilated - and the Fe-transporter gene (IRT1): this means an increase in iron uptake, reducing problems linked to the well-known ferric chlorosis (Euzen *et al.*, 2008).

Thus, the above described processes lead to multiple biological effects, which determine higher leaf chlorophyll content (Klarzynski *et al.*, 2005), and, consequently, a better plant growth and development (Klarzynski *et al.*, 2005; Klarzynski *et al.*, 2006).

Unraveling the mechanisms of action of the extracts is essential to understand the observed effects and to allow integrating them in sustainable and profitable production programs by better controlling plant nutrition, which ends in increased crop performance.

Keywords: tomato, wheat, corn, Goëmar algae extract, plant growth, plant nutrition, mannitol, nitrate reductase, root phosphatase, ferric chelate reductase, iron transporter.

Introduction

Liquid extracts from marine algae have been used to improve the growth of agronomically beneficial crops for several decades. Although these products are still far from having yielded all their secrets, the experience acquired over this period has improved the understanding of their mode of action. Algae extracts are obtained by grinding and filtering the brown alga *Ascophyllum nodosum* (Fucales, *Phaeophyceae*), harvested in Brittany (France), using a process that keeps the active substances present in the algae intact. The specifications defining the harvest period and location, and the specifications of the finished product guarantee manufacturing reproducibility. The development of algae extracts for agriculture is a domain in which the Laboratoires Goëmar have been operating for over 30 years. The following text describes the latest advances in our research that enables the term characterising Goëmar algae extracts to be validated: Physio-activator®.

Mode of action of algae extracts: state of the art

Analysis of the large volume of studies conducted on algae-based products shows their multitude of actions on plants, regarding the biological effects induced in plants as well as in the range of plant species stimulated (Jolivet *et al.*, 1991). However, few studies have precisely demonstrated the mode of action of algae extracts on plant metabolism. Indeed, these studies remain dependent on progress in the knowledge of regulation phenomena within the plant, and despite some advances obtained in recent years, many of the physiological mechanisms of plants still remain obscure.

The first studies conducted led to the conclusion that the mode of action of the algae extracts originates from the natural presence of active signals in the filtrates. In fact, this conclusion is based on the low effective doses of the product that invalidate the possibility of action as a direct source of nutrition.

Hence, some active substances present in the extracts open up possible avenues of investigation in which the mediators of the algae extracts effects may not directly be hormones.

The concept of “elicitors”, molecules acting as activator signals for enzymes or specific metabolic channels, is a good illustration of this point (Klarzynski *et al.*, 2006).

Our research approach is based on a close monitoring of advances in the knowledge related to the plant's internal regulation mechanisms in order to target key biological processes of plant metabolism, to use them as tools for understanding the effects of algae filtrates and to select active molecules in algae.

Effect of Goëmar algae filtrates on the growth and nutrition of treated plants

<u>Effect on the growth of treated plants</u>:

The investigation began by assessing the effect of the extracts on the growth of young wheat, tomato and corn plants. Sequential applications of the algae filtrate in addition to a nutrient solution (1 g/L for wheat and corn, and 0.2 g/L for tomato) made it possible to observe a spectacular increase in the growth of all treated plants as compared with control plants watered only with the nutrient solution (1 g/L for wheat and corn, and 0.2 g/L for tomato) (fig. 1, 2 and 3). A dose effect (data not shown) has notably been demonstrated both on green parts and roots.

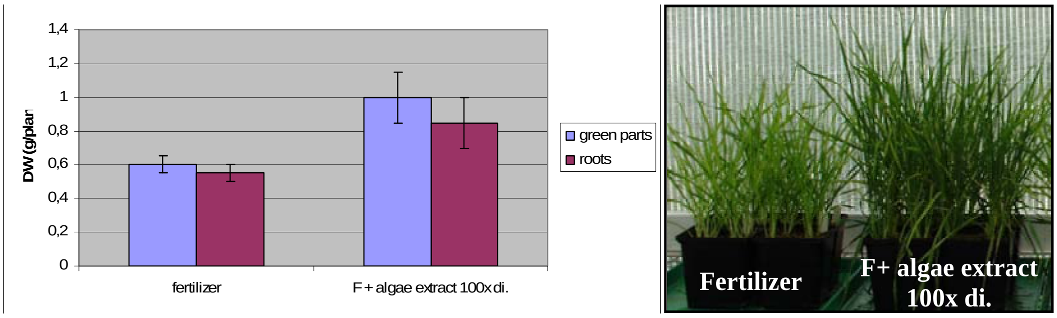

Figure 1: Effect of the Goëmar algae filtrate on the growth of green parts and roots of wheat plants (30 days after pricking out, expressed as DW in g/plant).

N.B. For all graphs, the wording "F + algae extract Ax di." means a nutrient solution at 1 g/L for wheat and corn and 0.2 g/L for tomato, complemented with an algae filtrate diluted Ax times.

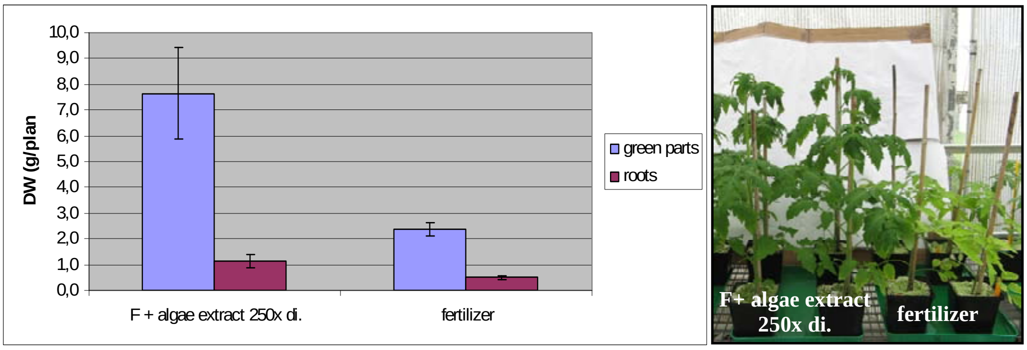

Figure 2: Effect of the Goëmar algae filtrate on the growth of green parts and roots of tomato plants (30 days after pricking out, expressed as DW in g/plant).

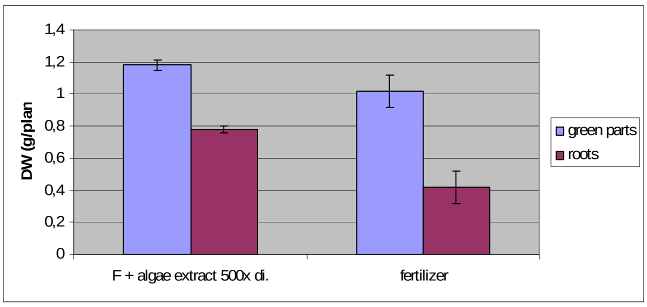

Figure 3: Effect of the Goëmar algae filtrate on the growth of green parts and roots of corn plants (30 days after pricking out, expressed as DW in g/plant).

Effect on the nutrition of treated plants:

The principal macronutrients and chlorophylls contents of the plants used in these experiments were also measured. As shown in figure 4, the increased growth of wheat and tomato plants is naturally accompanied by an increase in the quantity of the main elements (nitrogen N, phosphorus P, potassium K, expressed in relation to the dry weight of the plants). Similar effects were observed in corn (data not shown).

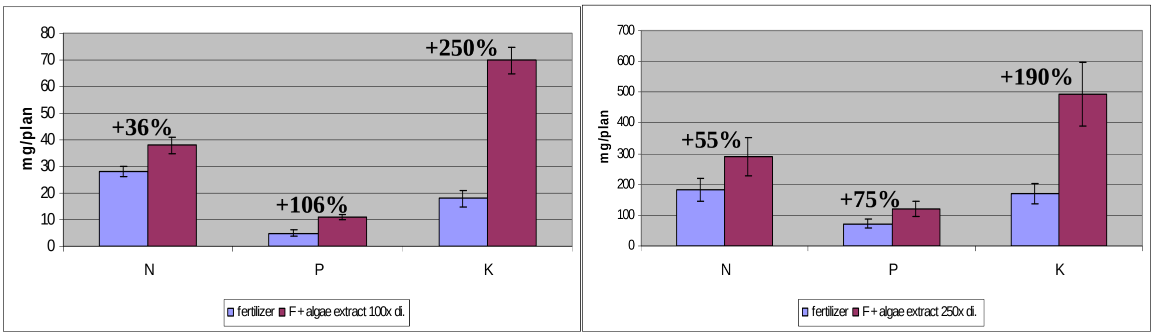

Figure 4: Effect of the Goëmar algae filtrate on the macronutrient content of treated wheat (left) and tomato (right) plants (30 days after pricking out and expressed in relation to the dry weight of the plants in mg/plant).

Total chlorophyll levels of treated plants also increased significantly (x2, data not shown), which confirms that the stimulation of nutrition by the algae filtrate, and notably the increased nitrogen and phosphorus levels, are directly used by the plants for their growth.

Mechanisms of action of the Goëmar algae filtrates on the absorption of nutrient elements

The results shown above show a wide-ranging effect of Goëmar algae filtrates on plant nutrition. The fact that, at the dose applied (diluted several hundreds of times), the algae filtrates nutrient content is not high enough to serve as an effective source, substantiates the hypothesis of a signal effect of the product. The tomato model grown in a hydroponic system was used as a base to study the Goëmar algae extracts mechanisms of action.

Effect on nitrogen nutrition, activation of nitrate reductase

Goëmar's research on the mode of action of algae filtrates has shown their capacity to stimulate the activity of Nitrate Reductase (NR), the key enzyme of nitrogen nutrition in plants. Indeed, the latter is responsible for the first step necessary in assimilating the nitrate absorbed by the roots (Morot-Gaudry, 1997; Klarzynski *et al.*, 2005) (fig. 5 and 6). Nitrogen nutrition conditions the synthesis of free amino acids, structural components such as DNA and

RNA but also chlorophyll. Interestingly, an increase in the chlorophyll content has already been noted for plants treated with the algae filtrate in comparison with untreated plants. Thus, this indicates that the Goëmar algae filtrate induces a significant stimulation of the NR at the level of the entire plant, which results in an increase in the content of chlorophyll, a key element in the growth, which is thus increased.

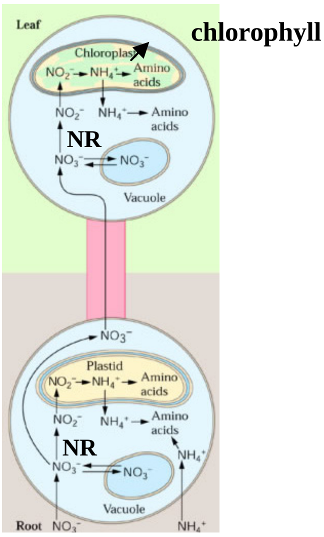

Figure 5: Action of nitrate reductase in nitrogen nutrition.

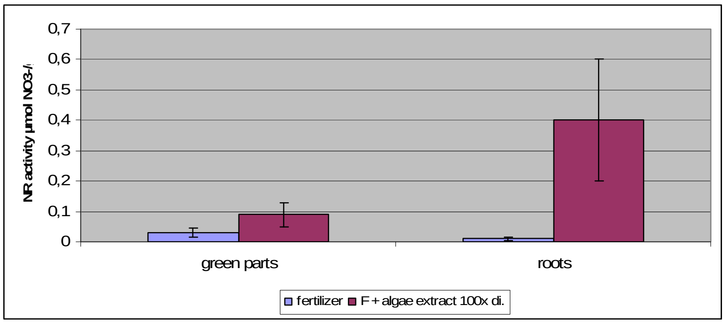

Figure 6: Effect of the Goëmar algae filtrate (100x dilution) on NR activity in the green parts and roots of treated tomato plants (4 days after pricking out, expressed in µmol $NO3^-$/g FW).

Several studies have shown that nitrogen nutrition is a phenomenon that is sensitive to internal signals of the plant, like the abundance of photoassimilates, such as carbohydrates (positive regulation) or certain free amino acids (negative regulation) (Kaiser and Hubert, 2001). However, during the trials, it was observed that one component of the Goëmar algae filtrates, mannitol (14% of the dry matter), is capable on its own of activating the NR and of increasing the chlorophyll content in the tissues of the treated plants. However, the effect of mannitol on the chlorophyll level, although significantly different from that in the control, is lower than that of the algae filtrates. This indicates that it may not act alone but in conjunction, or even in synergy, with other components of the filtrates. The origin of the mannitol activity probably lies in the fact that this molecule is synthesised as a photoassimilate in parallel with sucrose. It is thus a photoassimilate that can activate the NR.

Effect on phosphate nutrition, activation of root phosphatases

In addition to research on nitrogen nutrition, quantifying phosphorus in plants treated with the Goëmar algae filtrates has shown that this treatment enables the plant to make better use of the phosphorus present at the root level (fig. 4). The capacity of the algae filtrates to stimulate the physiological mechanisms involved in the absorption of phosphates in plants was therefore determined. These are essential ions for the plant (Bieleski, 1973; Raghothama, 1999) but are mostly not available in soil. In many agricultural systems, only 20% of the phosphorus used is available for the plant during its first year of growth, the rest being fixed on soil particles, a third of which in organic form (Russell, 1973). Plant roots phosphatases are enzymes that play a role in the absorption of phosphate since their activity is to free the phosphorus fixed on the organic matter of soil, which is then absorbed by the plant (Haran *et al.*, 2000, Baldwin *et al.*, 2001, Hunter and Leung, 2001) (fig. 7). These enzymes play a crucial role in the plant/soil competition for phosphate. Focus was therefore directed towards the effect of the Goëmar algae filtrate on root phosphatase activity.

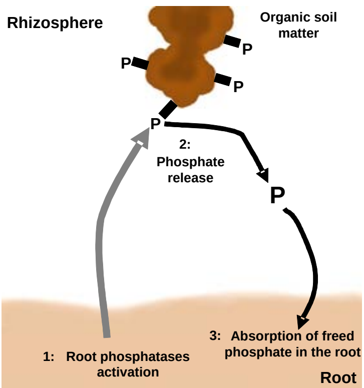

Figure 7: Mode of action of the root hosphatises.

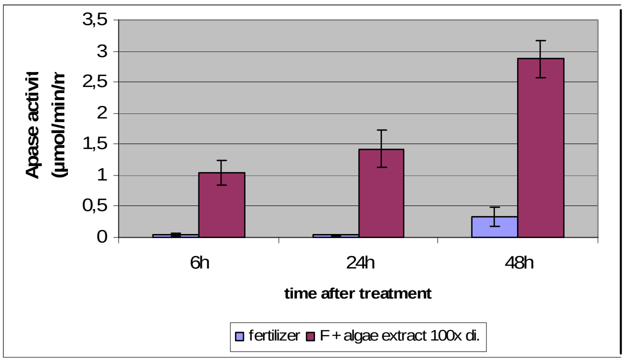

Figure 8: Effect of the Goëmar algae filtrate (100x dilution) on the activity of the root phosphatises in the treated tomato plants (expressed in µmol/min/mL).

The tests performed have shown that treatments with the algae filtrates noticeably increase the activity of these enzymes (fig. 8) (Klarzynski *et al.*, 2006). They may therefore enable the plant to uptake a greater number of phosphorus atoms from the soil's organic matter, which is confirmed by the measured quantities of phosphorus accumulation in the whole plants (fig. 4). These results show that the increased phosphorus content of the treated plants is a consequence of the algae filtrates stimulating the plant's phosphorus absorption mechanisms.

Effect on the iron nutrition: activation of the ferric-chelate reductase and the iron transporter gene in the plants

In addition to the macronutrients content, the use of Goëmar algae filtrates causes a rise in the micronutrients contents of treated plants, and notably iron in tomatoes (+60%, data not shown). The capacity of the algae filtrates to stimulate the plant physiological mechanisms involved in the absorption of iron via the roots and its transport to the roots was therefore determined. Iron is indeed an essential trace element for plants through its direct action on basic metabolic and enzymatic processes (oxidation-reduction, respiration, photosynthesis). Iron deficiency therefore has a direct impact, particularly on photosynthesis (chlorosis symptoms) and yield (Molassiotis *et al.*, 2006). However, this metal is hardly available for plants as it is mostly fixed to the mineral and organic particles of soil in ferric form, Fe(III). Therefore, overcoming the low availability of iron in the soil through fertilizers is not totally efficient as this is not a problem of abundance but of availability. To be absorbed by the roots of grapevine-type plants (sensitive to chlorosis), the iron fixed to soil particles in ferric form must be solubilised and then transformed into ferrous iron Fe(II). The NADH-dependent Fe(III)-chelate-reductase (EC 1.16.1.7) enzyme, located in the plasma membrane of the root epidermis cells, carries out this first stage. The ferrous iron thus produced in the root space is then taken up by a specific membrane transporter, the *IRT1* transporter (Iron Regulated Transporter), that enables the iron to pass from the rhizosphere to the root cells for subsequent use (fig. 9). The activity of this transporter is essential for iron absorption in grapevine-type plants (Eckhardt *et al.*, 2001).

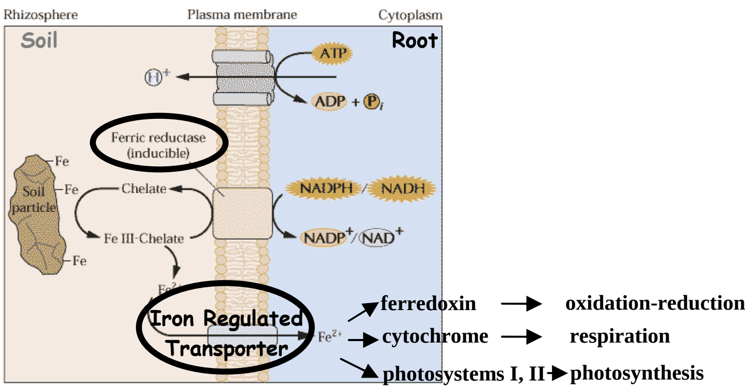

Figure 9: Iron assimilation and translocation mechanisms in roots.

Effect of the treatments on the ferric-chelate reductase activity

Tomato plants were cultivated in a hydroponic system containing only the ferric form of iron and were treated with the algae filtrate diluted 500 times. The medium was analysed after 48, 72 or 96 hours to measure the quantity of ferric iron transformed into ferrous iron by the root activity. The measurement of the ferric-chelate reductase activity is therefore indirect. Figure

10 shows that 72 hours after treatment, the average quantity of Fe(II) calculated for the samples treated by the algae filtrate is more than two times greater than the quantity obtained for the controls. At 96 hours, the difference is still noticeable (Euzen *et al.*, 2008). This very sensitive effect indicates a significant increase, following treatment with the algae filtrate, in the activity of the ferric-chelate reductase, single possible cause for the transformation of ferric iron into ferrous iron.

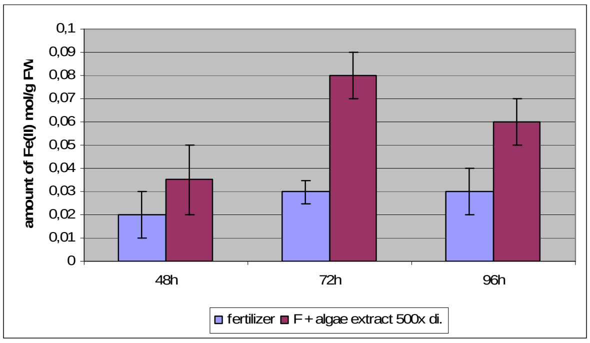

Figure 10: Effect of the Goëmar algae filtrate (500x dilution) on the activity of the ferric-chelate reductase in the roots of treated tomato plants (expressed in amount of Fe(II) in mol/g FW).

***Effect of the treatments on the expression of the LeIRT1 gene.**

Molecular biology was used to determine the effect of the algae filtrates on the *LeIRT1* gene expression in tomato plant roots. Actin, which has a constant expression in plants, was used as a reference in the semi-quantitative RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) method. Two experiments, using five plants per treatment in each experiment, were performed. The results presented in figure 11 show a significant increase in the expression of the *LeIRT1* gene in the treated plants treated as compared to the control plants (thickness of the electrophoresis bands) (Euzen *et al.*, 2008). Given that no difference was observed for actin between the control treatment and the filtrate treatment and that the expression of the gene is similar for the five treated plants, the differences observed for the *LeIRT1* gene can clearly be attributed to the filtrate treatment. The results indicate that the expression of the *LeIRT1* gene, involved in a better absorption of iron by roots, is two times greater in plants treated by the algae filtrate than in control plants (data not shown).

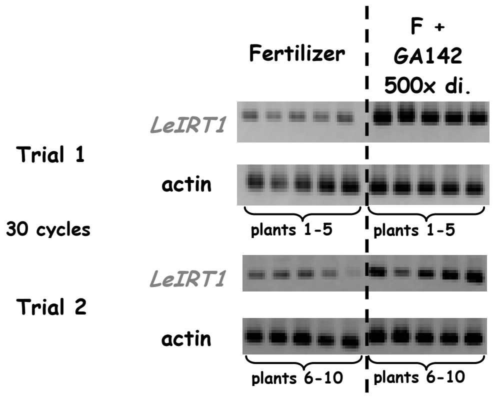

Figure 11: Effect of the Goëmar algae filtrate (500x dilution) on the activity of the LeIRT1 gene in the roots of treated tomato plants (48 hours after treatment).

The results of these trials thus show that the increased quantity of iron observed in plants treated with the algae filtrate is explained by the activation of the two mechanisms implemented by plants to uptake iron from soil; the ferric-chelate reductase enzyme and the ferrous iron transporter gene.

Conclusion and agronomic applications of the Goëmar algae filtrates

In conclusion, the variety of actions described above, which characterise the effects of the Goëmar algae filtrates on plants, is illustrated at the macroscopic level by the stimulated growth of young treated plants. This shows a direct or indirect activation for all the physiological processes involved upstream, notably in the nutrition pathways of plants. The stimulation of the plant enzymes involved in the absorption of nitrogen, notably by mannitol (active substance present in the algae filtrates), and of phosphates as well as in the assimilation of iron are a few specific examples for this. For iron nutrition, the originality lies in the fact that the mode of action is dual with an effect not only on the ferric-chelate reductase enzyme that makes it possible to assimilate the iron from soil but also the *LeIRT1* transporter gene that increases the penetration of iron into the plant.

Their activation then brings about a multitude of downstream actions in the plant. Positive actions on key stages in plant metabolism, which condition the growth of the plant, are notably observed, such as the synthesis of chlorophyll (rich in nitrogen and dependent on iron) or the energy metabolism of the plant cell (requiring phosphates) that will probably be improved.

The overall understanding of the effects of these filtrates will be acquired when the main organic compounds involved in the observed biological effects are identified. This is an ambitious approach, both in the number and diverse nature of the potential candidates and in the fact that they can act separately or through the effects of synergy with other molecules. However, this research now makes it possible to draw some initial explanations on the effects of the Goëmar algae filtrates and can be taken into account for new proposed strategies for growing plants. Indeed, the different results obtained have enabled the algae filtrates to be qualified as Physio-Activator® and original agronomic solutions to be defined, such as:

- Ecofert GA EU, a product containing 50% algae filtrate that is used in Italy.
- Dionyfer (sold in France) which is used to limit chlorosis problems that occur frequently on grapevine.

In addition to the nutrition effects, other properties of algae filtrates were highlighted, notably their capacity for stimulating the synthesis of polyamines in perennial crops such as apple trees (+25% in relation to untreated plants). These molecules, qualified as "natural flowering hormones", play a vital role in the blooming cycle and in reproduction. The correct biosynthesis of polyamines particularly determines the harvest quality and quantity. The use of the algae filtrate (marketed under the name of BM 86 in Italy) for in the pre- and post-flowering periods positively affects the polyamine synthesis metabolism, which is seen in the field by an improvement in economic qualitative criteria (fruit uniformity, increased average grade).

A promising new path, still largely untrodden, is therefore emerging: plant physiology. The natural origin and harmlessness of Goëmar Physio-activators® together with the knowledge of their mode of action indicate that they are in accordance with a sustainable production system that respects the environment and the user.

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OSMOPROTECTANS AMELIORATE TOMATO YIELD PERFORMANCE UNDER SALINE ENVIRONMENTS

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Abstract

Improving crop tolerance to salinity is critically important in many agricultural areas, including Mediterranean, which are increasingly affected by salinization problems. In this study, the effect of PHYT-actyl (Timac Agro Italia S.p.A., TIMAC) treatment on tomato plants exposed to salinity was assessed. It was hypothesized that the addition of PHYT-actyl (a commercial extract from the seaweed *Ascophyllum nodosum)* to the irrigation water may ameliorate plant performances in hyperosomotic environment through the osmoprotective action of several molecules that naturally accumulate in the "osmo-adapted" *Ascophyllum nodosum* (a large, common brown alga belonging to the *Fucaceae* family). The research was conducted during two growth seasons 2007 and 2008 in a long-term salinized field (a clay loam soil that had been exposed during the summer to saline irrigation since 1988) and in a short-term salinized field (a clay loam soil that had not been previously irrigated with saline water). Leaf gas exchanges, plant water relations and crop yield (quantity and quality) were measured on tomato for processing (Tampico F1) grown under three different irrigation treatments: a non-salinized control (NSC) and two concentrations of commercial sea salt in the irrigation water: 0.125% (SW1) and 0.25% (SW2), corresponding to electrical conductivities of 0.5 (NSC), 2.3 (SW1) and 4.4 dS/m (SW2). Furthermore, two different fertirrigation treatments were compared: the conventional system (Control) and the conventional system plus 1% of PHYT-actyl. Leaf total, osmotic and pressure water potentials decreased at increasing salinity of the irrigation water. Lower values were observed for the TIMAC treated plants, in both fields and both years. The stomatal resistance increased with salinity and was higher in the long-term salinized field and upon TIMAC treatment. Salinity stress reduced both total and marketable yield. However, PHYT-actyl applications increased tomato yield of 10%. Although salinized tomato fruits were smaller than non-salinized control fruits, they had higher dry matter content, soluble solids and sugars content, which are all highly requested qualities for the processing tomato industry.

Keywords: salinity, irrigation, leaf water potential, osmotic potential.

Introduction

In coastal regions of Mediterranean areas, growth and productivity of summer crops are often limited by irrigation with saline water. Although tomato is considered moderately tolerant to salinity, it is typically cultivated in areas which are particularly susceptible to salinization (Maggio et al., 2004). Improving crops performance in these environments is currently one of the major challenges in agriculture since salinization, drought and heat stress are the primary effects of climate change on agricultural crops. As a consequence, there is an urgent need to re-introduce in commercial varieties those tolerance traits that have been lost throughout breeding programs essentially aimed at improving yield and quality. Recent advancement in molecular genetics and functional biology of abiotic stress tolerance has shed some light on the fundamental mechanisms underlying plan adaptation to hyperosomotic environment, opening new opportunities to develop and/or fine-tune cultural techniques that are based on the acquired scientific knowledge (Zhu, 2001). Ion compartimentation and ion/water homeostasis are two critical mechanisms in salt stress adaptation and as such have been the primary targets to improve tolerance via genetic engineering. Constitutive overproduction of compatible solutes has been obtained in several plant species, including tomato, with a moderate tolerance improvement (Maggio and Saccardo, 2008). The possibility of manipulating plant response to salinity via irrigation-feeding or foliar spraying of molecular effectors that may improve salt tolerance has been also demonstrated (Foolad et al., 2003). However, in most cases these techniques have not been further developed to field applications (De Pascale et al., 2001). In this study we assessed the effects of PHYT-actyl on tomato performance in response to salinity. PHYT-actyl is a concentrated and lyophilized extract from the seaweed *Ascophyllum nodosum*, a large, common brown alga belonging to the *Fucaceae* family which is particularly rich of stress *protective* molecules including betaine and abscissic acid (ABA). Main agronomic and physiological responses measured over a two-year field experiment are here presented and discussed in light of possible strategies to improve crop adaptability to low quality (saline) irrigation water.

Materials e methods

The research was carried out at the University of Naples Federico II Experimental farm (43°31’ N; 14°58’ E) during two growth seasons 2007 and 2008 on tomato for processing

(*Lycopersicon lycopersicum* Mill.). The experiments were conducted on a clay-loam soil (42% sand, 27% silt, 31% clay, and trace amounts of lime) in a long-term salinized field (S) and in a field that had not been previously irrigated with saline water (C). Salinity treatments consisted of three NaCl concentrations in the irrigation water (w/v): 0% (NSC), 0.125% (SW1) and 0.250% (SW2) equivalent to electrical conductivities at 25°C (EC_w) of 0.5, 2.3 and 4.4 dSm^{-1}, respectively. The long-term salinized field (S) had been irrigated during spring/summer with saline water since 1988. When the soil salinization was begun the salinity treatments were arranged in a randomized block design replicated three times. In the following years, to assess the cumulative effects of salinization, the salinity treatments have been reassigned in each year to the same experimental field plots. During the growth seasons of 2007 and of 2008, the effects of saline irrigation were assessed on tomato plants. To specifically compare plant performances under salt stress in a long-term salinized field and in a short-term salinized, the same irrigation treatments were imposed to tomato plants in a field that had not been previously irrigated with saline water (C). Furthermore, two different fertirrigation treatments were compared: the conventional system (Control) and the conventional system plus 1% of PHYT-actyl (Timac Agro Italia S.p.A., TIMAC) added to the liquid fertilizers. The experimental design was a split-plot with three replications. The fields treatments were assigned to the main plots, the salinity treatments were assigned to the sub-plots, whereas different fertigation were assigned to the sub-sub-plots. On 23 April (2007) and on 26 May (2008), tomato plantlets (Tampico F1) were transplanted in rows 0.9 m apart with an intra-row spacing of 0.25 m. In both years, prior to transplanting 54 kg N ha^{-1} and 138 kg P_2O_5 ha^{-1} were applied to the soil (as di-ammonium phosphate 18-46-0). Subsequently, plants were fertilized with an additional applications of 33 kg N ha^{-1}, 66 kg P_2O_5 ha^{-1} and 48 K_2O ha^{-1} (as NPK fertilizer 11-22-16) 3 weeks after transplanting. Saline irrigation was initiated 4 weeks after transplanting (on 22 May 2007 and on 23 June 2008) after ensuring the establishment of the plantlets with a single irrigation with non-salinized water (330 mm). Plants were irrigated at 4 d intervals using a drip irrigation system with 2 L h^{-1} emitters. Amounts of water applied at each irrigation event were determined using a USDA Class A pan evaporimeter. For all salinity treatments the cumulative amount of water applied from the beginning of the growing season to harvest was 355 mm (2007) and 360 mm (2008) (distributed in eighteen irrigation events). Fertigation treatments were initiated 29 days (2007) and 28 days (2008) after transplanting (DAT). For both fertigation treatments the total amount of nutrients applied from the beginning of the treatments to harvest were: 103 kg N ha^{-1}, 87 kg K_2O ha^{-1} e 93 kg CaO ha^{-1} in 2007 and 105 kg N ha^{-1}, 90 kg K_2O ha^{-1} e 99 kg CaO ha^{-1} in 2008 (applied at 4 d.

intervals via fertigation in both years). Stomatal resistance, leaf total, osmotic and turgor potentials were measured at approximately 30-day intervals on three plants per treatment (one per each replication) between 12:00 a.m. and 1:00 p.m. Stomatal resistance was measured on the abaxial surface of the youngest, fully expanded leaves with a diffusion porometer (AP-4, Delta-T Devices, Cambridge, UK). Leaf total water potential (Ψ_t) was measured on tissue discs punched from the first, uppermost, fully expanded, healthy and sun-exposed leaf using a dew-point psychrometer (WP4, Decagon Devices, Pullman, WA). The osmotic potential (Ψ_π) was measured on frozen/thawed samples and pressure potential (Ψ_p) was estimated as the difference between Ψ_t and Ψ_π, assuming a matric potential equal to zero. Fruit harvest occurred on 6 August, 2007 (105 DAT) and on 5 September, 2008 (102 DAT). Fruits were counted, weighed and ranked for their marketability (the non-marketable yield included yellow fruits and fruits having lesions and/or weight <50 g); fruit dry weights were determined upon dehydration in an oven at 60 °C (until steady weight).

Results and Discussion

The leaf water potential (Ψt) and osmotic ($\Psi\pi$) potential decreased at increasing salinity (EC) of the irrigation water (table 1). Leaf water potential and osmotic potential were -1.15 and -1.43 MPa, respectively, at the highest salinity (4.4 dSm^{-1}). A significant decrement of Ψt and $\Psi\pi$ were also observed in long term salinized field and upon Timac fertigation, while the effect of the year (due to the different climatic conditions, data not shown) was significantly only for the osmotic potential. Salinity caused an increment of leaf stomatal resistance. Higher leaf stomatal resistances were measured also in Timac treated plants and in the long-term salinized field (S), with no significant difference for the two years (table 1). For these physiological parameters, it was observed a significant interaction between salinity and fertigation treatments. The leaf water potential and osmotic potential were both lower in presence of 1% PHYT-actyl (Timac) at all the salinity levels. However, the decline of these parameters in response to salinity was much stronger in PHYT-actyl treated plants respect to their relative control (conventional fertigation), indicating a specific response to salinity mediated by PHYT-actyl (data not shown). Salinity decreased plant growth. Increasing EC significantly reduced leaf area, leaf number and total dry matter (data not shown).

The decrease in leaf area of SW1 and SW2 plants was attributable mainly to a decrease in leaf number as opposed to a reduction in leaf size. A significantly reduced number of leaves were measured under Timac treatment and in the long-term salinized field (S). However, no difference was observed in terms of plant leaf area. In contrast, increasing salinity caused an increment of the specific leaf weight (SLW, dry weight/leaf area ratio) in salinized plants. A significant increment of SLW was also observed in 2007, but it was not affected by fertigation or long-, short-term salinized fields. The yield and its components were significantly reduced at increasing EC of the irrigation water (table 2). The total and marketable yield of SW2 plant were approximately 22% smaller than their relative non salinized-control. Yield reduction was in general attributable to both a smallest fruit mean weight and a reduced number of fruits per plant. Yield was also reduced respect to different years but it was not affected by short-long term salinized fields. The Timac treatment improved tomato yield of 10% respect to the conventional fertigation treatment. Overall these data indicate that is possible to ameliorate plant response to salinity by feeding through the irrigation water a *natural* mix of organic molecules that are physiologically produced by the plant under stress conditions. In this respect, an extract from physiologically osmo-adapted seaweeds may have a quite broad mixture of stable molecules with complementary properties that may be easily uptaken by the plants, if fed through the irrigation water. These may act as stress protective molecules and/or activate adaptation responses with protective functions in stressful environments. The

Table 1: Leaf total (Ψ_t), osmotic (Ψ_π), pressure (Ψ_p) potentials and leaf stomatal resistance (R_s) in response to years (2007; 2008), salinity treatments [EC of the irrigation water = 0.5 (S0); 2.3 (SW1); and 4.4 (SW2) dS m^{-1} at 25 °C]; fields [the long-term salinized field (S) and the short-term salinity field (C)] and fertigation (the conventional system (Control) and the conventional system plus 1% of PHYT-actyl (TIMAC). ns, *, ** = not significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

	Ψ_t *MPa*	Ψ_π *MPa*	Ψ_p *MPa*	R_s cm/s
Year				
2007	-1.02	-1.33	0.31	1.23
2008	-0.95	-1.23	0.28	1.20
	n.s.	*	n.s.	n.s.
Field				
S	-1.04	-1.34	0.30	1.32
C	-0.93	-1.23	0.30	1.10
	**	**	n.s.	**
Salinity				
NSC	-0.82	-1.13	0.32	0.89
SW1	-1.00	-1.28	0.29	1.19
SW2	-1.15	-1.43	0.29	1.55
	**	**	n.s.	**
Fertigation				
Control	-0.90	-1.20	0.30	1.08
Timac	-1.08	-1.37	0.29	1.35
	**	**	n.s.	**

implementation of physiology-based strategies in agricultural productions should be further considered to improve resource use efficiency and to reduce the environmental impact of agricultural practices.

Table 2: Tomato yield, Fruit mean weight, fruit per plant, yield plant and fruit dry matter percentage (DM) in response to years (2007; 2008), salinity treatments [EC of the irrigation water = 0.5 (S0); 2.3 (SW1); and 4.4 (SW2) dS m^{-1} at 25 °C]; fields [the long-term salinized field (S) and the short-term salinity field (C)] and fertigation (the conventional system (Control) and the conventional system plus 1% of PHYT-actyl (TIMAC). ns, *, ** = not significant, significant at *P*≤0.05 and *P*≤0.01, respectively.

Year	Total yield	Marketable yield	Fruit mean weight	Yield	Yield	Fruit dry matter
	tha^{-1}	tha^{-1}	g	fruit per plant	kg $plant^{-1}$	%
2007	62.40	57.30	63.7	21.2	1.35	6.0
2008	49.56	45.92	62.1	17.6	1.09	6.5
	**	**	n.s.	**	**	n.s.
Field						
S	55.18	50.83	62.9	19.1	1.20	6.5
C	56.78	52.39	62.8	19.7	1.23	6.1
	n.s.	n.s.	n.s.	*	n.s.	n.s.
Salinity						
NSC	63.22	58.76	67.4	20.5	1.38	5.7
SW1	55.20	50.89	64.0	18.9	1.20	6.1
SW2	49.52	45.18	57.2	18.8	1.08	7.0
	**	**	**	*	**	**
Fertigation						
Control	53.76	49.30	61.8	18.7	1.15	6.1
Timac	58.21	53.92	63.9	20.1	1.28	6.4
	**	**	n.s.	**	**	*

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SLOW RELEASE NITROGEN FERTILIZERS BY HYDROPHOBIC POLYMER COATING OF CLAY/UREA NANOCOMPOSITES

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Abstract

Large losses of nitrogen fertilizer by leaching and volatilization require the use of excessive amounts of fertilizers and cause economic and environmental impacts, which require the use of controlled or slow release fertilizers so these losses are minimized. In this work, slow release nitrogen fertilizers were obtained by montmorillonite clay and urea nanocomposites formation with polysulfone (PSF) and poly(vinyl chloride) (PVC) coatings. Coatings were formed by phase inversion and fluidized bed techniques and the kinetic of nitrogen release was evaluated by analysis of released urea to aqueous media. The urea/clay composites did take 60 min for the total losses of urea in water and the systems prepared with polyssulfone and with poly(vinyl cloride) coatings presented a total loss in about 240 and 180 min respectively, while pure urea dissolved immediately.

Keywords: slow release fertilizers, hydrophobic polymeric coatings, clay/urea nanocomposites.

Introduction

Nitrogen fertilizers are used in excessive amounts because a large portion of the used fertilizer is lost by leaching and volatilization. These losses cause contamination of soil, groundwater and atmosphere, which causes several economic and environmental impacts (Entry & Sojka,). In order to minimize the inadequate management of fertilizers, it can be used the so called slow or controlled release fertilizers.

Slow or controlled release fertilizers are systems where the nutrients are released gradually in accordance to the plants growing needs (Hanafi et all, 2000), minimizing losses and enabling the use of lower amounts. So, these specialty fertilizers are a good alternative to solve the economic and environmental problems caused by the inadequate management of fertilizers.

Slow or controlled release fertilizers can be obtained by the hydrophobic polymeric coating of water soluble fertilizers. Hydrophobic coatings reduce the water diffusion and the resulting release of the nutrients (Tomaszewska et all, 2002). Polymeric coatings can be formed by different techniques in order to be obtained membranes without cracks that will protect the water soluble fertilizer to immediate water exposition.

In order to reduce the fertilizer release, the nutrients could be stored in nanostructured materials. The montmorillonite clays have a crystalline structure with characteristics that lead to the expansion of its interlayer when in contact with water, making them a nanostructured material extremely convenient to store fertilizers (Liu et all, 2006).

In this work, slow release nitrogen fertilizers were obtained by montmorillonite clay and urea granules coated with polysulfone (PSF) and poly(vinyl chloride) (PVC). The kinetic of nitrogen release of the nanostructured fertilizers obtained was evaluated.

Material and Methodos

Materials: Polysulfone (Sigma-Aldrich, Mw 35,000) Poly(vinyl chloride) (Sigma-Aldrich, Mw 233,000), Bentonite (Bentonorte), Urea (Vetec) and N,N-dimethyl-acetamide (Vetec)

Adsorption of urea in montmorillonite clay: Aqueous urea solutions were prepared in different concentrations and 36 mL of each solution were mixed with 1 g of montmorillonite clay (bentonite). The mixture was maintained under agitation for 1 h. So, the mixture was centrifuged to separate the clay from solution and the clay was dried in oven at 60 ° C for 48 h. The clay samples were characterized by elementary CHN analyzer and X-ray diffraction (XRD).

Grains preparation of clay/urea nanocomposites: Grains of clay/urea nanocomposites were prepared by two techniques: pressing into perforated plates and granulation. In the first technique, 107 g of urea were dissolved into 100 mL of water. The solution was mixed with 93g of bentonite and the resulting mass was pressed into rectangular plates perforated with holes of 3 mm diameter and 3 mm height (figure 1). The mass was dried in oven at 60 °C for 48 hs and then removed from the plates. In the second technique, the same mass of bentonite and aqueous urea solution was used and dried in an oven at 60 °C for 48 h. The dried mass was crushed and granulated by a rotation dish granulator (figure 2) with water spray. The grains were sieved by sieves from 4 to 8 mesh.

Figure 1: Perforated plates used for grains preparation of clay and urea.

Figure 2: Rotation dish granulator used for grain preparation of clay and urea.

Polymeric coating: Polymeric coatings were prepared by two techniques: phase inversion and fluidized bed. In both of cases were used polysulfone (PSF) and poly(vinyl chloride) (PVC) solutions in N,N-dimethyl-acetamide at 10 %. In the phase inversion process, grains of clay/urea were dipped into a polymeric solution and then dipped into water in order to precipitate the polymer. For the fluidized bed coatings it was used a micro fluid bed (Freund-Vector), figure 3, with top spray application by cycles of 0,5 min at spray on and intervals of 1 min to drying. The spray worked pumping the solution at 50 rpm and air pressing at 1.2 psi.

Figure 3: Micro fluidized bed.

Nitrogen release in aqueous media: One gram of nanostructured obtained fertilizers was mixed with 20 mL of water, and the mixture was maintained in repose for different intervals of time and then centrifuged for 1 min at 5000 rpm. An aliquot of 1 mL from the supernatant was diluted to 100 mL and the contents of released urea was analyzed by using a kit for urea determination in plasma and urine by enzymatic colorimetric method (Human do Brasil). The solution absorbance was read at a spectrophotometer (Biocristal) at 578 nm.

Results and discussion

Adsorption of urea in montmorillonite clay:

The amount of nitrogen adsorbed on bentonite particles increased with the increase of the urea concentration in the used solutions rearching a plateau at a concentration of approximately 30 %. Figure 4 shows the percentage of adsorbed nitrogen against the urea solutions concentration. The X-ray diffraction analyzes results show an increase in the interlayer space of clay. The clay sample treated without urea presented an interlayer space of 12,68 Å while the one treated with an urea solution at 20% presented an interlayer space of 17,26 Å (figure 5). The increasing of the interlayer space by the urea adsorption indicates that urea is inserted in sites of bentonite structure, which contributes to reduce the speed of the urea release.

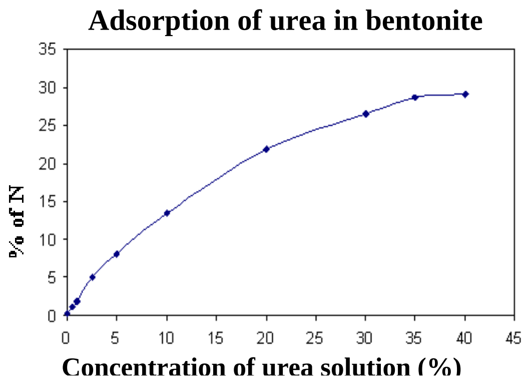

Figure 4: Percentage of adsorbed nitrogen versus urea solutions concentration.

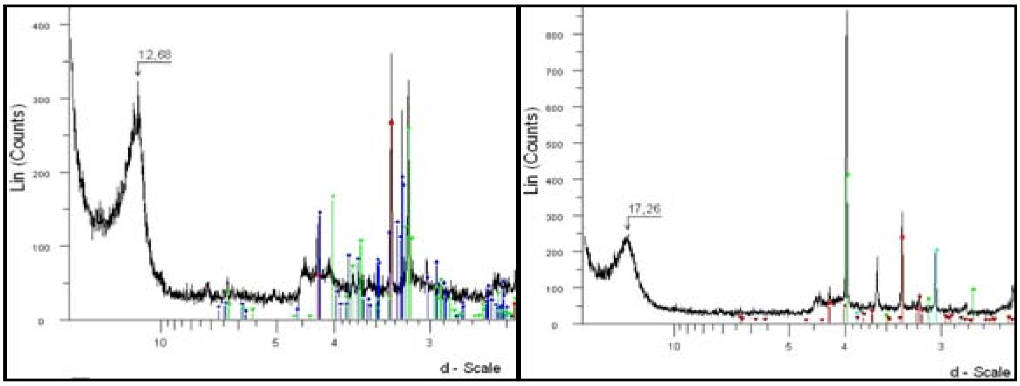

Figure 5: DRX of sample treated without urea (left) and sample treated with urea solution at 20 % (right).

Grains of clay/urea nanocomposites with polymeric coatings:

The process of production that used pressing of clay/urea masses through perforated plates produced regular cylindrical grains that enabled more uniform coatings, as compared with the grains prepared by granulation, which presented an irregular form. Figure 6 shows grains prepared by the two mentioned processes, coated with polysulfone by fluidized bed.

Figure 6: Grains prepared by granulation (left) and grains prepared by pressing in perforated plates (right) both coated with polysulfone.

Figure 7 show the kinetic of nitrogen release of samples coated with PSF. All samples coated with PSF presented a decrease in nitrogen release against the time of exposition in water, as compared with the uncoated sample. Samples of cylindrical grains coated with PSF at 5 % and 10 % by the fluidized bed technique presented the same kinetic profile and the sample coated with the same polymer at 5 % by phase inversion presented slightly higher amounts of nitrogen release, but the difference is negligible. The Samples of granulated grains presented a lower decrease of nitrogen release, as compared with the uncoated sample, than the cylindrical grains samples.

Samples coated with PVC also presented a decrease in nitrogen release when compared with the uncoated sample. The sample coated with PVC at 5 % by fluidized bed presented lower amounts of nitrogen released as compared with the sample coated by phase inversion, but both samples reaches the total nitrogen release at the same time. Samples of granulated grains presented a smaller decrease of nitrogen release, compared with uncoated sample, than cylindrical grains samples. Figure 8 shows the kinetic of nitrogen release of samples coated with PVC.

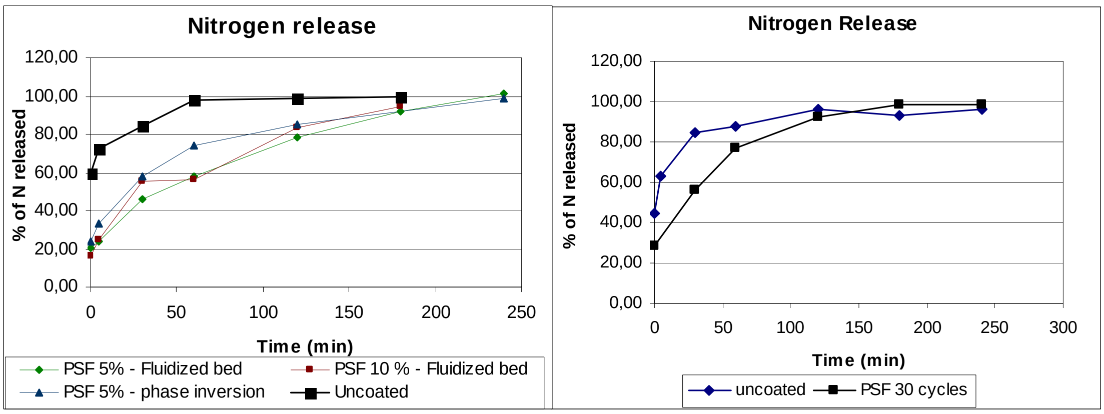

Figure 7: Kinetics of nitrogen release of cylindrical grains (left) and granulated grains (right) coated with polysulfone.

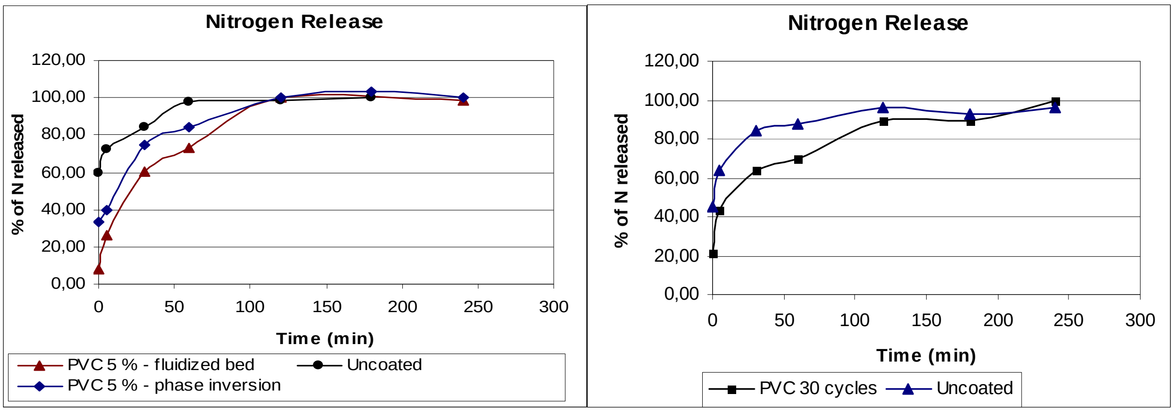

Figure 8: Kinetics of nitrogen release of cylindrical grains (left) and granulated grains (right) coated with poly(vinyl chloride).

Conclusions

Nanocomposites of clay/urea coated with polysulfone and poly(vinyl chloride) proved effective to reduce nitrogen released. Cylindrical grains coated with polysulfone by fluidized bed proved to be the more effective among the studied systems.

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BIOSTIMULANT ACTIVITY OF TWO PROTEIN HYDROLYSATES IN PEROXIDASE AND ESTERASE ACTIVITY OF MAIZE SEEDLINGS

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Abstract

Two protein-hydrolysate-based fertilizers (PHFs), one from legume (LH) and one from the mixture of Spirulina spp. and alfalfa (SA), were studied chemically and biologically. LH and SA showed a different chemical composition and different degree of hydrolysis. The biostimulant activity was investigated using two specific and sensitive bioassays of auxins (IAA) and gibberellins (GA). Result showed that both extracts of LH and SA elicited an auxin and gibberellin-like activity. To improve our understanding on the biostimulant activity of PHFs, LH and SA were supplied to maize plants and their effect on growth, chlorophyll and sucrose content was studied. Besides, the effect of PHFs was tested by the activity of peroxidase and esterase, enzymes notoriously used as markers in growth, differentiation and organogenesis- related processes. The biostimulant products increased leaf growth and induced morphological changes in root architecture similarly to growth factors. LH and SA positively affected peroxidase and esterase activities, suggesting their important role in the induction of growth and cell differentiation.

Introduction

The intensification of agricultural practices that make large use of fertilizers has determined an increasing impact on the quality of waters and soils (Hirel et al 2001). Phosphorus and nitrate represent the main agricultural water contaminants and have been associated with environmental pollution through the eutrophication of waters (Hirel et al 2001). High nitrates (NO_3^-) come from fertilization, as about the 50 % of nitrogen-fertilizers applied to crops, are left behind as residues, which can move throughout the soil profile into groundwater (leaching). In the last years, the production innovations have been evolving towards low cost, organic, sustainable and environmental friendly tools, which must contemporarily assure the yield and the quality of crops. A number of authors have suggested the use of biostimulants in plant nutrition instead of inorganic fertilizers to enhance the uptake and utilisation of nutrients by plants (Kauffman III et al 2007). Biostimulants are organic molecules that are often regarded as positive plant growth regulators or as metabolic enhancers, and are presently recognized by the legislation ruling the market of fertilizers. When applied in small amounts, biostimulants can promote plant development, increase yields and support plants to overcome stress situations by acting directly or indirectly on plant physiology (Sahain et al 2007). They can also be used as chelating agents of different cations for the cure of plant deficiencies (Ashmead, 1986), or as adjuvant for pesticides or herbicides (Cavani, 2006). Biostimulants include three groups of molecules: plant hormones, humic substances, and free amino acids/peptides. The third group is also known as protein hydrolysates (PHFs). They are natural biostimulants, comprising of oligo- and poli- peptides, and free amino acids, which can be obtained through chemical and/or enzymatic hydrolysis of organic matrix from plant or animal sources. Although the active molecules and the metabolic pathway target of PHFs are still mostly, it has been shown that foliar application of these products usually leads to the improvement of plant nutrition and metabolism, and increases the tolerance of plants to abiotic stress. In present study two products provided by ILSA S.p.A. have been tested for their biostimulant properties. The analysis performed included the Audus test, the measurement of leaf and root fresh weights, sugars and chlorophyll content, peroxidase and esterase activities.

Materials and Methods

Characterization of the protein hydrolysate. The protein-hydrolysate-based fertilizers (PHFs), used in this study are one from legume (LH) and one from the mixture of Spirulina spp. and alfalfa (SA), were produced by fully controlled enzymatic hydrolysis (FCEH®). Total organic carbon (TOC) was determined by wet oxidation method with potassium dichromate; total nitrogen (TKN) via Kjeldahl method. Total sulphur was determined by induced coupled plasma atomic emission spectroscopy (Spectro CirosCCD, Kleve, Germany), after plant tissue acid digestion with ultra pure nitric acid (Merck, Darmstadt, Germany)

Audus test. The biological activity of the protein hydrolysate (PHFs) was assessed by measuring the root growth reduction of watercress (*Lepidium sativum* L.) and the increase in the shoot length of lettuce (*Lactuca sativa* L.) as described by Schiavon *et al.* (2008). The values obtained were the means of 20 samples and five replications, with the standard errors always ≤5% of the mean.

Plant material. Maize seeds (*Zea mays* L.) were soaked in distilled water for one night in running water and germinated for 60 h, in the dark, at 25°C on a filter paper wet with 1 mM $CaSO_4$. Seedlings were then raised in a hydroponic set-up using a Hoagland no. 2 modified solution, inside a growth chamber with a 14 h light/10 h dark cycle, air temperature of 27/21°C, relative humidity of 70/85% and at a photon flux density (PFD) of 280 mol $m^{-2}s^{-1}$. Twelve-days old plants were treated for 48 h with different concentrations of either LH or SA: 0 (control), 0.01 or 0.1 mL L^{-1}.

Soluble sugars. Foliar tissues (100 mg) of five independent plants were dried for 48 h at 80° C, ground in liquid nitrogen and then extracted with 2.5 mL 0.1 N H_2SO_4. Determination of soluble sugars was performed according to Schiavon et al 2008. Sugar concentration was expressed in g Kg^{-1} d.wt. Three independent experiments were performed for each determination.

Chlorophyll content Chlorophyll were determined by the method of Lichtenthaler and Welburn (1984).

Esterase and peroxidase activities. Enzyme activities were determined according to Junge & Klees (1984).

Results and Discussion

With respect to the analyses performed on the products, the content of nitrogen and sulphur was similar in the two protein hydrolysates, LH and SA (table 1), while the amount of total carbon was significantly higher in LH (plus 50%).

Table 1: Chemical properties of the PHFs, from legume (LH) and from the mixture of Spirulina spp. and alfalfa (SA), TOC: total organic carbon; TKN: total nitrogen; Total S: total sulphur. Values are the means of 5 replicates, with standard errors ≤ 5% of the mean. Different letters indicate significant differences between treatments (P<0.05).

Property	unit	LH	SA
TKN	%(w/w)	2,07a	1,9a
TOC	%(w/w)	23,6a	11,6 b
Total S	%(w/w)	0,6a	0,4a

In order to establish the biostimulant properties of the two products, the Audus test was used (Audus, 1972), which revealed the auxin- and gibberellin- like activity of LH and SA (table 2). With respect to the IAA- and GA- like activities, the application of pure indolacetic and gibberellic acid reduced the root length of watercress and increased the epicotil of lettuce respectively, in a similar way of LH and SA application (data not shown). When the PHF was supplied to plants at the concentration of 1 ppm, the IAA-like activity in LH was three times higher than in SA, whereas the GA-like activity was comparable between the two biostimulants (table 2).

Table 2: Auxin and gibberellin-like activities of the PHFs, from legume (LH) and from a mixture of Spirulina spp. and alfalfa (SA) measured at 1 ppm PHF. Both hydrolysates were evaluated via Audus test, i.e. by measuring the reduction of root length (mm) of watercress and the increase in shoot length (mm) of lettuce, respectively. The values obtained were the means of 20 samples and five replications, with the standard errors ≤5% of the mean. GA, gibberellin acid; IAA, indolacetic acid.

product	GA activity (ppm)	IAA activity (ppm)
LH	0,1	1,075
SA	0,1	0,3

Following these findings, LH and SA were furnished to maize plants for 48 h at the concentrations of 0.01 and 0.1 mL L^{-1}, and their effects on a number of physiological parameters related to plant productivity were evaluated.

Results indicated that both concentrations of the products caused a significant increase of the leaf biomass (figure 1). As a consequence, the ratio root to shoot decreased from 1.42 (control plants) to 0.51 and 0.48 in plants grown with LH or SA, respectively. Increased plant biomass following PHFs applications was also observed by Ertani et al 2009 in mays.

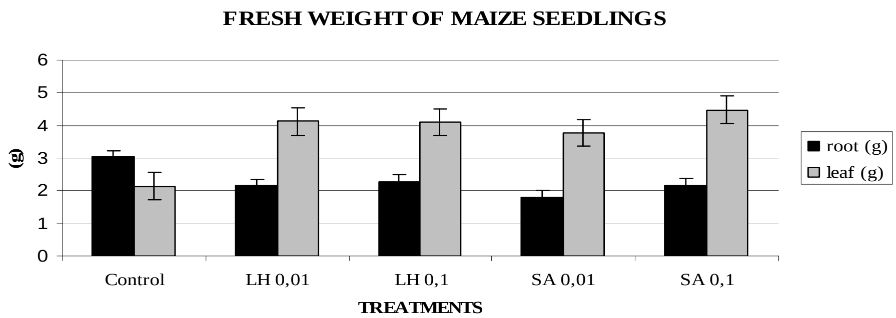


Figure 1: Effect of LH and SA treatment on root and leaf fresh weight of *Z. mays* plants grown for 12 d in a Hoagland modified complete nutrient solution and treated for 2 d with LH and SA at 0.01 or 0.1 mL L^{-1}. Data are the means of 10 values each from three independent experiments.

Plants supplied with LH and SA showed a decrement of the sugar content in their foliar and root tissues (table 3) according to Schiavon et al 2008. To evaluate the effects of LH and SH on plant metabolism, the chlorophyll content was evaluated in maize plants.

Table 3: Effect of LH and SA treatment on root and leaf sugars content in Z. mays plants grown for 12 d in a Hoagland modified complete nutrient solution and treated for 2 d with LH and SA at 0.01 or 0.1 ml L-1. Data are the means of 10 values each from three independent experiments.

product	total sugars (mg/g/pf)	
	root (g)	leaf (g)
Control	50,05 a	31,63 a
LH 0,01	19,3 c	6,48 b
LH 0,1	25,29 b	7,11 b
SA 0,01	20,42 c	6,28 b
SA 0,1	24,52 b	1,43 c

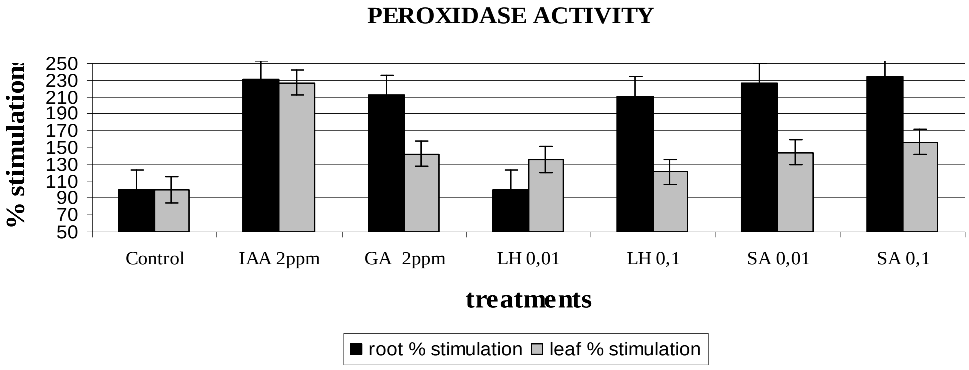
Total chlorophyll levels were higher in plants grown with PHFs than no treated plants. The highest increase was recorded in plants supplied with 0.01 mL /L SA (table 4).

Table 4: Effect of LH and SA treatment on chlorophyll content of Z. mays plants grown for 12 d in a Hoagland modified complete nutrient solution and treated for 2 d with LH and SA at 0.01 or 0.1 mL L^{-1}. Data are the means of 10 values each from three independent experiments (±SE). Different letters on bars indicate significant differences between treatments (P<0.05).

product	diluition (ml/l/tq)	total chlorophyll (µg Chl/ml)	stimulation (%)
Control		8,77 d	100
LH 0,01	0,01	14,46 b	165
LH 0,1	0,1	10,91 c	124
SA 0,01	0,01	20,24 a	231
SA 0,1	0,1	11,36 c	129

The two products increased the activities of the esterase and peroxidase enzymes. As it is known from the literature, the esterase activity represents a precocious indicator of the differentiation of the cells and organs, while the peroxidase activity is correlated to the morphological changes in leaf and root plants (Coppens et al 1990). The two biostimulants enhanced the activity both in leaves and roots in a similar way to gibberellic acid and auxin (Figure 2 and 3). The peroxidase activity was more stimulated in roots.

In conclusion, the obtained results demonstrated that the tested products were able to promote the plant growth and to influence the tissue concentrations of soluble sugars. Furthermore LH and SA showed to positively affect the peroxidase and esterase activities, suggesting their important role in the induction of growth and cell differentiation. Our data suggest that the two protein-hydrolysate-based fertilizers may be a valid alternative to the traditional inorganic fertilizers and encourage further research.



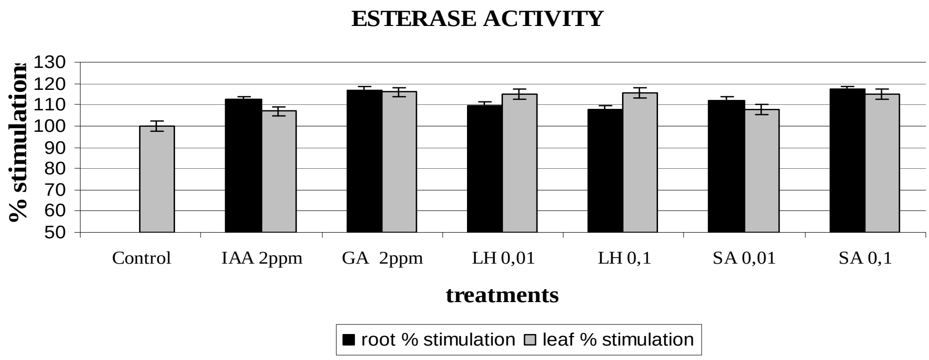


Figure 2 and 3: Root and leaf enzyme activity of peroxidase and esterase in Z. mays plants grown for 12 d in Hoagland modified complete nutrient solution (control) and treated for 2 d with LH at 0.01 or 0.1 ml L^{-1} and with SA at 0.01 or 0.1 mL L^{-1} . Data are the means of 5 values each from three independent experiments (±SE).

Acknowledgements

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SULFUR-INDUCED RESISTANCE (SIR): BIOLOGICAL KNOW-HOW FOR ENVIRONMENTALLY SOUND DISEASE CONTROL

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Abstract

Environmentally sound methods for disease control imply for instance soil tillage measures, crop rotation, mixed cropping systems and cultivation of resistant varieties. The targeted use of minerals offers yet another possibility to enhance resistance against pathogens. Here, the direct toxicity of nutrients and indirect impairment by minerals needs to be distinguished from nutrient-mediated, resistance mechanisms. Soil-applied sulfate fertilization proved to significantly reduce infection rate and severity of crops by fungal diseases. Up-to-date research in the field of (Sulfur Induced Resistance) SIR is summarized in relation to different host/pathogen systems. In addition, recommendations for stimulating SIR by targeted fertilizer practices will be given.

Keywords: Fungal infection, sulfur metabolism, Sulfur-Induced Resistance (SIR).

Introduction

Sulfur (S) is a major plant nutrient and S deficiency not only impairs crop productivity and quality, but also interferes with environmental demands (Haneklaus *et al.*, 2006a and 2006b).

The beneficial effects of S for plant health comprise various aspects such as the use of elemental S as a fungicide, the cultivation of glucosinolate-containing plants against pathogens and the targeted S nutrition for enhancing the natural resistance against pests and diseases. The fungicidal effect of elemental S was discovered by *William Forsyth* (1802) and has been widely used in agricultural production since the end of the nineteenth century (Hoy, 1987). Elemental S proved to be most efficient against numerous diseases such as rust and powdery mildew, but was also successfully used against other diseases, for instance downey mildew in cereals, common scab of potato and Alternaria black spot of oilseed rape (for references see Haneklaus *et al.*, 2007a). Only recently Haneklaus *et al.* (2007b) showed that repeated foliar-applied elemental S applications significantly reduced the infection rate with Fusarium head blight by 30% under field conditions after artificial inoculation. Next to its fungicidal effect, elemental S is an acaricide and used to combat mites (Hoy, 1987).

The allelopathic effect of plants from the orders Cruciferae, Resedaceae and Capparidaceae on weeds and soil-borne diseases usually focuses on the release of volatile isothiocyanates (ITCs) after degradation of glucosinolates (GSLs). Their effect on soil-borne pathogens is resumed by the term and phenomenon of biofumigation. Research in this field has shown that the GSL content and patterns vary in relation to plant species, plant part, growth stage and S supply. Biofumigation might advance to a promising and ecologically sound alternative for crop protection if its efficiency can be directed as disease control regularly proved to be unsatisfactory and irreproducible under field conditions. The major reasons are a restricted content of bioactive substances in the herbal plant, pathogen-related fungitoxic effects of different glucosinolates and failure of matching the spatio-temporal coincidence between pathogen and bioactive compound. The innovative solution to all three problems is the development of a functional bio-fertilizer, which consists of flexible, tailor-made mixtures of herbal plants with view to the prevailing pathogens, a maximization of the glucosinolate content through advanced cultivation, harvesting and preparation procedures and last but not least a mixed formulation of the fertilizer in terms of a short, medium and long-term release of bio-active compounds (Haneklaus *et al.*, 2006b).

Several anthropogenic and environmental factors influence crop performance and gradation of pests; S and nitrogen fertilization influenced the infestation of winter oilseed rape with insects and obviously a higher S supply favored specialist insects such as cabbage seed weevil, while N fertilization had no effect on *Ceutorhynchus obstrictus* (Haneklaus *et al.*, 2008). In comparison, N fertilization promoted the abundance of seed corn maggot and predators similarly (Haneklaus *et al.*, 2008). For pollen beetle larvae a close relationship between weight of larva and total S concentration was found; S fertilization had no significant effect on the biomass of the larvae (Haneklaus *et al.*, 2009a).

Little attention has been paid so far to the significance of the S supply for improving the natural resistance of crops against diseases (Haneklaus *et al.* 2007a and 2009b). It is important to differentiate between the fungicidal effect of elemental S and nutrient-mediated resistance mechanisms. Schnug (1997) predicted that the decline in atmospheric S depositions and S nutritional status of agricultural crops since the mid 1980s might have serious consequences for the stability of current ecosystems and in this regard referred to the advancing susceptibility of plants against fungal pathogens. Soil-applied sulfate fertilization proved to significantly reduce infection rate and severity of crops by fungal diseases and Sulfur-Induced Resistance (SIR) denotes the reinforcement of the natural resistance of plants against fungal pathogens through triggering the stimulation of metabolic processes involving S by targeted sulfate-based and soil-applied fertilizer strategy. The potential efficacy of SIR expressed as a reduction of the disease index ranged from 5 - 50% and 17 - 35% in greenhouse and field experiments, respectively (Haneklaus *et al.*, 2009). Noteworthy is in this context that nutrient-induced resistances against fungal diseases are meanwhile an acknowledged constituent of the complex phenomenon of induced resistance (IR) (Walters and Bingham2007; Datnoff *et al.* 2007).

Materials and Methods

The experimental designs and analytical methods employed, which are presented in the results section, are comprehensively described in Salac (2005), Bloem *et al.* (2007) and Haneklaus *et al.* (2007a).

Results and Discussion

Interactions between S fertilization, S-containing metabolites and SIR are comprehensively depicted, quantified and discussed for individual host/pathogen systems by Bloem *et al.* (2007) and Haneklaus *et al.* (2007a, 2009b). For the following S metabolites an involvement in resistance against fungal pathogens was found: total S, elemental S, cysteine, glutathione, H_2S, PR-proteins, phytoalexins and glucosinolates (Fig. 1).

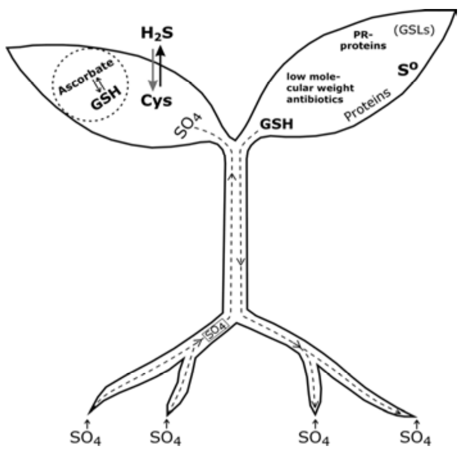

Figure 1: S-containing metabolites involved in SIR (bold notation indicates direct involvement in defense) (adapted from Haneklaus *et al.*, 2009).

S fertilization has been shown to increase significantly the content of stress-related S-containing metabolites such as cysteine, glutathione (GSH) and H_2S (Bloem *et al.*, 2007). So, S fertilization and infection with *Pyrenopeziza brassicae*, respectively increased the cysteine content to a similar extent of about 0.3 µmol g^{-1} cysteine (Fig. 2). Noteworthy is the fact that the expected increase in cysteine by a summative effect of S fertilization and fungal infections was exceeded by more than 20% (Fig. 2). These data support the assumption that an S supply, which is higher than the metabolically required amount of plant available S, has the potential to enhance mechanisms of SIR.

Targeted S fertilizer practices for disease control

Crop-specific and timely S fertilization offers the chance to strengthen the natural resistance of crops against diseases when soil-applied as sulfate, or to directly combat infections when foliar-applied as elemental S.

Infections with Fusarium spp. are a serious threat for food safety and repeated applications of elemental S with the start of flowering provided a good chance to reduce the infection rate with *F. culmorum* (Haneklaus *et al.*, 2007b). Yet, further research is required to quantify the influence of this measure to reduce the mycotoxin content in the harvest product. However, first results indicate that elemental S applications before and at flowering reduced the deoxynivalenol content efficiently (Bäck *et al.*, 2008).

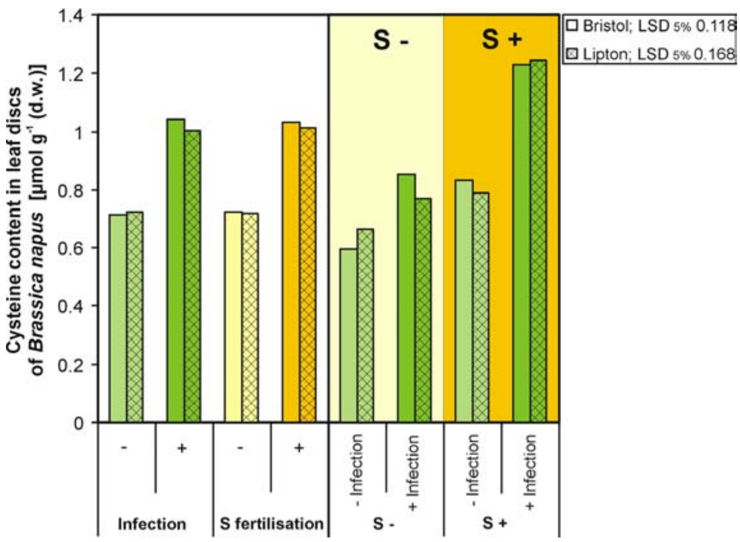

Figure 2: Influence of infection with *Pyrenopeziza brassicae* and S fertilization on the glutathione contents in leaf discs of young leaves of two varieties of winter oilseed rape.

[data represent analyses from two years and two sites with visual symptoms of infections with *P. brassicae*; sampling was carried out on the basis of visual scoring; n=384; Bloem *et al.* (2007)]

The mineral nutrient supply is supposedly the primary and pivotal barrier against infection, which also influences the course of pathogenesis. In general, the greatest benefit to the plant in terms of health can be expected when full nutrient sufficiency is provided; however, the response to a particular nutrient may be different when going from deficiency to sufficiency than from sufficiency to excess (Huber and Haneklaus, 2007). The basic difficulties to correctly determine the S nutritional status of a crop, to precisely evaluate the S demand for a projected productivity level and appropriate methods for a timely diagnosis are comprehensively discussed in Haneklaus *et al.* (2006a).

It was shown that an increasing S supply to plants was associated with a higher concentration of cysteine, glutathione, glucosinolates and H_2S and it was hypothesized that plants with a higher content of phytoanticipins might not only have a priori a better protection against pathogens, but also be able to activate resistance mechanisms more rapidly and intensely (Haneklaus *et al.*, 2007a). In return this would imply that a sufficiently high availability of sulfate in the soil after fungal attack might be crucial to satisfy an elevated S demand. And this S demand might well exceed the nutrient demand (Haneklaus *et al.*, 2007a and 2009b). These assumptions are supported by the results of Bloem *et al.* (2007), which reveal firstly that the cysteine content increased significantly with fungal infection and by S fertilization (Fig. 2). Secondly, the differentiation between both factors confirms that the strongest increase was observed when infected plants had been fertilized with S. Practically these findings connote for S fertilization that S has to be applied at crop-specific and adequately high rates, which ensure a sufficiently high availability during the vegetation period. In case of winter oilseed rape, which is grown under humid conditions, S should be applied at rates of 20-40 kg ha^{-1} S before winter and 60-100 kg ha^{-1} S starting with the beginning of the main vegetation period (Salac, 2005; Haneklaus *et al.*, 2006a). A possible over-supply with S needs to be taken into account, but can be expected to be rare as yield depressions of 10% may occur if the total S content in younger leaves of winter oilseed rape at start of the vegetation period exceeds 14 mg g^{-1} S (Haneklaus *et al.*, 2006b). In contrast, particularly on light sandy soils sulfate, which is highly mobile in soils, might be leached from the root zone after extreme precipitations so that plants will not be supplied sufficiently high with S despite an adequate S rate.

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THE IMPACT OF CONTROLLED UPTAKE LONG TERM AMMONIUM NUTRITION ON WINTER RAPE

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Abstract

The Controlled Uptake Long Term Ammonium Nutrition (CULTAN) indicates local placement of ammonium fertilizer into the soil. High NH_4^+ concentrations at fertilized spots inhibit nitrification, and N mobility is thus reduced. In CULTAN method, the total nitrogen supply is not split into smaller doses. The effectiveness of this method was examined in small plot field experiments with winter rape (Artus). The experiments were established in 2006 at 3 different research sites, and consisted of 2 conventional (plot1, plot 3) and 2 CULTAN treatments (plot 2, plot 4). In each treatment, 200 kg N ha^{-1} was applied, and sulphur was added in treatments 3 and 4. The whole amount of fertilizer was split into 3 doses in the conventional treatments. In CULTAN treatments, all fertilizer was placed to 5 cm depth using the injector applicator, during winter rape vegetation phase BBCH 26. The winter rape yields were always higher in conventional treatments. Their average yields were 4.08 t ha^{-1} (plot 1) and 4.34 t ha^{-1} (plot 3). The average yields of CULTAN treatments reached 3.49 t ha^{-1} (plot 2) and 3.81 t ha^{-1} (plot 4). The addition of sulphur caused increases of yields in both system of fertilization.

Keywords: winter rape, injection fertilization, CULTAN, small plot experiment.

Introduction

Winter rape is the dominant oilseed crop in central and northern Europe. Adjust the amount of nitrogen and the timing of N-doses is important for effectively increases of winter rape oil seeds (Rathke et al. 2006). Winter rape require high amount of nitrogen mainly in early spring. As a consequence, the risk of nitrogen losses and environment contamination is very high. The alternative nutrition CULTAN system (Controlled Uptake Long Term Ammonium Nutrition) is more environmental friendly, and provide higher efficiency of nitrogen fertilizer, because the nitrogen in ammonium form is locally placed into the soil (Sommer 2005). Ammonium fixation on the soil particles and its toxicity on the soil microorganism allow all nitrogen application in one dose (Kücke and Scherer 2006; Sommer 2003).

Materials and methods

A small-plot trial was set up in 2006 at three experimental sites in Caslav, Hnevceves and Humpolec (Czech Republic). The characteristics of the sites are given in Tables 1.

Table 1: Characteristics of experimental sites.

Site	*Altitude (m)*	*Annual average*		*Soil type*	*Soil texture*	*pH/CaCl$_2$*
		precipitation (mm)	*temperature (^{o}C)*			
Caslav	240	555	8.9	Phaeozem	loam	6.6
Hnevceves	265	597	8.1	Luvisol	clay loam	6.9
Humpolec	525	667	6.5	Cambisol	sandy loam	6.6

In the trial, the conventional treatments fertilized with split nitrogen doses were compared to CULTAN treatments, in which all the fertilizer was applied in a single rate. Each treatment had 4 replications, and the scheme of the trial is shown in Table 2.

Table 2: Fertilization scheme of the field experiment.

Plot	*Treatment*	*Amount of added N in kg ha^{-1}*			
		1st spring application	2nd spring application	*BBCH 26*	*BBCH 45*
1	conventional I	57 (CAN)	93 (CAN)	-	50 (CAN)
2	CULTAN I	-	-	200 (UAN)	-
3	conventional II	57 (AS)	93 (CAN)	-	50 (CAN)
4	CULTAN II	-	-	200 (UAS)	-

AS – Ammonium Sulphate, CAN – Calcium Ammonium Nitrate, UAN – Urea Ammonium Nitrate, UAS – Urea Ammonium Sulphate

Winter rape (*Brassica napus* L.) cultivar Artus (hybrid) was used in the trial. The size of each fertilized plot was 39 m^2 (3 * 13 m), of which 15 m^2 (1.25 * 12 m) was harvested. CULTAN fertilization was applied at the BBCH 26 growth stage with the GFI 3A injection machine (f. Maschinen und Antriebstechnik GmbH Güstrow), with 12 injection wheels working at a width of 3 m. Each wheel disposes of 12 hollow-spikes that apply fertilizer to a depth of 5 cm. The harvest was done with a small plot combine harvester; yield was determined by weighing seeds from individual plots and converting them into 8 % moisture. The samples were taken from the harvested seeds. The N-content was determined with the Kjeldahl method on the

KJELTEC AUTO 1030 Analyzer (f. Tecator). To evaluate the results, one-factor ANOVA analysis at the $p < 0.05$ level of significance was used. The computations were done using the Statistica 8.0 programme (f. StatSoft).

Results and Discussion

The yields of winter rape seeds from all sites and both experimental years are shown in Table 3. When comparing CULTAN I and conventional I treatments, the yield of winter rape seeds was always significantly lower in CULTAN I treatments on Caslav and Humpolec site in both experimental years. On Hnevceves site, CULTAN I treatment achieved higher yield of winter rape seeds in experimental year 2008, but there were not observed significant differences between CULTAN I and conventional I in both years. The average yields from all sites and both years were 4.08 t ha^{-1} in conventional I and 3.49 t ha^{-1} in CULTAN I treatment. CULTAN II treatments reached always lower yields in comparison with the conventional II treatments, and the differences were statistically significant in both experimental years on Caslav and Humpolec site. The average yield from all sites and both years were 4.34 t ha^{-1} in conventional II and 3.81 t ha^{-1} in CULTAN II treatment. The addition of sulphur in fertilizer led to a yield increase in each fertilization system, but the increase was not statistically significant. Boelcke (2003) found out the high dependance on the site of the winter rape yield in CULTAN systems. At Gülzow site (Germany), the lower yield of seeds was achieved in CULTAN treatment than in split doses fertilization. At Vipperow site (Germany), the higher yield of CULTAN treatments was shown. However, the differences were not significant anywhere. According to Felgentreu (2003), injection application increased the yield of winter rape seeds in both experimental years.

Table 3: Yield of the seeds (8 % moisture) in t ha^{-1}.

Plot	*Treatment*	*Caslav*		*Hnevceves*		*Humpolec*	
		I	*II*	*I*	*II*	*I*	*II*
1	convention I	4.47^{a}	3.08^{ac}	5.51^{ab}	4.04^{a}	4.26^{a}	3.13^{a}
2	CULTAN I	3.64^{b}	2.55^{b}	4.82^{a}	4.15^{ab}	3.36^{b}	2.44^{b}
3	convention II	4.35^{a}	3.43^{c}	6.44^{b}	4.46^{b}	4.39^{a}	3.45^{a}
4	CULTAN II	3.58^{b}	2.75^{ab}	5.44^{ab}	4.17^{ab}	3.88^{c}	2.55^{b}

I - experimental year 2007, II – experimental year 2008, ^{abc} data marked with the same letter in the column did not significantly differ at $\alpha = 0.05$

N-content of winter rape seeds from all sites is showed in Table 4. The lower N-content was always observed in CULTAN treatments in comparison with the conventional surface application, and the differences were statistically significant with few exceptions. The average

N-contents in seeds from all sites and both years were 3.14 % in conventional I, 2.78 % in CULTAN I, 3.16 % in conventional II, and 2.89 % in CULTAN II. The supply of sulphur fertiliser caused the increase of N-content in seeds only in CULTAN system. However, the effect of sulphur addition on N-content increase was not statistically significant in any case. Felgentreu (2003) determined higher protein content in injection application in comparison with splitted application in line and hybrid sorts. However, the protein content is negatively correlated with the oil content (Ali et al. 1996).

Table 4: N - content in the seeds (% in dry matter).

Plot	*Treatment*	*Caslav*		*Hnevceves*		*Humpolec*	
		I	*II*	*I*	*II*	*I*	*II*
1	convention I	3.32^{a}	3.22^{a}	2.96^{a}	3.14^{a}	3.22^{a}	2.98^{a}
2	CULTAN I	2.81^{b}	2.98^{b}	2.60^{b}	2.82^{b}	2.84^{b}	2.62^{b}
3	convention II	3.35^{a}	3.23^{a}	2.94^{a}	3.09^{a}	3.43^{c}	2.93^{a}
4	CULTAN II	2.90^{b}	3.17^{a}	2.67^{b}	2.87^{b}	3.13^{a}	2.58^{b}

I - experimental year 2007, II – experimental year 2008, ^{abc} data marked with the same letter in the column did not significantly differ at $\alpha = 0.05$

N-content in winter rape straw from all sites is shown in Table 5. The lower N-content was always observed in CULTAN treatments in comparison with the conventional application. The average N-contents in straw from all sites and both years were 0.64 % in conventional I, 0.51 % in CULTAN I, 0.66 % in conventional II and 0.56 % in CULTAN II. The addition of sulphur caused a small increase of N-content in straw in CULTAN method. In conventional system, the dependence of sulphur application and N-content in straw was not observed.

Table 5: N - content in the straw (% in dry matter).

Plot	*Treatment*	*Caslav*		*Hnevceves*		*Humpolec*	
		I	*II*	*I*	*II*	*I*	*II*
1	convention I	0.60^{a}	0.54^{a}	0.55^{a}	0.90^{a}	0.68^{ab}	0.60^{a}
2	CULTAN I	0.42^{b}	0.48^{a}	0.41^{a}	0.57^{b}	0.61^{a}	0.54^{b}
3	convention II	0.50^{ab}	0.65^{b}	0.55^{a}	0.84^{a}	0.85^{b}	0.54^{b}
4	CULTAN II	0.47^{ab}	0.49^{a}	0.48^{a}	0.65^{ab}	0.69^{ab}	0.54^{b}

I - experimental year 2007, II – experimental year 2008, ^{abc} data marked with the same letter in the column did not significantly differ at $\alpha = 0.05$

N- uptake by the winter rape seed is showed in Table 6. The lowest N-uptake by seed was always determined in CULTAN I variants and the differences were always significant in comparison with the conventional system. Opposite, the highest N-uptake by seeds were almost always in conventional II system. The average N-uptake by seeds from all sites and both years were 118 kg N ha^{-1} in conventional I, 90 kg N ha^{-1} in CULTAN I, 129 kg N ha^{-1} in conventional II, and 99 kg N ha^{-1} in CULTAN II. The supply of sulphur fertiliser caused an increase of N-uptake by seeds in both nutrition systems.

Table 6: N - uptake by winter rape seed (kg N ha^{-1}).

Plot	*Treatment*	*Caslav*		*Hnevceves*		*Humpolec*	
		I	*II*	*I*	*II*	*I*	*II*
1	convention I	137^{a}	92^{a}	151^{ab}	117^{b}	127^{c}	86^{b}
2	CULTAN I	95^{b}	70^{b}	116^{a}	108^{a}	88^{a}	59^{a}
3	convention II	135^{a}	103^{ab}	175^{b}	128^{c}	139^{d}	94^{b}
4	CULTAN II	96^{b}	81^{ab}	134^{ab}	111^{ab}	113^{b}	61^{a}

I - experimental year 2007, II – experimental year 2008, ^{abc} data marked with the same letter in the column did not significantly differ at $\alpha = 0.05$

Our results did not confirm the higher efficiency of applied nitrogen fertilizer in CULTAN method. We mostly achieved the lower yield of winter rape seeds, lower N-content in seeds and straw, and lower N-uptake by seeds in CULTAN treatments. The “late spring” CULTAN fertilization seems to be unsuitable for our conditions, because of regular drought in that time. The results show that further detailed examination with the earlier time of application in CULTAN method is necessary before it can be recommended for usage in common agricultural practice.

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THE EFFECT OF INJECTION AMMONIUM FERTILIZATION (CULTAN) ON WINTER WHEAT YIELD AND QUALITY OF GRAIN

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Abstract

The effect of the CULTAN system (Controlled Uptake Long Term Ammonium Nutrition) on winter wheat yield and quality characteristics was studied in 2-year small plot field experiments, which were set up on four experimental sites with different climatic and soil conditions in the Czech Republic. The experiment was carried with control and CULTAN treatments. The total dose of 150 kg N ha^{-1} was divided into three applications at the control treatment. For the CULTAN treatment, the whole nitrogen dose of 150 kg N ha^{-1} (in Urea Ammonium Sulphate) was applied in a single application in the wheat growing period of BBCH 29-30 in 5 cm depth into the soil using the injection applicator. The average yield of winter wheat was 9.56 t ha^{-1} for the control treatment and 8.78 t ha^{-1} for the CULTAN treatment in year 2007 and 9.91 t ha^{-1} for the control treatment and 9.63 t ha^{-1} for the CULTAN treatment in year 2008. Differences were not statistically significant in year 2008. Nitrogen and gluten content were significantly lower for the CULTAN treatment. The Falling number, Zeleny index and bulk density reached similar rate in both treatments.

Keywords: nitrogen, winter wheat, grain quality, ammonium injection.

Introduction

High mobility of mineral forms of nitrogen in soil do not allow the application of huge amount of nitrogen fertilizers in one dose, but all dose of nitrogen fertilizer must be divided in several application in conventional nutrition of winter wheat. By contrast, in CULTAN method (Controlled Uptake Long Term Ammonium Nutrition), it is possible to apply all dose of required nitrogen fertilizer for winter wheat vegetation in single application. This method is based on injection application of ammonium fertilizer into the 6 - 10 cm depth in soil, where the spots (called “depot”) with high ammonium concentration are made. Ammonium ion is fixed into clay and organic matter in depot and the toxicity of ammonium inhibits the nitrification and the nitrogen movement out of the range of the winter wheat root system (Sommer 2005). Then the winter wheat plants control the quantity of received nitrogen themselves by root penetration into diffusion zones around the depot (Kücke and Scherer 2006). On the basis of information about CULTAN method, the higher nitrogen utilization by winter wheat plants is assumed in injection application than in conventional broadcast fertilization (Sommer 2005). According to Balík (1985), the nitrogen utilization from the top dressing applied fertilizer is about 15 - 60 % in dependence on the form of fertilizer and the weather conditions. Nyord *et al.* (2008) proved 30 % reduction of nitrogen losses in volatilization at application of ammonium in 3 cm depth into the soil than in top dressing application. According to Kücke and Scherer (2006) the crops grown using the CULTAN method achieve comparable yields and quality of the main product as in conventional growing.

Materials and methods

The 2-year small plot experiment with winter wheat (*Triticum aestivum L.*) sort Sulamit (E) was set up on 2006 at the fields of 4 crop research institutes in Caslav, Hnevceves, Humpolec, Ivanovice na Hane, Czech Republic. The impact of all nitrogen fertilizer in single dose in CULTAN variants was compared to nitrogen split doses fertilization of winter wheat in control variants. CULTAN treatments were fertilized in growing phase BBCH 29 by injection applicator GFI 3A (f. Maschinen und Antriebstechnik GmbH Güstrow). Each treatment had four repetitions and the scheme of experiment fertilizing is showed in Table 1.

Table 1: System of fertilizing of the field experiment.

No.	*Treatment*	*Amount of added N per ha (in fertilizer form)*			
		BBCH 22	*BBCH 29*	*BBCH 33*	*BBCH 52*
1	control	43 kg (AS)	-	87 kg (CAN)	20 kg (CAN)
2	CULTAN	-	150 kg (UAS)	-	-

AS – Ammonium Sulphate, CAN – Calcium Ammonium Nitrate, UAS – Urea Ammonium Sulphate

The yields were determined as the grain weighing from the harvested plots and then convert into ton par hectare in 14 % moisture. The samples of grain were taken from each plot and the quality characteristics were measured after the cleaning on the laboratory sifter Swing 160 (f. Mezos). The bulk density was determined on the laboratory equipment Meopta model 1938. Sedimentation index as the Zeleny test and the gluten content were determined on NIR OmegAnalyzer G (f. Bruins Instruments). Falling number were tested from the crushed grain on the 0.8 mm size of particles and the measurement were carried out on Falling number 1400 machine (f. Perten). The protein content was determined according to Kjeldahl method on KJELTEC AUTO 1030 Analyzer (f. Tecator) and the results were multiplied with coefficient 5.7 for the milling wheat. To evaluate the results, one-factor ANOVA analysis was used followed with the Scheffe's test at the $p < 0.05$ level of significance.

Results and Discussion

In the experimental year 2007, the yield of winter wheat grain was significantly lower in CULTAN variants on all sites (Table 2), what was caused by more than month lasting drought in the spring all over the Czech Republic. The precipitation were about 2 mm instead of 40 mm (long term average) in April 2007. The negative effect of extreme drought on CULTAN variants was observed by Walter (2001) too, who find out lower yields of winter wheat in CULTAN treatments in comparison to split doses fertilizing treatments in the year 2000.

Table 2: Yield of the grain (14 % moisture) in t ha^{-1}.

No.	*Treatment*	*Caslav*		*Hnevceves*		*Humpolec*		*Ivanovice n. H.*	
		I	*II*	*I*	*II*	*I*	*II*	*I*	*II*
1	control	7.46*	9.51*	11.69*	12.87*	9.41*	7.61*	9.70*	9.64*
2	CULTAN	7.09**	9.11*	10.09**	12.47*	9.15**	7.53*	8.80**	9.41*

I - experimental year 2007, II – experimental year 2008, * data marked with the same symbol in the column did not significantly differ at $\alpha = 0.05$

In experimental year 2008, the both control and CULTAN treatments achieved similar yields of the grain, the differences were not statistically significant on any sites. The conditions of year 2008 were very suitable for the winter wheat growing and the yields of both treatments were much higher in Caslav and Hnevceves this season. The yield trend had opposite direction on Humpolec site. Research site Humpolec is situated in the area with worse soil and climatic condition, which can not provide the yield stability, therefore this area is less suitable for winter wheat growing. The average yield from all sites in 2007 was 9.56 t ha^{-1} in control treatment and 8.78 t ha^{-1} in CULTAN treatment. In 2008, the average yield was 9.91 t ha^{-1} in control and 9.63 t ha^{-1} in CULTAN treatment. During the years 1987 – 1991, Sommer (1992) reached slightly lower yields of winter wheat by using UAS (Urea Ammonium Sulphate) in CULTAN nutrition in comparison to CAN (Calcium Ammonium Nitrate) in conventional system. However the differences were significant only in 1991. On the other hand, Weber *et al.* (2008) made out 0.8 t ha^{-1} higher yield of winter wheat (sort Enorm) in CULTAN variant fertilized with 180 kg ha^{-1} of nitrogen in UAS in comparison to the conventional split dose fertilization of CAN with sulphur addition in regeneration fertilization. However, the higher yield was statistically significant only in one year of their 2-year experiment. Kücke (2003) achieved even about 26 % higher yield of winter wheat (sort Ohrum) by application 150 kg N . ha^{-1} in UAS in CULTAN treatment in comparison to the split application of urea.

Protein content, gluten content, sedimentation index, falling number and bulk density belong among the main quality characteristics of winter wheat. All these characteristics of our experiment are showed in Table 3. The nitrogen nutrition influence most the protein and gluten content. The protein content was always higher in control variants in both years on all sites. The average protein content of all sites is 12.4 % in control and 11.5 % in CULTAN treatments. According to Czech norm CSN 46 1100-2, both treatments get over the limit 11.5 % of protein content for the milling usage of winter wheat. Weber *et al.* (2008) reached similar results with using UAS fertilizer, the CULTAN treatment achieved significantly lower protein content in comparison to the split dose fertilization treatment and the difference was almost 2.5 %. On the contrary to our results, Sommer and Fischer (1993) as well as Kücke (2003) reached the same protein content of CULTAN and conventional broadcast treatment. In comparison the experimental years 2007 and 2008, the protein content was higher in 2008 at Caslav site. The increasing of protein content was explained by the dilution in the higher yield of grain at the same time. It matches to results of Kučerová (2005), who found out very strong negative dependence between yield and protein content in the grain. At the Hnevceves site, the trend of grain yield between the years had the same direction as at the Caslav site, but the trend in nitrogen content had opposite direction. This is explained by the positive effect of the peas as the foregoing crop for the experimental year 2008. In other cases the rape was foregoing crop.

Gluten content is tightly connected with protein content, therefore gluten content was also lower in CULTAN treatments in both experimental years on all sites. In the contrary, Weimar (1996) determined higher gluten content and higher rate of sedimentation index in CULTAN variants. Sedimentation index by Zeleny test show the quantity and quality of gluten proteins. In our experiment, Zeleny test was lower in all CULTAN variants and the differences were mostly statistically significant (Table 3). The average rate of Zeleny test from all sites and both years was 50 ml in control and 44 ml in CULTAN treatment. All values of Zeleny test except the Hnevceves in 2007 were adequate for the marking as the milling wheat. The minimum rate of Zeleny test from the Czech norm ČSN 46 1100-2 is 30 ml. Weber *et al.* (2008) reached also slightly lower sedimentation index in CULTAN treatment. However, Weimar (1996) determined the same rate of Zeleny test of winter wheat (sort Ibis) in conventional and CULTAN treatment with application of 172 kg N ha^{-1} in 1994. In 1995, Zeleny test (sort Batis) was even higher in CULTAN treatment with application 162 kg N ha^{-1}.

Falling number shows the activity of alfa amylase enzyme and is most influenced by genetic factors and climatic conditions, especially by the weather in the time of ripening. According to UKZUZ experiment, the average rate of winter wheat (sort Sulamit) Falling number is 349 s (Horáková *et al.* 2006). In our experiment, the Falling number ranged from 283 to 389 s and the average rate from all sites and experimental years was 345 s in control and 340 s in CULTAN treatment. According to the Czech norm ČSN 46 1100-2, the minimum rate for the milling wheat 220 s was reached in all cases.

Bulk density is indicator of flour yield in flour-milling processing. In our experiment, no big differences between control and CULTAN treatment were determined. The average rate of bulk density from all sites and experimental years was 79.5 kg hl^{-1} in control and 79.4 kg hl^{-1} in CULTAN treatment. According to the Czech norm ČSN 46 1100-2, the minimum rate of bulk density for the milling wheat (76.0 kg hl^{-1}) was fulfilled in all cases.

Table 3: Quality characteristics of the grain.

Characteristics	*Treatment*	*Caslav*		*Hnevceves*		*Humpolec*		*Ivanovice n. H.*	
		I	*II*	*I*	*II*	*I*	*II*	*I*	*II*
Protein content (%)	control	12.8*	11.8*	11.1*	13.8*	11.8*	12.0*	13.2*	13.1*
	CULTAN	12.0*	11.4*	9.7**	12.2**	10.8**	10.7**	12.6**	12.3*
Gluten content (%)	control	28.8*	28.1*	24.2*	35.0*	24.6*	27.9*	31.9*	31.7*
	CULTAN	26.1*	26.2*	20.3*	29.6**	21.0**	22.3**	28.0**	28.5**
Zeleny test (ml)	control	54*	51*	34*	53*	51*	54*	50*	51*
	CULTAN	50**	49*	24**	45**	44**	46**	46*	46**
Falling number (s)	control	389*	283*	369*	304*	392*	320*	322*	382*
	CULTAN	377*	305*	346*	310*	385*	323*	318*	361*
Bulk density (kg . hl^{-1})	control	78.6*	79.8*	80.9*	82.8*	78.1*	77.6*	79.0*	79.2*
	CULTAN	78.0*	79.7*	79.9*	82.8*	78.2*	77.9*	78.8*	79.5*

I - experimental year 2007, II – experimental year 2008, * data marked with the same symbol in the column did not significantly differ at $\alpha = 0.05$

Despite of the lower values of most characteristics in CULTAN treatment, the differences were usually not statistically significant. Therefore we could say that CULTAN method is suitable alternative winter wheat nutrition system. From the results, it is evident, that the further detailed study is necessary before the implementation of CULTAN system in wide agriculture practice.

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NEW FERTILIZERS AND SOIL NITROGEN CONTENT IN NO TILLAGE

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Abstract

As nitrogen contamination related to the incorrect use of fertilizers is one of the main environmental problems in Europe, the European Commission adopted the Directive 91/676 related to pollution caused by nitrates from agricultural sources. Guadalquivir river valley hosts the main cereal region in Andalusia. It is considered vulnerable to nitrate pollution, situation that restricted the nitrogen fertilization at farm level.

In the study, the nitrogen evolution in the soil was studied by soil sampling at different depths, up to 70 cm, during 2006-07 and 2007-08 seasons, in two different fields located in the cerealandalusian areas. The experimental design is a randomized complete block, with 4 repetitions and 8 treatments, with different fertilizers, not only conventional (urea, ammonium sulphate, etc.), but also new generation formulates (fertilizer with nitrification inhibitor, micronutrient or liquid fertilizers). The surface of the elemental plot is 50x7.5 m^2, cropped with wheat under no tillage. Results show that the diverse fertilizers do not produce important differences in the nitrogen evolution in the soil. In contrast, the structural improvements caused by conservation agriculture, produce the nitrogen surface accumulation, and enhance the assimilation by the crops.

Keywords: conservation agriculture, no till, fertilizers, wheat, nitrogen leaching, environment.

Introduction

The harmony between agrarian production and the environment is crucial to avoid its degradation. For this reason, there is an increasing awareness in many European, National and Regional politics about the importance of the environmental preservation.

Anyway, many farmers still practice inappropriate soil managements as intensive tillage, that produces many adverse effects to the natural resources. Tillage aerates the soil, and increases the proportion between macro and micropores, which decreases the retaining capacity of water and nutrients in the topsoil (Márquez *et al.*, 2009 a), and increases moisture loss through evaporation and nitrogen leaching (Márquez, 2008).

Due to the problem of nitrates leaching, the European Union adopted the Directive, 91/676, related to the protection of waters against pollution caused by nitrates from agricultural sources. This document establishes the criteria to designate as vulnerable zone those areas of land whose drainage could pollute surface and groundwater with nitrate, and the obligation of the governments to identify these zones affected by pollution. Thus, for example, most of the Guadalquivir Valley, one of the largest cereal producing regions of Spain, is included in these vulnerable zones, and the use of nitrogen fertilizers has undergone many restrictions.

No till can help to limit these polluting processes, as it reduces water run-off and percolation, and improves soil structure and pores distribution (Triplet and Warren, 2008). Furthermore, the appearance of new fertilizers as slow release and micronutrient starter effect allowed a more rational and appropriate use of nitrogenous compounds. Nitrogen and phosphorus fertilizers are the main pollutants related to the agricultural sector (Rodriguez *et al.*, 2008).

The objective of this work was to study the effect of different combinations of fertilizers on the temporal evolution of the nitric nitrogen content in wheat production under conservation agriculture (no till).

Material and methods

The study period covers two seasons, 2006-07 and 2007-08, in two fields located in different cereal areas of Andalusia (Spain), with a typical Mediterranean climate; more specifically in the village Las Cabezas de San Juan (Sevilla) and Jerez de la Frontera (Cadiz). Their physico-chemical characteristics are described in Márquez *et al.* (2009 b). It is important to remark that in Las Cabezas field the soil is vertic, deep and fertile, while a calcareous ground is present in Jerez, in a relatively mountainous area. No till has been practiced in both fields 2001; therefore, they present high levels of organic matter**,** as we can appreciate in table 1.

In the study were used conventional fertilizers (urea, ammonium sulphate, etc.), and new generation formulates (slow release, micronutrient impact starter, and liquid), whose characteristics and compositions are described in another communication published in this Congress (Márquez *et al.*, 2009 c).

Table 1: Main physicochemical characteristics of the soil of experimental fields.

		pH H_2O	pH ClCa	M.O. (%)	CO_3^{-2}(%)	N (ppm)	P (ppm)
La Pluma	0-3 cm	8,07	7,67	1,66	3,06	15,65	24,08
	3-13 cm	8,09	7,64	1,57	3,56	18,51	18,90
	13-26 cm	8,11	7,64	1,43	3,09	23,36	16,66
	26-52 cm	8,18	7,68	1,89	2,58	18,25	10,98
	52-70 cm	8,19	7,64	1,57	4,84	15,01	13,48
El Castaño	0-3 cm	8,21	7,56	3,33	29,49	39,03	15,52
	3-13 cm	8,30	7,88	3,30	29,64	30,26	8,72
	13-26 cm	8,38	7,78	2,60	29,67	27,07	6,49
	26-52 cm	8,40	7,88	1,92	35,04	18,41	3,83
	52-70 cm	8,44	7,81	1,78	36,72	15,92	2,87

The first season, the field of Las Cabezas was sown with *Triticum durum* L. and the second one with *Triticum aestivum* L. The area of each plot was of 50x7.5 m^2, and sowing density 360 seeds/m^2. The experimental design was a randomized complete block, with 8 treatments and 4 repetitions. Different fertilizer combinations, application rate and nitrogen fertilizer units (NFU) are shown in table 2. The first application was done in January in both years, and the second one in March.

Table 2: Type of fertilizer and application rate per hectare in the different treatments.

Treatment	Presowing	Sowing	Incorporated	1st aplication	2nd aplication	NFU
1	0	0	0	Urea 227 kg	Urea 109 kg	150
2	0	0	Diammonium phosphate 125 kg	Urea 168 kg	Urea 109 kg	150
3	0	0	Diammonium phosphate 125 kg	Ammonium nitrate 287 kg	Ammonium nitrate 185 kg	150
4	0	0	Diammonium phosphate 125 kg	Ammonium sulphate 369 kg	Ammonium sulphate 238 kg	150
5	Urea 109 kg	0	Diammonium phosphate 125 kg	Urea 168 kg	0	150
6	0	0	Starter 43 kg	Urea 200 kg	Nitrogen solution 100 kg	130
7	0	0	Starter 43 kg	Ammonium sulphate 425 kg	Nitrogen solution 100 kg	130
8	0	Slow release 310 kg	0	Ammonium sulphate 320 kg	0	130

Soil nitrogen content was studied by taking monthly samples in each treatment, and measuring their nitrate concentration. In each sampling campaign, two points in each treatment were taken, always in the same area at 20 and 40 m from the beginning of the plot, mixing it and obtaining a composite sample. We used the 6 cm of diameter Edelman drill. The depths studies were: 0-3 cm, 3-13 cm, 13-26 cm, 26-52 cm and 52-70 cm.

The analysis of nitrate was performed by reducing this anion in a copperized cadmium column, by the modified Griess-Illosvay method. The results were evaluated using the analysis of variance (ANOVA), and means were compared with the Tukey test at the $p<0,05$ significance level.

The temporal stability, a concept defined by Vachaud *et al.* (1985), allowed us to study the variation of the soil nitrate content of each treatment respect to the others combinations of fertilizers. This indicator was defined as the persistence of some parameter of the temporal distribution of a soil property. The variable δ_{ij} is the normalized value of the deviation of the nitrate measured values, S_{ij}, respect to the mean, $\overline{S_j} = (1/N)*\sum S_{ij}$,

$$\delta_{ij} = S_{ij} - \overline{S_j} / \overline{S_j}$$

Its mean is $\overline{\delta_i}$ and its typical deviation $\sigma(\delta_i)$,

$$\overline{\delta_i} = (1/m)\sum \delta_{ij} \qquad \sigma(\delta_i) = [1/m-1\sum(S_{ij} - S_j)^2]^{1/2}$$

Results and discussion

Table 3 shows the average of the nitrate content per centimeter of soil, in each sampling depth and treatment. The amount of this anion the first year was lower than the second one in both experimental fields, maybe because of the warmer climate of the second campaign, 2007-08. That situation could have increased the mineralization rate of the organic matter. The concentration of nitrate decreases with the sampling depth, because no till improves the structure and the porosity of the soil, increasing the retention capacity of water and soluble substances in the topsoil, and enhancing their uptake by plants. No till also reduces the leaching of polluting substances as nitrogen.

Table 3: Average content of nitric nitrogen per centimetre of soil of each sampling depth. Each data represents the mean of the 7 field sampling. Units (kg ha^{-1}).

			Treatment							
			1	2	3	4	5	6	7	8
Jerez	Campaign 2006-07	0-3 cm	2,10	1,92	1,56	2,33	2,33	1,40	2,78	1,54
		3-13 cm	1,32	1,26	1,55	1,22	1,22	1,03	1,17	1,17
		13-26 cm	0,91	0,91	1,10	1,03	0,87	1,01	0,95	0,85
		26-52 cm	0,65	0,70	0,61	0,65	0,54	0,54	0,58	0,53
		52-70 cm	0,40	0,41	0,47	0,57	0,47	0,40	0,35	0,40
		0-70 cm	**0,79**	**0,80**	**0,84**	**0,85**	**0,76**	**0,70**	**0,77**	**0,69**
	Campaign 2007-08	0-3 cm	6,12	4,61	5,21	6,56	3,53	2,94	3,75	2,74
		3-13 cm	3,44	2,45	3,41	4,04	2,51	1,83	2,12	2,25
		13-26 cm	1,72	2,11	2,31	2,21	1,71	1,79	1,71	1,63
		26-52 cm	1,17	1,59	1,69	1,84	1,46	1,11	0,96	1,70
		52-70 cm	1,03	1,33	1,64	1,35	1,25	1,13	0,97	1,04
		0-70 cm	**1,77**	**1,87**	**2,19**	**2,30**	**1,69**	**1,42**	**1,39**	**1,64**
Las Cabezas	Campaign 2006-07	0-3 cm	0,82	1,14	1,57	1,75	3,34	0,98	0,82	2,10
		3-13 cm	0,59	0,67	1,29	0,75	0,77	0,53	0,54	0,82
		13-26 cm	0,52	0,49	0,53	0,51	0,49	0,41	0,46	0,47
		26-52 cm	0,34	0,37	0,40	0,37	0,32	0,31	0,29	0,32
		52-70 cm	0,21	0,25	0,26	0,22	0,22	0,22	0,20	0,23
		0-70 cm	**0,39**	**0,44**	**0,56**	**0,47**	**0,52**	**0,37**	**0,36**	**0,48**
	Campaign 2007-08	0-3 cm	2,44	3,55	2,62	3,38	1,94	3,18	3,31	2,02
		3-13 cm	1,99	2,02	1,89	1,48	1,37	1,70	2,24	1,51
		13-26 cm	1,46	1,40	1,62	1,25	1,23	1,24	1,69	1,21
		26-52 cm	0,89	0,79	0,76	0,82	0,79	0,85	0,96	0,82
		52-70 cm	0,77	0,62	0,58	0,85	0,77	0,68	0,74	0,67
		0-70 cm	**1,19**	**1,15**	**1,11**	**1,11**	**1,00**	**1,10**	**1,32**	**1,01**

Figure 1 shows that in the field of Jerez the nitrogen content evolution in soils treated with conventional fertilizers (mean of the 5 first treatments) is higher than starter combinations (mean of treatments 6 and 7) and slow release (treatment 8). In the experimental field of Las Cabezas this situation is less pronounced.

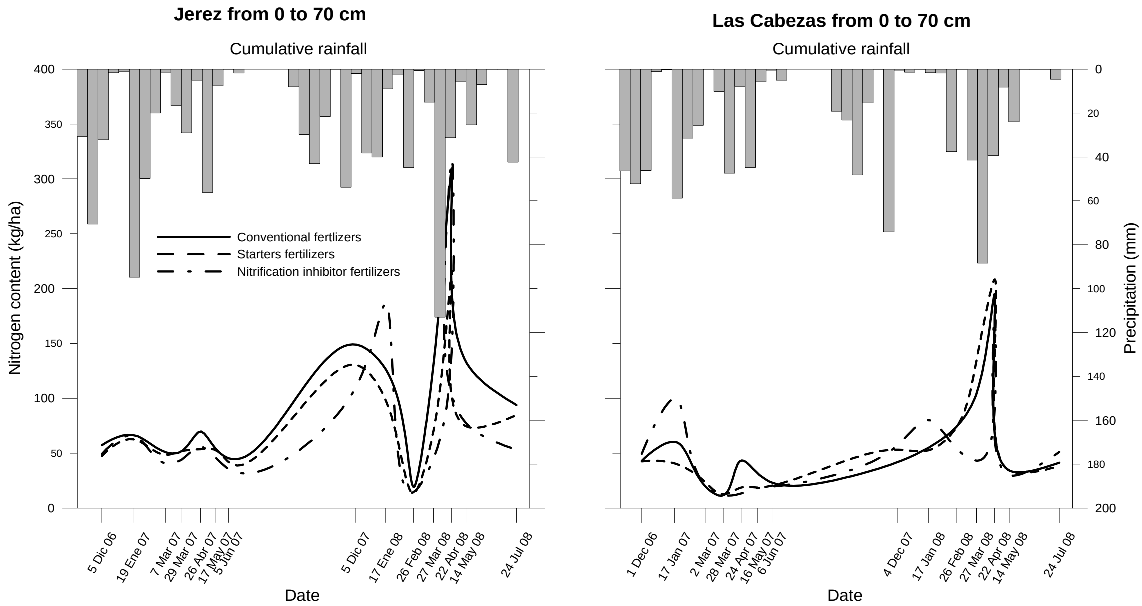

Figure 1: Temporal evolution of the nitric nitrogen content.

Table 4 shows the results of the study of the temporal stability. The less the nitrogen content of the treatment, the more to the left appears the position of the treatment in the table. In most cases, treatments with nitrogen in form of nitrate (trat. 3) or ammonium (trat. 4), contribute the soil with higher nitrate concentration, as they occupy the position most to the right in most of the depths. Treatments 1, 2 and 5 with ureic nitrogen occupy intermediate positions, except in treatment 5, with urea applied 20 days before the sowing. This situation produces an increased concentration of this nutrient in deeper soil layers (52-70 cm), and reduces the effectiveness of the fertilization, due to possibly increased leaching processes. Furthermore, starter combined with liquid fertilizers (trat. 6 and 7), and the slow release (trat. 8) show the lowest content of nitrogen in all depths sampled, and in the total, 0-70 cm. In the second year, in the experimental field Las Cabezas this situation was reversed, especially in treatment 7.

Figure 2 shows that relative differences of nitrate contents in comparison to the mean show important variations between experimental fields and years, as reported for other soil properties such as moisture (Martínez-Fernández and Ceballos, 2003). The greater the deviations from the average, the more the standard deviation increases, as happens in the campaign 2006-07 in Las Cabezas, 2007-08 in Jerez, and the set of two years (2006-08) in Las Cabezas.

Table 4: Position occupied by each treatment at each sampled depth, experimental field and campaign.

			Nitrogen amount →							
			Position							
			1	2	3	4	5	6	7	8
Jerez	Campaign 2006-07	0-3 cm	6	8	3	7	5	4	2	1
		3-13 cm	6	7	8	4	2	5	1	3
		13-26 cm	8	7	5	1	2	4	3	7
		26-52 cm	8	6	5	1	7	3	2	4
		52-70 cm	6	7	8	2	1	3	5	4
		0-70 cm	**8**	**6**	**5**	**7**	**2**	**1**	**3**	**4**
	Campaign 2007-08	0-3 cm	6	7	5	8	2	3	1	4
		3-13 cm	6	7	2	8	5	1	4	3
		13-26 cm	8	1	7	6	5	2	4	3
		26-52 cm	7	6	1	8	2	3	4	5
		52-70 cm	7	8	1	2	6	4	5	3
		0-70 cm	**7**	**6**	**8**	**1**	**2**	**5**	**3**	**4**
Las Cabezas	Campaign 2006-07	0-3 cm	6	7	1	2	8	5	3	4
		3-13 cm	6	7	1	8	5	2	4	3
		13-26 cm	6	8	7	5	2	4	1	3
		26-52 cm	5	7	6	8	1	4	2	3
		52-70 cm	7	1	8	4	6	2	3	5
		0-70 cm	**6**	**7**	**1**	**8**	**5**	**2**	**4**	**3**
	Campaign 2007-08	0-3 cm	1	5	6	3	8	4	2	7
		3-13 cm	4	5	6	2	8	1	3	7
		13-26 cm	5	4	8	6	2	1	7	3
		26-52 cm	2	5	6	3	1	8	4	7
		52-70 cm	2	3	7	8	6	1	5	4
		0-70 cm	**5**	**2**	**4**	**8**	**6**	**1**	**3**	**7**

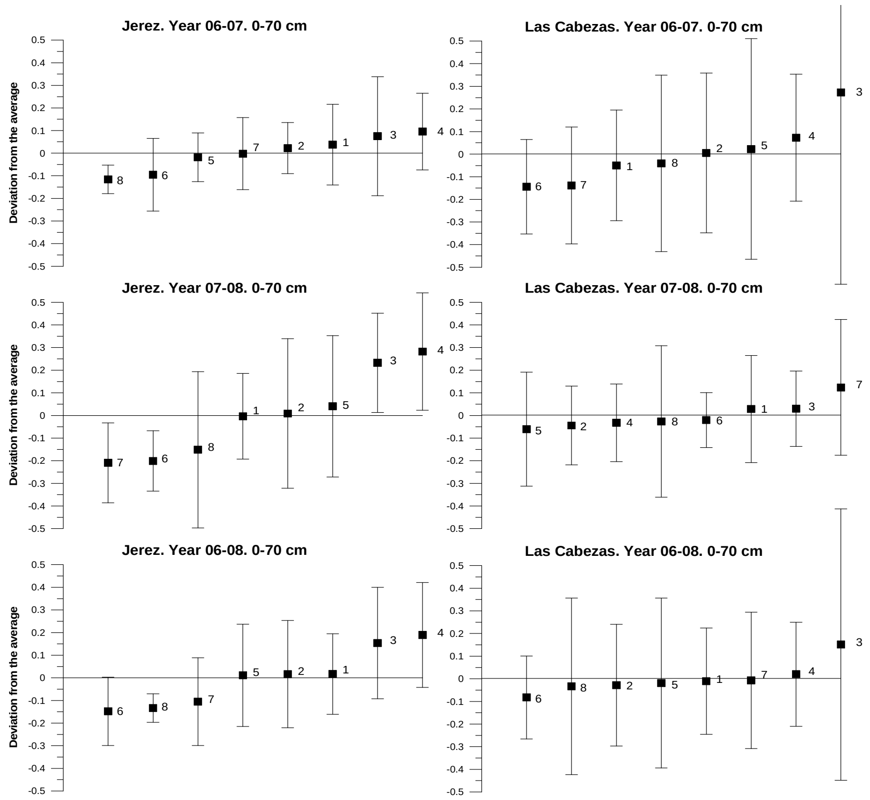

Figure 2: Normalized value of the deviation of the measured nitrate values, S_{ij}, respect to the mean, (δ_{ij}), and its typical deviation ($\sigma(\delta_i)$), for all the sampling depths (0-70 cm).

In the set of two years in both experimental fields, the treatments with new generation fertilizers, using 20 NFU less than the conventional one, have nitrogen amounts below the mean, occupying then positions to the left. Moreover, despite using less nitrogen, there are not significant differences in grain production respect to the conventional fertilizers (Table 5). Referring to the grain quality, starter combination obtains good results; the treatment with slow release has the worst results in relation to protein amount in this conditions and soils.

Table 5: Summary of production (Prod., kg ha^{-1}) and grain quality (Prot., %). Different letters show significant differences (p<0,05).

			Treatment							
			1	2	3	4	5	6	7	8
Jerez	Campaign 2006-07	Prod.	4.197 A	4.304 A	4.560 A	4.057 A	4.356 A	4.651 A	4.364 A	4.334 A
		Prot.	11,9 AB	12,5 A	12,2 AB	11,5 AB	11,1 B	11,8 AB	11,2 B	10,9 B
	Campaign 2007-08	Prod.	3.525 A	3.450 A	3.692 A	3.130 A	3.405 A	3.597 A	3.837 A	3.632 A
		Prot.	15,1 A	14,9 A	15,0 A	14,6 A	14,8 A	14,6 A	13,7 A	13,6 A
Las Cabezas	Campaign 2006-07	Prod.	4.523 A	4.745 A	4.791 A	4.909 A	4.948 A	4.535 A	5.335 A	4.888 A
		Prot.	15,1 A	14,6 AB	14,8 A	15,3 A	14,3 AB	14,5 AB	14,3 AB	13 B
	Campaign 2007-08	Prod.	4.031 A	4.316 A	4.464 A	4.117 A	3.995 A	4.103 A	3.911 A	4.361 A
		Prot.	14,7 A	13,5 ABC	13,9 AB	13,4 ABC	13,2 ABC	12,6 BC	12,7 BC	11,8 C

In conclusion, this study shows that the utilization of new formulations of fertilizers not only maintains the yields with less fertilizer, but it also produces an important reduction in the soil nitrogen content, possibly producing significant environmental benefits in terms of nitrate leaching.

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FROM ANAEROBIC DIGESTION PLANTS A RENEWABLE POTENTIAL ORGANIC MATRIX FOR FERTILIZERS

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Abstract

Anaerobic digestion is a renewable energy production process been diffusing in Italy over the last years. One of the major problem of the operating or under construction 1 MWh electricity plants is related to disposing some 15,000 m^3 of the 10% DM fluid digested phase (DP), containing all the mineral components (P, K, Ca, Mg, etc.) and most Nitrogen of the feeding materials. Problems concerning DP are related to: 1) its dilution and actual composition; 2) continuous production throughout the year, but mostly seasonal soil application, with nitrates regulations imposing a larger area for application (higher logistic costs); 3) necessity to set clear rules for its use, in a consistent legislative framework. DP could be easily formulated with nutrients to produce low and medium grade suspension fertilizers, or filter pressed to 35-40%DM and then almost dried by the recovered heat of the same biogas plant, to produce organic-mineral fertilizers. DP could be a traceable and renewable material for formulation of organic-mineral fertilizers, according to annex 5.

Keywords: anaerobic digestion, digested phase, organic-mineral fertilizers, organic matrix.

Introduction

Anaerobic digestion (AD) is a renewable energy production process which has been diffusing in Italy over the last few years, at the agricultural level, as a consequence of depressed cereal prices and incentives for production of electricity from biomass resources (very recently increased to 0.28 €/kWh), which minimize investment risks for farmers. Several on-farm 1 MWh electricity plants are planned, under construction or already operating, using ag feedstocks (maize, sorghum, triticale, alfalfa, and Lolium silages), on-farm ag-process byproducts, and livestock wastes. These materials are high-rate anaerobically digested, mostly under mesophilic conditions, to produce biogas, which is later purified and burned to cogenerate electricity and heat. After some 30-35 dd. the residual slurry (some 15,000 m^3 of 7-10%-DM DP) consists of the microbial population and undigested feed. It has potential as an animal feedstuff additive and as a fertilizer/soil amendment and may be subject to some degree of solid/liquid separation prior to use. After storage in lagoon or vertical tank, the raw DP or its supernatant liquid (0.1-0.2% N) are generally applied onto soils nearby the AD plant. So far, DP has been considered more a problem than a resource for energy producing farms. Problems concerning DP are related to: 1) its dilution and actual composition, which varies quite a lot according to feedstocks and storage conditions; 2) continuous production throughout the year, but generally seasonal soil application, although some examples of its use for compost production help reduce seasonality problems; however, nitrates regulations may impose a larger area for its application, determining higher logistic costs; 3) necessity to set clear rules for its use, in a consistent legislative framework, as regional and local norms may vary quite a lot, slowing down plant authorization process.

Depending upon the processed feedstocks, DP may pose some potential problems for its safe agricultural use. According to Effenberger (2006), feedstocks can be: a) harmless (cereal, grass, and beet silages; manure, sugar beet leaves, cut grass, fourth-range vegetable residues, brewer grains, starch, etc.); b) pathogen risky (biowaste, spoiled foodstuffs, rumen contents, contents of grease-skimming tanks, stomach/gut contents, blood meal, canteen kitchen and household wastes); c) processing contraries (biowaste, spoiled foodstuffs, canteen kitchen wastes); and d) pollutants, mainly for heavy metals and solvent residues (sewage sludge, biowaste, roadside grass, wastes from vegetable oil production). Excluding wastes (out of the incentives), processing problems at the farm level may arise mostly in case of mycotoxin affected grains and silages, chicken manure, and some selected ag residues, high in polyphenols and terpenes (e.g. citrus pulps) – Miele *et al.*, 2007; Ceccaroni, pers. comm.

Materials and Methods

Tests on selected DPs were directly run or collected by several biogas plants, plant manufactures, and literature sources. Most AD plants use, as feedstocks, cereal and grass silages, manures, and -in some cases- so-called “supplements”: hydrolyzed protein slurry (e.g., Idrobios® of Mantovagricoltura and Energom), crude glycerol from biodiesel FAME process (Miele *et al.*, 2008). Schittenhelm (2008) has recently indicated that differences concerning composition and biomethane yields among maize hybrids are minimal, so varietal differences were not considered. The major physical-chemical and biological aspects of these DPs were investigated, especially those more directly related to their potential as a renewable material for formulation of organic-mineral fertilizers. Some selected results are presented.

Results and Discussion

1) DP composition

The biogas production process digests some 60% of the silage and manure/slurry OM, i.e. starches, fats, and proteins, and 70% of the hydrolyzed protein supplements. The recalcitrant OM (mostly cellulose and lignin) and minerals remain in the DP slurry. Some examples of DP compositions are indicated in Table 1-2. FOS/TAC is the ratio between Volatile Organic Acids (*Flüchtige Organische Säuren*), expressed as acetic acid, and Alkaline Buffer Capacity (*Totales Anorganisches Carbonat*), expressed as $CaCO_3$. It is an index of how the AD plant runs, with an optimal range of 0.3-0.6 for the silage feedstock fed ones (Weiland *et al.*, 2006). Attention should be paid at the presence of livestock slurry high in sodium chloride and ammonia: at 20 g/l of sodium ion, the methane gas production is reduced to about 50% of the theoretical gas volume (Kim *et al.*, 2000), and a general inhibition of the digestion process happens when free ammonia concentration exceeds 0.8 g-N/l (Angelidaki *et al.*, 1999). Table 3 reports one typical microbiological analysis of a DP from the biodigestor 1 and 2 of a mesophylic AD plant, and Table 4 a typical heavy metal composition of a crop silage based DP. Actual composition of a DP of a given plant may vary only slightly through the year because only wise mixtures of feedstocks and minimal variations of the "diet" keep the process in full flow.

Table 1: Tests of DPs from different feedstocks (courtesy Guido Rota S.r.l., and Energom S.r.l.).

ANALYSIS	UNIT	RESULTS					
		1	2	3	4	5	6
Total Solids	% w/w as-	10.0	6.8	3.9	10.6	4.5	7.2
Organic Total Solids	% w/w as-	6.76	1.8	1.2	2.00	1.4	1.7
Total N	% w/w as-	1.27	2.47	2.31	1.66	2.13	2.27
NH_4^+-N	mg/kg as-	6,105.4	2,228.0	2,173.0	892.0	16,782.0	2,770.0
pH (water)		8.20	7.98	8.00	7.02	7.51	7.86
EC	mS/cm	n.d.	16.81	17.55	13.37	12.15	15.78
FOS/TAC		n.d.	0.17	3.45	0.94	0.22	0.17
Feedstocks							
1: corn, sorghum, and triticale silages and hydrolyzed protein supplement							
2: cattle slurry and maize silage							
3: pig slurry, maize silage and crude glycerol supplement							
4: cattle slurry and sorghum silage							
5: pig slurry and maize silage							
6: cattle slurry and grass cuts							

Table 2: Typical P_2O_5 and K_2O tests of a 10% DM, crop silage-based DP (courtesy Energom S.r.l.).

ANALYSIS	UNIT	RESULTS
Total P_2O_5	% w/w as-	0.230
Total K_2O	% w/w as-	0.620

Table 3: Typical microbiological tests of DPs (courtesy Energom S.r.l.).

Characters	*Units*	*Results*		*Analytical Methods*
		Digestor 1	**Digestor 2**	
Temperature at sampling	°C	41.6	41.2	Instrumental
Colony count at 30°C	UFC/g	$2.2.10^{12}$	8.10^{12}	ISO 4833:2004
Thermophilic colony count	UFC/g	$2.8.10^{10}$	3.10^{10}	ISO 4833:2004
Escherichia coli	UFC/g	<10	<10	ISO 16649-2:2001
Salmonella spp	25g	none	none	ISO 6579:2004
Enterobacteriaceae (*)	UFC/g	<10	<10	ISO 21528-2:2004
Sulphite-reducing Clostridia	UFC/g	$2.1.10^4$	2.10^3	UNI EN ISO 7937:2005
Sulphite-non reducing Clostridia	UFC/g	3.10^6	2.10^4	UNI EN ISO 7937:2005
Moulds	UFC/g	<10	<10	ISO 7954:1987
Yeasts	UFC/g	$1.5.10^2$	$1.3.10^2$	ISO 7954:1987

(*) Mostly *Proteus sp.* and *Klebsiella sp.*

Table 4: Typical heavy metal test of No. 1 DP of Table 1 (courtesy Energom S.r.l.).

ANALYSIS	UNIT	RESULTS	D.lgs. 217/2006
Cd	mg/kg DM	0,062	1,5
Cr VI	mg/kg DM	0,029	0,5
Pb	mg/kg DM	1,634	140
Ni	mg/kg DM	0,022	100
Hg	mg/kg DM	< 0,01	1,5
Cu	mg/kg DM	0,56	150
Zn	mg/kg DM	53,79	500

2) DP production and soil application

Reusing DP on soils under production makes the AD process more environmentally friendly, as fertilizer inputs onto feedstock crops can be conveniently reduced, and sequestered CO_2 in DP's OM, high in lignin, is slowly mineralized, with a progressive release of nitrogen and most mineral nutrients, once removed by the harvested crops. DP is generally applied to soils before crop planting. In farms where only Summer silage crops are grown this requires large storage (vertical tanks or lagoons) and heavy duty application equipment, as there is usually little time for spreading/injecting during Spring time. Where a triticale/sorghum(maize) rotation is carried out, storage can be reduced accordingly, as two application seasons are possible. A 1 MWh silage-fed AD plant produces typically some 18-19,000 kg/y of nitrogen as DP, therefore permitting to treat some 85-90 ha (210 kg N/ha rate), and save some 40 t/y of urea. However, the Nitrates Directive 91/676/EEC, concerning the protection of waters against pollution caused by nitrates from agricultural sources, may pose severe production constraints to farmers running plants in sensible areas (permitted rate: 170 kg N/ha) with increasing costs for application at a greater distance. So far, in very few cases, a 35-40% w/w DM DP is produced by sedimentation, filter pressing, and drying with the effluent plant heat. This makes easier both handling and longer range transport.

3) Legislative framework

It's a hard task to precisely define DP on the basis of present legislation. Although some Regions have a positive attitude toward this product (e.g. Lombardia, where it is considered as an organic amendment; Veneto, where similar transport documents are required for manure, cattle slurry, and DP) a comprehensive and uniform normative position still lacks. This sometimes negatively affects authorization process of new AD plants, and several aspects of DP handling. In Umbria, at a recent *Conferenza dei Servizi*, triggering the AD process with manure was evaluated negatively, as "*manure is a waste, and triggering the AD process with a waste produces wastes, to be disposed of in landfills*". Also, road transport, e.g. from a cooperative AD plant to members' fields, may raise issues. On the basis of the Italian Law 217/2006, the definitions of "green composted amendment" and "mixed composted amendment" do not precisely apply to DP. Although the AD process is a "transformation and controlled stabilization" one, it is not a composting process. Also, it is hard to define crop silages "organic wastes", as the feedstocks of a composted amendment are indicated in the law. A new amendment category should be required! DP could also be included among the organic matrices for organic-mineral fertilizer production, according to Annex 5. It could be a traceable and renewable material, instead of peat and lignite, for the formulation of both liquid and solid fertilizers. For example, DP can be easily formulated with at-plant dissolved nutrients (plant effluent heat keeps formulation costs low) to produce low and medium grade suspension fertilizers. Filter-pressed DP can be mixed and pelleted with solid fertilizers, using the same plant effluent heat for fertilizer manufacturing. Some tests are in progress, both on formulation and agronomic performance issues. However, a technical file for a new product category is to be prepared, and who cares for this? Presently, the Italian law on fertilizers requires a sponsor, who introduces and completes the technical file, but he/she is generally an industrial or commercial sponsor. In this case, the technical file should be prepared by a scientific institution, and the major AD plant manufacturers and the regional Agencies for agriculture could give their own contributions.

We feel that DP resource-oriented management will be a key-factor for further development of this technology at the farm level.

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TARGETING NUTRIENT MANAGEMENT OPTIONS TO ADDRESS SOIL FERTILITY CONSTRAINTS IN WESTERN KENYA

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Abstract

Widespread decline in food crop yields in Western Kenya is associated with soil fertility loss, aggravated by intensified land use in the absence of external inputs. The implementation of past approaches to soil fertility restoration failed as the availability of production factors in different farm types was generally not considered. We hypothesized that technology adoption depends on the effectiveness of addressing the soil-specific production constraints and the resource endowment of individual farms. Initially, a farm typology based of surveys on 192 farms was developed, taking into account the soil fertility status and production system attributes. Subsequently, technologies were evaluated in on-farm trials on the dominant soil types of Kakamega District, combining nutrient balances, yield gaps and productivity studies. Technologies comprised combinations of reduced tillage, mineral fertilizers and organic amendments. Key soil constraints were determined to be low organic C and N in Alfisols, and low P availability on Ultisols. These were most severe on small subsistence-oriented farms with mean maize yields 0.6 Mg ha^{-1}. Nutrient balances were generally negative for N and K while being neutral for P. The efficacy and adoptability of technologies differed by soil type and production system. Mineral fertilizers corrected the nutrient constraints and increased grain yields by 3.8 Mg ha^{-1}, but are unaffordable to low-input farmers. Farmyard manure and green manure legumes significantly increased maize yields on all soil types but differ in their resource requirements (labor implements, seeds). Based on this field research, a decision tool for site- and system-specific technology targeting is being developed.

Keywords: manure, nitrogen, organic matter, phosphorus, *Zea mays*.

Introduction

Despite high precipitation and a favourable rainfall distribution pattern, the agricultural production environment of Kakamega district in Western Kenya is characterized by poor resource availability and a low resource base quality (Tittonell *et al.*, 2005). Demographic growth and the prevailing real-split inheritance system have resulted in a rapid decline in average farm size, and households must meet their subsistence needs and much of their livelihood requirements from an ever-declining land resource base. This development has led to an intensification of the crop production over the last decades. Harvesting two instead of only one maize crop per year is further intensifying today's permanent land use systems. Without the use of external inputs, such intensification strategies have lead to severe soil nutrient depletion and declining soil organic matter contents. This has provoked a downward spiral of productivity decline, rural poverty and low livelihood levels across the Kakamega district. However, the dominant processes and mechanisms underlying degradation phenomena as well as their extent vary by soil type, production system and the resource endowment of individual farms.

In the Kakamega farmland case, the systems are being highly destabilized and have in some instances reached a state wherein the agricultural production system is unable to meet a growing human demand and/or the provision of ecosystem services (i.e. C sequestration), and a weak socio-economic and political environment further prevents a desired reorganization. Therefore, we assumed that a reorganization or increased agricultural production potential is only possible through technical change (Becker and Johnson, 2001). However, the type and intensity of resource base degradation appears to be highly site-specific, while the availability of farm resources and production factors to counteract biophysical degradation processes are highly system-specific. As an example, while some large, well-endowed farms can compensate fertility decline phenomena by purchased inputs, most farmers cannot follow the recommended application rates of mineral fertilizer or hire manual labour for regular weeding due to lack of funds (Shepherd and Soule, 1998). Therefore, the introduction and adoption of agronomic technologies that address key livelihood demands and correct key production constraints must be site- and system-specifically adapted to the resource endowment of the farmers.

Hence, the presented study aimed at identifying the prevailing farm types of Kakamega taking into account the soil fertility status as well as production system attributes, and evaluated technological option to overcome the identified constraints.

Materials and methods

The study was carried out in Kakamega District (34° 20´and 35° E and 0° 15´and 1° N; 1250 m to 2000 m above sea level) in Western Kenya, where the annual rainfall and mean daily temperature range from 1200 – 2100 mm and 18 - 21°C, respectively. The dominant soil types are sandy Alfisols in the North, clayey Ultisols in the South and small pockets of Nitisols in the central parts of the district, respectively.

Farm typology

The farm typology was based on a questionnaire (192 households) in a total of eight sites. It covered socio-economic factors (household size, level of education, occupation of the household head), land ownership, soil fertility management and species cultivation. On all surveyed farms soil samples were collected in maize, sugarcane, tea and vegetable fields and analysed for total nitrogen, and available phosphorus and potassium. The collected data was subjected to principal component and discriminant analyses.

Nutrient balances

Apparent input-output NPK nutrient balances for maize at the field level were established for 8 major farm types distinguished by the farm typology taking into account inputs by (1) mineral fertilizers sources, (2) organic fertilizer sources, and (3) contributions of biological nitrogen fixation and outputs by (4) crop harvest products and residues.

Evaluation of technological options

For the evaluation of technological options, the following treatments were established during two seasons in 5 sites with contrasting soil type and inherent fertility levels:

(1) Farmers' practice / Control (FP): tillage, no fertilizer application, clean-weeding once at 14 days after seeding (DAS); (2) Clean weeding (CW): Treatment (1) + hand weeding at 28, 56 and 84 DAS; (3) Mineral N & P fertilizer (NP): Treatment (2) + 100 kg ha^{-1} N (urea; 1/3 basal application, 1/3 at 28 DAS, 1/3 at 56 DAS) and 100 kg ha^{-1} P (TSP; basal application); (4) Farmyard manure application (FYM): FYM applied basally at a dry matter rate of 5 t ha^{-1}; (5) Seed priming (SP): Treatment (2) + maize seeds soaked in 50mM monopotassium phosphate (KH_2PO_4) solution for 12 hours followed by air-drying prior to planting; (6) Zero-tillage with N & P fertilizer (ZNP): Treatment (3), no tillage; (7) Zero-tillage with N & P fertilizer and *Arachis pintoi* cover crop (ZANP): Treatment (6) + cover crop; spacing: 0.75 m x 0.25 m, application of 20 kg ha^{-1} P (TSP) to facilitate establishment; (8) Green manure incorporation (GM): Treatment (2) + incorporation of 8-week old *Mucuna pruriens* plants prior to maize planting; seeding rate 150 kg ha^{-1}, spacing: 0.75 m x 0.25 m spacing, application of 20 kg ha^{-1} P (TSP) to facilitate establishment.

Results and discussion

Farm typology

The analyses revealed a total of 10 hierarchy clusters. The main characteristics of clusters with more than 3 members are given in Table 1. The farm typology indicated that low soil fertility is a major issue. Soil analysis showed that low N and P soil levels are a major concern within Kakamega District. The most limiting nutrient is P with only cluster 4 in the Alfisols region exhibiting a sufficient soil level and having only 20% of it members with values below the critical soil limit for maize. N levels are below the critical limit for maize within four clusters, of which three are in the Alfisols region. Potassium levels are generally well above the critical limit for maize in all clusters. Cluster 4 had the lowest yield (on average 1.0 Mg ha^{-1}) due to N levels well below the critical soil limit. Since both N and P fertilizer application rates were far

Table 1: Attributes of major farm types in Kakamega District, Western Kenya.

Variable	Cluster					
	1	2	4	5	7	8
Number of cases in cluster	52	9	5	51	8	3
Gradient						
Soil type	Alfisols (100%)	Alfisols (78%) Ultisols (22%)	Alfisols (100%)	Ultisols (98%) Alfisols (2%)	Ultisols (100%)	Ultisols (100%)
Land size (ha)	0.9 ± 0.53	1.3 ± 0.53	2.6 ± 0.49	0.6 ± 0.50	0.9 ± 0.54	0.9 ± 1.04
Presence of industrial crop	Sugarcane (73%) None (27%)	Sugarcane (56%) None (44%)	Sugarcane (100%)	Tea (2%) None (98%)	Tea (100%)	Tea (100%)
Soil fertility	None above critical soil limits	11% above critical soil limits	None above critical soil limits	None above critical soil limits	13% above critical soil limits	None above critical soil limits
Labour						
Hired labour index	0 to 4 hired activities	2 to 3 hired activities	0 to 4 hired activities	0 to 4 hired activities	0 to 3 hired activities	2 to 4 hired activities
Highest hired activity share	62% No activities	67% 3 activities	60% 3 activities	47% 3 activities	63% 3 activities	67% 2 activities
Capital						
Occupation of household head	64% Farmers 27% Skilled labourer 9% Others	67% Farmers 22% Skilled labourer 11% Others	80% Farmers 20% Retired	57% Farmers 33% Skilled labourer 10% Others	100% Farmers	67% Retired 33% State employee
Know how						
Nitrogen application rate (kg $ha^{-1}year^{-1}$)	27 ± 25.9	100 ± 25.9	27 ± 34.7	29 ± 29.0	43 ± 42.1	60 ± 31.0
Application rate below recommendation	65%	11%	80%	90%	63%	67%
Contact with extension service	33% Never 27% Rare 33% Average 7% Regular	2% Never 9% Rare 67% Average 22% Regular	0% Never 0% Rare 40% Average 60% Regular	65% Never 24% Rare 7% Average 4% Regular	38% Never 50% Rare 10% Average 2% Regular	33% Never 33% Rare 33% Average 1% Regular

lower than the recommended rates (75 kg N and 25 kg P ha^{-1} $year^{-1}$, respectively), the high P values in cluster 4 may be explained by the application of farmyard manure from the on average 5 cows owned by farmers in this cluster. The highest maize yield is found in cluster 8 on the Ultisols with on average 2.4 Mg ha^{-1}. In this cluster only P seems to be a limiting factor.

Nutrient balances and fluxes

The partial nutrient balances differed largely among farm types (Figure 1). The highest negative balance was found in the market-oriented, food crop cultivating, high-input farms on Alfisols (AFMS) with -81 kg N ha^{-1}, while they are close to neutral at -1 kg N ha^{-1} in the market-oriented, sugarcane-cultivating (AIMS) farms. The average P balances were positive, with the highest enrichment occurring in the market-oriented tea-growing farms on Ultisol (UIMS) with 24 kg P ha^{-1} and sugar-cane growing farms on Alfisol (AFMS) with 18 kg P ha^{-1}. A model simulation run based on a single farm type in the East African highlands indicated that P inputs of 20 kg P ha^{-1} $year^{-1}$ were sufficient to maintain soil P stocks (Shepherd *et al.*, 2005). However, the interpretation of P balances should be done with care. They are often not very reliable (Palm, 1995), as P availability is influenced by several factors including soil pH, the ratio of N to P and soil C:N ratio. The generally negative K balances result from a near complete absence of K application resulting in net mining of K in all but those farms, where

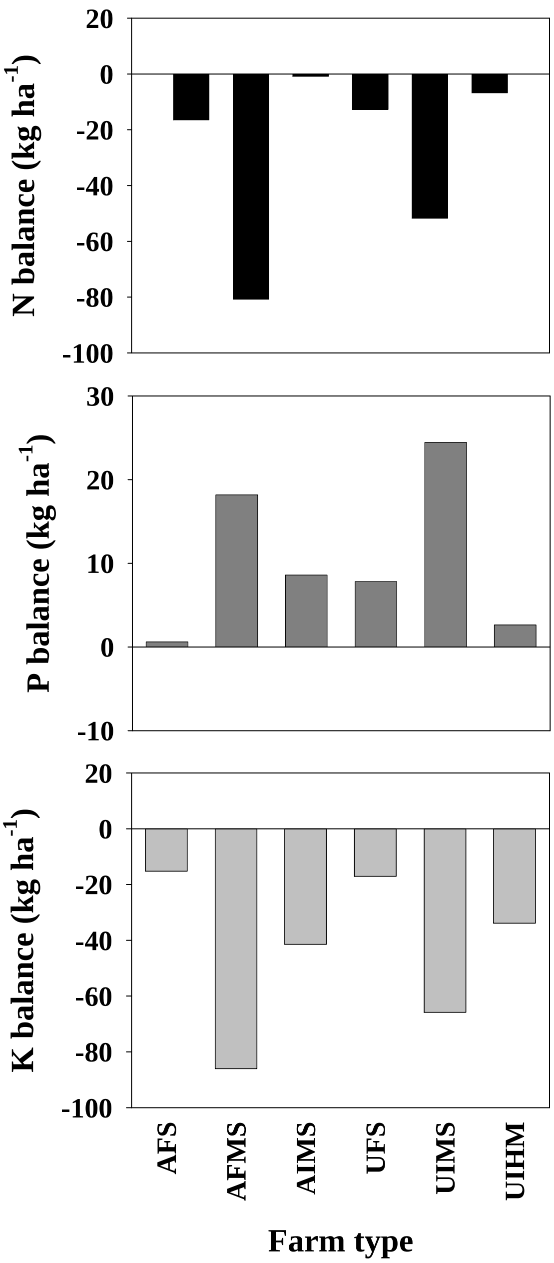

Figure 1: Partial NPK balances of maize fields in major farm types (AFS=subsistence-oriented farms; AFS=market-oriented farms specializing in food crops, AFMS=market-oriented farms cultivating sugarcane, AIMS=subsistence-oriented farms on Alfisols, UFS=market-oriented farms growing tea, UIMS= market-oriented farms growing tea and food crops, UIHM=highly market-oriented farms growing tea and maize in Kakamega District in 2006.

substantial amounts of farmyard manure were applied.

Evaluation of technological options

The responses of maize to the tested agricultural technologies varied widely and there was a highly significant interaction between treatment and site (P<0.01) confirming earlier reports of high levels of heterogeneity in the smallholder systems of Western Kenya (Vanlauwe *et al.*, 2005). The responses were more pronounced during the long rainy season, as soil moisture availability is less likely to restrict yields. During this season, mineral fertilizer application corrected the existing nutrient constraints and resulted in the highest grain yields of on average 6.2 and 5.9 Mg ha^{-1} on the moderately fertile Nitisols and Alfisols, respectively, while on the Ultisols yield levels were lower ranging from 4.2 to 4.8 Mg ha^{-1} (Figure 2).

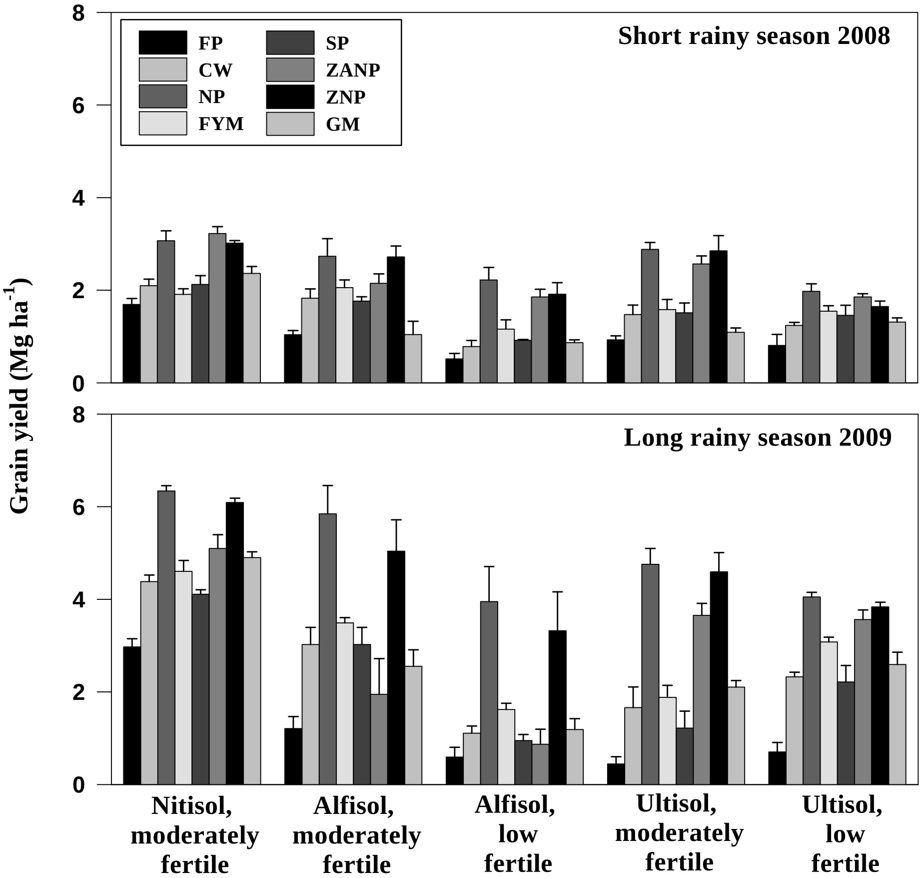

Figure 2: The responses of the grain yield of maize to technological options (FP=farmers' practice, CW=clean weeding, NP=N&P fertilization, FYM=Farmyard manure application, SP=seed priming, ZANP=zero-tillage + N&P fertilization + *Arachis pintoi* cover crop, ZNP=zero-tillage + N&P fertilization, GM=incorporation of *Mucuna pruriens*) imposed on 5 sites differing in soil type and inherent soil fertility during 2 seasons in Kakamega District, Western Kenya. Bars indicate standard error of the mean (n=4).

But the required fertilizer rates are unaffordable to low-input farmers. However, this can be partly compensated for increasing the weeding frequency when sufficient on-farm labour is available. Depending on site and season, clean-weeding alone increased maize yields across sites by on average 124%, while more then tripling them on the Ultisol sites. Farmyard manure and green manure legumes significantly increased maize yields over the control in all sites but differ in their resource requirements (labor implements, seeds).

We conclude that due to the high levels of heterogeneity in the smallholder systems of Kakamega District any successful and effective technology adoption has to address both, the soil-specific production constraints and the resource endowment of individual farms. Therefore, based on our findings, we currently develop socio-ecological fit indicators for individual technologies that will lead to the development of a decision tool for site- and system-specific technology targeting.

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AUTOMATED SCREENING OF LEAF TREATED ZUCCHINI PLANTS TO ASSESS GROWTH AND ABIOTIC STRESS

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Abstract

Drought stress is a significant problem affecting agriculture production worldwide and it is predicted to have a larger impact in the future, as a consequence of climatic changes. Organic leaf spraying, contributing to nutritional effects, could be used also to enhance plant's tolerance under drought stress conditions. This activity seems related to the reduction of stomatal opening and to the increase of antioxidant compounds.

An experimental test was conducted to evaluate nutritional and inhibitor leaf transpiration effects of eight organic formulations (Valagro S.p.A.) on zucchini plot plants, after a leaf treatment. Zucchini plants were grown in a greenhouse under drought stress conditions. Non-destructive measurements were conducted by using an automated plant phenotyping system, that uses image capture and processing technologies (Scanalyzer 3-D system). Results indicated that two tested products seemed to be able to reduce plant stress and to enhance plant growth.

Keywords: imaging analysis; drought stress; organic formulations; zucchini.

Introduction

Irrigation is an important mean of raising production in agricultural crops. It is essential in arid environments, and is often used to increase crop productivity in semiarid and humid areas. Drought stress is an important problem affecting agriculture worldwide and is predicted to become a larger trouble in the future. To fight this problem, the drought stress tolerance of crop plants needs to be increased, so enabling to growth in arid environments. As a result, new management strategies have been proposed, such as controlled deficit irrigation, to ensure low water loss with minimum yield reduction. Nevertheless, the use of new products will be required to reduce plant stress under drought conditions. Organic compounds, obtained from plant tissues, seem to enhance plant's drought stress tolerance, inhibiting stomatal opening and increasing antioxidant compounds content (Salisbury *et al.*, 1994). Organic matrix content in these formulations could determine the formation of film on leaves and, consequently, reduce leaf transpiration. The aim of this work was to evaluate nutritional and inhibitor leaf transpiration effects of several organic compounds (produced by Valagro S.p.A.) on zucchini plot plants under drought stress conditions.

Materials and methods

The trial was conducted in a greenhouse, equipped with the Scanalyzer 3-D system platform, located in Metaponto (South of Italy). Zucchini plants were grown in pots during the spring-summer period. The experimental design was a randomized block, with four replicates, 12 plants for each treatment, for a total of 120 plants. For up to 10 days after transplant, standard agronomic irrigation was applied. After this period plants were leaf treated by using eight different formulations, identified as 1708, 1709, 1710, 1711, 1708F, 1709F, 1710F and 1711F. The products were applied lonely on vegetations, with a backpack spray equipment. Afterwards, plants were grown under a total drought stress condition for 9 days. Treated plants were compared with two controls, one in which plants were grown under standard irrigation condition (Control) and the other without irrigation management (Control stress). Plant's stress and phenotypic responses were assessed by using an automatic plant phenotyping platform equipped with image capture and processing technologies (Scanalyzer 3-D system; http:/www.lemnatec.de/) that combines quantification of growth and phenotyping with high reproducibility, allowing long term data storage for data mining. The system consists of three parts: a conveyor unit, an imaging unit with two imaging modes, NIR (near infrared) and RGB (visible) camera, and software to analyse morphometric parameters. NIR imaging was used to obtain information on watering status of plant tissues and their reaction to limited water availability (Rajendran et al., 2009). RGB imaging was used to analyse zucchini plants in dimension, morphology and architecture measurements. Assessments were carried out after 1, 3 and 9 days after drought stress (DADS) and 3 days after water recovering, using images obtained from three photographs from 0°, 90° and 120° sides. Quantitative data obtained by NIR imaging expressed on diagram representation as false colour classes (blue, yellow and orange), of several absorption band between 1450 and 1600 nm. Plant growth and morphometric parameters were evaluated by using RGB imaging and plant images were taken from two sides (90 and 120°) and from the top view. Statistical test as ANOVA and Duncan's Test were used to elaborate data obtained from image analysis.

Results and Discussion

In the next table is reported the index of water content, obtained by image analysis, evaluating the response of treated zucchini plants under drought stress conditions. Results showed that after 3 days from treatment and under drought stress conditions, zucchini plants treated with 1710 and 1711 compounds showed the higher water content, with a smaller amount respect to the control, which was standard irrigated, but significantly different respect to the control stress, which was not irrigated at all.

Table 1: Index of water content after conditions of drought stress (1, 3 and 9 days) and of water plant recovery.

Treatment	1DADS	3DADS	9DADS	Water Recovery
1708	0.100 ns	0.218 bc	0.346 bc	0.448 b
1709	0.088 ns	0.199 dc	0.313 bc	0.412 b
1710	0.084 ns	0.271 ab	0.418 ab	0.508 a
1711	0.101 ns	0.283 ab	0.341 bc	0.426 b
1708F	0.082 ns	0.223 bc	0.339 c	0.403 b
1709F	0.078 ns	0.209 c	0.382 c	0.435 b
1710F	0.076 ns	0.188 dc	0.320 c	0.420 b
1711F	0.085 ns	0.209 c	0.313 c	0.436 b
Control	0.087 ns	0.289 a	0.448 a	0.459 ab
Control stress	0.081 ns	0.150 d	0.225 d	0.263 c

Ns=not significant. Within each column, different letter significant differences according to Duncan Test ($p<0,05$)

Particularly the 1710 treated plants were characterized by a significative difference in the water content respect to the control stress, during all the drought stress condition, indicating a probably effect in the reduction of leaf transpiration. Most likely, the 1710 product induces a greater accumulation of abscissic acid (ABA), inhibiting stomatal opening. Furthermore the test substance could determine the development of a film on leaves and consequently a reduction of transpiration processes. After the water recovery, treated plants and Control did not show significative differences. Probably in the treated plants, under drought stress condition plants activated responses, as higher stomatal closing, inducing a reduction of transpiration and water lost by leaves as reported by Salisbury et al., 1994. Furthermore, in treated plants, in response to the water deficit, it would be accumulate a greater content of ABA (Zhang J. *et al.*, 2006).

In the next figure is reported an image comparison of 1710 treated and Control stress plants obtained by using NIR imaging. In the pictures, taken at different days after drought stress, blue areas correspond to part of a plant with higher water content. After 9 days of drought stress plants treated with the 1710 compound showed a higher water content (blue false colour) respect to control stress.

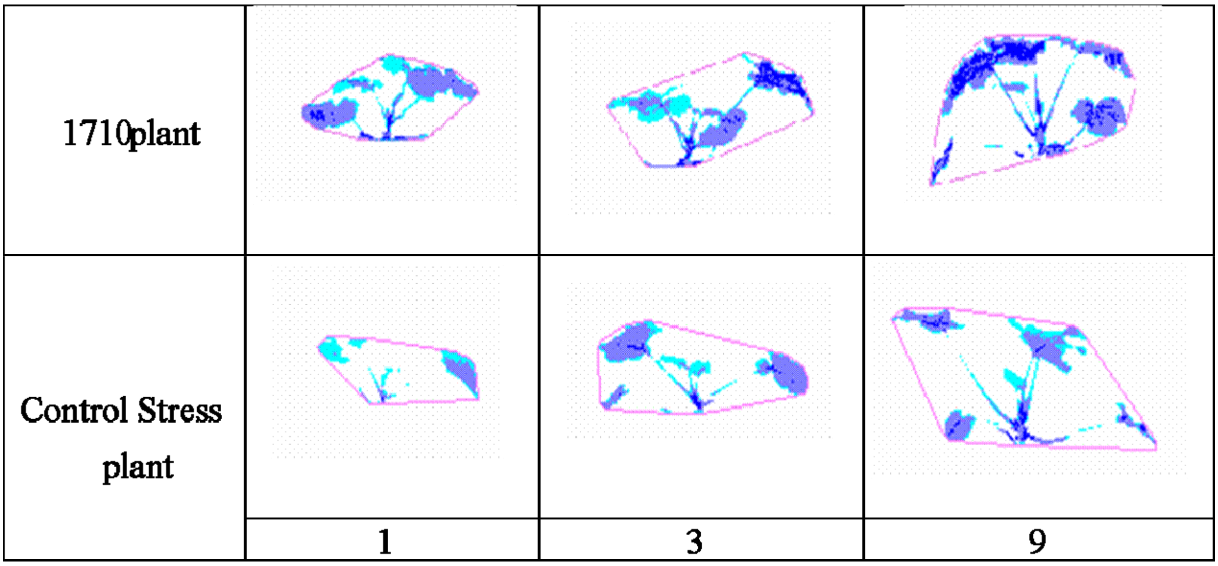

Figure 1: Images of 1710 treated plant and Control stress plant obtained by using NIR imaging. Pictures were taken 1, 3 and 9 DAI (Days After drought stress).

Interesting results were obtained concerning plant growth by using RGB imaging. As reported by Vandenhirtz, J. et al. 2006 the plants biomass can be determined in a non-destructively way with an accuracy of 10,0% just by using the 3-D image data. As described in Figure 2, zucchini plants treated with 1711 compound showed, under the drought stress condition, a higher growth (increased number of pixel of image), respect to Control stress and Control plant. Probably this compound has a direct nutritional effects as well as inducing the reduction of leaf transpiration.

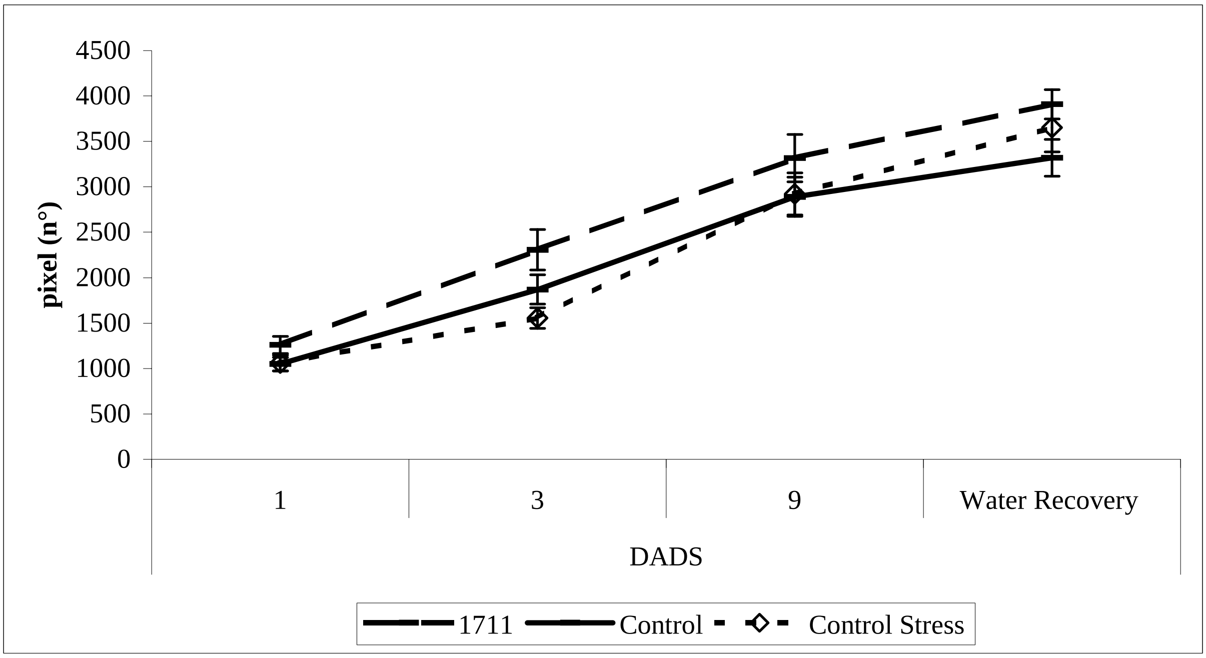

Figure 2: Zucchini plant growing dynamics of 1711 treated, Control and Control stress in the four assessments.

In conclusion image analysis allowed individuating the products which responses better to drought stress conditions, considering the index of water content and the plant growth, as number of pixel of images. Consequently, the phenotyping platform could provide effective and meaningful information on the efficacy of plant foliar treatment with new molecules, identifying in a quick screening the most excellent treatment.

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FERTILIZATION OF RICE: ASSESSING POTENTIAL N MINERALIZATION OF DIFFERENT FERTILIZERS IN TWO ITALIAN SOILS

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Abstract

This work would investigate, by studying N potentially mineralisation in laboratory conditions, the influence of aerobic/anaerobic environment on nitrogen release from some selected fertilizers, often used in rice cultivation in Northern Italy.

The experimental trials were performed taking into account two soils from Northern Italy, Carmagnola soil (TO) and Castello D'Agogna soil (VC): the first one was under durum wheat-maize rotation, while the second one sustained rice cultivation. Different nitrogen fertilizers were added to the tested soils and then putted under controlled aerobic (Stanford and Smith method, modified by Benedetti, 1994) and anaerobic conditions (Canali, 2005). Tested fertilizers were: calcium-cyanamide; mineral-N fertilizer containing urea and condensed urea; organo-mineral fertilizer from leather meal origin; organic fertilizer from horn and nails. The mineralized N was detected on time and the release N-curves were obtained for each fertiliser.

Experimental results indicated that both the aerobic/anaerobic conditions and the intrinsic characteristics of added fertilizers were effective in released N-fractions, being different and not complementary the N pools mineralized in presence or absence of oxygen supply. Also the soil played a key-role, since the alternative crops and management (cereals rotation or rice as continuous crop) probably selected the active microflora able to sustain the mineralization process.

Keywords : Potential N mineralization, soil, rice, incubation.

Introduction

Fertilisation of rice is usually conducted by applying N-fertilizers: however, the nitrogen supply to this crop is strongly affected by the field water-management, since the anaerobic conditions and the related soil microflora have significant effect on nitrogen mineralization.
Thus, when we have to optimize N fertilization in rice cultivation, we cannot do that only on the basis of the crop N need, but we have to take into account the specific soil biological fertility, the pedo-climatic conditions, that represents "cropping system" on general terms, in order to define the actual nutritional crop needs, in terms of fertiliser rate, typology of fertilizers to be applied, suitable agronomical interventions.
The considered cropping system plays a key role in the correct choice of fertiliser: in fact, for a submerged crop such as rice, the way by which N supply is released results strongly influenced by the alternation of aerobic/anaerobic conditions which could be found in soil. These changes in soil environment induce alternatively the preponderance of different naturally-selected population of microbial communities, which strongly influence the nutrient mineralisation and availability to the crop. In this direction, it is particularly interesting to evaluate how different soils, differently managed from agronomical point of view, could affect the mineralization of organic N, especially in relation to the aerobic or anaerobic conditions which more frequently they sustained.
The objective of this work was to attain the influence of aerobic/anaerobic environment on nitrogen release from some selected fertilizers, often used in rice cultivation in Northern Italy, by studying their potential N-mineralisation in laboratory conditions.

Materials and methods

The experimental trials were performed taking into account two cultivated soils from Northern Italy of Piedimont region (Figure 1): Soil 1 (Carmagnola, Turin), under durum wheat- maize rotation, and Soil 2 (Castello D'Agogna, Pavia), under rice as continuous crop.

Figure 1: Piedimont region and sites of the considered soils.

Biochemical characteristics of the two soils are reported in Table 1.

Table 1: Main characteristics of the two soils from Piedimont Region (Italy).

	C_{org}*	*OM*	C_{mic}	qCO_2	*qM*
	%	%	$mg\ C \times kg^{-1}_{soil}$	$mg\ CO_2\text{-}C \times mg\ C_{mic}^{-1} \times h^{-1}$	%
Soil 1 - Carmagnola (Turin)	1.2	2.1	172.1	1.85	4.3
Soil 2 - Castello D'Agogna (Pavia)	1.2	2.0	299.7	0.23	1.5

* Corg = soil organic carbon; OM = total organic matter; Cmioc = C-microbial biomass; qCO_2 = metabolic quotient; qM = mineralization quotient.

Four different N-fertilizers were considered in the experimental scheme:

- Calcium-cyanamide (Ca-CYAN; N_{tot}= 19,8%);
- Urea + urea-formaldheyde (UREA-FORM; N_{tot}= 43,0%);
- Organo-mineral fertilizer from leather meal (OM-LM; N_{tot}= 12,5%);
- Organic fertilizer from horn and nails (OM-HN; N_{tot}= 14,0%).

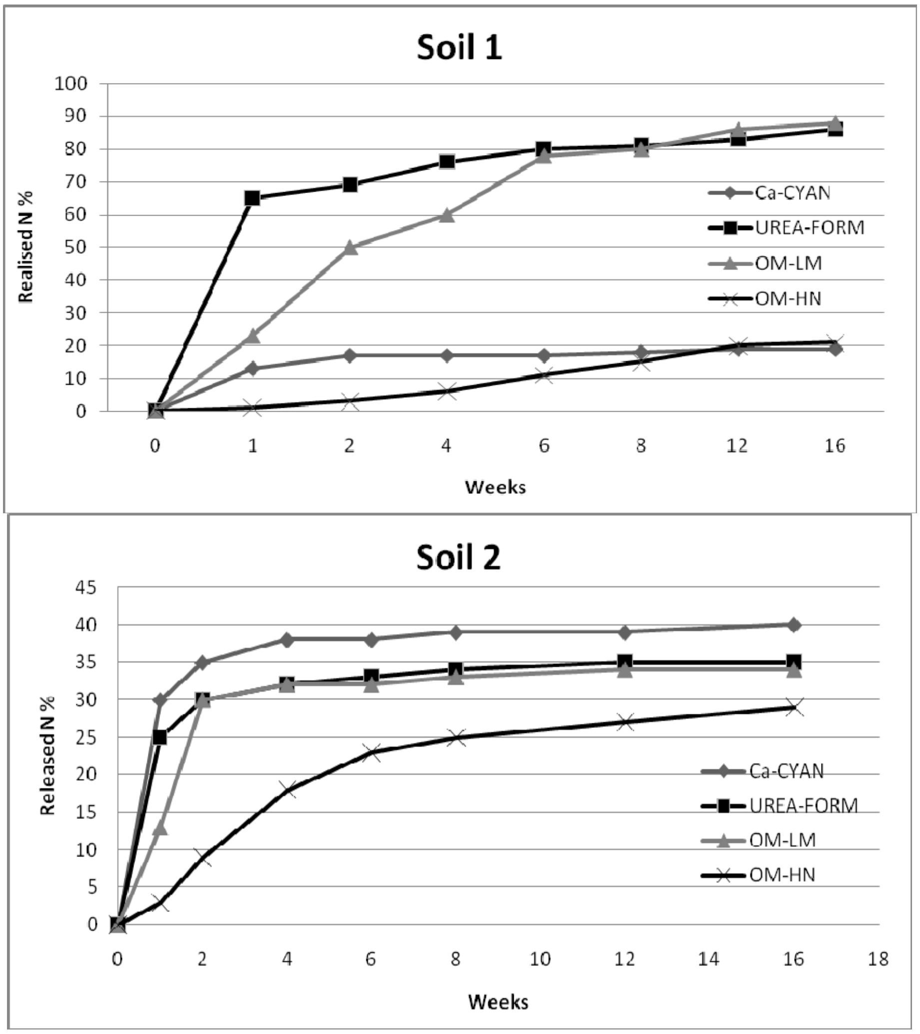
Considered N-fertilizers were added to the soil 1 and 2; the mineralized N in potential conditions on time was determined under controlled aerobic and anaerobic conditions. In relation to aerobic conditions, fertilizers were added to 50 g of air-dried soil at the rate of 250 mg N kg soil^{-1}) and mixed with quartz sand in a 1:1 ratio and the mixtures were incubated at 60% WHC (pF 2.5) at 30°C for different periods of time (Benedetti et al., 1996; Dell'Abate et al., 2003). Soil mixtures were eluted with 0.01M $CaSO_4$ after 1, 2, 4, 6, 8, 12^{h} and 16th weeks and NO_2-N, NO_3-N and NH_4-N, produced during the incubation were determined by a continuous flux analyser (Autoanalyser Technicon II).

Potentially mineralisable N (NPM) under anaerobic conditions was estimated from the NH_4-N (mg×kg^{-1}) accumulated after 7 days of anaerobic incubation at 40°C, by mixing 16 g of soil sample with an amount of each fertilizer (250 mg N/kg_{soil}) in 50 mL test tubes containing 40 mL of distilled water, and then incubating at 40°C for 8 days. After incubation, soil + fertilizer was extracted with KCl 2N and 40 ml KCl 4N was added to the suspension in order to keep the soil:solution ratio to 1:5 (Sahrawat and Ponnamperuma, 1978, slightly modified by Canali et al., 2000). The difference between the N-NH_4^+ released by the "soil + fertilizer" sample after incubation and the N-NH_4^+ released by the non-incubated sample was taken as mineralized nitrogen (NPM). All determinations were performed in triplicates.

Results and discussion

In the first aerobic test (Figure 2), for Soil 1 under durum wheat –maize rotation, we have recognized two group fertilizers: the first one, Calcium-cyanamide (Ca-CYAN), and horn and nails organic fertilizer (OM-HN), which released poor quantities of N (not more than 20% of the total N was mineralised in Soil 1 after 16 weeks of the experiment), and the second one, in which the condensed urea (UREA-FORM) and leather meal (OM-LM) released almost 100% of added N in two following steps (0-2 weeks the first one, 3-16 weeks the second one).

Figure 2: N-release curves from tested fertilizers under aerobic conditions into 1 and 2 soils.

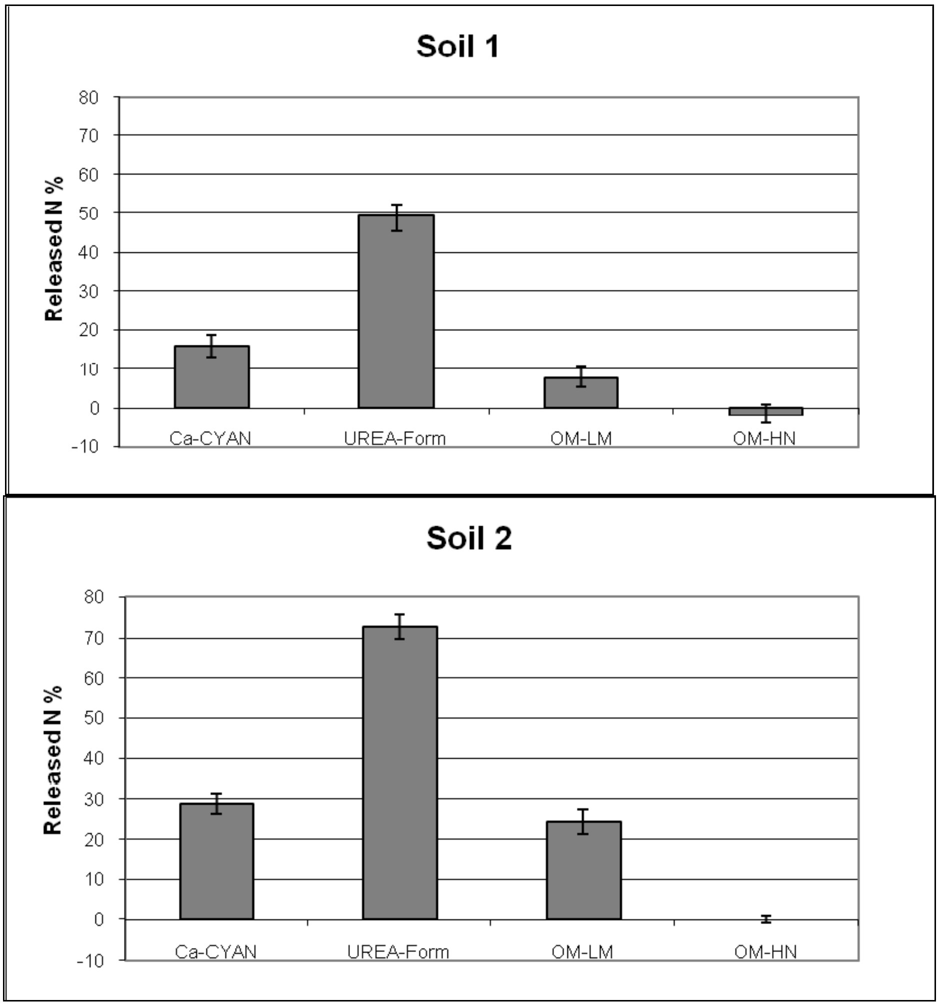


This behaviour was less clear in Soil 2 (Figure 2), under rice as continuous crop: in this specific soil, there is a reduction of N mineralization on general terms, since both the UREA-FORM and the organic fertilizers (OM-LM and OM-HN) release not more than 40% of the total added N. We interpreted these results on the basis of the different agronomic management of the considered soils: Soil 1 is under cereal cropping sequence, while Soil 2, under rice as continuous crop, is usually in conditions of water excess, thus determining alternative periods of anoxia / aeration for soil microbial pool.

Probably, the periodic reduction of O_2 availability in Soil 2 forced soil microflora to select species more adaptable to these stressing conditions , so to be less active the microbial pool when returned to the aerobic conditions. For these reason in soil 2 was released a lower rate of N.

In relation to the anaerobic test (Figure 3), information arisen from both the experimental trials indicated that the fertilizers were more mineralised in Soil 2 with respect to Soil 1.

Figure 3: N-release curves from tested fertilizers under anaerobic conditions into 1 and 2 soils.



In particular, N released from condensed urea (UREA-FORM) in Soil 2 was 25% more than that released in Soil 1, such as also Ca-CYAN (+15%), OM-LM (+20%) and OM-HN (+2%). We interpreted these results as a confirmation of our hypothesis: in this test, the higher microbial mineralizing activity was realized in continuous cropped rice soil (Soil 2), even if the organic substrates were the same ones. The microbial population selected in Soil 2 was more able to degrade organic matter or hydrolyse condensed molecule in anaerobic conditions respect to the Soil 1 microflora, usually active in aerobic conditions.

What emerged from our tests indicated that both the aerobic/anaerobic conditions and the intrinsic characteristics of added fertilizers were effective in released N-fractions, being different and not complementary the N pools mineralized in presence or absence of oxygen supply. Also the soil played a key-role, since the alternative crops and management (cereals rotation or continuous cropped rice) probably selected the active microflora able to sustain the mineralization process.

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GLASS-MATRIX BASED FERTILIZERS ON PLANT DEMAND: FIRST RESULTS

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Abstract

Glass-matrix based fertilizers represent a typology of fertilizer able to release nutrients on the basis of plant-demand, being nutrients not soluble in water, but only in metal complexing solutions (~99%), similar to those of root exudates. Nutrient release could be increased by mixing the glass-matrix fertilizer to different organic biomasses, such as leather meal, digested vine vinasse, etc.

A short-term pot trial on *Zea mais* L. was conducted to verify the effect of different mixtures of glass-matrix fertilizer (GMF) with increasing percentages of digested wine vinasse (DVV - 5%, 20% and 50% w/w) on maize growth, also performing nutrient release-test for all considered mixtures, in different extractive conditions (H_2O, citric acid 2% and HCl 1%).

Best results were obtained in relation to the mixture 80% GMF + 20% DVV, which gave the highest maize shoot weight and total produced biomass. Nutrient release was comparable in 2% citric acid and 1% HCl, confirming the importance of acidic root exudates in making available primary, secondary and micro-nutrients coming from GMF + DVV mixtures.

A parallel study was realized on "Tarocco scirè" orange trees [Citrus sinensis (L.) Osbeck] grafted on two different rootstocks [*Citrange carrizo* (L.) and *Citrumelo swingle* (L.)]. The research was conducted in 50 liter pots, utilizing tree different soils, fertilized with GMF and GMF mixtures, to verify the effects of treatments on growing plants. First results show that the mixture GMF + DVV seems to increase nutrient availability and plant development.

Keywords: Glass-matrix based fertilizer, vine vinasse, maize, nutrient release.

Introduction

Glass-matrix based fertilizers (obtained by altering the crystalline structure of a mineral natural substance through a physical process) represent a new typology of fertilizer, characterized by the specific attitude to release nutrients on the basis of plant-demand. More in deep, in these fertilizers nutrient elements are not soluble in water, but only in metal complexing solutions (~99%), which are similar to those exuded by plant roots (Pinton et al., 2007). Such products are assumed to have positive effects, due to the optimization of nutrients (Roll-Hansen, 1975), lowered environmental impact, increased soil fertility and improved end-user acceptance (Sequi et al., 2007). Effectively, the possibility to reduce the risk of nutrient elements leaching in water represents an important effort in order to guarantee environmental protection on long term: with this aim, these new glass-matrix based fertilizers can match the crop needs by minimizing the impact of mineral fertilization to soil. Moreover, the technology development allows to put on the market such formulates, further improved by mixing them with different organic biomasses, such as leather meal, digested vine vinasse, etc.: the main aim of this mixing process is to better modulate the nutrient availability, by utilizing the organic matrices as an "activator" of nutrient release.

In this paper, previous results obtained by testing as a fertilizer a glass- matrix based formulate, also mixed to digested vine vinasse at different ratios, are reported.

Materials and methods

A glass-matrix based fertilizer (GMF), alone or mixed with increasing percentages of digested vine vinasse (DVV) was tested as fertilizer by applying a multiple approach:

Nutrient release tests – A nutrient release tests for all the considered mixtures was performed, by extracting 2 g of the mixtures GMF 100%, GMF 95%+DVV 5%, GMF 90%+DVV 10%, GMF 80%+DVV 20%, GMF 50%+DVV 50%, DVV 100%, at increasing acidic conditions: H_2O, citric acid 2% and HCl 1%, followed by determination of extracted nutrient elements (P, K, Ca, Mg, Fe) by ICP-AES;

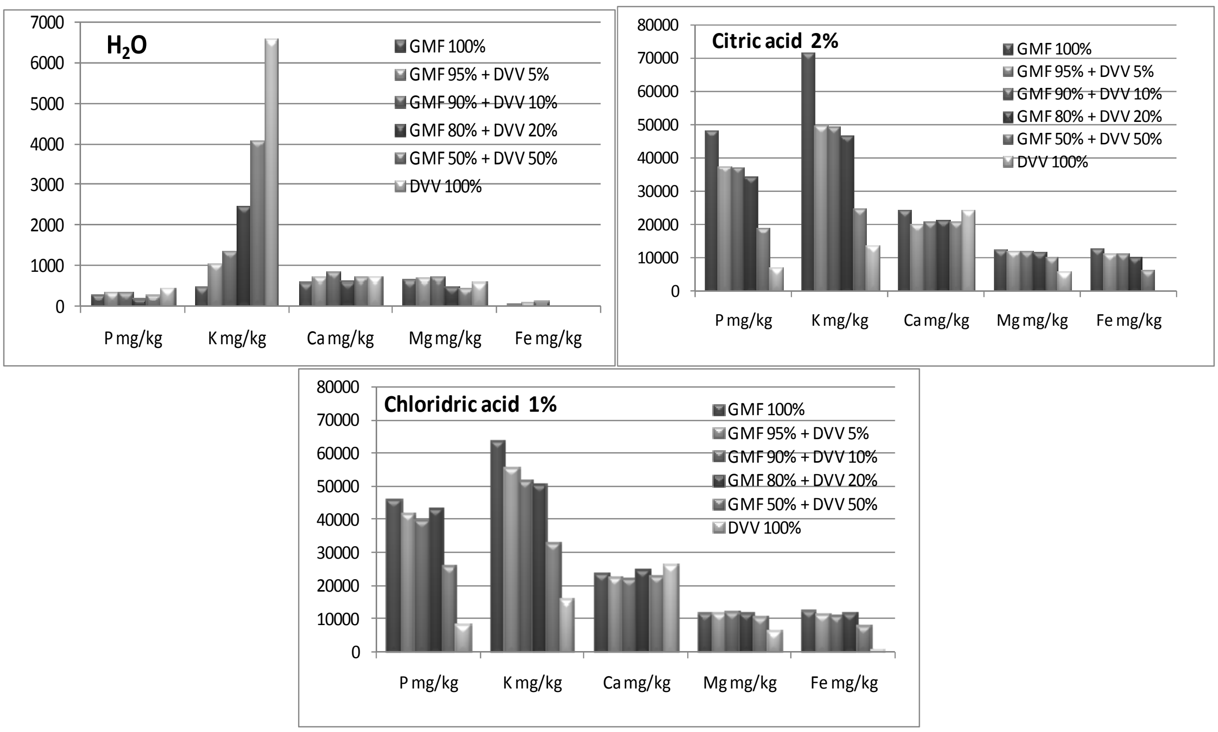
Short-term pot trial – A short-term pot trial (Subler et al., 1998; Tattini et al., 1991) on *Zea mais* L. was conducted to verify the effect of the different mixtures GMF 100%, GMF 95%+DVV 5%, GMF 90%+DVV 10%, GMF 80%+DVV 20%, GMF 50%+DVV 50%, DVV 100% on maize growth; pots were filled with an inert substrates (quartz sand) and maize seedlings were grown in a growth chamber for 2 weeks. Fertilizer application was 1.5 g/L for all the tested formulates. At the end of the trial, maize root weight (g), shoot weight (g) and total biomass produced (g) were recorded.

Agronomic test - From 2007, a parallel agronomic test was realized in Acireale (CT-Italy) on "Tarocco scirè" orange trees [Citrus sinensis (L.) Osbeck] grafted on two different rootstocks [*Citrange carrizo* (L.) and *Citrumelo swingle* (L.)]. The research was conducted in 50 liter pots, utilizing two different soils, a calcareous soil (9% CaO) and a volcanic soil (0% CaO), fertilized with the mixture GMF 70%+DVV 30% and with GMF 100%, compared to the unfertilized control (5 replicates × treatment), with the aim to verify the effects of treatments on growing plants. All plants received the same rate of ureic N, P, K and Fe (Rombolà and Tagliavini, 2005; Torrisi and Intrigliolo, 2009). Fertilization was made each year on the 1° decade of March, for three years. After 2 years, measurement of average stem elongation (in cm) and trunk circumference (in cm) were performed.

Results and discussion

Nutrient release test – Obtained results from the nutrient release tests indicated a low solubility of all nutrient elements in H2O (not more than 5% of the total contents), while both in citric acid and in HCl 1%, nutrients release increased notably (Figure 1). In particular, it should be remarked that in citric acid 2%, the amount of extracted nutrients was quite similar, or sometimes higher, to that obtained in HCl 2%.

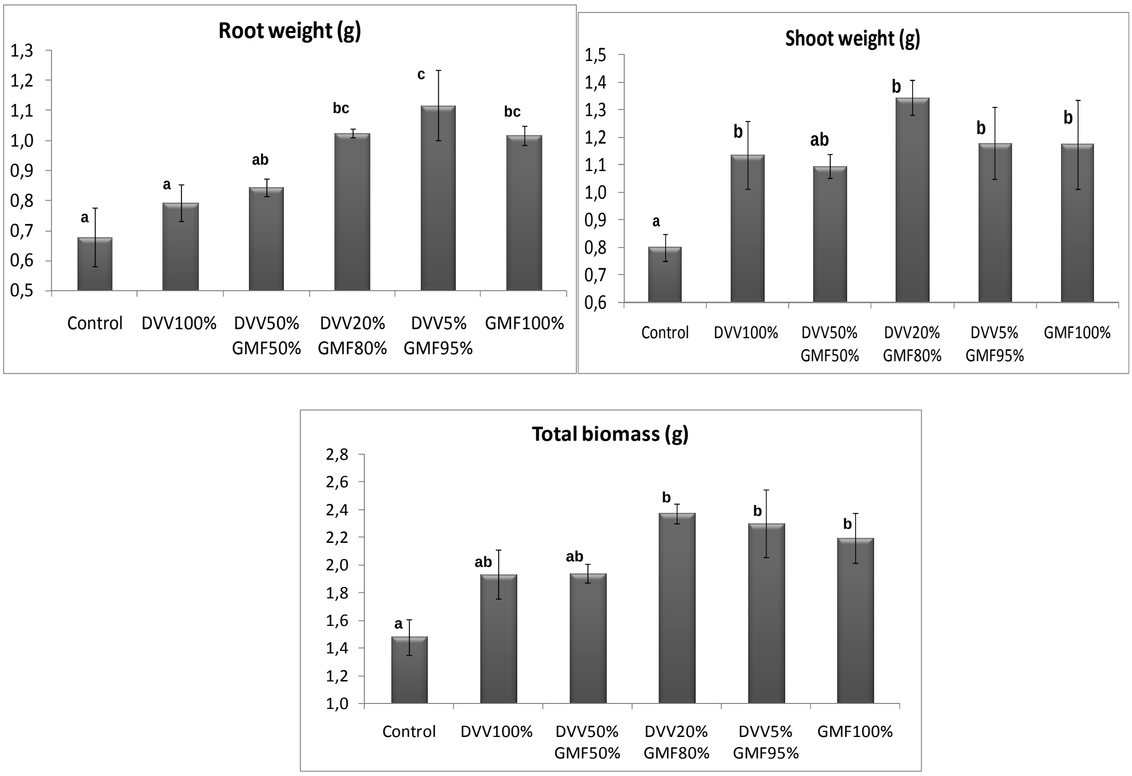
Figure 1: Nutrient release of tested mixtures in water, citric acid and chloridric acid.



It is important to point out that P, K, Fe solubility was the highest in citric acid 2%, which is an organic acid with lower acidic properties with respect to the chloridric acid: this finding indirectly confirmed the hypothesized mechanism of nutrient release based on plant demand, being citric acid one of the typical complexing root exudate: so, the extraction by citric acid simulates clearly what happens in chemical mobilization operated by plants in soil (Classen et al., 2001).

Short-term pot trial – In relation to the different treatments, maize root weight was the highest at GMF 95%+DVV 5%, while shoot weight resulted higher respect to the control for all the tested mixtures. As a consequence, total biomass produced gave the best results at the mixture 80% GMF + 20% DVV (Figure 2).

Figure 2: Maize Root weight, shoot weights and total biomass (g) of maize in relation to the different treatments.



What emerged was a synergic effect, due to the contemporary application of GMF and DVV: even if an increase in biomass production respect to the control was recorded also for GMF 100% and DVV 100%, the possibility to add the digested vine vinasse to the glass-matrix based fertilizer further promoted maize shoot growth, especially when the DVV was mixed at the lower amounts (5-20%). Probably, the DVV addition determined an improvement in nutrient availability, due to the complexing properties of the organic matter coming from the vine vinasse matrix.

Agronomic test - First results obtained after 3 years of experiment showed that the mixture GMF 70% + DVV30% improved plants development only in calcareous soil, being the stem elongation (Figure 3) and the trunk circumference (Figure 4) higher respect to those obtained for untreated plants.

Figure 3: Average stem elongation (cm) of citrus orange plants (2007-2009)

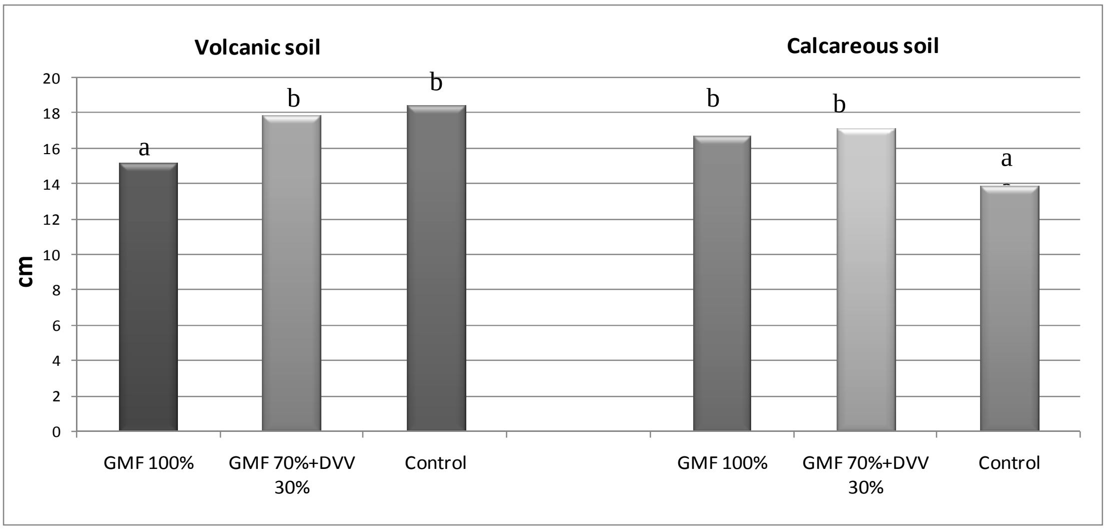
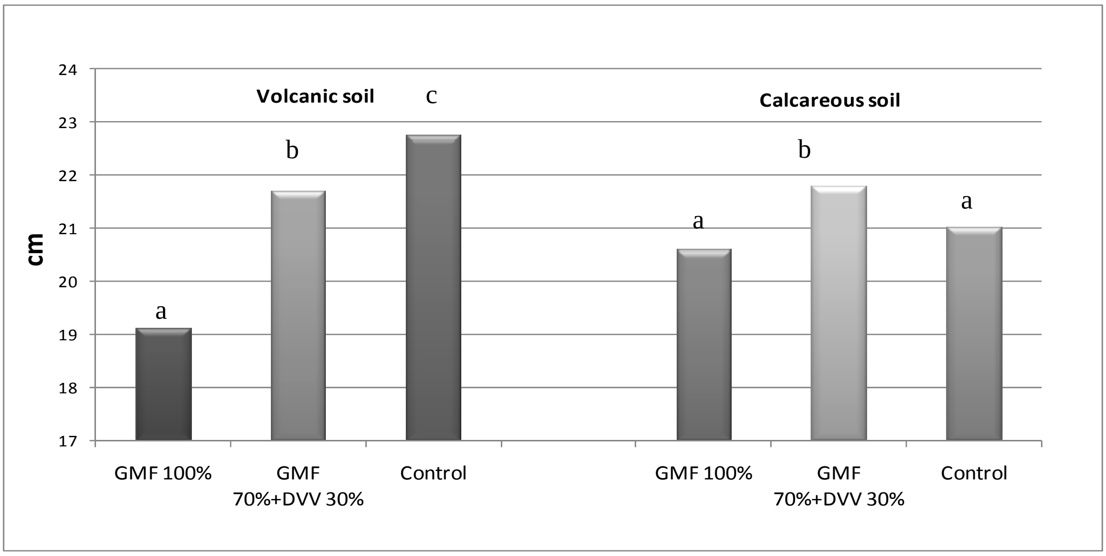


Figure 4: Trunk circumference (cm) of citrus orange plants (2007-2009)



Results obtained showed that probably the glass-matrix based fertilizer, especially when mixed with DVV, implemented its action in the worst conditions, that means in highly calcareous soil, while it didn't work well in soil where the CaO in absent: we hypothesized that, in a soil-plant environment characterized by a reduced availability of nutrients (i.e. Fe, Mg), an activation of the mechanism of nutrient plant-demand took place, by increasing plant root exudation and thus favoring the release of nutrients by the mixture GMF 70% + DVV 30%.

In conclusion, even if the reported trials gave only indicative information, results obtained were positive and seemed to attest that the hypothesized mechanism of action go through the increase of fertilizer nutrients availability by root exudation: this release-system became active particularly in critical conditions, when soil characteristics did not favor the nutrient element uptake.

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INFLUENCE OF BIOLOGICAL FERTILITY OF SOIL ON METHYLENUREA BIODEGRADATION

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Abstract

The objectives of this paper were to study the behaviour of different methylen-urea in soil, in comparison with other nitrogen mineral fertilizers.
The study was carried out in laboratory, by adding n.2 methylen-urea fertilizers, having the same molecular average-size, but obtained by different polymeric reactions, in comparison with urea and ammonium sulphate, to n.3 different soils, characterized by different biological fertility (on the basis of soil CO_2 evolution, C-microbial biomass and total organic matter contents, metabolic and mineralization quotients, etc.). The N-release fractions were determined according to different methodologies: (i) quartz send column leaching, (ii) EN 13286 sequential extraction and (iii) by biochemical leaching method (Stanford and Smith method, modified by Benedetti, 1994).
Obtained results showed the strong influence of soil biological fertility on nitrogen release: in the high fertility soil, during the first weeks of the experiment, the amount of nitrogen released by the methylen-urea fertilizers was significantly higher than that released in the median and low fertility soils, also in relation to the different typology of tested methylen-urea fertilizers. This information could be usefully utilized to make the best fertilizer choice when we need to reduce N leaching in soil, particularly active in microbial degrading of organic matter.

Keywords: Nitrogen, methylen-urea fertilizers, slow release, soil fertility.

Introduction

In the last decades appeared on the market a large number of new fertilizers defined "environmental friendly", conventionally able to guarantee a nitrogen slow-release trend, to avoid the nitrate leaching in the waters (Marashs, 2001). The methylen-ureas are of sparingly soluble products which evolved during the 1960 to 1970. The degree of polymerization of these products depend largely on the ratio of urea and formaldehyde during the condensation process (Alexander and Helm, 1999).

The objectives of this paper were to study of the behaviour of methylen-urea fertilizersin the soil, in comparison with other commonly-used nitrogen mineral fertilizers.

Material and Methods

The study was carried out in laboratory, by adding two methylen-urea fertilizers to three different soils, characterized by different biological fertility: Lodi soil, Pavia soil and Como soil, from Lombard Region (Italy).

The soil classification was made on the basis of soil organic carbon (C_{org} %), total organic matter (OM %), C-CO_2 evolution, C-microbial (C_{mic}) biomass and total organic matter contents, metabolic (qCO_2) and mineralization (qM) quotients (Benedetti et al., 2006). The applied methylen-urea fertilizers had the same molecular average-size, but they were obtained by different polymeric reactions, being characterized by different Activity Index (AI = hot water solubility / cold water solubility): 44% and 51%. Tested fertilizers were named AI 44 and AI 51, respectively.

In our lab-tests, the condensed fertilizers were compared to urea (U) and ammonium sulphate (AS), taken as controls.

Their N-release fractions were firstly determined according to the well-known method of sequential extraction in water (EN -13286).

The biochemical lab-test was based on a leaching method (Stanford and Smith method, modified by Benedetti, 1994). In particular, each fertilizer was added to 50 g of air-dried soil at the rate of 250 mg N $kg \times soil^{-1}$) and mixed with quartz sand in a 1:1 ratio. The prepared soil mixtures were incubated at 60% WHC (pF 2.5) at 30°C and then eluted with 0.01M $CaSO_4$ after 1, 2, 4, 6, 8, 12 and 16 weeks, by determining the leached NO_2-N, NO_3-N and NH_4-N by colorimetric method. These different N forms, recorded after each leaching, constitute released N and allow to obtain a cumulative N-release curve for each fertilizer on time.

Results and Discussion

The biochemical characteristics of three different soils are show in Table 1.

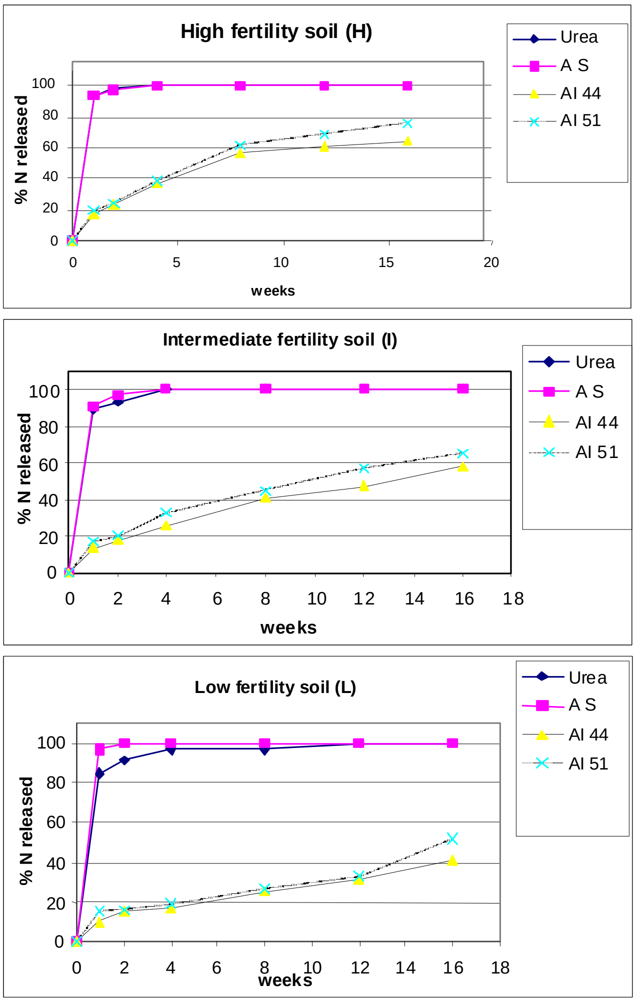
The recorded differences in C_{mic} and, particularly, in metabolic quotients (qCO_2) and mineralization quotients (qM) indicated clearly that the considered soils are characterized by decreasing microbial activity coming from H to L soils.

Table 1: Biochemical characteristics of the three considered soils.

Soil	C_{org}	OM	C_{mic}	qCO_2	qM	Biological fertility
	%	%	$mg\ C \times kg^{-1}_{soil}$	$mg\ CO_2\text{-}C \times mg\ C_{mic}^{-1} \times h^{-1}$	$[(mg\ CO_2\text{-}C \times kg^{-1}_{soil}) \times mg\ C_{org}^{-1} \times d^{-1}]$	
Lodi	1.1	2.0	135.7	0.091	11.2×10^{-4}	High (**H**)
Pavia	1.2	2.1	221.3	0.043	7.9×10^{-4}	Intermediate (**I**)
Como	1.3	2.2	170.1	0.033	4.3×10^{-4}	Low (**L**)

Obtained results in relation to the N-release curves showed the strong influence of soil biological fertility on nitrogen availability on time, as emerged from Figure 1.

Figure 1: N-release curves for the tested fertilizers in the H, I and L soils by biochemical test.

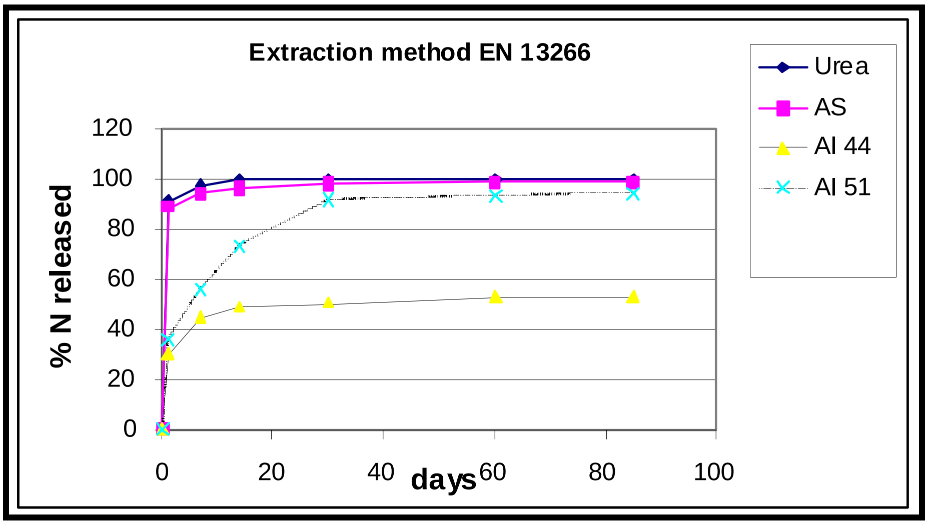


In the high fertility soil (H), during the first weeks of the experiment, the amount of nitrogen released by the methylen-urea fertilizers was significantly higher (~60%) than that released in the intermediate (I) and low (L) fertility soils (~30-40%). In relation to the different typology of tested methylen-urea, the fertilizer AI 44 released less N respect to the AI 51, in all the considered soils. The differences in nitrogen release were more emphasized in the low fertility soil C), where at the end of the experiment, AI 51 released 10% more than AI 44.

Furthermore, released N from methylen-urea fertilizers appears lower than released N from urea and A.S. in the three considered soils.

When we compared results coming from biochemical tests to those obtained by sequential extraction method (Figure 2), we observed that, while urea and ammonium sulphate released 100% of N just after the first week of extraction, the two methylen-urea modulated N release in a different way: at the end of the experiment (90 days), AI 44 released not more than 50 % of the total amount of nitrogen, while AI 51 released about 90% of the total N.

Figure 2: N-release curves for the tested fertilizers by EN method.



This results allow to affirm that the behaviour as N slow-release fertilizer of the considered two methylen-urea fertilizers was influenced by chemical and biochemical factors with different weights: water availability certainly constitutes one important factor in fertilizers' N solubilisation, as emerged by the application of EN method (water sequential extractions), but indubitably the microbial activity in soil assumes a key role, thus reducing or increasing N release on time in relation to the different soil biological fertility.

Information coming from these results could be usefully utilized to select the best fertilizer when we need to reduce N leaching in soil, particularly active in microbial degrading of organic matter.

A correct choice should be based not only taking into account the *routine* chemical tests, based on sequential water extractions or Activity Index calculation, but also by taking into consideration the specific biochemical characteristics of soil and its microbial biomass, active in degradation and mineralisation processes.

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REACTIONS IN SOILS AND FERTILIZERS AFFECT THE AVAILABILITY OF MICRONUTRIENTS TO PLANTS

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Abstract

Accessory compounds in mineral fertilizers can have a marked influence on the availability of nutrients to plants. When zinc (Zn) sulphate is incorporated into granulated NPK fertilizers, formation of sparingly soluble ammonium zinc phosphates may decrease the fertilizer effect of Zn. Granulation with superphosphate or phosphogypsum keeps Zn easily soluble, owing to the low pH upon dissolution of the carrier. These carriers can also be used with copper (Cu). Separate application of manganese (Mn) salt to soil commonly has no fertilizer effect but manganese sulphate in NPK fertilizers has been successfully applied to sugar beet to correct Mn deficiency in Finland. Applied with the placement method, the NPK granule gets into the zone of root proliferation, where lower redox potential and slightly lower pH enhance Mn solubility. Also selenium (Se), incorporated into fertilizers as Na_2SeO_4 in Finland, substantially increases the Se content of the crop. Fertilizers can also have indirect effects on the solubility of soil micronutrients by manipulating the pH in the dissolution zone. Nitrogen application as ammonium lowers soil pH and decreases molybdenum (Mo) uptake by the grass while Cu and Zn uptake can be enhanced.

Keywords : granular fertilizers, solubility, micronutrients, selenium.

Introduction

In basic research of plant nutrition, nutrients are usually added as separate readily soluble compounds or solutions which guarantee their immediate availability. They are often mixed evenly into the growing media. This setup is far from the practical use of mineral fertilizers in agriculture. Fertilizers are usually applied as granules, which can contain sparingly soluble compounds formed during manufacturing. Dissolution of a granule, particularly after localized application, manipulates the pH and element concentrations around the granule and may impact the solubility of nutrients from the granule and in the surrounding soil. In particular micronutrients can behave differently when they are applied separately or when they are incorporated in multinutrient compound fertilizers. Application of micronutrients to acid soils can be used to correct physiological deficiencies of plants or to increase the concentrations of these nutrients in the crop. The small amounts of the micronutrient compounds are difficult to be spread evenly in the solid state. Therefore carriers may be used to dilute the micronutrient concentrations. Selection of the carrier is critical, because some alternatives, such as $CaCO_3$, can have an adverse impact on the solubility of the nutrient concerned. The reactions mentioned above are mostly unknown to fertilizer dealers and users, who make decisions only on the basis of physical quality and total concentrations of nutrients in the fertilizer. Fertilizers are no more a topic of intensive research in public institutes and few papers on their behaviour are published in scientific journals. Therefore, there is little awareness of the impacts of these reactions also among the current generation of researchers. It is the manufacturer that has to take the responsibility of the proper functioning of their products.

This paper contains results of the effect of Cu, Mn, Se and Zn, applied to soil in different fertilizers. Particularly, the results show the potential of phosphogypsum ($CaSO_4$•2H2O) as a carrier for micronutrients. Additionally, the effect of fertilizer applications on the solubility of some soil nutrients is demonstrated. The paper is partially based on published results and partially on original material.

Materials and Methods

In all the pot experiments two vegetative yields of barley were grown in 8 dm^{-3} Kick-Brauchmann pots with four replicates. Two soils originated from plough layers of limed non-calcareous fine-sandy Dystric Regosols (pH 6.2 and 7.3). In one experiment commercial sedge peat (pH 5.6, intermediate degree humification) was used. The fertilizers containing Zn included zinc sulphate granulated with superphosphate (Zn-SSP, 3.6% Zn) or phosphogypsum (Zn-gypsum, 2.7% Zn) and a NPK fertilizer fortified with 0.3% Zn as zinc sulphate incorporated into the fertilizer granule upon manufacturing. As for Cu, copper sulphate granulated with superphosphate (Cu-SSP, 6.0% Cu) and gypsum (Cu-gypsum, 4.7% Cu) were tested. Separate application of copper sulphate, zinc sulphate and a compound NPK + Mg, S fertilizer were used as references. Soil pH was measured in water (1:2.5) and the fertilizer pH stands for the values in 10% water solutions. The fertilizers were mixed with the soil before sowing the first barley and additional NPK + Mg,S fertilization was applied to the second crop.

Zinc fertilizers were studied also in a 2-year field experiment with timothy on a Vertic Stagnosol, consisting of non-calcareous (pH 5.7) clay loam. The original results have been published by Yli-Halla (1993). Zinc was applied as a single dose of 1) zinc sulphate or 2) Zn-gypsum, drilled into the soil upon sowing the timothy in the previous autumn, or 3) as a top dressing of Zn-gypsum in the first spring of the experiment. Also, 4) the Zn-fortified NPK fertilizer was top-dressed to all the four grass yields harvested during the two experimental years. All the plots received the same amounts of NPK. To study Mn fertilization of sugar beet, seven field experiments were carried out in southern Finland on limed non-calcareous (pH 6.9-7.6) Regosols, Cambisols and Stagnosols of loamy or clayey textures. The original results have been reported by Erjala (1986). Using granular compound fertilizers, 115 kg N ha^{-1} and adequate quantities of other nutrients except Mn were applied with broadcast or placement method. Mn was supplied either as foliar application of manganese sulphate solution or incorporated into the compound fertilizer (0.7% Mn). The effect of Se-fortified NPK fertilizers on the Se concentration of barley was reviewed from the monitoring reports (Eurola *et al.*, 2003, Ekholm *et al.*, 2005), based on a large number of grain samples collected from farms. The impact of nitrogen fertilization (0 – 600 kg N ha^{-1} annually) on soil pH and Cu, Zn and Mo contents of meadow fescue – cocksfoot silage grass in 18 three-year field experiments was collected from Rinne *et al.* (1974). - All the results were subjected to analysis of variance followed by the Tukey's test to identify the statistically significant differences.

Results and Discussion

Zinc (Zn) sulphate is the most common compound used as Zn fertilizer. In Finland, Zn fertilization is recommended to increase the Zn concentration of fodder crops. To get a product which can be easily spread (e.g. 200-400 kg ha^{-1}), it has been granulated with phosphogypsum ($CaSO_4 \cdot 2H_2O$) or with superphosphate (SSP). Low pH (2.7 - 2.8) of SSP prevents the precipitation of poorly soluble zinc phosphates while phosphogypsum, even though having a higher pH (3.9 - 4.4), depending on the amount of phosphoric acid residues, does not have constituents to precipitate with Zn. Indeed, about 90% of Zn in these fertilizers was extracted with water, and these products increased Zn concentration of barley as effectively as did zinc sulphate alone (Fig. 1). Utilization of added Zn was 4.5-6.4%. Because Zn supply to the plants was above the deficiency level also in the control, Zn applications had no quantitative impact on plant growth. In soils of high phosphorus (P) status, application of SSP as the carrier is no more ecologically acceptable and in Finland phosphogypsum is currently used as the carrier in the separate Zn fertilizer.

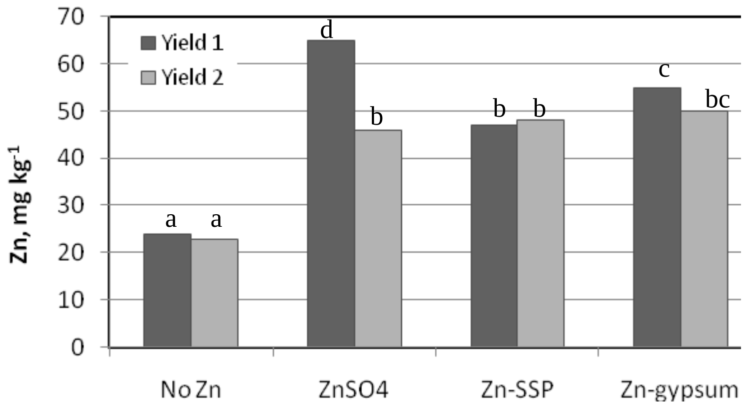

Figure 1: Zinc concentrations of two vegetative yields of barley in a pot experiment. The fertilizers were incorporated into the fine sand (pH 6.2). Zn application was 54 mg/pot. Columns not marked with a common letter differ from each other at P<0.05.

In a field experiment on Zn fertilizers (Table 1), the sum of four timothy yields (11.2 tons ha^{-1}) over two years were not affected by Zn applications. Zn concentration of the grass was markedly elevated upon Zn applications, except with Zn-fortified NPK fertilizer. In spite of an increase of Zn uptake, the utilization of added Zn was as low as 0.3%, which reflects the usual ineffectiveness of micronutrient fertilization applied to soil. A commercial Zn-fortified NPK fertilizers, which had pH values of 5.0 – 5.4 and about 10% of Zn in water-soluble form, had a negligible effect on Zn uptake of timothy in the field, when applied in granular form. When zinc sulphate is incorporated into the slurry upon the manufacturing of NPK fertilizers, sparingly soluble ammonium zinc phosphates and hydroxides may be precipitated. Addition of NH_3 increases the pH of the slurry and decreases the solubility of Zn to values less than 20% of non-ammoniated one (Mortvedt, 1968).

Table 1: Zinc concentration and uptake by four yields of timothy in a field experiment as a response to different Zn applications in a clay loam soil (pH 5.7). Zn fertilizers were surface-applied (surf) or incorporated (inc) into the soil. Numbers in a given column not marked with a common letter differ from each other (P<0.05). Results from Yli-Halla (1993).

Fertilization	*Zn-rate, kg ha^{-1}*	*Zn, mg kg^{-1}*	*Zn uptake, g ha^{-1}*	*Zn utilization*
No Zn	0	28^{a}	80	-
$ZnSO_4$, inc	6 (one dose)	34^{b}	92	0.20%
Zn-gypsum, inc	6 (one dose)	36^{b}	98	0.30%
Zn-gypsum, surf	6 (one dose)	37^{b}	100	0.33%
Zn-NPK, surf	4 x 1.3	30^{a}	82	0.03%

The impact of granulation was displayed in another pot experiment (Fig. 2) where the Zn-fortified granular NPK fertilizer had a slower fertilizer effect than the pulverized ones. Instead, the pulverized Zn-NPK was equally efficient as zinc sulphate applied alone. The insoluble Zn compounds formed upon fertilizer manufacturing seem to be solubilised in a circumneutral soil.

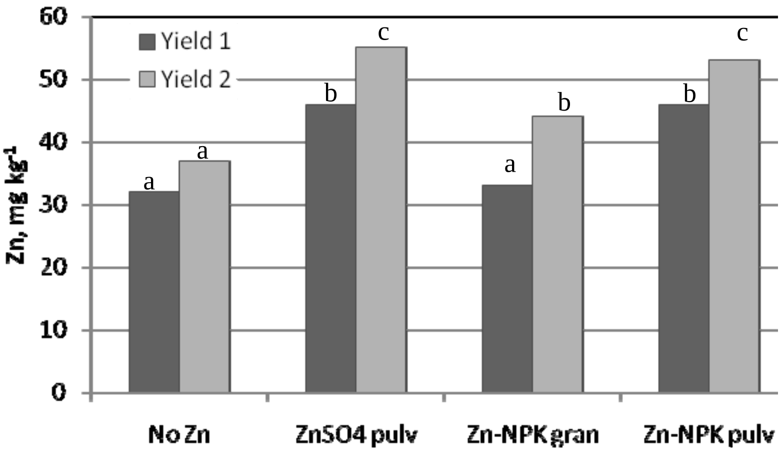

Figure 2: Zn concentrations of two vegetative yields of barley in a pot experiment. The fertilizers were incorporated into the fine sand (pH 7.3) as granules (gran) or in pulverized (pulv) form. Zn application was 28 mg pot^{-1}. Columns not marked with a common letter differ from each other at $P<0.05$.

Manganese Separate application of manganese (Mn) salt to soil has commonly no fertilizer effect. Mn incorporated as manganese sulphate in NPK fertilizers has been successfully applied to sugar beet in Finland. When incorporated into the NPK granule and applied according to the placement method, Mn gets into the zone of root proliferation. In the rhizosphere, lower redox potential and slightly lower pH enhance Mn solubility. The results in Table 2 (Erjala, 1986) show that placement of a Mn-fortified NPK fertilizer (pH 5.5) resulted in the highest yields and Mn concentrations in leaves and least deficiency symptoms of Mn. Even the NPK application alone without Mn seemed to mobilize soil Mn. Indicating higher supply of Mn to the crop, this fertilization practise resulted in higher concentrations of Mn in the tops than did broadcast application of Mn-fortified NPK fertilizer incorporated with harrowing.

Selenium (Se) has been incorporated into compound fertilizers as Na_2SeO_4 since 1984 in Finland. All fertilizers sold for field crops in Finland in 1998-2007 contained 10 mg Se kg^{-1} and in 2007 the concentration was elevated to 15 mg Se kg^{-1}. According to regular reviews (Eurola *et al.*, 2003), the concentration of Se in barley has increased from about 0.01 mg kg^{-1} up to 0.11-0.13 mg kg^{-1} in 1999-2001. Se concentration of crops has responded closely to the changes of Se applied to soil in compound fertilizers (Ekholm *et al.*, 2005). The utilization of added Se is usually 5-10%.

Table 2: Effect of different fertilizer applications on the yield and Mn supply to sugar beet in seven field experiments. Mn application was 2.6 kg Mn ha^{-1}. Numbers in a given column not marked with a common letter differ from each other (P<0.05). Results from Erjala (1986).

Fertilization	*Root yield (t ha^{-1})*	*Symptoms of Mn deficiency (scale 1-10)*	*Mn concentration of tops (mg kg^{-1})*
Top dressing of NPK	28.2 (100)[a]	3.0[c]	44[a]
Placement of NPK	28.8 (102)[ab]	1.0[a]	82[b]
Top dressing of NPK + foliar Mn	28.1 (100)[a]	2.3[b]	n.d.
Placement of NPK+ foliar Mn	29.7 (105)[bc]	0.7[a]	n.d.
Top dressing of Mn-NPK	28.2 (100)[a]	2.0[b]	56[a]
Placement of Mn-NPK	30.8 (109)[c]	0.8[a]	111[c]

Copper (Cu) cannot be incorporated into ammonium nitrate based NPK fertilizers because of a danger of decomposition. On the basis of a pot experiment, phosphogypsum and superphosphate can be used as carriers of Cu as well (Fig. 3), even though the utilization of added Cu (0.8-1.6%) was even lower than that of Zn. Cu additions had no quantitative effect on the growth.

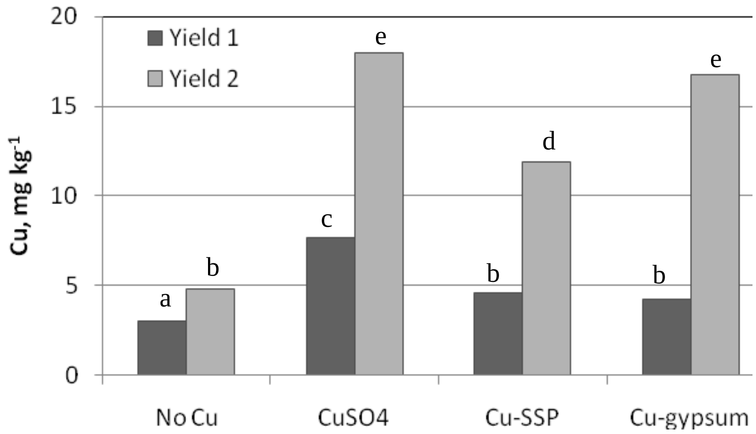

Figure 3: Cu concentrations of two vegetative yields of barley in a pot experiment. The fertilizers were incorporated into the sedge peat (pH 5.6). Cu application was 40 mg pot^{-1}. Columns not marked with a common letter differ from each other at P<0.05.

Manipulation of pH by fertilizer applications

Fertilizer compounds can have indirect effects on the availability of soil micronutrients by manipulating the pH in the dissolution zone. In field experiments heavy nitrogen applications lowered soil pH and decreased molybdenum (Mo) uptake by the grass while it increased Zn and Cu concentrations (Table 3, Rinne *et al.*, 1974). None of these micronutrients were added in the fertilizers.

Table 3: Soil pH and concentrations of Mo, Cu and Zn in meadow fescue – cocksfoot grass in 18 three-year field experiments upon different nitrogen applications. Results from Rinne *et al.* (1974).

Annual N application	*Initial soil pH*	*Soil pH after three years*	*Mo, mg kg^{-1}*	*Cu, mg kg^{-1}*	*Zn, mg kg^{-1}*
No N	5.79	5.69	1.94[b]	10.0[a]	30[a]
3 x 50 kg ha^{-1}	5.79	5.69	1.11[a]	11.1[b]	32[a]
3 x 100 kg ha^{-1}	5.78	5.63	0.87[a]	11.1[ab]	35[b]
3 x 150 kg ha^{-1}	5.78	5.49	0.74[a]	11.4[ab]	38[bc]
3 x 200 kg ha^{-1}	5.77	5.36	0.66[a]	12.1[b]	39[c]

Conclusions

Separate Cu and Zn fertilizers can be manufactured by granulation of copper and zinc sulphates with single superphosphate or phosphogypsum. Manganese and selenium can be incorporated into NPK without losing their availability to plants. Decrease of pH around a NPK fertilizer granule enhances the solubility of soil Cu and Zn and decreases the solubility of soil Mo.

Acknowledgements

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VII SESSION

Advances in fertilizer characterization and legislation - Miscellaneous

COMPOSTING OF RABBIT BY-PRODUCTS: EXPERIMENTAL TESTS AND DESIGN CRITERIA FOR A TURN-OVER/AERATING MACHINE

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Abstract

Rabbit manure and slaughtering (treated in autoclave at 133 °C and 3 bar, per 20 min) by-products have been used in a composting process. Three heaps of 4700 kg each have been investigated and experimental tests were carried out in an industrial horizontal axe reactor: 1) rabbit manure + rabbit slaughtering by-products + straw (C/N = 18,15); 2) rabbit manure + rabbit slaughtering by-products + straw (C/N = 16,2); 3) rabbit manure + rabbit slaughtering by-products (C/N = 14,9). The composting heaps were turned by means of a protoype of turning machine. The composting time lasted 85 days. For each examined heap, we examined the progression of fermentation process, so also the plant limitations that did not allow a correct composting process. The results allow for the chemical, physical and mechanical identification of the studied biomass. These are also useful for the development of appropriate mixtures, machines and plants assuring continuance and reliability in the composting of the biomass coming from rabbit industry. Thereby, producing compost which conforms to the law.

Keywords: rabbit by-products, heaps with several C/N, industrial scale composting plant.

Introduction

The production rabbit cycle should produce a commercial meat cut of approximately 2,5 kg, in approximately 70 days. During this period the animals are given a diet based on the following: products and by-products of oil-rich seeds, dry forage, grains, by-products of the sugar processing with the addition of dietary protein supplements, alpha-tocopherol, iron sulfate pentahydrate. In the first 25 days of the growing period the diet is also supplemented with iron, zinc, organic selenium, vitamin A and D3. The average feed intake is approximately 120 g/day x head with a production of manure approximately 195 g/head x day (King, 1984).

The scientific research carried out in the rabbit industry investigates primarily all the production aspects; however many breeders point out the need to study the environmental aspects concerning the intensive production and processing of rabbit meat. This is due to the fact that on many industrial farms the costs of the waste management, in terms of the law, have become very high in comparison to the company's turn-over, reaching even 3% of the total costs (ASIC, 2008).

Extensive data can be found concerning the research into composting process, but the available data refers to manure of fowls (Tiquia and Tam, 2000), hogs (Larney et al., 2002, 2006), bovines (Saludes et al., 2007) and other domestic animal species. Similar studies have not been carried out until now on rabbit manure, however laboratory tests have been carried out on the composting of organic mixtures which also contain chicken and rabbit carcasses, in order to monitor the development of the process (Barrena et al, 2009).

Therefore, a research study is in progress at an industrial farm breeding rabbits, with an annexed slaughtering house processing the on-site-bred animals, situated in Martina Franca area, province of Taranto (Southern Italy), in order to study the process of complete stabilization and humification of the farm's waste and by–products. In the present paper results will be shown about chemical-physical biomass characterization and designing for specialised composting plants.

Materials and methods

The experimental tests were carried out from 4/02/09 to 29/04/09 and from 11/07/09 to 11/10/09. The rabbit breeding farm consists of 20.000 heads and 2.000 breeding rabbits; the annexed slaughtering plant has CE licence and 1.500 heads/week are slaughtered. The average manure production is 3.000 kg/day, while the slaughtering by-products correspond to 1.500 kg/week. Before mixing with the manure, slaughtering by-products undergo treatment in an autoclave at 133 °C and 3 bar per 20 min. The slaughtering waste water is treated in an active sludge purifying plant, then it is distributed on the land. The sludge is almost completely re-used in the purifying process.

An industrial scale plant with simplified design criteria has been developed, based on a plant adapted for other mixtures, in the course of a previous study.(Fig. 1). Three heaps with a width of 2,0 m were designed on the plant's assembly chain. The starting mixtures were each with a mass of approximately 4,7 tons, but with different compositions:

1. Mixture: purifying sludge and slaughtering by-products with C/N=15,90 (Tab. 1);

2. Mixture: purifying sludge and slaughtering by-products + straw with C/N=16,20 (Tab. 2);
3. Mixture: purifying sludge and slaughtering by-products+ straw with C/N=18,15 (Tab. 3).

Width of the turn-over machine	4,2 m
Length of the turn-over machine	1,9 m
Height of the turn-over machine from the work surface (go/come back)	1,25 m/2,25 m
Height of the mixture into the tunnel	0,6 m
Advancing passage	1,2 m
Volume turned for each passage	2,88 m^3
Mass moved for each passage ($\gamma \approx 980\ kg/m^3$)	2822 kg
Advancing speed (go)	25 m/h
Fit power	15,0 KW
Deep dimension (length x width x depth)	89,0 m x 4,0 m x 0,60
Roof height (filled up line)	4,0 m

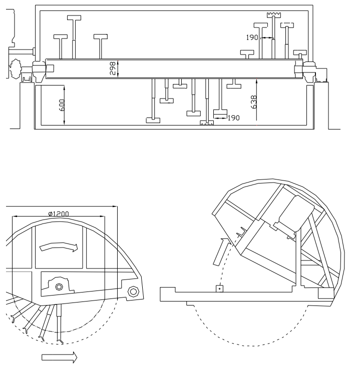

Figure 1: Diagram of the prototype turning-over and aerating machine and main technical characteristics of the composting plant.

The straw, with a width of 5 cm, was chopped by a common agronomic straw shredder, while all components of each mixture were mixed together with a rototiller.

Using the turning-over and aerating machine, each of these heaps was turned and aerobic conditions were maintained by way of advancement of the mass of approximately 1 m/day, for a period of 85 days; during this period the ambient temperature, outside and inside the plant, was monitored as well as inside the mass, at a distance of 10,0 cm from the bottom of the pit and 10,0 cm at the top of the heap. At variable intervals, between twenty and thirty days, the following analytical evaluations were carried out on the composting heap: total nitrogen (Kjeldahl method), total organic carbon (TOC), humidity (%), pH in H_2O, electrical conductivity. From the final sample taken from each heap the following values were measured: iron, nickel, lead, zinc and fertility parameters. The determinations were effected according to the official protocols of fertilizers analysis, by the Ministry of the Agricultural and Forest Politics. These assessments were executed according to official methods and according to the law, (Reg. CE n.2003/2003, D.L n° 217 of the 29/04/2006), repeating each analytical evaluation 3 times. In order to analyze the fraction of organic nitrogen and ammoniac, an automatic steam flowing distiller was used UDK 130 D- Velp scientific, integrated with a DK 6 Heating D digester. The specific electrical conductivity in a liquid sample was determined with the use of a conductivity meter "Crison Conductivity meter 524"; the measurement of the pH was carried out on the watery suspension, prepared in order to determine the conductivity, using a pH meter "Crison micro TT 2050".

Results and Discussion

The control mixture has a very high level of nitrogen and a relatively low level of carbon (Tab. 1); the humidity is slightly higher than the optimal limits and should be critical in order to the possibility of the studied by-products being mixed with other moist materials.

Table 1: Monitoring of the main characteristics of the "mixture 1" during composting (C/N = 15,90).

Parameters	Initial Sample 06/02/2009	Sample 27/02/2009	Sample 26/03/2009	Sample 26/04/2009
Moisture %	75,74	58.70	55.10	48.89
pH	8.70	8.40	8.85	8.90
Total Organic Carbon (%)	38.20	38.50	35.20	33.40
N_{tot} (%)	2.40	2.50	2.40	2.70
C/N	15.90	15.40	14.70	12.37
Electrical conductivity (dS/m)	3.46	3.56	3.58	4.06

An increase of the temperature profile along the cross-section, starting from the lowest layer of the heap, points out that the fermentation starts more rapidly in the upper layers and that inadequate mixing takes place as well (Fig. 2). In the control mixture the difference in temperature between the upper and the lower layers reaches 7 °C (Fig. 2A), while in the other mixtures the difference does not exceed 3 °C (Fig. 2 B-C).

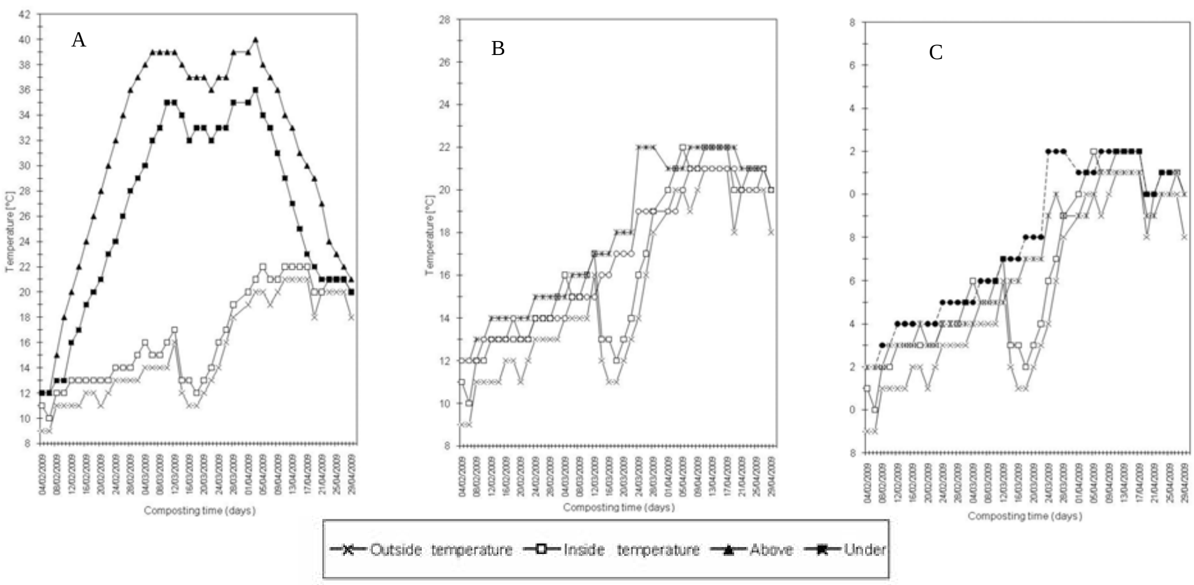

Figure 2: Temperature profiles registered during composting test (winter time). A: mixture 1 (C/N = 15,90); B: mixture 2 (C/N = 16,2); C: mixture 3 (C/N = 18,15).

During the winter trials (Fig. 2) the temperature remained for a long period of time rather low and it rained a lot. Anyway, the building that shelters the heap from the outside ambient does not offer adequate thermal insulation, considering that the inside ambient temperature was at the most 2 °C higher than the outside temperature (Fig. 2). On the contrary, in the specific case, the used perimetral and covering materials must be able to retain the heat produced by the biomass.

The control mixture is the only heap where a bio-oxidation process took place (Fig. 2). In the first 30 days the temperature rose from 12 to 39 °C, then remained constant for a week, to then decrease in the following 10 days, increasing again from 36 to 40 °C in the next 10 days and finally to decrease during the last 20 days of the trial. The final mass temperature was the same as the ambient temperature which, due to the fact that the seasons changed, resulted in being higher compared to the temperature at the beginning of the trial. In comparison, the mixtures with straw did not show a temperature increase in relation to the ambient temperature; the heap temperature profile followed that of the ambient temperature with deviations between 2-4 °C. It also did not show any significant signs of microbial activity (Fig. 2 B-C).

In the control mixture an incomplete fermentation process is reached, limited only to the mesophilic phase, with a maximum temperature much inferior to 60 °C. The process is influenced by the ambient temperature and when the temperature remains under 15 °C it tends to block the metabolism of the micro-organisms, however when there is an increase from 13 to 22 °C the increase leads to a re-activation of the bio-oxidation activity, despite the mass being in a phase of cooling-down. This also points out that on the thirty-first day of the trial there is a not completed stabilisation of the product evidenced by an organic sub-layer which is set for an intensive bio-oxidative activity.

During the whole process, including the phase of maturation, the humidity level of the control mixture remained superior to 55%; these values can be considered compatible with the evolution of biological reactions (Tab. 1). Despite periodic irrigation, the mixtures with the added straw dried out excessively after approximately fifty days of processing. At a humidity level of 30-35%; the microbial activity proceeds with difficulty or very slowly (Tab. 2-3).

Table 2: Monitoring of the main characteristics of the “mixture 2” (C/N ratio = 16,20).

Parameters	Initial Sample 06/02/2009	Sample 27/02/2009	Sample 26/03/2009	Sample 26/04/2009
Moisture %	73.04	65.60	37.30	25.93
pH	8.02	8.10	8.06	8.31
Total Organic Carbon (%)	34.05	39.30	29.30	20.90
N_{tot} (%)	2.10	2.50	1.90	1.50
C/N	16.20	15.70	15.42	13.90
Electrical conductivity (dS/m)	3.52	3.59	3.61	3.56

Table 3: Monitoring of the main characteristics of the "mixture 3" (C/N ratio = 18,15).

Parameters	Initial Sample 06/02/2009	Sample 27/02/2009	Sample 26/03/2009	Sample 26/04/2009
Moisture %	74.02	59.70	32,60	31,00
pH	8.05	8.60	7.70	7.80
Total Organic Carbon (%)	34.50	37.10	25.00	23.80
N_{tot} (%)	1.90	2.10	1.70	1.70
C/N	18.15	17.70	14.70	14.00
Electrical conductivity (dS/m)	3.75	3.77	3.88	3.81

The activity was absent in the previous trial as well, even though the mixtures had an adequate amount of humidity. The C/N ratio of the studied biomasses (Tab. 1-2-3) were not at all at such levels in order for the process of stabilisation to slow down; in fact, even though they were low, these values corresponded perfectly with the limits determined by D.L. 217/06. That is C/N values fluctuating between 25 and 50 are allowed for all typologies of amends (mixed compost amend).

The lack of variation of pH in mixture 1 does not only confirm that the complete thermopilic phase has been reached, during which there is the maximum development of the ammoniac bacteria, but also that during the maturation phase there has not been any significant activity of the nitrificant bacteria which transform the ammoniac subsequently into nitrous and nitric acid (Saludes et al., 2007) with a decrease of pH level. In any case, the pH values can be considered optimal with regards to the national and international regulations.

The studied mixtures present average values of electric conductibility: from 3,56 to 4,06 dS/m with a tendency to assume maximum values towards the end of the process (Tab. 1-2-3) when the humidity level diminishes. Nevertheless, there is no risk of high salinity for these mixtures; even though the D.L 217/06 does not set limits for this parameter, international regulations allow for maximum levels not exceeding 5 dS/m. Therefore, the obtained data can be considered within the norm.

The research carried out on the other chemical parameters present in the composted material show that in the starting mixtures there is a considerable quantity of organic substances which has the potential of being composted (Tab. 4).

Despite the optimal chemical composition, the compost processes did not proceed as expected. In fact, in the first heap the temperature, due to excessive compressing of the biomass and therefore insufficient aeration, did not exceed 40 °C. Also in the other two heaps microbiological problems occurred, which inhibited the active phase of the process. Nevertheless, the chemical parameters show levels of T.O.C which are only slightly inferior to the minimum values allowed by D.L. 217/06. Also the C/N ratios correspond to the legislative requirements, that is, they were inferior to the allowed maximum values (Tab. 4). The percentage of humic and fulvic acids was just below 7% for the first and second heap and more than 7% for the third heap; therefore the data can be considered not negative.

Table 4: Heavy metal residues and main fertilizer parameters of the mature compost.

Parameters	Limit (D.L. 217/06)	Mixture 1 – C/N = 15,90 (26/04/2009)	Mixture 2 - C/N = 16,20 (26/04/2009)	Mixture 3 - C/N = 18,15 (26/04/2009)
T.O.C. (%)	> 25	33.40	20.90	23.80
N_{tot} (%)	/	2.40	2.50	2.40
C/N	< 25	12.37	13.9	14
(HU +FU) (%)	> 7	6.64	6.23	7.17
HR (%)		16	21	22
Cu (mg/kg)	150.0	82.20	55.59	55.90
Ni (mg/kg)	100.0	3.21	2.33	2.21
Pb (mg/kg)	140.0	< 0.1	< 0.1	< 0.1
Zn (mg/kg)	500.0	459.30	290.5	291.10

For the above reasons the humidification rate (HR) resulted to be very low (Tab. 4), but considering the not regular composting process, one can hypothesize that by modifying the process parameters and the operative conditions this index could increase. Saying this, from an agronomic point of view, an aspect noteworthy to be pointed out with regards to the studied mixtures is the content of humidified organic substance (Tab. 4); in fact, even though the composting process was incomplete, the corresponding values are approaching the allowed limits defined by law, exceeding the limit in the heap with the added straw. Therefore one can conclude that it is sufficient to increase the C/N ratio in order to improve the biochemical yield of the process. No excessive values of heavy metals were shown and these were within the limits fixed by Italian low (D.L. 217/06). However, the amount of zinc should be monitored, given that the amount of zinc in the carried out tests is approaching the maximum limits (Tab. 4).

Graphs in Fig. 3 refer to the experimental test carried out in summer. The analytic evaluations are not complete, but the temperature profiles show a much better fermentation when the outside temperature is favourable.

In the control mixture the thermophilic phase was reached (Fig.3A) and in the two other mixtures the mesophilic phase was in an advanced state (Fig. 3 B-C). Also in this experimental test, the temperatures in the top of the biomass were higher than the lower layers. On the contrary, against to the previous test, no significant decreases in humidity were shown; therefore in these simple plants, the humidification system could be considered not very important, if the fermentation is carried out correctly.

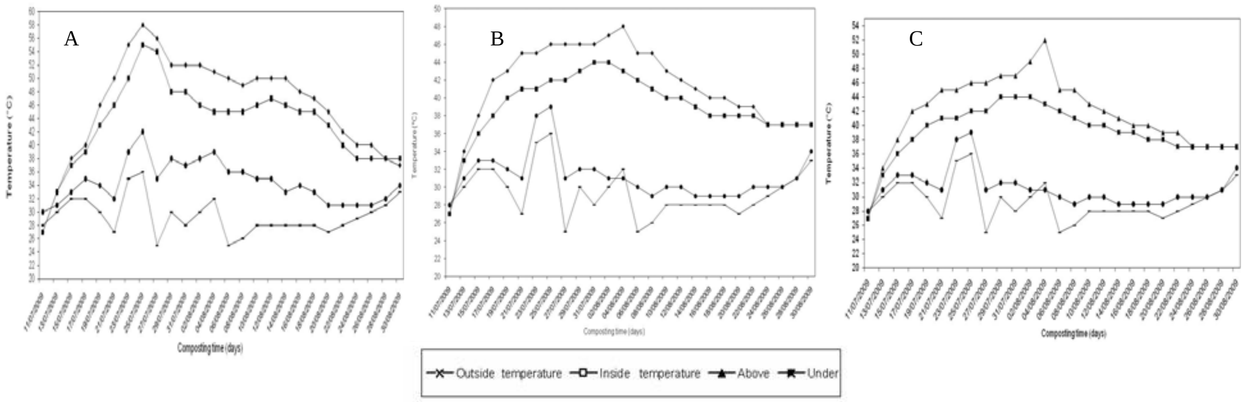

Figure 3: Temperature profiles registered during composting test (summer time). A: mixture 1 (C/N = 15,90); B: mixture 2 (C/N = 16,2); C: mixture 3 (C/N = 18,15).

Technical modifications on the plant and prototype of turn-over/aerating machine

In the trials which were carried out during the winter months the composting process could have been inhibited and in some cases blocked considering the following technical/operative parameters:

1 low temperature;
2 particles of organic matrix of dimensions which are too large;
3 insufficient aeration.

A solution could be to implement specialized equipment for each of these parameters, but at the same time one should take into consideration the average economic/ productive capacities of Italian rabbit farms; therefore big investments can not be made.

Regarding point 1), in the specific case, the used perimeter and covering materials must be able to retain the heat produced by the biomass, expecially in winter; moreover when the ambient temperature is too low, the possibility to heat the entering mass by way of pipes with circulating warm water on the bottom of the trough, could be considered. The adaptation would be limited to only 1/3-1/4 of the total length.

A specialized machine would be more suitable for point 2), in order to obtain pieces of straw with dimensions inferior to 5,0 cm; moreover, the prototype of the turn-over machine has to be modified with mechanical adaptations, to have an adequate mixing of the elements present in the organic matrixes both while entering and during the turning over of the mass in the trough. The possibility to improve the C/N ratio with easily available waste products and better mixing potentiality should not be neglected. In Apulia a source of carbon could be olive pomace if de-humidified correctly.

In order to resolve point 3) and partially also point 2) the following mechanical adjustments to the prototype of the turn-over/moving machine, have been programmed:

- reducing of the length of the turnover elements (Fig. 4), which have been welded to the traversal reel and a lowering of the above relative to the ground in order to allow for a better penetration into the mass and to obtain a better turn-over over a shorter distance; in this way, besides a reduction in energy consumption, it is also possible to effect more trips compared to the covered distance, thereby mixing better the upper layers as well as the lower layers;
- modifying the shape and the profile of the turn-over elements (Fig. 5) in order to improve the mixing and to aerate the mass still further when the machine is returning, therefore effecting a rotation in the opposite direction of the reel without moving back the material that has been pushed forward, therefore simply lifting it up.

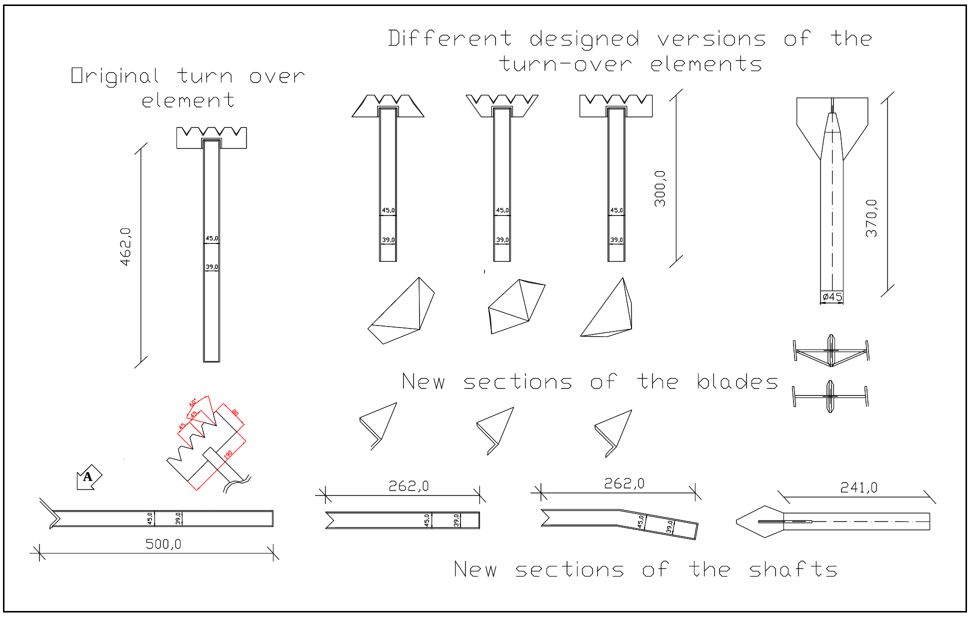

Figure 4: Technical modifications on the prototype of turn-over/aerating machine.

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AVAILABILITY OF SELENATE AND SELENITE ADDITIONS IN PEAT SOIL

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Abstract

The behaviour of selenium (Se) added to peat soils is unclear. The content of oxides that would adsorb Se is typically low, but also organic matter has been shown to adsorb Se. We examined the effect of these binding sites on Se behaviour and bioavailability by manipulating the oxide content of poorly humified sphagnum peat in a pot experiment. Two successive harvests of Italian ryegrass (*Lolium multiflorum* L. cv. Meroa) were grown on twelve combinations of three iron oxide and four selenium additions. We determined the amounts of Se in the leaf mass and Se remaining in the soil as easily soluble (NH_4Cl-extractable) or adsorbed (phosphate buffer-extractable) forms at the end of the experiment. Selenate had remained mainly salt soluble throughout the experiment and the iron oxide addition had no effect on its bioavailability. In contrast, selenite was adsorbed very efficiently into the soil, most likely in organic compounds. The iron oxide enrichment significantly increased plant selenite uptake, but from both untreated and moderately oxide enriched pots only <10% of added selenite was recovered in plant biomass and soil extracts. However, ample oxide enrichment resulted to >70% recovery of selenite from the adsorbed soil fraction, suggesting that adsorption on oxide surfaces was weaker than adsorption to organic matter. – These results reflect the complex behaviour of Se in soil, dependent on soil properties and on the form in which Se is added.

Keywords: selenium, selenate, selenite, peat, adsorption, plant availability**.**

Introduction

Since 1984, adequate selenium (Se) nutrition of man and domestic animals is secured in Finland by supplementing fertilizers with easily plant available sodium selenate (Yläranta, 1985). However, the acidic soil conditions favour selenate reduction into selenite (Koljonen, 1975) which tends to strongly adsorb onto soil surfaces, namely on iron and aluminium oxides (Parfitt, 1978). In many peat soils these inorganic sorption sites are scarce, and exceptionally high responses to Se fertilization have occasionally been observed in these soils. However, also organic matter has been shown to adsorb selenite (Hamdy and Gissel-Nielsen, 1976). The relative impact of the organic and inorganic adsorbents on the availability and behaviour of soil Se is not known. We examined the effect of iron oxide content on Se bioavailability in a peat soil in a pot experiment.

Materials and methods

The plant availability of selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) additions was studied on practically pure sphagnum peat (Kekkilä Luonnonturve) which had a low degree of humification. There were three artificially constructed oxide contents according to Table 1. The inherent oxalate-extractable Fe content of the peat was 0.2 g pot^{-1}. Peat was homogenized and accurately weighed into 3.5 dm^3 plastic pots. Iron oxide, [$Fe(OH)_3$], was precipitated directly into each pot by mixing ferric chloride ($FeCl_3$), solution and calcium hydroxide [$Ca(OH)_2$], thoroughly into the peat matrix. Sufficiently $Ca(OH)_2$ was applied to increase the peat pH to 4.7. After one week of incubation, the pots were fertilized and Se was added as sodium selenate [Na_2SeO_4], or sodium selenite [Na_2SeO_3], solution. Each of the 12 treatments had four replicates.

Table 1: Iron oxide and selenium treatments on sphagnum peat.

Iron oxide additions	**(g Fe pot^{-1})**	**Selenium fertilization**	**(μg Se pot^{-1})**
Fe_0	0	Se_0	0
Fe_1	0.7	$Selenate_1$	17.5
Fe_2	6.8	$Selenate_2$	3500
		$Selenite_2$	3500

Italian ryegrass (*Lolium multiflorum* L. cv. Meroa) was grown for six weeks, during which the leaf mass was harvested twice. Some of the pots were left unplanted. The harvested plant material was freeze dried, weighed, and analysed for total Se by an electrothermal atomic absorption method (Kumpulainen *et al.*, 1983). At the end of the experiment, soil from each pot was extracted sequentially with a salt solution (1 M NH_4Cl) and a phosphate buffer (1 M KH_2PO_4/ K_2HPO_4, pH 8.0) (Keskinen *et al.*, 2009). Salt solution extracts easily soluble Se, mainly selenate, whereas the phosphate buffer targets adsorbed selenite (Wright *et al.*, 2003).

Results and discussion

Selenate fertilization increased the Se uptake into ryegrass leaves multifold in comparison to an equal selenite addition (Table 2). In the leaf harvests of $selenate_1$ and $selenate_2$ treatments

25% and 40% of the added selenate was recovered, whereas the leaf mass of selenite treatments contained only less than 2% of the addition. Such superiority of selenate in increasing the yield Se content has long been known and is the reason for selenate being the form of Se added in fertilizers in Finland (Yläranta, 1985).

Increasing the inorganic Se adsorption capacity of peat by Fe oxide additions had no effect on the selenate uptake efficiency of ryegrass (Table 2). Since selenate is only weakly adsorbed in soil, this result was expected assuming that selenate reduction during the experiment was insignificant. In contrast, plant selenite uptake increased over fivefold in response to the moderate iron oxide enrichment. Possibly selenite associated with iron oxide was more available to plant than Se that was absorbed by organic compounds. Also competition in binding between the added oxides, organic matter and selenite might have increased selenite availability. High chloride content caused by the ample $FeCl_3$ addition in Fe_2 treatment hampered the germination and growth of ryegrass so severely that the results of the planted Fe_2 pots were omitted. The effect of Fe_2 addition on soil Se behaviour was examined solely by soil extractions of the unplanted pots (Fig. 1).

Table 2: Cumulative Se uptake of two ryegrass leaf harvest (µg Se pot^{-1}) on two iron oxide contents and four Se addition treatments of peat soil.

	Cumulative ryegrass Se uptake (µg Se pot^{-1})			
	Se_0	**$Selenate_1$**	**$Selenate_2$**	**$Selenite_2$**
Fe0	0.3	4.6	1400	11
Fe1	0.3	4.7	1300	64
$LSD_{0.05}$	0.1	1.1	340	15

At the end of the experiment, on average 55% of the added selenate was recovered from the soil with a salt extraction (Table 3), which confirmed that selenate had remained mainly unchanged in the soil. Only a minor part of the selenate addition was found in the adsorbed pool of the soil. The total recovery of added selenate was on average 90 ± 23%. Of the selenite addition, in contrast, mere 2% was found salt soluble and only a slightly bigger proportion recovered as adsorbed. Selenite had clearly immobilized very efficiently, most likely into the organic matter. Hamdy and Gissel-Nielsen (1976) suggested that proteins, fulvic acid or other organic compounds chelate selenite from the available soil fraction.

Table 3: Selenium (µg Se pot^{-1}) extracted from peat with a salt (NH_4Cl) solution and a phosphate buffer (KH_2PO_4/K_2HPO_4) after growing of ryegrass.

		Soil Se (µg Se pot^{-1})			
		Se_0	**$Selenate_1$**	**$Selenate_2$**	**$Selenite_2$**
NH_4Cl	Fe0	nd	11	1100	76
	Fe1	nd	14	1500	79
	$LSD_{0.05}$		4.3	200	19
KH_2PO_4/K_2HPO_4	Fe0	7.4	5.3	310	210
	Fe1	3.4	8.0	330	170
	$LSD_{0.05}$	3.0	4.4	63	110

nd = not detected

The total recovery of selenite in Fe_0 and Fe_1 treatments was only 8.1 ± 1.3%, but in the unplanted Fe_2 pots over 70% of the selenite was recovered with the phosphate buffer extraction (Fig. 1). This substantial increase in recovery may be explained by reversible adsorption of selenite on oxide surfaces, which would indicate that organic compounds bind Se more strongly than iron oxides.

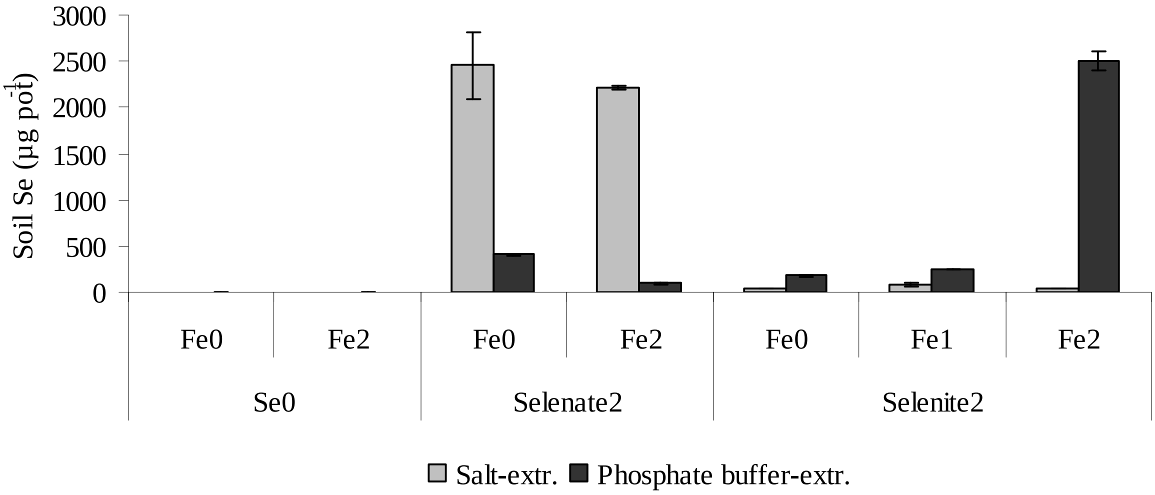

Figure 1: Selenium (µg Se pot^{-1}) extracted from unplanted peat with a salt (NH_4Cl) solution and a phosphate buffer (KH_2PO_4/K_2HPO_4). The bars represent the mean of two replicates and the error bars indicate the range of the values.

We conclude that selenate addition, as long as remaining as selenate, is easily plant available in peat soil regardless of the oxide content. Selenite, however, seems to be adsorbed very efficiently into organic matter and iron oxide addition increased its bioavailability. Therefore it seems likely, that in peat soils, the residual fertilizer Se, once reduced, accumulates in the organic substances.

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PRECIPITATION AND MINERAL FERTILIZATION EFFECTS ON POTATO (*SOLANUM TUBEROSUM* L.) YIELD AND QUALITY

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Abstract

Precipitation quantity, distribution and nitrogen (N)-, phosphorus (P_2O_5)-, potassium (K_2O)-, and magnesium (MgO) mineral fertilization effects were studied on a sandy acidic lessivated brown forest soil; WRB: Haplic Luvisol in the 47 year old Nyírlugos Field Trial (NYFT) in a Hungarian fragile agro-ecosystem in Nyírség region (N: 47^0 41' 60'' and E: 22^0 2' 80'') on potato (*Solanum tuberosum* L.) tuber production from 1962 to 1979. The experimental years were characterized by frequent extremes of climate under vegetation seasons. Drought and over rainfall negative effects were decreased by increasing N- treatments with combinations of potassium, phosphorous and magnesium from 13% to 32%. With the help of regression analysis it was found the polynomial correlation between rainfall quantity and yield could be observed in the case of NK ($Y' = 381.65 - 2.95x + 0.0056x^2$, n = 72, $R^2 = 0.95$), NPK ($Y' = 390.87 - 3.07x + 0.0060x^2$, n = 72, $R^2 = 0.96$) and NPK+Mg ($Y' = 390.45 - 3.06x + 0.0059x^2$, n = 72, $R^2 = 0.96$) nutrition systems. The optimum yield ranges between 17÷20 t ha^{-1} at 280÷330 mm of precipitation. Starch and crude protein contains of tubers were decreased of 22% and 18 % under a rany (556 mm rainfall y^{-1} in 1979) climate conditions in compared with an average (452 mm rainfall y^{-1} in 1969) year type on average of experimental (control -18.9 and -10.8%, N: -21.0 and -15.4%, NP: -17.9 and -15.8%, NK: -24.6 and -16.8%, NPK: -23.9 and -21.6%, NPK+Mg: -23.4 and -24.0%) treatments. And in 1969 minimum (control) and maximum (NPK+Mg combination) starch yield was 1.9 t ha^{-1} and 3.3 t ha^{-1}, 0.5 t ha^{-1} and 1.8 t ha^{-1} in 1979. Crude protein minimum (control) and maximum (NPK+Mg combination) yield was 0.22 t ha^{-1} and 0.47 t ha^{-1} in 1969, 0.07 t ha^{-1} and 0.24 t ha^{-1} in 1979. From 1962 to 1979 the weather was highly variable with particularly frequent droughts and over rainfall effects resulting maximum losses in yield of 42%, in starch of 22% and in protein of 18% under this period. Thus it is important to analyze the consequences of possible future climate change on this crop in Hungary.

Keywords: precipitation, mineral fertilization, potato, yield.

Introduction

"Climate Change" are recognized as a serious environmental issues (Johnston 2000, Estáquio *et al.*, 2001). Presently the build up of greenhouse gases in the atmosphere and the inertia in trends in emissions means that we can expect significant changes for at least the next few decades and probably for the 21th century too (Márton, 2001ab). It would badly need to understand what might be involved in adapting to the new climates (Márton, 2001f). A decade ago, researchers asked the „what if " question. For example, what will be the impact if climate changes. Now, we must increasingly address the following question: how do we respond effectively to prevent damaging impacts and take advantage of new climatic opportunities (Johnston, 2000). This question requires detailed in information regarding expected impacts and effective adaptive measures. Information on adaptation is required for governments, landscape planners, stakeholders, farmers, producers, processors, supermarkets and consumers. Not only the local effects and options, but also the spatial implications must be understood. Will yields be maintained on the present range of farms. Where new crops will be grown new processing plants will be required. There will be competition for water. Most recent agricultural impact studies have concentrated on the effects of mean changes in climate on crop production, whilst only limited investigations into the effects of climate variability on agriculture have been undertaken. The paucity of studies in this area is not least due to the considerable uncertainty regarding how climate variability may change in the future in response to greenhouse gas induced warming but also as a result of the uncertainty in the response of agricultural crops to changes in climate variability, effected most probably through changes in the frequency of extreme climatic events. It has been shown that changes in variance have a greater effect on the frequency of extreme climatic events than do changes in the mean values. Hence, it is important to attempt to include changes in variability in scenarios of climate change. Weather change at Hungary was started about of 1850 (Márton, 2001b,c,d,e,f). Among the natural catastrophes, drought and flooding caused by over-abundant rainfall cause the greatest problem in plant nutrition and in field crop production nowadays too (José *et al.*, 2001ab; László *et al.*, 2000a,b,c; László, 2001a; Márton, 2000a,b; Márton, 2001c,d,e; Márton *et al.*, 2000). It is why we found it necessary to revise and to analyze this problem. Potato (*Solanum tuberosum* L.) is one of the most important crop of many World countries (Kádár *et al.*, 2000; László 2000, 2001b) but little research in the field of climate change impact assessment has been undertaken. Potato is sensitive to the prevailing weather conditions (rainfall) and, hence, it is important to evaluate the effects of anthropogenic climate change on its production. This crop is demanding indicator of soil nutrient status also. It has a particularly high requirement for supply of soil nitrogen, phosphorus, potassium and magnesium (José *et al.*, 2001c,d). Tubers remove 1.5 times potassium as nitrogen and 4 or 5 times the amount of phophate. From 1962 to 1979 this paper describes climate-rainfall-change and N, P, K and Mg- mineral fertilization effects on potato yield and tuber quality on a acidic sandy brown forest soil at long term experiment scale under temperate climate conditions at Hungary.

Materials and methods

The effect of rainfall quantity and distribution on certain crop fertilization factors (N, P, K, Mg and yield, tuber quality) were studied in a long- term field experiment on acidic sandy brown forest soil at North- Eastern Hungary set up in 1962 and 2002. Ploughed layer of the experiment soil had a $pH_{(KCL)}$ 4.5, humus 0.5 %, CEC 5÷10 cmol (+) kg^{-1} soil. The topsoil was poor in macronutrients N, P, K and Mg. The mineral fertilization experiment involved 2 (Gülbaba and Aranyalma genotypes) x 2 (20 and 40 cm ploughed depths) x 16 (N, P, K, Mg fertilizations) = 64 treatments in 8 replications, giving a total of 512 plots. The gross and net plot size was 10 x 5 = 50 m^2 and 35.5 m^2. The experimental designe was split- split- plot. The N- levels were 0, 50, 100, 150 kg ha^{-1} y^{-1} and the P-, K-, Mg- levels were 48, 150, 30 kg ha^{-1} y^{-1} P_2O_5, K_2O, MgO in the form of 25% calcium ammonium nitrate, 18% superphosphate, 40% potassium chloride, and magnesium sulphate. The forecrop every second year was rye. The groundwater level was at a depth of 2÷3 m. Ecological (rainfall) and experimental dates were estimated by Hungarian traditional (Harnos, 1993), RISSAC-HAS (Márton, 2001b) new standards and MANOVA (SPSS). From the 64 treatments, eight replications, altogether 512 experimental plots with 7 treatments and their 16 combinations are summarised of experiment period from 1962 to 1979.

Results and Discussion

The experimental years (1963, 1965, 1967, 1969, 1971, 1973, 1975, 1977, 1979) were characterised by frequent extremes of regional climate changes. Seven years had an average rainfall, one year had an over rainfall and one year had a very dry. The unfavorable effects of climate anomalies (drought, over-abundance of water) on the yield formation, yield quantity of potato depended decisively on the time of year when they were experienced and the period for which they lasted. Droughts in the winter or summer half-year had much the same effect on yield. Rainfall deficiency in the winter could not be counterbalanced by average precipitation during the vegetation period, and its effect on the yield was similar to that of summer drought. It was concluded that economic yields could not be achieved with poor N, P, K and Mg nutrient supply even with a normal quantity and distribution of rainfall. Yield was influenced by rainfall to a greater extent than by combinations of NK, NPK, NPK+Mg. Drought and over rainfall negative effects were decreased by increasing N- treatments with combinations of potassium, phosphorous and magnesium from 13% to 32%. With the help of regression analysis it was found the polynomial correlation between rainfall quantity and yield could be observed in the case of NK ($Y' = 381.65 - 2.95x + 0.0056x^2$, $n = 72$, $R^2 = 0.95$), NPK ($Y' = 390.87 - 3.07x + 0.0060x^2$, $n = 72$, $R^2 = 0.96$) and NPK+Mg ($Y' = 390.45 - 3.06x +0.0059x^2$, $n = 72$, $R^2 = 0.96$) nutrition systems. The optimum yield ranges between 17 - 20 t ha^{-1} at 280 - 330 mm of precipitation. Starch and crude protein contains of tubers were decreased of about 22% and 18 % under a rany (556 mm rainfall $year^{-1}$ in 1979) climate conditions in compared with an average (452 mm rainfall $year^{-1}$ in 1969) year type on average of experimental (control:-18.9 and -10.8%, N:-21.0 and -15.4%, NP:-17.9 and -15.8%, NK:-24.6 and -16.8%, NPK:-23.9 and -21.6%, NPK+Mg:-23.4 and -24.0%) treatments. And in 1969 minimum (control) and maximum (NPK+Mg combination) starch yield was 1.9 t ha^{-1} and 3.3 t ha^{-1}, 0.5 t ha^{-1} and 1.8 t ha^{-1} in 1979. Crude protein minimum (control) and maximum (NPK+Mg combination) yield was 0.22 t ha^{-1} and 0.47 t ha^{-1} in 1969, 0.07 t ha^{-1} and 0.24 t ha^{-1} in 1979. From 1962 to 1979 the weather was highly variable with particularly frequent droughts and over rainfall effects resulting maximum losse in yield of 42%, in starch of 22% and in protein of 18% under this period. Thus it is important to analyse the consequences of possible future climate change on these crop yield and quality in Hungary.

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OCCURRENCE OF TRACE ELEMENTS IN MINERAL FERTILIZERS

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Abstract

There is a current debate in the EU on setting common thresholds for trace elements in mineral fertilizer. The elements As, Cd, Co, Cr, Cu, F, Hg, Mo, Ni, Pb, Se, Th, Tl, U, V and Zn were analysed in 145 mineral fertilizers marketed in Denmark. The samples represent straight and complex phosphorus fertilizers (P, 9; NP, 9; PK, 33; NPK, 27), sulphur-containing nitrogen fertilizer (39), straight potassium fertilizer (23) and multi-micronutrient fertilizers (5).

The concentration of trace elements varies significantly, but most of the variation is linearly related to the P-concentration. Several P-containing fertilizers had a content of Cd that exceeds the Danish threshold of 110 mg Cd/kg P, whereas some of the NPK and NP-fertilizer had a Cd-concentration below the detection limit.

Several NPK-fertilizers exceeded the proposed EU threshold of 120 mg Ni/kg, and straight P-fertilizers had high concentrations of Cd, Cr, F, V Zn and U. In contrast the concentrations of As, Hg and Pb were well below the proposed EU thresholds. It seems likely that trace elements are not linked to the N, K or S components in the fertilizer. The results of this study may be taken into account in future control programmes.

Keywords: mineral fertilizer, arsenic, cadmium, cobalt, chromium, copper, fluride, mercury, molybdenum, nickel, lead, selenium, thorium, thallium, uranium, vanadium, zinc.

Introduction

The marketing of mineral fertilizer has been subjected to legislative control of nutrient content since the beginning of the 20^{th} century in most European countries. Later, environmental concerns have introduced legislative thresholds for trace elements of xenobiotic character. In Denmark a threshold on cadmium in fertilizers containing >1%P was issued by statutory order from the Ministry of Environment in 1989 (110 mg Cd/kg P). Today, there is a debate in the EU on setting common thresholds for trace elements in mineral fertilizer, often denoted heavy metals, which is a misleading and inaccurate term. This presentation is based on a study by Petersen *et al.* (2009), where the occurrence of trace elements in mineral fertilizers marketed in Denmark was investigated.

The literature reveals a large variation in the content of trace elements in terms of both the origin and the market region of the fertilizer. The number of elements analysed has increased with improvements in analytical techniques and detection limits. Several recent papers describe in detail the analysis of mineral fertilizers, but without describing the sampling strategy of the analysed samples with regard to origin and market representativity. Straight N- and K-fertilizers appear to have the lowest concentration of trace elements. For P-containing fertilizer both the origin and processing of phosphate ore affects the concentration of trace elements. Arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), molybdenum (Mo), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se) and zinc (Zn) are the elements receiving most attention. These elements are characterized by acute toxicity at high concentrations (Cu, Zn, Cr, Se and Ni), accumulation in the food chain and accumulated toxicity (Co, Cd, Mo and Pb), or perceived carcinogenic effect (As, Cd, Co and Ni).

About half of the European countries have introduced legislative thresholds for Cd, Hg and Pb, and a quarter to a third of them has thresholds for As, Cr, Cu, Ni and Zn. The thresholds vary considerable, reflecting a heterogeneous basis for setting up threshold values. Thus, widely accepted thresholds for trace elements in mineral fertilizers have not yet been established.

Materials and Methods

To analyse the occurrence of trace elements in mineral fertilizers marketed in Denmark, a subset of 145 samples were selected from the 809 samples collected during 2006-2008 by The Danish Plant Directorate in the official annual control of nutrient content. The subset of samples where trace elements were likely to occur was established using two criteria in the selection: (i) fertilizers produced outside Scandinavia and (ii) P-rich fertilizers excluding those originating from the Kola Peninsula. The samples represent straight and complex phosphorus fertilizers (types P, 9; NP, 9; PK, 33; NPK, 27), sulphur-containing nitrogen fertilizer (type S, 39), straight potassium fertilizer (type K, 23) and multi-micronutrient fertilizers (type M, 5). The fertilizers were analysed for As, Cd, Co, Cr, Cu, Mo, Ni, Pb, Se, vanadium (V) and Zn using ICP-OES, and in the same sample extract the elements thorium (Th), thallium (Tl) and uranium (U) were analysed using ICP-MS and mercury (Hg) by CV-AAS. Fluoride (F) was analysed using a fluoride selective electrode in an acid solution.

Results and Discussion

The type S- and K-fertilizers (without P) were very low in trace elements, and in most cases the concentrations were below the detection limits. For Cr, Ni and Pb the concentrations were in all cases below national threshold values introduced in other European countries. This was also true for multi-micronutrient fertilizers (M-type).

The occurrence of trace elements was associated with the P component of the fertilizers. We have distinguished between the different types of P-fertilizer, but a substantial variation in the occurrence of trace elements and their concentration was recorded within each type. Straight P-fertilizer had a high concentration of Cd, Cr, F, V and Zn, and the P-related concentration of Cd exceeded the Danish threshold for all nine fertilizers. The concentration of As, Hg, Ni and Pb was below the thresholds proposed by EU.

The concentration of trace elements in NPK-fertilizers was generally well below that of the straight P-fertilizer but showed a large variation (three fertilizers contained 300-500 mg Cr/kg and seven fertilizers >120 mg Ni/kg). The Cd-content was close to the Danish P-related threshold, and two of the 27 fertilizers exceeded the threshold.

Seven of the nine NP-fertilizers had concentrations of trace elements below the P- and NPK-fertilizer, but for two NP-fertilizers the concentrations of most elements were significantly higher. Thus, the Danish P-related Cd threshold was exceeded for these two NP-fertilizers. The PK-fertilizers were more homogenous and the concentration of trace elements was comparable to the NPK- and NP-fertilizers having the lowest concentrations of trace elements. Nevertheless, five of the 33 PK-fertilizers exceeded the Danish P-related Cd threshold.

The elements Th, Tl and U were analysed in 53 of the 145 selected fertilizers representing P-, NP-, PK-, NPK- and K-fertilizers. The concentration of these elements was low in the straight K-fertilizers and, in general, below the detection limit for Th and U. The concentration of Tl was well below the current German threshold of 1 mg/kg.

In general the concentration of As, Cd, U, V and Zn were linearly related to the P concentration (R^2>0.79), and for NPK-fertilizers the concentration of Co, Cr and Ni were linearly related to the iron (Fe) concentration of the fertilizer (R^2>0.89).

A future sampling strategy for control should clearly include NPK-fertilizers due to their potential concentrations of trace elements and their widespread use. Despite the smaller consumption of NP- and PK-fertilizers these fertilizers should be included in a control programme because of their potential high concentration of trace elements. Although the highest concentrations were obtained for straight P-fertilizers, their very low consumption does not necessitate a particular effort in a future control programme. The fertilizers of type S and K may be excluded in the control even with a widespread use of type S fertilizers.

In conclusion, the content and concentration of trace elements in mineral fertilizers marketed in Denmark varies significantly, but most of the variation is related to the P-concentration. Several P-containing fertilizers had a content of Cd that exceeds the Danish P-related Cd threshold, whereas some of the NPK and NP-fertilizer had a Cd-concentration below the detection limit. The P-containing fertilizers account for 40% of the annual consumption of fertilizers in Denmark.

Several NPK-fertilizers exceeded the proposed EU threshold of 120 mg Ni/kg, and straight P-fertilizers had high concentration of Cd, Cr, F, V, Zn and U. In contrast the concentration of As, Hg and Pb were well below the proposed EU thresholds. It seems likely that trace elements are not linked to the N, K or S components of the fertilizer.

Acknowledgements

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NUTRIENT LEGISLATION IN FLANDERS (BELGIUM)

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Abstract

The implementation of the Nitrates Directive has lead to the set up of nutrient legislation and policy in all EU countries. As Belgium is a federal state, the Flemish policy and legislation is completely different from the south of the country, i.e. Wallonia. The legislation has been introduced already in 1991 and after several changes and adaptations, a new Manure Decree has been adopted the 22th of December 2006. The most important measures to reduce nitrate losses (and to a certain extent also P-losses) will be discussed in this paper. They include:

- application standards for N and P_2O_5;
- periods of allowed application;
- methods of application of animal manures;
- storage capacity of animal manures;
- calculation of N and P production on farms;
- calculation of N and P surpluses on a farm basis;
- nutrient emissions rights;
- levies.

The strong and weak points of the legislation will be discussed with some references to the legislation and policy in other EU countries.

Keywords: nutrients, legislation, Nitrate Directive, Manure Decree.

Introduction

The natural availability of most nutrients is insufficient to optimize yields. Therefore, the input of nutrients is necessary for agriculture to remain economically feasible. Intensification of agriculture, however, leads to an increased input of nutrients and because nutrient efficiency never reaches 100%, higher losses by intensification are inevitable.

Nutrient losses are harmful for both ground- and surface waters as well as for the quality of air. These losses can be:

-ammonia volatilization;

-emissions of nitrous and nitric oxides by nitrification and denitrification;

-leaching losses of nitrates and phosphates;

-nitrogen and phosphorus losses by runoff and erosion.

An example of a direct harmful effect is a too high nitrate (NO_3^-) and/or phosphorus (P) concentration in surface waters. Indirect effects are euthrophication of surface waters, losses of biodiversity, acidification, higher concentrations of greenhouse gases, destruction of the ozone layer, etc. To preserve our health, welfare, nature and environment, losses of nutrients to the environment should be as low as possible.

To cope with the negative effects of too high nitrogen (N) applications and its negative effects on the environment, the European Union has introduced the Nitrates Directive in 1991 (91/676/EEG, Anonymus, 1991). It has taken Flanders, via the new Manure Decree of December 22, 2006 (Anonymus, 2006), 15 years to fully implement the European Nitrates Directive for its entire territory. Furthermore, the European Parliament approved the “Water Framework Directive” (Anonymus, 2000) which establishes a global framework for Community action in the field of water policy. In this article, the most important parts of the nutrient legislation in Flanders will be discussed with reference to strong and weak points of this legislation.

The New Manure Decree (2006)

In accordance with Article 3 of the Nitrates Directive all waters in the Flemish Region are defined as "waters that are affected by pollution or that could be affected if actions pursuant to Article 5 of the Directive are not taken. This implies that the entire territory of the Flemish Region is designated as 'Nitrate Vulnerable Zone Water (NVZ)' and consequently that at maximum 170 kg N ha^{-1} from livestock manure can be applied. Interesting is that some countries in the EU designated the all territory as NVZ while other countries did not. To overcome the 170 kg N-animal $(ha\ y)^{-1}$-application standard, Flanders has requested for derogation. This request was based on *inter alias* the conditions mentioned in Annex III 2b of the Directive (high N uptake/long growing season/high net precipitation). Though other countries were already granted derogation, Flanders was the first to get derogation on parcel level and not on farm level. This derogation is especially important for dairy farmers as it allows them to apply 250 kg N-animal $(ha\ y)^{-1}$ on grassland and on a grass-maize combination.

Depending on the crop grown, there are application limits for N and P_2O_5 as given in Table 1 for which N-other means N from other organic sources like compost or other secondary products. These limits are rather stringent because it does not account for the effectiveness of N in all kind of organic manures as is the case in some other countries like Denmark and The Netherlands (Van Dijk and Ten Berge, 2009). It has to be further noticed that as a general rule a maximum of 20 kg P_2O_5 ha^{-1}, out of the total of 85 till 100 kg P_2O_5 ha^{-1}, can only be applied via chemical fertilisers, the so-called starting phosphorus. Though Flanders has argued for a higher N-application rate on grassland, the European Commission enforced Flanders to come to terms with the Dutch legislation. The Commission's arguments were based on the fact that the Netherlands have highly productive cows and the weather circumstances do not much differ between Flanders and the Netherlands. However, Flanders has an on-average significantly longer growing season than the Netherlands and hence a higher N-application was justifiable.

Apart from the application rates, both timing and the way of land application of fertilizing products is also rather strict. The way of application is subject to a multitude of rules, dependent on the crop, slope of the field, weather conditions, etc. Concerning the timing, the prohibition period generally extends from September 1st till February 15th, but dependent on fertilizer type and texture, other, less strict dates are imposed (Figure 1). Because of the occurrence of a rather long closing period, a storage capacity for livestock manure is also imposed and can be as long as 9 months for in-door housed animals. This rule prevents 'manure dumping' at inappropriate moments -just emptying the storage tanks- and not to use it as a valuable fertilizer. These measures are again quite different between countries (Van Dijk and Ten Berge, 2009).

To enforce the regulation, the Flemish government works on a field basis, rather than on a farm level. Therefore, they use the 'nitrate nitrogen residue stick', whereby at maximum of 90 kg NO_3^--N ha^{-1} (0-90 cm) (October, 1st - November 15th) is allowed. In case of misdemeanour, rather severe fines can be imposed.

Table 1: Fertilization limits (kg ha^{-1}) in function of crop type (N-total = maximum allowable N-amount; N-animal = N from livestock manure; N-other = N from other organic fertilizers; N-chemical = N from chemical fertilizers).

Crop	**P_2O_5**	**N-total**	**N-animal**	**N-other**	**N-chemical**
Grass	100	350	170	170	250
Maize	85	275	170	170	150
Maize on sandy soils					
2009	85	265	170	170	150
2010	85	260	170	170	150
Low N-need crops*	80	125	125	125	70
Leguminosae, not peas or beans	80	0	0	0	0
Sugar beets	80	220	170	170	150
Other crops	85	275	170	170	175
Cereals on sandy soils					
2009	85	265	170	170	175
2010	85	260	170	170	175

*Belgian endive, chicory, shallots, onions, flax; peas and beans

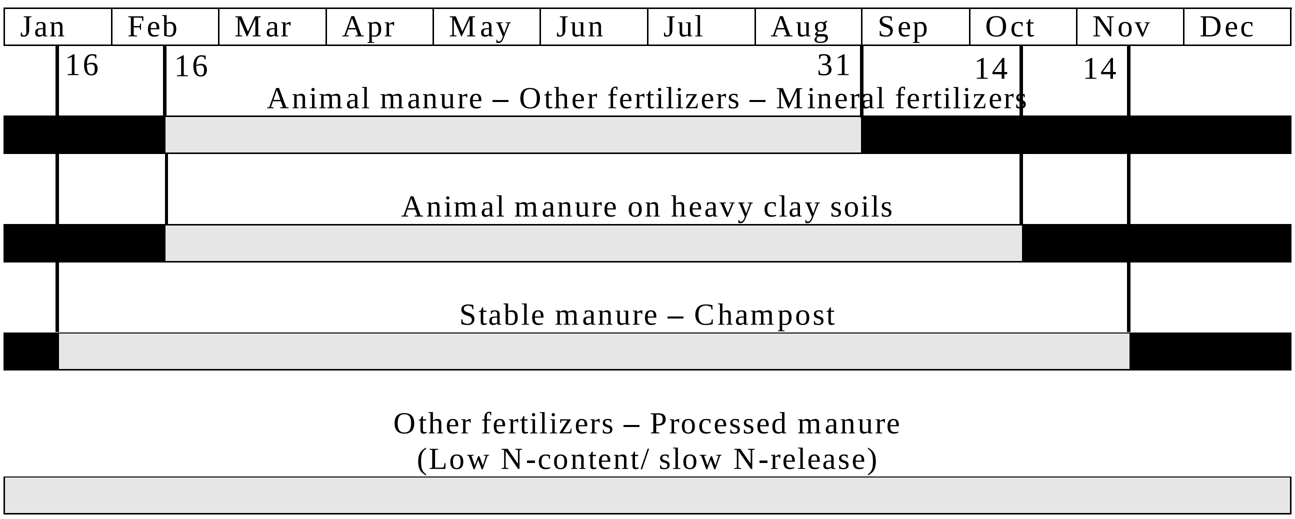

Figure 1: Application periods dependent on fertilizer type and texture (black = prohibition).

As the final goal of the Nitrates Directive is a nitrate concentration below 50 mg NO_3^- L^{-1} in ground and surface waters, Flanders has set-up specific measuring networks for both ground- and surface waters. The results for groundwater are still fragmentary, but the results of surface water (± 800 measuring points) show a clear downward trend (Table 2).

Table 2: Percentage of measuring points with at least 1 exceeding the norm of 50 mg NO_3^- L^{-1} during the winter period.

	1999 2000	2000 2001	2001 2002	2002 2003	2003 2004	2004 2005	2005 2006	206 2007	2007 2008	2008 2009
Flanders	59	50	41	31	42	40	41	43	37	27

In a third part of the Decree, the nutrient excretion by animals is dealt with. Farmers can choose between two systems, (i) the fixed rate system, based on default excretion standards or (ii) the nutrient balance system, whereby exact excretion values can be taken into account. With these data a farmer has to calculate, on a farm basis, if he has a certain nutrient surplus or not. These surpluses must be either disposed off to other farmers or processed. The obligation to process manure in some cases is flexible and realistic. Although there are certainly variations in excretion rates between countries, due e.g. to differences in feeding material, the differences are large which lead to some discussions between countries.

The latter parts deal with nutrient emission rights, growth of holdings, transport of nutrients, control measurements and penalties. Though an important part of the legislation, they are of minor importance in view of nutrient management *sensu* stricto.

Conclusions

Since 1991 Flanders started to impose the Nitrates Directive via the Manure Decree, but only since 2007 the whole territory of the Flemish region (Belgium) has been designated as NVZ. Besides this delineation, new and stricter rules have been imposed. These rules apply to application limits, timing and way of application and manure storage. The effect of these more stringent rules can be seen from the results of the surface measuring points, showing a clear drop in percentage (27% during the winter period 2007-2008 compared to 59% in 1999-2000). exceeding the threshold value of 50 mg NO_3^- L^{-1} . The last two parts mainly deal with 'technical matters', but are none the less important to come to a correct nutrient management in Flanders.

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CLOSING ADDRESS

at the 18th International Symposium of CIEC

Cristian HERA
President of CIEC

Distinguished participants,

Ladies and Gentlemen,

Dear Colleagues,

We are coming to the end of the 18th Symposium of International Scientific Centre of Fertilizers (CIEC) with the great satisfaction to have attended this very interesting meeting organized through the joint efforts of the **Italian Agricultural Research Council** through **Research Centre for Soil-Plant System, Rome, Research Unite for Cropping System in Dry Environments, Bari, Research Unit for Study of Cropping Systems, Metaponto (Matera), Institute for Environmental Protection and Research (ISPRA), Rome** and **International Scientific Centre of Fertilizers (CIEC)**.

The satisfaction is even greater having in mind that this Symposium is the **fifth CIEC meeting held in Rome** during 76 years of existence of this international organization and because **Italy *was the main initiative country among the 29 founders countries of CIEC,*** as I mentioned in my opening address.

In time, almost all CIEC meeting have enjoyed the participation of Italian delegation sharing other specialists their high competence.

Now, thank to Prof. Paolo Sequi who took over the Presidency of the 18th CIEC Symposium, and his very active and efficient colleagues, the gates to this marvellous City, Rome, have been opened again for our benefit.

Thank you Prof. *Polo Sequi* and please receive our heartedly gratitude.

We are also very grateful to all members of the Local Organizing Committee. Please allow me to express on behalf of the CIEC Presidium as well as on behalf of all participants at the 18th CIEC Symposium our thanks and gratitude

I am taking this opportunity to cordially express our thanks and gratefulness to all attendants for offering their kind support for the benefit of outstanding scientific level during 18th International Symposium of CIEC.

We are very thankful not only to the members of the Organizing Committee but also to the co-organizers of this significant scientific event.

Please allow me dear participants to express again my thanks and gratitude to my colleagues from the CIEC Presidium, Prof. *Ewald Schnug,* Deputy President and *Silvia Haneklaus*, Deputy Secretary General, who has been in the permanent contact with Prof. *Paolo Sequi, with the Secretariat of the Symposium* and members of the Symposium Local Organizing Committee, participating actively at the success of our Rome CIEC meeting.

During the 18th International Symposium of Fertilizers **52 papers were presented as Plenary lectures and 61 as posters.**

The great majority of these reports deserve to be appreciated as high scientific contributions in the fields of fertilizer production, utilization and effects on soil fertility, crop productivity and environmental impact especially under different soil and climatic conditions.

I am very pleased to express my warm thanks to all speakers and to those who presented posters, as well as to all who took part at the discussions, wishing them new successes and satisfactions in the future.

Dear Colleagues,

On the occasion of Pretoria Symposium we decided to elect CIEC-Country Representatives among the best qualified scientists in soil fertility – fertilizers and their uses, as well as in plant nutrition domains, in order to strengthen CIEC connections with all countries in the world, in addition to the CIEC National Branches.

This decision has proved to be very well-inspired and positive examples are represented by Prof. *Paulo Sequi* and Dr. *Francesco Montemuro*. As a consequence, the 18th International Symposium of CIEC was organized this year Rome, Italy.

During the 17th International Symposium held in Cairo, Egypt, **CIEC Presidium meeting decided to organize *15th CIEC World Congress of Fertilizers in my country of origin, Romania***.

I would like to take this opportunity to invite you all to attend at end of August/beginning of September 2010 this very important event, hoping to share new great successes in the sciences of soil fertility, plant nutrition, fertilizers production and utilization.

Many thanks to all of you dear participants, dear colleagues and I wish all the best and great successes in the future.

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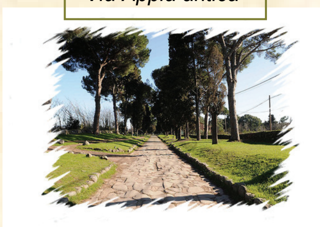

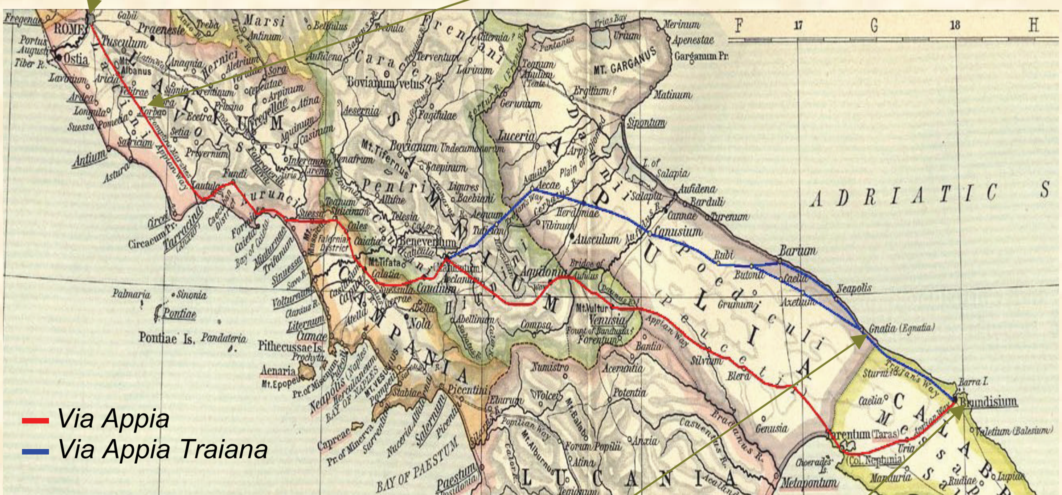

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