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- Prof. Elena Maestri, Department of Environmental Sciences, University of Parma, Parma………………………………………………………………………………………… Italy

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(peter.schroeder@helmholtz-muenchen.de)

**COST FA 0905 aims at:**
• identify bottlenecks limiting the content of bioavailable minerals (Fe, Zn, Mg, Se) in the consumable crop part.
• provide solutions for 3-fold increase in bioavailable food/feed mineral content.
• assess ways to limit the entry of Cd and As into the food chain.
• disseminate this knowledge to stakeholders and the public. European dimension and added values COST.

**COST FA 0905 will strive to:**
• maximise European synergy in research cooperation,
• provide innovative ideas to improve the mineral status of food and feed,
• find methods to exploit genetic variability of food and fodder crops,
• enhance scientific know how to improve minerals in food under modern processing,
• create training and exchange programs, for students, postdoctoral fellows and young researchers, especially women,
• identify key institutes and personnel for efficient assessment of food and feed mineral quality across Europe.
## Second Annual Conference and MC Meeting Cost Action FA 0905
Mineral Improved Crop Production for Healthy Food and Feed

### What's for lunch? Nutrients and minerals in every day food
How the knowledge on mineral nutrition of plants can improve human nutrition

**Wednesday, November 23rd, 2011**

*Registration desk open from 15:00 to 18:00 in Sala Giardino.*
*Posters cannot be displayed at this time*

**Thursday, November 24th, 2011**

*Registration desk open from 8:15 in Sala Giardino.*
*Posters must be set up in Mezzanino*

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<td>9:00</td>
<td>Welcome address</td>
<td>Bal Ram Singh – Chair of COST ACTION FA0905 Nelson Marmiroli for the Local Organizing Committee, Director of CINSA, Italy. Introduction to the Conference Paolo Cescon – President of CINSA, Italy Region Veneto, Italy CRA Agricultural Research Council, Italy</td>
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<tr>
<td>9:30</td>
<td>Session 1 – WG1 + WG3</td>
<td>Chair: Satish Gupta, Rapporteur: Edita Baltrenaite</td>
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| 9:30  | KEYNOTE LECTURE                  | Maria Greger, Stockholm University, Sweden How to influence the Cd content in food crops

10:00- Umit Baris Kutman, Bahar Yildiz Kutman, Yasemin Ceylan, Ismail Cakmak, Turkey
Combining nitrogen and zinc fertilization for improved mineral content in wheat

10:20- Anja Gramlich, Susan Tandy, Emmanuel Frossard, Rainer Schulin, Switzerland
Zn uptake by wheat in the presence of the ligands citrate, histidine or EDTA

10:40- Lidiya Moklyachuk, Orest Furdrycko, Yaroslav Chabanyuk, Viktor Shynkarenko, Andriy Vdovchenko, Yuriy Ternoviy, Olga Togatcynska, Volodymyr Strelko, Volodymyr Trychlib, Irina Maletina, Ukraine
Use of zeolite nanocomposite materials of "host-guest» type in growing of vegetable cultures
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<th>Time</th>
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| 11:00 | Katie L. Moore, Malcolm Hawkesford, Peter Shewry, Steve P. McGrath, Fang-Jie Zhao, Chris R. M Grovenor, United Kingdom
NANOSIMS: a technique for subcellular localisation of essential and toxic trace elements |
| 11:20 | Coffee break                                                                                   |
| 11:40 | Marta Marmiroli, Veronica Pigoni, Elena Maestri, Nelson Marmiroli, Italy
How different tomato cultivars (*Solanum lycopersicum* L.) respond to arsenic and arsenic + silicon treatments: uptake and translocation |
| 12:00 | Rubén López-Nicolás, Carlos A. González-Bermúdez, Carmen Frontela-Saseta, Victoria Gómez-Gómez, Patricia Peso-Echarri, Carmen Martínez-Graciá, Gaspar Ros-Berruezo, Spain
In vitro mineral availability in peel and pulp of prickly pear cactus fruits (*Opuntia ficus indica*) |
| 12:20 | Alessandra Salvioli, Ines Zouari, Michel Chalot, Paola Bonfante, Italy and France
The arbuscular mycorrhizal status has an impact on the transcriptome profile and amino acid composition of tomato fruit |
| 12:40 | Søren Husted, Daniel Persson, Thomas H. Hansen, Jan K. Schjørring, Denmark
Recent advances in compartmentation and speciation analysis of iron and zinc in the cereal grain |
| 13:00 | Lunch break                                                                                    |
| 14:00 | Poster Session
*Posters will be exposed all throughout the meeting (two days)*
Chair: Peter Schroeder, Rapporteur: Elena Maestri
Authors are invited to stay on poster side. |
| 15:00 | Session 2 – WG2 + WG4
Chair: Mark Aarts, Rapporteur: Erika Nehnevajova |
| 15:00 | KEYNOTE LECTURE
Elisabetta Lupotto, CRA Agricultural Research Council, Italy
Rice quality and nutritional value and the environment |
| 15:30 | Anna Barabasz, Lorraine Elisabeth Williams, Danuta Maria Antosiewicz, Poland and United Kingdom
Modification of a plant response to Mn, Zn and Ca due to the *AtECA3* expression |
| 15:50 | Stefan Rensch, Stephan Clemens, Germany
Substantial natural variation in micronutrient content of barley grain |
<p>| 16:10 | Coffee break                                                                                   |</p>
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<td>16:30</td>
<td><strong>Zeshan Hassan, Sangita Talukdar, Henk Schat, Mark G.M. Aarts</strong>, The Netherlands</td>
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<td></td>
<td>Expressing ( \text{Ncznt1} ) and ( \text{Ncztp1} ) from ( \text{Noccaea (Thlaspi) caerulescens} ) enhance Zn and Cd tolerance and accumulation in ( \text{Arabidopsis thaliana} )</td>
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<td>16:50</td>
<td><strong>Pauliina Halimaa, Viivi Ahonen, Attila Gyenesei, Sirpa Kärenlampi, Asta Laiho, Petri Pekkonen, Juha-Pekka Pursiheimo, Henk Schat, Marjo Tuomainen, Arja Tervahauta</strong>, Finland and the Netherlands</td>
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<tr>
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<td>Adaptive mechanisms of hyperaccumulator ( \text{Noccaea caerulescens} ) ecotypes with contrasting metal hyperaccumulation and hypertolerance traits</td>
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<tr>
<td>17:10</td>
<td><strong>Tristan Eagling, Fangjie Zhao, Andy Neal, Steve McGrath, Peter Shewry, Susan Fairweather-Tait</strong>, United Kingdom</td>
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<td>Size exclusion chromatography coupled with ICP-MS is an effective method of screening for and quantifying bioavailable minerals in cereal grain. This provides a useful tool for breeding strategies aiming to produce new wheat varieties that can be used to combat mineral deficiency.</td>
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<td>17:30</td>
<td>Management Committee (MC) meeting (1 hour)</td>
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<tr>
<td>8:30</td>
<td>Management Committee (MC) meeting (1 hour)</td>
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<td><strong>Session 3 – WG2 + WG3</strong></td>
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<td>Chair: <strong>Francesca Sparvoli</strong>, Rapporteur: <strong>Fernando José Cebola Lidon</strong></td>
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<tr>
<td>9:30</td>
<td><strong>KEYNOTE LECTURE:</strong> ( \text{Howarth Bouis, HarvestPlus, CGIAR, USA} )</td>
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<td>The HarvestPlus project</td>
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<td>10:00</td>
<td><strong>KEYNOTE LECTURE:</strong> ( \text{Luke Beesley and Marta Marmiroli, The James Hutton Institute, United Kingdom, and University of Parma, Italy (15min+15min)} )</td>
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<td></td>
<td>The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar</td>
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<td>10:30</td>
<td><strong>António Eduardo Leitão, Maria Paula Duarte, Maria Manuela Abreu da Silva, Benvindo Maçãs, José Prates Coutinho, Ana Sofia Almeida, Ana Luísa Fernando, Paula Scotti-Campos, Isabel P. Pais, José Cochicho Ramalho, Luís Filipe Goulão, Ana Isabel Ribeiro, Fernanda Simões, Ana Rita Costa, José Matos, Fernando Henrique Reboredo, Fernando Cebola Lidon</strong>, Portugal</td>
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<tr>
<td></td>
<td>Simultaneous biofortification of Zn and Fe in ( \text{Triticum aestivum} ) L. – Seed</td>
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nutritional evaluation

10:50  Viviane Mary, Magali Schnell Ramos, Jérôme Giraudat, Hicham Khodja, Sébastien Thomine, France
Characterization of Arabidopsis mutants affected in seed iron storage

11:10  Anna Manara, Giovanni Dal Corso, Antonella Furini, Italy
PCP1 and AtOSA1: proteins involved in chloroplast iron homeostasis in Arabidopsis

11:30  Coffee break

11:50  Martina Landini, Silvia Gonzali, Claudia Kiferle, Massimo Tonacchera, Patrizia Agretti, Antonio Dimida, Paolo Vitti, Amedeo Alpi, Aldo Pinchera, Pierdomenico Perata, Italy
Metabolic engineering of the iodine content in Arabidopsis thaliana

12:10  Avi Golan-Goldhirsh, Israel
From enzymatic browning in fruits and vegetables to modification of proteins by reaction with copper, ascorbate and oxygen

12:30  Carlos A. González-Bermúdez, Rubén López-Nicolás, Carmen Frontela-Saseta, Patricia Peso-Echarri, Mª José Bernal-Cava, Carmen Martínez-Graciá, Spain
Influence of the addition of different concentrations of thickening agents on in-vitro mineral availability in infant formula

Progress in the understanding of cadmium tolerance and accumulation

13:10  Lunch break

14:10  Poster Session
Posters will be exposed all throughout the meeting (two days)
Chair: Peter Schroeder, Rapporteur: Elena Maestri
Authors are invited to stay on poster side.

Session 4 – WG1 + WG4
Chair: Richard Hurrell, Rapporteur: Laszlo Erdei

15:00  KEYNOTE LECTURE
Thomas Schmülling, Freie Universität Berlin, Germany
Root enhancement for crop improvement

15:30  Luke Beesley, Eduardo Moreno-Jimenez, Jose L Gomez-Eyles, United Kingdom, Spain and USA
The role of biochar in the phytoremediation of metal/metalloid contaminated soils

15:50  Edita Baltrėnaitė, Arvydas Lietuvninkas, Pranas Baltrėnas, Lithuania and Russia
Use of dynamic factors to improve practical knowledge in metal uptake
<table>
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<tr>
<th>Time</th>
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<tbody>
<tr>
<td>16:10</td>
<td>Coffee break</td>
</tr>
</tbody>
</table>
| 16:30 | 16:30- Marta Marmiroli, Luca Pagano, Maria Luisa Savo Sardaro, Nelson Marmiroli, *Italy*  
Physiological characterization and transcription analysis of two *Arabidopsis* mutants resistant to CdS nanoparticles  
16:50- Søren Borg, Behrooz Darbani, Birgitte Tauris, Shahin Noeparvar, Preben B. Holm, *Denmark*  
Transport and Deposition of Iron and Zinc in the Wheat and Barley Grain |
| 17:10 | 17:10- Gijs Du Laing, Pradeep Alava, Filip Tack, Tom van de Wiele, *Belgium*  
Arsenic in rice: gastrointestinal bioaccessibility and speciation are affected by food matrix |
| 17:30 | General discussion and concluding remarks (1 hour)  
Chairs: Bal Ram Singh and Nelson Marmiroli  
Annunciations of Poster Awards |
| 20:00 | Social Dinner in Restaurant S.Trovaso  
and official distribution of poster awards |

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**Saturday, November 26th, 2011**

*Any other administrative leftovers*

*Departure of guests and meeting participants*
PLATFORM LECTURES
HOW TO INFLUENCE THE Cd CONTENT IN FOOD CROPS

Maria Greger¹,²

¹Faculty of Applied Ecology and Agricultural Sciences, Hedmark University College, Blæstad, 2418 Elverum, Norway.
²Department of Botany, Stockholm University, 106 91 Stockholm, Sweden.

Cadmium is a heavy metal which is toxic also in low concentrations. It reaches our body via the daily intake of food crops such as wheat, potatoes, lettuce etc. This paper takes up possible methods that may change the uptake of Cd into the food crops. Phytoremediation is a useful tool to decrease the level of metals in the soil and thereby would decrease the uptake in the post cultivated crop. Silicon has been shown to increase the biomass production as well as tolerance against various stresses. The accumulation of metals is also influenced. Macro algal compost as fertilizers has been used earlier in agriculture. Since algae takes up various elements from the water and are able to bind up metals in its body it is possible that these metals may be transferred to the crops or that the available metal concentration in the soil will decrease.
COMBINING NITROGEN AND ZINC FERTILIZATION FOR IMPROVED MINERAL CONTENT IN WHEAT

Umit Baris Kutman, Bahar Yildiz Kutman, Yasemin Ceylan, Ismail Cakmak

Sabanci University, Faculty of Engineering and Natural Sciences, 34956, Istanbul, Turkey

Biofortification of cereal grains with zinc (Zn) and iron (Fe) is a global challenge. In order to investigate the potential of N fertilization in biofortification of wheat grain, wheat was grown with different N and Zn treatments under growth chamber, greenhouse or field conditions. Grain Zn and Fe concentrations exhibited significant responses to increasing N applications not only in the whole grain but also the endosperm, the most widely consumed part of wheat grain. In the case of Zn, the positive impact of N was dependent on Zn availability, disappearing at low Zn and becoming more pronounced at high Zn availability. Nitrogen fertilization was much more effective than Fe fertilization for improving the Fe concentration of wheat grain. Partitioning experiments showed that better N nutrition enhances both the direct uptake and the retranslocation routes for grain Zn and Fe accumulation. Experiments with the radioisotope $^{65}\text{Zn}$ demonstrated that higher N is associated with improved uptake, root-to-shoot translocation and remobilization of Zn. Combination of Zn fertilization with optimized N applications are not only necessary for the synergistic improvement of grain Zn but also for the restriction of Cd uptake. Nitrogen appears to be a critical component in agronomic biofortification, which can rapidly alleviate the Zn and Fe deficiency problems in the developing world.
ZN UPTAKE BY WHEAT IN THE PRESENCE OF THE LIGANDS CITRATE, HISTIDINE OR EDTA

Anja Gramlich¹, Susan Tandy¹, Emmanuel Frossard², Rainer Schulin¹

¹Institute of Terrestrial Ecosystems, ETH Zurich, Switzerland
²Institute for Plant, Animal and Agroecosystems Sciences, ETH Zurich, Switzerland

Keywords: Free Zn, Organo-Zn-Complexes, Wheat, Zn Bioavailability

Organic ligands are known to increase the mobility of metals in soils, but it’s not clear whether organo-zinc-complexes in the soil solution can also play a role in the direct uptake of zinc (Zn) by plants. Using hydroponic cultures, we investigated the short-term effects of the three ligands citrate, histidine and EDTA on the uptake of radio-labeled Zn by wheat, comparing treatments with the same free Zn but varying ligand concentrations. The free Zn concentration chosen was 50 nM. At this concentration no saturation of Zn influx was observed in a pre-experiment. The required amounts of ligands were calculated using the speciation program MINEQL. In the EDTA treatments a Zn flux of 46±3 nmol g⁻¹ root dry weight h⁻¹ was found. Root Zn uptake and Zn translocation into the shoots were enhanced by a factor of 3-4 in the presence of citrate and by a factor of 8-9 in the presence of histidine. As the experiment did not allow to decide whether these ligand effects on Zn uptake were due to direct uptake of the ligands or due to enhanced transport towards the roots, a second experiment with double labeling of Zn and ligands was carried out. The samples are currently analyzed, and the results will be presented at the conference.
USE OF ZEOLITE NANOCOMPOSITE MATERIALS OF "HOST-GUEST" TYPE IN GROWING OF VEGETABLE CULTURES

Lidiya Moklyachuk1, Orest Furdycko, Yaroslav Chabanyuk1, Viktor Shynkarenko1, Andriy Vdovychenko1, Yuriy Ternoviy1, Olga Togatcynska1, Volodymyr Strelko2, Volodymyr Trychlib2, Irina Maletina2

1Institute of Agroecology and Environmental Economy
2Institute for Sorption and Problems of Endoecology

Keywords: nanocomposite materials, microfertilizers, trace elements, zeolites

Zinc, copper and manganese are obtained by plants in ion form. Use of metal salts as components of solvable microfertilizers is inefficient because salts, as well as complex metal compounds, are easily washed out. We studied the possibility of prolonged support of plants with microelements by introducing ions and complex microelement compounds in soil in form of nanocomposite materials of the "host-guest" type.

Objectives. Our main objective was to obtain nanocomposite materials of "host-guest" type based on the research of natural zeolites and complex ions of zinc, copper and manganese, and to study their effect in real-world conditions in grain and vegetable crop rotation (results of the first stage (1 year) of the 3-year study are presented.).

Methods. Natural zeolites were impregnated by complex ions of zink and copper \([\text{Cu(NH}_3\text{)}_4]^2+\) and \([\text{Zn(NH}_3\text{)}_4]^2+\) in aqueous solution. Zeolite-manganese nanocomposite was obtained using the aqueous solution of MnCl₂. Both zeolite portions were mixed and introduced into soil. Field experiments were conducted on carrot and beetroot. We compared our results with seeds treated with microfertilizers.

Results. Use of nanocomposite materials increases concentration of zink by 50% in carrot (up to 3 mg/kg) and by 100% in beetroot (up to 4 mg/kg). Copper concentration in both of these plants increases by 30% compared to control samples. Manganese concentration in vegetables is not affected by change of technology. Processing of seeds with microfertilizers has no influence on concentration of microelements in vegetables.

Conclusions. Use of nanocomposite materials allowed decreasing mobility of microelements in soil which led to the increase of zinc and copper concentration in carrot and beetroot.
NANOSIMS: A TECHNIQUE FOR SUBCELLULAR LOCALISATION OF ESSENTIAL AND TOXIC TRACE ELEMENTS

Katie L. Moore¹, Malcolm Hawkesford², Peter Shewry², Steve P. McGrath², Fang-Jie Zhao², Chris R. M Grovenor¹

¹ Department of Materials, University of Oxford, Parks Road, Oxford, OX1 3PH, UK
² Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

Keywords: Arsenic, Iron, NanoSIMS, Selenium, Subcellular

Determining the subcellular localisation of beneficial elements such as Se and Fe and toxic elements such as As is analytically challenging but can help to understand their mechanisms of uptake and how processing will affect the amount consumed. Co-localisation with associated elements such as Si, S and P in various parts of the plant will help to understand the uptake mechanisms. The NanoSIMS is a state-of-the-art microscope capable of high resolution chemical imaging (down to 50 nm) and detecting very low elemental concentrations (ppm levels) making it ideally suited for trace element localisation in biological materials. The NanoSIMS has been used to determine the localisation of Se and Fe in wheat and As in rice grain, roots and stem samples. This presentation will also show how complementary techniques such as transmission electron microscopy and synchrotron X-ray fluorescence have been used to give a better understanding of key problems in trace element analysis by combining key characteristics of these three techniques. The effect of Si mutants, such as Lsi2 on the As distribution in rice roots, will also be shown. The subcellular distributions determined by the NanoSIMS give a clearer understanding of trace element distribution and uptake with some unexpected results.
HOW DIFFERENT TOMATO CULTIVARS (*SOLANUM Lycopersicum* L.) RESPOND TO ARSENIC AND ARSENIC + SILICON TREATMENTS: UPTAKE AND TRANSLOCATION

Marta Marmiroli, Veronica Pigoni, Elena Maestri, Nelson Marmiroli.

*Department of Environmental Sciences, Viale G.P. Usberti 33/A, University of Parma, 43100 Parma, Italy.*

**Keywords:** arsenic, crop safety, inorganic contamination, tomato, silicon.

Elevated arsenic in soils raises concern regarding plant uptake and entry into wildlife and human food chains. Silicon is added to tomato plants to improve water stress resistance. In this work we have evaluated the effect of As (III) and As(V), with or without Si, on the germination of eight cultivars of *Solanum lycopersicum* L. We have determined the number of seeds germinated and shoot lengths to find the most resistant cultivar and the more toxic species of arsenic and how Si affected germination and growth. In order to assess the phylogenetic distances between the considered cultivars we also made an SSR analysis utilizing eleven tomato specific SSR primer combinations. All the tomato cultivars were also grown in garden soil for three months, then supplemented with As, with or without Si. After two weeks they were harvested and fresh biomass was measured and As content was determined. The cultivars showed a remarkably different behavior towards the treatments. In a further experiment, As was supplemented (with or without Si) to the plants at the fruit production stage. The ripened fruits were collected after three months and the content of As in tomato fruits was measured.
Prickly pear cactus (*Opuntia sp.*) is an important fruit crop in countries with hot environment like the south of Spain. Several studies have shown that this fruit is a good source of vitamins, minerals and fiber, based on the content of these nutrients. However, there is a little scientific information about the availability of these nutrients, and no attention has been paid to the peel, that could be used as by-product source of food ingredients in the functional food industry. Therefore, the aim of this study was to determine the fiber content and the *in vitro* intestinal absorption (by solubility and dialysis methods) of different minerals contained in the pulp of prickly pear fruits as well as in the peel. Results showed these fruits are a good source of calcium (1323mg/100g), potassium (811mg/100g), magnesium (125mg/100g) and sodium (50mg/100g), being higher the contents in peel than in the edible portion. Despite the high calcium solubility observed in the whole fruit, only 0.3% was dialyzable in peel probably related with its higher fiber content (20% vs. 10%); however iron, zinc, sodium and magnesium showed the highest absorption (dialyzability) in the whole fruit. On a regular basis, the intake of 2-3 prickly pear fruits could provide the RDI of calcium and magnesium, 1/3 of the RDI of potassium and a minimum proportion of the recommended sodium intake.
Arbuscular mycorrhizal (AM) symbiosis is the most widespread association between plant roots and fungi in natural and agricultural ecosystems. The success in time and space of AM symbiosis is mostly due to the benefits that both partners gain, which are above all due to a reciprocal nutrient exchange. As a consequence, AM fungi are currently considered key players in agronomic practices as they may lead to a reduction in the use of chemical fertilizers and pesticides, and are therefore potentially important component for the sustainable management of agricultural ecosystems.

This work investigated for the first time the influence of mycorrhization on the economically relevant part of the tomato plant, by analyzing its impact on the physiology of the fruit. To this aim, a combination of phenological observations, transcriptomics (Microarrays and qRT-PCR) and biochemical analyses was used to unravel the changes that occur during the ripening of fruit from Micro-tom tomato plants colonized by the AM fungus Glomus mosseae. Mycorrhization has exerted a positive effect on fruit productivity and accelerated the flowering and ripening processes. Eleven transcripts were differentially regulated in the fruit upon mycorrhization, and the mycorrhiza-responsive genes are involved in nitrogen and carbohydrate metabolism, signal transduction and response to hormonal stimuli. Mycorrhization has increased the amino acid abundance in the fruit from mycorrhizal plants, with glutamine and asparagine being the most responsive amino acids.

The obtained results offer original and novel data on the systemic changes that are induced by the establishment of AM symbiosis in the plant, and confirm the hypothesis that AM fungi extend their influence from the root to the fruit.
Recent advances in compartmentation and speciation analysis of iron and zinc in the cereal grain

Søren Husted, Daniel Persson, Thomas H. Hansen, Jan K. Schjørring

University of Copenhagen, Faculty of Life Science, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Key-words: Deficiency, Bioavailability, Speciation analysis, Multi-dimensional Chromatography, LC-ICP-MS

Iron (Fe) and Zinc (Zn) deficiency in humans is a serious problem in major parts of the world, not least in regions where people live on a cereal based diet. Iron and Zn deficiency leads to a number of diseases including anemia, mental retardment, stunted growth, immune dysfunction and various skin diseases. Consequently, there is an urgent need to increase the density and bioavailability of Fe and Zn in the edible parts of cereals. In this presentation we will provide a state-of-the-art overview on the current methods to study compartmentation of Fe and Zn between different tissue types of the cereal gain and present the most advanced methods to study their chemical speciation. This information is essential in order to study and improve the bioavailability of Fe and Zn.

Recent developments in compartmentation analysis using micro X-ray fluorescence Spectroscopy (µXRF), nano-Secondary Ion Mass Spectrometry (nano-SIMS) and LA-ICP-MS (Laser Ablation - Inductively Coupled Mass Spectrometry) have significantly expanded our knowledge on how Fe and Zn are distributed between the key-tissue types of e.g. wheat rice and barley grains. Moreover, novel information about the chemical binding forms of Fe and Zn are emerging, using a combination of multi-dimensional chromatography coupled to ICP-MS. This has recently shown that Zn predominately is bound to thiol-rich water soluble peptides, whereas Fe is bound to phytate oligomers. Moreover, it has been shown that the bioavailability of Fe and Zn in grains can be markedly improved by increasing the biosynthesis of low molecular weight ligands such as nicotianamine (NA), due to a change in Fe and Zn speciation.
RICE QUALITY AND NUTRITIONAL VALUE AND THE ENVIRONMENT

Elisabetta Lupotto1, Anna Benedetti1, Gianattilio Sacchi2

1CRA – Consiglio per la Ricerca e la sperimentazione in Agricoltura, Milan, Italy
2Università degli studi di Milano, Milan, Italy

Italy is the first European rice producer, with more than 50% of the total paddy production. Increased productivity and quality stability, resistance to actual and emerging diseases, resistance to old and new environmental constraints such as cold, drought stress and salty soils are major requirements to provide Italian rice production chain to sustain competitiveness at the international level. Industry and consumers’ request are driven by market, tradition, and well recognized search for quality. Farmers need new and more competitive varieties, adapted to the market’s request and tailored to sustain economically profitable cultivation joined to an environmentally friendly agrosystem: the actual rice production chain does not completely meets such requests important traits and associated mechanisms conferring élite characters to the plant.

A strong agriculture is vital for the EU food industry and global food security. The pressure on agricultural income is expected to continue as farmers are facing more risks, a slowdown in productivity, and rising input prices: these considerations are even more important for rice growers, being related to a well defined area almost completely devoted to paddy fields in a territory bound to the specific crop since centuries. It is therefore a major need to maintain income support and to reinforce instruments to better manage risks and respond to crisis situations. At the same time, agriculture and rural areas are being called upon to step up their efforts to meet the ambitious climate and energy targets and biodiversity strategy that are part of the Europe 2020 agenda. It has to be underlined that paddy fields are also one of the greenhouse gases producing areas, due to the type of the agrosystem adopted in anaerobic conditions. Farmers will need to be supported in adopting and maintaining farming systems and practices that are particularly favourable to environmental and climate objectives in order to operate in accordance to policy of strategic importance for food security, the environment and territorial balance.

Rice is not only one of the most important cereal species, but it is the model cereal species, with its genome completely sequenced and deciphered; indica and japonica varieties have been subjected to genome sequence thus offering the possibility to get information in wide genomic database. The completion of the accurate, map-based rice genome sequence in 2004 was a significant milestone for rice research. This indispensable tool has already helped rice researchers to identify the genes that are responsible for many important traits associated with rice growth and development. Currently, in addition to genomics, several new fields of study or the so-called “-omics” address various aspects associated with the genome including transcripts, proteins, and metabolites. Using the information revealed by the sequencing of the rice genome, techniques such as marker-assisted selection allow new varieties to be bred in a fraction of the time required as recently as 20 years ago. Considerable progress has been made in understanding the structure of the rice grain and its composition. Identification of genotypic differences in structure and nutrient composition and knowledge of the impact of pre- and postharvest factors on them are essential. Such knowledge will enable the development of
production, breeding and processing strategies and technologies to improve not only the processing and sensory quality of rice but the nutrition and health-beneficial characteristics of the grain.
Genomic approaches can be expected to further promote ground-breaking research that could lead to unprecedented improvement of rice as food or carbon source. Rice genetics, structural and functional genomics, comparative genomics, molecular biology and physiology, will all necessarily be integrated for a significant advance in final molecular assisted breeding strategies for a new crop ideotype.
Integration of genomics and breeding with the application of agrosystems and agricultural practices are essential tools to develop the agriculture of the future. Environmental quality is the main factor of food quality. In particular soil quality, expression of soil fertility, is the key factor for sustainability of crop production. The term ‘soil quality’ conveys well the important concept that the soil is a living system, which contains vast assemblages of organisms responsible for a variety of functions such as decomposition and recycling of nutrients from dead plant and animal tissues, fixation of nitrogen, maintenance of soil structure, regulation of the quality of air and water, and detoxification of pollutants by acting as a sink. Currently the European Union (EU) and many countries all over the world are developing legislation on soil conservation, soil quality and protection of soil biodiversity.

Italy has recently issued two large consortiums targeted to research on rice, from genetics and genomics to agronomy, agricultural practices and soil management. The project Risinnova coordinated by CRA-Agricultural Research Council, is a project targeted to genetic and genomic research, from the description of biodiversity in the European rice germplasm to the search for molecular markers for major traits, to assist breeding, and the discovery of genetic resistance to biotic and abiotic stress. The project Biogesteca, coordinated by the University of Milan, has a specific focus on the identification of sustainable agricultural practices for the major cereal crops of the Po plane: maize, rice and wheat. Both projects develop specific workpackages devoted to the study of soil fertility, association between the rice root system and the rhizosphere microorganisms, and the impact of cultural conditions on whole plant health, productivity, and the nutritional value and composition of the grain.
MODIFICATION OF A PLANT RESPONSE TO Mn Mn, Zn and Ca DUE TO THE AtECA3 EXPRESSION

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Keywords: AtECA3, calcium, manganese, P2A-ATPase, transformation, zinc,

Engineering plants with improved nutrient content and growth under unfavourable mineral conditions is a goal in biofortification.
In this study, ECA3 cDNA from Arabidopsis thaliana (P2A-ATPase) under the CaMV35S promoter was introduced into a model plant tobacco to examine its value for modifying responses to Mn, Zn and Ca. Plants were exposed in hydroponics to a range of metals (low-moderate-high), and their tolerance and accumulation were evaluated. AtECA3 expression did not produce one unique pattern of Mn and Zn accumulation, distinct from the wild-type. Instead, it depended on the external metal level. Transgenic plants grew better at moderate levels of Mn (2 µM) in the medium. Moreover enhanced tolerance to high Mn (100 µM) was noted though not accompanied by alterations of Mn accumulation. Transgenic plants were also more tolerant to Ca-deficiency conditions. In conclusion, the enhancement of plant productivity at moderate Mn levels and increased Mn tolerance at high (toxic) Mn supply, as well as the increase in Ca-deficiency tolerance and higher uptake of Zn at low Zn supply seen in ECA3-transformed plants indicates that this gene could be useful in plant biotechnological strategies aimed at improving crop production.


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SUBSTANTIAL NATURAL VARIATION IN MICRONUTRIENT CONTENT OF BARLEY GRAIN

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Keywords: Barley, breeding, diversity, QTL analysis

The potential to breed for biofortified crops depends on the extent of natural variation and the genetic architecture of micronutrient content traits. To address these questions we conducted one of the largest screenings of natural variation in barley grain micronutrient concentrations to date. Barley is a major crop and serves as a genetically less complex model for other graminaceous plants including wheat. Two different barley populations were analyzed for variation in grain micronutrient concentrations by ICP-OES: 1. a collection of about 140 cultivars assembled by us from the Barley Core Collection; guiding principle was maximization of geographic origins and soil types represented; 2. an extensively genotyped collection assembled by ICARDA. Plants were grown in multiple field environments and in greenhouses. Our results demonstrate substantial diversity. We obtained up to 4fold differences between genotypes for the biofortification target micronutrients Zn and Fe. Remarkably, ranges were similar for the two entirely independent collections and under a variety of conditions, indicating a large genotype contribution. Based on our phenotype data and marker information contributed by collaborators we initiated QTL mapping for micronutrient content in the ICARDA collection. Grain loading in extreme genotypes is being analyzed in transcriptome experiments using barley microarrays.
EXPRESSING \textit{NcZNT1} AND \textit{NcZTP1} FROM \textit{NOCCAEA (THLASPI) CAERULESCENS} ENHANCE Zn AND Cd TOLERANCE AND ACCUMULATION IN \textit{ARABIDOPSIS THALIANA}

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Keywords: Biofortification, Cadmium (Cd), hyperaccumulation, \textit{Noccaea caerulescens}, Zinc (Zn).

Zinc is a vital part of human nutrition and there is a global Zn deficiency in human diet being reported. Biofortification is an important tool to improve the nutritional value of food crops by using plant breeding and biotechnology. \textit{Noccaea (Thlaspi) caerulescens} is a model metal hyperaccumulator species that can accumulate up to 3\% of zinc, but also high amounts of nickel and cadmium. \textit{NcZNT1} is a member of IRT1 like protein family in \textit{N. caerulescens} and is localized to plasma membrane while \textit{NcZTP1} is a member of the CDF gene family, predicted to localize to the vacuolar membrane. We have expressed both these genes separately into \textit{Arabidopsis thaliana} under the control of constitutive CaMV 35S promoter to investigate their role in Zn accumulation and tolerance. Transgenic plants with high expression of the transgenes were grown hydroponically under high Zn, Cd, or low Fe supply. Transgenic plants were more tolerant to high Zn, Cd and low Fe, and accumulate more Zn and Cd. We conclude that the over expression of \textit{NcZNT1} and \textit{NcZTP1} play a role in high Zn, Cd and low Fe tolerance and enhanced Zn and Cd accumulation probably through enhanced Zn uptake inside cells and compartmentalization in the vacuole respectively. This can be one step further towards the progress of a viable GMO-based Biofortification technology.
ADAPTIVE MECHANISMS OF HYPERACCUMULATOR *NOCCAEA CAERULESCENS* ECOTYPES WITH CONTRASTING METAL HYPERACCUMULATION AND HYPERTOLERANCE TRAITS

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**Hyperaccumulator, next-generation sequencing, *Nocccea caerulescens*, transcriptome**

Studies on the metal hyperaccumulator *Noccaea caerulescens* have revealed several mechanisms that contribute to adaptation to metalliferous soils. Populations, however, vary in their abilities to tolerate and accumulate metals, but the lack of genomic sequence has hindered the discovery of the genes responsible for this variation. *N. caerulescens* is a close relative of *Arabidopsis thaliana* with 88.5% sequence identity in coding region, which has facilitated gene expression studies in *N. caerulescens*. The application of deep sequencing technologies to transcriptomics permits a large-scale comparison of transcriptomes for organisms without genomic sequence. We used SOLiD sequencing to characterize root transcriptomes of three *N. caerulescens* accessions with contrasting metal tolerance and accumulation patterns. Sequencing produced 250 million reads from which 70% were mapped to the *A. thaliana* genome. The 30% not mapped reads may comprise a source for identifying novel genes from *N. caerulescens*. The *A. thaliana* genome has 33518 genes (TAIR9), and *N. caerulescens* reads mapped to 97% of them. Transcriptional differences were found e.g. in metal ion homeostasis, defense response and secondary metabolite biosynthesis. The data provide a comprehensive picture of root processes among three *N. caerulescens* populations.
SIZE EXCLUSION CHROMATOGRAPHY COUPLED WITH ICP-MS IS AN EFFECTIVE METHOD OF SCREENING FOR AND QUANTIFYING BIOAVAILABLE MINERALS IN CEREAL GRAIN. THIS PROVIDES A USEFUL TOOL FOR BREEDING STRATEGIES AIMING TO PRODUCE NEW WHEAT VARIETIES THAT CAN BE USED TO COMBAT MINERAL DEFICIENCY

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Iron and zinc deficiency remain a prevalent nutritional disorder worldwide, disproportionately affecting people of low and middle income countries. Cereals account for over 50% of the energy intake in developing countries, and are a potentially important target for biofortification strategies aimed at improving dietary Fe and Zn content and utilisation. The effectiveness of a crop in combating Fe and Zn deficiency is largely dependent on Fe and Zn speciation, as different forms vary in their bioavailability. Size exclusion chromatography, coupled with inductively coupled plasma-mass spectrometry (SEP-ICP-MS) is a valuable approach to identify and quantify different forms of Fe and Zn in the grain. Adaptations of the method to quantify and identify nutritionally relevant forms of Fe (Fe-phytate, mono-ferric phytate and Fe-nicotianamine) and Zn-nicotianamine are described and demonstrated in the assessment of speciation differences in transgenic wheat (low phytic acid) and rice (over expressing nicotianamine synthase).

Six cultivars of wheat were analysed for total Fe and Zn in both the bran and endosperm fractions to identify any genotype differences. Two of these cultivars (Rialto and Riband) were further analysed for total Fe and Zn content in grain tissue fractions and speciation of Fe using SEC-ICP-MS. We found previously reported genotype differences in total mineral content of the whole grain to be consistent in both the endosperm and bran fractions over three locations. ICP-OES analysis showed that total Fe in Rialto endosperm (11.9 ± 0.6 mg/kg) was almost twice that of Riband (6.6 ± 0.4 mg/kg). Speciation analysis also showed a marked difference between the cultivars which may have important implications for the assessment of bioavailability.

Funding: Tristan Eagling has a BBSRC studentship and additional funding is provided by Harvestplus.
BREEDING CROPS FOR BETTER NUTRITION

Howarth Bouis

CGIAR, USA

Hidden hunger, or micronutrient malnutrition, afflicts more than 2 billion people around the world. It is caused by a lack of essential vitamins and minerals (such as vitamin A, iron and zinc) in the diet. Hidden hunger impairs the mental and physical development of children and adolescents and can result in lower IQ, stunting, and blindness; women and children are especially vulnerable. It also reduces the productivity of adult men and women due to increased risk of illness and reduced work capacity.

People who eat large amounts of cheaper staple foods (such as maize or cassava) and low amounts of more nutritious, but expensive, foods (such as fruits, vegetables, and animal products) are at greatest risk from hidden hunger.

HarvestPlus leads a global effort to breed and disseminate micronutrient-rich staple food crops to reduce hidden hunger among malnourished populations. This strategy, called biofortification, complements dietary diversification, fortification, and supplementation. Biofortification has three main advantages. First, it is targeted: 75% of the poor in the developing world live in rural areas where most of them eat large amounts of stale foods daily. Second, it is cost effective: after an initial investment in breeding micronutrient-rich crops, recurrent costs of improving these varieties are relatively low as the high-nutrient trait is ‘fixed’ in the crop. And third, it is sustainable: by improving the nutrient content of the staple foods that poor people already grow and eat, biofortification provides better nutrition using familiar foods. Farmers can save and share most micronutrient-rich seeds, roots etc. freely with their neighbors.

For biofortification to be successful high nutrient content must be combined with high yields and other attributes desired by farmers. Levels of nutrients in the crops, must also be sufficiently high to improve nutritional status when consumed by target populations. The biofortified crops must also be adopted by widely adopted by farmers and consumed by those suffering from micronutrient malnutrition to improve public health.

This presentation provides an overview of progress made in breeding for vitamin A, zinc, and iron in several staple food crops by HarvestPlus and its partners that are in the delivery pipeline. Recent advances in genomics, such as marker assisted selection, that have help advanced breeding will also be discussed.
THE IMMOBILISATION AND RETENTION OF SOLUBLE ARSENIC, CADMIUM AND ZINC BY BIOCHAR.

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Keywords: biochar, cadmium, zinc, arsenic, heavy metals, sorption

Water-soluble inorganic pollutants may constitute an environmental toxicity problem if their movement through soils and potential transfer to plants or groundwater is not arrested. The capability of biochar to immobilise and retain arsenic (As), cadmium (Cd) and zinc (Zn) from a multi-element contaminated sediment-derived soil was explored by a column leaching experiment and scanning electron microanalysis (SEM/EDX). Sorption of Cd and Zn to biochar’s surfaces assisted a 300 and 45 fold reduction in their leachate concentrations, respectively. Retention of both metals was not affected by considerable leaching of water-soluble carbon from biochar, and could not be reversed following subsequent leaching of the sorbant biochar with water at pH 5.5. Weakly water-soluble As was also retained on biochar’s surface but leachate concentrations did not duly decline. It is concluded that biochar can rapidly reduce the mobility of selected contaminants in this polluted soil system, with especially encouraging results for Cd.

Introduction

Biochar is a low-density charred material produced by burning biomass under conditions of low temperatures and minimal oxygen. Experimental application of this material to soils to sequester carbon has been encouraged by its very high organic carbon content (Yin Chan et al., 2009). There are wider agro-environmental benefits of adding biochar to soils, for example, increasing soil pH and reducing leaching of soluble macronutrients (Novak et al., 2009). Application of biochar to real world contaminated soil systems has received little systematic investigation to date but initial trials are encouraging regarding the retention of both inorganic and organic pollutants (Beesley et al., 2010). The present study evaluates the efficacy and permanence of As, Cd and Zn by biochar in a column leaching test and by microanalysis (SEM/EDX).

Materials and Methods

Triplicate bulk soil samples were sourced from a canal embankment in Kidsgrove, Staffordshire, UK, with a known history of heavy metals and As contamination (Beesley et al., 2010). Six glass leaching columns (XK50, Pharmacia Biotech, UK), 20 cm in length and 5 cm in internal diameter, were packed with 400g of air-dried soil (two columns) whilst the remaining four columns were packed to ¾ of their length with biochar (biochar had far lower density than soil).
Fig 1, 190x showing biochar’s variously sized pores (A.) and longitudinal, vertically cut cross-section of those pores at 550x magnification (B.).

Columns were leached upwards from their base continuously with de-ionised water (pH 5.5) at 0.1 ml min⁻¹ flow rate, maintained by a peristaltic pump. Two of the columns containing biochar were linked in parallel to the preceding two columns containing soil to allow biochar to intercept contaminated eluate directly leached from the soil columns. Eluate samples (10 ml) from soil were collected prior to passing through the biochar columns, by means of a small outlet at the joint between columns which remained closed at all times except during sample collection. The remaining two biochar filled columns were leached by the same method, but independently of the soil columns, to provide a biochar control. Columns were leached for 8 weeks in total. For the first 5 weeks (fractions 1-5) eluate was collected from all of the columns. In fractions 6, 7 and 8 (weeks 6, 7 and 8) leaching of soil columns was terminated, but both the intercepting biochar and biochar control columns continued to be leached as previously described. Arsenic, Cd, and Zn concentrations in the eluate samples were determined by ICP-MS. For the SEM/EDX analyses samples of soils and biochar both before and after leaching were dried and encased in epoxy resin, stuck to microscope slide, covered with colloidal graphite to ensure conductivity and analysed with a Jeol 6400 SEM microscope equipped with an Oxford detector. Images of EDX dot maps of As, Zn, Cd and SEM images of biochar structure were acquired and processed with INCA software (Oxford Inst.).

Results
Arsenic concentrations were by far the lowest of all elements measured in the eluate from the soil (< 10 µg l⁻¹), but did increase slightly when soil eluate was passed through biochar (Fig 2a & b), suggesting a cumulative effect given that concentrations were also very low from the biochar alone. Unlike arsenic, cadmium and zinc concentrations in eluate from soil were high, especially in the first 2 leaching fractions (200-500 µg l⁻¹ in each fraction) which resulted in cumulative removals of ~ 4 mg Cd per kg⁻¹ and ~ 2.5 mg Zn per kg⁻¹ of soil over the 5 fractions of the column test. Biochar rapidly and significantly (p < 0.05) rapidly reduced concentrations of both Cd and Zn as it intercepted contaminated eluate from the soil columns (Fig 2e & h). Given biochar’s large surface area (Fig 1) SEM/EDX detected greater sorption of As, Cd and Zn on biochar’s surface following the column test, corroborating the leaching test results. Furthermore, this microanalyses also confirmed de-sorption from the surfaces of soil, illustrating the relocation of soluble contaminants from soil to biochar.

Discussion
Biochar alone was free from significant amounts of As, Cd and Zn in water-soluble form and would be safe to use as a soil treatment without itself contributing to any environmental toxicity effects. Its structure might explain some of the effects observed as oxidation could occur most rapidly on outer surfaces, followed by interior pores, which might result in differential element retention between surface and interior sites as a result of shifting CEC for example. Nonetheless, biochar has proven to be effective at reducing high concentrations of soluble Cd and Zn originating from a contaminated soil and we can now more affirmatively say that sorption is one of the mechanism by which those metals are retained.
Fig 2. Concentration of arsenic (a, b and c), cadmium (d, e and f) and zinc (g, h and i) in eluate from soil, soil leached through biochar and biochar control. Note the differing scales between soil and biochar for Cd and Zn. Dotted, vertical line indicates where the leaching of soil was terminated and the leaching of the intercepting biochar and biochar control continued (mean n = 3 and error bars represent ± s.e.m).

In wider environmental terms the results of this study allow us to visualise biochar assisting the interception and arresting the migration of toxic elements to groundwater or its deployment in shallow, immature polluted soils to reduce soil-plant transfer of metals in the rhizosphere.

References


V. SIMULTANEOUS BIOFORTIFICATION OF Zn AND Fe IN *Triticum aestivum* L. – SEED NUTRITIONAL EVALUATION

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**Keywords**: Glicids, Nutritional value, Proteins, *Triticum* biofortification.

Certified seeds of *Triticum aestivum* L. cv Nabão (0Ts) were grown in walk-in growth chamber (EHHF10000, Aralab, Portugal), under environmental controlled conditions (80% RH; 24/20°C day/night temperatures; PPFD of ca 700 µmol m⁻² s⁻¹, 12 h photoperiod), in pots irrigated with a standard solution (1Ts), during 1 month following germination. Thereafter, until the end of the reproductive phase, half of the pots kept the same irrigation solution (1Ts), but the others were submitted to a five fold concentration of all nutrients (1T5s). The seeds obtained from 1T5s plants were grown again under the initial standard solution of nutrients (2Ts) and to 5, 7 and 10 times higher concentration of all the nutrients (2T5s, 2T7s and 2T10s). It was found that, relatively to 0Ts, in the seeds of 2Ts, 2T5s, 2T7s and 2T10s, Zn concentration increased about 9.92, 8.87, 4.73 and 9.00 fold, whereas Fe augmented 3.91, 4.95, 5.10 and 8.98 times. Moreover, the yield of the ears strongly decreased and the seeds became shrunken in 2T7s and 2T10. In the grains, relatively to 0Ts, raffinose, sucrose and starch showed, respectively, a 1.05, 1.29 and 1.04 fold increase in 2Ts, followed by a decrease to 96% / 86% / 76%, 66% / 59% / 53% and 45% / 35% / 47%, in 2T5s, 2T7s and 2T10s. The highest concentration of glucose and fructose was found in 2T7s (11.3 and 15.3 fold) and 2T10s (8.0 and 14.7 fold). Relatively to 0Ts, the contents of total protein increased 1.95, 3.01, 3.03 and 3.02 in 2Ts, 2T5s, 2T7s and 2T10s, respectively. Moreover, the amount of organic matter reached 98.3% in 0Ts, but in 2Ts, 2T5s, 2T7s and 2T10s was about 96.9, 95.9%, 96.3% and 95.5%, respectively. Relatively to 0Ts, the ashes in 2Ts, 2T5s, 2T7s and 2T10s showed 1.82, 2.12, 2.41 and 2.68 fold increases. The results suggest that at a nutritional level, 2T5s is the best option for simultaneous biofortification of Zn and Fe.
CHARACTERIZATION OF *ARABIDOPSIS* MUTANTS AFFECTED IN SEED IRON STORAGE.

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Keywords: Iron, Mutants, Seed, Transport, Vacuole.

To improve seed iron (Fe) content and availability, it is crucial to decipher the mechanisms which control Fe storage during seed development. In *Arabidopsis thaliana* seeds, Fe is stored in the vacuoles of cells surrounding the vasculature of the embryo. The AtVIT1 transporter is involved in Fe influx into vacuoles (Kim et al., Science 314:1295-98), while AtNRAMP3 and AtNRAMP4 function redundantly in Fe retrieval from vacuoles during germination (Lanquar et al., EMBO J. 24:4041-51). When germinated under Fe deficient conditions, *nramp3nramp4* double mutant development is arrested as a consequence of impaired Fe mobilization.

To identify novel genes involved in seed Fe homeostasis, we screened an EMS mutagenized population of *nramp3nramp4* for mutations restoring the growth of *nramp3nramp4* seedlings on low Fe. In parallel to their genetic characterization, we classified the mutant candidates based on their seed Fe content and Fe localization pattern. Among them, two mutants display a pattern of Fe localization similar to the *vit1-1* knockout mutant and carry mutations in the *AtVIT1* gene. This result establishes a functional link between Fe loading in vacuoles by AtVIT1 and its retrieval by AtNRAMP3 and AtNRAMP4. Other mutants with distinct Fe localization patterns and contents are currently under investigation.
PCP1 AND ATOSA1: PROTEINS INVOLVED IN CHLOROPLAST IRON HOMEOSTASIS IN *ARABIDOPSIS*.

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**Keywords:** Activity-of-bc1-complex family, chloroplast, Fe

This work is aimed at investigating the involvement in metal homeostasis of two *Arabidopsis thaliana* proteins belonging to the Activity-of-bc1-complex family. The first, PCP1, is homologous to a *Brassica juncea* gene modulated upon cadmium treatment; the second, AtOSA1, is a stress-related protein involved in plant response to oxidative stress. With the exception of a pale-green phenotype and a reduced total chlorophyll content in *atosa1* and *atosa1/pcp1* mutants, knock-out plants do not show any morphological/developmental abnormalities under standard conditions. Lack of PCP1 and AtOSA1 have no effects on photosynthetic performances, even though both are localised in chloroplasts. No differences in PSI and PSII protein composition were observed in mutants, with the exception of a reduction of Fe-containing Rieske and Cytf proteins. These differences suggest that mutant plants could be impaired in homeostasis of metal ions integrated into the photosynthetic apparatus. Indeed, mutant plants present a decrease in thylakoid Fe content in comparison to WT and showed a reduced growth and pronounced leaf chlorosis when cultured under Fe deficiency. Conversely, plants overexpressing PCP1 or AtOSA1 have an increased Fe content in thylakoids. In WT plants the expression of both genes is reduced by Fe deficiency. These data indicate a possible involvement of PCP1 and AtOSA1 proteins in chloroplast Fe homeostasis.
METABOLIC ENGINEERING OF THE IODINE CONTENT IN
ARABIDOPSIS THALIANA

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Keywords: Arabidopsis, biofortification, iodine, NIS, volatilization

Plants are a poor source of iodine, an essential micronutrient for human health. Several attempts of iodine biofortification of crops have been carried out in the recent years, but the scarce knowledge on the physiology of iodine in plants makes results often contradictory and not generalizable. In this work, we used a molecular approach to investigate how the ability of a plant to accumulate iodine can be influenced by different mechanisms. In particular, we demonstrated that the iodine content in Arabidopsis thaliana can be increased either by facilitating its uptake with the overexpression of the human sodium-iodide symporter (NIS) or through the reduction of its volatilization by knocking-out HOL-1, a halide methyltransferase. Our experiments show that the iodine content in plants results from a balance between intake and retention and that the increase of the uptake could be useless without a concomitant removal of the volatilization process. A correct manipulation of this mechanism could improve iodine biofortification of crops and prevent the release of the ozone layer-threatening methyl iodide into the atmosphere.
FROM ENZYMATIC BROWNING IN FRUITS AND VEGETABLES TO MODIFICATION OF PROTEINS BY REACTION WITH COPPER, ASCORBATE AND OXYGEN

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Keywords: ascorbic acid, copper, enzymatic browning, histidine, polyphenol oxidase

Enzymatic browning is common in many fruits and vegetables. The problem is acute especially in food processing. If not taken care off, it may lead to undesirable taste and color and loss of nutritive value. The reaction is catalyzed by polyphenol oxidase, a metallo-enzyme, containing copper as a prosthetic group. Prevention of enzymatic browning by ascorbate is generally thought to involve reduction of the product, a benzoquinone, to \( O\)-dihydroxyphenol, the substrate of the enzyme reaction, with the oxidation of ascorbate to dehydroascorbate. However, incubation of polyphenol oxidase with ascorbate alone, without the phenolic substrate, still led to the loss of enzymatic activity, suggesting a non-enzyme catalyzed reaction. It was hypothesized that free copper in solution in the presence of ascorbate catalyzed the reaction. Copper is an essential trace element in most fruits and vegetables. It is necessary for a complete diet. Ascorbate is a vitamin found in most of our plant derived foods. Ascorbate in the presence of added \( \text{Cu}^{2+} \) was effective in inactivation of the enzyme significantly more than either ascorbate, dehydroascorbate or copper, each alone. \( O_2 \) was required for the reaction. There were changes in the amino acid composition of polyphenol oxidase following treatment with ascorbate-\( \text{Cu}^{2+} \). The most distinctive change was a decrease in histidine from 4 to 1 mol/mol enzyme subunit. A similar modification, relatively specific to histidine, was obtained by treatment of other proteins by ascorbate-\( \text{Cu}^{2+} \).

The site-directed specificity toward histidine residues of proteins can best be explained by postulating that the \( \text{Cu}^{2+} \) involved in the reaction is bound to the imidazole group of histidine prior to reaction with ascorbate ion and \( O_2 \). There is indirect evidence that the reactive system leading to degradation of histidine residues in proteins is a quaternary complex between the imidazole group, \( \text{Cu}^{2+} \), ascorbate and \( O_2 \) reacting to form reactive species in proximity to the imidazole group leading to its degradation. The involvement of heavy metals, especially copper, in modification of proteins in food, is it beneficial or damaging?
INFLUENCE OF THE ADDITION OF DIFFERENT CONCENTRATIONS OF THICKENING AGENTS ON IN-VITRO MINERAL AVAILABILITY IN INFANT FORMULA.

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Keywords: Infant formula, mineral solubility, mineral dialysability, thickening agent.

Thickening agents such as carob bean gum or modified starches have been frequently added to infant formulas to increase their viscosity. These products, commercialized under the name of antirreflux (AR) formulas, are claimed to have beneficial effects on infants with gastroesophageal reflux. Nevertheless, it has been suggested that bioavailability of some minerals may be affected by thickening agents. The objective of this study was to determine the way in which the addition of different concentrations (7.5%, 15%, 50% and 100% respect maximum permitted) of carob bean gum (CBG), pregelatinized corn (PCS) or rice (PRS) starches, affect calcium, iron and zinc solubility and dialysability from infant formulas. A total of 12 AR infant formulas were prepared mixing each concentration of thickening agent with a commercial infant formula (reference). Each sample was in-vitro digested and the mineral content in soluble and dialyzable fractions was determined by AAS. Calcium solubility and dialysability were impaired by the addition of CBG, PCS and PRS, showing a negative correlation with their concentrations. Regarding to iron and zinc, only high concentrations of CBG decreased their solubility and dialysability, though no effect was found with PCS or PRS. The study of the effect of different combinations of CBG, PCS and PRS on mineral availability, as well as their effect on viscosity of formulas, could be an interesting target for future researches.
PROGRESS IN THE UNDERSTANDING OF CADMIUM TOLERANCE AND ACCUMULATION

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Keywords: accumulation, cadmium, hyperaccumulation, tolerance

Cadmium is one of the most toxic trace metallic elements for living organisms. Cadmium toxicity in plants is due to its capacity to react with thiol groups of proteins and of glutathione and to compete with essential elements. Cadmium accumulation in soils, mainly due to anthropogenic activity is a worldwide environmental problem and a threat for health.

Our aim is to understand detoxification and accumulation mechanisms of cadmium in plants. This research can provide novel tools in phytoremediation and food security.

Our models are the hyperaccumulators Noccaea caerulescens and Arabidopsis halleri, two close relatives to A. thaliana. They are Zn hyperaccumulators and some of their populations are also Cd hyperaccumulators. Genetic, transcriptomic and physiological analysis of those species allowed to understand major mechanisms of Zn tolerance and accumulation. The knowledge for Cd is less complete and will be reviewed in the presentation.

Recently we have shown that a 7 days Mg deficiency pre-treatment can alleviate Cd toxicity in Arabidopsis, without affecting Cd contents in roots or shoots. This observation opens a new pathway of research to understand Cd toxicity. Functional analysis of Mg deficiency responsive genes, with a potential role in Cd toxicity alleviation is in progress and will be reported.
ROOT ENHANCEMENT FOR CROP IMPROVEMENT

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The root system is an important plant organ, and optimized root system architecture is relevant to overcome yield limitations in crop plants caused by shortages in water or nutrients. Development of the root system is under control of both environmental and genetic factors. Classic breeding approaches for optimizing root systems are difficult because the trait is governed by many genes and is difficult to score. We have chosen a metabolic engineering approach to generate transgenic Arabidopsis, tobacco and oilseed rape plants with enhanced root-specific degradation of the hormone cytokinin, a negative regulator of root growth. Compared to wild type plants, these transgenic plants form a larger root system, whereas growth and development of the shoot are similar to the wild type. Elongation of the primary root, root branching and root biomass formation was increased by up to 80% in transgenic lines, causing an enhanced root-to-shoot ratio. Thus, it was demonstrated that a single dominant gene could be used to regulate to a great extent a complex trait, root growth. Plants with a larger root system had a higher survival rate after severe drought treatment. The accumulation of several elements, including micro- and macronutrients, was significantly increased in the aerial plant parts. This indicates that our approach could be used for the biofortification of crop plants or for phytoremediation of metal-contaminated soils.
THE ROLE OF BIOCHAR IN THE PHYTOREMEDIATION OF METAL/METALLOID CONTAMINATED SOILS.

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Keywords: Biochar, contaminated soils, heavy metals, phytoremediation, uptake.

Biochar has been evaluated for its role in improving soil quality and sequestering carbon, with much less attention paid to soil clean-up and remediation. In the following study a hardwood biochar and greenwaste compost alone and in combination were mixed with two contaminated soils (As, Cd, Cu, Pb and Zn) from former industrial sites and, following environmental exposure, pore water was collected and ryegrass (L. perenne L. var. Cadix) germinated to determine the amendments affects on i) solubility/mobility of the elements and ii) uptake and phytotoxicity to ryegrass.

Biochar was most efficient at reducing Cd and Zn in pore water, decreasing phytotoxicity to ryegrass, but mobilized small concentrations of As. Copper in pore water was also reduced by a decrease in dissolved organic carbon (DOC) and co-mobility. Greenwaste compost was more efficient for immobilizing Pb than biochar alone but combining greenwaste compost and biochar provided the best conditions for ryegrass growth and yield because of the immobilization of metals and the input of N and P from compost. Despite some reductions in ryegrass shoot concentrations of metals after amendment, the large biomass increase raised harvestable amounts of the metals, increasing food chain transfer potential. An assessment should therefore be made as to whether maximum reductions in plant concentrations or maximum reduction in harvestable amounts of metals are required to ensure amendments may be suitably deployed to maximize their effects.
USE OF DYNAMIC FACTORS TO IMPROVE PRACTICAL KNOWLEDGE IN METAL UPTAKE PROCESSES

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Keywords: dynamic factor of bioaccumulation, dynamic factor of biophilicity, dynamic factor of translocation, metal uptake

One of the milestones established by the WG1 “Soil-plant interactions and physiology (rhizosphere, bioavailability, agronomy, mineral fertilizers, use of nano-particles)” of the COST FA0905 is “to study selected soil and plant factors affecting root uptake and grain accumulation of heavy metals in crops”. Metal uptake by plants is a complex physicochemical process, which cannot be explained without taking into account plant physiology and plant existing conditions.

Since element bioaccumulation depends on physiological sensitivity on the total contamination of soil we use a new biogeochemical parameter – dynamic factor of bioaccumulation – to analyze change in bioaccumulation. Dynamic factor of translocation can be used to estimate changes of element translocation in plant. Change of element participation in metabolism can be expressed in dynamic factor of biophilicity.

Factor values express changes in uptake and translocation processes and are sensitive to changes in plant and soil conditions.

A case study of factor use with conclusions will be presented and discussed in the presentation.
PHYSIOLOGICAL CHARACTERIZATION AND TRANSCRIPTION ANALYSIS OF TWO ARABIDOPSIS MUTANTS RESISTANT TO CDS NANOPARTICLES

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Keywords: Arabidopsis thaliana, CdS nanoparticles, microarray, resistance, transposon mutation.

Nanoparticles have become widely used materials because of their unique structural, optic, electromagnetic and reactive properties. CdS quantum-dots are utilized in miniaturised hardware and optics equipment, they are synthesized through an inexpensive wet-chemistry process, and have a diameter of 5 nm. The CdS NPs small size make them particularly prone to enter in human and plant cells, but their toxicity potential has not been assessed yet. In this study we screened two mutagenised collections of Arabidopsis thaliana (L.) Heynh, to identify CdS NPs resistant individuals; we recognised two: Atnp01 and Atnp02, resistant to lethal concentration (for the wild type) of NPs. They showed the higher photosynthetic and respiration efficiency in the presence of the contaminant. We determined the integrity of the inserted Ac/Ds transposon, the number of transposon copies, and their positions within the genome of each mutant. We identified two putative insertions for Atnp01 and one for Atnp02. The next steps were the expression analysis of genes visited by the Ds element and the whole genome effects on the transcription using Real-Time PCR and Affymetrix microarray approaches. The relevance of the results will be discussed in the context of developing a risk assessment procedure for nanoparticles based on the model plant A. thaliana.
TRANSPORT AND DEPOSITION OF IRON AND ZINC IN THE WHEAT AND BARLEY GRAIN
IDENTIFICATION OF THE TRANSCRIPTOME CORRELATED WITH HEAVY METAL TRANSPORT GENE MANIPULATION FOR IMPROVED NUTRITIONAL VALUE OF THE WHEAT AND BARLEY GRAIN

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The research area comprises molecular characterization of genes encoding zinc and iron transporters and genes involved in zinc and iron binding and deposition in the developing barley grain and the manipulation of these genes by transformation to improve the zinc and iron content of the barley endosperm. We have previously performed microarray studies based on laser capture microdissection and have established the first roadmap for zinc and iron transport into the barley grain (Tauris et al.2009; Borg et al.2009). We have as one of our primary goals to develop this roadmap further by introducing next generation sequencing on laser capture microdissected tissues comprising the transport pathway from the vegetative tissues into the endosperm. Our second objective is to use this knowledge to improve iron and zinc content and bioavailability of the endosperm by gene manipulation by transformation. Our hypothesis is that it is possible to increase the content of essential minerals in the wheat endosperm by transgenic modulation of mineral transporters and deposition in the endosperm.

Gene manipulation:

- Bypass the normal deposition of zinc in the protein storage vacuole of the aleurone by tissue-specific down-regulation of the vacuolar zinc transporter, MTP1.
- Generate barley plants over expressing the HvHMA2 zinc transporter using unloading zone specific promoters.
- Generation of transgenic wheat overexpressing the wheat TaFer1 gene under the control of the wheat high molecular weight glutenine 1Dx5 promoter in modern CIMMYT wheat lines.
- Bypass the normal deposition of iron in the protein storage vacuole of the aleurone by tissue-specific down-regulation of vacuolar iron transporter, Vit1.
- Cross the transgenic lines with lines expressing phytase to improve the bioavailability of divalent mineral cations in the digestive tract of humans and livestock.

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References
ARSENIC IN RICE: GASTROINTESTINAL BIOACCESSIBILITY AND SPECIATION ARE AFFECTED BY FOOD MATRIX

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Rice significantly contributes to As uptake in Asian and Western diets. Several factors and processes may affect As bioaccessibility, bioavailability, speciation and toxicity upon rice ingestion. We assessed effects of the food matrix differing between diets. Two different matrices containing As-polluted rice were subjected in vitro to gastrointestinal digestion processes: a Western diet matrix high in fat and protein, and an Asian diet matrix low in fat and protein but high in carbohydrates. Gastrointestinal digestion processes were mimicked using the IVG method to which a colon digestion step was added. For use in colon incubations, microorganisms were grown in a validated simulator of the human intestinal microbial ecosystem, i.e. the SHIME system. In the Asian diet matrix, 85% of As was released from the rice matrix into the liquid fraction of the stomach and small intestine, and assumed to be bioaccessible. The remaining 15% is considered to move towards the colon. In the Western diet matrix, the bioaccessible fraction amounted to only 65%, suggesting that rice As will be less bioaccessible in presence of fat. In this diet, however, more As will move towards the colon and be transformed by colon microorganisms. Colon incubations reveal preferential formation of more toxic species (MMA³ and MMTA⁵) in the Western diet.
POSTERS
Wheat is a main staple crop in Iran and grain Zn concentration is a critical quality feature for human nutrition. Soil is the primary Zn source for plants and factors such as soil Zn availability, plant genotype, mineral nutrition and activity of microbes in the rhizosphere (especially the mycorrhizal fungi), all affect Zn uptake by plants. In a series of pot experiments using local wheat genotypes, we showed that the following soil factors (in order of importance) were limiting growth of wheat at Rudasht, central Iran: 1. High salinity, 2. Low N status, 3. Low P availability, and 4. Low Zn availability. Furthermore, we showed that management interventions like soil flushing in order to reduce salinity as well as different fertilization treatments had profound effects on the community structure of indigenous arbuscular mycorrhizal fungi in wheat roots. These induced shifts in community structure may have translated into different mycorrhizal activities that could explain a differential response to different soil salinity levels by two wheat genotypes in Zn use efficiency.
SELENIUM IN PORTUGUESE WHEAT GROWN UNDER FOLIAR SUPPLEMENTATION REGIME

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Keywords: Selenium, Triticum aestivum L., Triticum durum Desf., biofortification

Selenium is an essential micronutrient for humans and animals, yet it is deficient in at least one billion people worldwide. Plants and plant-derived products transfer the soil-uptaken selenium to humans; therefore, the cultivation of plants enriched in selenium can be an effective way to improve the selenium status on humankind. This paper focuses on determining the ability of bread and durum wheat to accumulate selenium after supplementation. One of the methods for supplementing this element in plants is foliar application with selenium solutions. These supplemented crops of wheat samples, bread wheat; Triticum aestivum L. (Jordão cultivar) and durum wheat; Triticum durum Desf. (Marialva cultivar), were used to determine if there is an increase of selenium content in cereal grains by comparing them with cereals cultivated in 2009 and harvested in 2010 with no supplementation. The experiments were done in two different growth stages – booting and grain filling – using sodium selenate and sodium selenite at three different selenium concentrations: 4, 20 and 100 g per hectare. Total Se is assessed by cyclic neutron activation analysis (CNAA), through short irradiations on the fast pneumatic system (SIPRA) of the Portuguese Research Reactor (RPI-ITN). Preliminary results show that the experiment was successful, since the selenium concentration increased in the cropped grains and reached values up to 35 and 50 times the non-supplemented crops of Jordão and Marialva, respectively. Supplementation in booting stage seems to be more effective for Jordão cultivar, while for Marialva grain filling stage shows more effectiveness.
ALIMURGIC HERBS AS POTENTIAL SOURCE OF MINERALS IN HUMAN NUTRITION

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Technological progress and food standardisation in the last few decades has narrowed the number of species supporting human diet, causing a loss of genetic resources (spontaneous herbs) and popular knowledge. The use of edible spontaneous species is known as phytoalimurgy.

In this study, 18 alimurgic species spontaneously growing in the Veneto region and belonging to 10 different families were screened for their mineral contents. The herbs were grown in silty-loam soil in a phytoalimurgic garden at the experimental farm of the University of Padova (Legnaro) during 2010-2011, and the edible parts (leaves, shoots or roots) were harvested in May 2011. The highest concentrations of 6 nutrients (Ca, Fe, Cu, Mn, B, Li) were found in *Borago officinalis* L. leaves, Cu and Li being particularly elevated (126 and 2.9 mg kg\(^{-1}\) d.w., respectively). *Campanula rapunculus* L. provided high concentrations of Co and Mo, but was poor in Ca (5 times lower than in *B. officinalis*).

Within species ranking, sprouts of *Humulus lupulus* L. were lowest in Fe (83 mg kg\(^{-1}\) d.w., i.e., 13× lower than the maximum), Li and Mo, but richest in P (6,970 mg kg\(^{-1}\) d.w.) and Zn (77.4 mg kg\(^{-1}\) d.w.). P and Mg were markedly concentrated in *Silene alba* (Miller) Krause. Half of the species belonged to the Asteraceae family and only rarely did they accumulate high levels of nutrients aboveground, probably due to root retention; the few exceptions were *Crepis vesicaria* L. for Ni (5.5 mg kg\(^{-1}\) d.w.) and *Pichris echoides* L. for Cr (3.18 mg kg\(^{-1}\) d.w.), both twice as high as the mean for the species. Selenium was not detected in any species, whilst S, Na, P, K, Mo and B had high bioaccumulation factors.

These preliminary results show that alimurgic herbs have an interesting natural nutritional value, which may be further investigated and exploited through specific agricultural practices.
MINERAL ACCUMULATION IN NATURAL POPULATIONS OF 
ARABIDOPSIS THALIANA

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Keywords: ionome, Arabidopsis, hydroponics, soil culture, elemental correlation

In order to grow on soils that vary widely in chemical composition, plants have evolved mechanisms for altering the elemental composition of their tissues. The variation that exists within a species can be exploited to understand how individual elemental pathways interact with each other and to identify genes important for these processes. We analyzed the elemental composition (ionome) of 96 accessions of Arabidopsis thaliana grown in hydroponic culture and soil using inductively coupled plasma mass spectrometry. The levels of 17-19 elements were analyzed in roots and leaves from the hydroponic experiment and leaves and seeds from the soil experiments. Significant genetic effects were detected for almost every element measured in every experiment. We observed very few correlations between the elemental composition of the leaves and either the roots or seeds. There were many pairs of elements that were significantly correlated with each other within a tissue and experiment, but almost none of these pairs were consistently correlated across tissues and growth media, a phenomenon observed in several previous studies. These results suggest that the ionome is highly dynamic, yet tightly controlled by genes and gene x environment interactions. The dataset provides a valuable resource for mapping studies to identify genes regulating mineral concentration in plant tissues.
EFFECTS OF BIOCHAR AND GREENWASTE COMPOST AMENDMENTS ON MOBILITY, BIOAVAILABILITY AND TOXICITY OF INORGANIC AND ORGANIC CONTAMINANTS IN A MULTI-ELEMENT POLLUTED SOIL.

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Keywords: Biochar, compost, trace metals, PAHs, pore water

Elevated levels of inorganic and organic pollutants co-exist in soils from previously industrialised areas. The use of soil amendments can reduce the water-soluble fractions of these pollutants, but the effects are often contaminant specific. Trace element mobility and PAH bioavailability in an amended, contaminated soil was monitored over a 60 day period to establish whether biochar, derived from pyrolysed hardwood, was more advantageous than greenwaste compost at immobilizing soil pollutants. Toxicity was also assessed by a simple ryegrass seed germination test.

Copper and As concentrations in pore water were elevated by the addition of both compost and biochar, associated with a significant increase in DOC and pH. Biochar immobilised Zn and Cd, with sustained, 10 fold reduction of Cd in pore water. Biochar also reduced the total and bioavailable concentration of PAHs further than compost. Combined application of amendments was less effective than their individual application at reducing both total and bioavailable concentrations of PAHs but seed germination was significantly increased (p<0.05) equally by compost and biochar.

Biochar reduced the bioavailable fraction of Cd and Zn more effectively than green waste compost whilst also reducing concentrations of the heavier and more toxicologically relevant PAHs but some mobilisation of Cu and As means that biochar cannot be considered as a blanket solution to pollutant reduction in multi element contaminated soils without further investigation.
CARBON AND TRACE ELEMENT FLUXES IN THE PORE WATER OF AN URBAN SOIL FOLLOWING GREENWASTE COMPOST, WOODY AND BIOCHAR AMENDMENTS, INNOCULATED WITH THE EARTHWORM *LUMBRICUS TERRESTRIS*

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**Keywords:** Biochar, dissolved organic carbon, earthworms, heavy metals, soil carbon.

The benefits of adding composted organic materials to soils to enhance carbon storage and ecological functionality could be countered by the mobilisation of some harmful pollutants commonly found in frequently degraded urban soils. Carbon, nitrogen and trace element fluxes in an urban soil’s pore water were studied in response to the surface mulch addition and the incorporation into of greenwaste compost and two non-composted amendments; a woody oversize material and biochar in the presence and absence of the earthworm *Lumbricus terrestris*.

Both composted and non-composted amendments enhanced dissolved organic carbon (DOC) in soil pore water to ~100-300 mg l\(^{-1}\) in the upper depth of the soil profile above which they were applied as a mulch and similarly within the mesocosms in which they were mixed. Dissolved organic carbon, dissolved nitrogen (DTN) and trace metals, especially Cu and Pb, where enhanced to the greatest extent by greenwaste compost, because of strong co-mobilisation of metals by DOC. Biochar enhanced As and Cu mobility in the field profile and, additionally Pb in the mesocosms, with no effect on Cd. The woody, oversize amendment neither greatly increased DOC nor As, Cu, Pb or Zn mobility although, unlike the other amendments, earthworms increased DOC and Cd mobility when soils were amended with this material.

Therefore the addition of non-composted materials to some urban soils, versus composted greenwaste could reduce the risk of mobilising potentially harmful elements, whilst usefully improving soil quality.
Abstract: In Italy, tomato plantations contribute a significant proportion of both economical prosperity and food capital. Measures to reduce uptake of contaminants from soils to plant and prevent risk to higher consumers are thus essential to maintain the integrity of this resource. Organic soil amendments have been applied to manage soil nutrition and element mobility, and ensure soil quality for many years. Recently biochars (biological residues combusted under low oxygen conditions, resulting in a porous, low density carbon rich material) have been demonstrated to immobilise soluble trace elements present in soils, and reduce their uptake to plants. Other potentially beneficial effects have been noted, such as increased cation exchange capacity, pH and soil moisture retention, which could also be beneficial to plant growth and success.

In the present study one *Solanum lycopersicum* L. cultivar, chosen from previous screening, was transplanted to biochar amended soils (30% by volume) and maintained in controlled conditions (23°C, 30% humidity and 16h photoperiod) in the laboratory. In the following 40 days, pore water samples were extracted from each replicate. Results were correlated with foliar As concentrations at the end of the experimental period to determine whether biochar had reduced As uptake. Plant growth parameters were also examined to establish the likely effects on fruit biomass and yield.
CULTIVATION OF VEGETABLES IN TWO AS-SOILS

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Arsenic (As) is widely distributed in nature, with arsenate and arsenite being the most common species. Arsenic intake from food stuffs may contribute to the total amount of As intake. Greenhouse vegetables were analysed for As-species using HPLC coupled with an atomic absorption spectrophotometer. The aim of the study was to investigate how two different soils, alum shale and glassworks soil, influenced the As accumulation and speciation in vegetables. Results show that alum shale soil had twice as high As concentration but 20 times lower plant availability of As compared with the glassworks soil resulting in a higher accumulation of As in lettuce cultivated in the glassworks soil. The concentration of plant available As was twice as high in rhizosphere soil compared with bulk soil for both soils. Arsenate was the predominating species in both rhizosphere and bulk soil, while arsenite predominated in both the roots and shoots of lettuce. Arsenate predominated in carrot and spinach when cultivated in alumshale soil while arsenite predominated when grown in the glassworks soil. In conclusion, the As availability in the soil influenced the As accumulation in plants and inorganic As predominated in the vegetables. Also, lettuce was able to modify the rhizosphere making As more plant available. The latter is interesting since lettuce is shown to accumulate high As levels.
EFFECTS OF SELENIUM ON THE GROWTH PARAMETERS OF TOMATOES AND BASIL CROPS

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Keywords: Bioaccumulation, Basil, Micronutrients, Selenium uptake, Tomatoes.

Selenium (Se) is an essential micronutrient for mammalian nutrition; yet the margin between its beneficial and harmful levels is quite narrow. Diet is the main selenium source for humans and animals. Increasing tissue Se concentrations in edible crops through Se fertilization strategies would improve the overall contribution of Se to the human diet. However, it is not known if Se is a required element for plant growth, but it may inhibit plant growth at various concentrations if accumulated in plant tissues. Anthropogenic Se contamination of ground water was documented in the Jezreel Valley in Northern Israel. A high content of Se (up to 37 µg L⁻¹) caused the shutdown of two wells in the surrounding area. Our objective was to evaluate the effects of Se concentration in water on the development of tomatoes and basil. Plants were cultivated in greenhouse pots and irrigated with water supplemented with Se 0.25-1.5 mgL⁻¹. The results showed that Se upto 1 mgL⁻¹ didn’t affect tomatoes’ roots or shoot biomass; however, basil was inhibited by all Se concentrations compared to the control group. The Se accumulation in roots, shoots, and fruits was linear; however, in basil the accumulation showed a saturation curve. Tomatoes plants are relatively resistant to Se compared to basil.
HMA2 EXPRESSION IN METALLICOLOUS AND NON-METALLICOLOUS NON-HYPERACCUMULATOR METALLOPHYES

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Keywords: Cd tolerance, HMA2, Silene paradoxa, Zn tolerance

The role of HMA2, a heavy metal transporting 1b P-type ATPase, in Cd or Zn hypertolerance was examined in the non-hyperaccumulator metallophyte Silene paradoxa L.. Cd and Zn tolerance and HMA2 expression levels before and after metal exposure were checked in nine Tuscan (Italy) populations from different substrates (non-contaminated, serpentine and mine soil). Plants were cultivated in hydroponics and tolerance was assessed from root length growth after Cd or Zn exposure. Metal concentrations in plants were measured by AAS, after acid digestion. RNA isolated from roots was used for cDNA synthesis. HMA2 expression levels were checked using real time qPCR. Only two mine populations showed metal hypertolerance, Fenice Capanne to Cd and Zn and Campiglia Marittima to Zn, while all the others were sensitive to both metals. Constitutive transcript levels of HMA2 were much higher in these two populations relative to all the others. This enhanced level of expression was maintained also under Cd and Zn exposure, showing a strict correlation of HMA2 over-expression with Zn hypertolerance. In the F2 and F3 progenies of segregating crosses between calamine (Fenice capanne) and non-metallicolous (Colle Val d’Elsa) plants, however, the enhanced constitutive HMA2 expression co-segregated strictly and consistently with Cd hypertolerance, but not with Zn hypertolerance. The same was found in intra-specific crosses of the congeneric metallophyte S. vulgaris.
EFFECT OF ADDITION OF SULPHUR FERTILIZER AND ITS FORM ON ZINC BIOFORTIFICATION OF WHEAT

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Keywords: zinc, wheat, elemental sulphur, sulphate, grains

The effect of sulphur on the uptake of zinc by wheat is much less studied than the influence of nitrogen. Increasingly soils are becoming sulphur deficient due to reduction in air pollution among other factors. Elemental sulphur ($S_0$) is commonly used to acidify alkaline soil and thus release micro-nutrients such as Zn. However no work has been carried out to distinguish if pH only or the metabolic function of S in the plant results in higher Zn uptake by plants. In this study we compared the addition of $S_0$, sulphate and zinc to a deficient soil on their effect on zinc uptake and yields. Soil pH was found not to be reduced by the 100 mg kg$^{-1}$ $S_0$ added to the soil and hence the available Zn was not increased. Due to the addition of fertilizers only at the start of the experiment, SO$_4$ addition resulted in a great deal of vegetative growth but low grain biomass and delayed maturity due to it having been consumed by grain filling time. $S_0$ released SO$_4$ more slowly so plants had a higher grain biomass, reached maturity faster and had longer for grain filling. This resulted in a greater total content of Zn and S in grains per pot. If only the treatments with sufficient Zn and S were considered a correlation between Zn and S concentrations in grains was found suggesting that Zn is associated with S-containing proteins in the grains.
CHANGES IN ELEMENT COMPOSITION IN *FUSARIUM*-INFECTED WHEAT GENOTYPES

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Keywords: *Fusarium*, micro-and macroelements, wheat genotypes

Wheat quality and consumer safety is threatened by *Fusarium* Head Blight caused by among others *F. culmorum* and *F. graminearum*. Infected grains contain *Fusarium* toxins, mainly deoxynivalenol (DON), nivalenol, but there are some minor components of still unknown physiological effects.

Our hypothesis is that *Fusarium*-infection influences physiological, biochemical and membrane-transport characteristics of the plant resulting in altered mineral composition in the grain.

Plant samples were collected from artificially *Fusarium*-infected resistant and sensitive wheat genotypes inoculated with *F. culmorum* and *F. graminearum*. After appearance of symptoms, samples of flag leaves, culm, green ear, and after harvest chaff and grain were wet digested and their Cu, Mn, Fe, Zn and K, Ca, Mg concentrations were determined.

Results show that gradients of element concentrations are characteristic for the element in question: from flag leaf to grain Cu, Mn, Fe concentrations are decreasing, Zn accumulates in the grain while Ca in the chaff, and K accumulates in the culm tissue. In some cases *Fusarium*-infection influenced element concentrations, mainly for Zn, K and Mg.

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I. SIMULTANEOUS BIOFORTIFICATION OF Zn AND Fe IN
Triticum aestivum L. – A TRANSGENERATION SURVEY

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Keywords: Iron, Triticum biofortification, Scanning electron microscopy, Zinc

Certified seeds of Triticum aestivum L. cv Nabão (0Ts) were grown in walk-in growth
chamber (EHHF10000, Aralab, Portugal), under environmental controlled conditions (80%
RH; 24/20°C day/night temperatures; PPFD of ca 700 µmol m⁻² s⁻¹, 12 h photoperiod), in
pots irrigated with a standard solution (1Ts), during 1 month following germination.
Thereafter, until the physiological maturity, half of the pots kept the same irrigation
solution (1Ts), while the other half was submitted to a five fold concentration of all the
nutrients (1T5s). The seeds 1T5s were sown and grown again with the initial standard
nutrient solution (2Ts) and with solutions containing 5, 7, 10 and 13 times fold the
concentration of all the nutrients (2T5s, 2T7s, 2T10s and 2T13s, respectively). It was
found that, relatively to 0Ts, in the grains of 1Ts, 1T5s, 2Ts, 2T5s, 2T7s, 2T10s and 2T13s,
Zn concentration increased about 7.18, 6.10, 9.92, 8.87, 4.73, 9.00 and 9.23 fold, whereas
Fe augmented 1.01, 1.35, 3.91, 4.95, 5.10, 8.98 and 11.06 times, respectively. Additionally,
by SEM it was established that seeds of the 2T7s, 2T10 and 2T13s became
increasingly shrunken. Although, a higher development of the spikelets occurred in the
ears of 2T5s and 2T7s, a genetic homogeneity was found to persist at DNA level, as judged
by AFLP markers. Nevertheless, differences at the transcription level are expected and will
be evaluated in a near future. Considering the wheat commercial relevance for the farmers,
it is concluded that, under the defined growth conditions, 2T5s is the treatment that might
fit best a simultaneous biofortification of Zn and Fe.
SELENIUM FERTILIZATION: AN EFFECTIVE STRATEGY FOR BIOFORTIFICATION OF DURUM WHEAT AND ITS END-USE PRODUCTS

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Keywords: biofortification, durum wheat, grain quality, pasta, selenium

Selenium (Se) is an essential trace element for human nutrition, with antioxidant, anti-cancer and anti-viral effects. One of the possible strategies to increase the Se concentration in wheat grains is represented by the development of new protocols of fertilization. A field study was conducted in replicated plots, over two years at Foggia, Italy, in order to evaluate, the effect of 11 rates of Se fertilization ranging from 0-120 g ha⁻¹ on Se accumulation in grains, semolina and pasta. The effect of Se on grain yield and quality of durum wheat was also investigated. The Se determinations were carried out by ICP-MS. Field trials on durum wheat showed that Se concentration in grains increased progressively by 20- to 60-fold with growing Se applications. The maximal grain Se concentration of 7 mg kg⁻¹ was achieved following the treatment with 120 g ha⁻¹ of Se. Grain yield, ash content, gluten index and protein content were not affected by applied Se. Conventional roller milling showed only a small effect on the Se concentration of flour fraction as well as pasta making and cooking. Further studies will be carried out to investigate the bio-accessibility in products derived from high-Se treatments.
Animals including ourselves have a broader array of essential elements than plants, and plants take up elements using a very restricted set of carrier ligands (far less than involved elements, even those essent. for plants), implying that nutrient transfer is not substrate-specific. Only for this reason herbivory can work at all, also delivering metals etc. not needed by the food plants as such but it also implies the co-transfer and probable resorption of unwanted elements, like Cd or Pb or REE, actinoid radionuclides. Research into details of ligands accomplishing the transfer into a plant thus are required to improve selectivity and, sometimes, quantities of uptake of nutrient (trace) metals and some non-metals.

Many quite different soil organisms (plants, fungi, soil bacteria including chemolithoautotrophs, earthworms, nematodes) are known to deliver organic (often N-containing) ligands to the soil liquid, which bring about more or less selective uptake of chemical elements from solid (mineral, metal-bearing organic) soil phases after complexation/etching, sometimes to the advantage of one’s neighbor (mykorrhiza). Now, that the chemical potential of metal ions in ecological surroundings (soil, water) can be precisely estimated, both empirical and theoretical studies on corresponding ligand effects – and thus the impact co-growing some selected kind of grass, fungus etc. has on biofortification of essential vs. withholding toxic metals in some food or fodder plant – are available which give a rationale for a simple strategy to achieve the ends of COST 905.
CHEMICAL COMPOSITION OF EIGHT CHESTNUT CULTIVARS FROM “IVANIK” PLANTATION - SOUTHWESTERN PART OF BULGARIA

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Keywords: chestnuts, chemical composition, moisture, starch, total sugar, mineral content

In this study, chemical composition of eight native and non-native cultivars from “Ivanik” nursery plantation - South-western part of Bulgaria, were investigated. High variability in chemical composition among cultivars was found, corresponded to the high genetic variability between cultivars. Cultivar I – 5/3, breaded from C. mollissima, produced nuts with lowest moisture content. The highest moisture content was found for C. crenata x sativa cultivars. Higher starch content showed cultivars, breaded from C. crenata. Cultivar Zlatarevo had higher total sugar content, much higher than the average values reported by the most of the researchers. Cultivars Marigoule and Hemus showed the lowest values in crude protein, but highest were recorded for C. crenata cultivars. Lower in potassium and phosphorous content were cultivars, breaded from C. mollissima and with higher content were those, breaded from C. crenata. All of the cultivars are needed of mineral supplementation in order to correct Ca:P imbalance.
A research on soil and plant contamination with heavy metals (Pb, Cd, Zn) in garden allotments and plots (fallow lands) located in the vicinity of non-ferrous metal plants in Piekary Śląskie, Poland was carried out. Representative samples of soil and plants (celery and parsley leaves plus carrot and celery roots) from 11 allotment complexes (each of them consisting of 50-200 individual allotment gardens) and also soil and grass samples from 20 fallow plots were collected. Levels of toxic metals were determined using a flame AAS technique. Concentrations in soil and plants were assessed based on the permissible values of these metals in particular environmental media. Pearson correlations ($r$) between the contents of metals in plants and their contents in soil were calculated and assessed. In majority of cases the correlations were statistically significant (positive correlations with a confidence level of 0.05), which confirms the strong impact of metal concentrations in soil on their concentrations in plants, as well as the existence of mutual correlations between the investigated metals.
LOSSES OF ESSENTIAL MINERAL NUTRIENTS BY POLISHING OF RICE DIFFER AMONG GENOTYPES DUE TO CONTRASTING GRAIN HARDNESS AND MINERAL DISTRIBUTION

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Key words: Bran, endosperm, ICP-MS, iron, micronutrients, polishing, rice, synchrotron X-ray fluorescence microscopy, zinc

The effect of different polishing techniques on losses of mineral elements from rice grain were quantified using a panel of indica and tropical japonica rice genotypes, empirically classified as differing in adhesion of the bran layers. Gradients in mineral elements across the bran-endosperm interface were further quantified by use of micro-scaled precision abrasive polishing in combination with inductively coupled plasma mass spectrometry (ICP-MS) and synchrotron X-ray fluorescence microscopy (SXRF). Rough frictional polishing of similar intensity as in commercial mills, i.e. 8-10% loss of grain weight, reduced the concentration of elements such as Fe, Mg, P, K, Mn and Se with 60-80% irrespective of whether grains were classified as easy of difficult to polish. Following gentle abrasive polishing (3-5% weight loss) genotypes classified as difficult to polish generally showed a smaller reduction in Fe, Mg, P, K, Mn and Se with 60-80% irrespective of whether grains were classified as easy of difficult to polish. Following gentle abrasive polishing (3-5% weight loss) genotypes classified as difficult to polish generally showed a smaller reduction in Fe, Mg, P, K, Mn and Se compared to genotypes classified as easy to polish. The concentration of other elements, e.g. Zn, S, Ca, Cu, Mo and Cd, was much less affected by polishing and their concentration upon milling showed comparable reductions (<30%) irrespective of polishing technique or ease of polishing. The different patterns of polishing losses of mineral elements closely reflected their distribution within the grain. There was a good agreement between semi-quantitative SXRF images and the quantitative data obtained by ICP-MS analysis of micro-polished grain fractions. Both techniques showed that Mn was more confined to the surface layers of the grain than Fe, while the Zn concentration declined much less steeply towards the core of the endosperm. Five-fold differences in the reduction of Zn and Fe concentrations during polishing were observed for different genotypes, showing a clear potential for selecting genotypes with reduced losses of Zn and Fe after polishing.
ZINC FLUXES INTO THE DEVELOPING BARLEY GRAIN: USE OF STABLE ZN ISOTOPES TO SEPARATE ROOT UPTAKE FROM REMOBILIZATION IN PLANTS WITH CONTRASTING ZN STATUS

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Key words: Barley, grain, isotope, flux, root, remobilization, zinc

Improved knowledge on Zn fluxes in plants is important for the development of agronomic and genetic strategies to address the problems of Zn deficiency in humans. Zn import into developing grain is the result of two processes integrated over time: translocation of Zn absorbed de novo from the root medium and remobilization of Zn from vegetative tissues. Little information is available on the relative importance of these two Zn pathways in plants and how they are affected by the nutritional Zn status of the plant. Here we used the stable isotope $^{67}\text{Zn}$ to characterize and quantify fluxes of Zn uptake and remobilization in barley plants with different Zn status. Barley plants were grown in complete nutrient solutions with 3 different zinc levels, i.e. 100 nM (low Zn), 1500 nM (medium Zn) or 5000 nM Zn (high Zn).

When grain development reached 15 days after pollination, the Zn concentration was in all treatments changed to 1500 nM $^{67}\text{Zn}$ and plants were harvested after 6 h, 24 h or 48 h. The short exposure time was chosen to minimize the remobilization of new $^{67}\text{Zn}$ from leaves to the spike. Zn concentrations and isotope ratios were determined using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). During the 48 h experimental period plants, with low Zn status absorbed a total of 1760 nmol Zn which was 2.8- and 4-fold more than that in medium and high Zn status plants. Stems and spikes were the primary recipients of the de novo incorporated Zn with preferential allocation to the developing grains over time. The leaves received in all cases a very small proportion (<5%) of the newly absorbed Zn and the proportion did not increase over time. Leaves have very low sink strength for newly absorbed Zn during generative growth stages. The highest flux of newly-absorbed isotopically-enriched Zn into the developing grains occurred in plants with low Zn status which imported 18 pmol Zn grain$^{-1}$ h$^{-1}$. In plants with medium and high Zn status, the grains imported 5.5 and 4.8 pmol Zn grain$^{-1}$ h$^{-1}$, respectively, from de novo root uptake. Remobilization of previously accumulated Zn from stems and leaves delivered over the same period 19.2, 17.8 and 59.5 pmol grain$^{-1}$ h$^{-1}$ in plants with low, medium and high Zn status, respectively. It is concluded that stable isotopes in combination with ICP-MS provide a strong tool for quantification of Zn fluxes in intact plants and that the importance of Zn remobilization compared to de novo root absorption of Zn increases with increasing plant Zn status, while very little newly absorbed Zn is translocated to the leaves during generative growth stages.
BARLEY METALLOTHIONEINS: MT3 AND MT4 ARE LOCALIZED IN THE GRAIN ALEURONE LAYER AND SHOW DIFFERENTIAL Zn BINDING

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Key words: Immunnocytochemistry, size-exclusion ICP-MS, metallothioneins, transcriptional analysis, zinc

Cereal Zn homeostasis and Zn remobilization to grains have received much attention due to the low nutritional quality of human diets throughout the world. Metallothioneins (MTs) are low molecular weight, cysteine-rich proteins believed to function in cytosolic Zn and Cu homeostasis. Even though MT expressions have been reported in developing grains or seeds across multiple plant species, their physiological role is elusive. We show here that the MT family in barley consists of at least 8, possibly 10, functional metal binding MTs. When the barley MT cDNAs were expressed in Cu or Cd sensitive yeast mutants, a variable increase in Cu and Cd tolerance was observed with the highest complementation obtained by MT1a, MT2b1 and MT3. In developing grains we focused on MT3 and MT4 as both represent unique proteins in barley. Combined transcriptional and histological analysis showed that protein and transcript levels correlated both over time and space during grain development. MT4 was localized in the embryo and aleurone layer whereas MT3 was present in tissues of both maternal and filial origin. Using state-of-the-art speciation analysis by SEC-ICP-MS and ESI-TOF-MS on recombinant MT3 and MT4 we quantified their specificity and capacity for metal ion binding, showing preferential Zn binding relative to Cu and Cd. We suggest a house-keeping role in metal homeostasis for MT3 and a Zn storage function for MT4 in developing and mature grains. The localization of MT4 and its discrimination of Cd make it a good candidate for future biofortification strategies directed towards increasing food and feed Zn concentrations.
THE PRESENCE OF COPPER (Cu^{2+}) IN WINE FROM SUB-
MEDITERRANEAN SLOVENIA

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Keywords: copper, grape, heavy metal, wine

Objectives: Copper (Cu^{2+}), as a heavy metal in grape and wine, represents a health hazard being in strong contradiction with the growing demands for food from environmentally-sound cultivation in the EU today. The Cu content in wines has already been legally limited to 1 mg L^{-1}, however excesses in its content have been observed lately, although their proveniences mostly remain unexplained.

Methods: The 22 wine samples produced in the sub-Mediterranean winegrowing region of Slovenia were sampled randomly in the years 2010 and 2011. In 2011 additional emphasis was put on young wines, where the highest and excessive Cu contents were expected. The wines’ copper content was determined with atomic absorption spectrometry (AAS).

Results: Surprisingly at all studied wines the Cu content was below legal limitation (< 1.0 mg L^{-1}), furthermore 75% of the samples were below detection limit for copper (0.1 mg L^{-1}). On the other hand, young wines tended to contain higher Cu concentrations in comparison to ageing wines, means between 0.2 and 0.3 mg L^{-1}.

Conclusion: The excessive Cu content in wines can be explained by its potential provenience, from grapes’ intake of spraying or by the inappropriate use of oenological agents. Further studies should additionally focus on the Cu provenience in wines, as human health has to be the uppermost in the minds of all involved in wine production.
GRAMINACEOUS PLANTS DYNAMICS IN SOIL CONTAMINATED WITH RUNWAY DEICER

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Keywords: above-ground part, graminaceous plants, phytomass, runway deicer.

Technical granular sodium chloride (NaCl) treated with potassium hexacyanoferate, a road maintenance material most-widely applied in wintertime in Lithuania. As an alternative to it, patented under the name of Clearway F1, was selected for the experiment. Clearway F1 is an environmentally friendly runway deicer, based on a unique formulation of potassium formiate and environmentally friendly inhibitors. Three species of graminaceous plants, most frequently used for roadside planting in Lithuania when building new and reconstructing or renovating the exiting motor roads, were analysed: perennial ryegrass (Lolium perenne L.), fescue-grass (Festuca pratensis Huds.), meadow-grass (Poa pratensis L.). The paper presents experimental findings about the effect the runway deicer materials have on the parameters of graminaceous vegetation – the height of an above-ground part and a phytomass. The experiment’s findings show that the perennial ryegrass had the biggest (15.98 mg) and the meadow grass – the smallest (2.06 mg) phytomass of the analysed graminaceous plants in contaminated soil. During the experiment it was again the perennial ryegrass that reached the biggest (12.18 cm), while the meadow grass – the smallest (3.57 cm) height of the above-ground part. The experimental findings also show that the perennial ryegrass has the highest resistance to a toxic effect of potassium formiate Clearway F1.
DETECTION OF CADMIUM DISTRIBUTION IN ARABIDOPSIS PLANTS

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Keywords: Arabidopsis, cadmium localization, dithizone, galactoglucomannan oligosaccharides

Increasing cadmium pollution, its toxicity and enter the food chain (mostly from plants) are known. Cadmium is readily absorbed by plant roots, where it can cause growth inhibition, and then is transported from roots to aerial parts. For understanding the mechanisms involved in Cd tolerance, trafficking and accumulation, essential is to resolve its distribution in the plant. The present study investigates the distribution of Cd in plants growing in contaminated substrate and possible mediatory role of biologically active galactoglucomannan oligosaccharides in Cd distribution, accumulation and plants protection against cadmium toxicity, especially in roots.

The presence of GGMOs diminished the negative effect of Cd on Arabidopsis roots elongation. The prolongation of the elongation zone and enlargement of the meristem size results in significantly longer primary roots compared to the cadmium treated roots.

Cadmium distribution in plants, determined by histochemical technique using dithizone, depended on the duration of cadmium stress. Cadmium, absorbed by the root cap and root hairs, was at the beginning of incubation stored in the root and hypocotyl, later transported by xylem up to the leaves, and here stored in trichomes. GGMOs didn’t unambiguously influence cadmium localization in plants.
MICROBIOLOGICAL AND PHYSICOCHEMICAL ANALYSIS OF WATER OF ARTIFICIAL LAKE “LIVOCI” DURING SPRING SEASON 2010

Kemajl Kurteshi, Muharrem Ismaili

During autumn season samples of waters were collected from different localities in the artificial lake “LIVOCI” nearby city Gjilani and analyzed bacteriological and physicochemical using standards methods. Total viable count was by plate technique. The pH ranged from 6.9 – 7.3 for water samples while temperature ranged from 7.9 to 14.5 °C. We done analysis and with system VITEK, where determination different bacteria such as: E.coli with biocode 6004770633.
DETERMINATION OF POLLUTION OF DRINKING WATER THROUGH MICRONUCLEUS TEST

Kemajl Kurteshi, Kasum Letaj

Clastogenic effects of home wastes and agricultural contaminates of river artificial lake Badove nearby Prishtina, which supply the Prishtina city by drinking water, were investigated in peripheral erythrocytes of fish Carasius auratus. Examination of blood smears showed that the formation of micronuclei significantly increased during one year (MN=29 /1000 erythrocytes, with P value < 0.01) and were more abundant compared to the control group. This increase in the formation of micronucleus indicates that agricultural pollution increases the risk of clastogenic effects on peripheral erythrocytes of fish Carasius auratus and may have similar effects on the human population located around the artificial lake.
Iron deficiency is the most important micronutrient deficiency in the world especially in developing countries where more than 50% of the anemias are thought to be due to iron deficiency. Potato is an important staple food and constitutes a good source of potassium and vitamin C, but remains less valuable as a source of iron. Thus, iron biofortification of potato tubers is a crucial strategy for these populations. Ferritin proteins are known to be one of the main components of the iron transport and storage in plant. In potato, until now, the involvement of ferritins in tuber iron accumulation has not been well described. The present work aimed to study ferritin gene expression in tubers with low and high iron content.

Two *Solanum tuberosum* cultivars (Princess and Amandine) were evaluated for the iron content in tubers. In the peel, a higher iron content was observed in Princess (38.97µg/g DW) compared to Amandine (29.82µg/g DW). In the flesh, this trend remained the same with higher amounts (26.57µg/g DW) in Princess compared to 22.33µg/g DW in Amandine.

Using an *in silico* approach, three different ferritin isoforms were isolated from the EST databases and from each isoform, specific qPCR primers were designed. All the ferritin isoforms were expressed in the tubers and seemed to be correlated with iron content.
Selenium (Se) is an essential trace element for animals and humans, which enters the food chain through plants. High excess of this metalloid can be toxic, however Se plays an important role in preventing against a number of diseases. Copper (Cu) is an essential microelement in plants, but at a toxic level its symptoms are dangerous for human health as well. Its deficiency is rare but a high concentration of it may cause nausea and diarrhea, tissue injury and disease, liver damage etc.

In our experimental system we used two pea cultivars (Pisum sativum L. cv. Rajnai törpe = Petit Provencal and. cv. Lincoln) and wild type (Col-0) Arabidopsis thaliana as model organism to investigate the physiological responses to long-term copper and short- and long-term selenium exposure. After treatment with different concentrations of Cu and Se, we studied growth parameters, biomass, the level of nitric oxide (NO)-, reactive oxygen species (ROS) and viability in the meristem cells of the plants using in vivo and in situ microscopic methods. We concluded that the effect of treatments is time- and concentration dependent in this stress condition. The increasing concentration of Se/Cu decreased the viability and this lead to serious growth inhibition and decreased biomass.
IV. SIMULTANEOUS BIOFORTIFICATION OF Zn AND Fe IN *Triticum aestivum* L. – KINETICS OF PHOTOCHEMICAL REACTIONS IN ISOLATED THYLAKOIDS FRACTIONS ALONG THE LIFE CYCLE

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Keywords: Photochemical reactions, Photosynthetic electron transporters, *Triticum* biofortification

Certified seeds of *Triticum aestivum* L. cv Nabão (0Ts) were grown in walk-in growth chamber (EHHF10000, Aralab, Portugal), under environmental controlled conditions (80% RH; 24/20°C day/night temperatures; PPFD of ca 700 µmol m⁻² s⁻¹, 12 h photoperiod), in pots irrigated with a standard solution (1Ts), during 1 month following germination. Thereafter, until the end of the reproductive phase, half of the pots kept the same irrigation solution (1Ts), but the others were submitted to a five fold concentration of all nutrients (1T5s). The seeds 1T5s were grown again under the initial standard solution of nutrients (2Ts) and to 5 times higher concentration of all the nutrients (2T5s). It was found that, relatively to 0Ts, in the seeds of 2Ts and 2T5s, Zn concentration increased about 9.92 and 8.87 fold, whereas Fe augmented 3.91 and 4.95 times, respectively. In order to evaluate the yield of quanta conversion implicating ATP and reducing equivalents synthesis, the accumulation of Chl and carotenoids, as well as the Hill and Mehler reactions (H₂O-MV, H₂O-DCPIP, DPC-DCPIP, DCPIP₂-MV) and photosynthetic electron carriers (Cyt f, b₅₆₃, b₅₅₉LP, b₅₅₉HP and plastocyanin) were determined 52, 74 and 94 days following germination. Trends of Chl and carotenoids content were 2T5s>2Ts>1Ts in the 52nd day, but in the other periods the higher values were detected in the 74th day. The functioning of PSII-OEC, PSI and PSI+PSII in most of the experimental periods showed higher rates in 2Ts. The contents of all the electron transport carriers kept its proportionality relatively to Chl accumulation. In this context, specific targets in the thylakoid machinery, prompting negative impacts or downregulation processes are presented and discussed.
BUCKWHEAT PRODUCTION ON ACID AND LOW FERTILE LITHUANIAN SOILS

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Keywords: soil liming, alternative crops, nutrient concentrations

Objectives and methods: Buckwheat is suitable for cultivation on various soils and can be easily raised organically, at a premium price. Buckwheat tolerates very acid soil and even negatively responds to increasing of soil pH >5 or intensive liming. Limited information available how liming intensity reflects on crop quality and chemical composition of both – grain and straw. In attempt to provide insights into these buckwheat cultivation issues, datasets from long-term liming and mineral fertilizing experiments were compiled and statistically processed. These studies been carried out on Haplic Luvisols in Eastern Lithuania.

Results: Data indicate that buckwheat biomass yield and its quality had unstable correlations with soil acidity indices. Nitrogen and phosphorus fertilisers are gradually improving concentration of these elements in grain (0.13-0.15 % N, 0.02-0.03 % P). But there were no positive effect of fertilising on accumulation of NPK nutrients in buckwheat straw. In contrast, accumulation of Ca increased on intensively limed soil (up to 0.97% Ca).

Conclusions: In general we conclude that buckwheat biomass production might be strongly soil acidity dependent, if climatic conditions are unfavourable during some critical vegetation periods. The results indicate that soil acidity management did affect nutrients concentrations in buckwheat. Both, grain and straw differ in their chemical composition depending on soil acidity status, with higher NP and Ca concentrations recorded on limed plots.
ARSENIC CONCENTRATIONS IN EDIBLE PARTS OF
VEGETABLES GROWN ON SOILS LIMED WITH CALCIUM
CARBONATE, COAL BOTTOM ASH, AND COAL FLY ASH

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Keywords: cereals, food safety, leafy vegetables, liming, root vegetables

Liming is commonly used to increase acid soil pH in SW France. Three alkaline materials, i.e. calcium carbonate (CaCO₃, 3.8 and 15.9 t/ha), coal bottom ash (CBA, 7.8 and 30.4 t/ha) and coal fly ash (CFA, 7.1 and 17.1 t/ha) were incorporated into a humic podzol (P) and a luvisol (L) in field experiments. Depending on soil conditioners, pH in year 1.5 increased from 4.6 to 5.3-6.7 in soil P and rose from 5.3 to 5.5-6.4 in soil L. The CaCO₃, CBA, and CFA inputs of As were 7.3-30, 365-1417, and 302-722 g/ha in Soil P and 1.1-3.7, 351-501, and 90-206 g/ha in Soil L. Winter wheat, maize, cabbage, celeriac, French bean, leek, lettuce, potatoes, turnip and carrots were cultivated and harvest at market stage. Potato tubers, roots of turnip and carrots were peeled prior to be oven-dried. Weighed aliquots of plant materials were wet-digested under microwaves with HNO₃/H₂O₂. Arsenic concentrations were determined with GFAAS (Zeeman effect). Yields of edible plant parts were higher for lettuce, celeriac, carrots, and French beans in soil L. At high level, CBA affected yields of lettuce (soil P) and carrot (soil L) whereas CFA decreased yields of leek (soil P) and French beans (soil L). Arsenic concentrations (<0.01 – 1.9 mg/kg DW) depended on plant species, soils and alkaline materials. As in lettuce leaves peaked for CFA-amended soil P exceeding common As values.
THE IMPACT OF HEAVY METALS ON RED CLOVERS

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Keywords: heavy metals, red clovers Arimaičiai, soil pollution

Red clovers Arimaičiai have been chosen in this work for decontaminating soil from heavy metals. These plants were grown under artificial laboratory conditions in soil which was periodically contaminated with heavy metals. Plastic pots of 7x25x11 cm with double bottoms were used for the experiment. 0.5 kg of uncontaminated soil was put into each of the pots. Each pot with soil contained 10 g of seeds. Uncontaminated soil was used for growing control plants. Seeds of plants were seeded to uncontaminated soil and soil which was periodically contaminated with heavy metals. Since humidity is the main factor conditioning the growth of plants and the necessary physiological processes, grass plants were watered every 3 days. Control plants were watered with 100 ml of ordinary water while plants grown in the periodically contaminated soil were watered with 100 ml of mixture of heavy metals. The maintained temperature was 22-24°C and the lighting was natural. The experiment lasted six months.

Table 1. Heavy metals concentration in the soil

<table>
<thead>
<tr>
<th>Cu mg/kg</th>
<th>Cr mg/kg</th>
<th>Zn mg/kg</th>
<th>Pb mg/kg</th>
<th>Mn mg/kg</th>
<th>Ni mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>100</td>
<td>300</td>
<td>100</td>
<td>1200</td>
<td>75</td>
</tr>
</tbody>
</table>

After permanent soil application with Cu, it was noticed that after six months Cu concentration in soil was 3 times smaller than in the beginning of experiment, but in red clovers there were 25 mg/kg of Cu. When Mn concentration in soil reached 1200 mg/kg, after VI month red clovers had absorbed 30 % of it from soil, so it was noticed that concentration in plant was 355 mg/kg of Mn. When Ni concentration in soil reached 75 mg/kg it was noticed that after VI month red clovers absorbed 32 % of Ni from soil (24 mg/kg of Ni in red clovers Arimaičiai). When Cr concentration in soil reached 100 mg/kg after VI month red clovers sorbs 31 % of Cr (31 mg/kg in red clovers), and when Zn concentration in soil reached 300 mg/kg red clovers sorbs 17 % of Zn from the pollution soil (51 mg/kg in plant). When Pb concentration in soil reached 100 mg/kg red clovers removed 23 % of Pb from the soil, so there were 23 mg/kg of Pb in red clovers Arimaičiai.
IRON BIOAVAILABILITY FROM POTATO: A COMPLEX INTERPLAY BETWEEN ENHANCERS AND INHIBITORS FROM THE MATRIX

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Keywords: ascorbic acid, Caco-2 cells, chlorogenic acid, Iron, Potato,

Dietary iron deficiency is the most common nutritional disorder in the world. Increasing the amount of bioavailable dietary iron could prevent iron deficiency and anaemia. Potato is one of the most consumed crops worldwide; it has been described to be a good source of minerals, such as potassium and magnesium and some cultivars have been shown to be rich in iron and zinc.

The aim of this work is to evaluate iron bioavailability for human consumption from different potato cultivars and to study the impact of enhancers of iron bioavailability (e.g. ascorbic acid) or inhibitors present in potato, such as chlorogenic acid.

Caco-2 cells were used to evaluate the bioaccessibility of iron; the abundance of the ferritin protein was used as a marker of iron uptake. Investigations were done on in vitro digested potato extracts as well as on separated compounds exposed to Caco-2 cells. ICP-MS, Elisa and western blot results on Caco-2 cells show that Fe(II) is absorbed in presence or not of ascorbic acid; however, its absorption is enhanced by ascorbic acid. Fe(III) uptake is mostly due to the presence of ascorbic acid. Chlorogenic acid inhibits Fe(III) and Fe(II) uptake induced by ascorbic acid.

Different potato cultivars submitted to an in vitro gastro-intestinal digestion displayed different polyphenol profiles; results will be presented on the iron uptake by Caco-2 cells as influenced by these different profiles and thus illustrate the impact and nature of enhancers and inhibitors of iron uptake in potato.
HOW TO ENHANCE NUTRIENT ASSIMILATION IMPROVING BIOAVAILABILITY

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Keywords: bioavailability, Cupper, diet, micronutrients, Zinc.

Abstract
This work shows that, increase of availability of micronutrients, such as Cu and Zn, displays direct correlation between the accumulation capacity of a species (insertion of a gene increases the internal concentration of nutrients), and increased bioavailability. Therefore bioavailability can be increased by inserting this single gene.

Objectives
The ultimate goal is to find a method, transportable to food crops (human diet), to increase bioavailability

Methods
Wild type, and plants overexpressing a phytochelatin synthase, were grown in pots containing two types of industrial sludge. After several months of growth, the accumulation of micronutrients inside the plants, growth medium and in leachate were measured.

Results and conclusions
There is a greater presence of micronutrients in the leachate from pots containing overexpressed-gene insertion plants and a greater decrease in the concentration in the growth medium. Since the amount of micronutrient accumulated is greater in the plants which contain the gene insert, can be concluded that gene overexpression, results in higher bioavailability. The percentage reduction of micronutrient in the middle of the pot, ranged from 78 to 87%. Consequently, this gene insertion may be a way to increase the efficiency of accumulation of trace elements in a plant, reducing costs and increasing the nutritional value of a crop.
THE EFFECT OF ROOT ENHANCEMENT IN TRANSGENIC CYTOKININ-DEFICIENT *ARABIDOPSIS* AND *BRASSICA NAPUS* LINES ON NUTRIENT ACCUMULATION

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**Key words:** Arabidopsis, Brassica napus, CKX gene, cytokinin-deficient plant, metal accumulation, transgenic lines

The size and the architecture of the root system determine the plant’s ability to access water and nutrients. The optimization of root system architecture can overcome yield limitations and lead to increase nutrient concentration in crop plants that might be also useful for human nutrition.

Werner et al. (2001; 2003: 2010) have shown that cytokinin is a negative regulator of root growth and that manipulation of the cytokinin status might be a feasible approach to genetically engineer plants with an enhanced root system. One possibility how to reduce the cytokinin status *in planta* is the constitutive overexpression of cytokinin-degrading cytokinin oxidase/dehydrogenase (CKX) genes (Werner et al., 2001, 2003).

The aim of the present study was to generate cytokinin-deficient transgenic *Arabidopsis* and oilseed rape (*Brassica napus*) plants with a larger root system and to explore the relevance of the root enhancement for an improvement of uptake of mineral nutrients. Novel transgenic *Arabidopsis* lines were generated by overexpression of the CKX3 gene under control of a root-specific promoter and rapeseed transgenic lines were generated by constitutive overexpression of the CKX2 gene. Root development was assessed *in vitro*, in hydroponics and in soil in the greenhouse, exposed to different metal concentrations.

Elongation of the primary root, root branching and root biomass formation were increased by up to 60% in transgenic *Arabidopsis* lines. In addition, leaf concentrations of several elements, such as Zn, S and Mn were significantly higher in all lines than in the wild type on all growth substrates (Werner et al., 2010). Cytokinin-deficient *B. napus* transgenic lines also formed more lateral roots and showed a significantly higher shoot metal accumulation than the wild type.


CONTROLLING CADMIUM ACCUMULATION IN PLANTS

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Keywords: CzcCBA transporter, heavy metals, phytoremediation, Pseudomonas putida

This study is focalized on the expression \textit{in planta} of the trans-envelope pump CzcCBA, typical of gram-negative bacteria as \textit{Pseudomonas putida}, selected in this study. The complex is constituted of three components (CzcA, CzcB, CzcC) that arrange a chemiosmotic antiporter, conferring heavy metal resistance. Since CzcCBA transports heavy metals across the membrane, we investigated its application to modulate cadmium accumulation in plants.

Constructs were created to (i) identify the localization of proteins in the plant cell and (ii) to analyze their effect on shoot metal accumulation:

(i) \textit{CzcA}, \textit{CzcB} and \textit{CzcC} were fused to eGFP. Protoplasts transfection highlighted probable plasma-membrane and nuclear localization of \textit{CzcC}. Results concerning \textit{CzcA} and \textit{CzcB} need further investigation.

(ii) \textit{Arabidopsis thaliana} and \textit{Nicotiana tabacum} plants were stably transformed with the information driving the overexpression of the three genes separately. Preliminary data in tobacco, show that plants are phenotypically normal and that \textit{CzcA} and \textit{CzcB} alone are able to lower shoot cadmium amounts in transgenic plants, when compared to wild type. Considering that the Czc system is constituted of three subunits, transgenic plants harboring the single Czc genes are being crossed, to isolate individuals that carry the complete CzcCBA complex and to verify whether the entire transporter confer different cadmium transport into plants.
STUDY OF HEAVY METALS AND METALLOIDS IN SOILS AND CROP PRODUCTION IN THE REGION OF “ELATSITE-MED” AD

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Keywords: disturbed soil, food safety, heavy metals, risk assessment, vegetables

OBJECTIVES
The objectives of the present study were to evaluate the level of heavy metal contamination of the soils of the municipality of Mirkovo in the region of “Elatsite-Med” AD and the risk of growing vegetables on that territory.

METHODS
The vegetative and animal nutrients are direct source of toxic metals and metalloids for humans. Representative plant and soil samples were taken according ISO 10381-1 and BSS 17.4.5.01-85 for analysis of heavy metals and arsenic. Sample preparation and analytical measurements were made according standardized methods and AAS.

RESULTS
We determined that in the soils from studied region exist lack of vital nutrients and variation of pH. Regarding the soils was found that the contamination with Pb is low, medium to high for Cu and low for Cd. No presence of Zn, Ni or As was found. Important accumulation of Pb and Cr were found in vegetables (pepper and cabbage).

CONCLUSIONS
The cultivation of food plants on low or medium contaminated soils could be an important risk for humans and animals. There is a necessity of recultivation on this territory in order to diminish such risk.
RESPONSES OF SORGHUM PLANTS TO STRESS CAUSED BY METAL EXPOSURE

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Keywords: abiotic stress, plant enzymes, Sorghum bicolor, toxic metals

Sorghum bicolor L. is an important crop due to its wide use as food, feed and energy crop. It is unique due to a specific compounds content (phytates, tannins), which affect its nutritional value. These compounds form complexes with calcium, copper, iron, manganese and zinc, thereby worsen the availability of these essential elements. Increased concentrations of essential elements in agricultural soils are the result of human activity, abundant in nature. Higher concentrations of zinc have resulted in higher income of the plant. On the other hand, most of the land is contaminated with more than one element, especially with toxic metals, and the plant receives not only more zinc, but also more unnecessary toxic elements like cadmium, lead, nickel, cobalt and arsenic. What’s more, the concentrations of these metals are so toxic they can cause death of plants or reduce their production. Their toxicity is mediated by reactive oxygen species that cause oxidative stress.

In this study, we investigated the temporal sequence of physiological reactions, including changes in chlorophylls contents and antioxidative enzymes activities and also metal contents in roots and shoots of Sorghum bicolor seedlings after metal exposure.

Acknowledgement: This work was supported by project NPVII 2B08058.
THE ROLE FOR HMA5 IN COPPER HYPERTOLERANCE IN THE METALLOPHYTE *SILENE PARADOXA*

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Keywords: Copper, Hypertolerance, Metallophyte, *Silene paradoxa*.

This project investigated the role of HMA5 in copper tolerance in *Silene paradoxa*, an herbaceous adaptable to cupriferous soil. HMA5 is a univalent cation transporter, essential for normal levels of Cu tolerance and Cu root-to-shoot transport in Arabidopsis; this gene is mainly expressed in roots (Andrés-Colás et al., 2006). It is strongly induced upon exposure to excessive Cu (Andrés-Colás et al., 2006).

We studied Cu tolerance and HMA5 expression in three cupricolous, serpentine and non-metallicolous populations, stressing the plants with different concentrations of copper treatments. From plants grown at 0 and 4 µM CuSO₄, DNA and RNA were extracted from roots and shoots, RNA was converted to cDNA; we amplified parts of HMA5 cDNA and gDNA.

Copper tolerance was confined to two mine populations, while other populations were Cu-sensitive.

The Cu-concentrations in plants showed differences between populations, but the pattern of variation was neither related with soil type, nor with Cu tolerance.

Fenice Capanne mine has the highest expression of HMA5, second is in Colle Val D’Elsa non metallicolous population, the third place is Campiglia mine. This could mean that HMA5 over-expression is not a primary determinant, but a hypostatic modifier of Cu tolerance, or that the gene functions differently in CVD.
TO BE ECO, OR NOT TO BE? – SOME ASPECTS OF CROP SAFETY

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Keywords: barley cultivars, cadmium grain concentration

Some percent of barley cultivated in Poland is used for direct human consumption. It is well known that food free of artificial preservatives, fumigants, heavy metals, etc. is always awaited on the market. The goal of the presented study is to evaluate cadmium uptake under different soil conditions by five barley cultivars commonly available on the market.

Two samples of clean soil and one sample of soil medium contaminated with heavy metals were chosen for the pot experiment performed in a vegetation room. Soil properties were analyzed using ISO methods. Barley grains were collected and analyzed for Cd concentration.

The total cadmium concentration in clean soil samples ranged from 0.47 mg kg\(^{-1}\) up to 1.73 mg kg\(^{-1}\), while in the case of medium contaminated soil the mean concentration was about 12 mg kg\(^{-1}\). According to the standard value for Cd grain concentration only one cultivar might be recommended for food production. The rest of the investigated cultivars grown on the clean soil can be used only for feed purpose. The barley cultivars grown on the contaminated soil may be used as feed after application of soil chemostabilizers.
NECESSITY OF BIOFORTIFICATION WITH SELENIUM OF PLANTS USED AS FODDER AND FOOD IN ROMANIA

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² “Al. Ioan. Cuza” University Iassy, Romania

Keywords:  selenite; alfalfa; wheat; biofortification

Selenium deficiency in animals, but with major implication in humans, required to carry out experiments regarding Se enrichment of some fodder and food sources. The experiments conducted into green house were followed the selenium enrichment of alfalfa, by the administration of selenium as potassium selenite solution (K₂SeO₃) into soil, on seed and on plant. Administration of selenium in soil, with doses from 1 to 10 mg / kg has contributed to increase the Se content of alfalfa plants up to 6.5 times. Foliar fertilization with solutions containing 4 mg Se in a volume of 100 ml water, increased the selenium content of the plant up to 14 times, and the application of selenium on seed by spraying it with solutions that contained between 1 and 5 mg Se / l helped to increase the amount of selenium in the plant up to 11 times. The experiments carried out on the field have revealed a tendency to increase the accumulation of selenium into the wheat grain, with up to 10% as a result of spraying the plants on 9-10 state according to Feeks scale with a solution containing 1 g sodium selenite (Na₂SeO₃) per hectare. The experimental data obtained show the necessity and possibility of bio-fortification with Se of the plants used as fodder and vegetable origin foods.
III. SIMULTANEOUS BIOFORTIFICATION OF Zn AND Fe IN *Triticum aestivum* L. – IMPLICATIONS ON THE PHOTOSYNTHETIC MACHINERY DURING THE LIFE CYCLE

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Keywords: Fluorescence, Photosynthesis, Sc anning electron microscopy, Triticum biofortification.

Certified seeds of *Triticum aestivum* L. cv Nabão (0Ts) were grown in walk-in growth chamber (EHHF10000, Aralab, Portugal), under environmental controlled conditions (80% RH; 24/20°C day/night temperatures; PPFD of ca 700 µmol m⁻² s⁻¹, 12 h photoperiod), in pots irrigated with a standard solution (1Ts), during 1 month following germination. Thereafter, until the end of the reproductive phase, half of the pots kept the same irrigation solution (1Ts), but the others were submitted to a five fold concentration of all nutrients (1T5s). The seeds 1T5s were grown again under the initial standard solution of nutrients (2Ts) and to 5 times higher concentration of all the nutrients (2T5s). It was found that, relatively to 0Ts, in the seeds of 2Ts and 2T5s, Zn concentration increased about 9.92 and 8.87 fold, whereas Fe augmented 3.91 and 4.95 times, respectively. To understand the mechanisms of photoassimilates mobilization to the seeds in these treatments, Chl a fluorescence parameters (F₀, Fₘ, Fᵥ/Fₘ, ETR, Φₑ, qₚ, qₙ, NPQ and Fᵥ'/Fₘ') where monitored 55, 73 and 91 days following germination. A progressive increase from 1Ts to 2Ts and between 2Ts and 2T5s was observed in most parameters. In these experimental time periods the trend displayed by stomatal conductance (gₛ) was 1Ts>2Ts>2T5s, but no structural changes of stomata were found, as evaluated by SEM. Moreover, the rate of photosynthesis (Pₙ) and the leaf internal CO₂ concentration (Cᵢ) showed maximum values in 2Ts and 1Ts, respectively. The transpiration rate (Tr) displayed higher values only in 2Ts, 55 and 73 days after germination. Considering these *in vivo* determinations, specific targets prompting negative impacts or downregulation processes in the photosynthetic machinery are presented and discussed.
III. SIMULTANEOUS BIOFORTIFICATION OF Zn AND Fe IN Triticum aestivum L. – NUTRIENTES ACCUMULATION IN THE SEEDS

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Keywords: Nutrients localization, Triticum biofortification, X-ray microanalysis

Certified seeds of Triticum aestivum L. cv Nabão (0Ts) were grown in walk-in growth chamber (EHHF10000, Aralab, Portugal), under environmental controlled conditions (80% RH; 24/20°C day/night temperatures; PPFD of ca 700 µmol m⁻² s⁻¹, 12 h photoperiod), in pots irrigated with a standard solution (1Ts), during 1 month following germination. Thereafter, until the physiological maturity, half of the pots kept the same irrigation solution (1Ts), while the other half was submitted to a five fold concentration of all the nutrients (1T5s). The seeds 1T5s were sown and grown again using the initial standard nutrient solution (now called 2Ts) and solutions containing 5, 7, 10 and 13 times the concentration of all the nutrients (2T5s, 2T7s, 2T10s and 2T13s, respectively). In the seeds of 1Ts, 1T5s, 2Ts, 2T5s, 2T7s, 2T10s and 2T13s, Zn concentration increased about 7.18, 6.10, 9.92, 8.87, 4.73, 9.00 and 9.23 fold, whereas Fe augmented 1.01, 1.35, 3.91, 4.95, 5.10, 8.98 and 11.06 times, respectively, compared with the 0Ts Zn and Fe levels. The pattern of nutrient accumulation, as seen by SEM, coupled with X-ray microanalysis, through a line scan on transversal sections of the grains, revealed a non-homogeneous distribution of the nutrients, despite the distribution of the mean values shown were: Cu 0Ts<2T7s<2T5s<2Ts<1T5s<2T10s<1Ts<2T13s; Mn, 1Ts<0Ts<2Ts<1T5s<2T7s<2T5s<2T10s<2T13s; Mg, 2T7s<0Ts<2T10s<2T5s<2T13s<1T5s<1Ts<2Ts; Na, 0Ts<1Ts<1T5s<2Ts<2T7s<2T5s<2T10s<2T13s; K, 0Ts<1Ts<2T7s<1T5s<2T5s<2T10s<2T13s and 2Ts<2T5s<2T7s<2T10s<2T13s; Ca, 1Ts<2T5s<2T7s<1T5s<0Ts<2Ts<2T10s<2T13s. According to these trends the maximum values shown for Cu, Mn, Mg, Na, K and Ca, increased 2.99, 4.45, 2.48, 7.74, 4.31 and 2.39 fold, respectively. In the equatorial region, also following a SEM analysis coupled with X-ray, a heterogeneous accumulation was found between the centre and the peripheral region, yet relatively to 0Ts the average amounts (in %) of Fe and Zn decreased to 98 / 79, 92 / 68 and 29 / 14 in 2Ts, 2T5s and 2T7s, respectively, but thereafter increased 4.11 / 6.23 and 4.54 / 9.75 fold. Moreover, the ratio of the means of Fe/Zn in these treatments displayed an opposite trend (2T13s<0Ts<2Ts<2T5s<2T10s<2T7s). Considering nutrients accumulation in the grains, data suggest that 2T5s is the best option for simultaneous biofortification of Zn and Fe.
Diabetes mellitus is a major endocrine disorder responsible for poor metabolic control and increased risk of cardiovascular disease, renal failure, blindness or diabetic cataract, and other symptoms including atherosclerosis and AGE (advanced glycation end) products. Antioxidants play an important role to protect against damage by reactive oxygen species and their role in diabetes has been evaluated. Many plant extracts and products have been shown to possess significant antioxidant activity and are used for dietary purposes. But uptake of trace elements seems to be of equal importance. For example, patients with diabetes have significantly lower levels of zinc in their blood compared to the control subjects. On the other hand, chromium is a trace mineral that improves the action of the hormone insulin and is directly implicated in the metabolism of carbohydrates, protein and fats. Magnesium seems to play a role as a second messenger for insulin action, whereas insulin itself has been identified to be a regulatory factor of intracellular magnesium accumulation. A lack of magnesium has been linked to insulin resistance, glucose intolerance and diabetes complications. Vanadium or its derivatives mimic the metabolic actions of insulin. In rodents, vanadate also sensitizes peripheral tissues to insulin. Hence it may improve blood sugar control in diabetes. Only few studies have examined whether selenium status influences risk for diabetes, but the available results are conflicting.

There is also in vitro and clinical evidence that an abnormal glutathione status in diabetes patients might be involved in β-cell dysfunction and in the pathogenesis of long-term complications. Therefore some interest has developed in a possible modification of the glutathione status during the therapy of diabetes. Overall, information is urgently needed on the interactions of antioxidants and trace elements on diabetes, and on a possible role of fortified food ingredients in a healthy diet.
VI. SIMULTANEOUS BIOFORTIFICATION OF Zn AND Fe IN Triticum aestivum L. – SEED FATTY ACIDS COMPOSITION

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Keywords: Fatty acids composition in the seeds, Triticum biofortification

Certified seeds of Triticum aestivum L. cv Nabão (0Ts) were grown in walk-in growth chamber (EHHF10000, Aralab, Portugal), under environmental controlled conditions (80% RH; 24/20°C day/night temperatures; PPFD of ca. 700 µmol m⁻² s⁻¹, 12 h photoperiod), in pots irrigated with a standard solution (1Ts), during 1 month following germination. Thereafter, until the end of the reproductive phase, half of the pots kept the same irrigation solution (1Ts), but the others were submitted to a five fold concentration of all nutrients (1T5s). The seeds 1T5s were grown again under the initial standard solution of nutrients (2Ts) and to 5, 7 and 10 times higher concentration of all the nutrients (2T5s, 2T7s and 2T10s). It was found that, relatively to 0Ts, in the seeds of 2Ts, 2T5s, 2T7s and 2T10s, Zn concentration increased about 9.92, 8.87, 4.73 and 9.00 fold, whereas Fe augmented 3.91, 4.95, 5.10 and 8.98 times. The accumulation of fatty acids was studied in the seeds, being found that the pattern in all treatments was: C18:2>C16:0>18:1c+t>C18:3>C18:0. For all treatments the amounts of C20:1, C14:1, C10:0, C12:0, C13:0, C14:0, C20:0, C16:1c+t, C20:2, C15:0 and C22:0 in the seeds remained below 2%. The highest percentage of: 1) C18:1c+t, C18:2 and C20:1 was found in 0Ts; 2) C16:0, was detected in 2Ts; 3) C12:0, C13:0, C14:0, C14:1, C15:0, C16:1c+t and C22:0 was obtained with 2T7s; 4) C18:0, C18:3, C10:0, C20:0 and C20:2 prevailed in 2T10s. Moreover, in 2Ts none of the fatty acids displayed the highest amounts. Similar total fatty acids amounts were observed in 0Ts, 2T5s, 2T7s and 2Ts, contrarily to 2T10s which presented significantly lower values. As regards the double bound index, the highest values (ca. 7.40) were observed for 0Ts while the remaining treatments (2Ts, 2T5s, 2T7s and 2T10s) presented lower values (ca. 5.18-6.42). The results suggest that at a nutritional level, 2Ts is the best option for simultaneous biofortification of Zn and Fe, since it shows balanced amounts of these micronutrients associated to unaltered total fatty acids.
COMPOST INCORPORATION IN SOIL DECREASES CADMIUM ACCUMULATION IN SPINACH

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Keywords: compost, food safety, heavy metals, immobilization, spinach

OBJECTIVES
The main objective of present study was to immobilize the bioavailable fractions of heavy metals in soil, decreasing their content in spinach grown on industrially contaminated soil.

METHODS
Heavy metal contaminated soil was used mixing with industrial compost in two experiments: growth chamber immobilization experiment mixing soil and compost (1 or 10 %); greenhouse immobilization experiment, growing spinach plants in containers with contaminated soil with or without compost (1/4 or 1/2). Total and DTPA-extractable Cd in soil was studied before, during and in the end of experiments. Heavy metals content was analyzed also in plant tissue. During the experiments different plant and soil parameters were observed.

RESULTS
Generally, the incorporation of compost increased in fold the soil microbial enzyme activity and respiration and promoted the growth of spinach plants. A good immobilization effect only was observed in case of cadmium and as consequence the accumulation of that toxic metal in spinach was decreased.

CONCLUSIONS
The incorporation of organic matter end-product of pharmaceutical industry could be an interesting and cost-effective way to protect plants in low contaminated soils, but also could provide important nutrients.
PRELIMINARY EVALUATION OF IODINE AND SALICYLIC ACID INTERACTION ON YIELD, FRUIT QUALITY AND IODINE UPTAKE BY TOMATO PLANTS CULTIVATED IN A HYDROPONIC SYSTEM (NFT)

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Key words: iodine, salicylic acid, biofortification, tomato, biological quality

The aim of the study was to determine the influence of iodine application in two forms (I and IO$_3^-$) and salicylic acid (SA) on yield, iodine uptake by plants and fruit quality of tomato cv. ‘Rambozo F1’ cultivated in a glasshouse in Nutrient Film Technique system (NFT). The following treatments were applied in the experiment: 1./ Control; 2./ 1 mg I · dm$^{-3}$ nutrient solution in KI form; 3./ 1 mg I · dm$^{-3}$ nutrient solution in KIO$_3$ form. 4./ 1 mg I (in KI form) +1 mg SA per one dm$^3$ of nutrient solution; 5./ 1 mg I (in KIO$_3$ form) +1 mg SA per one dm$^3$ of nutrient solution.

In comparison to the control, a significant increase in iodine content in pinnate leaves and petioles from combinations 2 and 3 (the oldest – located lowermost; fully developed – first leaf above 3$^{rd}$ cluster; the youngest – above 6$^{th}$ cluster) was noted ranging between 780% and 8.870% for KI as well as 108% and 1.830% for KIO$_3$. Additional application of SA to nutrient solution increased iodine accumulation in tested parts of plants, particularly for KIO$_3$ (in the range of 47% to 539%) rather than KI application (1-25%) as well as for a greater degree in the youngest leaves than the oldest ones.

Introduction of iodine salts as well its simultaneous application with SA improved iodine accumulation in fruits. Addition of SA to nutrient solution contributed to an increase in the level of phenolic compounds and acidity of tomato fruits. It was revealed that tested factors had no significant influence on yield as well as the content of lycopene and β-carotene in fruits.

This work was financed by the Polish Ministry of Science and Higher Education - grant no. N N310 080238 „Effectiveness of iodine biofortification of tomato cultivated in hydroponic system with recirculating nutrient solution”.
INFLUENCE OF PUTRESCINE APPLICATION ON CROP TOLERANCE TO HEAVY METAL STRESS

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Nowadays, there are two major problems in crop growing – pollution and nutrition. During an industrial period numerous areas were heavily contaminated by heavy metals and they are still not suitable for food production. The consumption of contaminated food crops is posing health risk for humankind. On the other hand, low concentrations of some heavy metals are essential for living organisms. The population growth puts a strong pressure on quantity and quality of substantial food. The improvement of food nutrition by the increase of essential elements concentrations in food is one of the goals. The problem is that heavy metals generate free radicals in plant cells. Plants have many types of defense mechanisms that protect them against damage by reactive oxygen. These mechanisms comprise of different types of non-enzymatic compounds such as ascorbic acid, glutathione, carotenoids, \( \alpha \)-tocopherol, and enzymatic compounds such as APOX, GR, SOD, and CAT. Recently, many authors suggest the possible antioxidant role of polyamines as radical scavengers. For example, spermine is known for the plant tissues protection of the metal-induced oxidative damage.

The aim of this work was to affect the accumulation and the translocation of toxic metals and essential elements in crop plants by external application of putrescine.

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REDUCED N INPUTS BY SOIL-DELINEATED MANAGEMENT ZONES AND REMOTELY-SENSED FERTILIZER REQUIREMENT

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Key words: Cotton, ground sensors, nitrogen content, nitrogen-use efficiency, site-specific management

Division of heterogeneous agricultural fields into management zones and estimation of crop N requirement by ground-sensor remote sensing technologies have been recently used as independent management strategies to reduce fertilizer N inputs. This study evaluated the efficiency of N inputs and cotton N uptake when management zones were delineated by soil near-infrared reflectance and zone fertilizer application was based on a ground-sensor chlorophyll index. Fertilizer N application averaged 52 kg N ha⁻¹ in the pilot areas compared to 122 kg N ha⁻¹ in the conventional practice in three cotton fields of the Thessaly Plain (central Greece) during the 2010 growing season. These values translate to a reduction of N inputs by 58% and an increase of nitrogen-use efficiency by 134% with only 8% yield loss in the pilot areas compared to the conventional parts of the fields. Despite the great reduction of N inputs, leaf N content and leaf δ¹⁵N, as an indicator of fertilizer N uptake, were similar in the pilot and conventional parts of the fields.
POSSIBLE NICOTIANAMINE INVOLVEMENT IN CADMIUM METABOLISM IN TOMATO PLANTS

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Keywords: cadmium, nicotianamine, tomato

Due to cadmium toxicity the research into reducing its amount in edible plant parts is necessary. We have generated tomato plants expressing \textit{AhNAS2p::AhNAS2} from \textit{A. halleri}, encoding nicotianamine synthase (NAS), an enzyme involved in the biosynthesis of nicotianamine (NA), chelator of Fe$^{2+}$, Fe$^{3+}$, Ni$^{2+}$, Zn$^{2+}$, Cu$^{2+}$. Until now, there was no indication of NA involvement in the metabolism of Cd.

Transgenic and wild-type plants were exposed to Cd (0.25µM) and different Fe regimes: 0.1µM, 10µM, 100µM. Plant tolerance to growth conditions and accumulation of Fe, Zn and Cd were evaluated.

The difference in Fe-deficiency tolerance between plants not detected during the first 7-9-days of exposure (all lines had leaves with yellow areas) became apparent on the day 8-12$^{th}$. Leaves of transgenics turned from yellow to green and Fe level was higher than in WTs. Preliminary results indicate also that transgenics exposed to 0.1µMFe/0.25µMCd had lower Cd concentration. In total, they accumulated less Fe but being more efficient in translocation Fe to leaves, its level remained as in WT.

Switch in the phenotype of transformants after 10-day of growth at low Fe indicates developmental regulation of \textit{AhNAS2p::AhNAS2} in tomato and contribution of NA to increase in Fe-deficiency tolerance. Decreased Cd concentrations in leaves of transformants indicate possible involvement of NA in Cd-binding and regulation of Cd root-to-shoot translocation.

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NORTHERN ITALY CULTIVATED DURUM WHEAT: LOW INPUT NITROGEN FERTILIZATION COUPLED BY INCREASED GRAIN NUTRITIONAL VALUE

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Key words: durum wheat; low input nitrogen fertilization; protein quality

Durum wheat (Triticum turgidum L.) is an important agricultural crop that enters the human food chain. Effective nutrient management considering nutrient interactions is important in order to increase crop yield and improve grain nutrient concentration. The objective of this study was to investigate the effect of various N treatments (soil urea, vs. foliar) on the grain protein yield and nutritional value in different Italian cultivars of durum wheat.

Three Italian cultivars of durum wheat, Biensur, Ariosto and Liberdur, were grown in the field at the University of Padova with conventional topdressing N rate. Three levels of late fertilisation were applied at booting stage: control, urea at soil, foliar spray. Grain mineral content was evaluated by ICP-MS. Different seed storage protein fractions (gliadin; HMW-GS and LMW-GS) were also quantified in the three cultivars.

Late N fertilisation affected S concentration; varieties showed also a different affinity for some nutrients: Biensur had higher concentration of molybdenum, Ariosto had more copper, Liberdur more calcium. N:S ratio was not affected by fertilisation but Biensur was the only cultivar with N:S ratio lower than 17 (16,7), generally a favourable condition for protein quality (Zhao et al. 1999). Protein analysis were also performed in the cultivars. Differences in protein amounts were found between the cultivars, indicative of a different grain quality. Results will be discussed.

References

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NUTRITIONAL QUALITY OF SELECTED SPROUTS ENRICHED WITH MG

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Key words: functional food, improving nutritional value, Mg ions, sprouts

Introduction: High nutritive value and natural food products are response to consumers new demands and production of sprouts enriched with essential elements is receiving an increasing attention.

Objective: This study was undertaken to assess possibility of enrichment of pea, alfalfa and red cabbage sprouts with Mg ions.

Material and methods: Seeds and sprouts were cultured in growth chamber at 23°C with 0 (control), 25, 50, 100, 150, 200, 300 mg Mg/dm³ H2O. On 7th day dynamics and capacity of germination, sprouts length, level of ROS, activity of some enzymes of antioxidative system, dry matter and content of selected ions were determined.

Results: Addition of Mg improved germination in red cabbage. Sprouts of pea were longest in Mg free medium but of alfalfa and red cabbage when cultured with Mg at lowest concentration and 100 mg/dm³ respectively. Sprouts length was reduced in concentrations dependent manner. Culturing with Mg resulted in increased concentration of this element in sprouts and caused changes in potassium and calcium content. In presence of Mg level of anion-radical and hydroxyl radical in pea and alfalfa sprouts increased. Activity of ascorbate peroxidase and catalase was higher in sprouts of all species when growing in the presence of Mg.

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Nickel (Ni) acts as the cofactor of urease, which catalyzes the breakdown of urea to ammonia. Urea is the most commonly used nitrogen (N) fertilizer, due to its low cost and high N content. Beneficial effects of Ni have been mentioned in literature, but the potential of Ni fertilization for crop production needs to be investigated. In this study, soybean seeds with different Ni concentrations were obtained by growing soybean hydroponically at different Ni levels. Selected seeds were used in another experiment where plants were grown in growth chamber at limited N supply, with or without Ni from solution and with or without foliar urea application. Both seed Ni and externally-applied Ni alleviated toxicity symptoms of foliar urea and reduced the number of leaves lost due to urea toxicity. Ni-rich plants, especially those sprayed with urea, had greater biomass than Ni-poor plants. Moreover, Ni-rich plants exhibited a higher elongation rate, and SPAD measurements revealed higher chlorophyll concentrations in young leaves of Ni-rich plants, indicating improved N use efficiency by better Ni supply. Urease assay results suggest that the observed positive effects of Ni may at least partially be attributed to urease activity. A sufficiently high Ni availability is required to improve N use efficiency and the utilization of foliarly-applied urea in soybean.
HOW DOES THE GREEN REVOLUTION AFFECT MINERAL CONCENTRATIONS IN WHEAT GRAIN?

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Keywords: Rht alleles, Wheat grain, Mineral concentration, Nitrogen fertilizer, Green revolution

Using the Broadbalk long-term (>160 years) experiment at Rothamsted, we found that the concentrations of zinc, iron, copper and magnesium in wheat grain have decreased significantly since the mid 1960s, which coincided with the introduction of semi-dwarf, high-yielding cultivars, the so-called Green Revolution. Furthermore, mineral concentrations were also lower in new cultivars than in old ones in a study including 150 cultivars of diverse origin. Here we investigate the effect of dwarfing alleles on mineral concentrations in wheat under field and glasshouse conditions. In the field trial, the gibberellin-insensitive reduced height allele Rht-B1b increased crop biomass, dry matter (DM) harvest index and grain yield, but did not increase the uptake of Cu, Fe, Mg or Zn so these minerals were diluted in grain. In contrast, nitrogen increased both DM yield and mineral uptake so grain concentrations were either increased (Fe, Cu) or only slightly affected (Mg, Zn). In the glasshouse experiment, the Rht alleles decreased grain yield and increased mineral concentrations in grain. We conclude that the effects of Rht alleles on mineral concentrations in wheat grain are mostly indirect due to their effects on DM, resulting in either dilution or concentration of minerals in grain. In semi-dwarf varieties, increased N requirement and N applications partly offsets this dilution effect.
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