Proceeding of the 7th International Phytotechnologies Conference

26 -29 September 2010
Hotel Starhotels du Parc
PARMA, Italy

Phytotechnologies in the 21st century: Challenges after Copenhagen 2009. Remediation-Energy-Health-Sustainability

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- Italian Ministry of Agricultural, Food and Forestry Policy
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- Municipality of Parma, *Italy*
- Chamber of Commerce of Parma, *Italy*
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- **A.I.B.G.** Italian Association for General Biology
- **A.I.A.T.** Association of Engineers Environment and Territory
- **S.B.I** Italian Society of Botany
- **S.I.G.A.** Italian Society of Agricultural Genetics
- **S.It.E** Italian Society of Ecology

- **CINSA** National Interuniversity Consortium of Environmental Sciences
- **ENEA** Italian National Agency for New technologies, Energy, and sustainable economic development
- **E.N.E.P.** European Network of Environmental Professionals
- **ITALBIOTEC** National Consortium of Biotechnology

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PREAFACE by *Lee A. Newman* (President of I.P.S. Society)

Although phytoremediation and phytotechnologies have been around for many years, the very first conference devoted to this field was not held until 1986. Then in 1999, the first issue of the International Journal of Phytoremediation was published under the strong editorial direction of Dr. Guy Lanza from the University of Massachusetts. In 2005, Steve Rock of the US Environmental Protection Agency, always a proponent of the technology, proposed the formation of a society to advance the technology, and to enable people who are engaged in work in this field to interact and collaborate with each other more easily.

The International Phytotechnology Society decided at its onset to not limit itself to phytoremediation, but to also welcome members who were using any plant-based technology to mitigate environmental problems, including biofuel production, green-roof technology, treatment wetlands, landfill caps, and carbon sequestration among others.

The Society has many important goals with the foremost being to promote the development of the plant based technologies. To do this, we work to develop student interest in the field, we promote and support the Journal, and offer services to our international members in manuscript preparation to keep standards for the journal high.

The Society is young and still growing. We encourage our students to become active in the development of student chapters. We want members to reach out to the wider public and regulatory community, educate them about the potential of the technologies we all believe in so strongly. We have many lofty goals and plans, and hope that you will consider becoming a member and helping us to reach these goals.

Since its inception, the Society has taken on the role of presenting conferences, like you are now attending, and workshops around the globe to bring information to people who are just learning about the potential of phytotechnologies.

For this year's conference, we have once again seen an increase in the number and quality of abstracts submitted. The organizers many hours sorting them to come up with a program that will best cover the range of technologies in use today. I hope you will all agree with me that this will be both an interesting and informative conference.

I invite you to enjoy the presentations, get the chance to meet the people who were here at the beginnings of the technology, as well as the numerous students who are our future. I look forward to meeting friends and colleagues from past conferences, as well as greeting new ones. I hope everyone here has fun and has the chance to learn something new.

Enjoy!

I.P.S. Society:
Newman Lee A. - *President*
White Jason C. - *Exec. Vice-President*
Gerth Andrè - *Vice-President*
Zeeb Barbara - *Vice-President*
Nichols Elizabeth - *Secretary*
Rock Steven A. - *Treasurer*

Sunday, September 26th, 2010

15:00-18:00	**Opening ceremony and plenary lectures**
Room 1	Welcome from local authority
	Nelson Marmiroli *(University of Parma, Italy)*
	Introduction to the 7th Phytotechnologies Conference
	Lee A. Newman *(Brookhaven National Laboratory, Italy)*
	Introducing the Gordon Lecture
	Larry Erickson *(Kansas State University, USA)*
	Phytotechnologies and sustainability
	Yongming Luo *(Chinese Academy of Sciences, China)*
	Advances in phytotechnologies for contaminated soils in China

Monday, September 27th, 2010

8:30-9:30	**Introductory Plenary**
Room 1	**Markus Puschenreiter** *(Uniersity of Vienna, Austria)*
	A decision tool system for the selection of gentle remediation technologies based on soil characteristics
	Rock A. Steven *(USEPA ORD , USA)*
	Environmental health concerns and urban agriculture on potentially contaminated land
	Introduction to sessions Wastewaters/Wetlands
9:30-11:10	**Concurrent Sessions**
Room 1	Metals 1 – Chair: **Liz Rylott** *(University of York, UK)*
Room 2	Environment and Health 1 – Chairs: **Bill Suk** *(National Institute of Environmental Health Sciences, USA)* and **Jerry Schnoor** *(University of Iowa, USA)*
Room 3	Wastewaters - Chair**: Eugenia Olguin** *(Institute of Ecology, Mexico)*
11:10-11:30	*Coffee break*
11:30-13:10	**Concurrent Sessions**
Room 1	Metals 2 – Chair: **Jean-Paul Schwitzguébel** *(Ecole Polytechnique Federale de Lausanne, Switzerland)*
Room 2	Environment and Health 2 - Chairs**: Bill Suk** *(National Institute of Environmental Health Sciences, USA)* and **Jerry Schnoor** *(University of Iowa, USA)*
Room 3	Wetlands – Chairs**: Andrè Gerth** *(Bioplanta GambH, Germany)* and **Michel Mench** *(Universitè Bordeaux 1, France)*
13:10-15:00	*Lunch and Poster Session*
15:00-16:00	**Introductory Plenary**
Room 1	**David L. Russell** *(Global Environmental Operations, USA)*
	Rethinking sustainability - The technology link
	Jorge Gardea-Torresdey *(Univ. of Texas at El Paso, USA)*
	Evidence of the differential biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles on soybean (*Glycine max*) plants
	Jean-Paul Schwitzguebel *(Ecole Polytechnique Federale de Lausanne, Switzerland)* Introduction to Session Metals 3

16:00-16:30 *Coffee break*

16:30-18:10 **Concurrent Sessions**
Room 1 Metals 3 - Chair**: Alan Baker** *(University of Melbourne, Australia)*
Room 2 Sustainability - Chairs**: Larry Erickson** *(Kansas state University,USA)* and **Elena Maestri** *(University of Parma, Italy)*
Room 3 Nanomaterials - Chairs**: Jorge Gardea-Torresdey** *(University of Texas at El Paso, USA)* and **Tomas Vanek** *(Institute of Experimental Botany AS CR, Czech Republic)*

Tuesday, September 28th, 2010

8:30-9:30 **Introductory Plenary**
Room 1 **Fabio Monforti-Ferrario** *(JRC Institute for Energy, Italy)*
Biomass for transport, heat and electricity: scientific challenges and JRC contribution
Barbara Zeeb *(Royal Military College of Canada, Canada)*
Introduction to sessions POPs1 and 2
Nele Weyens *(Hasselt University, Belgium)*
Introduction to sessions Plant-microbe interactions

9:30-11:10 **Concurrent Sessions**
Room 1 Biomass 1 Carbon sequestration - Chairs**: Fabio Monforti-Ferrario** *(JRC Institute for Energy, Italy)* and **Elizabeth Nichols** *(North Carolina State University, USA)*
Room 2 POPs 1 Pesticides - Chair**: Barbara Zeeb** *(Royal Military College of Canada, Canada)*
Room 3 Plant-microbe interactions, inorganics - Chair**: Jaco Vangronsveld** *(Hasselt University, Belgium)*

11:10-11:30 *Coffee break*

11:30-13:10 **Concurrent Sessions**
Room 1 Biomass 2 Energy - Chairs: **Ron Zalesny** and **Paolo Carrera**
Room 2 POPs 2 PCB - Chair**: Jason White**
Room 3 Plant-microbe interactions, organics - Chair**: Nele Weyens**

13:10-15:00 *Lunch and Poster Session*

15:00-16:00 **Introductory Plenary**
Room 1 **Peter Schroeder** *(Helmholtz-Zentrum Munchen,Germany)*
Introduction to sessions Alpine BioCluster
Liz Rylott *(University of York, UK)*
Introduction to sessions POPs 3 Energetics
Elena Maestri *(University of Parma, Italy)*
Introduction to Teaching Roundtable*

16:00-16:30 *Coffee break*

16:30-18:10 **Concurrent Sessions**
Room 1 Alpine BioCluster - Chair**: Valerie Ayache** *(Alps Bio Cluster, France)*

Room 2	POPs 3 Energetics - Chair**: Liz Rylott** *(University of York, UK)*
Room 3	Teaching Roundtable* - Chair**: Steven Rock** *(USEPA ORD, USA)*

Wednesday, September 29th, 2010

8:30-9:30	**Introductory Plenary**
Room 1	**Charles M. Reynolds** *(U.S.AERDC-CRREL, USA)* Implementing and monitoring rhizosphere remediation in remote regions **Stanislaw Gawronski** *(Warsaw Univ. Life Sciences,Poland)* Air phytoremediation in urban areas and indoor: dream or reality? **Lou Licht** *(Ecoltree Inc., USA)* 20 years of poplar phyto performance on, under and around landfills
9:30-11:10	**Concurrent Sessions**
Room 1	BTEX, TPH - Chairs**: Heather Henry** *(National Institute of Environmental Health Sciences, USA)* and **Harrison Atagana** *(University of South Africa, South Africa)*
Room 2	Air - Chairs**: Stan Gawronski** *(Warsaw University of Life Sciences, Poland)* and **Rita Baraldi** *(CNR IBIMET, Italy)*
Room 3	Covers - Chairs**: Paul Deutsch** *(AMEC Geomatrix, USA)* and **Lou Licht** *(Ecoltree Inc., USA)*
11:10-11:30	*Coffee break*
11:30-12:30	**Concluding plenary and Student Awards**

* Teaching Roundtable:
Elena Maestri, Alan Baker, Elizabeth Nichols, Scott Angle, Joel Burken and **Lee Newman**

Effective teaching of phytoremediation and phytotechnology concepts and implementation is critical for the future of the field. With informative and inspiring lectures and classes, students become excited about the field, and want to learn more about how they can develop a career either in teaching and research, or in field application of the technology. This session will include brief presentations by leaders in the field, who have also been developing new courses and teaching materials to motivate the next generation of students. Panel members will talk about what has worked and, just as importantly, has not worked in the classroom. Panel members have developed a range of courses, from the individual class lecture to web-based international courses. Following the brief presentations, the floor will be opened up for discussion and questions. This is an opportunity for everyone to learn from one another, and for ideas on the development of classes, from the single lecture to multi-day workshops to full semester courses. All are invited to not only listen but also to participate in the exchange of ideas.

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Phytotechnologies and Sustainability

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Phytotechnologies are central to sustainable development. Vegetation and photosynthetic microbial processes convert significant quantities of carbon dioxide into plant biomass in forests, farms, pastures, and oceans. The sustainable management of the phytotechnologies that lead to food, biofuels, and other bioproducts should be based on an understanding of the various ecosystems that support plant growth and product formation. As the human population increases, the need to optimally manage ecosystems grows in importance. Phytosustainability should be an integral part of ecosystem based management; that is, agricultural lands, forests, urban green spaces, and oceans should be managed to maintain effective functional ecological units (environments), in which ecosystem services are at or near steady state and their optimal productivity. Contaminants in soil and water should be viewed as transient problems that need to be managed as part of a restoration process in which contaminant levels decrease with time until a satisfactory steady state condition is reached.
Results of a sustainability evaluation will be presented, illustrating benefits and tradeoffs of different management approaches to meet societal needs.

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Advances in Phytotechnologies for Contaminated Soils in China

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This report mainly introduces recent research and development in phytoremediation and microbial remediation and their potential application for remediation of metal and/or organics polluted soil and mined tailing in China. Advances in field-scale phytoextraction of metal-contaminated farmland soils by using native hyperaccumulator plants such as *Sedum plumbizincicola* (Cd/Zn hyperaccumulator) and *Pteris vittata* (As hyperaccumulator) are introduced. Field demonstration in phytodegradation and microorganism-inoculated clover rhizoremedaition of persistent organic pollutants such as PAHs and PCBs in contaminated agricultural soils are also described. The potential plants with large and deep root system like *Vetiveria zizanioides* and *Miscanthus sinensis* used for phytostablisation, biofuel production, carbon sequestration and conservation of polluted land and mining tailings are discussed as well. Besides, the report further prospects phytotechnologies research and application in China in the future. It is believed that phytotechnologies integrating phytoremediation with microbial remediation will be developed and promoted for cleanup and restoration of contaminated farmland soil in China.

A decision tool system for the selection of gentle remediation technologies based on soil characteristics

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Gentle soil remediation technololgies for trace element contaminated soils comprise approaches for removal (i.e. phytoextraction) or immobilisation (i.e. *in situ* immobilisation, phytostabilisation) of the contaminants in the soil. Great progress has been achieved in lab and greenhouse and some pilot field studies have indicated the efficiency and feasibility of these methods. A particular problem remains regarding the decision for one of these approaches. Due to specific soil chemical and physical characteristics, the bioavailability of the pollutants may vary considerably which may have a significant implication on the efficiency of phytoextraction or *in situ* immobilisation. In order to develop a simple decision tool system, we have tested the suitability of three gentle soil remediation technologies [(1) *in situ* immobilisation, (2) phytoextraction of total concentrations, (3) phytoextraction of bioavailable fractions = bioavailable contaminant stripping (BCS)] on a set of seven soils polluted with Zn and Cd. The soils differed strongly regarding pH, carbonate content, texture and many other characteristics, resulting in very different levels of bioavailability. All soils were treated with all three gentle remediation methods. (1) was performed with iron oxide, loam powder or a mixture of red mud and gravel sludge and evaluated by assessing the Zn and Cd uptake in barley straw and grain; (2) and (3) were tested by using *Salix x smithiana* and evaluating changes of total and extractable Zn and Cd fractions in soil. The *in situ* immobilisation was most efficient on moderately acidic carbonate-free soils which had a low buffer power (Kd value) for Zn and Cd. Here, the largest reductions of extractable Zn and Cd in soil and corresponding lower accumulation in straw and grain were found. In contrast, the immobilisation was much less efficient on slightly alkaline calcareous soils with high Kd values for Zn and Cd. In contrast, little differences between the soils were found regarding the efficiency of phytoextraction. The accumulation of Zn and Cd in leaves of *S. x smithiana* was relatively independent from the initial bioavailability, indicating that very efficient Zn and Cd mobilisation processes in the rhizosphere are involved. The most critical factor for feasible phytoextraction of total contents in soil seems to be the contamination level, regarding both the duration of phytoextraction and potential phytotoxicity on strongly polluted soils. The evaluation of BCS efficiency (i.e. reductions of extractable Zn and Cd) is still ongoing and no results are available yet. However, according to preliminary work we expect to find significant changes only in less buffered acidic soils, whereas in well-buffered alkaline soils the extractability likely remained unchanged. Our work shows that the buffer power for Zn and Cd is the most critical factor determining the efficiency of gentle remediation technologies, thus only a few characteristics including pH, total and dissolved concentration of the target elements are needed in this decision tool system to select the most suitable approach.

Keywords: phytoextraction, in situ immobilisation, soil characteristics, desicion tool system

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Environmental Health Concerns and Urban Agriculture on Potentially Contaminated Land

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Midwestern American cities often have decreasing populations and increases in vacant lots on formerly residential, commercial, and industrial sites. Using those vacant lots for developing greenspace has many potential environmental and social benefits including stormwater management, air and water pollution abatement, and providing a source for locally grown food.

Gardening in urban areas brings the concern that contaminants in the soil may be dangerous. The source of those contaminants may be from the previous use of the land, from the demolition of the buildings, and deposition from airborne particles. Contaminants in soil may potentially be taken up by the plants and consumed as food. Some contaminants are more likely to be eaten with soil clinging to unwashed fruits, vegetables, or hands; some may be inhaled as dust. There is no universal answer for the question "Is it safe to garden in this soil?" It depends on the soil, the presence and concentration of various elements and chemicals, and the type of gardening practiced.

Ideally the soil of any newly established urban garden should be tested for contaminants of concern. The specific contaminants depend on the location and history of the site. The tests that could be done vary in complexity and cost. If the first and inexpensive tests show that the site is relatively clean, then it can be considered safe to build a food producing garden. If not then other tests may be indicated. If tests show the presence of contaminants, there are several choices for the site. In order of increasing expense and complexity those choices are:

1. Do not use all or part of the site
2. Amend the soil
3. Add clean soil on top of the soil of concern
4. Remove the soil and replace it with clean soil
5. A combination of the above.

More than fifty sites in Cleveland, Ohio USA were chosen by a process that included city officials, developers, and neighborhood organizations for potential green redevelopment. The main contaminants of concern among the developers and gardeners were lead, cadmium, and PAHs in soil. These sites were tested for metals and organic contaminants. Development plans were adjusted based on the technological and economical options available. Bioavailability and plant uptake and the potential impacts on human health were considered. This paper will present this project as a case study for safely redeveloping urban lands for food production.

Keywords: bioavailability, health impacts, lead, phytoextraction

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CONCURRENT SESSION
METAL 1

Thlaspi caerulescens: the "green rat" for ecogenetic studies on metals hyperaccumulation

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Phytoremediation is a technology relatively new but which has already shown some very interesting applications. There are indeed levels of basic research which promise to support the growth of phytoremediation in the direction of science and technology (Marmiroli and McCutcheon, 2003). Among these, can be certainly included the researches on tolerant plants and on hyperaccumulators. In particular some plant species growing on metal-rich soils can accumulate high quantity in leaves. Hyperaccumulating plants have been described for Ni, Cd, Zn, Se, As whereas accumulation capacity towards other metals have not been clearly demonstrated. Studying heavy metal hyperaccumulation is becoming more and more interesting also for ecological, evolutionary, nutritional and environmental reasons. Among hyperaccumulators, *Thlaspi caerulescens* has been studied to a great extent: its physiological, morphological and genetic characteristics and its close relationship to *Arabidopsis thaliana* make it an excellent candidate to be the plant heavy metal hyperaccumulator model species (Milner and Kochian, 2008). In this research we utilized an integrated physiological, biochemical and molecular approach for the study of the natural Ni hyperaccumulating *Thlaspi caerulescens* (J&C. Presl.) combining laboratory studies and *in situ* studies with the aim to understand the complexity of the response to heavy metals in this plant.
Comparative genomic and trascriptomics analyses on "candidate genes" involved in uptake, translocation and sequestration of heavy metals revealed differences between this population and other European metallicolous and non-metallicolous *Thlaspi caerulescens* populations, evidencing a high degree of genotypic plasticity of this species in adaptation to different environments. In addition, by utilising an hightroughput comparative proteomic approach we also demonstrated how the Ni hyperaccumulator (metallicolous) phenotype and the modifications in the abundance of some specific proteins were correlated. These proteins can be used as functional biomarkers both for screening biodiversity in indigenous plants and for selection of suitable plants after large breeding programme for phytoremediation capacity.

Key words: *Thlaspi caerulescens*; model plant; phytoremediation; genetic and proteomic markers

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Impact of Biomolecules in the Formation and Reactivity toward Heavy Metals and Metalloids of Iron Precipitates at the Soil-Plant Interface.

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Root exudates and biomolecules produced by microorganisms as well as humic and fulvic acids are involved in the weathering of minerals and in the subsequent release and transformations of iron at root-soil interface. Organic compounds play a very important role in the hydrolytic reactions of iron and in the formation, nature, surface properties, reactivity, and transformation of Fe oxides. Organic substances present in the rhizosphere interact with Fe promoting the formation of ferrihydrite and organo-mineral complexes.
Iron plaque, a rusty-colored coating found on many wetland plant roots, often contains high concentrations of metals such as Cr, Pb, Cu, and Zn and metalloids such as As. The sequestration of metals and metalloids can reduce the availability of these potentially toxic elements. Indeed, Fe-oxides (mainly ferrihydrite) selectively adsorb divalent cations, even at the solution pH values lower than the pzc of oxides. Many factors, such as pH, nature and concentration of organic chelating ligands, surface properties of Fe oxides affect the sorption/desorption processes of heavy metals and metalloids on/from Fe oxides. Usually, organic chelating anions promote adsorption of trace elements in cationic form by forming stable surface-metal-ligand complexes, but prevent fixation of elements in anionic form competing for sorption sites onto the surfaces of Fe-oxides. The factors which affect the toxicity of heavy metals and metalloids in soil environments are still obscure, but extraction tests are widely used for assessing their mobility and phytoavailability.

Keywords: Rizosphere, Biomolecules, Fe oxides, Ferrihydrite, Adsorption/desorption, Heavy Metals, Metalloids, Bioavailability.

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Biodegradable Chelators AES and IDSA Induced Phytoextraction of Heavy Metals in Polluted Soils by Ryegrass (*Lolium perenne*)

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Chelator assisted phytoextraction has been proposed to improve the efficiency of phytoextraction of heavy metals in contaminated soils. To study the effects of 2 new biodegradable chelators on extraction of heavy metals in soils in comparison with EDTA and EDDS, a pot experiment was conducted. Ryegrass (*Lolium perenne*) grown in Pb, Zn, Cu and Cd contaminated soils were treated with EDTA, EDDS, AES and IDSA at 5 mg kg-1. All the chelators enhanced significantly the concentrations of the metals in the shoots of ryegrass (with exception of IDSA for Cd). EDTA was most effective for Pb and EDDS for Cu extraction, and interestingly, AES exhibited highest effectiveness for Zn and Cd uptake. AES was more efficient than EDTA and EDDS in improving the transport of Zn and Cd from root to shoot.
The possible mechanism is, the Zn-AES and Cd-AES are also active and can be transported easily to the shoot, which needs further study.

Keywords: biodegradable chelators; AES; IDSA; phytoextraction; heavy metal contaminated soils

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The Role of Of Phytochelatin Overproduction in Cd Tolerance of Arabidopsis and Tobacco Plants

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Phytochelatins (PCs) are metal binding peptides synthesized from gluthatione by the enzyme PC synthase (PCS1). PCs are able to form specific complexes with Cd and other heavy metals in the cytosol, which are sequestered into the vacuoles by specific membrane transport proteins where metals are not harmful for the cell. We previously demonstrated that in tobacco seedlings PCS1 overexpression leads to PC overproduction and increases Cd tolerance in the presence of exogenous GSH (1). We analysed Cd tolerance of *Arabidopsis* plants overexpressing AtPCS1 (AtPCSox lines) and found profound differences between *Arabidopsis* and tobacco. Based on comparative analysis of seedling fresh weight, primary root length and alterations in root anatomy, we show that at relatively low Cd concentrations, Cd tolerance of AtPCSox seedlings is lower than wt, in contrast with what observed in tobacco, whereas at higher Cd concentrations AtPCSox seedlings are more tolerant to Cd as compared to wt *Arabodopsis*. Measurements of PC content in untransformed *Arabidopsis* and tobacco seedlings revealed that at 30 µM Cd level is 3 times higher in the former than in the latter, and differences were found also in the PC polymerization classes (Brunetti et al. submitted).
The role of PCs in Cd tolerance will be discussed in view of recent data.

(1) Pomponi M., Censi V., Di Girolamo V., De Paolis A., di Toppi L.S., Aromolo R., Costantino P. and Cardarelli M.(2006). PLANTA 223(2):180-90

Keywords: Phytochelatins, Cd tolerance, Arabidopsis thaliana, Nicotiana tabacum.
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Micro-XANES Study of Arsenic Species in Hyperaccumulators *Pteris vittata* and *Pteris cretica*

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The biochemical transformation of As in hyperaccumulators is essential for better utilizing plants in the phytoremediation of environment As contamination. This study aims to investigate the As distribution and species change in tissues of *P. vittata* and *P. cretica* through XRF and micro-XANES experiments, conducted on BL4A beamline in Photon Factory, High Energy Accelerator Research Organization, Japan.
In roots of *P. vittata*, As concentration decreases in the order of vascular bundle> cortex> epidermis, indicating that As was transported against the concentration gradient. When As(V) is supplied in the nutrient solution, the main As species in epidermis and cortex is As(V), while As(III) is the principal form in the vascular bundle, mostly coordinated with GSH. It indicates that As(V) is partly reduced to As(III) in roots, mainly during the transportation from the cortex to the vascular bundle, while such process is not obvious in epidermis and cortex. The transportation of As against the concentration gradient may be related to the As transformation in roots. In pinnae, As concentration is the highest in vascular bundle, and lower in epidermis or cortex, implying that the As unloading in pinnae follows the concentration gradient. As(V) component in midrib of *P. vittata* decreases as the following order: vascular bundle> cortex> mesophyll, and it is significantly greater in vascular bundle and cortex than that in mesophyll, indicating that mesophyll also has great ability to reduce As(V) to As(III). It is surprising and interesting that As(III) in roots is mostly As(III)-GSH, while that in pinna is not coordinated with GSH. Therefore, As transformation also happens when it is transported from root to leaf.
The As distribution pattern in *P. cretica* is similar to *P. vittata* but the transformation of As species is slightly different. As(III) component in all tissues of *P. cretica* is greater than that in *P. vittata*, indicating the greater reduction ability of *P. cretica* than *P. vittata*. Compared to *P. vittata*, As(III) component in epidermis and cortex in roots of *P. cretica* is relatively great, indicating that epidermis and cortex in roots of *P. cretica* also have As(V) reduction ability. As(III) component in vascular bundle in midrib of pinnate leaf of *P. cretica* is greater than that of *P. vittata*, indicating that more As(V) is reduced before it is transported to midrib of pinnate leaf in this plant. Differences in the As distribution and species in tissues may be one reason for two hyperaccumulators to have different As uptake ability.
Results provide new clues for the As hyperaccumulation mechanisms.

Keywords: arsenate, arsenite, glutathione, phytoremediation

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CONCURRENT SESSION
ENVIRONMENT AND HEALTH

Public Health Problems, Phytotechnology Solutions – Potential for Growth

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Phytotechnologies have the potential not only to reduce the levels of toxicants in environmental media, but also to prevent exposure. Therefore, it is important to realize that phytotechnologies are, in essence, primary prevention within the context of public health. This presentation serves as an introduction to the "Environment and Health" sessions. The presenters will provide an introduction to key concepts in public health, identify intractable public health problems for which sustainable solutions are needed, and introduce a framework through which phytotechnologies may become a part of the solution. The session is funded by the National Institute of Environmental Health Sciences (NIEHS) Superfund Research Program (SRP) which advances its mission to reduce the public health burden of hazardous substances through interdisciplinary research.

Key Words: public health, primary prevention, interdisciplinary research

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Plant Sampling Techniques for Forensic Assessment of Potential Contaminant Exposure

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Plant tissue sampling offers a unique potential for assessing contaminated sites, as plants interact with the soil, groundwater, and atmosphere; and they actively extract all they need for survival from these environmental compartments. Plant sampling approaches have looked at local and global distribution of airborne pollutants such as POPs, showing connections to likely human exposure patterns of these atmospheric pollutants. Plants have also been used to delineate subsurface contaminant plumes, both in the saturated and vadose subsurface horizons.

Newer methods are starting to open a window to not only taking a snapshot in time, but also looking back in time using novel analytical techniques and a unique application of dendrchronology and dendrochemistry as forensics tools to assess potential contaminant exposure.

Examples of the plant sampling methods will be presented, with a focus of an ongoing case study in Verl Germany will be presented. At the densely populated, contaminated area in Verl, site investigations have been ongoing for well over a decade, and through the recent use of multiple plant sampling techniques applied in coordination, the contaminated area was found to be of greater extent than previously thought even though rigorous sampling had been carried out.

Analysis of tree cores rapidly elucidated the area of contamination at little cost or impact to property. Then the use of Energy-Dispersive X-ray Fluorescence (EDXRF) analysis of the tree cores revealed details regarding the timing of the contamination events in Verl. Contaminant releases were suggested at times going back to the 1940s and as recent at 2008, when the industrial site was dismantled. In addition to specifics of the site investigations and related novel analytic approaches, this talk will also discuss the legal aspects of this new tool in environmental forensics

Key Words: dendrochemistry, dendrochronology, tree coring, *Daubert* criteria, phytoforensics

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Use of water hyacinth as an effective method for removal of arsenic from contaminated water

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Naturally occurring arsenic contaminates drinking water in many parts of the world, especially in Bangladesh, where more than 60% of the groundwater contains arsenic concentrations in excess of the WHO guideline value. Using dried roots of the water hyacinth, a plant often thought of as a weed in the tropical and subtropical world, arsenic levels in contaminated water can be reduced to concentrations below the World Health Organization's guideline value of less than 0.01mg/L. Results from atomic absorption spectroscopy showed that more than 93% of arsenite [As(III)] and 95% of arsenate [As(V)] was removed from a solution containing 200 µg of arsenic per L within 60 minutes of exposure to the powder.
This simple, effective and cheap solution using phytotechnologies in novel applications has potential implications on public health problems in the developing world, where serious contamination of drinking water by natural arsenic in surrounding rocks is threatening the lives of millions. Long term health consequences of arsenic exposure are severe and include skin cancer, nervous system damage and miscarriage.

Key Words: Arsenic, Drinking water, water hyacinth

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Removal and Metabolism of pharmaceuticals from waste water –Options for phytotechnologies

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Personal care products, medicaments and their metabolites are detected ubiquitously in the aquatic environment and increasingly polluting our drinking water supplies. Although the need for high quality drinking water has been recognized as one of the most challenging problems of our times, only little knowledge exists on the impact of pharmaceuticals on ecosystems, animals and man. Diclofenac and Paracetamol are two of the compounds that can be regularly found in the European water cycle and are detected in low μgL^{-1} levels in influents and effluents of waste water treatment plants and surface waters. Both are non steroidal anti-inflammatory drugs that are extensively used in human medication and tend to be relatively persistent in the environment.

Even modern waste water treatment facilities fail to remove completely numerous pharmaceuticals and their metabolites from municipal waste water. A possible solution for an additional cleaning stage would be phytoremediation, where plants get in contact with pharmaceuticals and tackle their detoxification. The mechanisms for the detoxification of xenobiotics in plants are comparable to the mammalian system. Following an activation reaction (Phase I), detoxification is mediated by conjugation (Phase II) with hydrophilic molecules like glutathione or glucose. Phase III reactions finally will result in storage, degradation and transport of the xenobiotic conjugate.

We investigate the fate and metabolism of both compounds in plant tissues using a cell culture of *Armoracia rusticana* L. and several typical wetland plants as model systems.

Our first results point to the direction of plants being able to take up and detoxify pharmaceuticals. We were able to detect both substances in plants and to identify their metabolites with LC/MS. The formation of two independently formed metabolites in plants again revealed strong similarities between plant and mammalian detoxification systems. The detoxification via glucuronisation in mammals is mirrored by glucosidation of xenobiotics in plants. Furthermore, in both systems a glutathione conjugate is formed. P450 enzymes catalyze the formation of the highly reactive intermediates with potential toxicity for animals and plants.

These findings underline the great potential of plants for waste water treatments in constructed wetlands and again show the flexibility of plants in the process. Covering only the very first steps of acetaminophen and diclofenac detoxification in plants, we are still lacking data on long term exposure as well as the possible impact of pharmaceuticals on plant health and stress defence. Long time experiments need to be performed to follow the fate of pharmaceuticals in root and leaf cells in a whole plant system to evaluate future successful usage of plants for the remediation of medicaments from waste water.

CONCURRENT SESSION
WASTEWATERS

Comparison of two filter media in constructed wetlands with *Pontederia sagittata*treating stillage from ethanol production

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Filter media in constructed wetlands (CWs) play a key role regarding several aspects: specific adsorption of pollutans, contribution to the flow type within the wetland, facilitation of the root penetration and contribution to the biofilm formation and structure. Volcanic gravel has being used as filter media in constructed wetlands (CWs) planted with *P. sagittata* for effective treatment of water-diluted stillage (1:100) from sugar cane in a previous work (Olguín *et al*., 2008). In such work, organic matter and nutrient content were removed very successfully, except for potassium and phosphates. The objective of this work was to evaluate the performance of two different filter media, volcanic gravel and ceramic material for the removal of organic matter and nutrients in sub-surface constructed wetlands (SSCWs) mesocosms planted with *P. sagittata* treating diluted stillage from sugar cane. Four mesocosms (0.90 m2 each) built with glass fiber (3 m long, 0.3 m wide and 0.3 m depth) planted with *P. sagittata* and four unplanted controls, were established and acclimatized during 6 months by feeding them with a synthetic wastewater (SWW). Four mesocosms were filled with volcanic gravel and the other four with ceramic material, maintaining a similar size of particle diameter in both type of filter media (2.55 ±0.46 cm for gravel and 2.50 ±0.33 cm for ceramic media. All mesocosms were fed continuously with diluted stillage (1:50) at a hydraulic retention time (HRT) of 5 days. Biochemical Oxygen Demand (BOD5), Chemical Oxygen Demand (COD), ammonia nitrogen (N-NH4), nitrates (N-NO3), phosphates (P-PO4), sulfates and potassium were the monitored parameters every 5 days. Growth of the plants was also evaluated every 10 days. It was found that BOD5 was removed apparently more effectively in mesocosms containing ceramic media (54.01 ±1.11%) compared to those using gravel (47.07 ±0.6%) in planted mesocosms after 15 days. Nitrogen forms were also better removed with ceramic filter media (66.49 %). Sulfates were removed effectively and similarly regardless of the filter media in both, planted and unplanted mesocosms (in the range of 70.59 ±3.50 to 81.93 ±1.30% only after 15 days). Surprisingly, phosphates were removed in a very high percentage in planted mesocosms in both filter media, 79.88 ±4.6 % in gravel and 89.41 ±1.2% in ceramic media. Potassium was removed in a high percentage (35.86 ±2.36%) in the ceramic media planted mesocosms in contrast with the expected poor removal in volcanic gravel planted and unplanted mesocosm (4.61±0.33% and 5.26±0.99%). It was concluded that ceramic filter media performed better compared to volcanic gravel and that the smaller size of the particles compared to those used in a previous work, played a crucial role in the case of phosphate and potassium removal. Furthermore, the system *P. sagittata* and associated rhizospheric bacteria, proved to be very resistant to toxic compounds found in stillage, since such aggressive effluent was fed a lot more concentrated than in a previous work and no damage to plants' growth has been observed after 15 days of operation.

Key words: *Phytoremediation, rhizofiltration, ethanol production wastewater, vinasse.*

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Accumulation and detoxification of sulfonated aromatic compounds from dye and textile industries by different plant species

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Sulfonated anthraquinones are precursors of a large family of synthetic dyes and pigments, recalcitrant to biodegradation and thus not eliminated by classical wastewater treatments. In the development of a phytotreatment to remove sulfonated aromatic compounds from dye and textile industrial effluents, it has been shown that rhubarb (*Rheum rabarbarum*) and common sorrel (*Rumex acetosa*) are the most efficient plants. Both species, producing natural anthraquinones, not only accumulate, but also transform these xenobiotic chemicals. Even if the precise biochemical mechanisms involved in the detoxification of sulfonated anthraquinones are not yet understood, they probably have cross talks with secondary metabolism, redox processes and plant energy metabolism. The aim of the present work was to investigate the possible role of cytochrome P450s and peroxidases in the detoxification of sulfonated anthraquinones. Several plant species were thus cultivated in a greenhouse under hydroponic conditions, with or without sulfonated anthraquinones. Plants were harvested at different times and either microsomal or cytosolic fractions were prepared. The monooxygenase activity of cytochrome P450 toward several sulfonated anthraquinones was tested using a new method based on the fluorimetric detection of oxygen consumed during cytochromes P450 catalyzed reactions. Peroxidase was measured by spectrophotometry, with guaiacol.
Results indicate that the activity of cytochromes P450 and peroxidases increased in rhubarb leaves, but not in leaves from common sorrel, when plants were cultivated in the presence of sulfonated anthraquinones. On the other hand, cytochromes P450 were able to accept as substrates anthraquinones containing sulfonated group in different positions, indicating that this enzyme was probably the first step in the metabolism of these xenobiotic compounds. These results support the idea that natural biodiversity should be better studied to use the most appropriate plant species for the phytotreatment of a specific organic pollutant.

Keywords: hydroponics, rhubarb, cytochrome P450, peroxidase, sulfonated anthraquinones

Screening of green microalgae species for phycoremediation of olive-mill wastewater

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Olive oil industries, largely represented in Mediterranean area, produce a wide amount of byproducts. Among them, olive-mill wastewater (OMW) is considered the most critical waste since contains phenolic compounds that are resistant to degradation. However, in nature are present organisms capable to degrade or complete remove a lot of those pollutants. Microalgae have been suggested as bioremediation tool, since they have shown to degrade varying pollutants. Despite the fact that *Chlorophyceae* are classified in 2.500 species, seldom living in contrasting habitat under severe environmental conditions, only few of those are used for this purpose. Therefore, the main aim of this work was the selection and the characterization of algal strains useful for phycoremediation of OMW. One hundred algal strains were grown into Bold Basal Medium for 10 days; than, free-cell culture broth samples were assayed for phenol-oxidase (PO) activity. By ABTS enzymatic assay, only 16 samples showed a strong PO activity. Further enzymatic assays were carried out in order to evaluate (a) their enzymatic activity on 2,6-dimethoxyphenol and syringaldazine and (b) the capability to decolorize the azo dye RBBR. On the basis of these results, two algal species belonging to *Chlamydomonas* and *Scenedesmus* genera were selected, along with *Chlamydomonas reinhardtii*. They were grown in 1:20 (v/v) OMW diluted with growth medium and cell concentration and total phenols content were quantified weekly. At 21 days of culture, *Scenedesmun* #95 was able to grow faster than the other two genera; furthermore, its was able to reduce the phenolic content up to 60%, while the other two genera were able to reduce the figure up to 40%.

Keywords: microalgae, phenol-oxidase, screening, olive mill wastewater, phycoremediation

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Environmental risk by the use of surfactants: use of macrophytes as bioindicators and bioaccumulators

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Surfactants have a wide range of domestic and industrial applications, and they are an important group of contaminants in aquatic systems. Ecological problems can raise when the surfactants are present in the aquatic environment in relatively high concentration.
Recently some surfactants deserved the interest of the researchers for their ability to modify the behaviour of other, preexistent or co-disposed contaminants. Thus, depending on the type and concentration of surfactant and the charges of the sorbent surface and co-contaminant, the latter may exhibit either enhanced sorption or enhanced solubilisation. While such effects have obvious implications for the fate and transport of a variety of contaminants in aquatic systems, the main body of literature has focused on the potential for surfactants to remediate contaminated environments. In particular, the sorption of cationic surfactants to a variety of synthetic and natural solids is an effective means of immobilising neutral and ionisable organic chemicals in landfill leachate and contaminated waters, whereas the application of either non-ionic or ionic surfactants at concentrations above their critical micelle concentrations (CMCs) has been exploited to release neutral chemicals from aquifers and soils. Application of surfactants to enhance heavy metals removal has been also investigated. Some surfactants exhibit toxic effects, albeit at levels in excess of environmental concentrations, therefore their use to remove pollutants has to be considered very carefully. Despite their position as primary producers in the food chain in aquatic ecosystems, the macrophytes are among the first organisms reached by pollutants in aquatic environments. Such plants are used *in situ* as biomonitors because of their abundance and limited mobility. Floating macrophytes represent also a model system to forecast the impact of pollutants.
We determined the uptake and the effects of sodium dodecyl sulphate (SDS), a commercially important anionic surfactant on the duckweed (*Lemna minor* L.) and water velvet (*Azolla filiculoides* Lam.); these species have shown a remarkable effectiveness in phytoremediation. Furthermore, *Lemna* is regularly used in ecotoxicological studies. We studied the response of the above mentioned species to SDS considering as marker of abiotic stress, changes in ethylene production and the variation of the activities of the enzymes involved in oxidative stress response, such as guaiacol peroxidase (POD), as well as enzymes of phenylpropanoid metabolic pathway: phenylalanine ammonia-lyase (PAL), polyphenol-oxidase (PPO). Phenolics content was also determined, since they play an important role in defending plants against biotic and abiotic stresses and can be potential markers of non-visible plant damage. The obtained results showed that plant metabolism can be heavily affected by SDS, although both macrophytes were able to remove the surfactant.

Keywords: Aquatic macrophytes, surfactant, SDS, stress response.

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Application of Phytobiotechnology in Water and wastewater purification

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This report details a preliminary investigation on the application of phytobiotechnology as a lowcost,appropriate and ecological alternative in purifying water and wastewater in Africa through the coagulative and disinfective ability of seeds of Moringa oleifera, Jatropha curcas seeds, calyx of Hibiscus sabdarifa, sclerotium of Pleurotus tuberregium and Alum on wastewater samples from Yelwa settlement in Bauchi, Nigeria. Varying weights (0.5 to 59) of dried pulverized plant materials and Alum were placed in 200 mls each of the three-wastewater samples and left for 24 hours. The results showed well above 90% reduction in bacterial load of the water samples by Moringa oleifera. All the plant materials exhibited appreciable coagulative effect comparable to Alum. Moringa oleifera seeds, Jatropha curcas seeds and Hibiscus sabdarifa calyx reduced the bacterial load drastically and inhibited Escherichia coli *in vitro* using the Agar diffusion method. The turbidity of both plant Alum treated water samples drastically reduced. The PH of Alum treated water was observed to decrease from neutral to acidic as opposed to a constant PH of 7.0 for both plant treated and untreated wastewater samples. This preliminary report does not only suggest an alternative and possibly cheaper water purification opportunity for rural communities in third world countries but also suggest good starting materials for the synthesis of environment friendly natural coagulant and disinfectants.

Key words: Coagulative, Disinfective, Total aerobic Mesophilic Counts, E. coli counts, Coliforms counts, Waste water, Medicinal Plants.

CONCURRENT SESSION
METALS 2

A comparative study to evaluate the phytoremediation potential of two fern species for the remediation of an arsenic contaminated site under field conditions

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From early 1900s to 1955, arsenical drenches were lavishly used in cattle dip sites across northern New South Wales (NSW), Australia to control cattle ticks. This practice inadvertently led to large expanses of arsenic (As) contaminated soils adjoining the dip sites. Phytoextraction using As-hyperaccumulating ferns might be an environmental friendly and economically sound approach to remediate the contaminated soil. The present study was aimed to evaluate the phytoremediation potential of gold dust fern (*Pityrogramma calomelanos* var. *austroamericana*), against the well-known As-hyperaccumulator Chinese brake fern (*Pteris vittata* L.), under field conditions. The field site is a disused cattle dip site located at the Environmental Centre of Excellence, Primary Industries Agricultural Institute, Wollongbar, NSW, Australia. In January 2009, Chinese brake fern (plot A) and gold dust fern (plot B) were transplanted in two plots of equal size giving 42 ferns per plot. In June 2009, frond tips from both species were sampled and in December 2009, subsequent a major harvest took place where aboveground biomass was removed from all the ferns. Soil cores were also taken in June 2009 around each fern at 0–20, 20–40 and 40–60 cm depths. Surface samples were analysed for total soil As and samples from all the depths were measured for phosphate extractable or bioavailable As concentration in soil.

The results from preliminary fern sampling revealed that the mean As concentration in gold dust fern fronds was 1.74 times greater than the Chinese brake fern. The successive major harvest confirmed these results. The results from this major harvest showed a significant difference ($p < 0.01$) between the frond As concentration of the two fern species. Arsenic concentration in the gold dust fern fronds was 2.1 times higher than in Chinese brake fern. In gold dust fern and Chinese brake fern fronds the As concentration varied between 360 and 1545 mg kg–1 and 416 and 1307 mg kg–1, respectively. Although, frond dry matter yield of both fern species was not statistically different, however, gold dust fern (plot B = 5200 g) yielded 1.6 times higher total frond dry biomass as compared to Chinese brake fern (plot A = 3223 g). The frond As uptake was significantly different ($p < 0.05$) between the studied fern species with gold dust fern accumulating 2.2 times more As than the Chinese brake fern (4.98 g vs. 2.23 g). In addition, the bioconcentration factors of gold dust fern (1.3 and 20) were also higher than the Chinese brake fern (0.78 and 14), based on total and phosphate extractable As concentrations in soil, respectively. Furthermore, frond As concentration was strongly correlated with the total and phosphate extractable soil As concentrations for the two species.

The results from this field trial demonstrated that gold dust fern was better suited than Chinese brake fern in the remediation of As contaminated soil under field conditions. These results contradict our earlier glasshouse studies that showed greater As accumulation and larger biomass production in Chinese brake fern as compared to gold dust fern. The research shows that the performance of hyperaccumulators under field conditions should be a key consideration in determining their phytoremediation ability.

Keywords: Arsenic, cattle dip site, Chinese brake fern, gold dust fern, phytoremediation

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Bioaccumulation of As and heavy metals by *Pteris vittata* from soil contaminated by glass industry wastes: a small-scale field study

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This study was aimed at evaluating the phytoextraction efficiency of the fern *Pteris vittata,* recently identified as an arsenic hyperaccumulator and thus proposed as a suitable species for phytoremediation of As contaminated sites.
The experimental activity was carried out directly in the field, in a site located in the Murano island (Venice, Italy), where the Venetian glass manufacturing industry is operating since ancient times. The soil of the study area is contaminated not only by arsenic, but also by other metals and metalloids, such as Cd, Se, Cu and Pb.
Adult plants of *P. vittata* (one and two-years old) were transplanted in the study area in June 2009. Some of the ferns were also previously inoculated with arbuscular mycorrhizae, in order to investigate the effects of mycorrhizal symbioses on plant growth in the presence of contaminants and on arsenic phytoextraction efficiency.
Bioaccumulation in ferns was assessed by collecting monthly frond samples from each plant, and carrying out analyses for metal and metalloid content. Soil samples were collected at the beginning of the experiment and analyzed to determine both the total amounts and the bioavailable fractions of these contaminants.
In the winter period ferns were left on the field, in order to evaluate survival and growth after exposure to cold weather conditions, and the possibility of a second experimental cycle with the same plants.

Keywords: Phytoremediation, phytoextraction, *Pteris vittata*, arsenic, metal, soil

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As an Pb mobilization and phytoextraction from an industrial contaminated soil

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This research work is part of a remediation project at an industrial site located in Avenza (Tuscany, Italy). The contamination in the soil is mainly due to As and Pb. In-situ phytoremediation has been considered a viable technology since cost-effective, environmentally compatible and highly acceptable from the regulators. Before applying the technology full scale a preliminary feasibility test to verify phytoextraction applicability has been carried out. Several soil samples have been collected and analysed for total and bioavailable concentration of both target metals, showing a heterogeneous distribution of contamination. The limited bioavailability has suggested the use of assisted phytoextraction. Microcosm scale experiments have been carried out on soils selected from hot spots, in the contaminated site, characterized by different As an Pb concentrations with the following plant species: *Lupinus albus*, *Helianthus annus* and *Brassica juncea*. The phytoextraction process has been enhanced increasing the bioavailable fractions of metals by using two mobilizing agents: 2 mM EDTA for lead and a 0,1 M K2HPO4 for arsenic. Very positive results have been obtained in the case of arsenic for all the tested species. After treatment with K2HPO4, As concentration in plant shoots reached pick values of nearly 6000 mg/kg in two of the selected soils. The best results for Pb after the treatment with EDTA solution, have been obtained with *Lupinus albus*. These results support the potential of the phytoextraction as remediation technology to reduce contaminants concentrations leading to a positive environmental balance. This issue has seldom been addressed, but it is an essential feature for environment and human health. Work is in progress for the scale-up of the experiments to evaluate from mesocosm to field scale the remediation potential of the technology with the same plant species and with the use of the hyperaccumulating fern, Pteris vittata with or without phosphate application, to maximize the efficiency of arsenic removal. In the meantime alternative low molecular weight organic acids will be tested to substitute as complexing agents the low biodegradable EDTA. In addition some Sequential Extraction Procedures (SEPs) will be performed in order to define the most effective strategy for remediation, the achievable target of treatment and evaluate the mobility of the contaminants.

Keywords: Phytoremediation, Arsenic, Lead, EDTA, K2HPO4, microcosm and mesocosm

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Phytoremediation of mine soils using plants and earthworms

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Since the primary aim of the phytoextraction technique is to remove metals from polluted soils by concentrating them in the harvestable parts of plants, the removal yield depends on the amount of above-ground biomass and the bioavailability of metals. Thus, the enhancement of both the plants' ability to accumulate metals in shoots and biomass yields is the key to improve phytoremediation efficiency. Recently, roles of earthworm in heavy metal remediation have been reported, based on their beneficial effects for plant growth and the enhancement of soil metal bioavailability. We have conducted a research focusing on the phytoextraction of Pb, Zn and Cu from two soils polluted by mining activities using the combination of maize (*Zea mays*) or barley (*Hordeum vulgare*) and *Lumbricus terrestris* earthworm.

Phytoextraction experiments were carried out in plastic pots using five treatments for the polluted soil: earthworms (E), maize (M), barley (B), maize + earthworms (ME), barley + earthworms (BE). Pots without plants or earthworms were also used as control (C). Experiments were conducted by triplicate under artificial light and constant temperature (17ºC). After 4 weeks, samples of plants, earthworms and soils were taken. Analysis conducted were: (i) total metal concentration in shoots and roots of plant samples and earthworms tissues; and, (ii) for soil samples, water and CaCl2 extractable metals and geochemical partitioning of metals by means of BCR sequential extraction. Addition of earthworms to soil slightly increased water soluble Zn for both soils. Zn extracted by CaCl2 was significantly increased ($p<0.01$) in comparison with data from the control pots (C). Results from BCR sequential extraction procedure showed that Zn and Cu in the "bound to organic matter and sulphides" fraction (F3) was significantly higher for pots with earthworms (E) than those of control pots (C). As compared as experiments with only plants (M and B), earthworm presence increased shoot weight of plants in ME and BE treatments; however, that increase was not statistically significant.

In general, earthworm activities increased plant metal concentrations to some extent. For Soil 1, that increase was only statistically significant for Cu and Zn root concentration in maize and for total Pb concentration in barley. For Soil 2, Cu and Pb shoot concentrations were significantly increased for maize; Pb and Zn contents were significantly increased for both roots and shoots in barley.

Therefore, those results showed that *Lumbricus terrestris* earthworm can moderately enhance bioavailability of heavy metals in soils, thus improving their absorption by plants and the accumulation of the studied metals. Nevertheless, the effect of *Lumbricus terrestris* on both metal availability and plant uptake was lesser significant than that of previously reported by the authors for *Eisenia fetida* earthworm [1].

Keywords: barley, maize, *Lumbricus terrestris*, phytoextraction, lead, zinc, copper

[1] E. Ruiz, L. Rodríguez, J. Alonso-Azcárate (2009) Effects of earthworms on metal uptake of heavy metals from polluted mine soils by different crop plants. Chemosph.75, 1035–1041

Lead and cadmium phytostabilization potential of *Nerium oleander* L. in contaminated non-saline and saline soils

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Phytostabilization have attracted much interest over the last decades for the remediation of heavy metals contaminated soils where the best alternative is often to hold contaminants in place. In this study, *Nerium oleande*r L. which is a fast growing with high biomass, salt tolerant endemic plant of the Mediterranean region, North Africa and South Asia, has been investigated. In Greece this plant can be found along the side of heavily trafficked highways where Pb concentrations have been very high in the past, along the seashores or in the Mediterranean riverbeds that remain dry most of the year, suggesting that this plant is able to adapt to a wide range of stressors and tolerates considerable drought, high temperature, strong winds, poor drainage, high salt content in the soil and probably heavy metals. Furthermore, a more efficient performance of several basic biochemical tolerance mechanisms provides an advantage to salt tolerant plants with respect to several environmental factors including heavy metals. Therefore, salt tolerant plants like *N. oleande*r have been suggested to be naturally better adapted to cope with heavy metals compared to salt-sensitive crop plants commonly chosen for phytoremediation purposes and thus, offer a greater potential for phytoremediation research.

The main aim of this work was to explore *N. oleander* final effectiveness as a cleanup tool for the phytostabilization of soils polluted with lead and cadmium. For that purpose, four pot experiments were conducted under field conditions for a 10 week period with *Nerium oleander* L. grown in soil artificially polluted with: 0, 800, 1600 and 2400 ppm of Pb irrigated with two different salt solutions (0.0 and 0.5 % NaCl); 800 ppm of Pb irrigated with three different salt solutions (0.0, 0.5 and 3.0 % NaCl); 40 ppm of Cd irrigated with three different salt solutions (0.0, 0.5 and 3.0 % NaCl); 40 ppm of Cd and 800 ppm of Pb irrigated with three different salt solutions (0.0, 0.5 and 3.0 % NaCl). Measurements of chlorophyll content, shoot height, biomass, leaf specific activity of guaiacol peroxidase (GPX; EC 1.11.1.7), and Cd and Pb content in the plant tissues were performed.

The experimental data reviled that the main lead and cadmium accumulation site is the plant roots and especially for Pb when concentration in soil reached 2400 ppm, the accumulation in aerial parts remained notably at low levels indicating an important restriction of the transport of the metal from the roots towards stems and leaves. Moreover, soil salinity increased lead uptake by the plants and cadmium translocation from roots to shoots. Furthermore, the plant developed no visible toxicity symptoms as well as no growth or chlorophyll content reduction while the specific activity of guaiacol peroxidase was found to increase in metal treated plants suggesting that this enzyme serves as a defense tool against Pb and Cd induced oxidative damage. Therefore, *Nerium oleander* L. is a Pb and Cd-resistant plant which does not translocate the metals into its aerial tissues, and hence, it is suitable for phytostabilization applications.

Keywords: phytostabilization, *Nerium oleander* L., lead, cadmium, salinity, tolerance

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CONCURRENT SESSION
ENVIRONMENT AND HEALTH 2

Air Quality in Public Space as a Factor of Health

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The atmosphere is the dominant transport pathway for many chemicals of environmental concern. Air pollutants are a direct threat to people and a great challenge for the environmentalists. It so because due to technical and meteorological reason the level of air pollutants is difficult to reduce. As major air pollutants most often are listed nitrogen oxides (NO_x), carbon monoxide (CO), volatile organic compounds (VOC), ozone (0_3) and particulate matter (PM). Ozone and particulate matter are the main threats to public health. In the last years the top position on the list of the air pollutants is occupied by particulate matters (PM). The awareness of potential risk imposed on human and the environment due to urbanization has greatly increased recently with air pollution as major factor of the risk in urban space. Particulate matters air pollutants caused by urbanization exert harmful effect on human health. European Environment Agency recognizes PM as a most dangerous air pollutants and estimate that they are major cause of 348 000 death yearly of UE citizens to respiratory disease from this pollution. Exposure to PM has been estimated to cause an average, in Europe, loss of life expectancy for 9 months but in some sites it is estimated even up to 3 years . There appear to be number of associates between outdoor and indoor air polluted with hazardous chemicals and respiratory and cardiovascular diseases, cancer, asthma and allergies. Children seem too be particularly susceptible to the harmful effects of air pollution as they posses weaker ability to detoxify, metabolize and excrete of environmental agents. Children, in addition perform a greater level of physical activity than adults, hence their intake of air into the lungs is much greater .
One of the most dangers are PM emitted from heating of household and transport vehicle, among this last equipped with Diesel engine emitted 20 to 100 times more PM than those with gasoline.
The use of phytotechnologies has been proven to impact the levels in urban settings and this approach can have direct impacts on public health of public areas. In fact in open space there is no other way to clean up the air than phytechnology. Numerous studies will be summarized on the direct impacts on air quality, showing that phytotechnologies can have a beneficial role in improving urban air quality and therefore citizens quality of life. This talk will also relate this removal directly to potential public health impacts.

Key words: green walls, public health, particulate matter (PM), air pollutants

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Deliberate attacks on critical ecological infrastructures and effects on human health

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Terrorism aims to attack resources which are critical and vulnerable, but it often aims also at jeopardising aspects of human life which have the highest emotional impact. Among the sensitive targets we can surely consider the environmental resources and ecological infrastructures. Vulnerability of environmental resources is a fact actively exploited by terrorists. These resources can be used indirectly to carry on damages into human populations, or be the direct "targets" of an action. For instance, water can be used as a vehicle to carry on pathogens to affect human populations, but through the disruption of the water supply infrastructures it can determine water deprivation or water shortage.

Everything concerning safety and salubrity of the environment affects all strata of the population. In particular, when environmental contamination affects the production of food and the supply of water the impacts become higher: (i) it affects populations at large; (ii) it specifically affects the weak members of the populations, e.g. children and elderly; (iii) it addresses a fundamental need of people; (iv) its perturbation is of high psychological impact; (v) its manipulation can destroy the consumers' trust in industry, producers, and retailers; and (vi) it leads to massive economical impacts.

The recent example of dioxin contamination of cheese showed how impacting can be a case of environmental pollution, especially at the level of public opinion: only few cases have affected the whole market at worldwide level, evidencing a lack of experience and resources, manifesting a large economic impact on production and commercialisation. An additional complication concerns the possibility that the contaminated food can be disseminated and transported covering long distances before the contamination was discovered.

It is possible to describe instances of environmental contamination and to list possible sensitive targets for terrorism based on past history of threats and attacks, learning from experience how phytotechnologies can provide effective countermeasures. Innovative approaches are being developed to monitor resources, using molecular biology and analytical chemistry to detect biological and chemical hazardous agents. Monitoring techniques and sensors are currently available for deployment at critical sites. Satellite surveillance of critical infrastructures is also considered for application. Besides sensors and monitoring devices, it is also required to build an infrastructure for data collection, management, elaboration and storage. The project SITCEN financed within the NATO Science for Peace and Security Program has focused on the development of countermeasures against ecoterrorism.

Keywords: ecoterrorism, countermeasures, food safety, mitigation, biosensors, satellite monitoring

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CONCURRENT SESSION
WETLANDS

Are Monocots more Efficient than Dicots for Removing Trace Elements in Constructed Wetlands?

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Constructed wetlands (CWs) using macrophytes are an effective way to treat trace element (TE)-contaminated water. However, removal rates are specific for metals, ionic forms, seasons, and macrophytes. Here, we focus on the role of macrophyte class used in phytoremediation. Both monocots (e.g. *Phragmites australis, Eichhornia crassipes*) and dicots (e.g. *Lythrum salicaria, Salix viminalis,* and *Bacopa monnieri*) act as catalyst of biochemical reactions in substrates and offer habitat in their rhizosphere to micro-organisms, but a question arises: *Are monocots more efficient than dicots for removing TE in CWs*? We reviewed 21 studies reporting on TE removal in CWs. Difference between influent and effluent concentrations was used to compute removal efficiency. Removal efficiency would be class-dependent for Cu (Wilcoxon test, p= 0.02) and Zn (Wilcoxon test, p= 0.07). Monocots would be more efficient than dicots in TE removal. This trend is strong but not clearly significant. This could be due to: (1) monocot root system is fibrous and/or adventitious, thus offers more surfaces to treat TE effluent and habitats to micro-organisms than dicots which present a tap root system; (2) graminaceous species produce phytosiderophores (e.g. mugineic acids) that efficiently chelate ferric iron, but also other TE, i.e. Mn, Cu, and Zn, due to their amine and carboxyl groups (Kidd et al., 2009).
Under certain conditions, e.g. low Fe supply, co-cultivation of graminaceous plants with other plants may enhance metal uptake (Ma et al., 2003, Luo et al., 2008). Phytosiderophore production by monocots and their rhizosphere properties may contribute to higher TE removal rate in CWs. These results are not supported by works on cationic exchange capacity (CEC) (Woodward et al., 1984).

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Key words: constructed wetland, macrophyte, dicots, monocots, trace elements, phytoremediation

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Manure processing to reusable water using constructed wetlands

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Many regions with intensive agriculture face local overproductions of animal manure that require processing in an economic sound manner. The current state of the art in manure processing in Flanders involves three phases: (a) separation into a liquid and a solid fraction, (b) subsequent conversion of the solid fraction into an exportable product and (c) reduction of the nutrient content in the liquid fraction in a biological treatment reactor. In previous research we demonstrated that constructed wetlands are an effective way to further process pig manure after biological treatment to a dischargeable effluent on large scale according to Flemish legal standards (concentrations < 15 mg/l total N, 2 mg/l total P and 125 mg/l COD). This was to our knowledge the first successful full-scale application of constructed wetland technology to convert manure into water of sufficient quality. Currently, we are exploring the possibility of recycling the end water of the constructed wetlands in high-grade (e.g., live stock drinking water, irrigation water) and low grade applications (e.g., cooling water, cleaning) by full physico-chemical and bacteriological monitoring at a monthly basis for an overall period of (at least one) year. Physical-chemical analysis includes a wide range of parameters such as pH, salts, trace elements, nutrients, amongst others. Bacteriological analyses comprise colony count at 22°C and 37°C, *Salmonella* spp., *C. perfringens* total coliforms, *E. coli*, Enterococci and sulphite reducing Clostridia. Preliminary results indicate that effluent quality scores better than initially anticipated, both for the bacteriological as well as the physico-chemical parameters. The stringent discharge criteria were consistently met, as was expected, but other test variables determining the re-use potential of the effluent also exhibited positive results. Even for high grade applications, such as drinking water for livestock, constraints for reuse were limited to parameters such as iron content, water hardness and certain salts, which are easy to address using simple polishing steps. There is perspective that reuse of end effluent will be technically and economically feasible. As water sources become more and more scarce and expensive, reuse of constructed wetland effluent in various applications may have important economical and environmental benefits.

Keywords: manure processing, constructed wetlands, water

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Constructed Wetland: Efficiency in Arsenic Removal and Management of Arsenic Accumulated Plants, Soil and Sediment

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The arsenic contaminated waste water in Ron Phibun District, Nakorn Si Thammarat Province, Thailand, was efficiently removed by the constructed wetland (CW). The wetland system was designed and tested to get the highest ability in arsenic removal. *Cyperus* spp. and *Colocasia esculenta*, widely grown wetland plants, were compared on its efficiency in removing arsenic from water. Both plants showed similar efficiency through out the 1 year operation. The arsenic contaminated waste water, after passing through the constructed wetland, could be directly discharged into the natural reservoir.
The biomass of the wetland plants, soil and sediments were hazardous wastes which required proper disposal and management. The degradation in water of arsenic enriched *C. esculenta* was performed for 149 d. The concentration of arsenic leached out from the plant materials was found to be at 0.01-0.09 mg L-1 which was much lower than the standard of industrial discharges. For *Cyperus* spp., the utilization as ornamental plants was applied. The sediments contained very high arsenic concentration and solidification/stabilization (S/S) was used in the management of the sediments. The arsenic leached out from all S/S blocks after curing for either 14 or 21 day was low (0.01-0.61 mg L-1), which did not need to put the S/S blocks in the secured landfill. The success of this study proved that the constructed wetland was suitable for Thailand in removing metals from water and the management of the arsenic accumulated materials from the constructed wetland was not a problem.

Keyword: constructed wetland, arsenic, *Cyperus, Colocasia esculenta*, waste management

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Stricter Regulation Demand for Nutrient Degradation- The Phyto-Sustainable Solution

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Pollutants from agricultural operations can be a significant contributor to the impairment of surface and groundwater quality. Constructed wetlands (CW) are capable of successfully treating pollutants such as BOD, TSS and pathogens in household sanitary wastewater, however, the wastewater from dairy farms present a more difficult challenge due to the high concentration of organic loads and nutrients, both requiring large quantities of oxygen for treatment. In Israel as around the world, governments are raising the standards for allowing re-use of treated wastewater, challenging ecological engineering to create more efficient systems. The purpose of the paper is to present the design considerations for a Natural Biological System (NBS) for a small dairy farm of approximately 300 milking cows in Beyt-Yatir-Atzmona, Israel. The regulator's requirement for contaminant levels are 10/10/10, BOD, TSS and TN, respectively. The dairy farm wastewater was characterized by 200 mg/l ammonia and 1600 mg/l BOD. In order to treat ammonia, oxygenated conditions must be present for nitrification. The sewage characteristic figures were used to calculate the oxygen demand of the NBS. In order to create optimal conditions for nitrification, vertical flow (VF) beds were designed with stepped cascade aeration. Empirical values from other systems constructed by "Ayala Water & Ecology" as well as values reported in the literature were used to calculate the transfer rate of oxygen from aquatic plant roots, atmospheric diffusion and a passive artificial aeration system. To treat the nitrate products of the denitrification process, horizontal flow (HF) beds were designed to create anaerobic conditions, for completion of the nitrogen cycle. An enhancement of the denitrification process was planned by re-circulating a portion of the effluent from the HF beds, through a small holding tank containing raw sewage. This extra feature will ensure an adequate carbon source for denitrification, as well as reduce the excess oxygen in the system after passing through the oxygenated VF beds.

Keywords: Dairy farm, Nutrients, Nitrification, Denitrification, Constructed Wetlands

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PLENARY LECTURES

H9

Dynamic of trace element and biochemical properties in contaminated soils afforested with Salicaceas

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The use of fast growing trees that are well adapted to different edafo-climatic conditions is an increasingly common practice in many regions of the world. Many of the trees such as those of the Salicaceae family accumulate trace elements (TE) (especially Cd and Zn) in their leaves. Salicaceas are deciduous and their plantation in TE polluted soils would cause, after the autumnal fall, the presence of an extensive 'carpet' of litter loaded with heavy metals, which is not always viable to entirely remove. We studied influence of this heavy metal rich litter on the biochemical properties of the soil and TE availability (CaCl2 extraction) on three different forest soils: control (no affected by trace elements), and two sites polluted with TE, one pH=7 (AZ) and the other pH=3 (DO) at two different depths 0-20 cm and 20-40 cm. Trace element availability was significantly higher in affected DO soils compared to CO and AZ. Concentration of available TE was similar at both depths in the three soils. Chemical and biochemical properties related with the C cycle (microbial biomass carbon, hidrosoluble C and β-glucosidase activity) were affected either by soil pH and available TE concentration. Values of these properties are also linked with the C content and were higher at surface (0 20 cm) than at deeper layer (20 40 cm). Values of the parameters related with the N cycle, (N-NO3, microbial biomass N and Protease activity) were in general higher in CO than in polluted soil (DO and AZ) at both depths. In our conditions C and N cycles were more affected by pH and trace element availability than for the total TE content in soil.

Keywords: heavy metal, availability, microbial biomass, enzymatic activities

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Rethinking Sustainability – The Technology Link

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The subject of sustainability is on everyone's lips. However, one man's sustainability is another'sunsustainability. The problem is both in definition and in technology. The paper will review the current definitions of sustainability both from the American perspective and from the European perspective.
The differences between the two will be compared, and the shortcomings of both will be examined.
The author will attempt to illustrate that real sustainability is linked inexorably with a desired level of technology, and that unless the civilization wants to regress and abandon its' levels of technology, it must accept some amount of unsustainable activity, and suggest a way to measure the sustainability of our activities.
A recent technical website illustrates the point: The website is calling for moving away from nonsustainable technologies to those which are more sustainable. The call was for improving energy efficiency by better thermal insulation, more efficient processes, conservation, and by moving away from petroleum, coal, and fossil fuels to renewable fuels such as plant derived fuels, geothermal energy, solar, wave, and wind power technologies and *natural gas.* Natural gas is still a hydrocarbon fuel.
The paper will offer a different perspective on the true nature of sustainable activity and demonstrate how it is linked to technology, and how a better definition of technology can lead to a better definition of sustainable activity.

Keywords: Sustainability, Technology, CO2 emissions, and some global warming.

Evidence of the differential biotransformation and genotoxicity of ZnO and CeO2 nanoparticles on soybean (*Glycine max*) plants

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Concern and interest related to the effects of nanomaterials on living organisms are growing in both the scientific and public communities. Reports have described the toxicity of nanoparticles (NPs) on micro and macro organisms, including some plant species. Nevertheless, to the authors'knowledge there are no reports on the biotransformation of NPs by edible terrestrial plants. Here, shown for the first time, is evidence pertaining to the biotransformation of ZnO and CeO2 NPs in plant seedlings. Although the NPs did not affect soybean germination, they produced a differential effect on plant growth and element uptake. By using synchrotron X-ray absorption spectroscopy we obtained clear evidence of the presence of CeO2 NPs in roots, whereas ZnO NPs were not present. Random amplified polymorphic DNA assay was applied to detect DNA damage and mutations caused by NPs. Results obtained from the exposure of soybean plants to CeO2 NPs show the appearance of four new bands at 2000 mg L-1 and three new bands at 4000 mg L-1 treatment. In this study we demonstrated genotoxic effects from the exposure of soybean plants to CeO2 NPs.

Keywords*:* ZnO, CeO2, Nanoparticles, Nanotoxicity, Genotoxicity, XAS.

CONCURRENT SESSION
METALS 3

Phytoextraction of Zn and Cd from contaminated soil in the suburbs of Hangzhou City by *Sedum plumbizincicola*

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Heavy metal contamination in modern China is a ubiquitous problem under the combined pressure of economic and urban development and it can be ameliorated using efficient and environmentally friendly phytoremediation technology. *S. plumbizincicola*, a Zn and Cd hyperaccumulator native to China, has considerable potential for phytoremediation of soil polluted with both Zn and Cd. Field plot experiment results show that intercropping *S. plumbizincicola* with *Elsholtzia splendens* increased the removal of total Cu, Zn and Cd from soil with multiple metal contamination. Moreover, intercropping of *S. plumbizincicola* with *Sorghum bicolor* decreased the heavy metal concentrations in the grains of *S. bicolor*. Soil Cd and Zn availability decreased with increasing number of years of phytoextraction and Cd and Zn concentrations in *S. plumbizincicola* in the second year were half of those in the first year and heavy metal uptake by *S. plumbizincicola* declined. Continuous phytoextraction had no effect on the aboveground biomass of *S. plumbizincicola*. Yields of *S. plumbizincicola* increased with increasing plant density but there did not continue to increase when plant density increased to very high levels. When the density was 440 thousand plants per hectare the removal rates of Cd and Zn in the first year in contaminated soil were 18.0 and 3.93 %, respectively, and the corresponding values in the second year were 9.86 and 2.46 %. Heavy metal uptake by *S. plumbizincicola* from contaminated agricultural land decreased with increasing number of years of phytoextraction. Appropriate plant densities will enhance Cd and Zn uptake by the shoots of *S. plumbizincicola* and shorten the time period required for phytoremediation.

Keywords: Soil, Zn, Cd, *S. plumbizincicola*, phytoextraction

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Effects of soil management on phytoremediation of metal-polluted pyrite wastes with field crops

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An *in-situ* phytoremediation trial was carried out at Torviscosa (Udine, NE Italy) in marked As-Cd-Co-Cu-Pb-Zn contaminated wastes. Pyrite cinders had been discharged in the past and covered with an unpolluted 0.15-m thick layer of gravelly soil. *Helianthus annuus* L., *Medicago sativa* L., *Raphanus sativus* L. var. *oleiformis* Pers. and *Lolium multiflorum* Lam. were cultivated under two soil managements, ploughing vs. subsoiling (0.3 m depth), comparing plant growth and metal uptake.
Subsoiling caused undesired contamination by pyrite in top soil, but the ploughed cinders showed higher total and bioavailable metal concentrations. The tillage system was not critical for mass balance of phytoextraction, but the effect on above-ground productivity and its metal concentrations diverged, higher biomasses being reached at weaker contamination levels. *Raphanus sativus* was the highest biomass-yielding species, and reached the best metal offtake. Removals were generally poor and followed the order *R. sativus* > *L. multiflorum* > *H. annuus* > *M. sativa*, i.e., 33, 21, 17 and 9 mg m-2 per year, respectively, with Zn and Cu as main harvested contaminants. Root growth was a key trait in phytoextraction, as shown by the combined highest root length density and shoot metal concentrations in *L. multiflorum*.
We conclude that phytomanagement of pyrite wastes with crop species is a feasible option, although agronomic practices should be managed appropriately to improve plant growth, root deepening and metal translocation. Confidence to achieve better metal removals requires substantial soil amelioration and appropriate adjustment of the cultivation method applied.

Keywords: crops, heavy metals, phytoremediation, pyrite wastes, soil management.

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Using Mediterranean shrubs for phytoremediating a soil impacted by pyritic wastes in South Spain: a field experiment.

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Revegetation is the main aim of ecological restoration projects, and in Mediterranean environments native plants are desirable to achieve successful restoration. In 1998, a burst tailings dam flooded the Guadiamar river valley downstream from Aznalcóllar (Southern Spain) with sludges that contained elevated concentrations of metals and metalloids, polluting soils and waters. After the initial treatment of the spill, a restoration project, the Green Corridor of the Guadiamar River, with the eventual aim of revegetating the affected area with an open forest ecosystem, was initiated. A phytoremediation experiment to assess the potential of native shrub species for this project was performed from 2005 to 2007 using a moderately polluted area at Aznalcóllar, divided into soils with pH<5 and pH>5. Four native shrubs (*Myrtus communis*, *Retama sphaerocarpa*, *Rosmarinus officinalis*, *Tamarix gallica*) were planted and left to grow on without intervention. Trace element concentration in soils and plants, their extractability in soils, elemental transfer factors, and plant survival were used to identify the most interesting species for phytoremediation. Total As in soils exceeded the legal limit of 100 ppm in 20% of the sub-plots planted with the different shrubs. Total As was higher in soils with pH<5. (NH4)2SO4-Extractable Zn, Cu, Cd, and Al concentrations were higher in very acid soils, but As extractability was more efficiently extracted when soil pH>5. Extractable metals showed significant relationships to soil pH (inversely related) and total soil metal concentrations, but extractable As did not. Arsenic was fixed by Fe oxides in these soils, and some was retained as sulphide. *T. gallica*, *R. officinalis*, and *R. spharocarpa* survived better in soils with pH>5, while *M. communis* had better survival where pH<5. *R. sphaerocarpa* showed the highest survival (30%) in all soils. Trace element transfer from soil to harvestable parts was low in all species and elements. In a previous pot experiment *R. sphaerocarpa* and *M. communis* were shown to improve soil quality, decreasing metal(liod)s availability, and increasing C, N, and enzymatic activities. Our results suggest that *R. sphaerocapra* is a promising plant for potential use in phytostabilisation.

Keywords: phytostabilisation, mine soil, native plants, arsenic, metals, plant survival

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Lead Accumulation in Native and Naturalized Woody Species Grown on a Pb-contaminated Site

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Phytoremediation has been proposed as an economic alternative for remediation of metal contaminated soils. It can be applied over extended surfaces and hilly areas, and targets the "bioavailable" soil fraction of heavy metals, which is the most relevant fraction from an environmental risk assessment perspective. The most important drawback is the long remediation period required (from years to decades). In these condition, the goal is to create a vegetative cap using native and naturalized woody plants in order to 1) prevent wind and water erosion of soil, 2) stabilize metal contaminants in the rooting zone, and 3) encourage epigeous uptake of metal contaminant. The use of shrub and trees species induce a gradual removal of the contaminant at diverse soil deep, making available biomasses for the production of renewable energy. This study was designed to analyze the ability of native and naturalized plant species found in a Pb contaminated area in La Spezia, located in the North of Italy, as candidate species to manage and mitigate environmental contaminant health risks.

The polluted site, a little hill close to a factory that produces PbO since 1922, presents soil Pb concentration ranging from 170,5 to 4500,5 mg·kg-1. Plants able to survive in the presence of out-range levels of lead respect to the threshold limit (DM 152/2006) were identified, including trees (pine and *Ailanthus* sp.) and shrubs (*Arbutus unedo* L., *Erica arborea* L., *Myrtus communis* L., *Ilex aquifolium* L., *Laurus nobilis* L. and *Pteridium aquilinum* (L.) Khun). The content of lead in plant tissues was determined by ICP in ashed samples obtained from roots, stems + branches and leaves. A clear species peculiarity in the accumulation of Pb metal in tive organs was revealed and to investigate Pb uptake capacity of selected woody species, a nine months long pot experiment was performed. Tree of Heaven, Bay Laurel and Common Myrtle plants were grown in soils with 312,4 mg Pb Kg-1 (Pb-) and 4344,5 mg Pb Kg-1 (Pb+), collected from two different sites within the contaminated area, without addition of organic fertilizer or treatments with EDTA. The Pb plant accumulation (550, 787, 1300 mg Kg-1s.s.) increased in *Laurus nobilis*, *Ailanthus* sp. and *Myrtus communis*, respectively, cultivated into 4500 mg Kg-1 Pb soil. *Ailanthus* plants accumulated larger Pb metal quantities compared to two shrub species, as a large part of lead was translocated from roots to epigeous parts (47%). All the species included in this study might be considered as Pb tolerant plants and may be cultivated in soils presenting a medium or high lead contamination. Their different ability to accumulate lead in epigeous and ipogeous organs suggests the potential use for phytoextraction and/or phytostabilization. They were also discussed for the biomass production capacity, the possibilities for an economical exploitation and the phytostabilization efficiency in order to reduce the mobilization of Pb.

Keywords: Pb, phytoextraction, phytostabilization, polluted soil

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CONCURRENT SESSION
SUSTAINABILITY

Sustainable Urban design: Plaine du Var and the city of Nice

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The Var River in the French Riviera is characterized by a steep and narrow channel, soft sedimentary rocks, high turbidity, and seasonal and sometimes sudden and dramatic changes in discharge rate. These conditions are favorable for landslides and major flooding, causing tremendous damage to property and even loss of lives in the densely populated Plaine du Var. The last half century has been characterized by a decrease in the annual amount of rainfall as well as longer dry seasons. During the same time there has been considerable human development in the valley, leading to increased pressure on the eco-system: though the river's sources are relatively protected, the underground aquifer fed by the river is overexploited, and is under threat from the industrialized zones above it. In addition, continuous loss of sand in the Nice French Riviera region has led to degradation of the famous beaches. For the remediation of the valley, a complimentary, sustainable design approach is presented. The re-design of the urban and rural spaces include features to reduce the momentary water capacity of in-charging water to the river during heavy rain events, such as renewing flood plains along the river banks; minimizing surface water run-off from urban area using techniques such as green roofs, bio-swales, water harvesting treatment and infiltration and seasonal ponds; minimizing storm water runoff from large open areas such as agricultural fields, industrial zones, parking lots, etc; rehabilitate natural landscape to include semi-natural elements such as agricultural fields and mountain terraces. Sustainable pre-treatment of water from all sources will prevent further contamination of the aquifer zones, and creation of "Green Belts" in strategically placed zones will assist in reducing air pollution. Allowing free continuous flow of sediments downstream by opening upstream dams and more importantly, navigating the river flow to the open sea in its natural course will allow sea currents and waves to carry on the fine particles back to the beach, regenerating the Riviera. Following these planning guidelines will assure that the different ecological units in the valley work together to improve the environmental conditions in a sustainable manner.

Keywords: Sustainability, Urban design, Green roofs, bio-swales, water harvesting.

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Use of Environmental Friendly Technologies for the Implementation of the E.U. Water Framework Directive: The Experience of the LIFE-EnviFriendly Project

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The Water Framework Directive (WFD) requires the development of watershed management plans and remedial actions to improve water quality and ecological quality of surface, ground waters and coastal zone. To date, environmental technologies have been implemented in a "surgical" approach without any concern of the impact to the watershed area as a whole. The premise of the LIFE-EnviFriendly project was that the selection, design characteristics and implementation of environmental friendly technologies for the minimization of non-point source pollution from agricultural lands should be conducted in conjunction with the development of watershed and coastal zone management plans." The design of environmental technologies was integrated with the watershed management plans and applied to Evrotas River Basin in Peloponnese, Greece. The project demonstrated a series of environmental friendly methods and technologies that reduce agricultural pollution from point and non-point sources including phytoremediation based technologies such as drainage canal and riparian zone management.

The drainage canal was located in Skala and drained fields of an orange grove. Plants covered two distinct areas with *Phragmites australis* and *Arundo donax*. The objective of this demonstration was to evaluate the removal efficiency of nutrients due to natural attenuation mechanisms. The project investigated nutrients balance in groundwater, sediments, and reeds of the drainage canal. Field sampling was conducted in order to assess the fate and transport of nutrients as they move from the groundwater to the drainage canal. In addition, laboratory studies were used to assess the biogeochemical processes that control the Nitrogen and Phosphorous cycles and evaluate the efficiency of the sediments to attenuate pollutants. Finally, the nutrient (nitrogen and phosphorus) uptake fluxes by reeds were measured on a monthly basis in order to determine the timing of harvesting reeds that will maximize the removal of nutrients by plant uptake. The removal efficiency of the system was estimated at 76% for nitrate and 100% for phosphorous.

Temporary rivers are flashy in nature and upon extreme precipitation they might produce floods with extremely high erosion potential. A riparian forest of 200 poplar trees was planted to decrease nutrient loads due to uptake and enhanced denitrification. In this way, phytoremediation in conjunction with river bank erosion controls was demonstrated as a combined remediation tool for non-point source pollution of nutrients. Groundwater sampling was conducted in order to assess the fate and transport of nutrients as they move from the groundwater to the River and assess the efficiency of the technology. On the average, 70% of nitrates were removed by the system.

Keywords: Riparian restoration, drainage canal management, monitored natural attenuation, water management plans

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Gardening on Brownfields Sites: Evaluating Trace Element Transfer from Soil to Plants

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Tens of thousands of brownfields (abandoned or underutilized properties where known or potential environmental issues are an obstacle to redevelopment) can be found in cities, towns, and rural areas across the USA. Our work has focused, in part, on the conversion of brownfields to garden areas and is motivated by the increasing interest in locally produced foods. Challenges of converting brownfields to community gardening sites will be discussed using one newly established urban community garden site located in Kansas City as an example. This site has mildly elevated levels of lead (Pb) and some detectable levels of dichlorodiphenyltrichloroethane/ dichlorodiphenyldichloroethylene (DDT/DDE). Suitable safety/corrective measures were suggested and implemented after thorough evaluation of soil properties. Measures focused on reducing both direct (soil-human) and indirect (soil-planthuman) exposure of gardeners and their children to Pb and/or DDT/DDE. In addition, field test plots were established within the community garden and three crop types with three very different growth and contaminant uptake patterns were planted. Effectiveness of selected sitespecific soil amendments to reduce bioavailability of Pb was evaluated in summer 2009.
Samples from additional crop types planted by the gardeners were also collected and tested for Pb in plant tissue. Lead concentrations in all tissue samples were below the recently amended maximum permissible levels set by the Joint Food and Agriculture Organization/ World Health Organization food standard program for vegetables and were in the following order: Carrot > Swiss chard > Sweet potato ~ Mustard > Tomato. Research at this site for a second growing season is on-going. Different methodologies will be utilized throughout the project to understand the significance of potential soil-plant-human exposure pathway of contaminants while gardening on mildly contaminated sites. Efforts will also be made to understand relationships between key soil properties and contaminant bioavailability as measured by the physiologically based extraction test.

Keywords: brownfields, trace elements, bioavailability, soil-plant transfer

Exploratory analysis of phytorestoration and remediation data from a field application in an abandoned mining area using different amendments

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Abandoned mining areas constitute a relevant environmental problem all over Europe and particularly in Sardinia where poor management after mine closure determined dispersion of high amounts of heavy metals by wind and water erosion on extensive areas, as shown by the absence or scarcity of vegetation. The application of traditional disruptive technologies to these areas is inappropriate because of the high cost necessary for soil remediation and the potential impacts to the environment. Phytoremediation, being an *in situ* low cost technique, can also allow to restore the site reproducing the ecological conditions before mining activities, through the use of mediterranean native plants. Plant growth can be enhanced by the addition of amendments, which improve soil properties increasing biomass production and influence metals behaviour. This study aims at identifying Mediterranean native plants for the application of revegetation and phytoremediation projects to abandoned mining sites, and the best amendments to be used in order to reduce the toxic effects of heavy metals. A two-year field experiment was carried out on a contaminated area of 300 m2 in Campo Pisano mine, near Iglesias (Sardinia, Italy), where the most abundant toxic metals are Pb and Zn at concentrations of 3000 mg/kg and 12000 mg/kg, respectively. Two Sardinian native species were used: *Scrophularia canina* subsp. *bicolor* and *Pistacia lentiscus*. The area was divided into 10 plots of 6x5m, in which the two plant species and different soil amendments (compost, chemical fertilizer and zeolites, used individually and in combination) were applied. Two of these plots were left untreated and used as control for the two species. The plant resistance to heavy metals and poor soil was determined through monthly counting of vital or suffering plants in the different plots and observation of the growth of new plants of the same or of different species. The phytoremediation performances of the two selected plants were evaluated through the periodical determination of metal concentration in soil and in the different parts of the plant (roots and leaves). Soil was chemically and physically characterized and both total and bioavailable metal concentrations were evaluated in order to verify the influence of the different treatments. In order to analyze the complex amount of data acquired during repeated surveys, two different data analysis approaches were considered: at first, data regarding the behavior of the observed plants were analyzed according to a classical statistical analysis approach, in order to allow comparisons with other experimental analyses, found in literature. Subsequently, in order to try to predict, on the basis of a learning sample, the effects of future treatments on plants behavior, some exploratory data analysis methods were considered as well as supervised and unsupervised classification methods (i.e. classification and regression trees). In other words, following a data mining approach, some non parametric models able to explain the relations among experimental results under different treatments and meteorological data were derived. *P. lentiscus* demonstrated to be the most suitable species for revegetation and phytoremediation applications, for its resistance to heavy metals and for the ability to produce high biomass. The effect of amendments was in general that of reducing metals bioavailability and the translocation coefficient, so establishing a phytostabilization process.

Keywords: amendments, data mining, heavy metals, mining areas, phytoremediation, phytorestoration, Sardinia, supervised and unsupervised classification.
Acknowledgement – The research has been financed by the Italian Ministry of the Environment in the framework of the "Pilot Project on Desertification control in the five Italian Regions at higher risk – Sardinia".
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Concept of Plant Functional Types: applications in phytoremediation and revegetation of polluted environments

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Plant Functional Types (PFTs) are groups of plants that respond comparably to environmental changes. These plants are ecologically, rather than taxonomically, related and can be diagnosed based on their role in ecosystem processes and way of adaptation. Application of this concept in phytoremediation and ecological restoration of polluted environments has not been explored. In this research, plant groups, which are mostly used for phytoremediation purposes, have been extracted from literature and were classified taxonomically. These groups have been searched for common functional traits which make them ideal for environmental cleaning purposes. Current environmental condition of polluted site and interaction between environmental parameters should be considered in functional trait analysis. Plants growing in polluted environments usually suffer from other factors like salt, heavy metal and drought stresses. Totally we found some critical and useful functional traits which should be considered in plant selection for phytoremediation and revegetation purposes including: 1-xeromorphy 2- C4 photosynthesis 3- salt tolerance (being halophyte) 4- affecting contaminants (transformation, degradation or accumulation) 5-symbiosis with remediator microbial communities 6- root system 7- root depth 8-life cycle (annual or perennial). Based on these traits, remarkable occurrence of plant families like Gramineae (grasses), Fabaceae (legumes) and Chenopodiaceae (Chenopods) in phytoremediation and restoration studies sound reasonable.

Keywords: Plant Functional Types, Phytoremediation, Revegetation, Pollution

CONCURRENT SESSION
NANOMATERIALS

Phytotoxicity and Accumulation of Nanoparticles in Agricultural Plants

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The manufacture and use of nanoparticles has increased dramatically in the past decade. The potential risks posed by nanoparticles (NP) to humans and the environment have been investigated only recently. Although there have been several studies evaluating nanoparticle exposure to humans, mammals, aquatic invertebrates, and microorganisms, there is little information about their effects on terrestrial and specifically agricultural plants. We have previously shown that standard phytotoxicity tests such as germination and root elongation are not sensitive enough or appropriate when evaluating nanoparticle toxicity to terrestrial plant species and that hydroponic exposures are preferred. Different *Cucurbita pepo* subspecies were exposed to elemental (Ag, Cu) nanoparticles, as well as corresponding bulk material controls, at concentrations of 100-500 mg/L in Hoagland's solution amended with 0 or 50 mg/L humic acid.

Plant biomass, transpiration, solution content, and particle accumulation were determined. The Ag aqueous ion concentration was 4.4-10-times greater in NP than bulk particle solutions. NP Ag (500 mg/L) reduced plant biomass and transpiration by 84% and 66%, respectively, as compared to the bulk Ag powder. The presence of humic acid moderated this particle sizedependent difference but was largely mediated through increased toxicity of the bulk Ag. The Cu aqueous ion concentration was 1.4-4.4-times greater in bulk than NP amended Hoagland's solutions. Humic acid (50 mg/L) decreased the aqueous ion content of the bulk Cu solution by 38-42% but increased the aqueous ion Cu content of NP containing solutions by 1.4-2.9 times.

Bulk and NP Cu at 500 and 100 mg/L were highly phytotoxic; growth was negligible and transpiration volume was reduced by 60-70% relative to untreated controls. NP Cu phytotoxicity was unaffected by solution type, but humic acid (50 mg/L) completely alleviated the reductions in plant biomass and transpiration volume caused by bulk Cu at 500 and 100 mg/L. The data clearly demonstrate differential toxicity of Ag NP relative to respective bulk particles. The finding that humic acid and solution chemistry differentially impact bulk and NP elemental (Cu and Ag) behavior highlights the importance of evaluating nanoparticle fate and effects under environmentally relevant conditions

Keywords- Nanoparticles, nanomaterials, phytotoxicity, Ag, Cu, Carbon nanotubes

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Nanoparticles and Plants – What is New

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The use of nanoparticles (NPs) in commercial products and industrial applications has increased greatly in recent years although understanding of the interaction mechanisms at the molecular level between NPs and biological systems, is largely lacking.
Nanoparticles are now being used in the manufacture of scratchproof eyeglasses, crackresistant paints, anti-graffiti coatings for walls, transparent sunscreens, stain-repellent fabrics, self-cleaning windows and ceramic coatings for solar cells. At the nanoscale, the properties of particles may change in unpredictable ways. Nanoparticles of titanium oxide used in sunscreens, for example, have the same chemical composition as the larger white titanium oxide particles used in conventional products for decades, but nanoscale titanium oxide is transparent. Antimony - tin oxide provides another example since nanoparticles of this oxide are incorporated into a coating to provide scratch- resistance and offer transparent protection from ultra-violet radiation, not seen with larger size particles.
Our work is focused to the study of effect of nanoparticles to the higher plant metabolisms, both in laboratory and real conditions to elucidate potential of phytoremediation methodology for removing NPs from environment. In laboratory conditions nanoparticles of TiO2, ZnO2, AlO2, Fullerenes and Graphite fibers were tested using *Nicotiana tobacco* cells BY2 as a model system.
The following experiments were performed with the aim of elucidation of effect of NPs to plant cells:
• Microscopic observation of cells after NPs stress
• Viability test
• Ethylene formation (ACC)
• Peroxidases formation
• Cytokinins level increasing/decreasing
• Metabolomic study
• DNA Arrays study
Generally, all nanoparticles decreases plant cells viability – the most toxic effect was found for ZnO2, where only 67% of starting viability was detected. All nanoparticles (with exception of graphite fibers) decreased the production of ethylene. Part of their unfavorable effects might be disturbance of defense pathways in tobacco cells, probably via disturbance of ion homeostasis. All nanoparticles exhibit negative effect on cell division and stimulate various stress responses, e.g. antioxidant system and ethylene formation, while microaray data confirm stimulation of antioxidant system as well as general stress response and down-regulation of genes related to cell division.

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Keywords: phytoremediation, nanoparticles, constructed wetlands, waste waters

Impact of Gold Nanoparticles Following Plant Uptake

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While research on the health effects of exposure to nanoparticles is growing, so too is the recognition that environmental exposures can cause problems. Many studies are being done looking at the impact of seedling germination, and potential impacts of overall plant health. However, little work is being done on a mechanistic level. In this study, we are looking at how the uptake of gold nanoparticles, considered to be 'inert' and therefore used in multiple health related applications, effects plant metabolism. Microarray analysis has shown multiple genes being impacted by uptake of nanoparticles, and additional studies have shown that these particles impact multiple metabolic pathways. Thus, although production and use of nanoparticles continues to grow, we are still a long way from understanding how these particles will ultimately impact our environment.

Keywords: nanoparticle, synchrotron, electron microscopy, plant uptake

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Phenotypic and genotypic characterisation of two *Arabidopsis* mutants resistant to CdS nanoparticles

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Nanoparticles have become widely used materials because of their unique structural, optic, electromagnetic and reactive properties. CdS are utilised in miniaturised hardware and optics equipment, they are synthesised through a quite inexpensive wet-chemistry process, the bulk density is 4.82 g/cm3 and a single particle, also known as quantum-dot has a diameter of 5nm. The CdS small size make them particularly prone to enter human and plant cells, but their toxicity potential has not been assessed yet. In this study we screened the two mutagenised collections of *Arabidopsis thaliana* (L.) Heynh, Feldman and Soll-Johnson, to identify CdS and/or CdSO4 resistant plants. We recognised three individuals resistant to 80 mg/L of CdS and three resistant to 200µM of CdSO4 (both doses were lethal for the wild type), these plants were not the same. We focussed our study on two mutants from the Soll-Johnson collection resistant to CdS, named *Atnp01* and *Atnp0*2, because they showed the highest biomass production and flowering rate in the presence of the contaminant. We determined the integrity of the inserted Ac/Ds transposon by PCR and the number of transposon copies in each mutant with Southern Blot technique; identification of the transposon flanking regions was performed utilising the "genome walking" approach. We identified three putative clones for *Atnp01*, located on chromosome 2 and 3, and one in *Atnp02*, located on chromosome 3. So far we were able to determine that the sequences were highly homologous to putative functions related to chloroplasts and cell membrane transport.
The relevance of this discovery will be discussed in the context of devoloping a risk assessment procedure for nanoparticles based on model plants.

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

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PLENARY LECTURE

Biomass for Transport, Heat and Electricity: Scientific Challenges and JRC contribution

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According to several projections and a preliminary analysis of the Renewable Energy Action Plans presented by the member states to the European Commission, bioenergy in its several different forms is going to constitute the backbone of the renewable energy share for European countries.
Technological flexibility, diversification of resources and recovery of "unused" material make bioenergy a renewable source of energy potentially very attractive for the European union energy mix. Indeed, Bioenergy will play a crucial role in meeting the 20 % target for renewables by 2020 and GHG emissions reduction in the EU-27. Biomass is expected to contribute to around two-thirds of the renewable energy share in 2020 according to recent PRIMES projections (SEC(2009)503 final). About 236 Mtoe of biomass can be theoretically available in 2020 and 295 Mtoe by 2030 in the EU while the EREC EU Technology Roadmap estimates that biomass can contribute by 175.5 Mtoe to the primary energy supply, covering 12.7-13.9% of total final energy consumption.
In order to fully exploit such an interesting potential even beyond 2020, bioenergy R&D is currently focusing on three main challenges: - Availability: biomass is a low density RES needing large amounts of land, a commodity expecting to become more and more scarce in next decades because of the growing competition with settling food, feed and other RES. An appropriate assessment of the biomass resources, which takes into account various environmental constraints and competitive uses, is the key issue. The logistics related to high biomass volumes required (transport, storage requirements) are crucial for the operation of bioenergy plants.
- Feasibility: The cost competitiveness of bioenergy production technologies remains a key barrier in the further deployment of biomass as a profitable energy source. A fast move in the direction of demonstration projects at a relevant industrial scale, which are costly but crucial for improving and certifying technical performance and to achieve cost reduction is required.
- Sustainability: The overall sustainability of bioenergy production chains must be evaluated carefully also by means of complete life cycle analysis in order to verify their actual advantages in terms of GHG savings, fossil fuels substitution and other parameters. A special care has to be devoted to indirect and less immediate drawbacks of bioenergy production, like market shocks, side effects on water availability or indirect Land Use change.
The state of the art of these issues will be reviewed and the contribution of the JRC both in terms of active research and policy support will be highlighted.

Keywords: bioenergy, biofuels, land use change, biomass availability.

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CONCURRENT SESSION
BIOMASS 1 CARBON SEQUESTRATION

Potential of Different Plant Species of Natural Tropical Forest in Carbon Sequestration in India

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Terrestrial ecosystems like forests possess great potential to mitigate climatic changes mainly via carbon sequestration. Forests not only sustain their own carbon but also have the potential to absorb carbon from the atmosphere. In the present study the biomass densities and carbon stocks of natural tropical forest of Tadoba Andhari Tiger Reserve forest (TATR), Chandrapur, Maharashtra, India was estimated using standard carbon inventory methods. Present methodologies used to obtain carbon stock estimates for large forest areas are mostly based on forest inventory information as well as various factors, such as biomass equations, which transform diameter, height or volume data into biomass estimates. Among various plant species, *Tectona grandis* (teak) showed the highest tree diameter of 26cm and average height of 19.85m while the lowest tree diameter and height of 10.35cm and 8.25m was shown by *Ziziphus glaberrima,* respectively. In addition, the total above and belowground biomass of the trees in the natural forest of TATR was observed to be 178.09 Mg ha-1 and 46.30 Mg ha-1 respectively. The carbon stock accumulated in vegetation was found to be 112.19 MgC/ha. Carbon sequestration in trees depends not only on climatic conditions but also on their growth and age.

This study helps to understand the present status of carbon stored and sequestered in tropical dry forest ecosystem. Such study revealed that the natural forest have a potential for reduction of carbon pool from atmosphere. Although, more survey work on growth patterns and speciesspecific effects on above and belowground carbon storage are needed.

Keywords: Tropical forest; carbon sequestration, above ground biomass; below ground biomass; carbon stocks.

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Carbon Stock Evaluation From Forest Stands In NE Italy

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Gas emissions from anthropic activities are increasing atmospheric temperature and determine important consequences to the whole planet. Mitigation actions, therefore, are needed in order to reduce and absorb glasshouse gasses (particularly CO2), as stated in the Kyoto protocol. Soil is the major carbon sink in the earth, being global soil carbon sequestration over 2Gt /y.
In the present work, the objectives were to evaluate the potential carbon stock of different forest stands in NE Italy, in order to outline the relationships among litter typology, soil organic matter dynamics and actual carbon stock under different vegetation coverage. Five forest stands (spruce, scots pine, larch, swiss stone pine, hemloch) were selected in the Dolomites area (NE Italy). The humus forms were examined in the field and samples were carried to the lab for further analyses. Soil organic carbon (SOC) was determined, and humification parameters were calculated. Finally, the carbon stock for each soil wascalculated from the balance equation:

$$SOC = \sum_{n=1}^{k} (\mathrm{C} \times \rho \times \mathrm{T} \times (1 - \delta) \times 10)$$

The less developed humus forms, as the Dysmull at site H1 and the Hemimoder at site H3, presented the highest carbon storage capacity (168 t/y and 129 t/y, respectively), followed by Lithoamphimus at site H2 (123 t/y) and Eu-amphimus at site H5 (96 t/y), and by Oligomull at site H4 (86 t/y).
Organic horizons proved to recover 36% of the total carbon stocked along the soil profile, and this points to humus layers as a fundamental source in carbon stock evaluation. Positive correlation between elevation, humus type and carbon stock values was found. The increased humus mineralization rate, however, would transform the carbon sinks (forest stands) in carbon sources, therefore enhancing the global change.
Current uncertainty about key processes requires further investigations on this scenery.

Key words: SOC, forest stands, humus forms, carbon stock, global change.

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Soil Carbon Sequestration in Tropical Plantation Forestry: Effects of tree species and long-term intensive management

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Reforestation can effectively sequester carbon in plant biomass and soil organic matter, reducing atmospheric concentrations and improving soil quality. Tropical forest systems, in particular, can play a critical role due to high productivity, but rates of carbon sequestration vary widely. Understanding the processes controlling soil carbon dynamics will be critical to explaining the variance in sequestration rates, allowing improved management of these complex biological systems for renewable resources and climate change mitigation. In this study, we are analyzing soil carbon dynamics from reforestation of degraded pasture to coniferous and deciduous forest plantations in Brazil, elucidating relationships between current vegetation, landuse history, and soil properties.

Intensive plantation forestry is expanding rapidly in Brazil, though despite a significant land base, the long-term soil carbon dynamics have yet to be fully considered. First, we examine interactions between three common vegetation types and their effect on total soil carbon over time. This part of the study is based on a chronosequence at the Anhembi Biological Research Station in the state of Sao Paulo, Brazil and will test the hypothesis that grasses, conifers and deciduous tree species vary in their influence on total soil carbon and nitrogen. Secondly, we analyze the long-term effects of high intensity *Eucalyptus* plantations on total soil carbon. Based on the third sequential sampling in a twenty-year study, we will examine the effect of certain soil properties and past land-use on soil carbon dynamics from three hundred *Eucalyptus* plantations across three states in Brazil. We hypothesize that rates of soil carbon accumulation relate to previous land use and certain soil properties (clay content and mineralogy).

Forests are expected to sequester more soil carbon than pastures; however, accumulation rates vary with tree species, stand age, soil properties, and climate. A major challenge in quantifying soil carbon sequestration is whether changes occur in short-term, unstable pools, or humified, long-term pools. Additionally, new organic matter from trees can be offset by rapid losses from previous vegetation, showing no net change of total organic carbon, and must be elucidated with natural isotopic 13C abundance from C4 grasses and C3 trees. A greater understanding of these processes can inform climate change policy concerning the distribution of offsets for tropical reforestation projects, the role production species may play, and determine the long-term effects of plantation forestry on soil organic carbon pools across a broad geographic gradient.

Keywords: soil carbon sequestration, tropical reforestation, land-use change

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External benefit of phytoremediation: The marginal effect of potential CO_2 abatement on the price of biomass

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The purpose of this paper is to examine all of the potential benefits of phytoremediation as a sustainable remediation technology and to explore which policies would promote the efficient use of the technology. The analysis is based on a case study in the Campine region (Flanders, Belgium). The contamination of (agricultural) land in that region with Cd, Pb and Zn is so extensive (< 280 km²) that conventional remediation is not applicable. There is an obvious need for remediation and risk reduction alternatives in Europe which are environmentally sound and protective of human health. It is also now widely recognized that cleanup activities of hazardous waste sites may affect emissions of greenhouse gases. In addition, the European Renewable Energy Directive promotes an increase in renewable energy to 20% by 2020. Phytoremediation offers the opportunity, not only for the farmer, but also for society, to come up with an approach that efficiently uses agricultural land to address all of these problems. Specifically, using contaminated biomass for energy production may contribute to the reduction of carbon dioxide (CO2) emissions. Performing a Life Cycle Analysis (LCA), complementary to a Cost Benefit Analysis (CBA), we examined the energy and CO2 abatement potential of willow (*Salix* spp), energy maize (*Zea mays* L.), and rapeseed (*Brassica napus*). We analyzed whether subsidizing this biomass would be economically efficient. Our conclusions are based on current Flemish policy and several valuation techniques for CO2. The most efficient technology is the digestion of energy maize with combustion of the contaminated digestate. Short rotation coppice (SRC) of willow to replace coke-based heat comes second. Alternatively, SRC of willow can be used in a biomass combustion installation to produce heat and electricity. Pure plant oil (PPO) and biodiesel from rapeseed score rather low because of the need for fossil fuels as an input for fertilizer.
Current energy subsidies are already reflected in current crop prices. CO2 abatement potential cannot be counted again as this would be double counting. Instead of using current prices, we calculate the "true" price per ton of output and the price per GJ needed to internalize the CO2 benefit of €20 per ton. The results suggest that current subsidized prices are too high.
Implications for the phytoremediation technology are mixed. We find that the true prices are not high enough to support renewable energy whether contaminated or uncontaminated biomass is used. However, the analysis suggests that including CO2 benefits would not cause phytoremediation to lose its competitive advantage compared to conventional technologies.

Keywords: willow (*Salix* spp), energy maize (*Zea mays* L.), rapeseed (*Brassica napus*), sustainability, life cycle analysis, energy, policy

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Biodiesel production & CO_2 mitigation by microalgae

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SAIPEM

Biodiesel is usually produced from oleaginous crops, such as rapeseed, soybean, sunflower and palm but the production in large scale deals also with the problem of sustainability. An alternative is offered by the use of microalgae that can be a suitable alternative feedstock for next generation biofuels thanks to their fast growth rate, no need of agricultural land and potable water for their cultivation, high oil productivity (compared with other vegetable crops) which could be extracted, processed and refined into transportation fuels, using currently available technology and possibility of secondary products energy conversion (proteins and carbohydrates to methanol/ethanol).

SAIPEM, with collaboration of University of Urbino, develop a research sudy to evaluate the possibility of using unicellular algae to bind CO_2 (reducing emissions) and to produce biomass suited to be converted into biodiesel. The purpose of this experimental phase is to select microalgae that are optimal from the point of view of both the production of lipids (biodiesel) and the possibility of growth with different nutrients and physical conditions on a seawater type of substrate in modular vertical photobioreactors with a view to industrial development.

Between 2008-2010 SAIPEM was involved in ENI R&M R&D project finalized to build in GelaRefinery an experimental plant for biofixation of carbon dioxide producing microalge to use in biodiesel production.

Microalgae are microscopic plants that typically grow suspended in water and carry out the same photosynthesis process as higher plants: the conversion of water, CO_2 and sunlight into O_2 and biomass.

CONCURRENT SESSION
POPS 1 PESTICIDES

Feasibility of Treatment of CPDC An-Shun Site Contaminated by Dioxin through Phytoremediation in Taiwan

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China Petrochemical Development Corp (CPDC) An-Shun Site is located in Tainan, Taiwan. In 2000, the site was found seriously polluted by dioxin in the soil and sediment of ponds and creeks due to improper waste treatment and illegal waste sludge dumping and burying. The contaminated area is about 38 ha. According to the surveying results, the range of dioxin concentrations was from 260 to 97,900 pg-TEQ/g with an average of 57,000 pg-TEQ/g, while the dioxin concentration in the fish bodies caught from the seawater storing pond on the site was analyzed as high as 247 pg WHO-TEQ/g, which was over the guideline of 4 pg WHO-TEQ/g set by WHO. In 2004, the EPA of Taiwan declared that this site was listed as a "Soil Pollution Remediation Site" because both dioxin and Hg exceeded the Soil Pollutant Control Standards so much. In the first stage of treatment, CPDC planned to use thermal remediation techniques, such as thermal desorption and incineration, to decrease the level of dioxin in the soils down to 1000 pg-TEQ/g. After that, combining bioremediation and phytoremediation techniques would be preferred. The purpose of this study is to investigate the feasibility of applying ecotechnology of phytoremediation in the second stage of treatment for the An-Shun Site. Although there are no data reporting the ability of plants to transform dioxins in the literature, some pure cultures of microorganisms are found able to biodegrade dioxin. These researchers further indicated that the biological degradation of dioxin is conducted by the joint action of aerobic and anaerobic microorganisms. Anaerobes, *Dehalococcoides* sp. Strain CBDB1, carry out reductive dechlorination of dioxins leading to the formation of *p*-dioxins. Then, the *p*-dioxins undergo enzymatic transformation (benzene ring split) by the aerobes *Sphingomonas* sp. RWI with participation of dioxygenases and hydrolases. The National SunYat-sen University research team has found that the bacterial species of *Pseudomonas* sp. isoloated and purified from the contaminated soil sampled from the An-Shun Site also can biodegrade the dioxin. In addition, there are still few phytoremediation studies on dioxin-like compounds (furan) in lab scale. It has been found that the plant species of *Cucurbitaceae* presented significant adsorption effects on PCDD/Fs, which might be due to phytoextraction. Thus, combining rhizosphere microorganisms and plants, rhizoremediation might be the optimal mechanisms for phytoremediation applying to the An-Shun Site contaminated by dioxin.

Keywords: dioxin contaminated site, phytoremediation, rhizoremediation

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Obsolete Pesticides and Phytoremediation of Polluted Soil in Kazakhstan

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Acute necessity in phytoremediation technology occurred because Kazakhstan was granted with numerous sources of obsolete pesticides as an ecological heritage from dissolved USSR.

Resulting from two years of work (2005 and 2006 years) was identification of chemical substances stored in 76 former storehouses of pesticides. Results of research have shown that soil around storehouses polluted by POP's in particular metabolite DDT and isomers HCH where their concentration exceed MAC (maximum concentration limit) in tens - hundreds times. In two areas (Almaty and Akmola areas) of Kazakhstan, a total of 388.6 tones of obsolete pesticides and unidentified stockpile material were observed, along with 513 pesticide containers. These data demonstrate the potential ecological danger and health risk posed by the former pesticide storehouses, especially those located near populated areas. Resolution of this risk will require elimination of obsolete pesticide stockpiles and pesticide containers, including locations where pesticides have been buried. Further priorities include remediation of soil polluted by organochlorine pesticides. Screening pesticide polluted sites will provide a basis for development of an action plan to prevent or minimize ecological risk from pesticide pollution in Kazakhstan. Results of inventories and inspection of former pesticide storehouses provide an additional source of data for official inventory of obsolete pesticide stocks and for development and conduction of public and state programs and projects on preservation of the environment and maintenance of ecological safety.

Methodological basis of assessing of soil pollution condition by obsolete pesticides on the territories of former storehouses of chemical means of plant protection was developed from 2002 to 2006 years. Plant community structure has been documented at the hot points and plant species was identified that grow in pesticide-contaminated soil near the center of the sites. The type of vegetation was characteristic of early successional plant species dominated by annuals and biannuals. Many of the species would typically be considered weeds. Can plant species that naturally colonize abandoned storehouse sites play a role in restoration and recovery of these sites? So, selected plant species were grown in pesticide-contaminated soil in containers in a greenhouse, in small field plots, and at a pesticide contaminated site at a former storehouse. By tracking pesticide residuals soil and plant tissue, issues that will impact development of phytotechnology applications were identified. We investigated cultivation methods to enhance plant uptake of pesticides. Use of mineral fertilizers resulted in stimulation of growth and biomass accumulation that increased phytoextraction. The concentration of DDT metabolites and isomers of HCH in soil and the application of fertilizers lengthened the rate of phenological development increasing plant height and biomass. In a greenhouse experiment using fertilizer applications to pesticide-contaminated soil, tolerant species showed increased phytoextraction of pesticides and some species, for example *Xantium strumarium*, decreased pesticide concentration of rhizosphere soil 11-24 % more in treatments with fertilizer compared to treatments without fertilizer. Field experiments using selected wild species demonstrated reduction of pesticide concentrations in soil in excess of reductions observed without plants and without fertilizers.

Additional work is needed to determine if practically useful phytotechnology applications can effectively manage pesticide-contaminated soil at former storehouse

Keywords: obsolete pesticides, phytoremediation, metabolites, dichlorodiphenyltrichloroethane, isomers, hexachlorocyclohexane

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Phytoremediation of Soils Contaminated Persistent Organic Pollutants

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Persistent organic pollutants (POPs) are of great environmental concern because of their toxicity, global distribution, and resistance to remediation. Most plants have proven ineffective for remediating weathered POPs. However, work at CAES has discovered that *Cucurbita pepo* (zucchini, pumpkin) roots phytoextract significant amounts of contaminant from soil, followed by effective translocation to aerial tissues. The pollutants shown to be accumulated by *C. pepo* include DDT/DDE, chlordane, and PCBs, although the extracted amount is dependent both on the pollutant characteristics and plant genotype. The stem-to-soil bioconcentration factors (dry weight ratio of contaminant in the stems to that in soil) for DDE approach 15, with up to 5% contaminant extraction in a single growing season. Recent research at CAES has focused on two lines of investigation; laboratory studies intended to elucidate the physiological mechanisms governing this unique ability and field studies to explore/optimize the potential of this approach. A range of laboratory-based hydroponic and rhizotron studies with plants in the *Cucurbita* genera have implicated unique *ex planta* root exudation patterns that result in the accumulation of hydrophobic residues in root tissue, as well as an unknown transport system that permits contaminant entry into the xylem stream. Current mechanistic studies with *C. pepo* are focused on isolating, characterizing, and potentially transferring the unique transporter system to a more amenable (non-food crop) plant system. Field investigations have looked at the impact of planting density, intercropping, and soil amendments on the accumulation of a range of POPs from contaminated soil. An overview of these results, with an emphasis on the strategies maximizing remedial success, will be presented.

Keywords- Persistent organic pollutants, phytoextraction, bioaccumulation, zucchini

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Use of Plant Matter in Green Remediation of Pesticides in Soil at Large Scale

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Extended application of organochlorine pesticides (OCPs) such as Toxaphene, Dieldrin, and DDT has resulted in long-term soil impacts at many agricultural sites. Thus, land that is being converted from agricultural to residential use often requires rigorous soil treatment. Typically, such soils would be excavated and sent to an appropriate landfill ("dig-and-dump"). However, in many cases the treatment of such environmental problems can be realized through the use of plants which mitigate the contaminants without a need for excavation and disposal elsewhere. Affected soils can be treated on-site, at lower cost, and at reduced levels of greenhouse gas generation.

The technology described herein consists of adding an organic soil amendment composed of biodegradable carbon in the form of processed plant fibres and zero-valent iron (ZVI). This amendment is mixed into the soil, typically using a rotary tiller, and water is added to the soil at ≈90% of its water holding capacity. The ZVI component oxidizes to form ferrous iron and releases electrons in the process. The organic carbon component is consumed by microorganisms indigenous to the soil, resulting in release of additional free electrons. These electrons are transferred to the OCPs, resulting in the removal of chlorine from the compound's structure (reductive dechlorination), and ultimately complete destruction of the pesticides. After a period of at least five days under reducing conditions, the soil is tilled again and allowed to dry to create aerobic conditions. This represents one 'treatment cycle' and each cycle decreases the concentrations further. The number of cycles required to treat a given soil depends mainly on the initial concentrations and the remedial standards required.

The process is very efficient from an ecological perspective, and is an innovative form of phytoremediation. The soil is treated on-site with minimal use of machinery, so compared to excavation and removal a significant amount of greenhouse gas generation is avoided. The ZVI component of the amendment is often sourced from recycled materials, thus helping to keep waste iron out of landfill. The organic fraction is often a by-product of the wheat-milling industry, again making effective use of potential waste materials. Finally, the process is biological in nature. It harnesses the power of native microorganisms, rather than relying on potentially harsh chemicals made in industrial processes.

A case study is presented, where the overall site was approximately over 200 acres in size and 34 acres were impacted with OCPs, primarily DDT, DDD and DDE. The full-scale implementation phase commenced in May 2007 and was completed in three months. Representative soil samples were taken after each cycle, on each acre, to determine if remedial standards had been met. Approximately 60% of the areas were treated to the remedial standards after one cycle, while the remainder required a second cycle. All impacted areas were treated to regulatory residential standards after completion of the second cycle. For areas that were completed in one cycle, average percentage removal for total pesticides ranged from 38% to 53%. For areas requiring two treatment cycles, average percentage removal was between 65% and 68%. Although original DDD levels did not exceed the standards, in one treatment cycle they were reduced by an average of 57%.

The guaranteed fixed price of this turn-key project was less than US$35,000 per acre (€68,000 per hectare), which is equivalent to less than US$21 per ton of soil treated (€18.21 per metric tonne) at the going exchange rate of 1 EUR = 1.27 USD. Compared to a theoretical "digand- dump" cost of US$80/ton (€69/metric tonne) of soil removed, the savings on the project would be over US$59/ton (€51/metric tonne). This approach resulted in savings of over US$3 million (€2.4 million) compared to the "dig-and-dump" alternative and resulted in significant environmental benefits such as reduced energy use and reduced greenhouse gas production, without the need for significant amounts of new material use or harsh chemical production.

Keywords: plant fibres; pesticides; soil remediation; landfill disposal; contaminated brownfield
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CONCURRENT SESSION
PLANT-MICROBE INTERACTIONS, INORGANICS

Influence of rhizobacteria isolated from a nickel–hyperaccumulating plant on metal mobility.

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Recent studies indicate that the efficiency of phytoremediation processes can be improved by taking into account plants and their associated microorganisms. Different rhizobacterial strains associated with two subspecies of the Ni-hyperaccumulator, *Alyssum serpyllifolium* (subsp. *lusitanicum* and *malacitanum*) were studied for their effects on soil metal bioavailability. Plants and rhizosphere soil were collected from three serpentinitic areas in the Iberian Peninsula: Sierra Bermeja (S Spain), Melide (NW Spain) and Tras-Os-Montes (NE Portugal); as well as from the calcareous region of Sierra Nevada (S Spain) where the non-hyperaccumulator *A. serpyllifolium* subsp. *serpyllifolium* grows. Three rhizobacterial strains were selected according to their phenotypic (production of organic acids, siderophores, biosurfactants, or indoleacetic acid and Ni resistance) or genotypic (BOX-PCR profiles) characteristics, and their ability to mobilise or immobilise Ni from ultramafic soils. These strains were identified by 16s rDNA sequencing as members of the genera *Arthrobacter* (strains SA5 and SA36) and *Bacillus* (strain SNA92). Sterile minimal culture medium containing one of three minerals (olivine, chlorite or biotite) or ground rock (collected from the serpentinitic region) were inoculated with each strain on its own or in combination. The concentration of Ni, Fe, K and P were determined in the supernatants of filtered cultures after 6 weeks incubation. It is proposed that microbial activity induces mineral weathering and the release of necessary micro- and macronutrients, and that the dissolution of Ni is an indirect result of this weathering process. Rhizobacterial inoculants capable of solubilising soil Ni from non-labile forms could be beneficial in phytoextraction techniques.

Keywords: nickel hyperaccumulator; rhizobacteria; clay minerals; weathering; Bioavailability

Selecting potential phytoextraction-improving bacteria through their ability for competitive colonization of Cd and Zn accumulating willows

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Willows *(Salix)* are known to accumulate zinc and cadmium from contaminated soils. They are particularly interesting plants for phytoextraction because they are fast growing trees, thus building up considerably larger biomass than many other heavy metal accumulators. To improve the applicability of *Salix* we search for suitable inoculant bacteria that increase the amount of extracted pollutants in the harvestable parts by influencing uptake rate and plant growth or by mobilization of the heavy metals in the soil. An important property of any microbial inoculant is the ability to colonize the plant in competition with other microorganisms which are present in a field soil environment.
In this work we inoculated *Salix caprea* plantlets with mixed inocula of approximately 25 strains, originating from a collection of rhizosphere and endophytic bacteria from *S. caprea* growing on a Cd and Zn contaminated site in Austria. After five weeks growth in a pot with contaminated soil, bacteria were reisolated on Zn containing nutrient medium and compared to the inoculants strains by restriction fragment length polymorphism (RFLP) of the intergenic 16S-23S rDNA region. Positive results were confirmed by 16S rDNA sequencing. We identified ten competitive colonizers among the 74 tested strains. These isolates belonged to *Beta-* and *Gammaproteobacteria* or to the *Bacteroidetes/Chlorobi* group. The reduction of the number of candidate bacteria allows detailed testing of the individual strains for beneficial effects on phytoextraction based on a reasonable selection criterion. One strain per plant will be inoculated to assess changes in uptake and biomass production. A test including all individual strains from our collection would not have been feasible.
The fact that only around 10 % of the inoculant strains could be recovered after some weeks shows that examining the competitive colonization ability is useful before starting detailed studies with a large number of bacteria on their beneficial effects on plants.

Keywords: Phytoextraction, *Salix caprea*, heavy metals, bacteria, colonization

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Effects of selected bacterial strains on cadmium and zinc accumulation and on shoot proteome of the metal hyperaccumulator *Arabidopsis halleri*

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Heavy metal hyperaccumulation depends not only on the plant, but also on the interaction between plant roots and rhizosphere microorganisms. Root proliferation and effective root uptake mechanisms are among the key processes in the rhizosphere that distinguish metal hyperaccumulators from normal plants. The high bacteria density in the rhizosphere is probably due to the high level of nutrients exuded from the plant roots and supporting bacterial growth. On the other hand, rhizosphere bacteria can promote plant growth by providing compounds or by facilitating nutrient uptake from the environment. In this context an ecotype of *Arabidopsis halleri*, harvested in a heavy metal contaminated soil, is under investigation. Plants grown in hydroponic culture with 1 mM CdSO4 and 10 mM ZnSO4 and with or without rhizosphere–derived microbes or with the addition of eight bacterial strains, identified among the rhyzosphere bacteria, and further selected *in vitro* for the ability to grow in the presence of high Cd and Zn concentrations, were compared for their ability to accumulate Cd and Zn and their shoot proteome was analyzed. Plant cultivated with the rhizosphere derived microbes accumulated much higher level of these metals in shoot than plants treated only with metals. Moreover, the presence of the selected eight bacterial strains strongly reduced shoot metal accumulation. Proteome analysis reveals a general upregulation of photosynthesis related genes counteracted by a decreased expression of defense genes in shoots of plants accumulating higher metal concentrations, indicating that an increased energy demand for metal transport and accumulation in the shoot is counteracted by thrift defense system and that metal accumulation provides a protection system. On the other hand, shoot proteome of plants treated with metals and the selected bacteria suggests that the low metal accumulation is accompanied by the activation of proteins that could have a protective function (for example, a germin-like protein). Furthermore, the transcription analysis of several genes surely involved in metal transport and homeostasis, but undetected in proteomic analysis, revealed an increased expression of these genes in plants accumulating high metal concentrations, whereas in plants treated with metals and the eight selected bacteria the transcription of these genes was similar to that of control plants. These results emphasize the effect of plant-microbe interaction in shoot metal accumulation and suggest the possibility of modulating shoot metal content by acting on soil rhizobacteria. The interaction of plant with each single bacteria strain with regard to shoot metal accumulation and gene expression is under investigation and will be discussed.

Keywords: A. halleri, cadmium, plant-microbe interaction, proteome.

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Identification of specific interactions between bacteria and heavy metal accumulating plants

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Salix caprea trees accumulate Zn and Cd in their leaves, produce high amounts of harvestable biomass and are suitable for phytoremediation of moderately contaminated soils. Plant-associated bacteria have been reported to contribute to heavy metal accumulation. The objectives of this work were the characterization of bacteria associated with Zn/Cd accumulating *Salix caprea*, the identification of strains promoting metal accumulation and an elucidation of the underlying interaction mechanisms. Rhizosphere bacteria and endophytes were isolated from *Salix caprea* on a former mining site in Austria. Most of the isolates were affiliated with *Proteobacteria, Actinobacteria* and *Bacteroidetes*. Selected strains were screened for production of 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD), indole-3-acetic acid (IAA), siderophores and novel metal mobilizing compounds, because these substances are thought to increase heavy metal uptake and tolerance in plants. IAA, ACCD and siderophores were detected in many isolates. Some *Actinobacteria* mobilized Zn and/or Cd in a soil leaching experiment with bacterial culture filtrates. Inoculation experiments with *Salix caprea* plantlets identified two strains enhancing metal uptake and one strain promoting plant growth in contaminated soil. One of the uptake promoting bacteria had mobilized Zn and Cd in the soil leaching experiment. All three beneficial isolates were members of the phylum *Actinobacteria* and unable of siderophore, ACCD or IAA production. Our results highlight the importance of novel interaction mechanisms between *Actinobacteria* and heavy metal accumulating *Salix caprea* trees.

Keywords: Heavy metals, Phytoextraction, *Salix caprea*, metal mobilization, ACCD, IAA, Siderophores

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Modified endophytes for improving phytoremediation of mixed contamination of toxic metals (Ni) and organic contaminnats (Toluene or TCE)

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Especially in case of large-scale contaminated areas, phytoremediation is considered to be a cost-effective and sustainable alternative for conventional remediation techniques since it works *in situ*, is solar powered and demands minimal site disturbance and maintenance. However, phytoremediation still has to deal with some important shortcomings like phytotoxicity, a limited contaminant uptake, and evapotranspiration of volatile organic contaminants. Plant-associated bacteria can be exploited to overcome these constraints. In case of phytoremediation of organic contaminants, endophytes equipped with the appropriate degradation pathway can diminish phytotoxicity and evapotranspiration. To increase metal bioavailability and root to shoot translocation and to decrease metal phytotoxicity during phytoremediation of toxic metals, plant-associated bacteria that are provided with a metal resistance/sequestration system and that are capable of producing siderophores and/or organic acids can be used. However, at most contaminated sites, plants and their associated microorganisms have to deal with mixed pollutions of organics and toxic metals. In a first step towards field scale application, we investigated if engineered endophytes that are capable of degrading organic contaminants, and deal with -or ideally improve- uptake and translocation of toxic metals, can improve phytoremediation of mixed organic-metal pollution. As a model system, yellow lupine was inoculated with a Ni-resistant, toluene- and TCE-degrading endophyte and plants were exposed to combinations of Ni and toluene as well as to combinations of Ni and TCE. To examine if the inoculated endophyte could improve phytoremediation efficiency, Ni, toluene and TCE phytotoxicity, Ni uptake and toluene or TCE evapotranspiration were investigated. Inoculation of plants exposed to Ni and toluene with the Ni-resistant toluene-degrading endophyte resulted in decreases of Ni and toluene phytotoxicity and of toluene evapotranspiration. When plants were exposed to Ni and TCE, inoculation lead to a reduced toxicity of Ni and TCE in the roots of the host plant, a slightly decreased TCE evapotranspiration and a strong increase in Ni uptake. From these results it can be concluded that endophytic bacteria possessing the right features can improve phytoremediation of mixed contaminations by decreasing metal phytotoxicity and increasing degradation of organic contaminants.

In a next step towards field application, yellow lupine, our model test plant, was replaced by poplar cuttings [*Populus deltoides x (trichocarpa x deltoides) cv. Grimminge*] since poplar is an excellent tree species for both biomass production and also phytoremediation purposes. Poplar cuttings were inoculated with the plant growth promoting, Ni-resistant, toluene- and TCE-degrading endophyte *Pseudomonas putida* W619 and phytoremediation efficiency was evaluated.

Keywords: mixed contamination, endophytes, Ni, toluene, TCE, yellow lupine, poplar

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CONCURRENT SESSION
BIOMASS 2 ENERGY

Phytoextraction of heavy metal contaminated agricultural land using energy crops

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Natural as well as anthropogenic immissions are causing increasing levels of heavy metals in parts of the German arable farm land. This leads to negative impacts on groundwater and to enrichment of heavy metals in food crops. To reduce the heavy metal concentrations in agriculturally used soils is therefore of great ecological, health and economical importance. Besides the mobilisation out of heavy metal containing parent rock, in particular in the German upland regions, a major natural immission source is the accumulation of polluted riverine sediments on flood plains. By agricultural land use heavy metals are added to soils as by-elements of fertilisers. If the increase of heavy metals will continue, some farm lands will be on risk to exceed official limit values. The result would be the forbidding of cultivation of food crops on these sites.

An alternative land use form for these sites is the cultivation of highly heavy metal accumulating energy crops. The following positive effects are possible by the combination of vegetational heavy metal extraction (phytoextraction) and energetic utilisation of the harvested remediation plants:

• decrease of heavy metal pollution in soils
• production and commercial exploitation of renewable energy sources
• contribution to substitution of fossil energy fuels

Extensive laboratory and field investigations have been carried out, to enable site specific recommendations about suitable plant species and the optimal cultivation techniques for agricultural areas with different heavy metal compositions.

On heavy metal polluted sites, the phytoextraction performance of selected plant species has been investigated. Plant species that have been tested in field experiments included rape (*Brassica napus*), sun flower (*Helianthus annuus*), corn (*Zea mays*), reed (*Phragmites australis*) and willow (*Salix spp.*). Besides the selection of plant species, the effects of cultivation techniques (e.g. fertilisation, irrigation, harvest time) on the phytoextraction rates have been determined.

Additional investigations were conducted to measure the effects of artificially induced stress (by application of herbicides) and application of mycorrhiza stimulating tonics on heavy metal uptake of the plants.

It was proved, that the choice of the plant species has the greatest effect on the efficiency of heavy metal extraction. Reed, which is suitable for flood plains and also for biological sewage sludge treatment due to its tolerance to waterlogging, and sun flowers took up the greatest annual heavy metal amounts.

Through fertisation of the remediation plants, the harvested biomass could be increased. Thus a higher extraction of cadmium, copper, nickel and zinc has been achieved. The application of mycorrhiza stimulating tonics and the induce of stress by application of an herbicide resulted in significant higher transfer of several heavy metal species. The highest extraction rate, that has been achieved in field tests amounts 9.7 kg/ha*a-1 for the sum of cadmium, chrome, copper, nickel, lead and zinc. The harvested biomass was utilised for energy production in a biogas plant.

It could be demonstrated, that the heavy metal concentrations in agricultural soils can be lowered by passive biological phytoremediation and that it is possible to utilise the harvest energetically. The investigation results allow for site specific recommendations about the
appropriate species of remediation plant, its optimal cultivation and the expectable rates of heavy metal extraction. The choice of the most efficient form of energetically utilization (biogas, solid fuel) depends on the properties of the harvested biomass (heat value, energy content, moisture).

Sustainable management of metal contaminated soils in the Campine region using short rotation coppice

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In the 19th and 20th century (up to 1970s), historic atmospheric deposition of trace metals from metal refinery activities has caused elevated concentrations in agricultural soils in the Campine region. The soils in the region are characterized by a sandy texture and relatively low pH. This entails an enhanced risk for uptake of these metals in crops or leaching to the groundwater.
While the soils should be remediated, there is a need to preserve the income of the affected farmers. Both goals might be achieved using phytoremediation in combination with the growth of energy crops. Short rotation coppice uses high biomass producing woody species, such as poplar and willow, which are harvested in a cyclic period of maximum 8 years. To study the potential and constraints, 4 ha were planted with various poplar and willow clones on a moderately contaminated soil (5 mg Cd kg−1 soil and 220 mg Zn kg−1on average). The commercial willow clones were Tora, Inger, Loden, and Belgisch Rood and the poplar clones were Muur, Oudenberg, Grimminge, Vesten and Koster. In a separate screening plot the performance of 8 willow clones (Tora, Inger, Loden, Belgisch Rood, Christina, Jorr, Zwarte Driebast and Belders) during 4 years of growth were investigated. The results of the screening plot showed that the highest productivity results were obtained between 3-4 years of growth.
Biomass productivity of commercialized clones varied between 2-7 ton DM ha-1 year-1. Some of the experimental clones however achieved productivity levels in the order of 10 ton DM ha-1 year-1. The metal concentration measured in the different plant parts did not vary significantly over the different years. Highest concentrations were always found in the leaves and the lowest levels of metal concentration were observed in the wood. Up to 15% of the extracted metals was accumulated in the leaves. Harvesting before leaf fall may therefore allow to decrease the remediation period by 10-30%, depending on the frequency of removal and the choice of clones.
The capacity of metal uptake depends, amongst other factors, on the genetic background of the cultivar. Appropriate selection will therefore be important both in optimizing the productivity levels and the efficiency of the metal extraction. More aspects related to regrowth, root growth, economic viability (sustainable income being a prerequisite for implementation as a remediating crop), but also related to environmental risks, metal behavior and balances during subsequent processing of the biomass is needed to fully apprehend the feasibility of short rotation coppice as a sustainable phytoremediation approach.

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Keywords: willow (*Salix* spp.), Poplar (*Populus* spp.), Short rotation coppice, metal distribution, phytoremediation, soil

Sodium and Chloride Uptake of *Populus* Energy Crops During Early Establishment

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Over the last decade, we have been testing the response of *Populus* energy crops to elevated levels of sodium (Na) and chloride (Cl) in an effort to select favorable genotypes with increased establishment potential. Initial phyto-recurrent selection cycles were conducted under controlled conditions, with later cycles consisting of field testing. The current study consists of further greenhouse experimentation to refine previous Na and Cl thresholds from wastewater irrigation sources such as landfill leachate. Our objectives are to: 1) test the variation in Na and Cl uptake among five *Populus* clones known to be salt tolerant, 2) identify Na and Cl thresholds suitable for early establishment, and 3) test whether electrical conductivity (Ec) can be used as an early stress indicator, given its previous correlation with stomatal conductance. Beginning in mid-May 2010, the trees will be grown in a split plot design with 6 blocks (replications), 6 irrigation treatments (1 unfertilized well water control and 5 salt treatments at 600, 900, 1200, 1500, and 1800 mg Cl L-1), and 5 clones (NC14018, NC14104, DN5, NM2, NM6). Trees will be irrigated with the well water control until 10 days after planting (DAP), then irrigated three times weekly for the remaining 50 days. Sodium and Cl concentration and Ec of the irrigation treatments and leachate will be measured after each irrigation event. Soil Na and Cl concentrations will be determined at planting and 60 DAP. Tree height and diameter will be measured weekly. Stomatal conductance will be measured at 10, 33, and 56 DAP. At 60 DAP all trees will be harvested and separated into roots, cutting, stems, and leaves, then Na and Cl concentration and dry mass of each tissue determined. The results and practical implications will be presented in Parma.

Keywords: hybrid poplar, osmotic effects, *Populus deltoides*, *P. nigra*, *P. maximowiczii*, *P. trichocarpa*, salts

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Assessment of the tree species willow, poplar and birch for biomass production on TE contaminated land (BCL).

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Biomass production with the primary goal of producing energy or biochar on fertile agricultural land is of questionable economic and environmental benefit and displaces agricultural crops thus raising the price of food. In this study we investigated the possibility of producing Biomass on Contaminated Land (BCL). BCL could improve both the economic and environmental outlook of bioenergy, as most contaminated land does not produce a positive economic return. In a screening program we collected soil samples and plant tissues (leaves, wood and bark) of adult willow (*Salix sp.*), poplar (*Populus sp.*), birch and (*Betula pendula*) species from five contaminated sites, in Northern France and in South Germany, and analyzed them for Zn, Cd, Pb Cu, Ca, and K. We investigated these particular species, as *Salix sp.* And *Populus sp.* are already used for biomass production and *Betula pendula* is a pioneer plant, characterized by rapid growth and low demands on the soil it is growing on. In general, crops used for BCL should display a low trace element (TE) leaf and wood uptake and reduce contaminant fluxes by minimizing wind and water erosion and leaching. Zinc and Cu leaf uptake was similar among the three tree species. For Pb no particular pattern could be seen. In the case of Cd however, *Betula pendula* revealed significantly lower leaf concentrations than *Salix sp.* and *Populus sp.*, thus posing the lowest risk for TE contamination of the surrounding areas through water and wind dispersion of foliage. Trace elements have also a direct influence on the energy production procedure as they can lower the ash melting point reducing the plant lifetime by sintering or slag formation. Among wood and bark, bark displays the highest TE concentrations, thus besides low uptake, small bark proportion in relation to the whole trunk is desirable. *Betula pendula* displayed the lowest Ca, K, Zn and Pb uptake by bark. The bark proportion reduced with the age and size of the trees, with the exception of *B. pendula* on the area of South Germany, where it increased. The TE uptake, which is seen as a drawback for the production of biofuels, could be used as an advantage, when the production of biochar is aimed, as TE increase the char yield in the gasification process.

Keywords: biomass production, trace element, poplar, birch, willow

Carbon Management of Palm Oil Projects in Central Africa through Life Cycle Assessment (LCA)

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The European Commission (EC) identified biofuel as a tool to cut greenhouse gas (GHG) emissions of transport and electricity generation: given the vast range and the abundant yield of the crop, palm oil is one of the most promising sources.
The EC DIRECTIVE 2009/28 on the promotion of the use of energy from renewable sources aims to ensure that only sustainable biofuels, which generate a clear and net GHG saving and have no negative impact on biodiversity and land use, will be used to achieve emission reduction target.
The feasibility of projects for cultivating oil palms (Elaeis guineensis) within the central African states, both for socio-economic development of the local populations and for bioenergetic production lines on a broader scale, has been assessed and environmental sustainability resulted as a critical issue. Every phase of the palm oil production process results in the emissions of GHGs that need to be quantified and minimized. These include the soil CO2 balance, the production of methane during the extraction phase and the shipment from producing countries.
Life cycle Assessment (LCA) is a methodology that is already widely used both at a scientific as well as a managerial level to evaluate the environmental performances of products and systems.
LCA was choosen as a tool for environmental accountability and instrument for supporting decision making, in order to compare the different project options. LCA findings have also been used to evaluate the sustainability of palm oil within the meaning of Directive 2009/28 and for screening the CDM (Clean Development Mechanism) projects that can be financed.

Keywords: palm oil, Elaeis guineensis, Life Cycle Assessment, LCA, biofuel, Carbon Management, CDM

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CONCURRENT SESSION
POPS 2 PCB

Research of wild plant species for applying in phytoremediation for the territories polluted with persistent pesticides

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The application of DDT has been prohibited throughout the world since 1970's, however, the studies carried out in Ukraine on the pesticides conduct to the human health, proved that accumulation of persistent organic pollutants in human tissues is related first of all, with DDT and its derivatives. The problem of soil pollution around pesticide warehouses (these are the sources of environmental contamination by DDT and its metabolites) nowadays is the sharpest problem in Ukraine. The phytoremediation is recognized in the world as ecologically safe and economical way to treat polluted areas. It is sought to be more promising to use the local species of plants in phytotechnologies that are adaptable to the specific climate and soil conditions. With the purpose to establish the wild plant species, which are resistant to persistent pesticides' influence and suitable for POP's extraction from the soil, the evaluation of the phytocenosis structure (as well as the soil sample contents of *4,4-DDT, 2,4-DDT, 4,4-DDD, 4,4-DDE* and persistent herbicides as prometryn, simazine, atrazine) have been carried out on the base of non-functioning pesticide warehouses in the Kiev region. It was established that the presence in the sampled soil of the high levels of DDT, its metabolites and other long-life herbicides, prevented the normal growth and development of plants in the researched areas, therefore, these factors caused the decline of sensitive species and the simplification of vegetation structure.

The phytocenosis of the researched area with 50m radius is represented by 54 wild species of plants: it is established that there are 7 species, among them, resistant to the high contents of herbicides (60-332,5 mkg/kg, with the phytotoxitcity koefficient is 10mkg/kg) in soil. Total content of DDT, its derivatives and metabolits exceeds the maximum acceptable levels (established in Ukraine at 100mkg/kg) in 1,5-63 times in the sampled soil. These species are: *Elytrigia repens* L., *Artemisia vulgaris* L., *Artemisia absinthium* L., *Achillea millefolium* L., *Calamagrostis epigeioes* (L.) Roth., *Taraxacum officinalis* Wigg. *and Erigeron canadensis* L.

The following laboratory studies of various wild plant species grown on soil polluted with persistent pesticides have been shown that *Taraxacum officinale* Wigg., *Xanthium strumarium* L., *Artemisia absinthium* L., *Artemisia vulgaris* L., *Erigeron canadensis* L., *Achillea millefolium* L., *Elytrigia repens* L., *Daucus carota* L., *Oenothera biennis* L. and *Calamagrostis epigeioes* (L.) Roth. have significant phytotechnologies application potential.

These species are able to accumulate large amounts of DDT and its metabolites, particularly in the root system (1016,3±6,6–3872,4±109,9mkg/kg for dry weight of plants with DDT content in soil at a level of 388,5±1,7–6377,0± 45,7mkg/kg), in the conditions of high soil phytotoxicity. Also they promote DDT content decreasing on 15,2–30,7% at plant rhizosphere soil in comparison with initial pollution level. Basing on the research results the algorithm and action plan of phytoremediation for the territories polluted with persistent pesticides.

Key words: DDT and its Derivatives, Phytoremediation, Wild plant species, Phytocenosis, Prometryn, Simazine, Atrazine.

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Phytoextraction of Polychlorinated Biphenyls (PCBs): The potential for ecological risk.

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Studies have shown that certain plant species can be used to phytoextract polychlorinated biphenyls (PCBs) from contaminated soils. Benefits of this technology include lower costs than those associated with traditional remediation technologies, and the ability to preserve the soil matrix. However, as plants are repeatedly grown in contaminated industrial soils, the soil itself becomes enriched and capable of sustaining a variety of plant and invertebrate species. As the soil becomes more nutrient rich and soil invertebrates begin to migrate into previously uninhabited areas, there is a potential for increased ecological risk.
Preliminary field studies carried out in 2009 at two historically contaminated sites (site contaminated with Aroclors 1254/1260 and site 2 contaminated with Aroclor 1248), indicated that invertebrate populations of Lumbricus terrestris (earthworm) and Succinea putris (snail) were on average three times higher than the soil or compost in which they were found living. In 2010, soils collected from both sites were used in controlled greenhouse experiments to determine the PCB uptake in both a known PCB phytoextractor (Cucurbita pepo – a pumpkin) and in L. terrestris . Results from these experiments are being applied in summer 2010 in which earthworms will be purposefully placed into soil on both sites to determine PCB uptake and calculate the potential for ecological risk and ecological risk management.

Keywords: soil, sediment, earthworms, snail, ecological risk

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Phytoremediation Experiment at a Former Pesticide Storehouse in Moldova

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The territory of Moldova republic is characterized by the great number of old pesticide storehouses with high POPs pollution level in the soil. The objectives of this experiment were to assess phytoremediation technology for the cleaning of POPs polluted soil and potential implementation to reduce risk in the Republic of Moldova. One site (Balceana) with a high risk level and appropriate agriculture conditions was chosen for a phytoremediation case study under field conditions. Several cultivated plants were evaluated for determination of pesticide extraction efficiency: maize (Zea mays L.), zucchini (Cucurbita pepo L. var. pepo), pumpkin (C. pepo L. var. pepo), and carrot (Daucus carota L.), and sorghum (Sorghum bicolor L. Moench). POPs residuals in the different media were determined by gas GC Agilent 6890 equipped with a µECD detector using USEPA Method 8081A. The bioaccumulation factor (BAF) and total POPs extraction from soil were determined for the every sort of used plants. The overall conclusion is that Phytoremediation can be used for remediation of polluted sites; however, it needs to be designed based on local conditions.
The best phytoextraction efficiency was shown by zucchini and pumpkin. Time required for agricultural phyto-remediation might be long and require utilization of complex approaches that consider use of biotechnology as well as native and perennial plants to develop a successful strategy.

Keywords: soil, POPs, phytoremediation, gas chromatography,

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Risk Assessment and Site Closure Strategies for Phytoremediation Plots on Persistent Organic Pollutant (POP)-Contaminated Field Sites

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One specific type of phytoremediation, termed 'phytoextraction' uses plants to accumulate significant amounts of contaminants from soil and store them in the plant shoot, which can then be harvested and treated as contaminated soil. Although this process relies on traditional treatment methods, it leaves the soil on site intact and can reduce the volume of contaminated solids for transport and treatment when compared to the excavation of contaminated soil. In order for phytoextraction to function successfully, plants must be able to accumulate high concentrations of the contaminant in their shoots while simultaneously maintaining high biomass production. This is particularly difficult in the case of persistent organic pollutants (POPs) (e.g. PCBs, DDT, chlordane), however it has now been repeatedly demonstrated that certain plant species do have the ability to phytoextract these highly hydrophobic contaminants.

The effect that phytoextraction might have on the bioavailability of POPs to nontarget organism living in the soil is not yet understood. Existing research in this area is conflicting, and suggests that bioavailability must be assessed on a site-specific and species-specific basis. We have collected soil invertebrates from two field plots in southern Ontario, Canada and used them to develop ecological and human health risk assessments. The information gained is critical for developing realistic protocols for carrying out phytoextraction remediation work at POPs-contaminated field sites. In a corollary study, we are developing a 'finishing step' after the phytoextraction on site is complete to decrease the bioavailability of residual POPs that are inaccessible to our target plant species. This work involves the use of both granular activated carbon (GAC) and biochar.

Keywords: persistent organic pollutants (POPs), polychlorinated biphenyls (PCBs), field sites,
risk assessment, biochar

CONCURRENT SESSION
PLANT-MICROBE INTERACTIONS, ORGANICS

Hydrocarbon degradation and plant colonization by selected bacterial strains isolated from Italian ryegrass and birdsfoot trefoil

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The objective of this study was to assess the degradation potential and plant colonization capacity of four alkane degrading strains (ITSI10, ITRI15, ITRH76, and BTRH79) in combination with birdsfoot trefoil and Italian ryegrass and to evaluate the diversity of indigenous alkane-degrading soil bacteria in the rhizo- and endosphere. Contaminated soil was prepared by spiking agricultural soil with 10 g diesel fuel kg-1 soil. Italian ryegrass (*Lolium multiflorum* var. Taurus) and birdsfoot trefoil (*Lotus corniculatus* var. Leo) were inoculated with four alkane degrading strains. Hydrocarbon degradation (up to 57 %) was observed in all inoculated treatments of vegetated and unvegetated samples. Italian ryegrass in combination with compost and BTRH79 showed highest degradation, while birdsfoot trefoil performed best with compost and strain ITSI10. Cultivation-based as well as cultivation-independent analysis showed that both strains were competitive colonizers. The combination between vegetation, inoculation with well performing degrading bacteria and compost amendment was an efficient approach to reduce hydrocarbon contamination. Two *Pantoea* sp. strains, ITSI10 and BTRH79, established well in the plant environment despite the presence of a variety of other, indigenous alkane-degrading bacteria**.** The present study suggests that the application of degrading bacterial strains, which are able to compete with the native microflora and to tightly associate with plants, are promising candidates to be used for phytoremediation applications.

Keywords: bioremediation, rhizosphere, degradation

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The inoculation method affects colonization and performance of bacterial inoculants strains in the phytoremediation of soil contaminated with diesel oil

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Plants in combination with their associated microflora can remediate soils that are contaminated with organic pollutants such as petroleum hydrocarbons. Inoculation of plants with efficient degrading bacteria is one approach to improve remediation processes. However, microbial inoculation is often not successful, in many cases the inoculant strain is not able to compete with the resident microflora. Consequently, it is of high importance to address the persistence and colonization behavior of inoculant strains. The objective of this study was to determine whether the inoculation method (seed imbibement and soil inoculation) influences colonization and plant growth promotion, hydrocarbon degradation and bacterial colonization. Italian ryegrass was grown in non-sterilized soil polluted with diesel and inoculated with the alkane degrading strains *Pantoea* sp. ITSI10, *Pantoea* sp. BTRH79 and *Pseudomonas* sp. MixRI75. Strains were labeled with the *gusA* gene in order to be able to follow their colonization behavior. Two strains (ITSI10 and BTRH79) showed ACC-deaminase activity. Bacterial survival and persistence in rhizosphere soil and plant interior were monitored. Inoculation generally had a beneficial effect on plant biomass production and hydrocarbon degradation, however, strains inoculated in soil performed much better than applied by seed imbibement. Performance correlated with the colonization efficiency of the inoculated strains. The highest hydrocarbon degradation (79%) was observed in the treatment, where all three strains were inoculated in combination into soil. Strains with ACC-deaminase activity performed better than the strain without ACC-deaminase activity. Our study revealed that besides the degradation potential and competitive ability of inoculant strains the inoculation method plays an important role in determining the success of microbial inoculation.

Key words: phytoremediation, inoculation method, colonization, hydrocarbons

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Vegetation and *In-planta* Phytoforensics Methods for Directional Analysis

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Advanced tree coring and *in-planta* sampling methods have been developed to allow for high spatial resolution detection of contaminated groundwater and soil. Trees have long been known to interact with groundwater, which has been exploited in numerous phytoremediation projects, due to their ability to extract groundwater and the associated contaminants via the evapo-transpiration stream. Now, we have developed specialized sampling techniques that use trees as a sampling point for contaminated groundwater. These methods are faster, cheaper, less invasive, and easier to implement than traditional well drilling. Single sites have been sampled, with up to 100 samples per site, in a single day with a team of 3 people using increment borers. These data were then analyzed within a week and the plume was mapped. Methods include tree coring with solid phase microextraction (SPME), *in-planta* solid phase samplers (SPSs), and *in-planta* SPME. SPME fibers include polydimethylsiloxane (PDMS) and composite PDMS/carboxen fibers. Recent work includes direct GC-MS analysis connected to the plants themselves. The use of the advanced methods have allowed the use of multiple samples in one individual tree to gain gradient information for the root zone. The directional analysis provides even greater spatial resolution, even when there are a limited number of trees at a site to be sampled.

This work summarizes the advantages and disadvantages of each method, such as the ability to perform repeat sampling, increased sensitivity over headspace analysis of tree cores, and cost of each method. The development of these methods will allow for improved implementation for plume delineation and monitoring.

Key Words: Phytoforensics, Chlorinated solvents, SPME, tree coring

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Bioaugmentation with engineered endophytic bacteria improves phytoremediation and contaminant fate in the field

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Phytoremediation of volatile organic contaminants is often unsatisfactory because plants and their rhizosphere do not completely degrade these compounds resulting in evapotranspiration through the leaves causing secondary contamination of the ambient air and by consequence undermining the merits of phytoremediation. Under laboratory conditions, endophytic bacteria equipped with the appropriate degradation pathway can be used to improve the *in planta* degradation of organic contaminants resulting in decreased phytotoxicity and evapotranspiration.
We report the first *in situ* inoculation of poplar trees growing on a TCE-contaminated site with the TCE degrading endophytic strain *Pseudomonas putida* W619. Under laboratory conditions, inoculation of poplar cuttings with *P. putida* W619 resulted in (a) a remarkably high plant growth promotion, (b) decreased activities of glutathione reductase in the roots, and superoxide dismutase in the roots and the leaves, and (c) a strong reduction in stomatal resistance. Three months after inoculation, the amount of TCE evapotranspiring through the leaves was compared for non-inoculated and inoculated poplar trees. To verify if the inoculation with *P. putida* W619 was successful, all cultivable bacteria associated with the poplar trees were isolated and characterized genotypically and phenotypically.
The *in situ* inoculation with strain *P. putida* W619 resulted in 90% reduction of TCE evapotranspiration under field conditions. This encouraging result was achieved after the successful establishment and enrichment of *P. putida* W619-TCE as a poplar root endophyte, and by further horizontal gene-transfer of TCE metabolic activity to members of the poplar's endogenous endophytic population. Since *P* W619-TCE was engineered via horizontal gene transfer, its deliberate release is not restricted under European GMO regulations.

Keywords: endophytes, phytoremediation, trichloroethylene, poplar

PLENARY SESSION

CONCURRENT SESSION
ALPINE BIOCLUSTER

Ecosystem services for human health in the alpine space – An initiative of the AlpsBioCluster

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In 190.912 sq.km. within the Alpine arc we find the territory of seven countries, 83 regions and about 6,200 communities. The Alps are home to 13 million people. Besides, the Alps have a unique combination of natural and cultural history, and have become a living space, an economic area and a recreational playground of eminent importance at the heart of Europe. To preserve the Alpine space as an overall region, specific models and concepts are just as important as individual measures.

Care has to be taken to preserve the Alpine region as an independent economic space to meet the needs of the resident population through sustainable development; to shape and manage the cultural landscape in order to preserve the Alpine region as a living space based on diversity; to permit a greater role to be played by the forces of natural development without anthropogenic interference, and to refrain from making unsustainable use of the Alpine space.

Novel Biotechnologies will aid to improve human health and maintain quality of life in the Alps. They are the most rapidly growing and changing markets in Europe. Be they green, grey, white or red, they are of paramount importance to keep our environment healthy and worth living for future generations.

Today it is ever so important to find new options to detect pollutants and threats, to reduce risks, and to develop novel bio-based technologies for safe food, clean water and sustainable energy supply. For the Alpine Space, where environmental issues have been a permanent worry, new methods and ways of cooperation have to be found to match these increasing requirements. On this background the Alps Bio Cluster with its main topics “autonomy and healthcare”, “new diagnosis and therapies” and “ecosystem services for human health” focuses on specific problems of the Alpine Space. To bring key players, experts and end users together, Alps BioCluster organizes a series of events, where new trends and methods in the biotech and medtech sector are presented and discussed. With view to future developments, it will be important to join expertise from the Alpine Region, to gather the latest developments helping to solve environmental and health related problems in the Alps. Besides reporting on the latest challenges, methods and solutions for the future, a group of experts should advise regional companies to strengthen their profiles and implement exchange between research experts, competitors, and partners.

The aim will be to work towards solutions for the Alpine region and their implementation. In an area that provides us with such a multitude of valuable ecosystem services, we need to take action.

CONCURRENT SESSION
POPS 3 ENERGETICS

Fate of the explosive TNT in Scots pine (*Pinus sylvestris*)

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Most of German former military sites are covered by woodlands which are dominated by conifers. Many of these areas are diffusively contaminated with explosives, mainly with TNT residues (2,4,6-trinitrotoluene). This causes our question, if coniferous trees may contribute to natural decontamination processes in explosive polluted soils (natural attenuation, dendroremediation). Therefore, three-years-old trees of sand cultivated Scots pine (*Pinus sylvestris*) were subjected to a seven-days pulse application with 45 mg l-1 of water solved 14[C]-TNT.
It was shown that Scots pines were able to reduce the content of 14[C]-TNT equivalents (TNTeq) in the planting substrate by transpiration driven tree uptake. Relative mass distribution showed that 96% of 14[C]-TNT equivalents (TNTeq) taken up by pine trees remained in roots reaching concentrations up to 308 mg TNTeq kg-1 root dry matter. Only a small percentage was transported to above-ground tree compartments, i.e. wood (3%) and needles (2%). Extractability of TNTeq was low in roots (max. 14%) but higher in wood (31%) and highest in needles (40%). Thus, the bulk of TNTeq was obviously deeply metabolised and non-extractable bound in root tissue. Only low amounts of metabolites were translocated to above-ground tree parts. Extensive TNT metabolisation was verified by radio TLC analysis indicating that residual extractable TNTeq portions contained neither TNT nor known metabolites (e.g. amino-dinitrotoluenes and diamino-nitrotoluenes), but very polar unknown compounds. Distribution analysis of TNT derived radiolabel in non-extracted residues (NER) revealed that among cell wall components (lignin, hemicelluloses, and cellulose) lignin is preferred as final TNT residue deposition compartment.
It is concluded that long living coniferous trees like Scots pine are not only fitted for sustainable dendroremediation of TNT contaminated soils, but all-year transpiration, low nutrition requirement and intrinsing TNT tolerance also qualifies conifers to be superior in TNT remediation compared to deciduous trees.

Keywords: phytoremediation, dendroremediation, explosives, 2,4,6-trinitrotoluene (TNT), conifer, *Pinus sylvestris*, Scots pine

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Engineering plants for *in situ* remediation of the explosive RDX

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The explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is both resistant to degradation and highly mobile through soils and ground water. Decades of its use in military weapons, andassociated production and decommissioning processes, have resulted in severe contamination of land and ground water. RDX is now a significant threat to drinking sources such as those close to the US Massachusetts Military Reservation. Currently, there are no cost effective processes tocontain RDX or remediate these vast areas of contaminated, vegetated land on military training ranges. While plants have an inherently low ability to degrade RDX, we have established that expression of a unique bacterial cytochrome P450, XplA and its partnering reductase, XplB in the model plant species Arabidopsis (*Arabidopsis thaliana*) enables these plants to remove and degrade saturating concentrations of RDX from soil leachate.

The object of this project is to engineer these genes into selected perennial wheatgrass species (*Pascopyrum smithii*, *Elymus trachycaulus*, *Agropyron fragile*) for the *in situ* phytoremediation of RDX from soil leachate on military training ranges. These perennial wheatgrasses are low growing, fire resistant and able to withstand and recover rapidly from disruption by heavy equipment; key properties for successful establishment on training ranges. To test the system in a monocot species, we are using the readily transformable species creeping bentgrass (*Agrostis stolonifera*). In contaminated soil experiments, XplA-expressing creeping bentgrass removed 40 % of the net RDX from soil, whereas there was no significant net removal of RDX by the untransformed control plants. We are now testing larger scale glasshouse-based systems to assess RDX uptake, characterizing transgenic wheatgrass species and developing designs for upcoming field trial designs.

Keywords: RDX, Arabidopsis, creeping bent grass, wheatgrass, ground water

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Developing better adaptable plants to phytoremediate contaminates.

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Plants used for phytoremediation should be able to establish rapidly and be adaptable to the lands on which they are grown to provide sufficient contact time for them to uptake and breakdown the contaminants. Previously we developed three new cultivars of grasses for use in a military environment that are more resilient to training activities.
Before our efforts, there was little or no research on the genetics or resiliency of lowmaintenance rangeland plants. Plant materials typically used for land restoration were developed where there is little anthropogenic traffic and emphasized forage production or other commercial productivity. In our plant-breeding research, we were able to improve traits related to establishment and persistence to military training activities in introduced and native species of rangeland plants, compared to existing commercially available cultivars. Three of the improved plants we developed for military lands are being used, after insertion of bacterial genes, to phytoremediated RDX (heahydro-1,3,5-trinitro-1, 3, 5-triazone) in soils. To carry the project forward, after the transgenic plants are developed, requires other factors to be addressed prior to widespread field demonstrations. These factors include: bio-containment strategies for pollen, field testing of planting methods for unprepared soils, design and construction of test site, and development of protocols on the use of the plants for RDX degradation. We propose to further develop an establishment technique for seeding these plants at sites where no human entry is generally permitted and the land cannot be prepared properly for planting.
To help establish plants we have concurrent projects looking at using seedballs to establish grasses in remote sites and we are talking to various military departments on securing a site to demonstrate our plants.

Keywords: phytoremediation, plant adaptability, plant establishment

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Phytoremediation Field Study for the Treatment of Explosive Compounds at Eglin Air Force Base, Florida USA

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The phytoremediation field study underway at EAFB is making progress toward increasing sustainability on military testing and training ranges. Bahiagrass Pensacola was planted in May 2009 and has become well established, producing considerable biomass in just six months, even after near drought conditions in June and July of 2009. The sampling protocol used in the study has provided considerable background contamination data for both planted and unplanted areas near the OB/OD. RDX and HMX are the major contaminants present at the site along with lower levels of TNT and TNT metabolites. The explosives RDX, HMX, and TNT were also detected in the creek immediately down-gradient from the OB/OD site, raising concerns for off-site migration.

Although a significant reduction in soil concentration and explosives detection did not occur between May and November of 2009, there is considerable time left in this field study to demonstrate the potential of phytoremediation at EAFB. Evidence showing the uptake and translocation of RDX and HMX into Bahiagrass Pensacola provides an indication that uptake is occurring. This is the first time uptake and translocation of RDX and HMX has been documented during a phytoremediation field study on military testing and training ranges.

Future monitoring of explosives and metabolites in soil and vegetation samples will provide the basis for recommendations regarding the implementation of phytoremediation as a viable treatment technique at EAFB.

PLENARY LECTURES

Phytoremediation Really Does Work in Elizabeth City, NC, USA.

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We report on performance verification of a 5-acre, mixed tree phytoremediation demonstration site that was planted in 2006 and 2007 in Elizabeth City, NC. The goal of this project is to use trees to decrease groundwater recharge, flow, and mixed-fuel contaminant concentrations in a shallow, water-table aquifer that discharges to the Pasquotank River. The site was planted with 2,700 trees in 2007 and 300 in 2006. Trees species include: hybrid poplars (*Populus spp*.; 94%); willow (*Salix sp*.; 5%); and loblolly pine (*Pinus taeda;* 1%). The assessment of site performance involves monitoring individual tree mortality or growth, concentrations of fuel constituents in groundwater, mass amounts of volatile fuel constituents in soil gas wells, and hydrologic flux before and after installation of trees. Soil gas analyses show a 76% reduction of total petroleum hydrocarbons (TPH) mass at the site from February 2007 to January 2010; groundwater concentrations of BTEX and MTBE are significantly lower than historical data prior to tree installation work. Spatial analysis of tree density mortality proved an effective tool to identify areas of the site that were more contaminated than historical ground water monitoring indicated.
Soil gas data correlate significantly with tree density mortality data. The rapid reduction of contaminant mass and concentrations in soil gas and groundwater were unexpected but a welcomed result for this demonstration site. Ongoing work will assess attenuation of residual petroleum constituents such as PAHs.

Keywords: TPH contamination, groundwater, soil gas, GIS.

20 Years of Poplar Phyto Performance On, Under and Around Landfills

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Keywords: Landfill, Leachate, Hydrology, Regulation, Economics, Green-house gases, biomass

Water is highly regulated on, around, and under landfills. Since 1990, the Salicaceae family of trees – including *Populus spp*. and *Salix spp*. – were essential in redefining water management along with treating soluble pollutants and methane on landfills.
Around a 'skeleton' of Salicaceae trees that develop the pioneering roots and biomass, other plants are established to eventually perform all closure functions. The plant dynamics affect the biogeochemistry of the cover and perimeter soils. By removing significant water from a soil cover, this 'sponge-and-pump' action reduces water percolation. By adding exudates, the plants stimulate microbial activity that oxidize landfill gases and remove soluble compounds in irrigated leachate when recirculated back onto a cover phyto cover.
This paper will review tree systems now planted at landfills that perform mandated functions including: water-restricting covers, leachate intercepting perimeter berms, irrigated covers with recirculated leachate, establishment of wildlife habitat, and improvement of neighborhood ambiance related real estate value increase. Many phyto-covered landfills are now viewed as potential parks and a future asset for the owner or community.
Performance data will be provided where available from 20 landfill installations. Economic data will be compiled to help understand the value of this phyto-type cover in comparison with geomembrane covers required for modern landfill closure and for old pre-regulated landfill rehabilitation.

CONCURRENT SESSION
BTEX, TPH

Detection of *in situ* biodegradation of benzene in a model constructed wetland using stable isotope analysis

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Groundwater contamination with aromatic hydrocarbons, e.g. benzene, is a significant concern to the environment. Increasingly, wetlands are employed as remediation strategy since they can serve as natural filter systems for polluted groundwater. Benzene can be removed in wetlands by biotic and abiotic processes, however, many studies focus on overall benzene removal efficiencies whereas information on whether microbial degradation occurs or not is scarce. The consideration of wetlands as a sustainable option for groundwater remediation requires further knowledge on the ongoing biodegradation processes. Hence, the aim of our study was to identify and quantify *in situ* benzene degradation processes taking place in a model wetland system. Benzene-polluted groundwater, originated from a contaminated field site (Leuna, Germany), was continuously supplied to a model constructed wetland, thus, simulating the transition of anoxic groundwater to surface-near water layers. The two-dimensional design of the system (201 x 60 x 5 cm) and its operation under constant temperature (10-12°C) allowed for investigations of biodegradation under controlled conditions. Applying two-dimensional compound specific isotope analysis, we clearly demonstrated that benzene was biodegraded predominantly under oxic conditions. Moreover, the data revealed that benzene was principally removed by microbes; abiotic removal processes (eg. volatilization of benzene) were of minor importance. Additionally, different functional genes coding for enzymes taking part in aerobic BTEX degradation pathways (e.g. monooxygenases) were detected in sediment samples of the wetland. The combined use of stable isotope analysis and molecular tools allowed a detailed description of the microbial contribution to benzene degradation in this model wetland.

Keywords: groundwater, benzene, constructed wetland, biodegradation, compound specific isotope analysis

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Implementing and Monitoring Rhizosphere Remediation in Remote Regions

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Treating contaminated soils in cold remote regions is difficult. The US DoD has investigated using phytoremediation to treat petroleum contaminated at a number of sites, including Alaska. The basis for this technology is to take advantage of the enhanced microbial activity near plant roots. Although mechanisms for remediation based on plant associations are still being investigated, there is convincing evidence that can be effective. Depending on the contaminant, the range of mechanisms includes plant uptake, degradation within the plant, volatilization through the plant, rhizosphere enhanced remediation and specific enrichment due to chemical analogs release by root as exudates. One of the major challenges with plant-associated treatment is monitoring the site for success or indications of progress. In more aggressive schemes, decreases in contaminant concentrations are used. However, plant based treatments are used to treat surface soils, and surface soils are notoriously heterogeneous with respect to contaminant distribution. We have conducted remediation tests at locations in Alaska and Korea and monitored both total petroleum hydrocarbons and specific fractions. Our results indicate a significant plant and plantnutrient effect on many of the fractions. Interestingly, we also observed a negative effect on specific hydrocarbon fractions when we used fertilizer alone.

Keywords – petroleum, rhizosphere, cold, PAH, monitor

Phytoremediation of Soils Contaminated by Heavy Oils with Plants Infected by Mycorrhizal Fungi

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Petroleum and its refined products spills might pollute soils during the processes of production, transportation, and storage operations. Comparing with other refined oil products, heavy oils are characterized of high heat resistance, high chemical stability, low water solubility, and low biodegradation rates. Thus, once soils are contaminated by heavy oils, it is usually time consuming and costly to clean them up. Phytoremediation is a phytotechnology as well as an environmental friendly ecotechnology, which uses plants or vegetation to remove hazardous chemicals in polluted soils or transform them to non- or less-hazardous ones. The advantages of phytoremediation include low costs, low energy consumption, small ecological disruption, and easy acceptance by the public. Such type of phytotechnology has been found performing well to treat gasoline and diesel contaminated soils. However, quite a few studies were conducted for heavy oils. Arbuscular mycorrhiza (AM) is an ubiquitous association between soil fungi and the roots of most herbaceous plant species that permits the host plant to exploit nutrients in the soil beyond the rhizosphere through fungal transport. Mycorrhizal colonization can result in quantitative and qualitative changes in root exudation and in the soil microbial community, particularly in the rhizosphere. The purpose of this study is to investigate efficiencies of phytoremediation for heavy oil contaminated soils by plants innoculated with mycorrhizal fungi (MF). In this study, three plant species, vetiver (*Vetiveria zizanioides*), big bidens (*Bidens pilosa*), Goose grass (*Eleusine indica*), and the arbuscular mycorrhiza fungi *Glomus mosseae* (Gm) were selected for the tests. The experimental results showed that vetiver and big bidens could survive under the total petroleum hydrocarbon (TPH) concentration of 100,000 mg/Kg in soils, and exhibited significant removal efficiencies (80%). In addition, the plants infected with Gm could enhance the degradation rates with 9% of TPH concentration of 30,000 mg/Kg in soils. It was concluded that AMF could be used in phytoremediation to enhance the treatment efficiencies for the soils contaminated by high concentration of heavy oil.

Keywords: Heavy oil, arbuscular mycorrhizal fungi, phytoremediation, TPH

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Phytoremediation potentials of cowpea (*vigina unguiculata*) and maize (*zea mays*) for hydrocarbon degradation in organic and inorganic manure-amended tropical typic paleustults

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A field study on phytoremediation of petroleum hydrocarbon contaminated soil, located at the University of Nigeria, Nsukka, was designed to assess the effects of organic manures (poultry droppings (PD) and cassava peels (CP)) and NPK fertilization on the potentials of cowpea (*Vigina unguiculata*) and maize (*Zea mays*) for simulating biodegradation of hydrocarbon in soil. Total hydrocarbon (THC) concentration, primary nutrient elements (N, K and P) levels and crop grain yield were used as indicators of soil productivity restoration. Cowpea and maize crops were established, using monocropping system, on the hydrocarbon contaminated soil that was treated with three rates (0, 4, and 8ton/ha) of soil amendments (PD, CP and NPK fertilizer), and arranged in treatment combination of 3 X3 X 3 factorial in Randomized Complete Block Design (RCBD). The treatments were replicated thrice for each crop. In the second year, half of the rates of the amendments were applied to the plots following the same pattern of the first year. Soil samples (0 – 20 cm) were collected from the plots at 1 and 3 months respectively after crop planting, while crop grain yield were harvested at maturity. The respective and combined effects of the soil amendments on N, K and P, and crop grain yield were determined in relation to hydrocarbon degradation over a two-year cropping season. Results showed that the soil amendments reduced the THC concentrations in the cowpea and maize plots. This reduction was significantly high in plots treated with the combined forms of the soil amendments. Comparison of the effects of the respective amendments on the THC concentration indicated that THC decreased in the order; NPK < PD < CP, at the cowpea plots, and PD < NPK < CP at the maize plots. The cowpea plots treated with 4 t/ha PD + 8 t/ha CP + 4 t/ha NPK fertilizer resulted to the least value of THC, while maize plots treated with 8 t/ha PD + 4 t/ha CP + 8 t/ha NPK fertilizer gave the least value of THC. Nitrogen, phosphorus and potassium levels showed that applying the soil amendments in a combined form enhanced the soil fertility status more than sole application of the amendments. Cowpea plots showed greater potential to stimulate hydrocarbon degradation at the early stage of the study (first year). However, THC concentrations at the cowpea and maize plots indicated no significant difference in the potentials of the crops to stimulate hydrocarbon degradation at the second cropping season. The grain yield of cowpea increased by 87 % at the second year, while maize was unable to grow maturity in the first year.
Keywords: Total hydrocarbon, soil amendments, cowpea, maize, primary nutrient elements, hydrocarbon degradation

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Two Potential herb species for phytoremediation of hydrocarbon contaminated soil in Oil field areas of Assam (India)

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Large areas of land containing elevated levels of hydrocarbons exist in century old oil fields, refinery sites etc. of Assam, India. Hydrocarbon contaminated soil lack in effective vegetation cover and are prone to erosion with a high risk of spreading of contaminants into adjacent areas. Experiments have been conducted to determine the efficacy of some native grass species viz. *Cyperus brevifolius* (Rottb.) Hassk, and *Cyperus rotundus* Linn, belonging to the family Cyperaceae for reducing hydrocarbon contamination and their effectiveness in possible large scale field trial. The sterilized soil was spiked with different percentages of crude oil and plantation was done in pots. Monthly monitoring of root zone soil and plant biomass was done to test changes in the total petroleum hydrocarbon (TPH) content of soil and its accumulation in various plant parts. Assessment of TPH was done with GCMS and TLC-FID. The plants showed effective results in reducing TPH and could sustain in crude oil concentration up to 12% (w/w) in laboratory as well as in field conditions. The soil microbial population showed increase with the decrease in TPH level as the root exudates control the quality and the quantity of microbial population and they exert strong influence on the general health of plants.
These plants are candidate species for effective phytoremediation in petroleum contaminated soil in existing ecological condition.

Keywords: Hydrocarbon contamination, native herb species, phytoremediation, root Exudates

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CONCURRENT SESSION
AIR

Air purification by trees and their impact in urban environment

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The impact of vegetation in urban air quality is receiving increasing attention for large-scale tree planting programs. Trees can provide many environmental benefits removing CO2 from the atmosphere during photosynthesis and capturing particulate air pollutants. Trees can also emit gases known as volatile organic compounds (VOCs) that can contribute to the production or reduction of ozone and particles, depending on the nitrogen oxide (NOx) level in the atmosphere. Since Nox concentration is especially high in polluted urban airsheds the profile and emission rates of these VOCs play an important role in urban air quality. The present study investigated the effectiveness in carbon sequestration and the leaf VOC emission capacity of widespread ornamental broad-leaf species by using a dynamic leaf enclosure system. In order to provide useful information for the suitability of these trees to improve air quality in urban environment by removing particulates, the leaf surface structures of these species were also characterized by scanning electron microscope analysis.

Keywords: urban forest, volatile organic compound, CO2, particulate

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Particulate matter deposition on tree and shrub leaves in urban green areas in Norway

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Air pollution has had increasing attention as affecting human health. The effects of particulate matter (PM) can be especially serious for people with heart diseases and respiratory problems. The amounts of PM deposition on vegetation of different species have yet not been well studied. However, the choice of efficient species and plant designs may greatly improve the air purifying ability of the vegetation along streets and in heavily trafficked cities. In a Polish – Norwegian project differences in responses of shrubs and trees are characterized in two contrasting climates. Testing and analysis were done on species of trees and shrubs widely used under the climatic conditions of the South Western coast of Norway. Leaves were collected in 2009, just before leaf fall at two sites near Stavanger (58° 47` N, 5° 41´ E). One site was in the city centre of Stavanger, at one of the most polluted cross roads. The other site was in the rural site of the research centre (Bioforsk West) representing a clean site. Leaves were analyzed for PM in three fractions of water and the same for chloroform (PM10, PM2.5 and PM0.2). Filters with PM were weighed (Mettler Toledo XP6) and leaf areas were measured (Li-COR LI-3000A), so that deposited matter could be related to the leaf area.
There were large differences between the two sites in amount of PM on the leaves. The results also show diverse amounts of PM on the leaves of different species tested. Also heavy metals and PAHs on and in the leaves were analyzed. Further results will be given in the presentation. The choice of species, and probably also the design and placement of plantings in the urban environments should be studied further in order to maximize the effects on air quality.

Acknowledgements: The project is funded by the Polish – Norwegian Research Fund.

Keywords: Air quality, Particulate matter deposition, Shrubs, Trees

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Air Phytoremediation – Experience from the Czech republic

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The aim of the project "Green technologies for air protection" is to suggest appropriate methodology for air quality improvement as well as to decrease contamination both environment and inhabitants by toxic metals and xenobiotics using selected plants which can trap and immobilized dust particles (PM10 and 2.5), toxic metals and organic xenobiotics in the areas of heavy traffic and industrial activities.
For such purposes we study the affectivity of dust particles sorption to the leaves of differentwoody plants, including evergreens. The achieved data are summarized in table 1

Czech/latin name	Dust amount [g]	Leaf area [dm2]	Dust amount on [µg/dm2]
Borovice černá (*Pinus nigra*)	0.08512	20.10	4233.86
Šeřík obecný (*Syringa vulgaris*)	0.05424	19.71	2752.25
Jilm habrolistý (*Ulmus minor*)	0.03557	18.73	1899.09
Růže šípková (*Rosa canina*)	0.02394	15.70	1524.80
Rdesno aubertovo (*Fallopia aubertii*)	0.02896	19.49	1486.21
Slivoň obecná (*Prunus insititia*)	0.02637	18.04	1461.43
Jabloň (*Malus*)	0.02542	20.61	1233.11
Bez černý (*Sambucus nigra*)	0.02448	20.45	1196.99
Lípa srdčitá (*Tilia cordata*)	0.02264	19.42	1165.75
Javor klen (*Acer pseudoplatanus*)	0.01846	19.56	943.57
Pámelník bílý (*Symphoricarpos albus*)	0.01365	15.29	893.01
Hrušeň obecná (*Pyrus communis*)	0.01306	16.31	800.53
Javor mléč (*Acer platanoides*)	0.01432	20.92	684.41
Bříza bělokorá (*Betula pendula*)	0.00995	17.52	567.78
Třešeň (*Prunus avium*)	0.01101	21.02	523.88
Topol černý (*Populus nigra*)	0.01064	20.56	517.40
Ořešák vlašský (*Juglans regia*)	0.01039	21.81	476.45
Svída krvavá (*Cornus sanguinea*)	0.00571	20.54	277.96

Similar data are available for PAH and toxic metals sorption on leaf area.
The selected plants, which offer both high sorption efficiency and were reasonable resistant to drought and high salt concentration, were planted around main roads in 3 municipalities in Prague West region.

Acknowledgment: this work was supported by SP/1b7/129/08 Ministry of Environment project.

Keywords: phytoremediation, air, dust particles, PAH, toxic metals

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Plants to improve air quality and reduce pollution in indoor environments

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Indoor air in cities has been reported to be more polluted than outdoor air. About 90% of our time is spent indoors where we are exposed to chemical, physical and biological contaminants. Deterioration of indoor air quality can result in "multiple chemical sensitivity", "new house syndrome" and "sick building syndrome" and other adverse physical symptoms for those exposed. Pollutants in indoor air come from the outside or are released directly by materials within buildings. In this work we summarize available information on the capacity of air decontamination by ornamental plants and briefly describe experimental methods used to characterize the pollutant uptake rate in the plants. Studies on indoor pollution, even though they have been often reviewed in recent years by the scientific community, still suffer from the lack of comparison among data and results obtained so far. We collected the published data from 1980 to 2010 in a database and summarized all the available international literature on the different indoor pollutants and on the use of the major ornamental species to improve air quality in closed environments. The database, INDOOR, consists of two files, a Reference File and a Plant File: the Reference File is a bibliographic file of published research papers; the Plant File contains information about plants and air pollutants (organic and inorganic) derived from the papers listed in the Reference File. The database includes about 90 species of ornamental plants able to eliminate toxic substances in our homes.

Keywords: indoor air pollution – ornamental plants – sick building – air quality - phytoremediation – removal capacity

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CONCURRENT SESSION COVERS

Phyto-Assisted Landfill Closure

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Phytotechnology has been used as a key component of a landfill closure at a site located in the western United States. The site is a former Class II landfill that received liquid waste (sludge) from development of a nearby energy resource. The wastes were placed in a series of ponds and allowed to evaporate during the hot dry summers. The area has a Mediterranean Climate with winter rainfall and long hot summers. The resulting consolidated sludge, which consists of a low pH – high boron content material, was buried on site. In the late 1980s, the landfill ceased operations when the operator went bankrupt. The sludge from several years of waste was left in the ponds and the ponds eventually filled with rainwater. . Under order from the state regulatory agency, a landfill closure plan was prepared that incorporated draining the ponds and encapsulating the waste in a closure cell. The governing regulations for landfill closures require a 5-foot separation distance between the groundwater and the waste. Portions of the closure cell did not meet this requirement. In order to address this issue several engineering controls were implemented including lowering the spillway in a nearby lake and planting about 30 acres (22,000 seedlings) of *Eucaluptus camaldulensis* around the closure cell to help lower the water table. The trees were planted over a 3-year period beginning in late 2005. Evidence of hydraulic control is required by 2011.

The effectiveness of the hydraulic control is being evaluated by quantifying evapotranspiration using sap flow technology on representative trees in each plantation and by monitoring the water levels in numerous piezometers installed in the plantations. Each piezometer is equipped with a pressure transducer, allowing for continuous monitoring of water level data. Since the site is in a remote location data are transmitted real-time from site instrumentation via cellular technology to a web server and made available through an internet connection.

Keywords: hydraulic control, eucalyptus, landfill closure

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Remediation of Cover Soil of the Solid Waste Landfill

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Waste disposal sites are one of the main urban problem area in a lot of City for developing countries. Environmental problems in this kind of area are; dispersion of wastes and pathogens to the surrounding area, direct touch/handling, unattractive smells, gases, leaking waters, stabilization of wastes and visual pollution. In order to minimize those negative effects there is an "upper surface covering" implication which helped to control waste dispersion and to minimize unattractive smell and as a resultupper surface gas concentration have been lessened in considerable amount. But the bare surfaced areas are still open to erosion without any vegetation on. It is necessary good soil features to planting of landfill. This is a study of the use of leachate water from solid waste landfill sites as irrigation for plant species which normally grow in the wild. The species used include; *Althea rosea*, *Cynodon dactylon*, *Inula viscosa, Melilotus officinalis* and *Thymbra spicata.* During the two years (2003-2004) of the study the plants were irrigated with tap water and leachate water under Mediterranean drought climate. After the experiment the features of the soil samples were analyzed and it was seen that using leachate water to cultivate different kinds of plants increased the percentage of total N and Organic matter, and the amount of P, K, Fe, Zn in the soil.

Key Words : Solid Waste Landfill Site, Landfill leachate, Landfill restoration, Soil

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Persistence of smelter wasteland phytoremediation – evolution of plant cover

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Metal mining and smelting wasteland sites are serious sources of secondary metal pollution. The sites are usually barren due to metal toxicity and harsh physical conditions which causes metal dispersion through wind erosion and runoff. Reclamation of such environments is a substantial challenge and involves a number of technical and cost difficulties.
The pilot remediation project was aimed at assessment to what extent biosolids and limestone treatments along with pre-selected grass mixture would support persistent functioning of revegetated metal waste ecosystem. Experiments were located at a brownfield site resulted from the shutdown of a smelting plant in Piekary Slaskie, Poland. Total zinc content in top layer ranged from 6.9 to 128 g kg-1. The reclamation work which took place in 1994/1995 resulted in vegetation of 3 ha pilot areas. The site was reclaimed by application of municipal biosolids at the rates 150-300 tons per hectare (dry matter basis) combined with the incorporation of high rates of oxide and carbonate lime. The grass mixture consisted of the following local cultivars: Festuca rubra L. cv. Atra, Poa pratensis L. cv. Alicja, Festuca arundinacea Schreb. cv. SZD, and Festuca ovina L. cv. Sima. The most crucial question is how persistent and sustainable are such constructed ecosystems. Different chemical and biological parameters were used for monitoring of revegetated site including metals extractability, plant biomass and microbial activity – samples were collected in a regular grid. This paper will present trends in metal mobility, evolution of plant cover and biodiversity over 15 years period and their implications to risk to humans and wildlife.

Keywords: smelter wasteland, metals, pollution, remediation, biodiversity

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Evapotranspiration (ET) landfill cover using Scots pines holds challenges

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The former municipal solid waste landfill "Altablagerung Wannsee" (AAW) in southwestern Berlin was chosen for installation of an evapotranspiration (ET) landfill cover design. This design relies on a qualified soil-vegetation barrier to minimize or prevent water from infiltrating into the underlying waste, thereby minimizing leachate generation. Conifers are known to be promising candidates for an efficient year round reduction of water infiltration. Scots pine (*Pinus silvestris*) saplings were planted on the AAW into the grass-covered top soil layer (silty sand compost blend) in 2005; one year after placement and initial greening of cover soil.
Most of the 14,000 planted 2-yr-old pines died the same year. Three experimental field plots were established in 2006 to tackle this problem. The plot locations represent locally common topographical and exposure conditions. Ecotechnology means are being tested for increasing survival and growth of Scots pine. Environmental site conditions are characterized regularly, emphasizing key soil parameters, vegetation composition, and weather. Extreme drought and heat in summer 2006 had again caused very high tree mortality rates of up to 98%. The water shortage-related stress posed upon pine saplings was amplified by unfavorable soil conditions, microclimatic extremes, and strong competition by thriving grasses and spontaneous vegetation. In response, two alternative conifer species, namely Austrian pine (*Pinus nigra*) and Norway spruce (*Picea abies*), were planted in 2007 on the experimental plots in addition to *P. silvestris*. Two planting methods and soil treatments were applied and their effects on conifer vitality and growth were examined. Eventually, 96% of the conifers that had been planted on the experimental plots survived until October 2009 and they grew stronger than ever before. Growth and vigor varied among conifer species and depended on microsite conditions, planting method, and soil treatment near the tree. *P. silvestris* eventually performed best and especially if planted bare-rooted and when mulched with conifer bark chips on the south-facing slope.

Keywords: waste site, capping, compaction, vegetation, conifers, mulching, inoculation

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Evaluate the potential of evapotranspiration covers as a biomass feedstock in urban southeastern Ontario.

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Shrub willows (*Salix* spp.) and hybrid poplars (*Populus* spp.), that can be used as a renewable energy crop, have great potential to be used in alternative landfill caps. Currently many landfills that are present in the urban areas of southeastern Ontario do not have properly engineered leachate collection systems or covers to prevent deep percolation that can lead to groundwater contamination. The cost to close existing waste sites with conventional covers is often prohibitive. The durability of conventional covers composed of compacted fine-grained barrier layers is often insufficient to ensure longterm reliability. Evapotranspiration covers (ET), that may consist out of fast growing shrub willows and hybrid poplars, do not rely on a barrier layer, but instead manage water storage capacity and ET to reduce percolation rates. Evapotranspiration covers are a cheaper and more durable alternative and can potentially provide an array of other environmental and community benefits. One environmental benefit of ET covers is the potential to use the resultant biomass for bioenergy purposes. A major challenge for bioenergy is the realization of sufficient biomass feedstocks, which can be made available at low costs. ET covers can be part of the solution and function as a source of low cost biomass feedstock. The objective of this study is to designate landfills in southeastern Ontario that can potentially be used for ET cover projects and develop a GIS (Geographic Information System) to 'map' these potential landfills.

Key words: GIS, landfills, leachate, *Populus* and *Salix*.

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POSTERS

A-I

A1

Uptake of phosphorus and lead from chloropyromorphite by *Brassica juncea* and *Medicago sativa*

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In phosphorus- (P) deficient soils, plants may develop extensive root systems to access P in any P-containing minerals, thereby affecting the stability of pyromorphite. We grew *Brassica juncea* and *Medicago sativa* in sand culture to evaluate the stability of chloropyromorphite (CP) in the presence or absence of hydroxyapatite (HA) as P source. Treatments (per kilogram of sand) watered with P-nutrient solution were 0, 1, and 5 g Pb as CP [PC0, PC1, and PC5] and 0.45 g P as HA (PA), and those of watered with P-free nutrient solution were 1 and 5 g Pb as CP [NC1 and NC5], 5 g Pb as CP plus 0.45 g P as HA [NAC5], and 0.45 g P as HA [NA]. The leaves of *B. juncea* appeared dark- green in NC1, due to P-deficient condition, and light- green in NC5, probably because of Pb toxicity and or Pb interference with Fe and Ca. Among CP treated pots, the highest shoot Pb accumulation was observed in NAC5 treatment indicating that the added HA was more effective in Pb accumulation in the shoots of both plants than a soluble P source. The results suggested that Pb accumulation and translocation in the plants was markedly higher in Psufficient conditions than in P-deficient conditions.

Key words: Stability, sand culture, chloropyromorphite, indian mustard, alfalfa

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A2

Effect of the nickel resistant rhizosphere bacteria on the uptake of nickel by the hyperaccumulator *Thlaspi caerulesens*

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Heavy metals are an important class of pollutants deriving from agriculture, industrial activity and disposal of waste matter. Phytoremediation, using plants to remove metal pollutants from contaminated soils, is being developed as a new method for the remediation of contaminated land. Hyperaccumulator plants have the ability to accumulate very high metal concentrations from contaminated soils in their shoots. The rhizosphere provides a complex and dynamic microenvironment where microorganisms, in association with roots, form unique communities that have considerable potential for detoxification of hazardous waste compounds. The rhizosphere of hyperaccumulator plants have been shown to support high amounts of metal tolerant bacteria wich may play an important role in regulating the availability of metals for the plant. The objective of the current study was to determine whether two Ni resistant bacterial strains (10 mM Ni), originally isolated from the rhizosphere of *Thlaspi caerulescens* (Brassicaceae) grown in serpentine Ni-rich soil from the Vosges mountains (France), affected the nickel uptake and hyperaccumulation by this plant species. Cultures inoculated with a mixture of these strains showed a significant increase of the aerial biomass comparatively to the axenic control. Despite the drastic decrease of the water extractable-Ni in soils at the end of the growth, there was no significant effect on Ni accumulation by plants comparatively to the axenic control. In contrast, highly significant promoting effect on plant growth and increase of Ni accumulation by *T. caerulescens* were shown in cultures with native microorganisms (non sterile control). This implies that optimal growth and Ni uptake by *T. caerulescens* requires the presence of various microorganisms. The Ni resistant rhizosphere bacteria reduced Ni bioavailability in the soil suggesting a Ni resistance mechanism including binding of metals and formation of insoluble metal sulfides. They could then serve as an effective metal sequestering and growth promoting bioinoculant for plants in Ni-stressed soil. They may also contribute in reducing the phytotoxic effects ofthe metals by sharing the Ni load due to their ability of biosorption and bioaccumulation.

Key words: heavy metals, phytoremediation, hyperaccumulators, rhizosphere, nickel resistant bacteria

A3

Microbial ACC-deaminase- A Useful Approach for Reducing Stress-induced Ethylene in Plants

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Higher levels of ethylene are produced in plants in response to biotic and abiotic stresses, which inhibit plant growth and nodulation. Some PGPR contain an enzyme 1-aminocyclopropane-1-carboxylic acid (ACC)-deaminase which lowers the elevated levels of ethylene and therefore, result in better growth and nodulation in legumes. In the present study, five isolates each of PGPR containing ACC-deaminase (R6, K6, M2, F2 and F6) and rhizobia (FS2, MN3, MN6, MG6 and KH6), and their combinations were screened for their growth promoting activity under axenic conditions and thee most effective combinations were evaluated for improving nodulation in pot trial. Results of axenic trial showed that separate application of PGPR containing ACC-deaminase and rhizobia enhanced the plant growth.
However, the co-inoculation of PGPR containing ACC-deaminase and rhizobia produced more pronounced results for improving the growth and nodulation in mung bean grown under axenic conditions compared with un-inoculated control. The maximum number of nodules plant-1 (22), nodule fresh weight (0.313 g) and nodule dry weight (0.04 g) were observed in case of the combined application of rhizobial isolate MN6 and PGPR strain F2 followed by F6xKH6 and F6xMG6. These three combinations along with individual strains were evaluated for improving nodulation in chickpea in pot trial under natural conditions. The results revealed that the separate application of PGPR containing ACC-deaminase and rhizobia enhanced the plant growth but combined application was more effective treatment and the combination F2xMN6 gave the maximum growth, yield and nodulation. A classical "triple" response bioassay was also conducted to evaluate the effect of high ethylene concentration on the growth of etiolated mung bean seedlings and the performance of co-inoculation was evaluated for reducing the classical "triple" response. The results showed that the intensity of classical 'triple" response was decreased due to co-inoculation. So combined application of PGPR containing ACC-deaminase and rhizobia could be a useful approach for improving growth and nodulation in mung bean.

Key words: ACC-deaminase, stress-induced ethylene, co-inoculation, mung bean

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A4

Evaluation *ex-situ* of the potential of *Halimione portulacoides* for metal phytoremediation

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There is information that certain salt marsh plants can sequestrate and inherently tolerate high metal concentrations frequently present in sediments, which can be useful for phytoremediation purpose, particularly for phytoestabilization. However, most studies only evaluate metal burden in natural marsh systems or marsh plants capability for metal uptake through lab studies. Studies aiming at evaluating *in-situ* or *ex-situ* phytoremediation potential of salt marsh plants are scarce. In the present work the capability of *Halimione portulacoides* (a halophyte commonly found in Portuguese temperate salt marshes, which can accumulate significant amounts of metals) for metal phytoestabilization in salt marsh areas was studied *ex-situ*. For that purpose, the plant was transplant into cylinder litterbags, with a sleeve form, filled with metal polluted estuarine sediment and left in a cleaner estuarine area for a nine months period. Total metal content (Cd, Cr, Cu, Ni, Pb and Zn) in sediment and plant tissues were determined (by atomic absorption spectrometry after high pressure microwave digestion) at the beginning and after the period of study.
The obtained results will bepresented and discussed.

Keywords: sediment, salt marsh restoration, salt marsh plants, phytoremediation, metals

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A5

Phytoextraction of mercury in contaminated soil: analyses of Hg-species by different sequential extraction procedure (SEP)

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The reclamation of polluted soils by means of low impact technologies, as phyto-remediation, has been increased in the last decades so as to have a lot of case-studies on heavy metals contaminations. However, for several metals heavily critical aspects in the treatment still remain. Metals exist in several different forms and are associated with a wide range of components in soils. The particular behaviour of metals in the environment is determined not only by their total concentration but also by their specific physical-chemical forms. For this reason different protocols of Sequential Extraction Procedures (SEPs) have been developed. In this paper, the Hg-species in contaminated soils to be treated with phyto-remediation were investigated, in order to assess Hg bioavailability and mobility and then to correctly design and execute remedial strategy. In five soil samples the total concentration and three different SEPs focused on mercury were carried out. The chemical results were compared with mineralogical phases determined by means of XRD analyses. The SEPs were selected to integrate different Hg species bounded with defined groups as a function of their solubility in a solvent. The first SEP was the procedure of Tessier et al. (1979) modified by Salomon and Forstner (1980), the second the procedure of Miller and Dobb (1995) and the third the procedure of Boszke et al. (2008). The total concentrations of Hg in the soils samples range from 12.9 mg/kg d.w. to 25.4 mg/kg d.w. with an average content of 18.4 ± 5.1 mg/kg d.w..
The SEPs highlighted a low content of highly soluble mercury - between 1% (Tessier et al. 1979) and 5% (Miller and Dobb, 1995). No methylmercury and mercury bound with organic matrix (humic acids) were detected (Miller and Dobb, 1995 and Boske et al., 2008). The results showed that the total Hg may be distinguished into a moderately soluble fraction (46-56% of the total) and a residual one (38-42% of the total). Elemental mercury was identified as the moderate solubility phase. The residual phase did not show a significant content of sulfur (less than 1-2%), as detected by means of mineralogical determinations. Synoptically, the SEP resulted a good screening tool in order to define the most effective strategy and the achievable remediation targets; moreover, the identification with high precision of Hg species results much more efficient through the comparison of different SEPs.

Keywords: Mercury, SEP, Phytoremediation, Phytoextraction

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A6

The effect of organic amendments on uptake and allocation of heavy metals and plant nutrients in sunflower (*Helianthus annuus*)

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Pot experiments were carried out to evaluate effects of organic soil amendments (peat, compost and vermicompost) on uptake and allocation of heavy metals and plant nutrients (Pb, Cd, Zn, Cu, Fe, Mn, Ca, Mg, K, N and P) in sunflower (Helianthus annuus). Soils used in this experiment were sampled from the vicinity of the areas contaminated by the zinc and lead smelter near Plovdiv (Bulgaria). The total content of Zn, Pb and Cd is high (894 mg/kg Zn, 470 mg/kg Pb and 12.4 mg/kg Cd, respectively) and exceeding the maximum permissible concentrations. The soils were amended or not with 5.0, 7.5, or 10.0% of peat, compost and vermicompost. Heavy metals were measured in roots, stems, leaves and seeds. Soil metals were measured in a DTPA soil extracts to assess the effect of the amendments on metal bioavailability. Peat, compost and vermicompost application led to effective immobilization of Pb, Zn and Cd phytoaccessible forms in soils. A correlation was found between the quantity of the mobile forms and the uptake of Pb, Zn, and Cd by the sunflower.
The highest concentrations of Pb, Cd and Zn occurred in the leaves and/or roots. Organic amendments significantly reduced heavy metals concentration in sunflower seeds, but the effect differed among them. Also, there was a dose effect for amendments. The peat treatments had only slight effects on the uptake and allocation of plant nutrients and heavy metals. Thus, there was little benefit of peat treatments for phytoremediation purposes at these sites. The compost and vermicompost treatments had significant effects on the uptake and allocation of plant nutrients and heavy metals. The 7.5% compost and 5.0% vermicompost treatments led to the maximal reduction of Pb, Cd and Zn in sunflower seeds (70%, 51% and 40%, respectively).

Keywords: organic amendments, heavy metals, uptake, sunflower, polluted soils

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A7

New Composite Insulating panel from renewable material

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The more common insulating panels, currently used in construction sector, are made by synthetic foam material, as expanded polyurethane or polystyrene. The expected goal of this research is to develop an insulation material based on lignin-sulfonate, coming from waste of ligno-cellulosic production. This material must guarantee expansibility, homogeneity, high thermal and mechanical resistance, as well as a dramatically reduced environmental impact.
Lignin is a natural polymer, which constitutes the inner structural core of the wood.Commercial lignosulfonates are complex anionic polymers obtained as co-products of wood pulping. They are obtained from spent sulfite pulping liquors, for this reason, its structure is undetermined, but basically composed by different combination of three main alcohol (coniferilic, sinapilic, cumarilic), largely depending on the wood species (soft or hard).
The lignin insulation material could be used to make simple or composite panels, for application on wall and roof. Composites are sandwiches, in which wood or metal external skins are stuffed by an insulating lignin core.
In both the cases, the lignin vitreous-based layer is able to combine thermal properties with mechanical resistance, further improved by the rigid skins to meet the more severe requirements for specific applications.
Preliminary prototypes were made by using lignin sulfonate as starting material, having different grades of alkaline metals added to a silica sol-gel solutions obtained from TEOS (tetrahydroxysilicate) from areogels to xerogels. Pre-treated maize starch has been added as glue and thickener keeping homogeneous phase as much as possible an together with addition of kenaf fibers as mechanical binders to microcells. The introduction of controlled amounts of partially hydrolyzed vegetable oil may increase the hydrophobic sites, acting as plasticizers. Furter, solid CO2 or ammonium carbonate is added to the viscous material, as promoter for a proper expansion grade, strictly related to the drying procedures adopted (fig. 1).
Structure and physico-chemical characterizations of the meso or microporous material are still investigated to elucidate features and behaviour in evaluating its attitude as eco-friendly component for building insulation.

Keywords:green building, sustainability, insulation, renewable material, lignin

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Figure 1: Lignin insulation material – Preliminary prototypes

A8

Poplar plantations for treatment of food processing wastewaters

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Many food processors in Michigan rely on land application to treat high strength, high flow process wastewater. While properly operating fields can assimilate wastewater through biodegradation of organic carbon and biochemical oxygen demand (BOD), excess application of wastewaters can rapidly contribute to poor treatment and environmental deterioration. Ponding and continuous saturation can decrease oxygen concentrations and mobilize metals that can contaminate groundwater. At multiple sites in Michigan, elevated groundwater concentrations of iron, manganese, and arsenic have been attributed to land application of food processing wastewaters. The use of poplar trees to remediate hazardous waste sites is a well-developed, low-cost technology for hazardous waste sites. Poplar plantations utilize transpiration, microbial degradation of pollutants in the rhizosphere, plant uptake, and immobilization of metals in the rhizosphere to remediate metal-contaminated hazardous waste sites. Research, to be started in summer 2010, will quantify the effects of poplar trees on metal mobility by assessing metal leaching, BOD concentrations, water content, and the oxidation-reduction potential (ORP) in soil columns to which synthetic food processing wastewater is applied. Research will evaluate three poplar varieties: native *P. deltoides* (eastern cottonwood), native *Populus balsamifera* (balsam poplar), and hybrid *P. detoides* x *Populus nigra*. Poplar plantations are expected to be a low-cost, sustainable solution to metal mobilization caused by food processing wastewaters.

Keywords: Poplar, metals, arsenic, food processors

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A9

Risk Assessment of Triclocarban and Triclosan Phytoaccumulation by Pumpkin and Zucchini

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Triclocarban and triclosan, two widely used antimicrobials in products like soaps, deodorants, lotions, cosmetics and toothpaste, have been shown to have adverse effects to human and ecosystems. Pumpkin and zucchini in our experiment with land applied biosolids phytoaccumulated triclocarban and triclosan. This study compared the environmental occurrence of antimicrobials with known toxicity thresholds and evaluated the risk of eating pumpkin and zucchini produced from biosolid applied soil in experimental columns. Mean margin of exposure and aggregate margin of exposure for each triclocarban and triclosan were evaluated for worst case scenario. Margin of exposure values from eating vegetables were comparable to that of using bar or liquid soap containing triclocarban and triclosan and were substantially less than that of drinking water contaminated with triclocarban and triclosan. The study concluded that there is negligible risk of eating vegetables produced from land applied biosolids.

Keywords: Risk, Triclocarban, Triclosan, Antimicrobials, Phytoaccumulation

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A10

Screening of plant growth promoting rhizobacteria to assist phytoremediation process of petroleum contaminated soils

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Petroleum, the backbone of any country, now became a threat to environment as leaking of storage tanks, pipelines and accidental spills jeopardize the environment. Petroleum and its products contain carcinogenic aromatic compounds, such as benzene, which contaminate soil. Efforts are now focused on potential remediation techniques for the contaminated soil. Microbe's assisted phytoremediation of petroleum contaminated soil is getting attention compared to the alone use of either microorganism or plant. The challenging task for such efforts to be successful is not only the survival of microorganisms upon their inoculation into xenobiotic environment but also a positive plant-microbe interaction. Plant growth promoting rhizobacteria (PGPR) having ACC-deaminase activity are considered to be helpful for plants in stressed environment by reducing stress induced ethylene but the survival of such microbes in hostile environment like petroleum contaminated soil is another problem to be addressed. We have collected petroleum contaminated soil samples from various petroleum contaminated sites. About 300 hundred isolates were initially isolated from these soil samples. To assess their bioremediation capability and ACC-deaminase activity, bioremediation assay and ACC metabolism was carried out.

Among these 300 isolates only 27 isolates were positive for both, bioremediation and ACC metabolism activity. For the assessment of growth promoting activity of these 27 isolates in alfalfa, growth pouch assay was conducted. The growth parameters like root and shoot length, fresh and dry biomass were significantly improved compared to un-inoculated control plants. Results indicated that screening of PGPR with bioremediation capability along with ACCdeaminase activity could be a good tool to improve plant growth under petroleum contaminated soils and these bacteria could assists in phytoremediation process.

Keywords: Phytoremediation, Hydrocarbon, Contaminates soils, PGPR

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B1

Optimizing the phytoremediation capability of *Chromolaena odorata* on oil sludge contaminated-soil with rhizosphere organisms from a *Chromolaena* odorata population

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The present study attempts to optimize the interaction between *Chromolaena odorata* roots and microorganisms to remediate oil sludge contaminated soil under laboratory conditions. A consortium of rhizosphere organisms isolated from the root regions of a *Chromolaena odorata* population was enriched in the laboratory before being introduced into the rhizosphere of *Chromolaena odorata* plants growing in soil contaminated with different concentrations of oil sludge in pots. Control experiments were set up using plants growing in sludge contaminated soil that was sterilized and not inoculated with the microorganism. All experiments were set up in duplicates and placed in a tunnel with controlled temperature. The plants were watered three times a week using deionized water containing 5% NPK fertilizer. The experiments were monitored for 90 days. Soil samples and plant materials were removed for analysis of total petroleum hydrocarbons (TPH). The samples were extracted with suitable solvents and analyzed with Infrared spectroscopy for TPH and GC/MS for selected hydrocarbons. The results showed that the inoculation of the consortium of rhizosphere organisms into the root region of the plants enhanced the removal TPH in the soil. There was no significant difference in the amount of selected hydrocarbons accumulated in the plant tissues. The studies shows that rhizosphere organisms play a significant role in the phytoremediation of oil sludge contaminated soil by *Chromolaena odorata*.

Keywords: *Chromolaena odorata*, phytoremediation, rhizosphere organisms, oil sludge

B2

Phytorehabilitation of polluted soil to As. Presentation of experimental protocol in-situ and results obtained

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In recent years, the French and European policies on the management of contaminated land have greatly advanced, both by promoting greater transparency of health and environmental problems that may arise over time, but also by introducing legislative constraints stronger on liability and risk prevention. Although physico-chemical techniques are many and varied, the valorisation and requalification of polluted sites can be done by a set of biological processes respectful of environment and biodiversity of ecosystems that are meetings in the terms of phytoremediation. In the case of soil contaminated by metals or metalloids, the preferred method is the phytostabilization. It is a technique based on the reinforcement of vegetation cover that helps to reduce the transfer of pollutants. Besides, it improves the aesthetic of sites.
The work within our team has permit to acquire extensive knowledge of the effects induced, in different plant models, by heavy metals and metalloids. The objective is to implement the methodologies and results (dose-effect) obtained in the laboratory to field situations. This study focuses on the establishment of a process of phytoremediation *in situ* on an industrial site heavily contaminated with arsenic (As) and to shut down since 1969. This monitoring is done at two scales :
- Evolution of the content and speciation of As in the soil ;
- Monitoring of development of vegetable cover, taxa dominance and measurement of physiological activity of sentinel specie (*Vicia faba*).
This work began in spring 2010. The experimentation was conducted on plots of 10 m² put in place on the industrial site. Two plant covers were tested :
- A plants association composed of 50 % of Poaceae, 30% of Fabaceae and 20 % of other plant species ;
- A monoculture of hemp (*Cannabis sativa*), tolerant to As and permitting the valorisation of aerial biomass in the textile or isolation materials.
For to obtain a better development of vegetation, the immobilization of As by adapted amendments is preferred. These amendments have the ability to promote geochemical processes such as precipitation, sorption, ion exchanges or redox reactions so as to reduce the mobility of the pollutant and by consequently, its bioavailability.
- Contribution of iron shot (1 %) for the formation of ferrihydrite (Fe2O3) playing an important
role in the retention of As ;
- Contribution of organic matter for the plants association permitting a better plants development, and involved in the methylation reactions of As (methylated forms less toxic) ;
- Contribution of NPK fertilizers for growing hemp, to meet the demanding needs of the plant in mineral elements.
So the combination of adding of an immobilizing agent with the revegetation can limit the environment impact of contaminated areas.
The presentation of this work will permit to expose on the one hand the *in-situ* culture protocol applied in function of environmental conditions, on the other hand the results obtained on the plants development and impact on the availability and speciation of As in the soil.

B3

Remediation of canal sediment exposed to continued wetting and drying: effect on metal mobility by inorganic amendment addition

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The Woolston New Cut Canal sediment (Warrington, UK) is currently exposed to continuedwetting and drying. The sediment has been well studied from past phytoremediation research (King et al., 2006), being highly contaminated by toxic metals and As. Sincephytoremediation is a long-term solution, the greatest hazard is from metal leaching. Thepossibility of immobilising metals (Cd, Cu, Pb, Zn) in the sediment was explored by means of various inorganic amendments. In laboratory studies, the sediment was mixed with cement at 1, 20, and 50% w/w, lime at 1% w/w, and lime + iron sulphate at 0.5% w/w. In order to simulate natural conditions, a contiguous wetting and drying extraction (4 cycles) was performed, in comparison with untreated controls. Results showed that Zn was effectively immobilised by cement, at all concentrations, unlike lime + iron sulphate which resulted in high mobilisation during the first cycle. Lime + iron sulphate also solubilised substantial amounts of Cd, whilst high cement rates (> 20%) mobilised Cu. Lime alone and the lowest concentration of cement effectively reduced all metals. Cadmium mobility was not reduced by any of the amendments. It is concluded that reduced risks of metal leaching during phytomanagement of canal sediments should consider amendment with cement or lime, at small doses, but caution must be considered with lime, as this is known to mobilise arsenic.

Keywords: canal sediment, cement, iron sulphate, lime, metal pollution, metal stabilisation.

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B4

An attempt to improve phytoextraction through soil and foliar IBAapplication in fodder radish (*Raphanus sativus* L. var. *oleiformis*) grown in metal-polluted pyrite wastes

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The use of plant growth-promoting substances may potentially improve the phytoremediation of trace metals, as more roots are available for their uptake and increased shoot biomass is expected. In this view, fodder radish (*Raphanus sativus* L. var. *oleiformis* Pers.) was cultivated in pots for about 3 months in As-Co-Cu-Pb-Zn-polluted pyrite wastes. Starting from 28 days after sowing, indolebutyric acid (IBA) was given 5 times at 10-day intervals through foliar spraying (10 mg IBA L-1), and through irrigation (0.1 and 1 mg IBA kg-1 of wastes) in combination, or not, with foliar treatment, vs. untreated controls. With the exception of foliar spraying alone, IBA unexpectedly reduced the above-ground biomass at harvest – the higher the dose, the lower the growth. Root weight was also generally reduced, but not root length, foliar treatment alone leading to higher growth than in controls (+16%).
Concentrations of trace metals were always enhanced by IBA, probably through increased H+-ATPase activity, particularly for Co and Pb at the highest auxin dosage. Impaired aboveground yield was considered the main cause of reduced metal removals. At our tested IBA dosages, phytoextraction was not improved, but the promising effects on rooting observed for foliar applications should be examined more thoroughly in order to extend this phytotechnique to polluted sites.

Keywords: auxins, fodder radish, heavy metals, indolebutyric acid; phytoremediation, pyrite wastes.

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B5

Brassica juncea for the phyto-management of boron from sediments of cecina river basin: effects of fertilizer amendments

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Large areas of Cecina river basin (waters and sediments) show boron contamination attributed to both, hundred years of mining activities and the spilling of waste into the tributaries of Cecina river. The waste was produced by acid boric extraction from geothermal fluids and from B minerals. The reclamation of contaminated areas using the phytotechnologies needs a continuous investigation due to the site-specific parameters that can affect its efficiency. In a recent investigation using sediments collected along the Possera creek near the Bulera dump (Cecina river basin) promising results were found regarding the B phytoextraction power of *Brassica juncea* plants with a great reduction of boron contamination. Nonetheless, the feasibility needs to be evaluated on a larger scale, assessing also the best agronomic practices. This communication focused on the evaluation of the effect of different fertilizer amendments to improve the biomass production and the agronomic manipulation to obtain elevated roots density and increased evapotranspiration. In laboratory tests plants were allowed to grow for about 40 days. Five different fertilizer amendments were applied to sediments: *i*. mineral fertilization using a mix of ammonium and potassium phosphates; *ii*. mineral fertilization using the commercial formulation N.P.K_8.24.24; *iii*. mineral fertilization as before plus a chelating organic fertilization with compost, and *v*. organic fertilization with sodic salt of humic acid. The different treatments produced different effects on B uptake with significantly higher accumulation when the mineral fertilization plus the chelating agent was used. No difference in shoots or roots biomass were found probably due to the short growing time in this small scale experimentation.
Growing-up scale with lysimeter testsare in progress.

Keywords: sediment remediation; boron phytoextraction; *Brassica juncea*, fertilization.

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B6

Phytoextraction process optimization: characterization of the rhizospheric microbial activity of the hyperaccumulating plant *Arabidopsis halleri*

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Phytotechnologies are plant microbe based techniques that use living plants for the treatment of contaminated sites. In this approach, phytoextraction exploit natural physiological traits of certain plants able to accumulate high levels of metal in their aerial tissue. In such a process, the ideal plant is expected to reach high biomass production and high accumulation capacity together with the maintenance of high tolerance to soil metal concentration and fast rate of growth.

Previous studies have shown that soil microflora can enhance trace element mobility, resulting in higher metal concentrations in accumulating plants. In addition, soil microflora is also associated with plants biomass augmentation through the promoting action of PGPB (*Plant Growth Promoting Bacteria*).

The aim of this work is to study the microbial diversity associated with the hyperaccumulating plant *Arabidopsis halleri* growing on a Zn and Cd polluted soil and to identify bacteria that may be potentially involved in plant biomass production and/or plant Zn and Cd accumulation. For this purpose, three different soil samples were collected from an industrial site contaminated with Zn and Cd located in Auby (59, France). Each sample was choosen to reflect a different degree of soil contamination (ranging from less to highly polluted soil). For each sample, three sub-fractions of soil were defined and collected independently relatively to their distance to *Arabidopsis halleri*'s roots (global, rhizospheric and rhizoplan soil, respectively). The nine soil sub-fractions were simultaneously treated for total flora DNA extraction followed by PCR amplification of a 1300 bp fragment of the 16S rDNA gene. Amplicons were cloned to generate a mini library, the content of which was further analysed by restriction fragment length polymorphism (RFLP). RFLP profiles obtained were informatically compared and the major represented clones were subsequently sequenced. The results obtained by the RFLP approach have still to be confirmed by denaturing high performance liquid chromatography (d-HPLC) analysis on shorter PCR products (400 bp), which is actually under way. First results concerning microbial diversity in these nine samples will be presented.

The aim of this project is to identify and isolate bacteria that may enhance the metal extraction efficiency of *Arabidopsis halleri*. Therefore, bacteria species identified during the molecular approach described above will be further investigated for the presence of PGPB characteristics (ACC deaminase activity, Indol-Acetic Activity and siderophore productions). In this purpose, pots of *Arabidopsis halleri* growing on contaminated soil will be inoculated with PGPB identified bacterial species. Plant growth as well as bioaccumulation will be monitored regularly in order to determine which bacterial strains present a significative impact on *Arabidopsis halleri* mediated phytoextraction process.

Keywords: *Arabidopsis halleri*, phytoextraction, bacteria diversity, PGPB, bioaugmentation

B7

Ultrastructural damage and heavy metal accunulation in three aquatic plants

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Metal pollution in waters and soils is a major environmental and human health problem. Heavy metals are normally present at low concentrations in freshwaters, but due to the anthropogenic activities they are also the most common non biodegradable pollutant detectable at elevated concentrations in a majority of parts of the world.
Aquatic plants are known to accumulate and bioconcentrate heavy metals. In this study we tested with heavy metals three aquatic plants (the monocotyledons *Lemna minor* and *Elodea canadensis*, and the moss *Leptodictyum riparium*) by *in vitro* culture. The plants were exposed to several concentrations (from 10-7 to 10-3 M) of cadmium, lead, zinc and copper salts for several days. We evaluated classical toxicity endpoints (survival percentage, relative growth rate, dry to fresh weight ratio) at first, then we assessed metal bioaccumulation in total body, tissue and cell localization, ultrastructural alterations and chlorophyll content and chlorophyll a/b ratio; these parameters could be regarded as biomarkers of heavy metal stress.
L. riparium was the most resistant species (EC50 values of survival percentage were 10-4M for Cu and Zn, 10-5 M for Pb and 0.5 x 10-5M for Cd). Heavy metal bioaccumulation in total body was evaluated by Atomic Absorption Spectrometry; found values varied depending on the metals and their concentrations and the plant species tested. Heavy metal tissue localization was assessed by X-ray SEM microanalysis: this technique showed preferential metal localization in plant tissues of *L. minor* and *E. canadensis*. Ultrastructural observations were performed by a transmission electron microscopy (TEM): visible metal effects were shown on vacuolar and thylakoid organization in all the three aquatic plants. X-ray TEM microanalysis localized heavy metals in cell wall and cytoplasm vesicles. Chlorophyll was extracted and examined spectrophotometrically: differences were shown between plants and treatments.

B8

Design and Construction of Large Soil Columns for Tree and Bush Experiments

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Soil columns are widely used in phytoremediation experiments to evaluate diverse characteristics of treatment for numerous contaminants. Traditional soil columns used in research are less than 15 inches in diameter and 3 feet in length. Customary soil columns are difficult to grow larger plants such as trees and bushes for evaluation. This paper covers the structural assessment and procedures for constructing large soil columns for tree and brush experiments. Information presented will help researchers evaluate the structural integrity of large soil column construction to withstand environmental loading. Soil columns were designed to grow poplar and willow trees to evaluate the impact of industrial wastewater application for a three year experiment. Soil columns were constructed to the dimensions of 3 ft diameter and 5 ft deep. Soil column construction design was evaluated based on wind loading for structural design of the pipe, overturning, sliding, and bearing capacity assuming tree growth 15 feet above the soil column. This evaluation concluded that 3 ft diameter HDPE storm drain pipe is adequate to withhold soil pressure. The weight of the soil column is adequate to prevent sliding. Bearing capacity is satisfied by concrete or asphalt base. Overturning exceeded the allowable 1.5 factor of safety, therefore steel anchor supports were designed to meet overturning requirements.

Keywords: large soil column, poplar, trees, structural evaluation, wind loading, construction

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B9

Reducing Human Exposure to Toxicants from Air, Soil, and Water using Phytoremediation

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Humans are exposed to a variety of contaminants via different fate pathways including: airborne exposure and inhalation, dermal exposure from contaminated soil, and by drinking contaminated water. Here we include results on the potential for reducing human exposures by scavenging semi-volatile PCB congeners from air; on remediation of explosives compounds tightly bound to soil particles; and on the uptake and translocation by plants of mobile contaminants in groundwater. We also examine the uptake and transformation of natural steroid hormones and their derivatives which are used as growth promoters to cattle and applied to land as manure and/or sewage sludge amendments. Plants have been shown to scavenge semi-volatile compounds from air onto waxy cuticles and leaf surfaces; enhance the rhizosphere biodegradation of compounds tightly bound to soils; and uptake, translocate and metabolize water soluble chemicals from soil solution and groundwater.

This paper provides an overview of research results on semi-volatile PCB congeners (PCB 3, 15, 28, 52, 77), on explosives chemicals (RDX, HMX, TNT), and on natural steroid hormones (17B-estradiol), estrone, and the estrogen analog zeranol. We are working at demonstration sites in the field, in small-scale microcosms, and in hydroponic solution in the laboratory. Novel metabolites have been identified and pathways proposed for degradation of selected PCB congeners (PCB 3 and PCB 77) which form mono-hydroxy and di-hydroxy metabolites, some which may be more toxic than the original parent compound. TNT and RDX degrade in the laboratory and in-situ, but the propensity of RDX for leaching and mobilization in groundwater is a continuing concern.

We have been utilizing several different plant species for these investigations including hybrid poplar, *Bahiagrass pensacolum*, switchgrass, and water hyacinth. Hybrid poplar is a "model plant" from both the standpoint of genomics, and as a commercially important species widely used in silvaculture and phytoremediation. Bahiagrass is native to the Florida-site, a requirement for sustainable management at military testing and training ranges.

Keywords: Phytoremediation, biodegradation, PCBs, hormones, antibiotics

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B10

Groundwater desalination by phytoremediation in Central Tanzania.

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For a lot of populations in the world the access to the drinkable water is still very difficult; in the village of Kongwa, in Central Tanzania, groundwater chemistry is influenced by elevated salts, sulfate and metals concentration.
In the research of a economic and eco-sustainable solution for to improve the water quality, the answer has been thought can be the phytoremediation. In this project we have worked a system based on phytoreactor that reaches the groundwater's purification by alophyte plants (used together to other species more common such as Phragmites and Juncus). Alophyte species have the property to bioaccumulate to many salts in roots, stems and leaves, and the aim is to use this plants to decrease salt concentration in the water crosses the phytoreactor.
Species as *Atriplex Patula*, *Salicornia Europaea* e *Spergularia Canadensis* has very elevated ability of accumulation of salt in roots and leaves (Galvez, 2007), other species as *Suaeda Fruticosa* have an ability of desalination of around 2,646 Kg of NaCl for hectare in one year (Khan et al., 2000).
We have chosen to plant *Suaeda Fruticosa* in partnership whit Phragmites species to improve sulphate removal and metals accumulation; these species are naturally present in Tanzanian territory.
The phytoreactor has horizontal flow. We have tried to seek a filling whit similar characteristics of the project sand typology. This sand it would guarantee a hydraulic conductibility of 45 meters per day.
Up to now the state of the jobs has the built reactor and we are waiting for the first analytical comparisons to appraise the purification efficiency.

Keywords: phytoremediation, desalination, suaeda, groundwater, Tanzania

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C1

Phytoremediation of metal-polluted soils with *Dittrichia viscosa*. It is possible to extrapolate to field the results obtained in greenhouse?

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With the increase of anthropological practices, more and more toxic metal ions are being added to the natural environment disrupting the ecosystem. High soil concentrations of metals like Cd, Pb, Hg, Fe, Al, Zn or Cr have potential toxic effects on plant overall growth and metabolism. Phytoremediation is an environment friendly, technology that uses the ability of plants to extract metals from polluted soils and to concentrate them in their above-ground parts. The metal-accumulating plant material can be safely harvested and removed from the site without the costs and loss of topsoil associated with traditional remediation practices. The objective of this work is to study the growth and accumulation of heavy metals by *Dittrichia viscosa* when grown in greenhouse in a polluted soil and to compare these results with those obtained in field.

We selected an experimental area contaminated with heavy metals and pH 12.5. *D. viscosa* was planted on this soil due to its high Cd accumulation capacity, high biomass and adaptation to our regional soil and climatic conditions. The studies were initiated simultaneously in greenhouse and in field (slag heap) with different concentration of slag (0, 25, 50 and 100%) and we studied growth and development of the plants and its accumulation capacity. 0% slag denotes that the plants were cultured in the soil but their roots were only surrounded by peat; 25% slag indicates that roots were surrounded in 25% slag: 75% peat and 100% slag denotes that plants were cultured directly on the slag.

When the concentration of slag around the roots increased in greenhouse, dry weight and length of shoots decreased 63% and 40% respectively, whereas dry weight and length of roots was similar in all treatments after 90 days of culture. The plants cultured in 100% slag died. In the field, the dry weight and length of shoots were tion of slag rose. The length of roots did not change. Moreover, in 100% slag the rate of mortality was high (70%) but some plants survived. Analysis showed that *D. viscosa* accumulated different heavy metals, more in field than in greenhouse. Plants accumulated Al, Ti, V, Cr, Cd, Mn and Fe in concentrations higher than normal values. The concentration of Ni, Zn, As and Pb in plants was low because their levels in the soil were also low. In most cases, the accumulation of heavy metals was higher in shoots than in roots. Thus, a good transport rate of the metals through the xylem was observed, being the relation shoot to root metal concentration higher than 1.

So, we can conclude that the best results of growth and accumulation in field were obtained when the roots of the plants were surrounded only by peat, which should be taken into account when designing a phytoremediation program. Although the results in field were better than in greenhouse, in both cases plants followed the same tendency with little changes, so an extrapolation of greenhouse studies can be made. At the moment we are studying how long would it take to phytoremediate with *D. viscosa* a soil moderately contaminated.

Keywords: *Dittrichia viscosa*, heavy metals, hyperaccumulation, phytoremediation.

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C2

Role of Transgenic Poplar and Strengthening Measure in Atrazine-Polluted Soil Remediation

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The soil in many regions of China is contaminated by the unreasonable using of pesticide. Atrazine is one of the herbicides that are widely used in China. Its environmental effects and threats are becoming more of a concern. Phytoremediation which clean up the environment relies on plants and their associated microbes has gained acceptance in the past 10 years. It has the advantages of cost-effective, noninvasive alternative and no secondary pollution. So it has a bright future for application to the cleaning-up of contaminated soil.
Transgenic poplar was taken as plant materials to remediate atrazine-contaminated soil, with sawdust, peat, inorganic fertilizer and mycorrhiza as additives, in the Research Greenhouse of Chinese Academy of Forestry in this study. The degradation dynamic of atrazine, effects of additives, microbial community structure and microbial activity, and the soil enzyme activity were studied in this experimental research, for the sake of identifying the role of this poplar in phytoremediation, and offering references for the further practical application.
The main findings were summarized as follows. The effect of phytoremediation for atrazine-contaminated soil was noticeable. The change in total amount of atrazine's residue with time met a kinetic equation C=5.979e-0.02t. The phytoremediation efficiencies varied according to the kinds of additive. The effects of 1% peat and mycorrhiza were the most significant judging from degradation effect, tree height, ground diameter, biomass and etc. The microbial biomass carbon and microbial diversity were found to be promoted in atrazine-polluted soil, with more obvious performance for the phytoremediation treatment group. During the whole experiment period, the changes in Evenness index and Shannon index were not significant. Phytoremediation was also beneficial to the recovery of the enzyme activities, which was more significant during the late experiment period. The soil enzymes was significantly correlated with microbial biomass carbon under both control and phytoremediation treatment condition.

Keywords: Phytoremediation, Atrazine, Microorganism, Soil enzymes

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C3

Environmental risk by the use of surfactants: use of macrophytes as bioindicators and bioaccumulators

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Surfactants have a wide range of domestic and industrial applications, and they are an important group of contaminants in aquatic systems. Ecological problems can raise when the surfactants are present in the aquatic environment in relatively high concentration.
Recently some surfactants deserved the interest of the researchers for their ability to modify the behaviour of other, preexistent or co-disposed contaminants. Thus, depending on the type and concentration of surfactant and the charges of the sorbent surface and co-contaminant, the latter may exhibit either enhanced sorption or enhanced solubilisation. While such effects have obvious implications for the fate and transport of a variety of contaminants in aquatic systems, the main body of literature has focused on the potential for surfactants to remediate contaminated environments. In particular, the sorption of cationic surfactants to a variety of synthetic and natural solids is an effective means of immobilising neutral and ionisable organic chemicals in landfill leachate and contaminated waters, whereas the application of either non-ionic or ionic surfactants at concentrations above their critical micelle concentrations (CMCs) has been exploited to release neutral chemicals from aquifers and soils. Application of surfactants to enhance heavy metals removal has been also investigated. Some surfactants exhibit toxic effects, albeit at levels in excess of environmental concentrations, therefore their use to remove pollutants has to be considered very carefully. Despite their position as primary producers in the food chain in aquatic ecosystems, the macrophytes are among the first organisms reached by pollutants in aquatic environments. Such plants are used *in situ* as biomonitors because of their abundance and limited mobility. Floating macrophytes represent also a model system to forecast the impact of pollutants.
We determined the uptake and the effects of sodium dodecyl sulphate (SDS), a commercially important anionic surfactant on the duckweed (*Lemna minor* L.) and water velvet (*Azolla filiculoides* Lam.); these species have shown a remarkable effectiveness in phytoremediation. Furthermore, *Lemna* is regularly used in ecotoxicological studies. We studied the response of the above mentioned species to SDS considering as marker of abiotic stress, changes in ethylene production and the variation of the activities of the enzymes involved in oxidative stress response, such as guaiacol peroxidase (POD), as well as enzymes of phenylpropanoid metabolic pathway: phenylalanine ammonia-lyase (PAL), polyphenol-oxidase (PPO). Phenolics content was also determined, since they play an important role in defending plants against biotic and abiotic stresses and can be potential markers of non-visible plant damage. The obtained results showed that plant metabolism can be heavily affected by SDS, although both macrophytes were able to remove the surfactant.

Keywords: Aquatic macrophytes, surfactant, SDS, stress response.

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C4

Simultaneous absorption of Cooper, Zinc and Chromium on soils by *Sesbania virgata*

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Heavy metals (HM) incorporated to the ecosystems as a mixture can interact with each other and generate contamination. The negative effects caused by these HM mixtures could be mitigated through biological remediation strategies such as phytostabilization. Several species from *Sesbania* genus have elevated ability to tolerate heavy metals in soils. The tolerant behavior of native species from the Argentinean Pampas region is not documented until now. The goal of this work was to evaluate the effect of interactions between cooper, zinc and chromium, in binary form, on absorption and translocation in *Sesbania virgata* plants. The transfer of metal ions from contaminated soils to plants were discusses in term of Transfer Factor (TF). In order to determine the type of interactions existing between HM, a pot experiment was conducted under light and temperature controlled conditions. The HM were added in unary and binary mixtures solutions of Cu, Zn and Cr, with low and high concentrations. Also, an extraction procedure was developed to determine the content of metal available to plants. In general, in all treatments the TF of plants were <1. Therefore, the major accumulation of HM was in roots of the plants. The highest Cu, Zn and Cr concentrations in plants roots from different treatment were 557.7, 1469.8 and 160 mg kg-1, respectively. In binary mixture of Cu and Zn, Sesbania had taken major concentrations of these metals. Contrary, Cr was more absorbed by Sesbania in unary treatment. The bioconcentration factor average in roots were Cr (1.4) > Zn (1.3) > Cu (0.5), indicating that Sesbania virgata is more effective in removed Cr from soils. Our results suggest that plants of *Sesbania virgata* have capacity to tolerate and stabilized high concentrations of Cu, Zn and Cr. In view of its tolerance, they may be used for phytostabilization of metals in contaminated soils.

Keywords: soil, binary mixtures, heavy metals, phytostabilization

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C5

Characterization of plant-microbe associations from a heavy metal polluted area in SW Sardinia in view of their use in phytoremediation.

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The reclamation of metal polluted mine wastes by use of authoctonous bioresources combines the soil remediation with the restoring of biodiversity, habitat enhancement and conservation. Main purpose of our experiments were to assess heavy metal tolerance and accumulation, biomass and seed production of different plant species and to estimate the effects of symbiotic microbial populations on these plants, in order to select the most efficient plant-microbe associations for appropriate phytoremediation interventions in a severely contaminated area. Native plant species belonging to the botanic families Leguminosae and Compositae, plant growth promoting rhizobacteria (PGPR) and nodule bacteria, were collected from a Cd, Zn, Pb polluted dismissed mine area in SW Sardinia. Such species were characterized for heavy metal (HM) tolerance/accumulation, uptake of nutrient elements and interactions with the related symbiotic microbial populations using hydroponic culture, pot experiments and field plots. The content of nutrient elements and HMs was determined on plant samples using an atomic absorption spectrophotometer. Significant interspecific differences in the growth, HM relations and response to inoculation with microorganisms were found. Efficient and metal tolerant plant-microbe associations were detected. Synergistic and additive effects on plant growth and nutrition were observed in *Lotus* spp. grown in HM-polluted soil after combined inoculations with nodule bacteria *Mesorhizobium loti* and PGPR *Variovorax paradoxus*.
Phytoremediation of HM polluted soils using plant-microbe systems can be an important biological tool, provided that are solved the problems of limited HM bioavailability in soils and potential lack of competitiveness of inoculated microbial strains in field conditions, major constraints to the applications of such systems in phytoremediation interventions. A question needing further research is the role of microorganisms and plants in the cycling of metals in contaminated soils. A good way to integrate research and field applications can be germplasm collection from contaminated areas for establishing collections and facilities, useful for large scale seed and inoculants production for pytoremediation purposes, and for selecting suitable genotypes for phytoestraction or phytostabilization interventions in contaminated areas.

Keywords: heavy metals, Leguminosae, PGPR, phytoremediation, rhizobia, rhizosphere

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C6

Plant cover modification oil excavator (on an example of Perm region)

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Oil extraction in the Perm region is realized from 1929. Now in the territory region it is maintained an order of 6.4 thousand oil wells. The subject of research was phytocenoses and their technogenic modification under oil excavator. Research works took part in the Perm region in 2005-2009. The plant cover in territory of oil extracting changes under the influence of many factors such as mechanical force, changes of a water mode, salt pollution of substratum, oil pollution and atmosphere pollution.

By results of the conducted research maximum decreases and observe of species diversity take place under mechanical force. In primary stage the vegetative groupings generated on such sites, contain species of meadow plants (from 57,63 % to 90,48 %) and there are species of water plants (from 4,68 % to 29,06 %).

In the vegetative groupings generated under the influence of changes of a water mode of territory of a deposit, salt pollution of substratum, oil pollution and atmosphere pollution decrease in species diversity is noted.

The biodiversity of the vegetative groupings which are affected changes of a water mode, decreases, including a decrease in species riches, simplification of community's structure.

There is a decrease of community's species diversity under salt pollution of substratum. Here we can observe prevalence of nitrophillous forest plants and meadow plants.

At the raised maintenance of organic substances in soil that is a consequence of oil products, there is increase of species diversity for wood communities. In such places introduction of water plants and oligotrophic bog plants is characteristic.

In process of removal from a source of atmospheric pollution in wood communities there is a decrease in a species diversity and complication of structure of community. It is caused by introduction of meadow plants in edge sites located near to a source of influence and restoration of communities structure of vegetative groupings in process of removal from a source of influence.

Oil extraction leads to introduction of the sinantropic species concerning to meadow plants and water plants. At the initial stage of oil deposit operation mechanical influence on plant cover is played the main role. At this stage sinantropisation a plant cover leads to increase of species diversity. At long technogenic loading there is a receipt and accumulation geochemic active substances. At this stage decrease in species diversity is observed.

Results of work are used at a substantiation of functional zoning of protecred landscapes "Nizhnevishersky", "Kuedinsky"; by working out of the ecological monitoring program and its realization; by working out of the program of conducting biotic monitoring in territory of oil deposits with special conditions of Company "LUKOIL-PERM".

Keywords: Oil extraction, plant cover, species diversity.

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C7

Degradation of estrogenically-active compounds by crop and riparian plants

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Natural estrogens and synthetic estrogen mimics can enter the environment from manure-fertilized fields and may be harmful to aquatic systems, but the environmental fate of such hormonally-active compounds has not been fully elucidated. To assess the role of plants in the fate of two natural estrogens (estradiol and estrone) and one synthetic mimic (zeranol) and to evaluate the possibility of using phytoremediation to remove these compounds from agricultural runoff, we have examined estrogen and zeranol uptake and degradation by plants. Specifically, we evaluated degradation of these compounds by corn and pea seedlings and hybrid poplars in both hydroponic and soil experiments. In previous work we have explored the soil sorption of these compounds, and using those data we have completed mass balances for the fate of estradiol, estrone, and zeranol in soil uptake experiments in order to construct a holistic picture of the role of plants in the fate of these compounds. We observed rapid decreases in zeranol and estrogen concentrations in hydroponic media exposed to corn and peas, with concentrations decreasing by up to 96% over eight days. The parent compounds were detected in the hydroponic solutions, roots, and shoots of corn and in the hydroponic solution and roots of other species. Concurrent to the degradation of zeranol and the natural estrogens, metabolites were detected in hydroponic solutions as well as root and shoot tissues. Metabolites were identified using LC-MS-MS, and the primary metabolite of zeranol has been identified as zearalanone while the primary plant-mediated metabolite of estrone is one which was previously identified as an intermediate in bacterial degradation of the estrogen. Further, these metabolites have been tested for their estrogenic potency.

Keywords: Estradiol, estrone, zeranol, hybrid poplar, crop plants

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C8

O-acetylserine(thio)lyase (*OASTL*) a prominent enzyme in the heavy metals responses in *Scorpiurum circinatum*

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§ C.I.S.M.E.

The toxic effects of heavy metals in plants are related to the inhibition of enzyme activity and oxidative damage into the cell. Most metal ions present in the shoots and roots of plants are bound to low molecular mass ligands or to proteins. The major types of S-containing ligands potentially al binding are metallothioneins, phytochelatins, and glutathione (GSH), all derived from cysteine.Cysteine biosynthesis in the plant cell proceeds via a two-step reaction: in the first step the amino acid serine is activated by acetyl-CoA to form *O*-acetylserine (OAS), through serine acetyltransferase (SAT) enzyme. In a second step *O*-acetylserine(thio)lyase (OASTL) enzyme inserts sulfide into OAS to form the amino acid cysteine.
Scorpiurum circinatum (Brid.) Fleisch. & Loeske is a widespread epiphytic moss in Mediterranean areas. Moss gametophytes were collected from Botanical Garden of Naples and cultured in vitro in Mohr modified medium with concentrations of 10-5 and 10-4 M of Cd, Pb, Zn and Cu for 24 h. After metal treatment the samples were prepared for TEM observations or for assays of *O*acetylserine(thio)lyase (OASTL) enzyme. TEM observation showed that heavy metals caused ultrastructural alterations of vacuolar system and thilacoidal organisation. In *S.circinatum*, OASTL activity seems strongly correlated with the ability to hyperaccumulate heavy metals like Cd, Cu and Pb. Through the various metals tested, Cu and Pb at very low concentration (10-5M) effected OASTL activity more than to that observed in moss, Cd-treated where OASTL activity enhances at higher concentration (10-4). Increased cysteine synthesis associated with heavy metals, appears to be a necessary response for biosynthesis of GSH and the other ligands involved in metal binding.
The authors suggest the use of OASTL enzymatic assay in *Scorpiurum circinatum* as one of the used bioindicators of some heavy metals.

Key words: *Scorpiurum circinatum*, OASTL, heavy metals, TEM, GSH, moss

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C9

Endophytic bacteria for toxicity resistance and growth promotion in PAH contaminated soil

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Popular tree hybrids were inoculated with *Enterobacter* sp. strain 638 and *Mycobacterium* sp. strain PYR-1. The cuttings will be grown while exposed to various concentrations of PAH contaminated soil from a Manufactured Gas Plant. Growth and water usage are recorded to show the benefits of the endophytes and trees will be harvested and root, leaf, and stem samples obtained. We expect to recover the inoculants from the roots of trees, which better tolerated the PAH exposure. Contaminant concentrations will be measured in the soil before tree planting and after the experiment is complete and in the trees after harvesting. We hope to find an indication of which endophyte may offer the co-benefit of PAH treatment stress resistance and increased biomass production.

Keywords: Phytoforensics, Site investigation, tree coring, SPME Chlorinated Solvents

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C10

Poplar-based phytoremediation : the PHYTOPOP experience

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Phytoremediation is the direct use of green plants and their associated microorganisms to stabilize or reduce contamination in soils, sludges, sediments, surface water, or ground water. Work on the fast growing and large biomass producer poplar (*Populus* spp.) suggests that there is a great deal of potential for its use in phytoremediation The PHYTOPOP project aims at establishing large-scale field trials on contaminated sites, to test the most appropriate poplar management regime for phytoremediation. There is a growing consensus that carbon-dioxide emissions from burning fossil fuels is one of the main factors in altering the global climate. The EU aims to meet 15% of its real primary energy demand with energy produced from renewable sources. The use of poplar short rotation coppice (SRC) could help meet these targets by providing a renewable energy source that produces very low net carbon-dioxide emissions. The relevance of high-density SRC schemes needs to be tested for phytoremediation, in large-scale field trials. The PHYTOPOP project includes 5 ha poplar field plantations, including 14 poplar cultivars, on two contaminated sites. Growth parameters, trace element distributions and symbiotic development have been measured after two years of growth. Additionally, the development of valorisation paths is necessary, especially for the woody biomass treatment and for the destiny of metal-enriched ashes. Large-scale assays have been performed in an experimental wood-fired boiler to estimate trace element distribution during the combustion process.

Keywords : combustion tests, mycorrhiza, phytoremediation, poplar, trace elements

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D1

Treatment of groundwater contaminated by volatile organic compounds in pilot scale constructed wetlands – concept and first results of PhD work

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Recently, constructed wetlands have received considerable attention as a cost-effective alternative of treating groundwater contaminated by volatile organic compounds. Removal of volatile organic compounds can, in principle, be carried out in wetlands using different removal processes: 1) mainly microbial degradation; 2) volatilization and phytovolatilization; 3) plant uptake and metabolization; 4) sorption. Three different types of pilot scale constructed wetlands were established: the traditional horizontal subsurface flow wetland, the horizontal subsurface tidal flow wetland and the floating plant mat. The concept is to investigate the removal efficiency of volatile organic compounds (chlorinated hydrocarbons, BTEX aromatics, etc.) and its seasonal variability in these three systems. Scientific investigations include process characterization, identification of the microbial community as well as measurements to study volatilization and phytovolatilization during different seasons. Technological investigations include optimization of the treatment and comparison of the different water levels and inflow pollution loadings. First results are demonstrated.

Acknowledgement: Zhongbing Chen would like to thank the China Scholarship Council for a PhD scholarship and the Helmholtz Interdisciplinary Graduate School for Environmental Research (HIGRADE) for support.

Keywords: constructed wetlands, wastewater treatment, chlorobenzene, BTEX aromatics, volatile organic compounds

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D2

Investigation of Phytoremediation Potential of Plant Species (Brassica napus L. and Amaranthus cruentus L.) for Ni-contaminated Soils

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At present, biosphere pollution with heavy metals (HM), nickel in particular becomes increasingly actual problem. Ni contamination is harmful for vital activity and yield of most crops. The minimal toxic concentration of Ni in crops is about 1.0 mg/kg dry wt (Liphadzi a. Kirkham, 2006). However, many species of terrestrial plants could inhabit soil ecotops enriched in Ni. Most of them are Ni hyperaccumulators (from 1.0 to 30 g Ni/kg shoot dry wt) (Raskin, 2000 et al.). The obstacle for application of natural HM hyperaccumulators for phytoremediation is their relatively small biomass and low growth rate. Many investigations were based on assumption that some plants species may be found among which were wild plants growing naturally in contaminated areas or of some cultured species accumulating Ni. The aim of this project was to identify plant species with strong ability to take up Ni from contaminated soils.

Screening of Ni-accumulating species could be performed among many bioenergy crops (Brassica napus L.) and ruderal plant (Amaranthus cruentus L.) inhabiting megapolis waste burying in humidity zone of Russia (Madzhugina et al. 2008; Shevyakova et al. 2010). Both of these plants have been the subject of our investigations. Plants were grown in a growth chamber under water culture for 5 weeks were subjected to rooting media NiCl2 In 5 days after treatment (150-250 M NiCl2) damaging nickel action was manifested in reduction of the root system size, the decrease of the above-ground weight and the content of chlorophyll and especially Fe in leaves. However, the cultivation of the plant in presence of elevated Fe content in growth medium (100 M FeEDTA) or spraying leaves with polyamine putrescine (1mM) reduced markedly Ni toxic effect on plant growth and increased Ni content in young leaves. The results suggest that obtained data open a possibility to develop technology for use of rape and amaranth for soil decontamination from Ni.

Keywords: Brassica napus, Amaranthus cruentus, Fe EDTA , putrescine, phytoremediation

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D3

Oxidative and non oxidative stress responses of *Pteris vittata* and *Populus* hybrids grown on arsenopyrite ashes: a field and *ex situ* experiments.

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Iron-bearing sulphide minerals are largely responsible for the generation of mine drainage and hence the release of heavy metals and arsenic into the environment. Roasted arsenopyrite in dumped ash from the production of sulfuric acid has raised significant health and environmental concern. Current technologies applied for the remediation of arsenic-contaminated sites are expensive and environmentally disruptive. Nevertheless, phytoremediation through arsenichyperaccumulating ferns such as *Pteris vittata* L. and *Populus* hybrids has gained interest due to its cost-effectiveness and environmental soundness. In this respect, we have reported a preliminary study on plant-dependent restoration of a soil used as dumping site for arsenopyrite ashes in a industrial area in South Tuscany (IT). Pot trials *ex situ* and plot trials *in situ* have been set up in order to assess plant viability, arsenic hyperaccumulation capability, enzymatic and non-enzymatic oxidative stress responses (catalase activity, superoxide-dismutase activity, and thiolic acid) in leaves and roots. The data obtained were subjected to statistical analysis and some phytoexctraction parameters were considered in order to carry out an application of this technology, *in-situ,* with the installation of a pilot scale experiment.

Keywords: Heavy metal, arsenic, phytoremediation, Pteris vittata, Popolus hybrids, roasted arsenopyite, catalase, superoxide-dismutase, ascorbate peroxidase, thiolic acid.

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D4

A potential feedstock biomass for energy generation processes from seawater microalgal

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Renewable sources of power derived from biologically based fuels are biomass, methane, ethanol, and one possible biological fuel source is the microalgae. Their high photosynthetic efficiency (5% of the incident solar radiation in outdoor massive culture) and high areal productivities (15-25 g m-2 d-1), turned on the attention on these photosynthetic microorganisms as potential feedstock in energy generation processes. Algal CO2 sequestration, 1.8 g of CO2 per gram of biomass produced, by means of selected strains has been also proposed as a method for removing GHG from power plants's flue gases. Isolation of naturally occurring microalgae represents the firs step in selecting promising strains to be subsequently employed as biomass producers. Samples were collected along the Tuscany cost both from sea and brackish waters. Several diatoms (*Nitschia* sp.) and cyanobacteria (*Nostoc sp*.) strains were present in the collected water samples. The most interesting isolate was a chlorophyte of the genus *Tetraselmis* collected from the mouth of river Scolmatore (PI). 132 mg of *Tetraselims* sp. biomass were obtained from 150 ml air bubbled (3 % CO2 v:v)
glass tubes continuously illuminated. Thermogravimetric analysis (TGA) of the collected biomass was conducted to have a first characterization of its combustion properties. Three different combustion phases where individuated. The main thermal degradation (60% of weight loss) occurred between 250-500 °C. Residual humidity (3%), ash content (9.8%), volatile solids (82,3%) and fixed carbon (4,9%) were also determined.

Keywords: microalgae, renewable source, biofuel, energy, thermogravimetric analysis

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D5

Mix for forage growing on metal contaminated soil: uptake and translocation by eight plant species

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The aim of this paper it is to present the results from experimental tests on the accumulation of As, Cr, Co and Pb by eight species normally mixed in forage for animals. Most of the seeds of this mix are metals excluders, but others no. So the first target was to analyze separately the contribution of each species.
The species tested and the percentage composition in the seeds mix was: *Lolium perenne* Livree 26%*; Lolium multiflorum L.* 15%*; Trifolium pratensis* 13%*; Glomerata amba* 11%*; Festuca arundinacea Demeter* 11%*; Dactylis phleum Pratense climax* 10%*; Lotus corniculatus* 7%*; and Trifolium repens* 7%. We added in the solution 4 different metal concentrations : for As and Pb 10 µgL-1,100 µgL-1, 200 µgL-1 and 500 µgL-1, for Cr and Co 50 µgL-1, 500 µgL-1, 1000 µgL-1 and 2500 µgL-1 We focused the objectives of the research into: (ì) analyze the uptake by each species, (ìì) compare the metal concentration in each species and in the mix (data obtained in a previous research), (ìì) calculate BCF (bioconcentration factor) and TF (Transolcation factor), (ìv) determine the metal concentration in soils after the plants removal.
These metals and metalloids, present in the environment, come from natural source as well from human pollution. Consequently the soil cannot be used for crop production without cleaning up and/or before measuring metal concentration. When present in water and soil they can accumulate and translocate in plants. The metal that moves from soil to forage plants can enter in the food chain.
In the discussion we present the results in order to show the interaction between each plant and to suggest appropriate mix for forage to use in soil polluted by specific metals and/or metalloids.

Keywords: forage, animals, food chain, land contaminated, trace metals

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D6

Effects of heavy metal stress in *Leptodictyum riparium* Hedw.

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Heavy metals (HM) are very toxic environmental pollutants; their presence in the atmosphere, soil and water, even at low concentrations, can cause serious problems to all organisms. Plants can respond to HM showing different effects: some plant undergo morpho-physiological and/or genetic modifications at organism level, others show alterations at population or community level. Such effects depend on accumulation of HM. Furthermore plants have several defence mechanisms against HM. Plants respond to HM in a very complex way, by evoking a number of parallel and/or consecutive events at molecular, physiological and morphological levels.
The objective of this study was to evaluate the effects of HM on the aquatic moss *Leptodictyum riparium* (Hedw.). For this purpose, cell ultrastructure modification, bioaccumulation, fitichelatins and HSPs induction, PAL activity, proteomics and stress ethylene production induced by HM have been determined in both treated and untreated plants.
L riparium, collected at the Botanical Garden of the University of Naples Federico II, was cultured in Mohr medium and exposed from 10-4 to 10-5 M of the following metals: Zn, Cu, Cd, Pb.
Sampling was made at 6,12 and 24 hrs after the treatments. The metals were uptaken by the plants, dose-response accumulation was detected. Zn and Cu wereabsorbed more than other ions. Ultrastructural alteration affected thylacoidal and vesicular organization. Fitochelatin and HSP were induced by HM. PAL activity of *L. riparium* was enhanced by the presence of HM. The protein synthesis change when the moss was treated by Cd. No changes of ethylene production rates was detected in plants treated with HM except for the plant exposed to Cu.
This study demonstrated that *L.riparium* could be used as model organism in phytoremediation projects.

Key words: moss, *Leptodictyum riparium*, ethylene, PAL, HSP, fitochelatin, bioaccumulation,
proteomics

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D7

Effects of heavy metals on *Lemna minor* L.

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Plants have been suggested as good indicators of heavy metal pollution: a sequestration of heavy metals (HM) in particular tissues to preserve the cells from toxic effects has also been demonstrated. Plants exhibit different mechanisms to bind heavy metals, such as the synthesis of amino acids, citric acid, malic acid and phytochelatins. Among the different mechanisms of resistance to heavy metals stress, the induction and the increase of the Heat Shock Proteins play a primary role counteracting these toxic effects, protecting proteins from misfolding and proteolytic pathways. These pollutants can cause alteration both in ultrastructure and also in biochemical systems in *Lemna minor* L.. In our work the effects of Pb, Cu, Zn e Cd at two different concentration 10-4 M e 10-5 M were studied. In particular we report data on bioaccumulation, tissue and cell localization (ESEM), ultrastructural alteration, PAL activity, HSPs induction, chlorophyll concentration.
Data show that the metals were uptaken by the plants and dose-response accumulatted. Zn and Cu were absorbed more than other ions. Different tissues accumulated in different way HM. Ultrastructural alteration affected thylacoidal and vesicular organization. HSPs were induced by HM. PAL activity of *L. minor* was enhanced by the presence of HM.
This study show the high bioaccumulation ability and toxi-tolerance of *L. minor* anddemonstrated that *L. minor* could be used as model organism in phytoremediation projects.

Key words: Lemna, heavy metals, PAL, ESEM, HPS, bioaccumulation, water

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D8

Effects of arbuscular mycorrhizal inoculation and P supply on the growth of *Lactuca sativa* L. and As and P availability in a polluted soil

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DISSPAPA

Arsenic (As) toxicity has become a global concern owing to the ever-increasing contamination of water, soil and crops in many regions of the world. Elevated soil As concentrations generally decrease the ability of the soil to support an economically viable crop and safe for health on contaminated sites.
Arbuscular mycorrhizal fungi (AMF) can form symbiotic relationships with the vast majority of land plants, and are known to benefit the phosphorus (P) of the host plants by increasing P acquisition. Due to the physicochemical similarity between phosphate and arsenate, AMF are likely to have a strong influence on arsenate uptake and resistance. In this study, the role of P application and AMF on growth, As and P accumulation in lettuce plants growing in an Italian As-polluted soil (total As 250 mg kg-1), was investigated.
In particular, it was tested whether application of a commercial inoculum (CI) (*Glomus intraradices*), with (+P at 90 kg P ha-1) and without (-P at 0 kg P ha-1) P fertilizer, supported greater plant growth and provided more P, enhancing As tolerance, than indigenous fungi alone. The influence of these treatments on As and P availability in the rhizosphere and bulk soils was also investigated. Greenhouse pot experiments were established where plants were grown with and without commercial inoculum (+CI,-CI). Inoculation with commercial inoculum and P application together considerably increased plant biomass, by enhancing host plant P nutrition and lowering shoot and root As concentrations compared to plants inoculated only with native AMF. In the rhizosphere of +CI+P plants there was P soil depletion compared to -CI+P. The results evidenced that, with P addition, inoculation with commercial inoculum alleviated the toxicity of excessive As by improving P nutrition without increasing As concentrations in the plant, emphasizing the role of beneficial microbes and P fertilizer to improve soil fertility and to minimize the ingestion of As through the consumption of crops grown in As-contaminated soil.

Keywords: Arsenic, phosphorus nutrition, arbuscular mycorrhiza, yield reduction, soil unproductiveness, rhizosphere , health safety

D9

Non conventional water sources: a sustainable management strategy for oasis restoration in Southern Algeria

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Oases are the main spots of human development in desert areas. Biotic and abiotic constraints together with human actions determine the fragile equilibrium of the ecosystem that is threatened by desertification. The study presents an integrated, sustainable solution for the oasis recovery and development, which could access also to carbon credits market through the Clean Development Mechanism (CDM) of the Kyoto Protocol.
The main purpose of this study is to set up a new oasis management strategy based on recovering and treatment of wastewater effluents gathered from the nearby human communities. The no conventional water resource becomes a win-win solution for reducing risks of water table pollution and for promoting new agro-forest activities. Wastewater is treated by a constructed wetland system to enhance environmental benefits. The model uses a multipurpose forest plantation that, reclaiming unproductive and damaged land, protects oasis from ongoing soil erosion, curbing desertification as well as sequestering carbon from atmosphere.
The feasibility study is developed for the oasis of Brézina in the Wilaya of El Bayadh in Algeria.
We optimized, based on local weather and soil condition, a modular system composed by a constructed wetland sized for a rate flow of about 100 m3 per day and an agro forest module designed to match the quantity of treated wastewater available. The module is designed to be replicated several times in order to face potential increase of wastewater outflow (replication in parallel) as well as more stringent water quality requirements for treated effluent (replication in series). The criteria used for the specie selection of agro-forest module are the adaptability to extreme conditions such as aridity and salinity, the biomass production and the supply of nontimber products. We present cost/benefit analyses considering the ecological, social and economic aspects that demonstrate the viability and sustainability of the design as a model of development and the layout of a small-scale pilot study under realization.

Keywords: oasis, carbon sequestration, constructed wetlands, sustainability

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D10

Vetiver system for wastewater treatement and landfill leachate disposal

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The Vetiver System (VS) is based on the use of vetiver grass (*Chrysopogon zizaniodes* L.). Extensive research has established vetiver tolerance to elevated and sometimes toxic levels of salinity, acidity, alkalinity, sodicity as well as a whole range of heavy metals and agrochemicals. Latest research also shows its exceptional ability to absorb and to tolerate extreme levels of nutrients, capable of consuming large quantities of water under wet conditions and to produce a massive growth. These attributes indicated that vetiver is highly suitable for treating polluted wastewater from industries as well as domestic discharges. Application of VS for wastewater treatment is a new and innovative phytoremedial technology, researched and developed in Australia, China and Thailand over the last 15 years. It is a green and environmentally friendly wastewater treatment technology as well as a natural recycling method. Its end-product has several uses including animal fodder, handicraft and material for organic farming. Due to its effectiveness, simplicity and low cost, the Vetiver phytoremedial technology is being used very effectively and successfully in more than 100 tropical, subtropical and Mediterranean countries, including Italy, Spain and Portugal, for the treatment and disposal of municipal sewage effluent, domestic sewage effluent, landfill leachate, industrial wastewater from abattoir, food processing factories and contaminated water from land development and suburban sites.

Keywords: Vetiver grass, wastewater, effluent, landfill leachate

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E1

Phytotecnology for food safety and health benefit

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New phytotecnology can been used to approach emerging needs related to food need and human healthy. Tomato breeder are using wild tomato relatives, even non-cross compatibles ones, in order to obtain cultivars with highly commercial values bearing new traits. Breeding procedure foreseen a limited control on traits not linked with the ones submitted to selection. However, the introgression of a wild genome into the cultivated one produces a new gene combinations that may lead to the expression of undeliverable traits for human safety, perhaps not so easy to recognise. Our goal is to evaluate by a ionomic approch the modification of ions uptake and accumulation in tomato plant organs, related to breeding procedure. A complete Introgression Line population produced by interspecific crosses between *Solanum lycopersicum* cv. M82 and the wild specie *S. pennellii*, are available in our Department. Until now, the contribution of the genome of *S. pennelli* for ion biofortification and food safety in the tomato varieties is not studied. Tomato ionome was studied in *S. lycopersicum* cv.M82 and *S. Lycopersicum x S. pennellii* IL 6-4 plants, grown in hydroponics in a growth chamber (18 h light at 26°C, 8 h darkness at 18°C, 60% RH, 300 µmol m-2s-1 PPF) and treated for 15 days in the presence of non-letal concentration of Cd (10 µM), Pb (3 µM), Zn (100_µM) given separately or combined. Heavy metals and micronutrient distributions induced by treatments were revealed by ICP-MS performed on *S. Lycopersicum* cv.M82 and IL6-4. The ionome of *S. lycopersicum* M82 and IL 6-4 control plants resulted statistically indistinguishable; the ionome of treated plants resulted changed in a significative mode and showed that both respond to treatments modifying ions homeostasis.
Correlation studies carried out by comparing ionome profiles obtained for each metal treatment given separately to combined treatment ones showed a strong positive correlation between the presence of zinc and the exclusion of other toxic metals. The introgression of the wild genome into the cultivated one produces a new phenotype that lead to the expression of traits linked also to adsorption, translocation and accumulation of useful and/or toxic metal into plant tissues and organs, probably correlated to the up-regulation and/or down regulation of metal uptake proteins.

Keywords: *Solanum licopersycum* cv M82, Introgression Lines 6-4, ionomic approch, Inductively Coupled Plasma-Mass Spectrometry, food safety

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E2

Potential of Sunflower for Phytoremediation of Heavy Metals from Contaminated Soil

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Sunflower is a promising environmental crop candidate for extraction of heavy metal and radionuclide from water and soil. Soils are generally contaminated by a mix of heavy metals, that can cause problems of plant toxicity and interact with other elements modifying their bioavailability and the accumulation capacity of the plants. Based on analysis of contaminated soils in Sites of National Interest of Basilicata, we observed that in many areas there is a mix of contaminants, such as Cd, Zn and Cu. The present study has the following aims: a) to study the physiological behavior of sunflower, grown on soil artificially contaminated with Cd, Zn and Cu, and the effect of contamination on biomass production; b) to evaluate the phytoextraction capacity; c) to analyze the accumulation of heavy metals in different portions of the plant (roots, stem, leaves, head). The experiment was carried out on sunflower (*Helianthus annuus* L.) subject to four different treatments: uncontaminated soil ©; contaminated soil with respectively 5 mg/kg of Cd (Cd); 5 and 300 mg/kg of Cd and Zn respectively (Cd-Zn); 5, 300, 400 mg/kg of Cd, Zn and Cu respectively (Cd-Zn-Cu).
In the pluri-contaminated treatments was observed a greater alteration of water relations and chlorophyll content then in the treatment with Cd alone. Although there were not great differences in biomass production, in the treatments Cd-Zn and Cd-Zn-Cu a significant reduction of stem dry matter was observed. The first data show that sunflower has a good capability to accumulate heavy metals, particularly when the soil is pluri-contaminated, mainly in roots and in lower leaves.
Good biomass and oil production for technical purposes, such as lubricants or renewable energy (biodiesel), makes this crop interesting for phytoextraction use.

Keywords: sunflower, phytoextraction, cadmium, copper, zinc, water relation, plant growth

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E3

On the potential use of an *in vitro* screening: the case of three *Populus alba* L. clones

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The increase in organic pollutants and heavy metal concentrations in natural and agricultural ecosystems has become a widespread problem. Several techniques for removing heavy metals from contaminated soils have been developed, but most of them are expensive, can create long-term destabilization of the site and have the potential for increasing contaminant mobilization. The process of phytoextraction is one of the principal method adopted in plantations for reducing heavy metal toxicity. Among hardwood species poplars held promise for phytoremediation because they are adaptable to grow on contaminated areas and able to accumulate metals but the investigation of possible differences among poplar clones in metal tolerance and accumulation deserves to be deeply studied and exploited. Therefore, for improving the application of these plants to phytoextraction, our study aimed at evaluating the response of three clones of Populus alba L. to arsenic (0, 5, 50, 250 μM), cadmium (0, 5, 50, 250 μM), zinc (0, 250, 1000, 2000 μM) and copper (0, 5, 50, 250 μM), in terms of tolerance and accumulation in an *in vitro* system.
Data demonstrated that *in vitro* screening of cuttings really represents an easily reproducible test method and an helpful way of assessing the ability of different poplar clones to take up, tolerate and survive metal stress. In fact, for the metal concentration used in the experiments, clones of Populus alba showed variation in metal tolerance and metal content. In the three clones, the root responded more sensitively than the shoot to toxic metal concentrations and the reduction in shoot biomass production was never significant for all the clones and all the metals for the concentration used. Considering the non significant effect of metal treatments on shoot growth for the concentrations used, data showed that poplars can accumulate high levels of these metals with no adverse effect on their biomass production.

Keywords: poplar, metals, tolerance and accumulation variability, clonal selection

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E4

Particulate matter deposition on leaves depending on height in tree and traffic-related crown side

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Particulate matter (PM) is known as one of the major health-affecting pollutants of urban atmosphere. Phytoremediation as a plant-related technology of reducing ambient air pollution has been under research. Plants, especially deciduous trees and shrubs, are likely to accumulate big loads of harmful particles on their leaf surface. It is known that air purifying properties vary among plant species. However the data available is still quite limited,. One of the important factor is the possible variation in PM accumulation on leaves on different sides of plant crown (1) directly exposed to the traffic pollution (road side) and (2) opposite ones (pavement side). The next not evaluated parameter yet is the vertical distribution of particulate matter on tall trees – important for general assessment of urban trees effectiveness in air purification. In this study we attempt at evaluation of these factors. Leaves of *Cornus alba* L. plants, a common densely leaved shrub, often grown in urban sites, were harvested from the road side and from the pavement side of the plant. For effect of tree height on PM accumulation on leaves a four tall, column-shaped *Populus nigra* L. 'Italica' trees were used. Leaves from ten height levels ranging between 2 and 20 meters were collected for the assay. In both species amount of particles rinsed with water and chloroform, of three size fractions for each group of leaves were analyzed. The measured fractions were: (i) 0,2-2,5µm, named fine particles and widely known as very harmful fraction, (ii) 2,5-10µm, named coarse particles and (iii) 10-100µm. Amount of waxes and leaf area of samples taken for analysis were determined too. Particulate matter accumulation was significantly higher on leaves exposed to the traffic side than on the other side, which gives the evidence of filtrating ability of the plant. The analysis of vertical distribution of PM showed that differences were not significant, even between the lowest and highest level.

Keywords: air pollution, phytoremediation, PM

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E5

Production of biodiesel from plants irrigated with sewage water

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Utilization of sandy soil and sewage water in the production of biodiesel is sound resource management. In this work, pot experiment was carried out. Raw sewage in different ratios was used to irrigate canola and safflower cultivated in sandy soil. Soil and water physical and chemical parameters were determined. Plant morphology and growth features (branching, fresh weight, dry weight, no. of pods and flowers….etc.) were carried out. Seed yield for both plants were also determined. Heavy metals (Ni, Cu, Mn, Pb, Zn, Cd, and Fe) were determined in sewage water, soil, biomass and seed cake. Results were tabulated and discussed.

Key words: Biodiesel, canola, safflower, sewage and sandy soil

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E6

Bioaccumulation of heavy metals and arsenic in plants that colonize tailings: a phytoattenuation process

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We are presenting a synthesis of the data we have obtained in the research we have made, in terms of evaluating the contents of some heavy elements and arsenic present in different species of plants grown in the tailings and in the surroundings of an abandoned mine (Vale das Gatas Mine – Vila Real – North of Portugal). We have analysed samples of five species of authochtonous plants (*Agrostis castellana*, *Holcus lanatus*, *Pteridium aquilinum*, *Juncus effuses* and *Pinus pinaster*) grown in the tailings and in the zone of secondary dispersion of the mine, and we have taken as a reference samples of representative sites of the local biogeochemical background. The values present in the plants range from 30 to 1297 mg/kg (values on dry weight basis) for the Fe, from 17 to 877 mg/kg for the Mn, from 0.04 to 3.99 mg/kg for the W, from 1.3 to 117 mg/kg for the Cu, from 7 to 471 mg/kg for the Zn, from 1.5 to 77.7 mg/kg for the Pb, from 0.7 to 4.2 mg/kg for the Ni and from 2.12 to 51.01 mg/kg for the As. The potential for colonization of highly contaminated mine tailings revealed by these native species can be very interesting because it constitutes a natural attenuation of contamination (phytoattenuation) until they are taken advanced decontamination processes.

Keywords: Abandoned mine, native flora, soil contamination

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E7

Evaluation of phytostabilization of mine tailings after biochar amendment

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Phytostabilization is one of the possible interventions to reduce the risks of contamination posed by mine tailings. This technique aims at reducing the mobility of the pollutants by establishing a green cover on the wastes. Recent studies, have focused the attention on biochar application to soil.
Biochar is a C-rich product obtained when biomass is heated with limited oxygen supply and at relatively low temperature (<700°C). Some experiments revealed the potential benefits that biochar application induces to the soil in terms of property changes that turns into yield improvements. Biochar also seems to reducing the uptake of toxic elements by plants by reducing their bioavailability. The case study is represented by the past mining site of Cave del Predil (NE Italy) where about 20 hectares of dumping site lay undisturbed. Among the local species found on the site, *Poa alpina* and *Anthyllis vulneraria* were selected as possible candidates for phytostabilization.
Two other species were selected: *Festuca rubra* and *Buphtalmum salicifolium*. The pot experiment aims at testing the four species on the following substrates: control (untreated mine tailings); 3 levels of equal rate of biochar and compost amendments applied to the mine tailings (0.5, 1 and 2%dw); the previous 4 substrates with mineral fertilization (NPK). At the end of the growing period, the plants will be harvested and the heavy metals content will be analyzed in their tissues (roots and shoots). The data from the metal uptake and accumulation and the biomass production will give important hints on the feasibility of the remediation process.

Keywords: phytostabilization, mine tailings, biochar.

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E8

Cd Accumulation and Phytochelatin Synthesis in Two Ecotypes of the Hyperaccumulator Species *Dittrichia viscosa*

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Plants growing in heavy metal-polluted sites show several responses to this stress. However, the mechanisms involved in accumulation and tolerance are not yet fully understood. *Dittrichia viscosa* is a species with a great potential for phytoremediation, due to high Cd accumulation capacity, high biomass and adaptation to our regional soil and climatic conditions. In order to elucidate if the Cd accumulation capacity of this species is related to the synthesis of phytochelatins (PCs) and to original environment we compared two contrasting ecotypes of *D. viscosa*, as they would show different responses under a short-term exposure to Cd. Clone DV-A, capable of accumulate up to 1300 mg Cd kg-1 dry wt. in shoots when cultured *in vitro* for 40 days [1] and which comes from seeds of *D. viscosa* collected in Asturias in a heavy-metal polluted area. Clone DV-W comes from seeds kindly donated by Kew's Royal Botanic Garden (UK) that were collected in a non-polluted area. In contrast to DV-A plants, which have been exposed to a chronic Cd stress, plants of DV-W might not have developed strategies for coping with a heavy-metal stress. DV-W plants cultured *in vitro* in ½ MS medium supplemented with 3 and 90 µM Cd for 6, 12 and 24 hours accumulated equal and even more Cd than DV-A plants. In all cases Cd content in roots was higher than in shoots. According to these results, the Cd accumulation capacity shown by *D. viscosa* is not determined by the environmental conditions, as the ecotype coming from the heavy-metal polluted soil accumulated less than the non-adapted one.
The analysis of PCs showed that plants of *D. viscosa* synthesize glutathione (GSH), PC2, PC3 and PC4 and two other non-proteic thiols (CT1 and CT2) in shoots, and GSH, PC4 and CT2 were found in roots but in a lesser amount. No new compounds were observed neither between control plants and plants treated with 3 and 90 µM Cd nor between both ecotypes. A remarkable quantitative difference between ecotypes is the fact that PCs content in shoots of DV-A exceeded that of DV-W, whereas in roots of DV-W there was a higher PC content than in roots of DV-A. However, there was not a clear pattern of PC synthesis which could correlate with Cd accumulation, and moreover, PCs were found even at the beginning of the treatment. Thus, it seems that these peptides are constitutive and not directly related to the Cd accumulation capacity of *D. viscosa* and that there are other, alternative mechanisms contributing to Cd-accumulation in this species. Therefore, the Cd uptake and tolerance capacities of these two ecotypes are now being checked in greenhouse in a long-term exposure to Cd artificially-polluted soil in order to know if the results obtained in vitro will repeat under more realistic conditions.

[1] Fernández R, Bertrand A, Casares A, García R, González A, Tamés RS. (2008). Cadmium accumulation and its effect on the in vitro growth of woody fleabane and mycorrhized white birch. Environ Pollut ;152:522–529.

Keywords: cadmium, *Dittrichia viscosa*, ecotype, hyperaccumulation, phytochelatins.

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E9

Use of barley plants to study phytoremediation of sulfonamides

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The application of contaminated manure to fertilize agricultural soils is among the major routes through which antibacterial sulfonamides (SA) enter the environment.
In this research we studied the interaction between plants and SA with the aim of utilizing vegetation in SA remediation projects. To this purpose barley was chosen as the model plant. After germination, seeds were grown hydroponically in a climate chamber for two days, then sulfadimethoxine (SDM) or sulfamethazine (SZ) 40 mM (ca 12/11 ppm respectively), were added to the nutrient solution. At different times (0 – 48 h) SA concentrations were evaluated in the nutrient solution through spectrophotometric analysis, validated by TLC. Similar experiments were conducted with cell wall fractions obtained by treating fresh roots with organic solvents.
In the presence of plants, SA concentration in the nutrient solution declined with time. After 48 h, SDM and SZ were accumulated in the roots with a bioconcentration factor of ca 19 and 6 respectively, whereas in the leaves they were not detectable. Even if cell walls accumulated much less SDM than fresh roots, data show that this tissue fraction can be an important sink for SA. Part of the supplied SA (about 25%) was not recovered at the end of the experiments, probably for some kind of plant metabolic transformation. No visible effect was observed in plants during the 48 h of experiments. However, after 15 d roots showed reduced weight and higher cell differentiation as revealed by optical microscopy, while leaves appeared healthy and with unchanged chlorophyll content. Further studies aimed at ascertaining the possible involvement of root enzymes in the improvement of sulfonamide uptake/transformation are presently in progress in our laboratory.

Keyword: antibiotics, barley, phytoremediation, sulphonamides

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E10

The use of Stable Nitrogen Isotopes of Tree Tissues as a Proxy Indicator of Subsurface Nitrate-Nitrogen Contamination

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Swine waste lagoons and waste application fields are a potential source of NH4 and NO3 contamination in groundwater and surface waters. Stable nitrogen isotopes can be used to determine nitrogen sources and their contribution to groundwater and surface waters when sources are specifically known. Swine waste has a unique δ15N signature, usually in the range of +10‰ to +20 ‰, while δ15N signatures for trees are usually in the range of -4‰ to +2‰. This difference can be used to differentiate between an anthropogenic and non-anthropogenic nitrogen sources to waters. This study focuses on the use of stable nitrogen isotope ratios, 15N/14N, of tree tissues as an indicator of subsurface nitrogen contamination from swine waste lagoons and waste application fields. I hypothesize that the δ15N isotopic signature present in tree tissues will reflect the integrated δ15N isotopic signature in groundwater and soil; trees that utilize groundwater containing swine waste should have an enriched δ15N signature relative to trees that do not. My primary objective is to determine the δ15N signatures of tree tissues and their spatial locations relative to known nitrate concentrations in ground water. Two sites will be used to test my hypothesis; one of the sites is an active hog operation while the other site is an inactive hog operation. Groundwater and lagoon waters will be sampled at each site and analyzed for nitrate concentrations, bulk δ15N signatures, δ15N signatures of nitrate, δ18O of nitrate, as well as δ2H (δD). Oxygen and hydrogen stable isotopes can be used to determine the contribution of swine waste to groundwater. Trees leaves and stem core samples, groundwater monitoring wells, and lagoons will be spatially recorded using GPS and digitally mapped using GIS. Tree leaves will be collected at different height intervals ranging from 6 to 24 feet and analyzed for nitrate concentrations and bulk δ15N isotopic signatures. Kriging analysis will be used to generate proxy nitrate plumes based on tree tissue isotopic values and compared to known nutrient and isotopic groundwater data.

Keywords: Phyto-monitoring, δ15N, δ18O, δ2H, δD, Swine Lagoons, Nitrates, Groundwater

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F1

USEPA Initiatives Related to Phytotechnologies

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This presentation will provide an update on several EPA initiatives related to phytotechnologies, ecorestoration, and carbon sequestation. Several ongoing projects will be discussed that make current useful information on phytotechnologies readily available to project managers and others interested in the latest technology developments. Projects include recent updates to a websites on phytoremediation, evapotranspiration (vegetative) covers, and ecorestoration. EPA has also conducted an extensive update on its alternative landfill cover database, and recently released a fact sheet on the current state of phytoremediation and is finalizing a more detailed fact sheet on evapotranspiration covers. The presentation will also discuss a urban gardening project to develop tools to help community members analyze property and make intelligent decisions concerning how to determine if land is contaminated, and what crops would be the best to grow. Lastly, the presentation will describe a project to create a methodology for measuring carbon sequestration, and the results from tests of this methodology at three sites.

Keywords: phytotechnologies, phytoremediation, ecorestoration, carbon sequestation, evapotranspiration covers, vegetative covers, urban gardening

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F2

Climbers as green walls in cities - environmental engineering for air phytoremediation

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Polluted ambient air in our cities became one of the most severe environmental problems and a major threat to life quality of their inhabitants. Regrettably, there is not much being done when it comes to absorbing air pollutants after they are emitted. Vegetation needs to be put in areas where pollution is present: along the streets and around the facades of buildings. Green belts of vegetation separating roads with intensive transport, the main source of pollution, from our houses and offices are well understood and desirable. Laboratories started evaluation and selection of species most efficient in accumulation of air pollutants, which would be suitable for such protection. In some countries municipal authorities in the newly planned and designed settlements and districts reserve 30% or more of land for vegetation.
Much more difficult situation is usually in the older parts of cities where ground with soil is limited. Vegetation in these places can be introduced only in vertical dimension in a form of a green wall. The buildings' shroud of vegetation plays a major role in absorbing pollution from the atmosphere. Moreover, this vertical landscape absorbs heat and noise, provides shading to the buildings, enhances humidity and supports biodiversity in those parts of cities. Supporting constructions for green wall vegetation will be presented. Amount of PM10, PM2,5 and PM0,2 deposited on leaf surface and captured by waxes (rinsed first with water and next with chloroform respectively) were measured. Amount of waxes and leaf area taken for analysis were also determined. From the fifteen commonly cultivated in Europe climbing shrubs the most efficient in accumulation of particulate matter (PM) were: *Lonicera acuminata* and *Actinidia kolomicta*. Results for them and other species will be presented.

Keywords: air pollution, phytoremediation, particulate matter, climbing shrubs

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F3

The movement and biotransformation of PCB congeners phytoextracted by *Cucurbita pepo* ssp *pepo* – from soil to plant to compost

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Polychlorinated biphenyls (PCBs) are ubiquitous organic xenobiotics posing significant ecological and health risks as a result of their toxicity and environmental persistence. PCBs were sold as mixtures of congeners, possessing between one and nine chlorine atoms attached to a biphenyl ring. Traditionally incineration has been used, at great environmental and monetary cost, to dispose of PCBs. A significant market seeking alternative remediation technologies now exists. While it has been demonstrated that *Cucurbita pepo* ssp *pepo* cv. Howden (a pumpkin) can extract PCBs from contaminated soil and subsequently translocate PCBs from the plant roots to shoots (Zeeb *et al.,* 2006, Whitfield Aslund *et al.,* 2007), little is known of how PCBs are transported *in planta*. To better understand how specific congeners move within plants a survey was undertaken that compared congener profiles of soil, plant roots, plant shoots at various locations, and xylem sap. Principal components analysis revealed distinct separations between each compartment surveyed. Understanding the differences in congener movement through plants may lead to enhanced PCB phytoextraction. The effects of composting on congener biotransformation and biomass reduction were documented through a controlled greenhouse study. PCB-contaminated pumpkin biomass was harvested and analyzed for congener profile at the end of the 2009 growing season. After five months of composting, clear differences in the congener profile of an Aroclor 1248-contaminated biomass were observed, while no differences occurred using Aroclor 1254/1260-contaminated biomass. During this time, volume reductions of ~84% and 83% were achieved, respectively. Reducing biomass volume through composting will concentrate PCBs in humic material, and ultimately there will be less biomass requiring storage or incineration.
Finally, a bench-scale bioremediation study using composted PCB phytoextractors was undertaken to document the dechlorination of highly chlorinated PCB congeners under the possibility of subsequent aerobic PCB mineralization. Differences to congener profile were inconclusive after six months of treatment, however, together the above projects have led to a better understanding of PCB congener movement and transformation from contaminated soil to plant to compost.

Keywords: soil, phytoextraction, compost, bioremediation, PCBs

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F4

Protection against Gamma Irradiation by Natural Biomass: Algae

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Algae living in harsh environments can develop peculiar survival processes. Especially cyanobacteria (blue green algae) are capable of growing in extreme conditions. In order to evaluate radiation shielding properties of some green algae; *Chlorella vulgaris, Scenedesmus obliquus,* and blue-green algae; *Synechococcus sp., Planktothrix limnetica, Microcystis aeruginosa, Arthrospira maxima* were cultured in batch systems at continuous light at room temperature and harvested by centrifugation when the algae were two weeks old. Lyophilized biomass was tested for it is its high tolerance to radiations in terms of measurement of linear attenuation coefficients.
In the present work, the linear attenuation coefficients (μ) were measured at photon energies of 662, 1170 and 1332 keV using γ-ray spectrometer. The spectrometer contains a 3"x3" NaI(Tl) detector connected to the Multi-Channel-Analyzer (MCA). The results were found in the range of 1.788 and 4.125 cm-1 for 662 keV, 0.104 and 0.984 cm-1 for 1173 keV, 0.203 and 1.206 cm-1 for 1332 keV photon energy respectively.

Key words: Gamma irradiation, algae, environmental protection

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F5

Uptake and Phytotoxicity of Zero Valent Iron Nanoparticles to *Typha latifolia*

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Use of Nano Zero Valent Iron (nZVI) for treatment of different halogenated hydrocarbons, arsenic and various other contaminants has been proved successful. However, with so much diversified use of nZVI in the field and heighted attention to engineered nanoparticles, the environmental fate and impact of the nZVI remains unknown. The goal of this project was to evaluate the effects of different types of nZVI on *Typha latifolia*, a common wetland plant. Plants grown hydroponically in a green house were dosed with different concentration of bare or bimetallic nZVI (with 10% and 50% nickel coating) for one to four weeks. The results showed that bare nZVI was toxic in high concentrations but enhanced growth of plants at lower concentrations. Bimetallic nano particles are significantly more toxic and resulted in plant death within a week. Scanning electron microscope (SEM) clearly showed the adsorption of the nZVI on the plant root surface, confirmed by x-ray analysis. Transmission electronic microscope (TEM) and light microscope confirmed the uptake of nZVI by plants roots.

Keywords: phytotoxicity, uptake, fate and transport, nano zero valent iron, typha,

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F6

Effect of *Rhizobium* and *Azatobactor* on lead (Pb) Phytoextraction and Maize Growth in Pb Polluted Soil

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The effects of *Rhizobium* and *Azatobactor* on Pb phytoextraction and their subsequent effect on the growth of maize (*Zea mays* L.) plant was evaluated in a green house study. *Rhizobium leguminosarum* strain TAL-102 and *Azotobacter chroococum* were used as single cultures as well as co-culture. In single culture inoculations, the *Rhizobium* showed better reponse than *Azatobactor,* whilst the co-inoculation treatmnet showed highly significant increase in growth as well as in dry biomass of plant in Pb polluted soil. Accumulation of Pb in different parts of plant was analyzed using atomic absorption spectrophotometer. Highly significant increase in Pb accumulation was found in plant co-inoculated with *Rhizobium* + *Azatobactor* as compared to control. In single cultures treatments, the *Rhizobium* was superior than *Azatobacter* in enhancing Pb untake by the plant. In roots and stem all the treatments either in single culture or coculture inoculation showed significant increase in Pb accumulation as compared to control, however the Pb translocation into leaves was significant only in co-inoculated plant. Conclusively the co-inoculation was much better than single cultures inoculations in Pb phytoextraction along with increase in plant growth and biomass.

Keywords: phytoextraction, lead, Maize, *Rhizobium*, *Azatobactor*

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F7

Effects of phytohormone IAA and chelator EDTA on lead uptake by Zn/Cd hyperaccumulator *Picris divaricata*

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In this paper, the effects of indole-3-acetic acid (IAA) and/or ethylenediaminetetraacetic acid (EDTA) on lead uptake by a newly identified Zn/Cd hyperaccumulator *Picris divaricata* were studied. Plants were grown in nutrient solution amended with Pb at 100 μM and different concentrations of IAA and/or 100 μM EDTA for 12 d. Results showed that P. divaricata responded to Pb by an increased biomass, enhanced accumulation and translocation of Pb in presence of phytohormone IAA, which also reduced the inhibiting effects of Pb on transpiration without reducing the uptake of Pb. The application of 100 μM IAA increased Pb concentration in leaves by about 37.3 % as compared to treatment exposed to Pb alone, and the Pb uptake of leaves was also increased by about 46.5 %. The enhanced phytoextraction efficiency could be attributed to IAA through alleviating Pb toxicity, creating a better root system and biomass, promoting a higher transpiration rate as well as regulating the level of nutrient elements. On the contrary, inefficiency of phytoextraction was found with EDTA or the combination of IAA and EDTA probably because most Pb was in the form of Pb-EDTA complex which blocked the uptake by *P. divaricata*. The present study demonstrated that IAA was able to enhance the phytoextraction of Pb by Zn/Cd hyperaccumulator *P. divaricata*, providing a feasible method for the phytoremediation of polymetallic contaminated soils.

Keywords: EDTA; IAA; Lead; *Picris divaricata*; Root system; Transpiration

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F8

Microbiome of Aromatic Degraders in Aspen Rhizosphere –Aiming to Enhanced Phytoremediation

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Phytoremediation represents sustainable way to restore contaminated soils. Phytodegradation of pollutant occurs in the rhizosphere (soil surrounding plant roots), as well as within the plant tissues. Rhizodegradation mediated by soil organisms such as fungi or bacteria or via enzymes exuded from microorganisms or plants. Two months greenhouse experiment was performed to study the change in microbial populations in soil and plants after oil contamination taken place. Aspen seedlings were planted in soil containing 1 % of oil. Tree heights were measured, the stress symptoms were recorded and soil and plant samples were collected weekly. Microbial population was studied by amplifying the proteobacterial 16S rRNA genes and functional genes encoding ring cleavage dioxygenases. Ring cleavage enzymes catalyses a key reaction in the degradation of aromatic hydrocarbon and are frequently found from proteobacteria. We have previously found novel ring- cleavage dioxygenases cluster from birch rhizosphere and from polluted soils. The aim of this project is to identify key aromatic degraders in aspen rhizosphere and soil by combining analysis of proteobacterial 16S rRNA genes and ring cleavage genes in temporal analysis. The aspen seedlings were tolerant to oil pollution and minute stress reaction was observed. The differences in oil tolerance stress were observed between the clones. Amplification of both 16S rRNA and ring cleavage genes was successful from bulk and rhizosphere soil.
Endophytic bacteria were isolated from aspen leaves and roots. Bacteria will be inoculated to aspen seedlings in vitro to study their response for plant growth in sterile conditions. Improved stress tolerance of aspen clones and enhanced phytoremediation is developed within this project by ecosystem services mediated by combination of aspen variants and their associated bacteria.

Keywords: aspen, polyaromatic hydrocarbons, aromatic degraders, endophytes

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F9

Long Term Phytoremediation -Where Science meets Art

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Traditionally, contaminated sites have first been treated by scientists to remove or mitigate pollution and then handed as a clean site to the Landscape Architects. However, trees and other long term solutions are now being left in place well into the period of reuse, which necessitates the Landscape Architect being involved right from the beginning of the process. This paper first aims to establish where phytoremediation is being employed in practice in the United Kingdom and then examines some projects from Europe and North America undertaken with multidisciplinary teams treating sites in a more sustainable and long term manner allowing public or private reuse whilst the remediation continues and draw conclusions on what working practice or technological lessons can be learned from these.
Finally a critique will undertaken of a piece of my own design work and desk studies will be used to provide an early technical and design assessment of a post-industrial site in the United Kingdom.

Keywords: Design, Architecture, Contaminated Land

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F10

Growth and Developmental Enhancement of Poplar (*Populus deltoide*s x *nigra* OP367) by Associated Endophytic Bacteria

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The ability to produce biomass for biofuel on a large scale is one way in which the United States will be able to become more energy independent. With controversy surrounding the use of corn for bioethanol production, more novel methods for biofuel manufacturing must be found. To be able to produce biomass for biofuel on the scale needed, fast growing, non-edible woody-plants should be considered. Here, we report on the inoculation of one such plant species; Poplar, (*Populus deltoides* x *P. nigra* OP367) with 7 different endophytic bacterial strains. When inoculated with one strain, *Enterobacter* sp. 638, the OP367 line has significant growth over the control poplars while inoculation with the other endophytes. The biochemical mechanism by which *Enterobacter* sp. 638 is able to increase plant growth is the production of plant growth hormones. This production is linked to the presence of plant or plant sugars, showing that this is an adaptation to the organism's endophytic lifestyle.

Keywords: poplar, endophytes, growth enhancement, biomass, biofuel

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G1

Proteomic Analysis in Poplar Clones Treated with Cadmium

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Dispersal of trace elements, metals and organics is relevant for environmental contamination and for fresh water utilization. Plant species have been used to decrease or remove the contaminants in polluted sites and in particular, tolerant and hyperaccumulator species seem promising for removal of metals in contaminated soils. The practical application of natural hyperaccumulators in phytoremediation is difficult because of their small biomass at maturity and reduced growth rates. For these reasons the metal uptake capacity of plants with higher biomass, such as trees belonging to Salicaceae family like poplar and willow, has been investigated. Laboratory and field trials have found that different clones of the genus *Populus* have different tolerance and metal uptake in contaminated environments.
In this work we studied the behavior of three Italian selected poplar clones: *P.nigra* (clones 58-861 and Poli) and a hybrid *P.nigra x deltoides* (A4A), which showed different Cd tolerance and capacity and Cd uptake, accumulation and traslocation (1).
The modifications occurring in the proteome of the three clones subjected to Cd treatments were investigated by comparing also the physiological behaviour of the clones. Rooted cuttings of three poplar clones were grown in hydroponic cultures with 0 µM CdSO4 (control) and 20 µM CdSO4 for 48h (short term treatment).
(i) Different physiological parameters were analyzed: total leaf area, stem growth and elongation of the roots. Metal uptake and root to shoot translocation were observed by mineral analysis by Atomic Absorption Spectroscopy (ASS). Metal compartmentalization in leaves, roots and stems were analyzed by Scanning Electron Microscopy with microanalysis (SEM/EDX).
(ii) Proteomic analysis was performed on crude protein extracts, obtained from leaves and roots. Proteins with different isoelectric point (pI) and hydrophobicity were separated by a 2D liquid chromatography technique (ProteomeLab PF2D, Beckman) (2).
Qualitative and quantitative differences between protein profiles of treated and untreated samples were evidenced by DeltaVue Software (Eprogen). Proteins differently expressed in various conditions will be further characterized by MALDI-TOF/MS to infer on their possible role in metal response.
The results will be discussed with the aim to understand the different processes at the basis of accumulation and tolerance of metal by plant species.

Key words*:* Poplar, cadmium, proteomics, phytoremediation, 2D-LC technique

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Uptake of p,p-DDE from contaminated soil by grafted Cucurbitaceae

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Two Cucurbitaceae, Zucchini, Watermelon, and their heterografts, and homografts were grown in a field containing soil contaminated with dichlorodiphenylethanes (DDT and DDD) and –ethene (DDE), concentration ranging from, 172 to 1934.88 ng/g, 8 to 407.04 ng/g, and 107.17 to 3294.64 ng/g, respectively. Heterografted watermelon plants, watermelon scion on zucchini rootstock, are commonly used to produce watermelon in many countries.
The purpose of this research was to explore the possibility of uptake and translocation of weathered p,p-DDE by grafted Cucurbitaceae grown under field condition in p,p-DDE contaminated soil. Intact plants, homografted, and compatible heterografts of zucchini and watermelon plants grown in the contaminated soil were analyzed. Concentrations of p, p-DDE in roots, shoots, leaves, and fruits were also compared for intact plants, homografted, and heterografted zucchini and watermelon plants.

Keywords: p,p- DDE, DDD, DDT, grafted, watermelon, heterografted

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G2

Accumulation of Weathered p, p–DDE in Xylem Sap of Grafted Cucurbitaceae

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Pot experiments, each cultivar growing in 6 pots, were conducted to assess the effect of grafting on phytoextraction of weathered p,p-dichlorodiphenyldichloroethane (p,p'-DDE) from soil to plant. Fifty pots were packed with an average of 6.2 kg of p,p'-DDE contaminated soil and divided into 6 main categories based on the following scheme: Intact plants, homografted, and compatible heterografts of Zucchini and Watermelon plants, and their 4 vegetated controls. Movement of the *p,p'*-DDE through the soil-plant system was investigated by comparing *p,p'*-DDE concentration in the bulk soil, the rhizosphere soil pore water, the xylem sap, and aerial tissue. Concentrations of p,p'-DDE in the rhizosphere soil pore water ranged from 0.36 µg/L (heterografted zucchini) to 0.53 µg/L (Intact plant of watermelon) and there were no statistically significant differences between grafted and non-grafted plants. Conversely, p,p'-DDE concentrations in xylem sap of heterografted watermelon is much more higher than that of intact plant of watermelon and homografted watermelon. The bio-concentration factors were 344, 325, 197, 1.28, and 0.90 for intact plant of zucchini, homografted zucchini, heterografted watermelon (watermelon scion on zucchini rootstock), homografted watermelon, and intact plant of watermelon, respectively. It is the first time our experiment showed that weathered p,p'-DDE were accumulated in xylem sap of heterografted watermelon plant.

Keywords: grafted, homografted, heterografted, p,p-DDE, xylem sap , BCF, watermelon

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J-Z

G3

Role of pH in phytofiltration by using *Elodea canadensis*

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Submerged aquatic macrophytes take up metals both through their roots and shoots, therefore are promising candidates for phytofiltration. *Elodea canadensis* is able to change the surrounding water pH and by that influence the metal uptake, making it suitable for treatment of metal polluted waters particularly acidic waters. The influence of surrounding water pH on metal uptake by *E. canadensis* was studied with the aim to optimize the plant use for phytofilteration.
Cd uptake by *Elodea canadensis* shoots was investigated after cultivation of plants in hydroponics with starting pH of 4.0 and different concentrations of cadmium (0, 0.1, 0.5 µM) and by monitoring the role of free metal ions in triggering the plant to adjust the rhizosphere pH.
The result showed that the cadmium uptake by *Elodea canadensis* depends upon the cadmium concentration and surrounding pH. When *Elodea's* shoots were grown at fixed pH, a positive linear relationship between shoot cadmium contents and surrounding water pH was observed.
Another interesting observation was that *Elodea's* shoots increased the surrounding water pH with a maximum of 1.4 pH units at the highest cadmium concentration (0.5 µM) and highest plant density treatment (6 plants). We concluded that cadmium uptake by *E. canadensis* is highly dependent on medium pH and free cadmium ions have some triggering effect on *E. Canadensis* that then change the surrounding medium pH. The increase of pH by *E. canadensis* will also increase the uptake of Cd due to the decreased interaction between Cd2+ and H+ caused by the lowered [H+]. We started investigating the mechanisms operational behind it. By understanding plant-induced pH-changes and its mechanism in plants exposed to metal stress may provide an effective approach for optimizing strategies to handle the water pollution as well as illustrating fundamental aspects of plant stress physiology.

Key words: *Elodea canadensis*, pH increase, cadmium uptake, phytofiltration

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G4

Portulaca oleracea, a potential candidate for contaminated soil?

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Phytoremediation, an emerging green technology, is a cost-effective, nonintrusive technique that uses the ability of certain plant species to remove toxic metals from the soil. The objective of the present study was to compare the performance of commonly reported hyperaccumulators, *Helianthus annus*, *Brassica juncea* and the hitherto little reported *Portulaca oleracea,* in the presence of electroplating effluent. The plants were grown in pots containing 3 Kg soil each, spiked 1%, 1.5% and 2% of electroplating effluent. The uptake of zinc, iron and chromium, were evaluated on the 30th, 45th and 60th day. Shoot uptake of Cr by *B.juncea* was significant ($p \leq 0.05$), on day -60, for 1% effluent contamination. *H annus* and *B juncea* plants individually could not survive in the effluent contaminated soil for more than 30 and 45 days, respectively. On the other hand, when both were grown together, they were able to withstand a maximum of 2% of effluent contamination. *Portulaca oleracea* was unaffected and was able to withstand more than 2% of the effluent contamination and showed no wilting even on the 60th day.Translocation of metals to shoots was seen only in *P.olereaceae,* on day-60 for 2% effluent concentration.

Key words : Soil contamination, hyperaccumulators, heavy metals , electroplating effluent.

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G5

Sustainable Ecosystem-Restoration of Manganese Mine Spoil Dumps Through Microbe-Assisted Green Technology

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A holistic approach called Microbe-Assisted Green Technology (MAGT) was developed at National Environmental Engineering Research Institute (NEERI) through comprehensive laboratory and field studies to serve as a model for land reclamation and development of green vegetation on mine overburdens. This technology involves the use of native metal-resistant plant species and indigenous plant growth promoting microflora along with other amendments (mainly organic matter rich soil bulking agents eg. pressmud) for the revegetation of mine dumps and phytostabilization of metal pollutants. One year old seedlings of native tree species were planted on 6.3 ha area of manganese mine overburden at Gumgaon under Manganese Ore India Ltd., Maharashtra, India. Addition of organic amendment (pressmud) facilitated the rapid establishment and colonization of these "pioneer plants". Site specific microbial inoculants enhanced the biogeochemical cycles and the regeneration capacity of the spoil. MAGT resulted in fast recovery of ecosystem, soil formation, organic matter accumulation, recycling of nutrients, microbial activities restoration and finally a self sustainable ecosystem on the barren manganese mine spoil dumps. Continuous efforts resulted in nutrient rich soil with high N, P, K and organic carbon; well developed biodiversity, including bacteria, fungi, higher plants (more than 350 species) and different classes of animals. Planted trees accumulated 698 t ha^{-1} above ground biomass and 143 t ha^{-1} below ground mass. This was achieved in 18 years by MAGT, which otherwise takes hundreds of years. At the same time, large crown canopy of the trees encouraged immigration of animals and turned source for shelter. In the perspective of mine restoration the MAGT provides an eco-friendly, cost effective and rapid bio-network development.

Keywords: Ecosystem-restoration, Mine dumps, Green technology

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G6

Effects of Fruits and Leaves of *Melia azedarach* (L.) from Eastern Mediterranean on Soil Organic Matter Mineralization

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Present study was carried on soils of three different *Melia azedarach* L. (Neem tree) growing in Çukurova University Campus under the influence of Mediterranean climate. Carbon mineralization was investigated over 30 days using respiration method [Ba(OH)2 + Oxalic acid)] in control soil, and in soils added with the same and double amounts of carbon containing fruits and leaves of the plant separately. Mineralization was also observed in azadirachtin (0,15 g/100 g soil), a natural insecticide, added soil. Carbon mineralization was insignificant at all groups in the first three days, which showed a considerable increase in fruit and leave added groups on the following days. Although there was no difference in carbon mineralization between the control and azadirachtin added soils, and between the fruit and leave added soils, carbon mineralization decreased significantly in the latter two soils ($P<0.001$).
It was concluded that due to attenuating effects of fruits and leaves of *Melia azedarach* L. on carbon mineralization, they might lower the sera effect by sequestering carbon in soil.

Keywords: *Melia azedarach* L., azadirachtin, soil carbon mineralization, carbon sequestration

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G7

Copper Phytoextraction Using Mutant Lines of Sunflower and Tobacco

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Anthropic activities such as smelting, long term application of Bordeaux mixture and pig slurries, use of wood preservatives containing Cu salts and subsequent wood washings results in Cu-contaminated topsoils. To promote their decontamination, phytoextraction may be improved through various ways: (1) amendment or chelator incorporation into the contaminated soils to increase plant uptake; (2) selection of efficient cultivars and fast-track breeding mutant lines (non-genetically modified plants); (3) use of agricultural practices: irrigation, crop rotation, intercropping, fertilization, bioaugmentation, etc. Sunflower is a candidate to phytoextract Cu due to its relatively high Cu mineralomass. It can provide financial returns through oilseed production and be included in a sustainable crop rotation promoting soil development processes, nutrient cycles, microbial community, and soil ecosystem functions. Shoots can be converted by various processes, e.g. vacuum pyrolysis, solvolysis, anaerobic digestion, etc. Tobacco is another candidate with a considerable shoot biomass, especially the Cu-tolerant mutant lines. Furthermore the morphological and physiological traits of both plants are changed by various ecological factors, that allows their use for biomonitoring.

Researches focus on opportunities to use (1) sunflower and (2) tobacco for thephytoremediation of Cu-contaminated topsoils at the BIOGECO phytoremediation platform. Commercial cultivars and mutant lines of sunflower were cultivated in 2008 and 2009 in field plots. In parallel, a biotest using potted soils was carried out to reveal the most significant phenotype traits of the sunflower mutant line 1 exposed to the Cu-contaminated topsoils. In 2009-2010, sunflower and tobacco mutant lines were cultivated on potted soils from the field plots and on a soil series obtained by the fading technique using Cu-contaminated (832 mg Cu kg-1) and uncontaminated soils (21 mg Cu kg-1). In both cases, soil pore waters were sampled using Rhizon MOM moister samplers.

(1) Responses of sunflower to spatial variation of total soil Cu for 6 cultivars and 2 mutant lines: shoot FW and DW, capitulum and seed yields, and shoot elemental concentrations were determined. The addition of compost and dolomitic limestone reduced the labile Cu pool in amended soils, allowing sunflower growth and a full life cycling with relevant shoot and seed yields. Shoot Cu concentration was plotted vs. Cu concentration in the soil pore water. Shoot Cu removal was compared to Cu concentrations and other characteristics (pH, OM, etc.) of soils and soil pore waters. At high Cu exposure, Cu removal was higher using mutant lines and one cultivar.

(2) Responses of 1 month-old plants of sunflower mutant line 1 to Cu exposure:
chlorophyll and carotenoid densities were determined as well as stem length, leaf, stem and root biomasses, leaf area and asymmetry. Root systems were studied vs. Cu exposure.

(3) Tobacco mutant line grew even in the unamended Cu-contaminated soil showing a higher Cu tolerance than sunflower. Phenotype traits and shoot elemental concentrations were determined. Plant performances and phytoextraction efficiency are discussed in the perspective of thesustainable management of Cu-contaminated soils.

Keywords: field experiment, crop rotation, phytoremediation, soil solution, biomonitoring
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G8

Arbuscular Mycorrhiza Fungi and Bacterial Mixtures Lowered Cadmium Accumulation of Selected Economic Plants under Field Conditions

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A number of local plant species from the village of Baan Padae, Mae Sot district, Tak province, Thailand have been previously identified with cadmium phytoremediation potentials as an alternative in agriculture in the area. Nevertheless, most of the identified plants may be not of the villagers' interest due to their low economic value. Therefore, in this study, we applied arbuscular mycorrhiza (AM) fungi and bacterial mixtures to selected economic plants aiming to enhance phytoremediation effects under the field conditions of Baan Padae village, where a total cadmium concentration in the soil exceeds 500 mg.Kg-1.
Six species of economic plants, including African lily (*Agapanthus africanus*), Chinese chive (*Allium ramosum* L.), nugget marigold (*Tagetes eracta* and *Tagetes patula* hybrid), Chinese cabbage (*Brassica rapa* L.), water morning glory (*Ipomoea aquatica* Forsk.), and lettuce (*Lactuca sativa* L.), were selected for the study. The plant seedlings were dipped in either a mixture of AM fungi or bacterial mixtures before planting in the field. After two months, plant materials and soil samples were collected for analysis. We found that microbial diversity in the plots treated with bacterial mixtures was generally higher than those with AM and the control (without any microbial inoculation). The treatments with either AM fungi or bacterial mixtures appeared to accumulate lower cadmium concentrations than the control did. In addition, most treatments with AM fungi showed significantly lower cadmium accumulation than those with the bacterial treatments; thus, suggesting that microbial inoculation could lower cadmium accumulation in economic plants when grown in cadmium contaminated soil.

Keywords: cadmium contamination, arbuscular mycorrhiza, microbial diversity, phytoremediation, Baan Padae village, Mae Sot district

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G9

New Sustainability? The influence of sulphur nutrition on herbicide detoxification in winter wheat

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Yield and quality of crops are diminished without application of mineral fertilizers and pesticides, especially herbicides. Chemical weed control accounts for more than 50% of pesticide applications. Herbicides as xenobiotics need to be detoxified by the cultivated plants. During the detoxification process, xenobiotics are conjugated with glutathione catalysed by specific enzymes (glutathione-S-transferases). Aim of the study was to investigate, whether the detoxification reaction in plants could be improved concomitantly with yield and quality by varying the sulphur nutrition. A greenhouse and a plot experiment with winter wheat were set up under different sulphur nutrition conditions. In the plot experiment, top leaf samples were taken 18 h after herbicide application. Plants from the greenhouse experiment were harvested without herbicide application. After enzyme extraction, glutathione-S-transferase-activity was determined photometrically and glutathione contents (GSH, GSSG and total thiol) were determined by HPLC. GST- activities in the plot experiment increased up to five times compared to those from the greenhouse. Glutathionconcentrations in the plot experiment were three times higher than the concentrations measured in samples from the greenhouse. Ratio of GSH to GSSG was always 2:1. Yield and quality parameters were improved following the sulphur nutrition regime. The study showed that an intelligent sulphur nutrition management influenced detoxification in plants positively. As buffering stress is one of the central challenges in plant production, this could be an instrument for future agriculture practice. In order to secure yield and quality in future research is needed to quantify the crops detoxification capacities and responses to micronutrients under the emerging challenges of climate change.

Keywords: sulphur, detoxification, fertilisation, glutathione, enzyme, winter wheat

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G10

Regional Government of Tuscany (Italy) supports the R.E.P.E.T. Project for the reclamation of arsenic polluted soil through rhizosphere-enhanced phytoextraction at a dumping site contaminated by arsenopyrite roasting residues

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Content and aims of the Research Project R.E.P.E.T. (***R**hizosphere-**E**nhanced **P**hyto-**E**xtraction **T**echnology*) funded by Regione Toscana (*i.e.* the Regional Government of Tuscany, Italy) are illustrated here. The main objective of this investigation is to identify suitable and effective plant-assisted bioremediation protocols for the *in-situ* reclamation of soils contaminated by residues from the roasting process of arsenopyrite for sulphuric acid production. In particular, the present study refers to the experimental work still in progress at a dumping site located in Southern Tuscany within the industrial area of Nuova Solmine SpA, which has been producing sulphuric acid from roasted pyrite for more than thirty years between the 60s and the 90s of the last century. An interesting perspective for the treatment of As contaminated soils is given by phytoextraction, based on the use of plant species such as the poplar clone DVINA (*Populus deltoides*) and the hybrid ORION (*P. deltoides* x *Populus nigra*) as well as the fern *Pteris vittata*, which has demonstrated to be cost-effective and environmentally sound. Moreover, among the different phytoremediation strategies, exploitation of possible plant-microbes interactions has received great attention in the last decade, owing to the supposed role of rhizobacteria in enhancing phytoextraction.

Therefore, a preliminary study has been carried out in order to characterize the rhizobacterial cenoses of autochthonous plant species occurring in the contaminated area of interest and even to determine the phytoremediation efficiency of either poplars or *P. vittata* grown in arsenopyrite polluted soil. Enrichment cultures, starting from the rhizospheres of autochthonous pant species, were used to individuate bacterial strains able to cope with arsenite (AsIII) or arsenate (AsV). This latter procedure was adopted to identify bacteria isolates particularly resistant to high As concentrations and putatively capable of improving the phytoremediation efficiency. Different bacterial strains were obtained in axenic culture.

Sequencing of ribosomal genes indicated that the phyla of Firmicutes and γ-Proteobacteria were the most represented. In particular, *Bacillus* sp., *Pseudomonas putida*, *Stenotrophomans* sp., *Brevibacterium* sp. and *Arthrobacter* sp. were the main occurring genera. Meanwhile, both *ex situ* pot trials (lab scale) and *in situ* plot trials have been set up in order to assess plant viability, arsenic accumulation as well as enzymatic and non-enzymatic responses to the oxidative-stress through monitoring of catalase, superoxido-dismutase, ascorbate peroxidase, and –SH groups activities in leaves and roots. Once confirmed, these specific phytoextraction parameters will be considered for a possible *in-situ* exploitation of this technology.

Keywords: arsenic, bioremediation, hyperaccumulator plants, oxidative stress, phytoextraction, pyrite roasting residues, rhizosphere bacteria

* *Presenting Author* - § *Project Leader*

H1

Uptake kinetics of different arsenic species in lowland and upland rice colonized with *Glomus intraradices*

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Arsenic (As) is known to be present as different chemical species in the environment. The low and high affinity uptake kinetics of four As species (arsenate; arsenite; dimethylarsinic acid, DMA; and monomethylarsonic acid, MMA) were studied in two-month-excised rice roots with and without inoculation with *Glomous intaradices*. Concentration-dependent influx isotherms for the four As species of the mycorrhizal roots fitted better to Michaelis-Menten function than linear function. High-affinity uptake (0-0.05 mM) for the four As species with the lowland rice (Guangyinzhan) and the upland rice (Handao 502) showed there was significantly higher uptake ($p < 0.05$) of arsenate compared with arsenite, DMA and MMA in mycorrhizal roots of both rice cultivars. The mycorrhizal roots of lowland rice had a higher uptake of arsenate than the nonmycorrhizal roots, whereas the mycorrhizal roots of upland rice had a lower arsenate uptake than the nonmycorrhizal roots. Low-affinity uptake (0-2.5 mM) for the four As species with the lowland and upland rice suggested that the uptake of arsenate and arsenite by mycorrhizal roots were less than by the nonmycorrhizal roots in both cultivars. Therefore, the effects of *Glomus intraradices* on As uptake by rice depended on the genotypes of host plants, As concentration in the environment and the As species available for uptake.

Keywords*:* arbuscular mycorrhizal fungi, arsenate, arsenite, dimethylarsinic acid, monomethylarsonic acid

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H2

Rhyzo treatment project experience to predictably treat priority pollutants in water

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Plant-based treatment systems take advantage of natural processes that evolved to beneficially use nutrient, neutralize toxins and uptake water now discharged by human activity at rates which harm native ecosystems. To work properly, root zone reactors must provide one specific function –"Each drop of waste water passes within an inch of a root before percolating or drained by subsurface tiles". Before pollution treatment regulation, plants normally were used for basic functions like cycling nutrients in manure. Now, monitored systems document how phyto treatment can be a practical and economical alternative to 'conventional' water pollution control.

Since the U.S. Clean Water Act PL 92:500 passed in 1972 and other similar laws in many other countries, municipal waste water treatment is mandated accomplished by centralized Publicly Owned Treatment Works (POTW's). In the past 20 years, in-stream treatment capacity became more sophisticated; now the Total Maximum Daily Loads (TMDL) limits for several key pollutants are issued on most U.S. streams from the headwaters to estuary. Both point and nonpoint discharges are known contributors to in-stream pollutant loads that exceed known limits for sustained ecological health.

EWastewater takes advantage of the under-used pollutant treatment reactor – the plant root zone. Since 1988, poplar & willow trees to grow predictable rooted soil reactors to greatly affect regulated contaminants as deep as 5 meters below ground surface. Polluted water that requires further treatment is filtered, pumped and irrigated into the tree root zones. By design, the effluent is treated to specific discharge requirement before it leaves through the bottom of the rhizosphere.

Water flows through the root system where specific pollutants are adsorbed, entrained, mineralized or taken into the plant. With sufficient planted area, irrigated water can be applied into the rhizosphere with sufficient dwell time for removing nitrogen, phosphorous, pathogens, pharmaceuticals, metals, oils/greases, BOD, total dissolved solids (TDS), and total settleable solids (TSS). Depending on the flow, time of year and soils, drained water then percolates to the ground water or reaches a drain line that discharges to a stream. Winter water can be stored in existing lagoons or dripped through subsurface drip irrigation into the microbe-rich root zone.

Such phyto treatment concepts are ready to be deployed on a large scale based on data from prototypes and full-scale systems. This paper will explain the known process kinetics and summarize performance at mature phyto treatment systems. Owners justify this approach to projects due to the carbon sequestration, habitat value, low cost for labor and maintenance equipment, and potential for harvested value. Phyto systems that perform permitted water treatment are becoming more attractive as tax revenue and profit margins are strained in the global economic down turn.

Key words: Phyto treatment, performance data, research summary, effluent, storm water, leachate, treatment, poplar, willow

H3

Reducing the Negative 9-Billion Human 'Foot Print' Via Phyto Processes

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This presentation is divided into two segments:
1. A historical perspective in developing phytoremediation as a major pollution cleanup and treatment technique
2. A potential phyto future that contributes to a real potential challenge for good.

Phytoremediation technology that focused on regulated environmental pollution control started with a small university research project in 1988 and has evolved to global application with fullscale field applications, over 300 Ph.D. and M.S. theses awarded, and the International PhytoSociety.

Many of the modern phytoremediation projects started with poplar (*Populus spp*.).

Fundamentally, poplar physiology allowed root placement to a specific subsurface depth. With a defined a root zone 'reactor' volume, the plant dynamics, soil physics and microbial activity has pollutant treatment properties. As trees and the associated rhizosphere mature, this predictable reactor volume had treatment capacity that improved with time – essential to regulated sites.

Before the phyto concept began, poplar attracted commercial and academic interest because of its commercial fiber value. The poplar genetic pool was selected for fast growth over a large fraction of the earth's surface - thus a commercially harvested crop that is safe and renewable added to the phyto attraction. Because it is a plant system, the skilled labor and maintenance equipment is normally available – creating honest 'green collar' jobs for the community.

Treatment is accomplished by in-soil properties and plant-derived exudates requiring less capital construction cost, less refined chemical cost and less electrical energy for pumps and aeration.

On mature poplar, it is possible to remove 25 metric tons of carbon dioxide per hectare per year, which improves the 'carbon footprint' for waste water and pollutant treatment.

These phyto traits improve the economics of long-term pollution management programs for industry and communities. There has been a shift in phytoremediation acceptance due to several factors relating to a broader concern for global warming, river and estuary water quality, damaged wildlife habitat, and poor commercial economics. Overall, the phyto future appears strong when trees are part of the plant 'skeleton' around which the other plants, soils, amendments, and engineered modifications are placed.

The potential phyto future is built on our combined experience and technology that exploit plant photosynthesis, soil geochemistry, and redefined acceptable post-use product fate. Phyto helps create a more cyclic lifestyle with ever-growing industrial activity and population growth.

Keywords: Phyto history, Acceptance, Future Expansion, Global application

H4

Phytoforensic Techniques: Directional Uptake of Chlorinated Solvents

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Phytoforensic methods have been used to demonstrate directional uptake of trichloroethylene (TCE) and perchloroethylene (PCE) by trees. These methods have previously been shown to delineate contaminant plumes in the groundwater through tree coring and *in planta* sampling. In this study, advanced tree coring, *in planta* solid phase microextraction (SPME), and *in planta* solid phase samplers (SPSs) were used to evaluate contaminant levels on different sides of trees, both in the field and in the lab. In the field, contaminant levels on various sides of trees were shown to be correlated with groundwater concentrations in a directional manner. Likewise, trees in the lab dosed directionally exhibited core concentrations of TCE 4.5 times higher on the side of the tree closest to the source compared to opposite side. The crosssectional distribution of PCE and TCE was obtained through destructive sampling and then mapped in high resolution for the first time. Directional sampling promises increased resolution for determination of contaminant plumes, making directional Phytoforensic sampling a valuable site assessment tool.

Key Words: Phytoforensics, Chlorinated solvents, SPME, directional sampling

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H5

Street sweepings – phytoremediation prior to use in landfill greening

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Sweepings removed from roads in Berlin reach annual quantities of 60,000 to 80,000 or even more metric tons. This road cleaning material blend contains numerous constituents and some chemicals that exceeded certain levels pose risks to human health and the environment. The disposal of untreated street sweepings on landfills was thus prohibited in Germany in 2005. Recycling and beneficial use of this soil-like road cleaning waste in land application is being considered instead, e.g. for capping and greening of closed municipal landfills. However, pollutant concentrations above German federal regulatory limits require cleanup of Berlin street sweepings first. A greenhouse trial was conducted to investigate the potential of phytoremediation for removing critical pollutants from Berlin street sweepings down to levels that comply with the German landfill regulations (DepV). Six field crops and spontaneous vegetation grew rapidly and vigorously in pots with sieved street sweepings that were rich in nitrogen and provided an overall excellent plant growth substrate. Two months into phytoremediation, concentrations of several contaminants in street sweepings had already declined depending on plant species. After three months of growing spring barley, concentrations of heavy metals and organic contaminants and the values of all other parameters that are regulated complied with the DepV. Also, leaching of contaminants from street sweepings occurred to a lower extent beneath spring barley compared to the other plant covers. Uptake of heavy metals and arsenic by plants investigated herein had not exceeded regulatory limits set by the German forage crop quality regulation (FuttMV).

Keywords: road sweepings, phytoremediation, landfill, recultivation, landscaping

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H6

Metal partitioning in plant-soil-water compartments under EDDS-assisted phytoextraction with *Brassica carinata* A. Braun

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Chemically enhanced phytoextraction is proposed as an environmentally friendly approach for removing trace metals from contaminated soils by fostering their bioavailability and uptake in nonhyperaccumulator plants. The aim of this study was to measure residual Cu-Pb-Zn bioavailability in pyrite wastes – an index of leaching risks – and their uptake and leaching after one phytoextraction cycle. Cultivation of *Brassica carinata* A. Braun was assisted with [S,S]-ethylenediaminedisuccinic acid (EDDS) at various doses and application times. Treatments were: 2.5 and 5 mmol EDDS kg-1 of waste applied one week before harvest, and 5 applications of 1 mmol kg-1 of waste at 5- and 10-day (with earlier supply) intervals in comparison with untreated controls. Metal bioavailability in wastes was measured with the Lindsay-Norwell method, and metal contents in plant tissues and leached water by ICP-OES. In view of its higher chelating constant, Cu was the most easily solubilised and harvested metal. The residual Cu biovailability in wastes (after cultivation) and the OSM (overall solubilised metal, i.e., plant uptake + residual bioavailability + leaching) were increased at the highest EDDS harvest application dose, but repeated treatments caused significant Cu leaching during cultivation. Regardless of the dosage used, chelate harvest applications decreased residual Zn and Pb bioavailability compared with controls. Zn and Pb are also presumed to be efficiently solubilised by EDDS, as tissue concentrations generally tended to increase, but colloidal re-absorption may have occurred in the meantime, explaining the reduced residual soil availability. It is concluded that the smallest dose (2.5 mmol EDDS kg-1) of this new and less persistent chelator is recommended, to minimise phytotoxicity and achieve good metal removals, without raising significant leaching risks.

Keywords: [S,S]-EDDS, assisted phytoextraction, *Brassica carianata* A. Braun, heavy metals,
metal bioavailability.

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H7

Heavy metal accumulation in Rhyzobacteria-treated agricultural crops

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The problem of agricultural-soil pollution is closely associated with human heath. An important task, therefore, is producing ecologically pure foodstuffs and monitoring the content of heavy metals for the entire food chain – from seeds and fodder to final products.
An effective biotechnological method of plant protection is the inoculation of plants with plant-growth promoting rhizobacteria (PGPR) that can decrease the level of heavy metal translocation in cultivated plants.
From the rhizospheres of plants growing in areas with different levels of heavy metal pollution, we isolated microbial strains showing tolerance to heavy metals (*Micrococcus* sp. 21, *Micrococcus* sp. 22, *Arthrobacter* sp. L1, *Arthrobacter* sp. 31, *Aeromonas* sp. MG3, *Moraxella osloensis* 32, *Comamonas* sp. T3, *Alcaligenes* sp. П2). We assessed the potential activity of the isolated strains toward plants (phosphate solubilization and auxin synthesis) and the isolates` role in cadmium and lead accumulation by three widely used agricultural crops: sorghum (*Sorghum saccharatum*), sudan grass (*Sorghum sudanense* (Piper.) Stapf.) and sunflower (*Helianthus annuus* L.). The application of the isolates by seed coating overcame heavy metals toxicity: the plants showed lessened metal accumulation, extensive rooting, and enhanced plant growth.
Further research on and development of this promising strategy will be a potent tool to prevent the accumulation of heavy metals in agricultural crops, thereby ensuring food security for humans.

Key words: Cadmium, lead, phytoaccumulation, plant-growth promoting rhizobacteria, food Crops

Acknowledgment. This work was supported by the Federal Science and Innovations Agency (State Contract no. 02.512.11. 2210).

H9

Constructed wetlands are suitable to treat wastewater from Italian cheese productions

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The aim of this work was to verify the performances and efficacy of sub-surface flow constructed wetlands (SSF-CW) applied to cheese factory effluents. Because of the presence of milk's fats, proteins and carbohydrates (lactose), pollutants concentrations in wastewater coming from cheese production, not subjected to purification treatment, generally exceed the threshold values for discharge into surface waters.
Two horizontal sub-surface flow constructed wetlands (h-SSF CW) were set-up and controlled over 16 months to determine their efficiency in reducing the polluting load of wastewaters from Parmigiano-Reggiano and Grana Padano cheese production. Besides, comparisons were made of biomass production and nutrient uptake of cattail (*Typha latifolia*) reaped at different stages.
The average concentrations of the influent waters were about 250-320 mg/l TSS, 940-1060 mgO2/l COD, 600-700 mgO2/l BOD5, 35 mg/l NTK, 8-13 mg/l total P, 60-170 mg/l animal and vegetable fats and oils. The removal of COD, BOD5 and animal/vegetable fats and oils were above 95%, while resulted 60-65% for nitrogen and very different for phosphorus, varying from 75% in one CW to 20% in the other.
Results demonstrated that h-SSF CW could help to solve the problem of the cost-effective disposal of cheese dairy wastewater, being a suitable treatment for reducing pollutants to values in conformance with Italian standards for discharge to a watercourse. *Typha latifolia* showed considerable biomass yield, though the N and P uptake was quite low if compared with nutrients inputs in CW (about 10%). There is the possibility to maximise biomass yield and nutrient uptake reaping cattail twice a year instead of only one time.

Keywords: Typha latifolia, wetlands, dairy wastewaters

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H9

Potential Use of Macrophytes as Bio-indicators of Trace Element-Contamination along the Jalle River (France).

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We focused on the uptake of trace elements (TE) by macrophytes growing along the Jalle d'Eysines river (Bordeaux, SW France) in the vicinity of contaminating activities, i.e. golf using pesticides, cultures using CuSO4 as fungicides, sewage sludge plants and an abandoned military base. Previous researches indicated these freshwaters are contaminated by polar organic pollutants (Abadie and Budzinski, 2005), but no data are reported on TE contamination yet. We investigated four sites along the Jalle river to monitor TE concentrations in helophyte shoots (i.e. *Arum italicum, Scirpus lacustris, Juncus glaucus, Juncus effusus, Carex pendula, Carex riparia, Phalaris arundinacea, Iris pseudacorus,* and *Ranunculus repens*) and in leaves of submerged species (i.e. *Ludwigia palustris* and *Elodea canadensis*). This aims at identifying (1) potential TE (hyper-)accumulator species and/or ecotypes, (2) species and/or ecotypes as potential bioindicators of the river contamination by TE. Labile TE pool in water measured by DGT (0.9-2.1 µg Cu and 2.2-7.5 µg Zn L-1) and total sediment TE concentrations (5.61-39.5 mg Cu and 36.5-224 mg Zn kg-1) were determined to gain information on biogeochemical cycles. Shoot concentrations varied between 6-42 (*I. pseudacorus – E. canadensis*) mg Cu and 16-340 (*C. pendula - E. canadensis*) mg Zn kg-1. Submerged macrophytes accumulate more TE, e.g. 5-7 fold for Cu, than helophytes in their shoots confirming previous findings (Fritioff and Greger, 2003; Olette et al.,2008). Helophytes are generally reported as low TE accumulators, especially into aerial parts (Lee and Scholz, 2007). However shoot TE concentrations (in mg kg-1) of *P. arundinacea* (Cu: 12.3, Zn: 51), *Ranunculus repens* (Cu: 18.7, Zn: 47), *I. pseudacorus* (Zn: 189) and *A. italicum* (Zn: 174) are greater than labile TE concentration in water. Our data for *R. repens* are similar to those reported by Favas and Pratas (2009) for *R. peltatus*. *R. peltatus* and *R. repens* would be good TE accumulators.

Acknowledgements: This work was supported by AXA foundation for L. Marchand (PhD grant) and by EU Erasmus MUNDUS Lot 6 for Y. Vystavna and A. Kolbas (PhD grant).

Key words macrophyte, monitoring, bioindicator, water, contamination, *Ranunculus repens*

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I1

Responses of Tomato Cultivars (*Solanum lycopersicum* (L.)) to Inorganic Arsenic and Silicium Exposure

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Elevated arsenic in soils raises concern with respect to plant uptake and subsequent entry into wildlife and human food chains. Arsenic in irrigation water or in domestic and agricultural land has arisen from application of pesticides, lawn herbicides, and insecticides. Silicium is added to tomato plants to improve water stress resistance. In this work we have evaluated the effect of two inorganic species of arsenic (As (III) and As(V)) at two concentrations (0,2mM and 0,5mM), with or without 0,025 mM Si, on the germinability of eight cultivars of *Solanum lycopersicum* utilized to produce pasta sauce. We have determined the number of the seeds germinated and root lengths to indicate the most resistant cultivar and the more toxic species of arsenic and how Si affected germination and growth in vitro Arsenite was more toxic than arsenate at both concentrations. Germination but not root growth of the cultivar Aragon was uninhibited. Podium showed highest root growth of all cultivars in respect to the control. All the tomato cultivars were grown in garden soil for 3 month then supplemented with 5ppm of either As species, with or without 2,5mM of Si. After 2 weeks they were harvested and fresh biomass was measured. As content was determined through HGAAS.
The cultivars showed a remarkably different behaviour towards the treatments; in general As uptake is quite low, so is the translocation with few exceptions (cultivars Aragon, Axel, Frigio). Silicium in soil lowered As uptake , with only the exception of the cultivar Wally Red. The longerterm objectives are to select the most appropriate cultivars to limit human exposure to arsenic through tomato consumption.

Keywords: Tomato, Arsenic resistance, Silicium, Germination, Contamination.

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I2

Rapid recognition of virgin olive oil geographical origin by secondary electrospray ionization-mass spectrometry

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Virgin olive oil (VOO) is a high added value agricultural product in the EU from commercial, nutritional and health-promoting potential points of view. To date the EU has already registered 95 Protected Denomination of Origin (PDO) olive oils. Unfortunately, economic fraud, such as false claims of geographical origin on product labels, cannot be fully prevented. To protect the market from fraudulent practices, a wide range of analytical strategies have been developed to confirm olive oil authenticity with varying levels of success. Here we propose the analysis of the volatile fraction of olive oils by secondary electrospray ionization (SESI) and pattern recognition employing NIST MS search software. Our goal is to provide an alternative screening tool for geographical origin traceability. We have analyzed the headspace of 218 olive oil samples, comprising Liguria (n= 110; PDO) and other Mediterranean regions (n=108), and including harvests from 2005 and 2006. 1 mL of sample was introduced in a cell culture flask. We let the samples stabilize for 5 min at 40 ºC and the headspace was gently swept by a flow of nitrogen (0.5 L/min). The volatiles were drawn into the SESI chamber to be finally analyzed in positive ion mode with an ion trap mass spectrometer (HCT Bruker). Note that there is no sample preparation nor sample consumption, taking the analysis ~ 8 min/sample. The background-corrected mass spectra were used to create a NIST library containing two groups: "Liguria" and "non-Liguria". Our preliminary data show a rich volatile fingerprint for all the samples analyzed, with some characteristic peaks common to almost all of the samples (e.g. m/z 99) and whose identity is currently under investigation via MS/MS. In addition, upon visual inspection of the spectra, one can observe clear differences in the patterns among the different harvest years and among the different geographical origins. To test the feasibility of this approach for authentication purposes we interrogated each "Liguria" spectrum against the rest of the spectra (i.e. "leave one out" crossvalidation). The percentage of correct classifications was above 89%. These results are comparable to those provided by other off-line techniques such as GC-MS combined with alternative statistical analyses.

Keywords: virgin olive oil, geographical origin, traceability, authenticity, SESI, mass spectrometry, NIST

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I3

Naphtalene degraders active in diesel rhizoremediation with willow

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Rhizoremediation relies on plants to biostimulate microbial degradation of pollutants, but little is known about how degradative microbial populations in a contaminated soil change in response to rhizostimulation. Outdoor pot experiments coupled with DNA-based stable isotope probing (SIP) were applied to investigate how *Salix alaxensis* (felt leaf willow) affects naphthalene degrading populations during rhizoremediation of diesel-contaminated soil. The concentration of diesel range organics (DRO) and the most probable number (MPN) of culturable diesel degrading microorganisms in the rhizosphere were monitored in planted and unplanted (control) treatments, with and without fertilizer addition, over a summer in interior Alaska. SIP incubations were performed in microcosms constructed from soils following treatment to identify and compare 13C-naphthalene-utilizing bacteria in the willow-planted versus unplanted (control) treatments. Terminal restriction fragment length polymorphism (T-RFLP) of 13C-DNA isolated by isopycnic centrifugation was conducted to examine the diversity of bacteria that derived carbon from naphthalene. In the pot study, growth of *S. alaxensis* resulted in the greatest loss of DROs, although treatments amended with fertilizer contributed to a significant increase in MPN of culturable diesel degrading microorganisms. T-RFLP profiles of 13C-DNA suggest that the presence of willow may have enhanced the growth of a particular bacterial taxon. Further cluster analysis showed separate clusters between control and willow samples. To identify naphthalene-utilizers, sequencing of 16S rRNA clone libraries is underway. This research suggests that *S. alaxensis* can be a useful plant for rhizoremediation of diesel-contaminated soil and that growth of this willow altered the structure and/or composition of naphthalene-utilizing populations relative to that of unplanted soil.

Key words: Rhizoremediation, Phytoremediation, Diesel, Willows, Alaska, MPN, SIP, T-RFLP

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Rhizospheric mineralization of PCB mixtures by hybrid Poplar and Switchgrass

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Sequential redox cycles of microbial dechlorination followed by aerobic bio-oxidation are desirable to achieve complete degradation of a mixture of higher and lower chlorinated PCBs. Research in our laboratory has shown that hybrid poplar trees are able to take up some lower chlorinated PCB congeners in hydroponic solution. Coplanar PCBs are among the most toxic because they exhibit dioxin like properties and bind to the Ah receptor. The potential for achieving complete mineralization and transformation of a mixture of hexa and tetra chlorinated coplanar PCBs is investigated, by subjecting artificially contaminated soil, planted with hydrid poplar and switchgrass, to aerobic and anaerobic cycles. Anaerobic and aerobic cycles are achieved through varying the soil moisture content. We hypothesize that this cyclic manipulation of redox conditions along with plant- microbial interactions in the rhizosphere will facilitate more complete degradation of this PCB mixture. Treatments with poplar and switchgrass will be compared. GC mass spectrometry is used to analyze the PCB concentration in plant tissue and soil.

Keywords: PCB, redox, dechlorination, rhizosphere, poplar, switchgrass

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I4

Antibacterial sulfonamides: accumulation and effects in *Salix* plants

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The application of contaminated manure to fertilize agricultural soils is among the major routes through which antibacterial sulfonamides (SA) enter the environment. SA can be easily leached into groundwater, due to their persistence and low sorption to soil constituents. Previous results showed that barley plants are able to accumulate high amount of SA from nutrient solutions. *Salix* is currently under intensive research for its potential use in soil phytoremediation. In this study we evaluated the possibility to use these plants to remediate soils/groundwater contaminated with SA, by studying both the ability of willow to absorb sulfadimetoxine (SDM) and to tolerate it. The experiments were conducted with *Salix fragilis* cuttings grown hydroponically in a climate chamber. Once rooted and with leaves, plants were exposed to different concentrations of SDM (0.04 to 3 mM) for a month. At fixed time intervals photosynthetic (LI-COR 6400) and growth parameters were measured. At the end of the experiments plants were weighed and tissues were analysed for their content in sulfadimetoxine, N and P.
Results show that at the beginning of the experiment plants exhibit a high tolerance towards the antibacterial, as they did not show any modification of photosynthetic parameters. However, at the end of the test we observed negative effects of the treatment mainly at the level of roots, depending on SDM concentration. These effects were probably due to the accumulation of high amounts of the SA. Further studies are necessary to define the response of willow in the field, in long term experiments and with other kinds of SA. However, these preliminary results are promising, as they indicate that *Salix fragilis* can absorb high amount of SA.

Keywords: antibiotics, phytoremediation, soil pollution, sulphonamides, water pollution, willow

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I5

Use of soil amendments to alter trace element mobility in a soil polluted by pyritic-mining wastes: a pot experiment

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Trace element presence in soil is an ongoing topic in environmental restoration, and amendments can play an important role in dealing with this. A pot experiment was designed to evaluate the efficiency of different soil amendments on mine soil reclamation and revegetation. The amendments were: two sources of phosphorus, two iron amendments, and compost. As indicators of metal availability and phytotoxicity, plants of *Lolium perenne* L. var. Cadix were grown in the pots. *In situ* formation of iron oxide was more efficient in trace element retention and blocking plant uptake than the application of commercial oxide. Phosphorus amendment had slight effects on trace element behaviour in soil and on plant growth, indicating that phytotoxicity in polluted substrates was more important than nutrient limitations. Compost decreased plant concentration of trace elements but increased their mobility in the substrate, increasing risk of leaching. The simultaneous application of iron sulphate and calcium carbonate to the substrate was the most efficient amendment reducing trace element availability and transfer to the plants. Compost improved plant growth and hydro-physical properties in soil and decreased trace element concentration in plants, but did not reduce trace element availability or solubility. Both iron sulphate and compost amendments alleviated soil phytotoxicity. A mixture of iron oxide and compost could be the best alternative, improving both chemical and physical properties of the substrate, avoiding toxicity symptoms in plants.

Keywords: trace elements, soil remediation, iron oxides, phosphorus, compost, phytotoxicity

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I6

Using EDTA as a Chelating Agent in Phytoextraction of Cd and Ni from Multi Contaminated Soil and Its Effects on Plant Growth

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Heavy metal pollution of soil is a significant environmental problem. It has negative impact on agricultural products and human health. The clean up of metal-contaminated soils by traditional physicochemical methods is both very costly and destructive to the normal properties of the soil. In contrast, phytoremediation, that use of green plants to decontaminate the metals out the soil is an emerging technique with advantages of being in situ, cost effective and environmentally sustainable. Enhanced phytoextraction of heavy metals using chelating agents and agricultural crops is widely discussed as a remediation technique. The objective of this work, therefore, was to examine the effect of EDTA as a synthetic chelator, on the plant growth, the uptake and translocation of Ni and Cd within corn (Zea mays) grown on a contaminated soil. In this study, phytoextraction efficiency of Zea mays after applications of EDTA (3mmol kg-1 soil) was tested on the laboratory scale in a soil contaminated with Cd (10 mg kg-1 soil) and Ni (300 mg kg-1 soil) as pollutants. EDTA effectively increased the mobility of target heavy metals (Ni and Cd) in the soil solution. The results showed that the content of Cd was significantly lower in both, roots and shoots. However, biomass production, leaf area, root volume, and plant height in corn dropped after EDTA addition in the experiment. Moreover, the contents of Ni enhanced in corn shoots.
Application of 3mmol kg-1 EDTA to soil significantly decreased remediation factor of Ni and Cd in this experiment. Compared with this results, lonely increased The remediation factor of the metals studied was promoted using Z. mays in the absence of EDTA in polluted soil. This observation may make the use of EDTA not suitable for the remediation of severely heavy metal contaminated soils by Z. mays in a reasonable time frame and may result in substantial environmental pollution under these conditions.

Keywords: Phytoremediation, phytoextraction, EDTA, Zea mays, chelator

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I7

Effect of Cadmium on Antioxidative Defense System in Calluses From Safflower (*Carthamus tinctorius* L.*)* Varieties.

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Calli obtained from three varieties, Arak2811, 14-4 and Goldasht of safflower *(Carthamus tinctorius* L.*)* plant grown in the presence of different concentrations (0-100 µMol l-1) of Cadmium (Cd) were assayed for activities of antioxidative enzymes. A time, concentration and variety dependent response of Cd was observed. The results showed that the Safflower calli responded to Cd induced oxidative stress by modulating antioxidative enzymes. The activities of antioxidative enzymes, CAT and GPX, followed the same trends as antioxidants first increasing up to a concentration of 25 µMol l-1Cd and then decreased in all varieties after 4 days of culture while at longer duration(i.e. day 8) the activities of CAT and GPX decreased equivalent to increase of Cd. In all of varieties both APX and GR activities responded differently to Cd stress with attention to duration exposure of calli to Cd. These results suggest that how safflower callus withstand heavy metal stress by induction of antioxidative enzyme activities accompanied by increase of proline and protein.

Keywords: *Carthamus tinctorius* - antioxidant enzymes - cadmium - callus.

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I8

Oxidative Stress and Antioxidant Defense Mechanisms in Response to Cadmium Treatments in Two Safflower (*Carthamus tinctorius* L.) Cultivars

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In this study effect of Cadmium (Cd) stress was investigated in two cultivars, Arak2811 and Goldasht of safflower (*Carthamus tinctorius* L.). Effect of different concentrations of Cd (0, 25, 50, 75 and 100 µMol l-1) applied to the seedlings were detected by measuring changes in the lipid peroxidation, none enzymatic and enzymatic antioxidats activity and protein and proline content. A time, concentration and cultivar dependent response of Cd was observed. Plants accumulated substantial amount of Cd, the maximum being in roots of Arak2811 (mg g-1 dw after 4 days at 100 µM). Cd induced oxidative damage which was indicated by the reduced chlorophyll and increased malondialdehyde content in seedlings of both cultivars especially in Goldasht. Differences were noted in both cultivars for SOD, APX and GR activities under various Cd stress levels. ASA and GSH biosynthesis induction in seedlings of Arak2811 exposed to increasing Cd concentrations may be involved in Cd tolerance in this cultivar. Arak2811 ability to accumulate and tolerate high amount of Cd through enhanced level of protein, proline and various antioxidants suggest it to be a suitable candidate for phytoremediation.

Keywords: *Carthamus tinctorius*; antioxidative enzymes; cadmium.

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I9

Particulate matter deposition on leaves of *Tilia cordata* Mill. depending on leaf age

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Particulate matter (PM) has been reported as a great threat to human beings, especially in urban areas and along routes with intensive road traffic. High levels of PM pollution have significant influence especially on children, elderly people and those with respiratory and heart problems. Vegetation was discovered to have the ability to store particles on the leaf surface or in waxes covering the leaves. Analyses of different tree and shrub species indicated major differences among species in the amount of PM that is being deposited on the leaves. This study was undertaken in order to examine the relationship between leaf age and the amount of PM stored on leaves of *Tilia cordata* Mill. Leaves were collected in two consecutive years, 2008 and 2009, from five different trees growing along Rodowicza-"Anody" street in Warsaw (experimental field at WULS-SGGW campus), acting as replications. Plants developed nine leaves on a shoot, which were numbered 1-9, with no 1 being the oldest, and for each number leaves were collected from ten different shoots of two sides of a tree (side exposed to the road and internal side). Samples were washed off with distilled water and chloroform. Liquids were filtrated in order to determine the amount of PM in these two fractions and three filter pore size categories (10µm, 2,5µm and 0,2µm). Leaves area and amount of waxes were also determined. Results showed that the total amount of PM deposited per unit of leaf area was insignificantly higher for the internal side of trees, which might seemingly be due to lower wind speed influence on that side. Younger leaves show tendency to accumulate more PM than the older ones, with the highest amount accumulated on leaves no.7-9. That pattern was observed in all particle size categories and might presumably be attributed to the quantity and quality of wax cover.

Keywords: air pollution, phytoremediation, linden

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I10

Phytoremediation and Public Health Implications of Community Involvement

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Phytoremediation has long been touted as an environmentally and community friendly technology to regulators and to responsible parties. However, the public health aspects of phytoremediation are often overlooked. These benefits are not insubstantial, and should be considered more when planning and developing phytoremediation projects. Not only is phytoremediation removing environmental contaminants, but can also engage the community in both the planning and execution of the site clean-up. This engagement allows the community members to feel that they are a part of the solution, rather than passive bystanders to a process that is being controlled by the very entities that caused the problem. This increases community well-being, and in itself is an important part of the health of the community that the remediation action is working to protect. Here we will present firsthand accounts of how community members have taken active roles in phytoremediation projects, from inception to implementation to maintenance, and how this affected the community outlook about the work being done.

Keywords: public health, community involvement, groundwater remediation

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J1

A geostatistical model based approach to evaluate spatial variability of arsenic in soil and to compare arsenic-hyperaccumulation efficiency of two fern species

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The experimental site was a historical disused cattle dip site, in northern New South Wales, Australia, where arsenicals were used to treat ticks in cattle. A preliminary soil sampling was conducted to evaluate arsenic (As) content in soil. The results from this sampling indicated a high variability in soil As concentration around the cattle dip. This variation was possibly resulted from the dipping process, pumping-out of the dipping solution from the dip and the disposal of dip sediment. It was imperative to measure the small scale variability (few centimetre) in soil As around the cattle dip site. Additionally, it was important to isolate the effect of soil As variability to compare the As hyperaccummulation potential of two fern species, Chinese brake fern (*Pteris vittata* L.) and gold dust fern (*Pityrogramma calomelanos* var. *austroamericana*). Therefore, the objectives of this study were to estimate the spatial variability in soil As concentration around the cattle dip; and validate the differences in hyperaccumulation potential of the studied fern species, employing geostatistical models.The study area was divided into two plots of same size (3.15 m2) where these two fern species were planted on a grid keeping plant to plant distance of 30 cm and giving 42 ferns of each species per plot. Soil samples were taken at 0–20, 20–40 and 40–60 cm depths, around each fern. Surface soil samples were analysed for total As and samples for three depths were measured for phosphate extractable As. The aboveground portion (fronds) of ferns were harvested after 10 months, dried and ground to analyse for total As concentration. The data were kriged and area maps were generated to determine the spatial distribution of As in soilat surface and subsurface depths. Linear mixed models (LMM) were used to analyse the data for soilfern interactions. The fixed effects were the interaction between the (i) fern As concentration and fern species, and (ii) fern As uptake and fern species. The random effect was a spatial correlated term. Two models were fitted using total soil As and phosphate extractable soil As at 0–20 cm depth. The residual maximum likelihood (REML) approach was employed to fit these models. This was similar to an analysis of covariance (ANCOVA) but due to a systematic design we could not assume independent residuals. The data were natural log transformed to meet the assumption of normality in LMM. For the first two layers, As concentration was spatially correlated with the distance from the dip bath, and generally illustrated a decreasing trend when moving away from the dip. The spatial distributionmaps also showed a strong relationship between the As concentration of the first two layers. However, at the lowest depth no variability in phosphate extractable As was observed. The maps showed that As concentration and uptake in fronds of both species varied with the soil As concentration, however, gold dust fern demonstrated higher frond As concentration and uptake than Chinese brake fern. Fortotal and phosphate extractable As, each of the fern species possessed the same slope (simple model) for soil-fern As relationship, which meets the theory of ANCOVA. The results indicated that As concentration and uptake in both fern species varied with As in soil. The model based comparison confirmed that gold dust fern accumulated As at a higher rate than the Chinese brake fern. These results suggest that this trait of gold dust fern was due to performance of the species rather than theeffect of varying soil As levels. It is suggested that geostatistical methods including kriging and LMM can be useful to overcome high level spatial variation in contaminated soil and to discriminate soiland plant factors.

Keywords: Arsenic, Chinse brake fern, geostatistical models, gold dust fern, hyperaccumulation

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J2

Tree Core Sampling for Screening of Toxic Elements in Soils

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A screening method for the detection of heavy metals in subsurface plumes using tree core samples was tested. Concentrations measured in wood samples of alder (*Alnus*), birch (*Betula*), beech (*Fagus*), poplar (*Populus*), pine (*Pinus*) and willow (*Salix*) in the polluted sites were compared to those measured in soil samples collected around the tree and in wood samples from reference trees. The comparison was carried out on three polluted sites currently covered by vegetation: a former wood proofing area near Hillerød (DK) a steel production site in Frederiksværk (DK) and a former landfill in the Oslo fjord (NO).
The method was tested for the toxic elements arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) and soil and wood samples were analysed by ICP-OES.
For most elements, concentrations from the test sites were higher than those from the reference sites. Cd showed in all cases lower concentrations in the wood from the test sites. The total Cu concentrations in soil and in wood samples were significantly correlated. Cr and Zn showed significant correlation between the concentration in the soil liquid phase and in wood. Cd did not show any correlation, and As, Ni and Pb were undetectable in most wood samples. The examined trees showed different uptake and different mobility factors, depending on the location and the metal analyzed. Poplars and willows acted as accumulators of Cd and indicators of Zn in the reference unpolluted sites. Alder, birch, beech and pine excluded the mentioned metals. In contaminated sites all tree species excluded these metals.
Wood concentrations seem to depend on the bioavailable concentration in soil rather than on the total concentration. The tree core method might be used to detect elevated metal concentrations in soil. However, the basis for the assessment must integrate changes in wood concentrations, correlation analysis, and changes in the mobility factor of the trees.

Keywords: tree core, wood, plant uptake, arsenic; cadmium; chromium; copper, lead, nickel, zinc, heavy metals, contaminated soils.

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J3

Impact of the growth of *Abelmoschus esculentus* (L.) moench and *Corchorus olithorius* L on soil contaminated with mixture of petroleum products

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The impact of the growth of *Abelmoschus esculentus* and *Corchorus olithorius* on the soil contaminated with mixture of petroleum products (gasoline, diesel and spent oil) were investigated in this study. The soils were contaminated with 1%, 2%, 3% and 4% (w/w) of the petroleum mixture and the pH, moisture content, organic matter content, microbial load and total petroleum content of the soils were analysed before and after the growth of the plants. The growth of the plants affected the soils. Growth of *A. esculentus* generally led to higher soil pH than the growth of *C. olithorius*. More moisture accumulated in soils that had *A. esculentus* than in soils that had *C.olithorius*. In the 1% and 2% treated soils, the growth of *A. esculentus* led to higher accumulation of organic matter than the growth of *C. olithorius* and the reverse was the case in the 3% and 4% treated soils. More fungal load than bacterial load was found in soils with *A.esculentus* and soils without plants while in soils with *C. olithorius* more bacterial load was found than fungal load. Growth of *A.esculentus* led to more abundance of bacteria and fungi than the growth of *C. olithorius.* Growth of the plants led to greater loss of total petroleum hydrocarbon with more total petroleum hydrocarbon being lost from soils with *A. esculentus* than from soils with *C. olithorius*. There were statistical differences between the impacts of *A. esculentus* and the impacts of *C. olithorius* on the contaminated soil ($P<0.001$, $P<0.01$, $P<0.05$).

J4

***Centrosema pubescen* benth and *Mucuna pruriens* var. Pruriens in copper uptake and translocation-phytoremediation**

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In order to assess their practical capability for the absorption and accumulation of Cu, twocommon crop plants, i.e. Centro plants (*Centrosema pubescens* Benth) and Mucuna plants (*Mucuna pruriens* var pruriens) were tested in pot experiments using simulated crude oil polluted soil in the concentrations of 2, 4, 6, 8, and 10 % (v/w). A range of amendments of various types was tested for increasing the copper uptake with the test species. These included UREA fertilizer, NPK fertilizer and Chicken manure. Cu concentrations of the soil ranged from 201.1 to 271.5 mg/kg after spiking. Cu uptake and translocation into the shoots of Mucuna and Centro plants were 91 mg/kg and 6.25 mg/kg respectively, in the un – amended treatments at the highest contaminant dose of spill simulated. Amendments further took the observed levels to 90.1, 63 and 117 mg/kg and 8, 23, and 10.92 mg/kg for NPK, UREA and POULTRY amendments in Mucuna and Centro plants respectively. Cu root concentrations were markedly higher than those of the shoots for all Centro plants and the reverse for Mucuna specie respectively. WhilePOULTRY MANURE – assisted phytoextraction with Mucuna plants by all indices proved efficient, our study showed that Centro plants were not feasible to remediate the heavily or moderately contaminated soils simulated in order to achieve the target total metal soil concentrations irrespective of the amendments employed. Tests with NPK and UREA fertilizers indicated detrimental effects on copper uptake, biomass yield, and the translocation of copper from roots to shoots in Mucuna species.

Key Words: Phytoextraction; Toxicity; Amendments; Contamination; Bioconcentration factor (BCF); Uptake

J5

Capacity of several tree species and *Chlorophytum comosum* Thunb. for PM accumulation

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Particulate matters (PMs), air pollutants caused by urbanization exert harmful effect on human health. They can be suspended in the air even for weeks as aerosols, and when inhaled can have carcinogenic, allergic and mutagenic effects. It is estimated, that PMs reduce average life expectancy in Finland 4, in Poland 11 months and in some regions even 3 years (1). The only opportunity to lower/remove PM from atmosphere in open space is via environmental biotechnology - phytoremediation in which plants are employed to clean up the environment. In this study we attempt at evaluation of the potential of PMs deposition on leaf surface of tress growing in nursery garden and on the herbaceous plant *Ch.comosum* grown in indoors with 5 various activities.
Amount of PM10, PM2,5 and PM0,2 deposited on leaf surface (rinsed with water) and captured by waxes (immersing in chloroform) were measured. The water rinsed fraction comprises the PM deposited on the surface and not very deeply into the wax, refers to PMs that in nature, are removed with rain and probably also by wind. While the part removed by chloroform (removing all wax) refers to the remaining part of PM, which are too are capture by waxes due to lipophilic nature. Capability for accumulation of PM of 25 plant taxons of trees recommended for cultivation in urban area, growing under the same conditions in a nursery was evaluated in leaf samples collected at the end of the vegetation seasons of 2007, 2008 and 2009. The same measurement were performed for *Chlorophytum comosum* in indoors as follows: (i) house outside of Warsaw, (ii) apartment, (iii) office, (iv) dental clinic and (v) small cosmetic factory.
Tested tree species significantly differed in capabilities of PMs accumulation. There were also differences in PM washed by water and chloroform. Amount of deposited PM corresponded with amount of waxes and hairs on leaf surface. In case of *Ch. comosum* , the highest deposition of PMs was in small cosmetic factory followed by dental clinic, apartment, office and the lowest was on leaves of plants grow in house outside of Warsaw. Particulate matters are too big to penetrate to the plants tissue and are accumulated on hair of leaf surface and in waxes covering leaves and stems surface. Softly attached PM can be removed by water (rain) and wind, deeper located in the waxes can be removed by chloroform. Species with leaves covered with bigger amounts of waxes have tendency to accumulate smaller fraction of PM while bigger fractions are deposited leaves covered with hair.

References: 1.EEA Report 2/2007
Keywords: air phytoremediation, genotypic differences, particular matter, spider plant
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J6

Phyto-cover and phyto-treatment technologies for mitigating the environmental impacts of aluminum industry located at Angul in Orissa State of India

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In India the estimated mineral deposits are about 20,000 representing 60 different kinds. At most of these reserves mining activity is in progress. Abandoned mine sites and smelting areas have often been neglected for restoration. Ecorestoration is a necessary step failing which the detrimental effects would be loss forest cover, leaching to trace metals, contamination of cultivated land and ultimately posing threat to biodiversity. The National Aluminum Company Limited (NALCO) is Asia's largest integrated aluminum production complex, located at Angul in Orissa State of India. NALCO is involved in bauxite mining, alumina refining, aluminum smelting, casting, and production of aluminum ingots. In order to meet the power demand, NALCO had its own captive power plant (CPP) of 720 MW capacity that produce a variety of coal combustion residues (CCR) of which coal fly ash is the major waste. Establishment of phyto-cover for containment of hazardous waste (spent pot line) and phyto-treatment technologies for coal fly ash slurry using and biological are presented in this paper.

Keywords: Phyto-cover, phyto-treatment, hazardous waste, aluminium smelter, Coal combustion products, , fly ash, constructed wetland, engineered vegetative cover, Orissa, India

J7

Phytofiltration of uranium from contaminated mine waters

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The work presented here is a part the on going study on the uraniferous geochemical province of Central Portugal in which, the use of aquatic plants as indicators of uranium contamination is being probed using aquatic plants emphasizing their potential use in the emerging phytotechnologies. Several of the uraniferous deposits were exploited either by underground or surface mining methods. Many of the places were left in different stages of degradation. The samples were collected in running and in standing waters (lentic and lotic) in the places were it was possible to observe aquatic species. In these sites, samples of the waters and of the vegetable species were taken. The plants collected represented the free floating and the rooted emergent plants. In the ponds, only free floating plants were found growing. The methodology adopted for the determination of the U content in the water and plants was fluorometry.
Even though we have observed very low concentration of U in the fresh waters of the studied sites we found a set of vegetable species with the ability to accumulate U in concentrations which are orders of magnitude higher than the surrounding environment. We have observed that *Apium nodiflorum, Callitriche stagnalis, Lemna minor* and *Fontinalis antipyretica* accumulated significant amounts of uranium, whereas *Oenanthe crocata* excluded U. These results indicate substantial scope for proper radiophytoremediation and phytosociological investigation exploiting the native flora. These species show great potential for phytoremediation because they are endemic and easy to grow in their native conditions. *A. nodiflorum* and *C. stagnalis* have high bioproductivity and yield good biomass.

Keywords: Aquatic plants, bioaccumulation, Central Portugal, phytoremediation, uranium

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J8

Potential of *Callitriche stagnalis* for phytofiltration of Uranium-contaminated waters

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Aquatic plants are of considerable importance for investigating the quality of water. The work presented here is deals with the use of water plants for phytoremediation of U contaminated waters. Investigations made at 185 sampling sites in uraniferous geochemical province of Central Portugal revealed U presence in the superficial waters in a wide range of concentrations (0.23 – 1220 µg/L as against 1.8 µg/L of the natural background concentration. In most of the sampled sites, the U concentration was below this value. Differences were only observed when comparing non-contaminated sites with streams directly fed by mine drainage (139.4 µg/L, average). High bioaccumulation levels of U were observed in several some aquatic plants and in a magnitude much higher than the concentration in the surrounding water. The highest concentrations of U were found in the submerged species *Callitriche stagnalis* Scop (1948 mg/kg; all values on dry weight basis), in *Potamogeton pectinatus* L. (365 mg/kg DW), in *Potamogeton natans* L. (95 mg/kg DW), and in the free-floating *Lemna minor* L. (43 mg/kg DW). To investigate the U accumulation by *C. stagnalis*, these plants were exposed during 7 days to different concentrations. This test confirmed the ability of *C. stagnalis* to concentrate U and the bioaccumulation coefficient (BAC = concentration in plant/concentration in water) was 3.4 x 103 (average), confirming its high potential for uranium phytofiltration. We observed a decrease in U concentration in water of 82% (on average). For U concentrations in water between 40 and 125 µg/L, it was possible with this simple experiment to decrease the U concentration to levels below the limit established by EPA (30 µg/L). Prospects for development of phytofiltration systems to decontaminate U in mine water are very high and work is in progress.

Keywords: Aquatic plants, bioaccumulation, Central Portugal, mine waters, phytoremediation

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J9

Rhizodegradation of Phorate in the Presence of *Brassica juncea* and *Pisum sativum*

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Rhizodegradation is the enhancement of naturally-occurring biodegradation in soil through the influence of plant roots, leading to detoxification of contaminants. Pot studies were conducted to study the rhizodegradation of phorate (at 20 mg Kg-1 concentration) with *Brassica juncea* and *Pisum sativum* plants for 30 days, in presence and absence of added bacterial consortium. Three phorate degrading bacteria *Pseudomonas aeruginosa*, *Ralstonia eutropha*, *Enterobacter cloacae*, isolated by enrichment technique, constituted the consortium. In the presence of bacterial consortium, phorate degradation was highest in treatment with *Brassica juncea* + microbes (51.6%), followed by the treatment with *Pisum sativum* + microbes (46.8%) and then by treatment with microbes (41.7%), which indicated that the presence of plants stimulated the degradation process. Similarly, in treatments without added consortium, highest degradation was observed in presence of *Brassica juncea* (24.6%) followed by *Pisum sativum* (11.3%). Thus, among plants, *Brassica juncea* was more efficient in phorate degradation as compared to *Pisum sativum*. The total viable count and dehydrogenase activity of the soil was found to increase remarkably in the treatments with added consortium as well as in the treatments with *Brassica juncea* and *Pisum sativum* plants as compared to the respective controls. A significant negative correlation was found to exist between the phorate concentration and the cell count [R= -0.363, **(P<0.01)], and phorate concentration and dehydrogenase activity [R= -0.754, *(P<0.05)], which evidenced that phorate degradation is mainly due to the microbial consortium present in soil. In general, the acid and alkaline phosphatase activity of the soil was found to increase during the 30 days period. These observations suggest that the presence of plants aid the microbial biodegradation of phorate and these plant-microbe systems can be applied for bioremediation of phorate contaminated soils.

Keywords: Rhizodegradation, Phorate, *Brassica juncea*, *Pisum sativum*, Bacterial consortium

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J10

Uptake of Cd and growth and photosynthetic responses to Cd stress in an extreme halophyte, *Arthrocnemum macrostachyum*

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The remediation of heavy metal-contaminated sites using hyperaccumulating plants represents a promising alternative to currently used methods. The potential of *Arthrocnemum macrostachyum* (Moric) C. Koch (a halophytic C3 shrub) was examined to determine its tolerance and ability to accumulated cadmium for phytoremediation purposes. A glasshouse experiment was designed to investigate the effect of cadmium from 0 to 150 ppm on the growth and the photosynthetic apparatus of *A. macrostachyum* by measuring relative growth rate, chlorophyll fluorescence parameters, gas exchange and photosynthetic pigment concentrations. We also determined total ash, cadmium, calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sodium and zinc concentrations, and C/N ratio. *A. macrostachyum* survived to concentrations as high as 70 mg Cd kg-1 DW in stems and 350 mg Cd kg-1 DW in roots (with external Cd of 150 mg kg-1), although a biomass reduction of c. 50% was recorded over external 5 mg Cd kg-1. This reduction in the biomass was mediated by a decline in the photosynthetic function after 30 d of treatment. Also, pigment concentrations and quantum efficiency of PSII declined with external Cd. Finally, the results indicate that *A. macrostachyum* is capable of tolerating a very high and continued exposure to cadmium, since this species controls the ion transport into leaves. Therefore, *A. macrostachyum* could be useful in the phytorremediation of soils.

Keywords: Cd accumulator, growth rate, photosystem II, phytoremediation, tolerance

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K1

Effect of *Glomus mosseae* and Different Combination of Heavy Metals on Accumulation Factor of Heavy Metals in Alfalfa

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An experiment was set up for determination of accumulation factor of heavy metals, in a 2×8 factorial completely randomised design, with four replicates. The first factor was inoculation (M) with *Glomus mosseae* or un-inoculation (M0). The second factor had seven levels of contaminants: (Co, Cd, Pb, CoCd, CdPb, PbCo and PbCoCd) plus an uncontaminated control treatment (C). Total Co content =51.91 mg kg-1 dried soil, total Cd content =8.5 mg kg-1 dried soil and total Pb content =436 mg kg-1 dried soil. The heavy metal salts used included CoSO4, CdCl2 and Pb(NO3)2. The soil was contaminated before planting by adding the calculated amounts of heavy metal salts in distilled water and mixed throughout the soil profile. They were allowed to stabilise for 15 days. Then, 50 g *G. mosseae* inoculum was mixed with 5 cm of upper surface of soil and Alfalfa seeds planted as before. Plants were cut from soil surface in early flowering stage. Roots were extracted from pot. Aboveground materials separated into the stems and leaves were washed by distilled water. Plant material was dried at 70 °C for 48 hours. Heavy metals concentration in plant tissue and soil were measured by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Variant-Liberty 150AX Turbo). Accumulation factor of heavy metals were calculated by concentration of heavy metals in leaves (mgkg-1) / concentration of heavy metals in leaves (mgkg-1). Statistical analysis of data was performed by SAS software. The data were analyzed through one-way analysis of variance (ANOVA) and Comparisons between means were performed using Duncan's multiple range test at the significance level of $P<0.05$. The most amount of accumulation factor of Co, Pb and Cd was in MC, M0PbCd and M0Cd ($P<0.05$),
respectively. Results showed types of contaminants and combinations effected on accumulation factor of heavy metals.

Keywords: accumulation factor, heavy metals, alfalfa

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K2

The role of silicon in the detoxification of cadmium in wheat (*Triticum turgidum* L. cv. Claudio W.) plant.

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Due to industrial and agricultural development, contamination of agricultural soils with heavymetals is becoming a serious problem throughout the world. Cadmium (Cd) is a highly toxic heavy metal for both plants and animals. The presence of Cd in agricultural soils is of great importance because of its larger mobility than other heavy metals. Due to more solubility, Cd is more available and readily taken up by the plants and accumulate in different parts of the plants. Silicon (Si), on the other hand, is a quantitatively major inorganic constituent of higher plants but the element is not considered essential for them. However, it has been proved to beneficial for the healthy growth of many plant species such as rice. Silicon can enhance the resistance in many plants to toxic metals, in this regard, we report an experiment showing that Si as amorphous silica, applied directly onto the soil, was used to decrease Cd uptake and alleviate its toxic effects especially in wheat (*Triticum turgidum* L. cv. Claudio W.) plant. Plants were grown in pots in a greenhouse on a sandy loam (naturally contaminated with sewage sludge) soil. Four treatments (0, 1, 10 and 15 ton/ha) of Si were investigated without and with plant growth. Diatom was used as a source of Si. After harvesting, oven dry weight of plant roots and shoots was measured and also measured the concentrations of Cd and Si in roots, shoots, soil solution and in the soil. The result obtained showed that plant biomass (both shoots and roots) increased with Si treatments especially with 15 ton/ha Si. Silicon treatments increased Si contents in shoots and roots. Si treatments significantly increased Cd concentration in roots and decreased translocation to shoots. A full comprehensive description regarding this detoxification of Cd by application of Si will be presented.

Key words: Heavy metals, uptake, silicon, cadmium, wheat

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K3

Phytoremediation of salt soils polluted with Ni2+ and Cu2+

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One of the problems of soils of Volga-Caspian region in Russia is their pollution by heavy metals, for example, nickel and copper. Frequently the soils are not only polluted with the metals but also salted during of active land irrigation.
The purpose of this work is to study the opportunity of phytoremediation of soils polluted with Ni2+ and Cu2+ and salted by sodium chloride.
We studied the stability of different plants towards the action of sodium chloride and high concentrations of copper and nickel. The corn (*Heliantus annus L.*) and sunflower (*Zea mays L.*) were chosen as vegetative cultures most viable and distributed in agriculture. It was established, that activity of corn and sunflower to extract metals depends on the salt concentration in soil. At an average salt degree (0,3-0,6 %) ability of plants to accumulate copper and nickel increased up to 5-13 % and 10-20 %, consequently.
Using the corn it was determined, that the majority of the absorbed nickel and copper was located in roots. After accumulation the metals passed to stems and leaves, not getting in ears. In salted soils this process was intensified. It was revealed, that at the joint presence of Ni2+ and Cu2+ in soil copper was more preferable for the corn than nickel.
The results of laboratory experiments were confirmed in natural tests. During the full period of vegetation the most part of metals was collected in roots and stems, but not in ears, that allows using them for the food purposes. For the avoidance of secondary pollution of soils with heavy metals it is necessary to delete root system of corn after its cultivation.

Keywords: phytoremediation, salted soil, sodium chloride, nickel, copper

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K4

Phytoremediation of mine soils using plants and earthworms

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Since the primary aim of the phytoextraction technique is to remove metals from polluted soils by concentrating them in the harvestable parts of plants, the removal yield depends on the amount of above-ground biomass and the bioavailability of metals. Thus, the enhancement of both the plants' ability to accumulate metals in shoots and biomass yields is the key to improve phytoremediation efficiency. Recently, roles of earthworm in heavy metal remediation have been reported, based on their beneficial effects for plant growth and the enhancement of soil metal bioavailability. We have conducted a research focusing on the phytoextraction of Pb, Zn and Cu from two soils polluted by mining activities using the combination of maize (*Zea mays*) or barley (*Hordeum vulgare*) and *Lumbricus terrestris* earthworm.

Phytoextraction experiments were carried out in plastic pots using five treatments for the polluted soil: earthworms (E), maize (M), barley (B), maize + earthworms (ME), barley + earthworms (BE). Pots without plants or earthworms were also used as control (C). Experiments were conducted by triplicate under artificial light and constant temperature (17ºC). After 4 weeks, samples of plants, earthworms and soils were taken. Analysis conducted were: (i) total metal concentration in shoots and roots of plant samples and earthworms tissues; and, (ii) for soil samples, water and CaCl2 extractable metals and geochemical partitioning of metals by means of BCR sequential extraction. Addition of earthworms to soil slightly increased water soluble Zn for both soils. Zn extracted by CaCl2 was significantly increased ($p<0.01$) in comparison with data from the control pots (C). Results from BCR sequential extraction procedure showed that Zn and Cu in the "bound to organic matter and sulphides" fraction (F3) was significantly higher for pots with earthworms (E) than those of control pots (C). As compared as experiments with only plants (M and B), earthworm presence increased shoot weight of plants in ME and BE treatments; however, that increase was not statistically significant.

In general, earthworm activities increased plant metal concentrations to some extent. For Soil 1, that increase was only statistically significant for Cu and Zn root concentration in maize and for total Pb concentration in barley. For Soil 2, Cu and Pb shoot concentrations were significantly increased for maize; Pb and Zn contents were significantly increased for both roots and shoots in barley.

Therefore, those results showed that *Lumbricus terrestris* earthworm can moderately enhance bioavailability of heavy metals in soils, thus improving their absorption by plants and the accumulation of the studied metals. Nevertheless, the effect of *Lumbricus terrestris* on both metal availability and plant uptake was lesser significant than that of previously reported by the authors for *Eisenia fetida* earthworm [1].

Keywords: barley, maize, *Lumbricus terrestris*, phytoextraction, lead, zinc, copper

[1] E. Ruiz, L. Rodríguez, J. Alonso-Azcárate (2009) Effects of earthworms on metal uptake of heavy metals from polluted mine soils by different crop plants. Chemosph.75, 1035–1041

K5

Biomass production and phytoextraction of *Brassica napus* L. var. *oleifera* D.C. grown on contaminated soil

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Phytoextraction is a promising technique to extract heavy metals from polluted soils and *Brassica napus* L. var. *oleifera* D.C. is species known for its metal tolerance and accumulator useful.
The aim of this study was to evaluate the biomass production and the potential of phytoextraction of two commercial varieties, Betty (Apsob sementi) and PR46W31 (Pioneer Hi Breed), grown in pot on a multi-metal contaminated soil sampled in protected Area of Alta Murgia (Southern Italy). More than 400 ha of soils from this National Park, in fact, are polluted by heavy metal (chromium, in particular), for runaway disposal of different types of industrial wastes.
An uncontaminated agricultural soil collected nearly was used to fill control pots.
At the end of the experiment, the morphological parameters was measured to define biomass yield. The phytoextraction potential of the heavy metal concentrations in the plant parts were determined with ICP-EOS spectrometer and the bioaccumulation coefficients calculated.
The morphological data obtained show that plant growth increases on the multi-metal contaminated soil compared to control, producing considerable biomass. In our conditions, although both varieties are tolerant to heavy metals, *B. napus* PR46W31 demonstrated the best capacity of multi-metal absorption. In particular, in this experiment, 'PR46W31' behaves as Cr hyperaccumulator, even if Chromium is principally immobilized in the roots, like so the other metals. Probably metal bioavailability is reduced by the basic soil pH and the high content of organic matter in tested polluted soil.
The results obtained are very interesting considering that we are restoring a protected area where other methods may prove impractical. Moreover the biomass produced with low investment can be used as a source of energy, adding to phytoextraction an economical return.
To make some more suitable this technique specific agronomic practices, including the possibility to pull up the whole plant, could be to evaluate.

Key words: pollution, phytoremediation, heavy metals, metal tolerance plant

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K6

Plant & organochlorines, uptake *vs* phytotoxicity

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Organochlorines (OCs) are known to be persistant organic pollutants due to their high lipophilicity and stability. Therefore, solutions to restore polluted system by bio-technologies are studied especially using plant materials. For this purpose, phytoextraction of standard organochlorines (OCs) [lindane, monochloro-, dichloro-, trichloro-benzenes (MCB, DCB, TCB)] was experimentated. Screening of plant species adaptated to OCs exposition (biomass and morphological criteria) permitted to select *Zea mays* as a relatively tolerant plant. Therefore, 7-day-old plants were exposed to a concentration range of OCs to determine NOEC (7 days of exposure) and then, uptake rate (1 month exposure). OCs were in part phytoextracted and mainly localized in root tissues. Nevertheless, such an OCs exposure seemed to impact the viability of plant tissue. Therefore, ROS, CAT, GPX and GdR analyses shown that OCs could induce an oxidative stress on root tissues. Moreover, root cell viability was strongly dependent on the OCs concentrations. Using biomass and oxidative stress indicators, *Z. Mays* could be a good plant candidate to phytoextract those pollutants. Finally, OCs phytoremediation could be considered in area with a pollution range comprised between 0 to 30 mg kg-1.

Keywords : Organochlorines, *Zea mays,* uptake, oxidative stress, cell viability

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K7

Response to cadmium stress in lichen thalli

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The improvement of knowledge regarding the cytological and functional response of lichens to metals is essential for laying the basis for fundamental research and reliable applications in atmospheric pollution biomonitoring. To date, there is little information available concerning protection mechanisms exerted by lichen thalli, as well as their aposymbiotically-grown bionts, to contain Cd stress. In this context, my general mechanistic hypothesis concerning Cd detoxification and tolerance in lichens is that Cd toxicity could be counteracted by an integrated ‘‘fan-shaped’’ response, the details of which vary according to the species and the biont investigated. As far as this model is concerned, the ‘‘first line’’ mechanisms to cope with Cd stress can involve cell wall immobilization, phytochelatin synthesis and vacuolar compartmentalization. The ‘‘second line’’mechanisms can instead rely on other systems (essentially up- or downregulation of protein expression and activation of enzymatic antioxidant), which may be less direct, but still of considerable importance. These two “defense lines” would be crucial for organisms such as lichens, which are constantly exposed to a large number of simultaneous stress factors and should therefore be able to minimize the energy expended in self-protection against Cd stress.

Keywords: biomonitoring, cadmium, lichens, metals, phytochelatins
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K8

In-Planta Solid Phase Sampling Devices

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The detection and analysis of chemical contamination at contaminated groundwater sites has always been an expensive and time-intensive affair. The use of phytoforensics in order to estimate the levels of contamination has shown promising signs. Since plants growing on site interact with the water, air and soil in the surroundings, they can be used as sources of information to access contaminant plume size and concentration differences. Their use as monitoring aids can save time and money, as well as incorporate minimal impact to the surrounding ecosystems. These new techniques involve the placement of passive sampling devices in trees on-site followed by laboratory analysis post equilibrium. The performance and sensitivity of a number of different materials commonly used as solid phase sampling devices will be presented, to demonstrate their viability as in-planta samplers, in supplementing contaminated-site investigations for chlorinated solvents. The effects of temperature and sampling variations like exposure time will be accessed to gain a better understanding of which materials would be best-suited for use in field analysis. The results obtained using solid phase samplers will be compared to those obtained using Solid Phase Microextraction (SPME) and tree coring to determine if the results are consistent. If these in-planta solid phase sampling devices are capable of generating reproducible results, they may prove to be a viable option for use in the field of phytoforensics.

Keywords: Phytoforensics, passive sampling devices, chlorinated solvents, site investigations**.**

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K9

Evaluation of cadmium removal by micropropagated plants of *Narcissus tazetta* and its effect on alkaloidproduction

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Plant survival and growth performance are essential factors in phytoremediation of heavy metals. *Narcissus tazetta* is a plant of choice for heavy metal phytoremediation which is ornamental plant and can be used for the production of highly valuable alkaloids with anti-tumor, anti-viral and anticholinergic activities. In this study, micropropagation of this plant was done in three procedures: induction (mMS+4 mg l-1 BAP+ 0.12 mg l-1 NAA medium), proliferation (MS+2 mg l-1 BAP +0.12 mg l-1 NAA medium) and bulb production (MS + 9% sucrose + 0.1 mg l-1 NAA medium) from twin scales as explants. After 4 months, micropropagated plants were treated by two concentrations of cadmium chloride (0.5, 1 mM) in the MS + 9% sucrose solidified media. After 3 weeks, growth, total proteins, peroxidase activity, cadmium accumulation, quality and quantity of alkaloids were investigated. Results showed that this plant accumulated large amounts of cadmium in its roots (2778.13 µg g-1DW), bulbs (801.87 µg g-1DW) and leaves (162.8313 µg g-1DW), without any impact on growth.
This point suggests that this plant is a hyperaccumulator of cadmium and can be used for cadmium remediation. Peroxidase activity increased under cadmium stress. Also, isozyme pattern of peroxidase was changed and new anionic isoenzyme was appeared. Cadmium affects on both quality and quantity of alkaloids. In the presence of cadmium, alkaloid content was increased, however homolycorine was found instead of 9-O- demethylhomolycorine.

Keyword: *Narcissus tazetta*, cadmium, phytoremediation,
alkaloids, micropropagation

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K10

Can be the industrial side-products tools for sustainable agriculture?

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The most important factors for an individual site are sun, air, soil and water. Although air and sunlight are available everywhere on the Earth, crops depend on soil nutrients and the availability of water. When farmers grow and harvest crops, they remove some most of these nutrients from the soil. Without replenishment, the plants will suffer from nutrient deficiency which leads to reduced yields. Sustainable agriculture depends on environmental circumstances including the available nutrients while to minimize the use of non-renewable sources, such as energy used to convert atmospheric nitrogen into synthetic fertilizers or mineral ores is general task. The aim of our study is to give a brief overview about the effects of some industrial wastes on the ecosystem especially on the physiological parameters of plants. Sewage sludge, lime sludge and compost were examined is this study. Corn (*Zea mays L cvs. Norma SC*) seedlings were used in the experiments. The soil-plant and nutrient solution-plant system were examined. The filtrates of examined materials were added to the nutrient solution and the raw materials to the soil. These materials contain plenty of useful elements for plants e.g. calcium (Ca), zinc (Zn), magnesium (Mg), iron (Fe). Moreover these wastes contain also some heavy metals e.g. aluminium (Al), chrome (Cr), manganese (Mn), lead (Pb). Dry matter accumulation of shoots and roots, relative chlorophyll contents, the contents of elements were measured of the plants that were grown on nutrient solution. The daily growth (nigh and day separately) of roots was measured in rizobox experiment. We came to the conclusion that the sewage may have dangerous effects on the ecosystem because it contains some harmful microorganisms for plant development. The intensity of growth of roots was more intensive when lime sludge was used than it was applying sewage sludge in soil experiments, while the other examined parameters were better in case of sewage sludge treatments.

Keywords: plants growth, ecosystem, sustainable agriculture, wastes

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L1

Physiological examination on some industrial wastes

Toth B.*, B. Kovács, L. Lévai, S. Veres

Human efforts such as the burning of fossil fuel, clearing of forests, draining of wetlands, and fertilization of croplands have released huge amounts of carbon - dioxide, methane and nitrous - oxide into out atmosphere. The average yearly temperature, despite a great deal of year-to-year variation has risen about 0.05 °C per year. The average global temperature will increase between 1.4 °C and 7.7 °C by increasing greenhouse gases concentration. These changes will play an increasing role in the mineral nutrition of plants. Warmer soil temperature may accelerate chemical and biological processes, including the available nutrients, soil microbial activity, and root metabolism. Moreover, more and more wastes and by-products are produced from the developed countries, e.g. sewage sludge, plastic bags, hand-me down technical equipments, liquid and solid wastes equally.

The aim of our work is to give a brief overview about the effects of some industrial wastes on the ecosystem especially on the physiological parameters of plants. Sunflower and corn seeds were used in the experiments. The filtrates of examined materials were added to the nutrient solution in different quantities because of different solubility. Acidic division and oily emulsion were examined is this study. These materials contain plenty of useful elements for plants e.g. calcium (Ca), zinc (Zn), magnesium (Mg), iron (Fe). The contents of elements in the root and in the shoot of corn and sunflower were measured. The relative chlorophyll contents and the dry matter accumulation were also measured. The relative chlorophyll contents were around the control when oily emulsion was applied, but the plants died after one week, so we could not measure the relative contents just the dry matter accumulation, which remained below the control value.

Especially disadvantageous physiological effects of acidic division and oily emulsion were proved. The compensation effect of environment is excluded; however, the compensation effects of environment are not endless. Summery, the waste resulting acidic separation method has disadvantageous and hazardous effects on the plants and therefore on the ecosystem.

Keywords: plants growth, ecosystem, acidic division, oily emulsion, wastes

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L2

A Comparison of Three Different Remediation Techniques of Risk Elements Contaminated Soil *via* Lysimeter Trial

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Among existing low cost remediation techniques, in situ applicable ones for the clean-up ofsites contaminated with risk elements or organic pollutants are preferred. Three approaches have been proposed for remediation of risk elements (Cd and Zn): i) *natural phytoextraction*, ii) *assisted phytoextraction,* and iii) *chemical immobilization.* The first one is based on the use of natural fast growing plant species (willows). Willow is suitable plant for risk element phytoextraction due to high element accumulation, high Cd and Zn transport to the shoots and high biomass production. *Chemical immobilization* is a remediation method where inexpensive materials (e.g., fertilizer, waste products) are added to contaminated soil to reduce the solubility of risk elements. In the case of *assisted phytoextraction* the plant growth can be promoted by "external treatments" using inoculation (ectomycorrhizal – ECM symbioses). The application of ECM inoculum (*Paxillus involutus)* and calcareous sorbent were used for their putative ability to enhance phytoextraction and phytostabilization, respectively. Willow clone *(S. x smithiana Willd. S-218)* with high phytoextraction potential was cultivated in lysimeters (*high 30 cm and diameter 20 cm*) and leaching of Cd and Zn together with overall pot water balance was monitored repeatedly during all the vegetation period. In the end of the experiment the distribution of chosen risk elements and nutrients in plants biomass and plants tissue concentration of selected stress indicating-amino acids were assessed (e.g. free Proline: *i) = 83±12 mmol*g-1, ii) = 261±16 mmol*g-1, iii) = 192±21 mmol*g-1*). Calcareous sorbent and mycorrhizal inoculation alleviated the plant stress imposed by risk elements resulting in better plant growth and lower levels of stress markers yet through different mechanisms. The sorbent confirmed ability to suppress metals leaching from the soil substrate resulting in limited element uptake by plants whereas mycorrhizal treatment increased the effectivity of Cd and Zn phytoextraction by experimental plants. This research is part of project EEA-Norway Grants CZ0092.

Keywords: risk elements, lysimeter, soil leaching, inoculation, sorbent, willow clone

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L3

Application of phytotechology in the Niger delta, Nigeria

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Applications of phytotechnology are gaining importance in Europe and United States as an effective and low-cost alternative for treatment of sewage effluents in small cities and / or food & meat manufacturing companies. They have obvious advantages over the traditional wastewater treatment systems and inspite of its advantages and extended practice, its application in developing countries like Nigeria in general and the Niger Delta region, in particular, has not yet been reported anywhere. The authors are of the opinion that because of the apparent simplicity of design and management, coupled with its efficiency and cost effectiveness and with the presence of abundant natural aquatic macrophytes in the Niger Delta region, phytotechnology can be adopted to treat municipal / sewage, industrial wastes and oil spill sites in Nigeria.

Three sites in the Niger Delta region (Obiafu / Obrikom in ONELGA, Rivers State; Brass and Obama in Bayelsa State), Nigeria were therefore characterized with a view to establishing phytotechnology (phytoremediation and phytodepuration) plants. Soil physical parameters determined included texture and infiltration rate / permeability while chemical parameters included pH, organic matter, exchangeable cations, conductivity, nutrients and TPH. Infiltration characteristics of the soils revealed that penetration of water was initially faster (1 cm min-1), but reduces gradually until a constant value (1.6 cm hr-1) was reached at 75 minutes. Generally, water intake was slow with a cumulative intake of 43.10 cm3 hr-1. Moreover, soil THBC, TFC, HUB & HUF and predominant microbial isolates were determined. Indigenous / autochthonous species and economic importance of predominant aquatic macrophytes in each of the sites were identified. Habitat, the slope of the basin and indices of quality for each of the sites were determined. This article reviews the site characterization reports for the three locations for the establishment of phytoremediation and phytodepuration plants in the Niger Delta region, Nigeria.

Key words: Phytotechnologies, Niger Delta region, Infiltration characteristics, Aquatic macrophytes, microbial isolates

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L4

Potentials for the application of phytotechnologies in phytomonitoring and ecorestoration of mobil's aviation fuel spill in Ibeno, Nigeria

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With huge oil & gas activities in the Niger Delta region of Nigeria in the past four decades, environmental pollution has posed a threat to ecosystem health, food safety, sustainable agricultural development and human health and security in the area. The level of insecurity in the region was at its peak until the amnesty granted by the Nigerian Government. Recently, Ministry of Niger Delta Affairs, Nigeria concluded the study of major oil impacted and wastes dump sites within the nine states of the Niger Delta region. The next phase of work is to remedy as well as monitor these locations. One of such oil-polluted sites in the region is the Mobil Producing Nigeria's (MPN's) pipeline rupture that occurred on 8th August 2001 along the 30-year-old 4" Qua Iboe Terminal (QIT)-Jetty aviation fuel pipeline which resulted in the spillage of about 1,000 bbls of aviation fuel into the environment, at Inua Eyet Ikot, Ibeno Local Government Area of Akwa Ibom State, Nigeria. The baseline hydrocarbonoclastic and other microbiological and physico-chemical data of the soil and plant tissues analyses from this spill site after about 9 years have been reported in this article. The use of phytotechnologies for phytomonitoring and ecorestoration of this and other contaminated sites in the Niger Delta region has been advocated. This research presents an overview of the potentials of the use of phytotechnologies in the monitoring and restoration of the polluted sites in the Niger Delta region, Nigeria.

Key words: Phytotechnologies, Phytomonitoring, Ecorestoration, Niger Delta region, Oil-polluted and Wastes dump sites, microbiological and physico-chemical data

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L5

Effect of aeration on the treatment performance of constructed floating wetlands

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Three mesocosms simulating full scale retention basins (length: 1.5m; width: 0.8m; water depth: 0.9m) were constructed in January 2007. Two of them were equipped with floating macrophyte mats planted with *Carex* spp, mimicking constructed floating wetlands. The third mesocosm served as a control and did not contain a floating macrophyte mat. All three mesocosms were batch loaded (retention time 11 days) with domestic wastewater coming from a wastewater treatment plant. Aeration was provided by air diffusers at the bottom of one of the two CFWs at a rate of 3.1 L air min-1 m-3 water. The removal of total nitrogen (TN), ammonium-nitrogen (NH4-N), total phosphorus (TP) and carbon (TOC, COD) was evaluated.

Providing aeration resulted in an improved removal of NH4-N, total nitrogen (TN), total organic carbon (TOC), chemical oxygen demand (COD) and total phosphorus (TP) when compared to a non-aerated floating wetland. Removal efficiencies for the aerated and nonaerated wetland after 11 days were >99% and 43.2 ± 10.9% for NH4-N, 67.9 ± 5.7% and 43.0 ± 8.1% for TN, 69.0 ± 5.3 and 22.6 ± 18.5 for TOC, 68.7 ± 5.0 and 55.2 ± 4.4 for COD, and 59.4 ± 2.9 and 35.1 ± 11.7 for TP. In the aerated system all NH4-N was removed within 4 days whereas in the non-aerated wetland and control a gradual decrease was observed throughout the 11 day experiment. Furthermore, most removal occurred within the first 4 days in the aerated system. No significant difference in removal performance was observed in the aerated system after 4 or 11 days. Due to nitrification in the aerated wetland NO3-N concentrations increased up to 5 mg L-1 whereas in the non-aerated wetland and control NO3-N concentrations remained low (< 0.5 mg L-1). Measurement of dissolved oxygen concentrations indicated concentrations higher than 10 mg L-1 whereas no oxygen was detected in the non-aerated system and control. To obtain a reduction of NO3-N concentrations oxygen levels should be lower. Another steering factor for denitrification next to oxygen and temperature is the availability of an organic carbon source. Both TOC and COD concentrations were lower than respectively10 and 15 mg L-1 after 4 days of aeration and no significant removal of TOC was observed after that period. This could indicate that the carbon, still available in the wastewater, was not readily degradable and would, as such, not be suitable for the denitrifiers. The decrease of P in the aerated system was due to improved chemical precipitation of P with calcium, iron, magnesium and aluminium.

As pollutant removal is improved by aeration, shorter residence times and smaller installation footprints can be used. When using CFWs for treatment of combined sewer overflows or storm water, the foreseen buffer capacity can be used more optimally as the water can be quicker discharged.

Keywords: treatment wetlands, nitrogen removal, nitrification

L6

Selectivity and Efficiency of Organic Acids and their Salts on Risk Elements Concentration in Soil Leachate

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Phytoextraction, the use of engineered metal-accumulating plants to remove toxic metals from soil, could potentially be an environment-friendly and cost-effective technology compared to conventional remediation techniques [1]. Chemically assisted phytoremediation has been developed to induce accumulation of metals by high biomass plants. The use of natural compounds such as low molecular weight organic acids (LMWOA) which are easily biodegradable sounds better than synthetic chelate application to the public acceptance of phytoextraction technology. The low effectiveness of assisted phytoextraction using aliphatic LMWOA has been attributed to their fast mineralization by soil microorganisms. The short term leaching experiment (approximately 6 hours) was conducted to examine the potential and efficiency of four LMWOAs {acetic (AA), tartaric (TA), citric (CA), oxalic (OA)} and their ammonium salts (concentration both = 10 mM) on three risk elements (Cd, Pb and Zn) mobility in multi-contaminated cambisol soil. Ion species/solubility, adsorption and desorption equilibrium reactions was provided with the Visual MINTEQ model (US EPA). A Cadmium concentration increased 24x (AA), 28x (OA), 91x (TA) and 93x (CA), lead concentration increased 6x (AA), 42x (OA), 193x (TA) and 225x (CA) and zinc concentration increased 29x (AA), 69x (OA), 107x (CA) and 128 (TA) in comparison with control.
Ammonium salts showed lower effect. Oxalate (2x), acetate (3x), tartarate (45x), citrate (58x) increased concentration of Cd in solution, 0,3x (acetate), 26x (oxalate), 126x (tartarate), 150x (citrate) increased Pb concentration in solution and 3x (acetate), 16x (oxalate), 93x (tartarate) and 111x (citrate) increased Zn in solution. This study has been funded by the CzechUniversity of Life Sciences foundation (CIGA-21140/1313/3109).

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Keywords: soil, risk element, organic acid, mobility, leachate

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L7

Correlation between habitat and molecular features in the Ni hyperaccumulator *Thlaspi caerulescens* Monte Prinzera population.

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In the context of soil pollution, an important aspect is the treatment and management of sites contaminated by metals such as Ni, Cr, Co, Pb, Zn, Cd, Cu, Mn. In natural conditions many of these elements are present at high rates in ophiolitic soils which are widespread in the Italian territory. Thus the ophiolite territories represents natural sites in which heavy metals are concentrated and the vegetation growing in these sites, which are usually rare endemic plant species, have been forced to adapt to a series of ecological factors among which a peculiar physical- chemical composition of the soil (Baker et al., 2010; Maestri et al., 2010).
Monte Prinzera (MP) is one of the ophiolitic Italian territories, situated in the Tosco-Emilian Appennin and it is a Natural Reserve. The physical-chemical characteristics of this site allowed for the selection of some plant species tightly linked to these substrates, with specific adaptive traits, such as tolerance and accumulation of metals. In particular a metallicolous population of *Thlaspi caerulescens* is found in this site and studied and classified as a Ni hyperaccumulator population. The absence of this plant nearby, in non-ophiolitic soils, and its growth and reproductive traits render *Thlaspi caerulescens* MP an interesting model to study the adaptation to extreme environments.
By studying *Thlaspi caerulescens* population of MP, the presence of different morpho-types were observed in sub-sites which showed differences in the geo-morphology. A multivariate statistical analysis performed on morphological traits such as i) number of leaves of the rosettes; ii) dimension of leaves of the rosettes; iii) height of stalk; iv) number of inflorescences; v) weight of seeds; vi) dimension of seeds evidenced the significance of this phenotypic variability. Environmental soil parameters such as i) pH; ii) soil organic content; iii) metal content such as Ni, Fe, Co, Zn, Mg; Mn, Ca, K; iv) water content were also analysed in correlation with plants' growth behaviour. Plant phenotypic variability was analysed also at molecular level by a comparative high-throughput analysis of protein variations. Differences in protein abundance were observed between plants in the different sub-sites evidencing a strong phenotypic plasticity of these plants in the adaptation to specific micro-environments. In conclusion, in this work, the combination of phenotypic and molecular descriptors showed to be an useful approach for a better characterization of the biodiversity in a species adapted to grow on metalliferous soils.

Key words: *Thlaspi caerulescens*; serpentine soil; phenotypic plasticity; proteomic markers

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L8

Phytoremediation pilot at an industrial site heavily contaminated with aromatic hydrocarbons

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After the decommissioning of a former styrene production facility at an industrial site in the Netherlands, the shallow subsurface was found to be heavily contaminated with monoaromatic hydrocarbons (MAH), mainly benzene, ethylbenzene, and styrene. Although the contamination is not causing unacceptable risks and remediation is not required by the authorities, the owner prefers the site to be cleaned up.

Several remedial options have been assessed. Phytoremediation was identified as the preferred option for several reasons:

- in situ remediation is not feasible due to the poor permeability of the soil
- short-term redevelopment of the site is not foreseen
- evapotranspiration of water will minimize contaminant migration
- phytoremediation is sustainable and cost-effective.

To determine the feasibility of phytoremediation at the site, a pilot application is currently being performed at an area of 40 x 60 m. In this area 33 local type willows (*Salix alba*) have been planted. To establish the need of protecting the trees against the contamination, three different planting configurations have been used:

- typically planted trees with clean soil in the tree hole
- trees with the *TreeWell*® system, in which the sides and top of the tree holes are covered with an impermeable liner
- modified *TreeWell*® system, without using a impermeable liner

Prior to the tree planting, a baseline study was done to determine the contaminant situation and the nutrient status of the soil and groundwater. This involved analyses of soil and groundwater and PID measurements in boreholes. The contaminants were found to be unevenly distributed and two highly contaminated zones have been identified. MAH concentrations in soil ranged from < dl to 11.500 mg/kg d.w. MAH concentrations in the shallow groundwater ranged from 55 to 141,000 µg/l.

For the pilot, an intensive monitoring programme was designed. Monitoring activities include:

- measurement of contaminants and nutrients in soil
- measurement of contaminants, nutrients, and redox parameters in groundwater
- hydrological monitoring using pressure divers
- tree inspection
- measurement of contaminant evapotranspiration
- measurement of contaminant levels in the trees

The trees were planted in April 2010. Initially three trees, all located in a relatively clean part of the pilot area, showed poor growth and symptoms of watermark. Two months after planting these trees are recovering well. At the conference the first results of the pilot study will be presented.

L9

Bioprecipitation of heavy metals using *Calotropis gigantea* latex exudate

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Phytoremediation is a plant based remediation technique, used all around the globe for the bioaccumulation of heavy metals from various contaminated soils at sites where industries are abundant. *Calotropis gigantea* a milk weed plant belonging to the family Asclepiadaceae, is a potential plant, the latex from which can be made use of for the bioprecipitation of heavy metals like chromium, cobalt and copper. The presence of free phosphate groups in the latex supernatant obtained from the plant, play an important role in the bioprecipitation. The present study aims at using the supernatant of the latex obtained from the plant for precipitating the heavy metals at an alkaline condition for a time interval of 10 m, 20 m and 30 m, with the concentration of heavy metals varying from 1ppm to 5 ppm. On carrying out the experiments it is found that at a reaction time of 30 minutes maximum bioprecipitation of these heavy metals from a range of 80 -90 % is obtained. On comparing the three metals, chromium has shown the maximum percentage of precipitation. Hence we can infer from the study, that chromium a major component of the effluent from tannery industries can be treated using this method for its removal. Similarly the other metals copper and cobalt found in petroleum refinery waste and other sources can be treated with this method. The abundance of the plant in tropical regions makes it a cheaper and a commercial source for the removal of the heavy metals from the effluent streams.

Key words: *Calotropis gigantea*; bioprecipitation; phytoremediation; chromium; copper; cobalt.

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L10

External benefit of phytoremediation: The marginal effect of potential CO2 abatement on the price of biomass

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The purpose of this paper is to examine all of the potential benefits of phytoremediation as a sustainable remediation technology and to explore which policies would promote the efficient use of the technology. The analysis is based on a case study in the Campine region (Flanders, Belgium). The contamination of (agricultural) land in that region with Cd, Pb and Zn is so extensive (< 280 km²) that conventional remediation is not applicable. There is an obvious need for remediation and risk reduction alternatives in Europe which are environmentally sound and protective of human health. It is also now widely recognized that cleanup activities of hazardous waste sites may affect emissions of greenhouse gases. In addition, the European Renewable Energy Directive promotes an increase in renewable energy to 20% by 2020. Phytoremediation offers the opportunity, not only for the farmer, but also for society, to come up with an approach that efficiently uses agricultural land to address all of these problems. Specifically, using contaminated biomass for energy production may contribute to the reduction of carbon dioxide (CO2) emissions. Performing a Life Cycle Analysis (LCA), complementary to a Cost Benefit Analysis (CBA), we examined the energy and CO2 abatement potential of willow (*Salix* spp), energy maize (*Zea mays* L.), and rapeseed (*Brassica napus*). We analyzed whether subsidizing this biomass would be economically efficient. Our conclusions are based on current Flemish policy and several valuation techniques for CO2. The most efficient technology is the digestion of energy maize with combustion of the contaminated digestate. Short rotation coppice (SRC) of willow to replace coke-based heat comes second. Alternatively, SRC of willow can be used in a biomass combustion installation to produce heat and electricity. Pure plant oil (PPO) and biodiesel from rapeseed score rather low because of the need for fossil fuels as an input for fertilizer.
Current energy subsidies are already reflected in current crop prices. CO2 abatement potential cannot be counted again as this would be double counting. Instead of using current prices, we calculate the "true" price per ton of output and the price per GJ needed to internalize the CO2 benefit of €20 per ton. The results suggest that current subsidized prices are too high.
Implications for the phytoremediation technology are mixed. We find that the true prices are not high enough to support renewable energy whether contaminated or uncontaminated biomass is used. However, the analysis suggests that including CO2 benefits would not cause phytoremediation to lose its competitive advantage compared to conventional technologies.

Keywords: willow (*Salix* spp), energy maize (*Zea mays* L.), rapeseed (*Brassica napus*), sustainability, life cycle analysis, energy, policy

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M1

Spontaneous vegetation colonizing Zn-Pb waste heaps in Southern Poland

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Zinc and lead mining and smelting activities have caused environmental degradation worldwide and have created large amounts of waste deposits. Such waste heaps pose a serious threat to the surrounding areas, often densely populated, as a source of metal dispersion through wind and water erosion. The development of vegetation is extremely difficult and protracted in these areas because of the unfavourable physical and chemical properties of the waste as well as the limited pool of appropriate species able to colonize them. Nevertheless, some vegetation spontaneously colonizes these areas, which reduces erosion and accelerates the development of the initial soil-type layer. Such vegetation may be also a valuable source of species/ecotypes for devastated habitats restoration.
The aim of the present study was to examine the vegetation spontaneously populating the waste deposits produced by Zn-Pb ore mining and smelting industry in Upper Silesia/Southern Poland and to find factors determining spatial diversity of plant cover. The study was carried out on three heaps varying in the type (slag or flotation sediment) and age (20 – over 100 years) of the waste. Plant species composition and abundance, plant metal concentration as well as the properties of the upper waste layer were determined. Over 90 plant species were found within the study areas. The community composition and cover density varied significantly between the three heaps. They were influenced by the waste type and age, the concentration of Ca-chloride extractable Zn and Pb and total concentrations of Zn and Cd.
The metal content in aerial plant tissues varied between species and was relatively low whichcan be attributed to the avoidance of metal uptake and limited transport of metals to plant shoots.

Keywords: waste heaps, heavy metals, spontaneous vegetation, habitat restoration

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M2

Biological Intervention for Ecosystem Restoration of Heavy Metal Contaminated Mining Sites in India

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Pollution of the biosphere by the toxic metals is a global threat that has accelerated dramatically since the beginning of industrial revolution. Mining causes the destruction of natural ecosystems through removal of soil and vegetation burial beneath waste disposal sites. Barren dumps of iron, copper and zinc mine wastes comprising spoil or tailing often exhibit adverse physical and chemical properties, that inhibit microbial and plant sustainability. Revegetation/ Restoration of such sites are therefore essential in order to prevent erosion by the action of water or wind, runoff of metallic effluents, and creation of aesthetically environment. The purpose of this study was to assess the growth through pot trail and field scale experiment of selected native plant species on metalliferous wastes, along with organic materials and plant growth promoting rhizobacteria (PGPR) as soil amendments on plant growth and soil metal transformations. The amendments include: topsoil, farm yard manure (FYM) and isolated strains of *Azotobacter* and *Rhizobium* and VAM spores. Total five treatments namely T1 (spoil as such); T2 (topsoil as such); T3 (1:4 of topsoil and spoil); T4 (1:4 of topsoil, spoil and FYM @ 50 t ha–1) and T5 (1:4 of topsoil, spoil, FYM @ 50 t ha-1 along with biofertilizers which were site /plant specific nitrogen fixers and VAM) where tested in pot culture experiment with three different mine spoil dump materials such as iron, copper and zinc mine spoils. Among various amendments were tested the treatment T5 was found to significantly improve plant growth and proliferation of microbial communities in all three mine spoil/tailing and were similar to those present in good topsoil. Total soil metal concentrations had a marked influence on plant uptake. All plant species accumulated Fe, Cu and Zn to varying degrees. Top dressing versus incorporating treatment T5 had a significant ($p < 0.05$) effect on plant tissue metal concentrations. Soil metals concentration tended to shift towards less bioavailable forms after treatment with T5. Addition of organic amendments along with suitable microbial consortium to the soil resulted in excellent root system development for all plant species; it is therefore expected that dense plant cover and extensive root formation will significantly decrease leachate production and improve metal retention. These results indicated that the use of native plant species in combination with organic amendments and microorganisms as a heavy metal immobilizing agent, is excellent to restore a dense vegetative cover on heavy metal polluted soils. Therefore, treatment T5 (1 part of topsoil + 4 parts of spoil + 50 t ha-1 of FYM and inoculation with plant specific *Azotobacter, Rhizobium* and VAM spores) can be used as an ecofriendly treatment for revegetation/restoration of the barren metal mine spoil dumps/waste land.

Keywords: Mine spoil/tailing, heavy metal, immobilization, plant growth promoting rhizospheric microorganisms, farm yard manure and eco-restoration.

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M3

Relationship of Flora Diversity and Water Purification Efficiency in the Constructed Water Treatment System

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The ecological engineering methods was used to construct a water treatment system was to remove water pollutants through the various natural treatment mechanisms of soil, plant and microorganisms. In the experiment period, the change of flora diversity was observed to evaluate the water purification efficiency of the constructed water treatment system. The constructed water treatment system was consisted of a water channel of aeration ladders, an oxidation pond and flooding irrigation zone of plants growth. The results show that there were certain relationships between plant growth and nutrient removal. The removal rate of Phosphorus was occurred at blooming times of dominant plants. The removal rate of Nitrate Nitrogen was related positively to the photosynthesis rates of plants growth. However, the removal rate of organic compounds occurred mainly in the oxidation pond, and that was not related with plant growth.

Keywords: constructed water treatment system, water Purification, flora diversity

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M4

Application of Phytobiotechnology in Water and wastewater purification

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This report details a preliminary investigation on the application of phytobiotechnology as a lowcost,appropriate and ecological alternative in purifying water and wastewater in Africa through the coagulative and disinfective ability of seeds of Moringa oleifera, Jatropha curcas seeds, calyx of Hibiscus sabdarifa, sclerotium of Pleurotus tuberregium and Alum on wastewater samples from Yelwa settlement in Bauchi, Nigeria. Varying weights (0.5 to 59) of dried pulverized plant materials and Alum were placed in 200 mls each of the three-wastewater samples and left for 24 hours. The results showed well above 90% reduction in bacterial load of the water samples by Moringa oleifera. All the plant materials exhibited appreciable coagulative effect comparable to Alum. Moringa oleifera seeds, Jatropha curcas seeds and Hibiscus sabdarifa calyx reduced the bacterial load drastically and inhibited Escherichia coli *in vitro* using the Agar diffusion method. The turbidity of both plant Alum treated water samples drastically reduced. The PH of Alum treated water was observed to decrease from neutral to acidic as opposed to a constant PH of 7.0 for both plant treated and untreated wastewater samples. This preliminary report does not only suggest an alternative and possibly cheaper water purification opportunity for rural communities in third world countries but also suggest good starting materials for the synthesis of environment friendly natural coagulant and disinfectants.

Key words: Coagulative, Disinfective, Total aerobic Mesophilic Counts, E. coli counts, Coliforms counts, Waste water, Medicinal Plants.

M5

Application of Phytobiotechnology in the control of Tropical infectious Diseases in Cameroon

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In this report, an evaluation on the applications Phytobiotechnology in disease prevention, This report is based on an inventory of indigenous knowledge base on disease management in Cameroon and Nigeria using indigenous medicinal plants and the research built on this unclassified traditional knowledge and medicinal plants used in traditional medicines basis for experimentation, validation, development and application of appropriate Phytobiotechnologies for cheap, low tech disease control strategies with an equally high efficiency and ecological acceptability. The findings generally show that most African tribes as well as globally have a rich heritage of indigenous knowledge and medicinal plants that can be utilized through products formulation using simple and cheap biotechnologies to attend to public health problems rather than the over dependence on synthetic drugs with its attendant drug failure and uncomfortable side effects. Phytobased drugs were formulated from the selected screened plant directly as tinctures, tisanes and or galenical to control tropical infectious diseases such as salmonellosis, amoebiasis, tuberculosis, cryptosporidiosis, Malaria, toxoplasmosis, hepatitis, Leishmaniasis, dracucunliasis, HIV and AIDS. Plant extracts with insecticidal activity were formulated into pesticides/insecticides used for vector control, tick sprays for ectoparasites of man such as lice e.g. sarcoptic mange,pediculosis humanus var capitis. Formulation of the extracts with Silicon oil and petroleum jelly into phytocosmetics was used for skin problems amongst patients with skin ailments attending PRF Clinics and observed to have more 99% efficacy. Plants such as Allium sativum (garlic), Vernonia amygdalina (bitter leaf), Moringa oleifera (Horse raddish plant), Lantana camara, Persea Americana Occimum gratissimum (basil), Aspilia africana (Iodine plant), Carica papaya (pawpaw), Aloe barbadensis, Khaya senegalensis, Artemisia annua, Distemonantus benthamianus, Standia kamerunansi, Viscum album, Arctium lappa, Cucurbita pepo (pumpkin) and macrofungi such as Ganoderma lucidum, Pleurotus tuberregium, Termitomyces titanicus amongst several others were identified during the survey as important candidate plants and macrofungi with alcohol extracts that were used directly as biodegradable antiseptics to bring about hygiene and sanitation, disinfect pit toilets while controlling flies . It was found that the extracts can provide important leads/insights in the production of natural antiseptics, antibiotics, fungicides and insecticides for the control of diseases and their vehicles for transmission. Some of these plants (Persea americana, Gonoderma lucidum, Aspilia africana) have been formulated with starch from maize and paraffin to control cercaria,cyclops,and larvae of Annopheles and culex mosquitoes respectively.Extracts from Moringa oleifera and termitomyces have been used as nutriceuticals to boost immunity in HIV and AIDS patients.The immune boosters increased CD4 conts of the patients by 65% comparable to synthetic antiretroviral drugs like Triomune 40 and reduced the HIV viral counts by 60 %. Moringa oleifera seed extracts amongst others have been used in sand filter systems to purify domestic water and waste water by removing solids and microbes by 99.9%.From the survey phytodrugs produced from Phytobiotechnology research laboratory were useful in attending to cardiovascular diseases, hypertension, diabetes caused by complications from infectious diseases. The conclusion is made that Phytobiotechnology has a crucial role in bringing about sustainable health in Cameroon and Africa at large as it is a comparatively cheaper and effective technology, there is therefore the need to further develop and promote it rather that the exploitation of single compounds for patent drug development and over focus on vaccine development which have little prospect of sustainability.

Keyword index: Phytobiotechnology, applications, medicinal plants, Antibiotics, Antimicrobials, tropics, infections, public health

M6

Effect of *Glomus mosseae* and Different Combination of Heavy Metals on Accumulation Factor of Metals in Barley (*Hordeum vulgare* L.)

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Accumulation factor (ACF) represent ability of plants in accumulation of elements in plants leaves. An experiment was set up in a 2×8 factorial completely randomised design, with four replicates. The first factor was inoculation (M) with *Gomus mosseae* or un-inoculation (M0). The second factor had seven levels of contaminants: (Co, Cd, Pb and combinations) plus an uncontaminated control treatment (C). A sample of soil (clay 35%, silt 40% and sand 25%) were used. Total Co content =51.91 mg kg-1 dried soil, total Cd content =8.5 mg kg-1 dried soil and total Pb content =436 mg kg-1 dried soil. The heavy metal salts used included CoSO4, CdCl2 and Pb(NO3)2. The soil was contaminated before planting by adding the calculated amounts of heavy metal salts in distilled water and mixed throughout the soil profile. They were allowed to stabilise for 15 days. Then, 50 g *G. mosseae* inoculum was mixed with 5 cm of upper surface of soil and Alfalfa seeds planted as before. Plants were cut from soil surface in early flowering stage. Roots were extracted from pot. Aboveground materials separated into the stems and leaves were washed by distilled water. Plant material was dried at 70 °C for 48 hours. Heavy metals in plant tissue and soil were quantified by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Variant-Liberty 150AX Turbo). Accumulation factor (ACF) of contaminants calculated by concentration of heavy metals in leaves (mgkg-1) / concentration of heavy metals in leaves (mgkg-1). Statistical analysis of data was performed by SAS software. The data were analyzed through one-way analysis of variance (ANOVA) and Comparisons between means were performed using Duncan's multiple range test at the significance level of $P<0.05$. The results showed that maximum accumulation factor of Cd and Co were produced by M0Pb and MCo ($P<0.05$). The highest rate of ACF of Pb was in MC, MCo and MCd ($P<0.05$). According our experiment mycorrhizae and combinations had key role in ACF of contaminants.

Keywords: accumulation factor, heavy metals, barley

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M7

Effect of *Glomus mosseae* on Nutrients (N, P and K) Uptake by Alfalfa in Multi Metal Contaminated Soil

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A pot culture experiment was carried out to study the effects of arbuscular mycorrhizal (AM) inoculation on the nutrients (N, P and K) uptake of alfalfa (*Medicago sativa* L.) in heavy metals contaminated soil. This experiment was set up in a 2×8 factorial completely randomised design, with four replicates. The first factor was inoculation (M) with *Glomus mosseae* or un-inoculation (M0). The second factor was seven levels of contaminants: (Co, Cd, Pb, CoCd, CdPb, PbCo and PbCoCd) plus an uncontaminated control treatment (C). Total Co content =51.91 mg kg-1 dried soil, total Cd content =8.5 mg kg-1 dried soil and total Pb content =436 mg kg-1 dried soil. The heavy metal salts used included CoSO4, CdCl2 and Pb(NO3)2. The soil was contaminated before planting by adding the calculated amounts of heavy metal salts in distilled water and mixed throughout the soil profile. They were allowed to stabilise for 15 days. Then, 50 g *G. mosseae* inoculum was mixed with 5 cm of upper surface of soil and Alfalfa seeds planted as before. After germination, plants were thinned tomaintain a plant density of 5 plants per pot. Plants were cut from soil surface in early flowering stage. Roots were extracted from pot. Aboveground materials separated into the stems and leaves were washed by distilled water. Plant material was dried at 70 °C for 48 hours. P and K concentration were quantified by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Variant-Liberty 150AX Turbo). N concentration determined by Kjeldahl digestion method. In without contamination, IC treatment had significantly shoot N content more than I0C. Shoot N content was higher in ICo, ICd and Ipb than un-inoculated plants (I0Co, I0Cd and I0Pb) by *G. mosseae.* But, in the contaminated pots to PbCo and PbCoCd, un-inoculated plants had more N concentration than inoculated ones ($P<0.05$). Comparison of controls indicated IC produced the most amount of whole plant phosphorous. Inoculation of plants with *G. mosseae* fungi significantly increased P content of whole alfalfa plants at contaminated pots to Co, Pb and PbCd. In the rest of treatments, un-inoculated plants had more phosphorous than inoculated ones ($P<0.05$).
Results revealed that in the dual inoculation of C, Co, Cd, Pb, CoCd and PbCd and *G. mosseae* fungi, Shoot K was higher. But, in the PbCo and PbCoCd polluted and uninoculated pots shoot concentration of K was more than inoculated plant with *G. mosseae* ($P<0.05$). Generally, uptake of nutrients by plant depends on kind and combination of heavy metals.

Keywords: nutrients uptake, heavy metals, alfalfa

M8

Sustainable Production of Chickpea through Microbial ACC-deaminase

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High ethylene concentration under different environmental stresses is one of the contributing factors which inhibit plant growth. Microbial 1-aminocyclopropane-1-carboxylic acid (ACC)-deaminase is the enzyme which is present in some strains of plant growth promoting rhizobacteria (PGPR). These PGPR alone and also in combination with rhizobial strains promote plant growth by lowering the endogenous level of ethylene along with some other mechanisms. A pot trial under natural conditions was conducted to evaluate the potential of PGPR with/without ACC-deaminase for improving the efficacy of pre-isolated rhizobial strain in chickpea. The experiment was laid down under CRD with six replications. The results of the experiment showed that PGPR containing ACC-deaminase and rhizobium alone increased the growth, yield and nodulation in chickpea while PGPR strain without ACC-deaminase showed non significant result in all the parameters. But the combined application of PGPR containing ACCdeaminase and rhizobium was the most effective among all treatments and it increased the root length (93%), root fresh weight (95 %), root dry weight (70 %), plant height (73 %), fresh biomass (48 %), shoot dry weight (75 %), no. of pods plant-1 (59 %), pod weight plant-1 (88 %) and grain yield plant-1 (119 %) of chickpea grown under natural conditions in pot trial compared with un-inoculated control. The coinoculation of PGPR containing ACC-deaminase and rhizobium also significantly enhanced the nodulation in chickpea and the maximum increase in number of nodules plant-1 (79 %), nodule fresh weight (54 %) and nodule dry weight (85 %) were observed in case of the combined application of PGPR containing ACC-deaminase and rhizobium over the un-inoculated control. The nitrogen and phosphorus content in grain and straw was also maximum in the treatment where combination of PGPR containing ACC-deaminase and rhizobium was applied compared with un-inoculated control. The results imply that combined application of PGPR containing ACC-deaminase and rhizobium could be a useful approach for sustainable production of chickpea.

Key words: ACC-deaminase, co-inoculation, sustainable production, chickpea, stress-induced ethylene

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M9

Impacts of climate change and sea-level rise on soil carbon cycling in forested ecosystems of the lower coastal plain of North Carolina

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Soils of lower coastal plain forested wetlands contain large quantities of organic carbon that is vulnerable to mineralization due to climate change, and possibly sea level rise. Therefore, we are conducting a study to characterize the soil carbon cycle in three coastal plain forested ecosystems. Major carbon inputs include litterfall biomass and fine root turnover, while outputs are carbon loss through soil CO2 efflux and dissolved forms of carbon. Our two study sites consist of a relatively undisturbed lowland hardwood forest and a drained loblolly pine plantation, at which field studies and laboratory soil incubations are being conducted to study the soil carbon dynamics. Leaf litter is being assessed litter traps and fine-root dynamics are being measured using sequential-cores and ingrowth-core methods. Soil respiration is being quantified in the field using both automated and manual methods, and lab incubations of soil will give an idea of the sensitivity of soil organic mineralization to changes in temperature and moisture. The combined studies will clarify the environmental drivers affecting soil carbon cycling in lower coastal plain forests and improve ecosystem models to help predict the effects of climate change on terrestrial carbon sequestration.

Key words: wetland, climate change, carbon sequestration, soil,

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