Copper tolerance in macrophyte populations: Innate tolerance and/or phenotypic plasticity?

Lilian Marchand, Michel Mench, Florien Nsanganwimana

PREFACE

Phytotechnologies - plant-based strategies to clean water, soil, air and provide ecosystem services - have an effective power beyond their science when integrated into our managed landscapes.

The aim of the 9th IPS Conference on Phytotechnologies is to exchange information, experience and achievements in all plant-based technologies adopted to mitigate environmental problems, evaluate currently applied technologies and present innovative ideas. The presented plant-based technologies are not limited to phytoremediation, but also include biofuel production on degraded soils, green-roof technology, treatment wetlands, landfill caps, and carbon sequestration among others.

The organizers strongly believe in the active participation of young scientists in the conference. As a result, all participating students are invited to 'combat' for the student awards for the 3 best posters and 3 best platform presentations. An important aim of this and previous IPS conferences has been to give young scientists the opportunity to present their scientific results and exchange ideas with established researchers in the field of plant-based technologies.

With over 320 participants, 90 full platform presentations, 30 short platform presentations and about 170 poster presentations, the participation to the 9th IPS Conference on Phytotechnologies is more numerous and diverse than ever before. Indeed, besides an overwhelming participation from most European countries, many contributions have been received from plenty of countries around the world.

At this point, we would like to express our sincere thanks to all those who have worked with dedication in helping us put together this attractive and comprehensive scientific program, as well as to all participants for presenting their work.

We invite you to enjoy the presentations and hope that this conference will offer you the opportunity to meet colleagues from around the globe, including the people who studied phytotechnologies since the 'early years' as well as the numerous students who will continue this research in the future.

Welcome to Hasselt!

The Organizing Committee

ORGANIZING COMMITTEE

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Steven Rock (USA)
Lee Newman (USA)
Elena Maestri (Italy)
Renee Stoops (USA)

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Nele Witters (Conference Co-Chair)
Ann Cuypers
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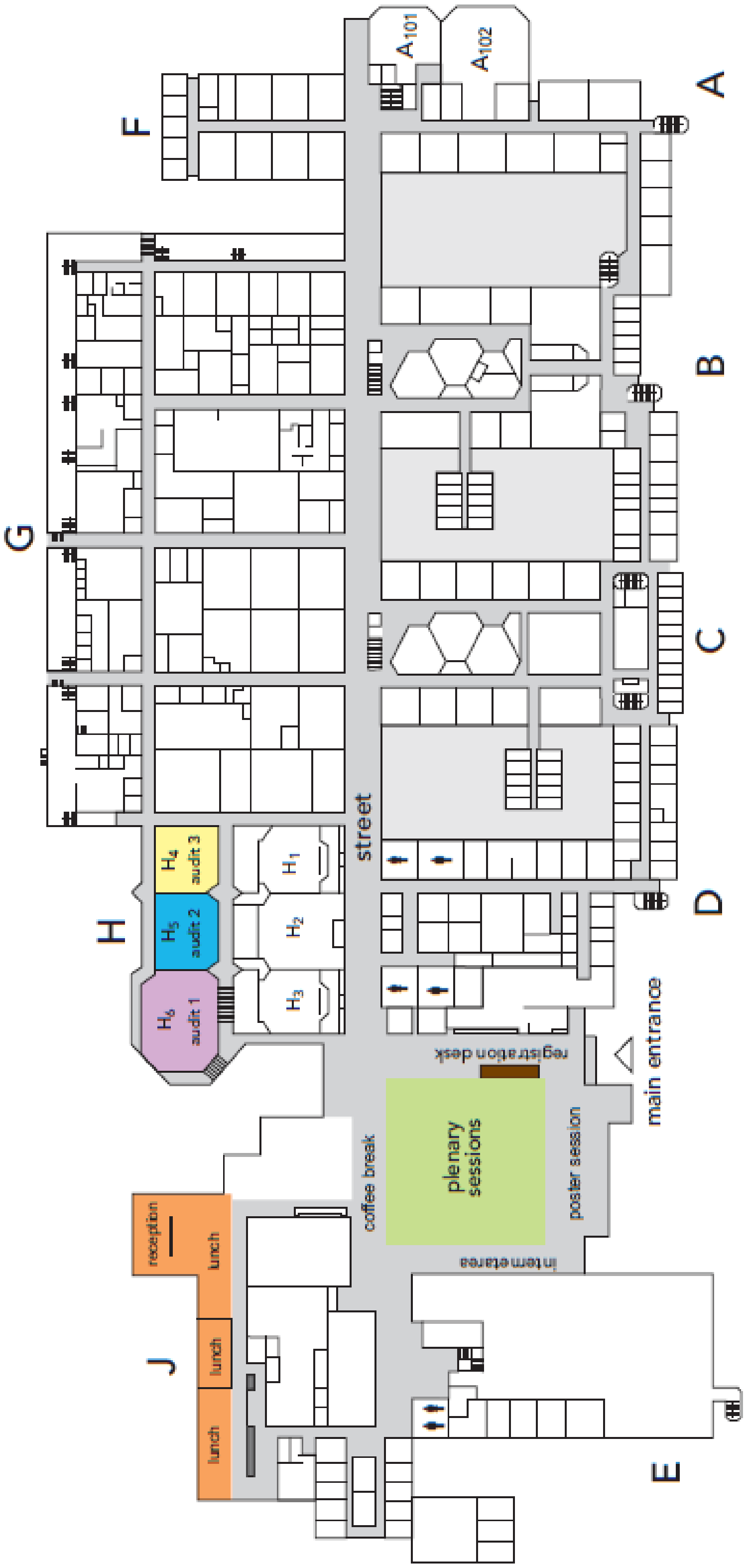
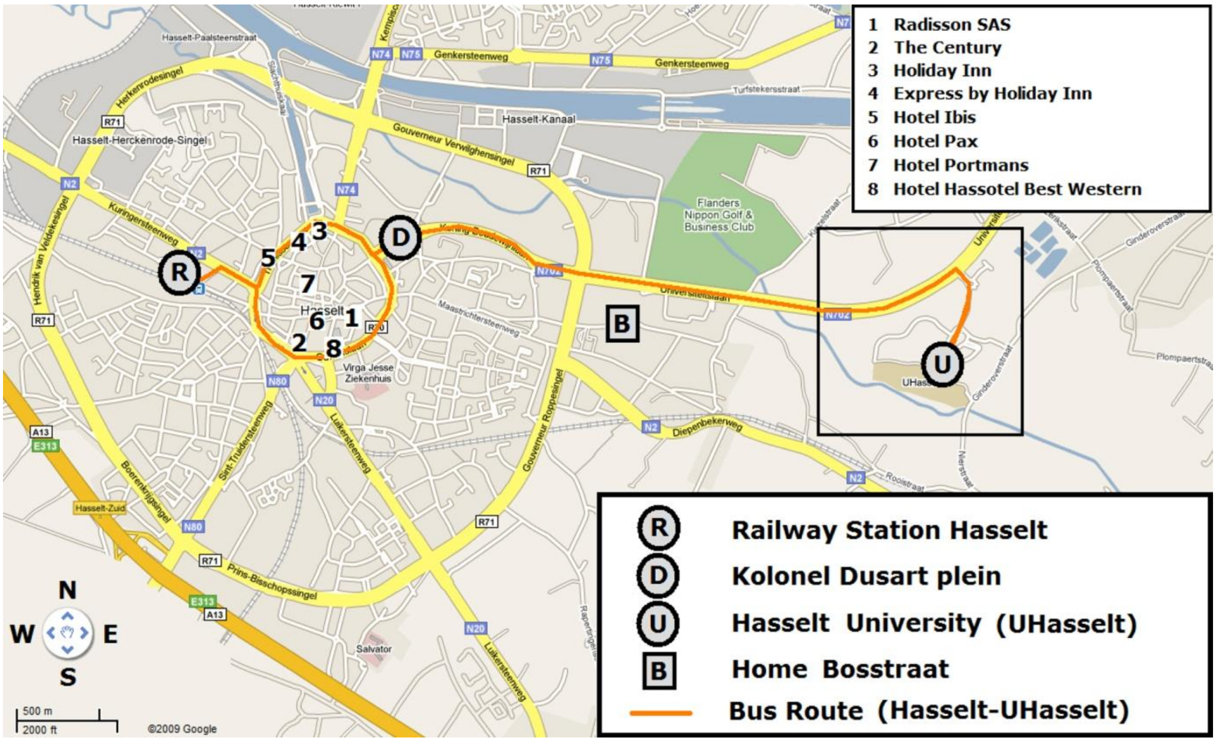
Andon Vassilev (Bulgaria)
Luo Yongming (China)

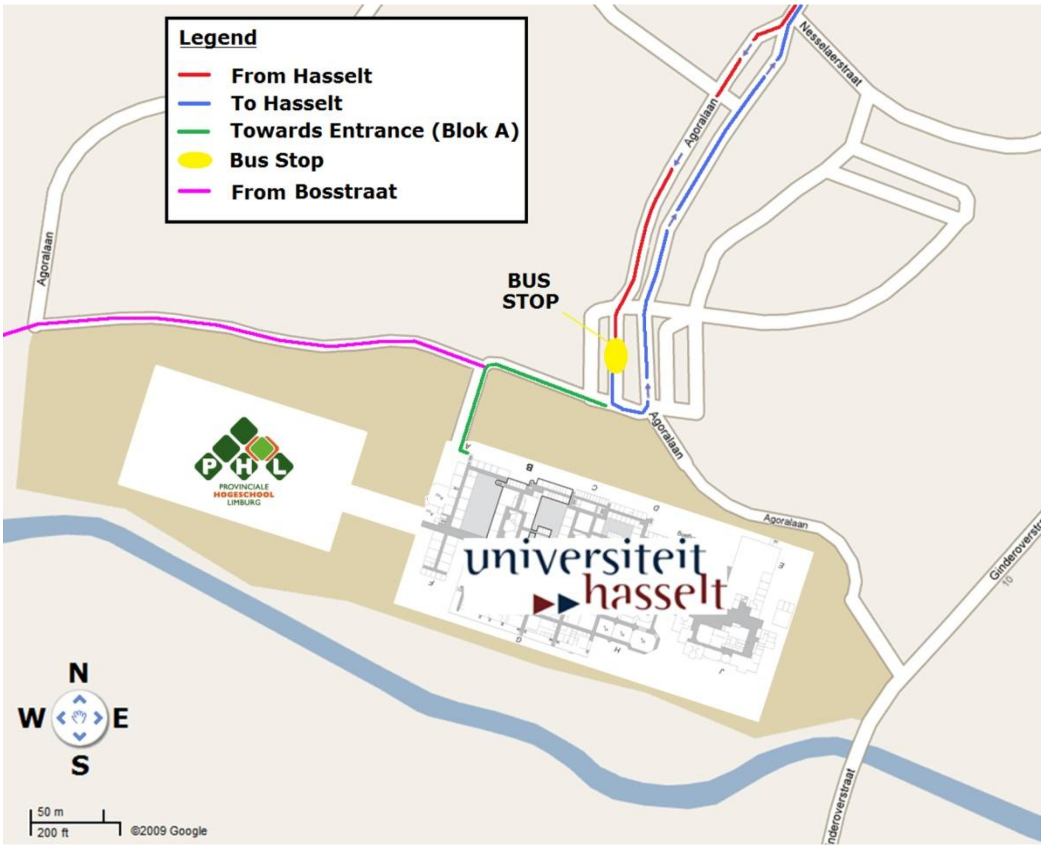
Map of the Building



MAP OF THE CITY



MAP OF THE CAMPUS



Tuesday, September 11		
13.00	17.00	Workshop 1 or Workshop 2 *Leaving at 13.00 at Kolonel Dusart Square*
17.00	20.00	Registration
18.00	20.00	Opening Reception with local drinks and appetizers

Wednesday, September 12				
8.00	8.45	Registration		
8.45	9.00	Opening Ceremony *Agora*		
9.00	9.40	Plenary: Gordon Award Keynote *Agora*		
9.40	10.15	**Mini-Plenaries** Nele Weyens Tomas Macek Jan Japenga *Agora*		
10.15	10.45	Coffee Break		
10.45	12.50	**Plant-Microbe Interactions: Metals** *Auditorium 1 (H6)*	**Persistent Organic Contaminants** *Auditorium 2 (H5)*	**Ecosystem Services** *Auditorium 3 (H4)*
		Session Chairs Nele Weyens Petra Kidd	**Session Chairs** Tomas Macek Lizz Rylot	**Session Chairs** Jan Japenga Silvie Daniels
		Phytostabilization of heavy metals and metalloids by rosemary: the mycorhizospheric contribution *FP Speaker: Marie Cécile Affholder*	Using plants to remediate TNT and RDX pollution *FP Speaker: Elizabeth Rylott*	Native plants for the phytoremediation of heavy metals and arsenic contaminated soils in an area with high ecological value in La Unión (Spain) *FP Speaker: Domingo Martínez-Fernández*
		Bacterial-induced weathering of ultramafic rock: implications in phytoextraction *FP Speaker: Cristina Becerra-Castro*	Effectiveness of organic amendments and plant cover for *In situ* remediation of DDT and Dieldrin residues in old orchard soils *FP Speaker: Tiziana Centofanti*	*In situ* amendments allow spontaneous establishment of plants on bare soil near the copper-smelter industry of Lubumbashi (DR Congo) *FP Speaker: Mylor Ngoy Shutcha*
		Improving phytoextraction efficiency of high-biomass crops using microbial inoculants and organic amendments *FP Speaker: Vanessa Álvarez-López*	Investigating differences in the root to shoot transfer of organic compounds between zucchini, squash and soybean using a pressure chamber method *FP Speaker: Bill Doucette*	Phytotechnologies in intensive agricultural landscapes in Canterbury, New Zealand *FP Speaker: Nicholas Dickinson*

FP = Full Platform presentation
SP = Short Platform presentation

			Long-term Cd exposure induces beneficial shifts in the seed endophytic population of *Arabidopsis thaliana* *FP Speaker: Sascha Truyens*	Effect of hydrogen peroxide on the uptake of POPs by *Cucurbitaceae* *FP Speaker: William Berger*	Floristic composition of the various physiognomies in shasha forest reserve in Ile-Ife area of South Western Nigera *FP Speaker: Samson Olajide Oke*
			Microbial consortia enhancing plant growth for the remediation of heavy metal contaminated sites *FP Speaker: Francesca Langella*	Advances in the interfacial processes and self-remediation of persistent organic pollutants in soil-plant systems: an example of polychlorinated biphenyls *FP Speaker: Luo Yongming*	Phyto-rehabilitation and economic development of open-pit coal mining residue areas in Quang Ninh province, Vietnam *FP Speaker: Jan Japenga*
			Improving biomass production of willow for phytoremediation of metal-contaminated soils using plant growth promoting bacteria and fertilization strategies *SP Speaker: Jolien Janssen*	DDX accumulation in grafted *Cucurbitaceae* grown under greenhouse and field conditions *SP Speaker: Mehmet Isleyen*	Organo-zeolitic biofertilizers: a new approach to the promotion of plant growth *SP Speaker: Peter Leggo*
			Exploitation of PGPB strains within phytoremediation protocols for the reclamation of soils contaminated by roasted arsenopyrite residues *SP Speaker: Silvia Lampis*	Atrazine phytoremediation in humid pampa plots (Argentina) under intensive agriculture management *SP Speaker: José Merini Luciano*	To sustain sustainability in agricultural using industrial by-products *SP Speaker: Brigitta Tóth*
			Contribution of the plant rhizosphere system to the phytoremediation of metals in estuarine areas *SP Speaker: Marisa Almeida*	Plant uptake and translocation of dieldrin: role of protein-like materials in xylem sap of *Cucurbitaceae* *SP Speaker: Otani Takashi*	CO_2 fixation increase by new effectors of photosynthetic enzymes, ecdysteroids *SP Speaker: Tomas Macek*

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				Aboveground biomass, standing floor litter and soils Carbon stock in a 10 year *Tectona grandis* plantation in Ile-Ife, Southwestern Nigeria *SP Speaker: Anthony Odiwe Ifechukwude*
				Improving ecosystem health and functioning through phytotechnologies at Freshkills Park, Staten Island, New York, USA *SP Speaker: Ron Zalesny*
12.50	13.45	Lunch		
13.45	14.30	Poster Session (A-E)		
14.30	15.15	**Mini-Plenaries** Nelson Marmiroli Kate Kennen David Tsao Joel Burken *Agora*		
15.15	16.35	**Metals: Toxicity and Uptake (I)** *Auditorium 1 (H6)*	**Plant-Microbe Interactions: Organics** *Auditorium 2 (H5)*	**Design** *Auditorium 3 (H4)*
		Session Chairs Nelson Marmiroli Stan Gawronski	**Session Chairs** Angela Sessitsch Bill Doucette	**Session Chairs** Kate Kennen Alan Darlington
		Proteomic characterization of phenotypic plasticity between metallicolous and non-metallicolous populations of *Agrostis capillaris* L. exposed to Cu *FP Speaker: Elena Hego*	Microbial inoculants improving phytoremediation of diesel fuel show efficient colonization, competitive ability and expression of hydrocarbon degradations *FP Speaker: Angela Sessitsch*	Botanical biofilters remove TVOCs from indoor air *FP Speaker: Alan Darlington*
		Role of NRAMP transporters in metal homeostasis in poplar leaves *FP Speaker: Mathieu Pottier*	The role of plant-associated bacteria for the improvement of phytoremediation of TNT-contaminated sites *FP Speaker: Sofie Thijs*	Thermal performance of tropical green roofs with different vegetation growth forms *FP Speaker: CY Jim*
		Characterization of ZIP and CDF proteins involved in the uptake and sequestration of trace elements in the ectomycorrhizal fungus *Laccaria bicolor* *FP Speaker: Laurence Lacercat*	Bioremediation of Cd and DDTs co-contaminated soil by Cd-hyperaccumulator (*Sedum alfredii*) and inoculation of DDT-degrading microbe *FP Speaker: XE Yang*	Integrating phytotechnologies with design and landscape architecture practice *FP Speaker: Kate Kennen*

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SP = Short Platform presentation

		Functional analysis of Poplar (*Populus nigra* L. and *P. nigra x P. deltoids*) during environmental exposure to cadmium *FP Speaker: Martha Marmiroli*	Diversity assessment of endophytic bacteria of the halophytic plant *Tamarix parviflora* and their role in BPA degradation *FP Speaker: Evdokia Syranidou*	Farming the pollution: a modular fence for phytoremediation of polluted soil *FP Speaker: Giacomo Piovan*
16.35	17.00	Coffee Break		
17.00	18.30	**Metals: Toxicity and Uptake (II)** *Auditorium 1 (H6)*	**Petroleum** *Auditorium 2 (H5)*	**Phytoforensics** *Auditorium 3 (H4)*
		Session Chairs Stephen Ebbs Andon Vassilev	**Session Chairs** David Tsao Richard Bentham	**Session Chairs** Joel Burken
		Consequences of Cd or Zn accumulation on element redistribution within leaves of *Zygophyllum fabago* and related impact on plant physiology *FP Speaker: Isabelle Lefèvre*	Rhizoremediation of polyaromatic hydrocarbon (PAH) contaminated soils *Lupinus* species *FP Speaker: Richard Bentham*	Utilization of trees (willow, poplar) for phytostabilization and biomonitoring of metal fluxes on contaminated land *FP Speaker: Michael Evangelou*
		Cd induced growth stimulation and differentially-expressed proteome in the Zn/Cd hyperaccumulator *Sedum alfredii* *FP Speaker: Lu Tang*	Engineered phytoremediation pilot study for petroleum hydrocarbons in a deep confined aquifer *FP Speaker: Edward Gatliff*	Transient uptake of chlorinated solvents by trees *FP Speaker: Matt Limmer*
		A novel gamma-glutamyl cycle in plants is involved in heavy metals and metalloids detoxification by maintaining GSH homeostasis and efficient recycling of glutamate *FP Speaker: Dhankher Om Parkash*	Influence of plant root exudates on the mobility of fuel organic compounds in contaminated soils *FP Speaker: María Balseiro-Romero*	Pollution investigation by trees -PIT- *FP Speaker: Chris Balouet*
		Platinum in plants and environment of urban area *FP Speaker: Stanislaw Gawronski*	Rhizoremediation of hydrocarbons in cold climate *FP Speaker: Kim Yrjälä*	Volatilization of trichloroethylene from trees and soil: measurement and scaling approaches *FP Speaker: Bill Doucette*

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			Ecological understanding and molecular mechanisms of plant root growth responses to excess metals, in support of growth improvement for safe biomass production or phytoremediation strategies *SP Speaker: Tony Remans*	Effects of soil clean-up using lemon grass on the interaction of selected soil minerals with organic pollutants: management implications *SP Speaker: Eucharia Nwaichi Oluchi*	Phyto test cells designed to mimic mature rhizosphere effect for regulatory permitting and construction *SP Speaker: Louis Licht*
			Growth and trace element accumulation of *Salix smithiana* on seven different trace element-contaminated soils *SP Speaker: Markus Puschenreiter*	Impact of metabolites on the phytoremediation of hydrocarbons *SP Speaker: Stéphanie Ouvrard*	
			Conference Dinner at Orangerie Krekelhof *Leaving at 19.45 at Kolonel Dusart Square*		

<table>
<tr><th colspan="5">Thursday, September 13</th></tr>
<tr><td>8.30</td><td>9.00</td><td colspan="3">Registration</td></tr>
<tr><td>9.00</td><td>9.30</td><td colspan="3">Introductory Plenary
Ronald Zalesny
Agora</td></tr>
<tr><td>9.30</td><td>10.00</td><td colspan="3">Mini-Plenaries
Nicholas Dickinson
André Gerth
Agora</td></tr>
<tr><td>10.00</td><td>10.30</td><td colspan="3">Coffee Break</td></tr>
<tr><td rowspan="7">10.30</td><td rowspan="7">12.50</td><td>Metals: Toxicity and Uptake (III)
Auditorium 1 (H6)</td><td>Wetlands
Auditorium 2 (H5)</td><td>Biomass for Bioenergy
Auditorium 3 (H4)</td></tr>
<tr><td>Session Chairs
Michael Evangelou
Hildegarde Vandenhove</td><td>Session Chairs
Michel Mench
Andre Gerth</td><td>Session Chairs
Ronald Zalesny
David Dowling</td></tr>
<tr><td>Mechanisms of chromium tolerance, uptake and accumulation in land plants

FP Speaker: Shira Corem</td><td>Constructed treatment wetlands: industrial case studies

FP Speaker: David Tsao</td><td>Electricity generation with a novel tubular plant microbial fuel cell

FP Speaker: David Strik</td></tr>
<tr><td>Development of practical rice cultivars for Cd-phytoextraction use (2) characteristics of developing rice lines with high-Cd allele

FP Speaker: Abe Tadashi</td><td>Potential of a plant commonly used in constructed wetlands to remove veterinary drugs from wastewater

FP Speaker: Pedro Carvalho</td><td>The fate of trace elements during combustion of woody biomass collected from a phytoremediation field trial

FP Speaker: Michel Chalot</td></tr>
<tr><td>Effect of different P supply on metal and nutrient uptake by co-cropped Ni-hyperaccumulator with Lupinus albus grown on two different soils

FP Speaker: Jiang Cheng-ai</td><td>Constructed wetland pilot project for FGD wastewater treatment

FP Speaker: Larry Erickson</td><td>Bacterial endophytes for improving sustainability of bioenergy

FP Speaker: David Dowling</td></tr>
<tr><td>Construction and effective expression of the synthetic merBps gene in tobacco

FP Speaker: Kavita Singh</td><td>Evaluation of floating plant root mat for the treatment of groundwater contaminated by benzene and MTBE

FP Speaker: Zhongbing Chen</td><td>Designing a multi-functional sustainable agricultural system at the farm scale using energy crops

FP Speaker: Gayathri Gopalakrishnan</td></tr>
<tr><td>Ligand enhanced heavy metal uptake by fast growing trees under controlled deficit irrigation – Results of batch and lysimeter studies

FP Speaker: Pinchas Fine</td><td>Development of a constructed wetland water treatment system for selenium removal: use of mesocosms to evaluate design parameters

FP Speaker: Norman Terry</td><td>Assessment of the phytoremediation and bioenergy potential in three cadmium-contaminated bioenergy crops: oilseed rape, oat and wheat

FP Speaker: Huayong Zhang</td></tr>
</table>

FP = Full Platform presentation
SP = Short Platform presentation

			Soil metatranscriptomics for mining eukaryotic heavy metal resistance genes *FP Speaker: Damien Blaudez*	Removal of pathogens and organic contaminants from wastewater using constructed wetlands and UV systems *FP Speaker: Hassan Azaizeh*	Phytotechnology opportunities in North Carolina for producing woody biomass for energy while accomplishing phytoremediation *FP Speaker: Dennis Hazel*
			Nitrate nutrition status modulates stress responses of alfalfa plants to mercury *SP Speaker: Luis Hernández*	Environmental services and economic opportunities of naturally occurring willows on the prairies *FP Speaker: Jaconette Mirck*	Valorization of plant biomass harvested at trace element contaminated sites managed by gentle (phyto)remediation options *SP Speaker: Valérie Bert*
			Cadmium-induced oxidative stress and mitochondria: a kinetic study in *Arabidopsis thaliana* *SP Speaker: Els Keunen*	Biological and sustainable treatment of sulphate loaded water *SP Speaker: André Gerth*	Application of the rejuvenate decision support approach *SP Speaker: Yvonne Andersson-Sköld*
			Diversity of lead hyperaccumulation in *Hirschfeldia incana:* perspectives for phytoremediation *SP Speaker: Mouna Fahr*	Organic acid content and copper uptake in two plant species used in a wetland system to treat copper mining effluents *SP Speaker: Claudia Ortiz*	Enhanced growth of bio-fuels plants on amended coal waste *SP Speaker: Peter Leggo*
			Relationships between chemical and biological factors in trace element-contaminated soils managed using Gentle Remediation Options (GRO) *SP Speaker: Jurate Kumpiene*		Effect of pyrolysis temperature on chemical and surface properties of biochar of rapeseed (*Brassica napus* L.) *SP Speaker: Angin Dilek*
12.50	13.05		Group Picture		
13.05	13.50		Lunch		
13.50	14.30		Poster Session (F-P)		

FP = Full Platform presentation
SP = Short Platform presentation

14.30	15.15	**Mini-Plenaries** Alan Baker Nicolas Kalogerakis Heather Henry Steve Rock *Agora*		
15.15	16.35	**Metals: Search for New Potential Phytoextractors (I)** *Auditorium 1 (H6)*	**Salinity and Drought Stress** *Auditorium 2 (H5)*	**Risk Management and Assessment for Public Health (I)** *Auditorium 3 (H4)*
		Session Chairs Alan Baker	**Session Chairs** Norman Terry Nicolas Kalogerakis	**Session Chairs** Heather Henry Jurate Kumpiene
		Botanical exploration for hyperaccumulating plants in a gold mine *FP Speaker: Pornsawan Visoottiviseth*	Selection of salt and boron tolerant selenium hyperaccumulator *Stanleya pinnata* genotypes and characterization of Se phytoremediation from agricultural drainage sediments *FP Speaker: John Freeman*	Uptake and distribution of metals in vegetation on metal contaminated dredged sediment derived soils *FP Speaker: Filip Tack*
		Gentle remediation at formerly "Pertusola Sud" zinc smelter: evaluation of native species for phytoremediation purposes *FP Speaker: Luca Marchiol*	Heavy metal phytoexcretion and phytoextraction using halophytic plants *FP Speaker: Eleni Manousaki*	Phytomanagement in case of a nuclear accident – lessons learnt from Chernobyl and possible application to Fukushima *FP Speaker: Hildegarde Vandenhove*
		Phytoremediation of lead (Pb) and arsenic (As) by *Melastoma malabathricum* L. from contaminated soil *FP Speaker: Abdullah Siti Rozaimah Sheikh*	Salinity effect on Zn uptake by *Brassica juncea* *FP Speaker: Luis Novo*	Managing the phytoaccumulation of cadmium using lignite *FP Speaker: Brett Robinson*
		Plants growing on mine tailings avoid dispersion and contribute to stabilization of potentially toxic elements *FP Speaker: Ariadna Sánchez-López*	Cadmium phytoextraction by the halophyte *limoniastrum monopetalum* *FP Speaker: Nicolas Kalogerakis*	Techno-economic assessment of fast pyrolysis for the valorisation of short rotation coppice cultivated for phytoremediation in the Campine *FP Speaker: Tom Kuppens*
16.35	17.00	Coffee Break		

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17.00	18.30	**Metals: Search for New Potential Phytoextractors (II)** *Auditorium 1 (H6)*	**Stakeholder Involvement: Round Table Discussion** *Auditorium 2 (H5)*	**Risk Management and Assessment for Public Health (II)** *Auditorium 3 (H4)*
		Session Chairs Nicholas Dickinson Rufus Chaney	**Session Chairs** Steve Rock Michel Mench	**Session Chairs** Nele Witters Steven Van Passel
		Is hybrid larch (*Larix* x *eurolepsis* Henry) a good candidate for cadmium phytoaccumulation? *FP Speaker: Gaëlle Saladin*	Stakeholder engagement and "gentle" remediation options: a critical review *Speaker: Andrew Cundy*	The adoption of gentle groundwater remediation strategies: a health risk based decision analysis *FP Speaker: Sarah Creemers*
		Cadmium and uranium phytoextraction potentials of young douglas fir trees *FP Speaker: Céline Faugeron*		Effect of plant root growth on Pb phytostabilization of East Helena, MT, smelter contaminated soils *FP Speaker: Rufus Chaney*
		The effects of cadmium on growth and accumulation of *Ipomoea aquatic* Forsk. (water spinach) and *Brassica campestris* var. chinensis (Pak choy) *FP Speaker: Pathitta Thumcharoen*		Accumulation of antimicrobials by vegetables *FP Speaker: Dawn Rheinold*
		Phytoremediation of a dumpsite at Moniya, Ibadan, in Nigera using *Chromolaena odorata* and *Portulaca oleraceae* L. *FP Speaker: Funke Ogundola Adijat*		Phytoremediation uncertainty tackled: a real option approach *FP Speaker: Tine Compernolle*
				Testing combinations of amendments for stabilization of metals in contrasting contaminated soils *SP Speaker: Grzegorz Siebielec*
19.00	…	IPS annual meeting and members dinner *Radisson SAS Hotel*		
21.00	…	Student Activity (not sponsored) Visit to Hemelrijk, bar with over 500 beers available *Het Hemelrijk*		

FP = Full Platform presentation
SP = Short Platform presentation

<table>
<tr><th colspan="5">Friday, September 14</th></tr>
<tr><td>8.45</td><td>9.00</td><td colspan="3">Registration</td></tr>
<tr><td>9.00</td><td>9.30</td><td colspan="3">Phytoremediation: Myth or Reality
Jean-Paul Schwitzguébel
Agora</td></tr>
<tr><td>9.30</td><td>10.00</td><td colspan="3">Mini-Plenaries
Jason White
Markus Puschenreiter
Louis Licht
Agora</td></tr>
<tr><td>10.00</td><td>10.30</td><td colspan="3">Coffee Break</td></tr>
<tr><td rowspan="6">10.30</td><td rowspan="6">12.10</td><td>Nanoparticles
Auditorium 1 (H6)</td><td>Field Projects
Auditorium 2 (H5)</td><td>Waste Water
Auditorium 3 (H4)</td></tr>
<tr><td>Session Chairs
Jason White
Jerry Schnoor</td><td>Session Chairs
Jaco Vangronsveld
Markus Puschenreiter</td><td>Session Chairs
Elisha Tel-Or
Louis Licht</td></tr>
<tr><td>Size, shape and surface property based phytotoxicity of gold and silver nanoparticles to plants

FP Speaker: Xingmao Ma</td><td>Evapotranspiration (ET) landfill cover in Berlin – field data on ecology and technology of successful pre-successional establishment of conifers

FP Speaker: Hans-Holger Liste</td><td>Field-scale phyto waste water treatment considering soil complexity

FP Speaker: Louis Licht</td></tr>
<tr><td>Accumulation of engineered nanoparticles in belowground vegetables: nutritional bioaccessibility and dietary exposure risks

FP Speaker: Stephen Ebbs</td><td>Field demonstration of energy plants production on heavy metal contaminated farmland

FP Speaker: Jing Song</td><td>Recent advances in phytoremediation technologies involving aquatic plants for the removal of toxic heavy metals from polluted wastes, soil and sludge

FP Speaker: Elisha Tel-Or</td></tr>
<tr><td>Fullerene-enhanced accumulation of p,p′-DDE in agricultural crops

FP Speaker: Roberto De La Torre Roche</td><td>Feasibility of phytoextraction soluble zinc decontamination of topsoil: results of a 5-year field scale experiment in Switzerland

FP Speaker: Rolf Herzig</td><td>Safe disposal of dairy/domestic wastewater via irrigation of reconstructed mixed forest- forage formation

FP Speaker: Pinchas Fine</td></tr>
<tr><td>Enhanced level of glutathione protects plants from silver nanoparticle toxicity

FP Speaker: Chuanxin Ma</td><td>Performances of gentle (phyto)remediation options at field scale in the GREENLAND network of large trace element-contaminated sites

FP Speaker: Michel Mench</td><td>Performance comparison between free surface and sub-surface flow systems in phytoremediation study for hydrocarbon removal by Scirpus grossus

FP Speaker: Israa Abdulwahab Albaldawi</td></tr>
</table>

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SP = Short Platform presentation

			Interaction of plants and engineered nanoparticles *FP Speaker: Jerald Schnoor*	Effect of plant cover on long term *in situ* attenuation of a multi-contaminated soil *FP Speaker: Corrine Leyval*	
12.10	12.30		**IPS Plenary and Announcement of Next Conference** Lee Newman and Jason White *Agora*		
12.30	13.00		Closing Ceremony and Awards *Agora*		
13.00	14.00		Lunch		
15.00	17.00		Visit to Jenever Museum		

Saturday, September 15			
9.00	17.00		Guided daytrip to Brussels or Bruges

FP = Full Platform presentation
SP = Short Platform presentation

Alan J.M. Baker – Environmental Earth Sciences International Pty Ltd.

Prof. Alan J.M. Baker gained a B.S. in Botany (1970) and a Ph. D. in Plant Ecology (1974) from Imperial College, London. He worked with the University of Sheffield before joining the University of Melbourne, Australia where he was Professor of Botany before he retired in July 2008. He still remains an Honorary Professorial Fellow in the School of Botany and is a Visiting Research Fellow at the University of Sheffield, UK, where he was awarded a D.Sc. degree in 2009. In 2011, he was also made and Honorary Professor in the University of Queensland's Centre for Mined Land Rehabilitation. Alan's research group was involved in restoration and revegetation projects of mineral wastes, remediation of contaminated land and phytocapping of landfill sites, in addition to carrying out fundamental research on heavy metal uptake and accumulation, and on the development of new phytotechnologies. As well as independent consulting work he is also the Director of Research for the Centre for Contaminant Geoscience of the Australian consulting/contracting group, Environmental Earth Sciences International Pty Ltd. He was Editor-in-Chief (Inorganic Contaminants) of the International Journal of Phytoremediation 1999-2009 and remains a member of its Editorial Board.

Joel Burken – Missouri University of Science and Technology

Dr. Joel Burken earned a B.S. in Civil and Environmental Engineering at the University of Iowa in 1983 and received his M.S and Ph.D. from Iowa as well in 1993 and 1996. Since that time he has been at Missouri S&T (formerly University of Missouri-Rolla), currently serving at Associate Chair and Professor of the Civil, Architectural and Environmental Engineering Department. At Missouri S&T he founded the Environmental Engineering Bachelor of Science Program and the Green Campus Committee. Joel is also the associate editor of both the International Journal of Phytoremediation and the Journal of Environmental Engineering and is the current President of the Association of Environmental Engineering and Science Professors (AEESP).

Nicholas Dickinson – Lincoln University

Prof. Nicholas Dickinson has a Ph.D. from the University of Keele, UK, followed by a an academic teaching and research career in Kenya, UK and New Zealand. He relocated from Liverpool to Christchurch in 2009, where he is currently Professor of Ecology and Head of the Ecology Department at Lincoln University. He specializes in ecological restoration of degraded and contaminated land. Joining a high-profile team of soil scientists and ecologists, the current focus of his research is on phytotechnologies, biodiversity and soil ecosystem services in the production landscapes of New Zealand.

André Gerth – Bioplanta GmbH

Dr. André Gerth has studied at the University of Leipzig. In 1998 he got his diploma as graduated agricultural engineer. Afterwards he studied for his Ph.D. at the University of Leipzig and the University of Santa Clara (Cuba). In 1991 he founded BioPlanta GmbH (Leipzig) and since then he acts as general manager. His main focuses are plant biotechnology and phytotechnologies. Dr. Gerth is managing projects on water treatment using constructed wetlands in Latin America (Peru, Mexico, and Chile), Vietnam, Hungary and South Africa. The range of application of constructed wetland includes passive biological treatment of wastewater from municipalities and industry, landfill leachate, mine water, and groundwater. He has experience in bioremediation of contaminated soils, sediments and sludge. He is coordinating environmental research projects on processes for the biological cleaning of contaminated soils, water and sediments.

Heather F. Henry – National Institute of Environmental Health Sciences

Dr. Heather F. Henry received a B.S. in Biology from the University of Rochester, which included a year of study in plant-derived medicine at universities in Australia and Ecuador. In 2004, she completed a Ph.D. from the University of Cincinnati, where she was a Superfund Basic Research Program trainee participating in numerous multidisciplinary research projects. Henry has recently returned from a Fulbright Postdoctoral Fellowship in Australia studying the role of arbuscular mycorrhizal fungi on arsenate acquisition by native grasses growing on gold mine tailings. Currently, she is working at the Center for Risk Analysis at the National Institutes of Health as a program administrator.

Jan Japenga –
Alterra Green World Research, Wageningen University and Research

Dr. Jan Japenga works for Alterra which is a non-profit market-oriented research organization that recently merged with Wageningen University. Dr. Japenga is educated as an organic chemist, but has been working in the field of soil science since 1985, mainly in soil biogeochemistry. Until recently he was the head of the soil contamination research group inside the Alterra Soil Department. This group works on all aspects of the soil/soil solution/plant interaction for heavy metals in the framework of groundwater protection, crop safety, polluted site management, forest acidification, and phytoremediation. At present dr. Japenga is mainly active in China and Vietnam. There he works together with local research organizations in applied research projects and technology demonstration projects in the field of ecological restoration of mine waste, including the use of biofuel crops.

Nicolas Kalogerakis – Technical University of Crete

Prof. Dr. Nicolas Kalogerakis got his diploma in Chemical Engineering in 1977 from the National Technical University of Athens. He continued on to his masters degree in chemical engineering from McGill University (Quebec, Canada). He earned a Ph.D. from the department of chemical engineering and applied chemistry from the university of Toronto (Canada) in 1983. From 1989 to 1995, he was the associate director of Pharmaceutical Production Research Facility at the University of Calgary (Canada). He continued on to be a Professor at the university before he became professor of biochemical engineering at the state university of New York. In 1997, he returned to Greece where he currently is Professor of biochemical engineering at the technical university of Crete.

Kate Kennen – Harvard University

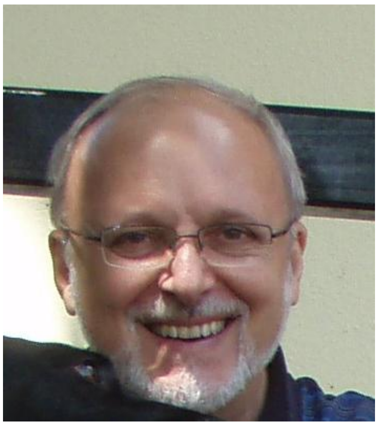
Kate Kennen earned her undergraduate degree from Cornell University (1998) and Master's Degree in Landscape Architecture with distinction (2005) from Harvard University. Kate teaches a research seminar on phytotechnology concepts for landscape architecture with Niall Kirkwood at the Harvard University Graduate School of Design. She is principal of Offshoots, Inc. (www.offshootsinc.com) and Kennen Landscape Architecture (www.katekennen.com) based in Boston. She works with landscape architects to integrate phytotechnology concepts into designed outdoor spaces. Having spent her childhood at her family's garden center in central Massachusetts, Kate is well versed in the plants of the Northeastern United States.

Louis Licht – Ecolotree Inc.

Dr. Louis Licht is founder and president of Ecolotree, Inc. Since 1990, Ecolotree has designed and installed phytoremediation systems at over 55 sites in America and one in Europe. These plant systems use trees (principally Populus spp.) that grow a usable crop while managing water and regulated pollutants. Many projects have been directly connected to ongoing university research, providing a demonstration field site to verify phytoremediation concepts. Projects have been planted in 22 states as alternatives for prescribed landfill covers, for treatment of municipal and industrial wastewater pollutants, as perimeter buffers around hazardous chemical spills, and as interim or final closure for polluted industrial sites.

Dr. Licht is a licensed engineer and serves as an adjunct professor at The University of Iowa, Department of Civil and Environmental Engineering. He has funded research and serves on Ph.D. and M.S. thesis committees. Past research at The University of Iowa has included water pollutant control by tree buffers, non-point pollution control, solid waste cycling, industrial wastewater management, and landfill water management using plant systems in buffers and caps.

Tomas Macek – Institute of Chemical Technology Prague



Born 1951 in Prague, Prof. Tomas Macek obtained his Master degree at the Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague in 1976. He then worked at the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, with interests ranging from enzyme immobilisation to whole cells, from natural product formation by plant tissue cultures to biotransformation of organic compounds. He obtained his PhD in 1983. His experience abroad includes a year in Biological Research Center in Szeged, Hungary (1977/78), and together with his wife Martina stays at Universite de Picardie, Amiens, France (1993/94), Utah State University, Logan, USA (1995/96) and the Federal Agricultural Research Center in Braunschweig, Germany (1997/98). Since the early nineties he has been working on preparation of genetically modified plants, with concentrated efforts on phytoremediation. In the last decade his research included plant-microbe interaction within rhizoremediation, exploitation of metagenomics for bioremediation and studies of plant enzyme effectors. Since 2010 he became full professor of biochemistry at the Department of Biochemistry and Microbiology of the Faculty of Food and Biochemical Technology, ICT Prague, which is his present address. Within the last 23 years he closely cooperated in all these topics with his wife Prof. Dr. Martina Mackova, who passed away in August 2012.

Nelson Marmiroli - University of Parma

Prof. Dr. Nelson Marmiroli obtained the degree in Biological sciences at the University of Parma (Italy) in 1971 and continued working there as a research assistant. He became Professor in Applied Genetics at the University of Parma after a period at the University of Udine and in 1986 he was nominated Full Professor in Genetics at the University of Lecce. In his career he has also held teaching and research positions at the Universities of Bologna, Verona, and Chicago. Currently he is Chair Professor of Environmental Biotechnologies at the University of Parma, and the Director of the Department of Life Sciences. He coordinates a research group of 15 staff units; the main research topics are the application of genomic and proteomic approaches to bioremediation and phytoremediation, the functional genomics of genotype-environment interactions in different organisms, new issues in food safety and quality and countermeasures against environmental terrorism. He authored over 200 publications and participates in the Editorial Board of the International Journal of Phytoremediation. As a member of the Executive Committee of the International Phytotechnology Society he organized in 2010 the first Phytoconference held in Europe.

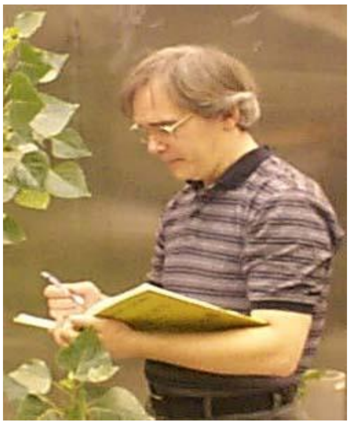
Lee Newman – SUNY College of Environmental Science and Forestry

Dr. Lee Newman earned her Ph.D. at Rutgers University and Robert Wood Johnson Medical School and is currently an Associate Professor of Plant Biotechnology at the State University of New York College of Environmental Science and Forestry. Her work has included the genetic engineering of plants for increased tolerance to heavy metal stress and increasing degradation of organic compounds, uptake and degradation of chlorinated solvents and aromatics, fuel additives, pesticides, energetic compounds, and nitrogen reduction in soil and groundwater; and has included a variety of trees, herbaceous plants and grasses. In addition to the laboratory work, she has worked on the installation of a number of phytoremediation sites. She is also working with NASA to develop hyperspectral imaging technologies to determine plant exposure to environmental contaminates and is doing research on the use of endophytic bacteria to increase plant growth for biomass and agricultural production. Her most recent area of research is the toxicological study of nanoparticles in the environment, and how crop plants respond to exposures to a range of nanoparticles. This work also includes the impact of nanoparticle accumulation on insects that might feed on exposed plants, and the impact of nanoparticles in consumer products on commensalistic skin microbes. Dr. Newman is co-Editor in Chief for the International Journal of Phytoremediation; founding member and currently Immediate Past President of the International Phytotechnology Society; founding member of the Northeast Phytotechnology Society; and Scientific Advisory Board member of the Association of Environmental Health Science.

Markus Puschenreiter – Universität Bodenkultur Wien

Dr. Markus Puschenreiter completed a postdoc at the Institute of Soil Science at the BOKU in Vienna from 2000 to 2007. In 2001, he was a visiting scientist at the Chinese academy of Sciences in Nanjing, China. Since 2008, he has been assistant Professor at the institute of Soil Science in Vienna. In 2011, he was rewarded with the award of the Venia Docendi for soil ecology. His area of expertise is situated within rhizosphere ecology, phytoremediation of soils contaminated by heavy metals, phosphorus, and low molecular weight organic compounds.

Steve Rock – US EPA



Steve Rock is an Environmental Engineer in the Remediation and Contaminant Branch at EPA's National Risk Management Research Laboratory in Cincinnati, Ohio and has worked for the EPA since 1994. Steve manages field projects using phytoextraction, phytodegradation, plume control and vegetative. He is the author of several phytotechnology publications, including acting as team leader on the EPA's Introduction to Phytoremediation, and a chapter in the Standard Handbook of Environmental Engineering. He co-chairs the RTDF Action Team on Phytoremediation, and has three subgroups researching the phytoremediation issues of petroleum hydrocarbons, chlorinated solvents, and vegetative covers for waste containment. He participates in EPA in-house research, and provides technical assistance to EPA regional staff on questions of phytoremediation. Steve was a member of the ITRC Phyto team and an instructor in the ITRC training classes. He is a member of the ITRC Phyto Revision Team. Steve earned a bachelor's degree in Energy Systems from The Evergreen State College in Olympia, Washington, and a master's degree in Environmental Engineering from the University of Cincinnati.

Jean-Paul Schwitzguébel –
Swiss Federal Institute of Technology at Lausanne

Dr. Jean-Paul Schwitzguébel is a French and Swiss citizen and a plant and microbial biochemist. After his studies at the University of Geneva (CH), he worked as a postdoctoral researcher at the Swiss Federal Institute of Technology (ETH) in Zurich (CH), at the Imperial College of Science and Technology in London (UK), and at the University of Neuchatel (CH), focusing on the metabolism and bioenergetics of higher plants and microorganisms. He moved then to the Swiss Federal Institute of Technology at Lausanne (EPFL) as a permanent senior scientist in the Laboratory for Environmental Biotechnology. Most of his work is now devoted to research and development in the fields of phytoremediation and bioenergy. His is the initiator and coordinator of the European COST actions 837 and 859 on phytotechnologies to promote sustainable land use and improve food safety. Dr. Jean-Paul Schwitzguébel is also the Swiss delegate on the COST Domain Committee on Food and Agriculture. Finally, he is subject editor for "Environmental Science and Pollution Research" and "Journal of Soils and Sediments", and member of the editorial board of the "International Journal of Phytoremediation" and of "Agrochimica".

David T. Tsao – BP

Dr. David T. Tsao is the Americas Technology Manager for the Remediation Engineering & Technology group in BP's Remediation Management function at their Naperville, IL office. He is a three-time chemical engineering graduate of Purdue University (B.S., M.S., Ph.D.) where his areas of research included plant biotechnology, pharmaceutical production, plant nutrition, and plant biomass production for space (NASA) applications. Upon graduation, David came to work for Amoco in the Environmental Technology Assessment and Development group where he specialized in the areas of phytotechnologies and the remediation of gasoline oxygenates. Currently, David is responsible for a team of technical specialists coordinating, developing, and implementing the technical aspects of clean up strategies for a broad range of sites around the globe. He is personally active in bioremediation, phytotechnologies, wetland technologies, native prairie restorations, ecosystem developments, biodiversity, and greenhouse gas emissions reduction. David is also responsible for evaluating the potential environmental impacts of new BP biofuel products.

Jaco Vangronsveld –
Centre for Environmental Sciences, Hasselt University

Prof. Jaco Vangronsveld is Director of the Centre for Environmental Sciences of Hasselt University. Originally, his work mainly focused on heavy metal uptake by plants and the effects of toxic metals on plants and their associated micro-organisms (mycorrhizae/bacteria). Since the early 1990s, his group has been involved in several projects concerning the effects of, and remediation options for, contaminated sites around metal smelters in Belgium. They began with laboratory and in situ studies on different aspects of phytoremediation (metals and organics, singly or mixed) and continue to be involved in numerous national and international projects and collaborations in this field. Special attention is targeted to the interactions between plants and their associated micro-organisms in remediation strategies. These investigations are performed from laboratory to field scale. In 2009, Jaco Vangronsveld, was awarded Doctor of Science ("Doctor Honoris Causa"), at the Agricultural University of Plovdiv, Bulgaria.

Nele Weyens – Centre for Environmental Sciences, Hasselt University

Dr. Nele Weyens is a Postdoctoral Fellow at the Environmental Biology Program in the Centre for Environmental Sciences at Hasselt University. Her mentor is Professor Jaco Vangronsveld, a world expert on phytoremediation/phytostabilization of polluted sites. During her Ph.D. studies she won "The best student presentation award" for her oral presentation entitled "Endophytic bacteria cut down evapotranspiration of TCE during phytoremediation in the field" during the International Phytotechnology Symposium in St. Louis, Missouri. She successfully defended her Ph.D. dissertation entitled "*Exploiting plant-microbe partnerships to improve plant growth and phytoremediation*" on December 18, 2009. Nele works mainly on unraveling the role of plant-associated bacteria in the remediation of soils and ground waters contaminated with toxic metals and/or organic chemicals. Her aim is to couple our fundamental understanding of plant-microbe ecology with phytoremediation applications at field scale.

Jason C. White – The Connecticut Agricultural Experiment Station

Dr. Jason C. White is the head of the Analytical Chemistry Department at the Connecticut Agricultural Experiment Station. His research has focused on the potential phytoremediation of weathered persistent organic pollutants such as DDT/DDE and chlordane from soil by Cucurbita species. Dr. White also has a research program evaluating the potential uptake of nanoparticles by agricultural plant species. He earned his B.S. in Environmental Science from Juniata College and his Ph.D. from Cornell University. Dr. White is currently Managing Editor of the *International Journal of Phytoremediation* and President of the International Phytotechnology Society. He is also on the Editorial Board of *Environmental Pollution*. Dr. White holds Adjunct Professor status at the University of New Haven, Quinnipiac University, and Post University and is also a research Affiliate at Yale University.

Ronald S. Zalesny Jr. – US Forest Service, Northern Research Station

Dr. Ronald S. Zalesny Jr. is Research Plant Geneticist and Team Leader of the Genetics and Energy Crop Production Unit at the Institute for Applied Ecosystem Studies in Rhinelander, WI. He studies short rotation woody crop systems (i.e., poplars and willows) for fiber, energy, and environmental benefits, with an emphasis on testing genetic and physiological mechanisms controlling tree development in the face of changing climates and the need for carbon sequestration. His primary phytotechnologies research includes the use of wastewaters as irrigation and fertilization sources for energy crops and other plants. He continues to develop and refine 'phyto-recurrent selection', a method used to match unique genotypes to specific contamination sources to optimize uptake into different plant tissues. He earned his Ph.D. in Forest Biology (Quantitative Forest Genetics) from Iowa State University, where he was the McNabb Research Fellow from 1999 to 2003. Currently he is the bioenergy science leader of the Northern Research Station's Climate Change Science Council, as well as one of the Station's energy experts.

PLATFORM PRESENTATIONS

PLANT-MICROBE INTERACTIONS: METALS

Phytostabilization of heavy metals and metalloids by rosemary: the mycorhizospheric contribution

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[2]*Aix-Marseille Université – CNRS, Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale, IMBE UMR 7263 / IRD 237, 3 pl. Victor Hugo, Case 97, 13331 Marseille Cedex 3, France*
[3]*Aix-Marseille Université – CNRS, Centre Européen de Recherche et d'Enseignement des Géosciences de l'Environnement, CEREGE, UMR 6635, Europôle de l'Arbois, BP 80, 13545 Aix en Provence Cedex 04*

The former smelting factory of l'Escalette (South East of Marseille, France) is a typical example of the current incidence of past industrial activity on the Environment. A subsequent diffuse heavy metals and metalloids (HMM) pollution occurred. Since this industrial wasteland is included in the perimeter of the future National Park of Calanques, a study started on the phytoremedial potential of autochtonous plant species. In this context, the phytostabilization potential of rosemary (*Rosmarinus officinalis* L.) and the potential role of its symbionts on HMM concentrations and locations in roots was investigated. Thereby experimental plots, into each of which 5 plants and respective mycorrhizospheric soils were collected, were selected along a pollution gradient. HMM (As, Pb, Sb, Zn) concentrations were analyzed in all samples (ICP-AES or GF-AAS). Elemental distribution in roots were determined by SEM coupled to EDX analyzes and X-Ray Fluorescence micro-spectroscopy (µXRF). Symbiont occurence was estimated under optical microscope after lactophenol blue staining.

The pollution gradient was confirmed with concentrations varying from 302 to 48 and 3031 to 62 $mg.kg^{-1}$ in soil and from 21.9 to 0.86 and 626 to 4.45 mg.kg-1 in roots, for As and Pb, respectively. Pb and Zn seemed mainly adsorbed/absorbed in peripheral root tissues, corresponding to symbionts colonisation area, the latter showing higher colonization under contaminated conditions. These results are in agreement with previous reports on the beneficial effect of arbuscular mycorrhizal fungi on plant HMM resistance.

In conclusion, with the help of its symbionts, Rosemary appears as a potential candidate for phytostabilization.

Keywords: Heavy metals and metalloids, rosemary, symbionts, phytostabilization

Bacterial-induced weathering of ultramafic rock: implications in phytoextraction

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[5]*University of Natural Resources and Life Sciences, Department of Forest- and Soil Sciences, Konrad Lorenzstraße 24, A-3430 Tulln*

Soil metal bioavailability is often cited as a limiting factor of phytoextraction (or phytomining). Bacterial metabolites, such as organic acids, siderophores, or biosurfactants, have been shown to mobilise metals, and the use of microbial inoculants to improve metal extraction has been proposed by several authors (Abou-Shanab *et al.,* 2003; Becerra-Castro *et al.,* 2011).

In this study, the weathering capacity and Ni mobilisation by bacterial strains was evaluated. Minimal medium containing ground ultramafic rock was inoculated with two strains of Arthrobacter spp.: LA44 (IAA-producer) or SBA82 (siderophore-producer and PO4-solubiliser). Both strains were isolated from the rhizosphere of the Ni-hyperaccumulator Alyssum serpyllifolium. Non-inoculated controls were also prepared. Trace elements and organic acids were determined in aliquots taken at different time intervals. Mobilisation of Fe and Si was observed in SBA82 cultures, but not in controls or LA44 cultures. Ni release was correlated with Mn solubilisation ($R=0.957$, $p<0.01$), and significantly higher concentrations of both elements were found in LA44 cultures. Sequential extraction, SEM and microprobe analyses of the rock samples suggest that the strains act upon different mineral phases. LA44 is a more efficient Ni-mobiliser, apparently solubilising Ni associated with Mn oxides and this appeared to be related to oxalate production. SBA82 also leads to release of Ni and Mn, albeit to a much lower extent. The concurrent mobilisation of Fe and Si indicates preferential weathering of serpentine minerals possibly related to siderophore production by this strain. Ni-mobilising inoculants could be useful for improving Ni uptake and accumulation during phytoextraction (or phytomining).

Keywords: rhizobacteria, *Alyssum serpyllifolium*, Ni-mobilisation, phytoextraction

References:
Abou-Shanab RAI, Angle JS, Delorme TA *et al.* (2003) New Phytologist, 158: 219-224.
Becerra-Castro C, Prieto-Fernández Á, Álvarez-López V *et al.* (2011) International Journal of Phytoremediation, 13: 229-244.

Improving phytoextraction efficiency of high-biomass crops using microbial inoculants and organic amendments

Álvarez-López Vanessa[1], *Prieto-Fernández Ángeles*[1], *Becerra-Castro Cristina*[1], *Rodríguez-Garrido Beatriz*[1], *Herzig Rolf* [2], *Puschenreiter Markus*[3] *and Kidd Petra*[1]

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Phytoextraction aims to remove trace metals from the soil through their uptake and accumulation by plants. Current phytoextraction practises employ either hyperaccumulators or fast-growing high biomass plants. One of the objectives of the EU FP7 "Greenland" project (266124) is to develop biotechnological methods for improving the phytoextraction process; based on the selection of appropriate plant species, soil amendments or microbial inoculants which can affect plant growth or soil metal bioavailability.

Within the framework of the Greenland project we evaluated the effect of soil amendments (compost elaborated from municipal solid wastes) and/or microbial inoculants on the growth and metal accumulation of *Salix caprea* (clone BOKU 01 AT-004) and *Nicotiana tabacum* (*in vitro*-bred clone NBCu10-8). Both have been described as efficient metal phytoextractors (Herzig et al. 2003; Dos Santos Utmazian and Wenzel, 2007). Plants were grown (for 20 weeks) in soil collected from an abandoned Pb/Zn-mine. Soils (<8 mm) were amended with or without compost (5% w/w) and inoculated with 5 bacterial strains isolated from the rhizosphere soil of metal-tolerant plants growing in the same mine. Isolates were selected according to phenotype (metal-tolerance, ability to mobilise soil metals or plant growth promoting traits), and identified as members of the genera *Pseudomonas* sp. (P29), *Rhodococcus* sp. (P30), *Streptomyces* sp. (P64), *Tsukamurella* sp. (P75) and *Massilia* sp. (P87). The effects of inoculants (alone or in combination with compost) on biomass production, plant photosynthetic efficiency and metal extraction (Cd, Pb, Zn), and changes in soil metal labile pools, will be discussed.

Keywords: phytoextraction, organic amendments, plant-associated microorganisms, trace metals, *Salix*, *Nicotiana*

References:

Dos Santos Utmazian MN and Wenzel W (2007). Cadmium and zinc accumulation in willow and poplar species grown on polluted soils Journal of Plant Nutrients and Soil Science, 170: 265-272.

Herzig, R et al. (2003) In: Phytoremediation Inventory: COST Action 837 View. Eds.: Vanek T, Schwitzguébel JP, COST 837.

Long-term Cd exposure induces beneficial shifts in the seed endophytic population of *Arabidopsis thaliana*

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Plant-associated bacteria can have beneficial effects on the growth and health of their host. Nevertheless, the role of endophytic bacteria present in seeds has not been investigated in depth. We isolated the cultivable endophytic population associated with the seeds of *Arabidopsis thaliana*. Two types of seeds were used: seeds from plants that were exposed to Cd for several generations ('Cd-seeds') and seeds from plants that were never exposed to Cd ('control seeds'). We observed differences between the 2 seed types concerning the genera present and the phenotypic characteristics of the different isolates. *Sinorhizobium sp.* and *Micrococcus sp.* were only found in control seeds, while *Pseudomonas sp.*, *Bosea sp.* and *Paenibacillus sp.* were only found in Cd-seeds. *Sphingomonas sp.*, *Rhizobium sp.*, *Acidovorax sp.*, *Variovorax sp.*, *Methylobacterium sp.*, *Bacillus sp.* and *Staphylococcus sp.* were occurring in varying numbers in both seed types. Metal tolerance and 1-aminocyclopropane-1-carboxylate deaminase activity were predominantly found in strains from Cd-seeds, while the production of siderophores, indole-3-acetic acid and organic acids was more present in endophytes from control seeds. The phenotypic characteristics tested in this study appear to be a suitable indicator for root growth promotion under Cd stress. Three out of 10 selected seed endophytes were able to increase primary root length and growth rate of the root. These data indicate that seeds can function as a vector to transfer certain beneficial endophytes from one generation to the next and that the presence of these beneficial seed endophytes might be important for subsequent germination and seedling development.

Keywords: *Arabidopsis thaliana*, seed endophytes, cadmium, plant growth promotion

Microbial consortia enhancing plant growth for the remediation of heavy metal contaminated sites

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[2]*Friedrich Schiller University, Earth Science, Burgweg 11, 07749 Jena, Germany*

The project aims to develop cost-efficient and sustainable measures for soil remediation at different sites in Europe: the former Uranium mining area in Ronnenburg (Germany), the Copper mine in Kopparberg (Sweden) and the tailing dam of Mica Valley in Zlatna (Romania). Microbially aided phytoremediation is proposed as an alternative for soil remediation as it offers in-situ soil restoration and partial decontamination combined with maintenance of the biological activity and physical structure of the soil. More than 200 different microbial strains native to each site were isolated and screened for plant growth promotion traits such as phosphate mobilization, nitrogen fixation or siderophore and phytohormone release. The resistance against the prevalent heavy metals in the soil was assessed in addition. Physiological characterization and 16S rDNA sequencing was performed to identify the isolated plant growth promoting bacteria (PGPR). In greenhouse pot experiments, the consortia of ten strains for each soil were applied with selected plants: *Helianthus annuus*, *Agrostis capillaris*, *Verbascum thapsus*, *Deschampsia flexuosa*, *Euphorbia pithyusa* and *Festuca rubra*. The effect of microbial inoculation on either metal uptake into plant biomass or metal stabilization in the soil was evaluated by assessing bioavailability of metals in soil, plant performance and metal uptake in above ground biomass. The best performing plant-microbe consortium for the German soil (*Helianthus annuus* with the German microbial consortium) was further tested in a field trial to investigate metal mobility at large scale and in environmental conditions.

Keywords: Phytoremediation, PGPR (Plant Growth Promoting Rhizobacteria), heavy metals contaminated sites

Improving biomass production of willow for phytoremediation of metal-contaminated soils using plant growth promoting bacteria and fertilization strategies

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Cleaning up metal-contaminated soils using short rotation coppice of willow is a very promising technique. Besides a gradually decontamination of the soil by extraction of metals, willow clones also have an economic advantage for farmers since the harvested biomass can be used for bioenergy purposes. This work aims to increase biomass production of willow by (a) selecting the best performing clones, (b) inoculating these clones with selected plant growth promoting bacteria and (c) testing different fertilization strategies.

A greenhouse experiment was set up using metal-contaminated soil. Two experimental willow clones, *Salix viminalis* (Clone1) and *Salix alba x alba* (Clone2), were used and inoculated with 5 selected PGPB: *Rahnella sp.* (Rh1), *Bosea sp.* (Ro1), *Caulobacter sp.* (Ro2), *Curtobacterium sp.* (St1) and *Pseudomonas sp.* (St2). Half of the plants was fertilized using 2 different techniques, (i) a commercial slow release fertilizer mixed with the soil and (ii) weekly pouring of the plants with a solution of N, K and Mg. The willow cuttings were planted the 17th of February and will be harvested the 17th of May.

Follow up revealed already that bacteria Rh1 and St2 and bacteria Ro2 and St2 increase biomass production of respectively Clone1 and Clone2. Fractionated fertilization with N, K and Mg had no significant effect until now. However, the commercial fertilizer decreased biomass production significantly. Measuring more biomass parameters at harvest will certainly demonstrate more effects of the PGPB and the fertilization strategies. The best performing combinations (clone-bacteria-fertilization) will be *tested in situ* on a contaminated area.

Keywords: Phytoextraction, willow, biomass, PGPB, fertilization

Exploitation of PGPB strains within phytoremediation protocols for the reclamation of soils contaminated by roasted arsenopyrite residues

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This study aimed at evaluating the effect of the bioaugmentation of PGPB strains, highly resistant to arsenic, on the efficiency of *Pteris vittata* - grown on an arsenopyrite contaminated soil - in phytoextracting this metalloid. The research was carried out in the frame of the R.E.P.E.T. (Rhyzosphere Enhanced Phyto-Extraction Technology) Project, granted by the Tuscan Regional Government, for the reclamation of a former dump site highly polluted with As. Initially, a characterization of the bacterial community acclimated to As was carried out starting from enrichment cultures. 109 bacterial strains were isolated in axenic culture and phylogenetically identified through 16S rRNA sequencing analysis. The bacterial isolates were then analyzed for the occurrence of PGP traits such as ACC-deaminase activity, IAA release, and production of siderophores as well as for the presence of As-resistance genotypes such as *ars* and *aox.* On the basis of the evidences gained, three bacterial strains - *Ochrobactrum* sp. E, *Pseudomonas putida* O and *Achromobacter xylosoxidans* P - were chosen for the bioaugmentation trials. Tests were performed in mesocosms under glasshouse conditions for six months. At the end of the experiment, both plant biomass production and total As content in plant tissues were measured. The persistence of bacterial inoculawas assessed by means of PCR-DGGE analyses. Results revealed that the bacterial inocula exerted positive effects on plant biomass production, with an increase up to 20% when strain O and P were added. Nevertheless, the total phytoextraction efficiency seemed not to be highly influenced by the inoculation of PGPB strains.

Keywords: *Pteris vittata*, PGPB, arsenic, phytoremediation, bioaugmentation

Contribution of the plant rhizosphere system to the phytoremediation of metals in estuarine areas

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Some salt marsh plants have already shown to have potential for metal phytoremediation in estuarine areas (Almeida *et al*., 2011) ,being important to study and test strategies to enhance that potential. The aim of this work was to evaluate how the rhizosphere of *Juncus maritimus* and *Phragmites australis* plants influenced Cd removal from sediments favoring environmental decontamination.

For this study, plants of both species, collected in Rio Lima estuary together with the sediment involving their roots, were placed in vessels and maintained in greenhouses, exposed to natural environmental and light conditions. A nutritive saline solution was added to all vessels through an automated irrigation system (2 daily cycles of flood / draught) to mimic the tides and maintain plants at optimum nutritional conditions. After 2 weeks of acclimation, all vessels were spiked with a saline $CdCl_2$ solution (20 mg L^{-1} of Cd). Solution was in contact with the plant rhizosphere for about 6h. Afterwards a solution containing an autochthonous enriched consortium of microorganisms resistant to Cd (prepared in the laboratory) was added to half of the vessels. Vessels were maintained for 2 months in the abovementioned conditions, being afterwards disassemble. For that, plants aboveground tissues were separated from belowground structures, which in turn were carefully separated from the sediment. Cadmium was determined as before (Almeida *et al*., 2011).

Analyses are still in course but obtained results will allow evaluating the role of the microorganisms present in the rhizosphere of both plants for Cd phytoextraction.

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Keywords: plant rhizosphere, cadmium, estuarine areas, autochthonous microorganisms

References:

Almeida C. M. R., Mucha A. P., Vasconcelos M. T. S. D. (2011). Role of different salt marsh plants on metal retention in an urban estuary (Lima estuary, NW Portugal). *Estuarine Coastal and Shelf Science,* 91, 243-249.

METALS: TOXICITY AND UPTAKE (I)

Proteomic characterization of phenotypic plasticity between metallicolous and non-metallicolous populations of *Agrostis capillaris* L. exposed to Cu

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Soluble proteome was analyzed in roots of metallicolous (M) and non-metallicolous (NM) plants of *Agrostis capillaris*, a pseudo-metallophyte with phenotypic plasticity reported for Cu tolerance (Symeonidis *et al.*, 1985) to investigate (1) differential protein expression in NM and M roots under increasing Cu exposure, and (2) molecular mechanisms underlying higher Cu tolerance in M plants.
Seeds were collected at a wood preservation site (M) and a forest edge (NM), then cultivated 2-month on perlite imbibed with a Hoagland nutrient solution spiked with $CuSO_4$. After extraction by the TCA/acetone procedure, soluble proteins were separated with a linear 4-7 pH gradient followed by 2D electrophoresis (Gion *et al.*, 2005), and stained with Colloidal Coomassie Blue. Image analysis was realized with Melanie 7.0 and protein identification made by mass spectrometry (LC-MS/MS).
At supra-optimal Cu exposure (15-30 µM), glycolysis was likely altered in NM roots with increased production of glycerone-P and methylglyoxal based on overexpression of TPI and FBP-aldolase. Changes in tubulins and higher MetE and SAMS abundances respectively underpinned impacts on the cytoskeleton and stimulation of ethylene metabolism.
Conversely, higher GAPDH abundance supported an efficient glycolysis flow in M roots; better detoxification of methylglyoxal and superoxide in line with the up-expression of GlxI and chloroplastic Cu/Zn-SOD. Increased AAT amount may facilitate production of L-methionine, needed for SAM and nicotianamine and L-cystein, for metallothioneins and GSH production.
This metallicolous *A. capillaris* population did not evolve a specific mechanism explaining their higher Cu-tolerance. It would merely result from simultaneous cooperation of various processes.

Keywords: *Agrostis capillaris*, root, phytoremediation, superoxide dismutase, tolerance

References:

Gion J, Lalanne C, Le Provost G *et al.* (2005) Proteomics, 5: 3731-3751.
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Role of NRAMP transporters in metal homeostasis in poplar leaves

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The possibility to take advantage of the high biomass production of poplar to rehabilitate toxic heavy metal polluted soils by phytoextraction has been investigated for over a decade. Previous studies showed that poplar accumulates a significant amount of metals in its leaves, which can limit phytoextraction because of the autumnal leaf abscission. In this context, experiments are conducted in a heavy metal contaminated field. Among 14 studied cultivars, we observed significant differences in leaf metal contents. Positive correlations between manganese, zinc and cadmium contents suggest the involvement of broad specificity transporters able to carry these three metals. NRAMP (Natural Resistance Associated Macrophage Protein) metal transporters have been associated with manganese, zinc and cadmium transport and we found that their expression levels display dramatic variations among the poplar cultivars investigated. *PtNRAMP3.1* and *PtNRAMP3.2* homologues of *Arabidopsis thaliana AtNRAMP3* were located in the same chromosomal area (32Kb) of the *Populus trichocarpa* genome and share 88,2% protein identity. We showed that they are both able to transport iron and manganese and we are currently testing their ability to transport zinc and cadmium. Strikingly, PtNRAMP3.1 and PtNRAMP3.2 exhibit different subcellular localizations: although PtNRAMP3.2 is targeted to the tonoplast like its homologue AtNRAMP3, PtNRAMP3.1 is localized in intracellular vesicles, yet to be identified. A prospective recent duplication of an ancestral PtNRAMP3 could have generated two different paralogues which acquired specific localizations, regulations and functions. We are generating *A. thaliana* that over-express PtNRAMP transporters to get a better understanding of their functions in the plant.

Keywords: poplar, phytoextraction, NRAMP, zinc, cadmium

Characterization of ZIP and CDF proteins involved in the uptake and sequestration of trace elements in the ectomycorrhizal fungus *Laccaria bicolor*

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Mycorrhizal fungi could play a major role in phytoremediation by protecting plants against trace element (TE) toxicity. They could act as biofilters by immobilizing TE in their hyphae and by limiting TE uptake by roots. Fungi possess potential extracellular and cellular mechanisms that are involved in both metal homeostasis and tolerance. These mechanisms include uptake of TE into the cytosol and their sequestration into intracellular compartments. However these molecular mechanisms are very poorly understood in ectomycorrhizal fungi.

In this context, we studied the role of ZIP (Zrt-Irt- like Proteins) and CDF (Cation Diffusion Facilitator) proteins in TE homeostasis/tolerance in the ectomycorrhizal model fungus *Laccaria bicolor*. Therefore in a post-genomic approach, three ZIP and three CDF genes were isolated from this fungus and functionally characterized. Members of the ZIP family are known to transport mainly zinc from the extracellular space or from organelles into the cytosol. Two ZIP proteins are located at the plasma membrane and may be involved in zinc uptake from the extracellular space, whereas the third ZIP protein, located at the vacuolar membrane, is involved in zinc remobilization from the vacuoles. Members of the CDF family are usually known to be involved in TE sequestration into intracellular compartments. The three CDF proteins are indeed located at the vacuolar membrane, and are involved in zinc tolerance, and to a lesser extent, to cobalt and manganese. Transcriptional regulation of these genes will also be presented and discussed in the context of zinc homeostasis/tolerance.

Keywords: trace elements, ZIP, CDF, tolerance, mycorrhizal fungi

Functional analysis of Poplar (*Populus nigra* L. and *P. nigra x P. deltoids*) during environmental exposure to cadmium

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The dispersal of heavy metals is a relevant factor in environmental contamination capable of compromising soil and water utilization.

In this work, cuttings of clones of the *Populus* spp. (clones 58-861 and Poli of *Populus nigra* L. and hybrid A4A *P. nigra x P. deltoids*) were investigated in relation to their response to contamination by cadmium, in order to understand and quantify the differences between the clones in terms of cadmium tolerance, accumulation capacity and translocation of the contaminant to the aerial parts and in order to evaluate the short (48h) and long term (14d) effects of the cadmium contamination. The evaluation of the phytoremediation potential of these clones has been performed combining chemical, physiological, proteomic approaches

Physiological parameters were analyzed: number of leaves, total leaf area, root elongation. Metal uptake, root to shoot translocation were observed by mineral analysis with ASS. Metal compartmentalization organs by SEM/EDX

Proteomic analysis was performed on crude protein extracts from leaves. Proteins with different isoelectric point (pI) and hydrophobicity were separated by 2D liquid chromatography and analyzed using DeltaVue Software, for qualitative/quantitative differentially abundant bands between treated and untreated samples. Proteins whose abundance was statistically different in response to various experimental conditions were identified by MALDI-TOF/MS to infer their role in plant metabolism response to metals.

This integrated study leads to a better understanding of some of the molecular mechanisms at the basis of cadmium stress response and the possibility for in field cadmium phytoremediation application.

Keywords: *Poplar, cadmium, proteomics, 2D-LC, SEM/EDX, MALDI-TOF*

References:

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METALS: TOXICITY AND UPTAKE (II)

Consequences of Cd or Zn accumulation on element redistribution within leaves of *Zygophyllum fabago* and related impact on plant physiology

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The aim of our research was to identify some new interesting accumulators which may associate an important biomass production with an effective absorption and translocation of heavy metals. The present study focused on the response to cadmium and zinc stress in a metallicolous population of the Syrian bean caper (*Zygophyllum fabago*), a succulent perennial shrub. Its goal was i) to determine the accumulation and spatial distribution of Cd and Zn in leaves of *Z. fabago* and the consequences of their accumulation and distribution on essential elements, ii) to determine the biochemical and resulting physiological parameters modified in relation to the distribution of essential and non-essential elements.
Seedlings of *Z. fabago* were exposed during 4 weeks to 10 µM $CdCl_2$ or 50 µM $ZnSO_4$ in a controlled environment. A detailed localization of elements within leaf tissues was determined through micro-PIXE and LA-ICP-MS and revealed a highly heterogeneous element distribution within leaf cross-section. A preferential accumulation of Zn occurred in spongy mesophyll while Cd appeared more uniformly distributed in leaf sections. Pattern of distribution of essential elements between leaf tissues were modified in response to Cd or Zn accumulation and are specific of the considered heavy metal.
These results will be discussed in relation to a combined physiological (photosynthetic activity, plant water and oxidative status), metabolic (accumulation of putative osmo-protecting compounds) and proteomic approach. All together, these results give some clues about adaptation processes of *Z. fabago* to Cd and Zn toxicity.

Keywords: heavy metal, ion mapping, osmo-protecting compounds, proteomics, Syrian beancaper

Cd induced growth stimulation and differentially-expressed proteome in the Zn/Cd hyperaccumulator *Sedum alfredii*

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Growth stimulating effects have been demonstrated in some Zn/Cd hyperaccumulators exposed to low levels of Cd, but it remains unknown how these effects are achieved in these special plants. To explain this, leaf development, photosynthesis and differentially-expressed proteome of the Zn/Cd hyperaccumulator *Sedum alfredii* were investigated in a hydroponic culture with 0, 1, 5 and 10 μM Cd. The development character of stomata, mesophyll tissue and chloroplast in leaves were studied by scanning electron microscopy. 2-DE and MALDI-TOF/TOF-MS were applied to identify proteins and their functions involved in growth improvement induced by Cd. Photosynthesis parameters and chloroplast size were improved especially under 5 μM Cd, at which a 32% increase of leaf biomass and better leaf development were observed. Also, addition of 5 μM Cd significantly increased the stomatal pore size and the thickness of mesophyll cross-sections in leaves. Moreover, Cd increased the concentrations of three photosynthesis related elements (S, Cu and Fe), while the concentrations of P, K, Ca, Mg, Zn were kept unchanged. Most of identified proteins were known to function in energy metabolism, cellular metabolism, protein metabolism, and so on. However, proteins involved in plant photosynthesis were specific or over expressed under all Cd treatments, such as photosystem II stability/assembly factor HCF136, carbonic anhydrase, ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, suggesting the responses of photosynthesis were the possible main process controlling plant growth under low Cd. The results suggest that low levels of Cd increases biomass in leaves dependent primarily on the active responses of photosynthesis in the Zn/Cd hyperaccumulator *Sedum alfredii*.

Keywords: Cadmium (Cd), Zn/Cd hyperaccumulator, leaf development, photosynthesis, proteome

A novel gamma-glutamyl cycle in plants is involved in heavy metals and metalloids detoxification by maintaining GSH homeostasis and efficient recycling of glutamate

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Plants detoxify arsenic and other thiol-reactive toxins through a glutathione (GSH)-dependent pathway. GSH homeostasis is maintained by the gamma-glutamyl cycle, which involves GSH synthesis and degradation, and the recycling of component amino acids. The enzyme gamma-glutamyl cyclotransferase (GGCT) is involved in glutamate (Glu) recycling. GGCT activity has been reported in plants, but the underlying gene(s) has not been identified. We have identified a small gene family encoding GGCTs functioning as gamma-glutamyl cyclotransferases. Heterologous expression of a GGCT in yeast strains produced phenotypes that were consistent with decreased GSH content due to the diversion of gamma-glutamyl cystein (g-EC) from GSH synthesis to 5-oxoproline (5-OP), and significantly increased the 5-OP content. 5-OP levels were further increased by the addition of arsenite and GSH to the medium, indicating that GGCT2;1 participates in the cellular response to arsenic and other toxic metals via GSH degradation. *GGCT* transcripts were upregulated in arsenic-treated *Arabidopsis,* and *ggct* knock-out mutants were more tolerant to arsenic and cadmium, but not to nickel, than the wild type. The constitutive overexpression of GGCT in *Arabidopsis* resulted in the accumulation of 5-OP and enhanced tolerance to arsenic, cadmium, and mercury. The As/N ratio was significantly higher in the roots of the GGCT overexpression lines, suggesting that Glu was efficiently recycled. Thus, our results suggest that GGCTs ensure sufficient GSH turnover during heavy metals and other abiotic stresses. Manipulation of gamma-glutamyl cycle in plants will be highly useful in developing strategies for detoxification and phytoremediation of heavy metals and xenobiotics.

Keywords: gamma-glutamyl cycle, GSH homeostasis, heavy metals and metalloids, phytoremediation

Platinum in plants and environment of urban area

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From early eighties modern cars equipped with catalyst significantly reduce emission to environment of NO_x, CO and PAHs. However, simultaneously emission of platinum group elements (PGE) takes place. Increase of platinum (Pt) is noted in different environment matrices such as road dust, soils along heavy traffic road, sediments of urban rivers and in plants growing in these sites. Pt is easy taken up by plants and enter food chain playing it's catalytic functions. Although PGE are a noble metals they might be a source of pollution if in high concentrations. Pt and its oxides can pose serious threat to human health as allergenic or carcinogenic agents.

Studies with *Arabidopsis thaliana* revealed that Pt is taken up by roots and effectively translocated to rosettes. It had diverse effects on plants being both stimulatory (hormesis) at low and toxic at high concentrations. At toxic concentrations level of membrane injuries increased while leaf area, biomass accumulation and effectiveness of photosynthetic apparatus were significantly reduced. We also confirmed an increase of Pt concentration in soils and in six plant species growing along roads with heavy traffic comparable to clean sites. Pt in soils was noted up to 8 m from edge of the road with relatively high concentrations to 3 m. Pt did not penetrate to deep layers of soil profile, most probably due to sorption by organic matter.

Keywords: platinum emission, uptake and distribution, biomass, photosynthetic apparatus efficiency, hormesis

Ecological understanding and molecular mechanisms of plant root growth responses to excess metals, in support of growth improvement for safe biomass production or phytoremediation strategies

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Diffuse contamination by excess metals affects large areas worldwide. Bringing these lands back into sustainable use can reduce detrimental environmental and socio-economic impacts. There are opportunities to use these contaminated lands for the production of renewable energy biomass and industrial feedstock applications.

Economically interesting, high biomass producing plants are subject to the toxic effects of the metals, which generally cause growth inhibition, so plant growth under these conditions needs to be optimized. Although the physiology and molecular mechanisms of metal uptake, sequestration and detoxification are under intense investigation, root system development under these circumstances is poorly understood. Yet, placement of roots is an important factor in phytoremediation (in which growth inhibition should be reduced to allow placement in contaminated soil patches), and in safe biomass production (in which root growth should be directed to less- or non-contaminated soil-patches).

Using root growth of *Arabidopsis thaliana* in vertical agar plates as a model, we had previously observed that excess of three different metals (cadmium, copper and zinc) caused distinct effects on lateral roots (LRs). These metal-specific morphological responses indicate the existence of underlying metal-specific sensing and signaling pathways. Using split-root systems, we also found distinct local and/or systemic responses that influence root growth distribution under metal stress. In a reverse genetics approach, mutants in genes related to root growth and hormonal signaling have been studied to identify underlying molecular components. Knowledge is generated that will support strategies for plant growth optimization for the purpose of phytoremediation or safe biomass production.

Keywords: roots, metal pollution, biomass, phytoremediation, growth response

Growth and trace element accumulation of *Salix smithiana* on seven different trace element-contaminated soils

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Risk assessment and phytomanagement of metal-polluted soils requires information on plant responses to metal availability in soil but the predictability of metal accumulation in plants may be limited by metal toxicity and inherent shortfalls of the bioavailability assays. The aims of this work were to: 1. evaluate the relation between soil properties and traditional metal availability assays (soil extraction, soil solution concentration, K_d) versus DGT in polluted soils with widely varying properties; 2. establishing relations between metal availability assays in soil and metal concentrations and accumulation in a metal-accumulating Salix smithiana clone; 3. evaluating as to whether soil properties, metal toxicity and competition with other ions for uptake affect these relations and may thus obscure the predictive power of the soil-based availability assays. Therefore we measured the uptake of Cd, Pb and Zn in a Cd/Zn- accumulating *Salix smithiana* clone grown on soils with differential characteristics and metal availabilities, determined by classical soil extraction (0.05 M Na_2EDTA, 1 M NH_4NO_3), soil solution obtained by centrifugation and diffusive gradients in thin films (DGT). While growth reductions were observed on soils with high chemical metal availability, metal toxicity apparently distorted correlation between DGT-measured concentrations (C_DGT) and metal concentrations in willow shoot biomass. The weaker correlation for Cd may be related to competitive Zn uptake. Except for Cd, metal uptake in willow was generally better predicted by C_DGT than by soil solution concentrations or extractable fractions.

Keywords: Salix, phytoextraction, trace element, bioavailability, DGT

PERSISTENT ORGANIC CONTAMINANTS

Using Plants to Remediate TNT and RDX Pollution

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The containment and remediation of the explosive compounds 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) on military ranges are of high priority to the US Department of Defense. We will present findings, funded by the US Strategic Research and Environmental Development Program, on the *in planta* detoxification routes and the development of a phytoremediation strategy to tackle these significant environmental pollutants.

While RDX is highly mobile in the soil column, where containment and remediation strategies are urgently needed, highly phytotoxic TNT remains tightly bound to the organic material in the soil, hindering the use of plant-based approaches for remediation. Our biochemical and genetics studies on TNT using *Arabidopsis thaliana* as our model species, have revealed response mechanisms, mode of toxicity, location of detoxification and the enzyme activities involved.

In addition to the phytotoxicity of TNT, plants have inherently low abilities to detoxify TNT or RDX. To overcome these problems we have identified, and expressed, bacterial TNT detoxifying genes in plants that confer enhanced resistance to and detoxification of TNT. Similarly, we have established that expression of a unique bacterial cytochrome P450, XplA and its partnering reductase, XplB in a range of plant species confers the ability to remove and degrade saturating concentrations of RDX from soil leachate. We are now testing this technology in a selection of grass species including switchgrass, wheatgrass and *Agrostis sp.* for trials on US training ranges.

Keywords: 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, phytoremediation, military range

Effectiveness of Organic Amendments and Plant Cover for *In situ* Remediation of DDT and Dieldrin Residues in old Orchard Soils

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This study tested whether addition of organic amendments and growing plants can reduce DDE and dieldrin bioaccumulation by earthworms exposed to contaminated historic orchard soil (10 mg DDX kg^{-1}). Highest environmental risk of soil DDE is dependent upon the bioavailability of the pesticide soil residues to earthworms which can then be transferred to birds and small mammals.

The effect of organic amendments on DDX and dieldrin bioavailability from historically contaminated orchard soil was assessed using *Lumbricus terrestris* without and with plant cover.

The plants were orchard grass (*Dactylis glomerata* L. 'Burenbrug') and perennial ryegrass (*Lolium perenne* L.'Paragon'). The experiment was carried out in a temperature and light controlled growth chamber. Five different organic amendments and two application rates (5 and 10% dry weight) were used: 2 months old dairy manure compost, 4 years old dairy manure compost, Orgro® composted biosolids, pine biochar, and limed biosolids. The amendments were thoroughly mixed with the contaminated soil and the mixture was incubated for one month before potting. Plants were grown for 45 days before 12 earthworms were introduced to each pot (6 L). The earthworm exposure lasted 45 days.

Results indicated that dairy manure compost was the most effective amendment in reducing bioaccumulation of DDX and dieldrin by earthworms; it reduced worm DDE bioaccumulation by more than 50%. There was no significant plant effect on worm DDX bioaccumulation. A field test is currently being undertaken to field-validate the effect of dairy manure in reducing DDX bioaccumulation by native earthworms.

Keywords: organic amendments, DDX and dieldrin bioavailability, earthworms, environmental risk

Investigating differences in the root to shoot transfer of organic compounds between zucchini, squash and soybean using a pressure chamber method

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Transpiration Stream Concentration Factors (TSCF) for 14C-caffeine (log Kow = -0.07), 14C-triclocarban (log Kow = 4.9) and 14C-endosulfan (log Kow = 3.83), relative to titrated water, were determined using a pressure chamber method for soybean (*glycine max L.)*, zucchini (*cucurbita pepo ssp pepo)*, and squash *(cucurbita pepo ssp ovifera)*. TSCF values for caffeine (TSCF = 0.8), endosulfan (TSCF = 0.2) and triclorcarban (TSCF = 0.05) were statistically equivalent for squash and soybean. For zucchini, the TSCF for caffeine was the same as for squash and soybean. However, for the more hydrophobic endosulfan and trichlorcarban, the TSCF values were 0.6 and 0.4, or about 3 and 10 times greater, respectively, than the soybean and squash. The greater difference in TSCF with increasing log Kow suggested a solubility enhancement in the zucchini xylem sap. To test this hypothesis, the solubility of caffeine and triclocarban in soybean and zucchini xylem sap was determined and compared to deionized water using a modified shake flask method. Caffeine solubility in the xylem saps of soybean and zucchini was equal to deionized water (22000 mg/L) triclocarban solubility in the zucchini xylem sap was double (20.6 mg/L) that of the soybean xylem sap (10.6 mg/L) and deionized water (11.2 mg/L). This indicates that the enhanced root to shoot transfer of hydrophobic organics reported for zucchini may be partly due to increased solubility in the xylem sap.

Effect of hydrogen peroxide on the uptake of POPs by *cucurbitaceae*

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Zucchini (*Cucurbita pepo* L. ssp. *pepo)* is capable of removing percent level amounts of chlordane from soil. It is known that aquaporins in plants and algae can mediate the transport of certain organic molecules and that these transport proteins are closed in the presence of hydrogen peroxide (H_2O_2). The aim of our study was to investigate the effect of H_2O_2 exposure on the uptake of chlordane by *C. pepo.*

C. pepo was grown hydroponically for six weeks. The growth solution was then amended with chlordane, with selected plants also receiving H_2O_2. Following 18 h exposure, xylem sap was collected and its chlordane content determined. *C. pepo* was grown in chlordane-contaminated soil with selected plants irrigated with H_2O_2 solution. Plant tissues were analyzed for chlordane content.

Significantly less chlordane was observed in saps of plants exposed to H_2O_2, an effect found to be reversible. Significantly more chlordane was detected in the tissues of plants grown in contaminated soil when irrigated with H_2O_2.

H_2O_2 decreases the uptake of chlordane by *C. pepo* in hydroponic growth solution. This effect implicates aquaporins in the uptake mechanism of *C. pepo.* Release of chlordane from soil organic matter by exposure to H_2O_2 masked this effect for *C. pepo* grown in contaminated soil.

In current experiments, the effect of H_2O_2 on chlordane and p,p'-DDE uptake is being compared in zucchini known to uptake these POPs well versus squash known to be non-uptakers. The POPs uptake abilities of *Arabidopsis* aquaporin knockouts will also be investigated.

Keywords: Persistent organic pollutants, Chiral compounds, Chlordane, Aquaporins, Phytoextraction

Advances in the interfacial processes and self-remediation of persistent organic pollutants in soil-plant systems: An example of polychlorinated biphenyls

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The soil-plant system is a very important subsystem in the earth surface ecosystem, playing a key role in ensuring food security and human health. Polychlorinated biphenyls (PCBs) are one of the priority persistent organic pollutants (POPs) in soil environment. The interactions among soil colloids, microbes and plants change physico-chemical and biological processes of PCBs in soil-plant system, result in either decrease of pollutant bioavailability and toxicity or enhanced degradation, then reduce a risk to food chain through pollutant bio-transfer, and lead to decontamination and self-remediation eventually under natural environment. This paper presents an overview on advances in the interfacial processes of polychlorinated biphenyls in soil-microbe-plant systems, and put forward a theoretical principle of self-remediation of POPs contaminated soil.

Keywords: soil-microbe-plant system, interfacial processes, self-remediation, persistent organic pollutants, polychlorinated biphenyls

DDX Accumulation in Grafted *Cucurbitaceae* Grown under Greenhouse and Field Conditions

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Large pot and field experiments were conducted to assess the effect of grafting on accumulation of weathered DDX (the sum of *p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE) from soils to plant systems. In two-year study, intact squash (*Cucurbita maxima x moschata*) and watermelon (*Citrullus lanatus*), their homografts, and compatible heterografts were grown in the soil contaminated with weathered DDX at 150-1760 ng/g. DDX concentrations in plant compartments were measured and compared in the grafted and nongrafted plants.

In the pot and field study, the highest stem DDX concentrations measured in heterografted watermelon plants were 140 and 19 times greater than contaminant concentrations in the intact watermelon, respectively. Grafting watermelon onto squash rootstock significantly increased DDX uptake into the watermelon shoot system. Similar to stem DDX content, the highest DDX concentration was measured as 6.10 μg/L in xylem sap of heterografted watermelon plants grown in the pots. The DDX concentrations were 0.5-9 ng/g in fruit of the cultivars. Data show that the DDX concentrations in the xylem sap, stems, leaves, and fruit of watermelon plants were significantly increased by squash rootstocks.

Keywords: grafted watermelon, homografted, heterografted, *p,p′*-DDE, DDX

Atrazine phytoremediation in Humid Pampa plots (Argentina) under intensive agriculture management

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Humid Pampa, the main agricultural region in Argentina, covers about 600.000 km^2 largely dedicated to intensive agricultural practices. As one of the main world atrazine consumers (7000 tons/year), developing of biotechnological strategies to minimize the environmental impact associated to this herbicide application is necessary. In order to achieve this aim, a pilot scale open field experiment was carried out to assess the phytoremediation performance of the recently reported atrazine tolerant ryegrass species (*Lolium multiflorum*) in a corn field. Accordingly, a 16 hectares field was selected in Ramallo city vicinity (30°40'35,2'' S – 60°09'21,0'' W), as representative of the intensive agricultural practices of this region. Soil was sampled before sowing and 36 plots of 100 m^2 were delimited in order to representatively cover the plot. Then, corn crop was conventionally sowed and 2 L.ha^{-1} of atrazine (Atrazine500-Dow) applied postemergence. After corn harvest, ryegrass was sowed at 22 Kg.ha^{-1} rate in a paired plots fashion. Composite core soil samples were monthly collected from every plot and separated in 5, 15 and 25 centimeters deep in order to assess either the horizontal or the vertical atrazine movement. Soil samples were extracted and atrazine and metabolites content analyzed by HPLC-UV. Parallel, soil agronomical properties were assessed. Results indicate that ryegrass implantation significantly improves atrazine degradation, soil agronomical properties and reduce the herbicide movement caused by rainfall. Concluding, the especially designed ryegrass intercropping strategy fits as biotechnological strategy for atrazine environmental impact associated to intensive agronomical practices of the region.

Keywords: Atrazine, Phytoremediation, Field scale, Ryegrass

Plant uptake and translocation of dieldrin: Role of protein-like materials in xylem sap of Cucurbitaceae

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Cucurbitaceae has been known to take up a large amount of hydrophobic organic chemicals (HOCs) like persistent organic pollutants. To understand the HOCs uptake mechanisms of Cucurbitaceae, we compared dieldrin uptake potentials of several plant families. Corn, sunflower, soybean, tomato, broccoli, cucumber and zucchini were grown in a dieldrin contaminated soil, and the amount of dieldrin in their shoots and roots was measured. Dieldrin was similarly detected in all roots, but high concentrations of dieldrin were detected in the shoots of Cucurbitaceae (zucchini and cucumber) only. The dieldrin distribution ratios in the shoots of zucchini and cucumber were 47.5 and 30.2%, respectively, while that in non-Cucurbitaceae plants was <1%. Thus, Cucurbitaceae have unique HOCs translocation mechanisms that differ from those in non-Cucurbitaceae, and the amounts of HOCs in the shoots are affected by the root-to-shoot translocation ability. Xylem saps of the tested plants were collected, passed through the column packed with C8 granules adsorbed with dieldrin, and the amount of dieldrin in the leachate was measured. Xylem saps of zucchini and cucumber leached dieldrin adsorbed on C8 granules, but those of the other plants did not. Xylem saps of zucchini and cucumber eluted high amounts of dieldrin from the size-exclusion chromatography (SEC) column before the fractions of Apotinin (6.5 kDa). The enhancement of dieldrin mobility by xylem sap was reduced by proteinase and heating. It was suspected that the protein-like materials in xylem sap of Cucurbitaceae have a role to translocate dieldrin from roots to shoots.

Keywords: Cucurbitaceae, dieldrin, translocation, uptake, xylem sap

PLANT-MICROBE INTERACTIONS: ORGANICS

Microbial inoculants improving phytoremediation of diesel fuel show efficient colonization, competitive ability and expression of hydrocarbon degradation

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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. Several plant-associated bacteria, residing in the rhizo- or endosphere, have the capacity to degrade hydrocarbons, to promote plant growth or to alleviate plant stress. Although the potential of bacterial inoculation in phytoremediation applications has been demonstrated, the outcome of inoculation is sometimes not successful or leads to variable results. In many cases this is due to the fact that inoculant strains may insufficiently interact with or colonize plants used for phytoremediation and / or cannot compete with the resident microflora under certain environmental conditions. However, colonization and competitive ability of inoculant strains is generally rarely addressed, but is essential to deduce serious knowledge about the efficiency of an inoculation. We will present our results obtained from various experiments on the colonization capacity of several inoculants strains as well as catabolic gene expression in various plant environments (rhizosphere, root and shoot interior). Furthermore, we addressed the question how environmental parameters affect gene expression and colonization and to which extent inoculant strains compete with native bacteria. We will show how efficient colonization and expression of hydrocarbon degradation genes correlate with plant growth and efficient remediation of diesel fuel.

Keywords: rhizosphere bacteria, endophytes, alkane monooxygenase, gene expression, competitive ability

The role of plant-associated bacteria for the improvement of phytoremediation of TNT-contaminated sites

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The persistence, toxicity and mutagenicity of nitro-aromatics in the environment has increased worldwide concern about these wide-spread pollutants. Phytoremediation is an attractive cleanup strategy because it is both affordable and sustainable. However to overcome limitations of high phytotoxicity and recalcitrance, efforts have been directed towards bio-augmentated stimulated phytoremediation. Both techniques rely on either the introduction of competent bacteria into the rhizosphere or application of nutrients to stimulate degradation by the native bacteria. Until now, characterization of plant-associated bacteria from a TNT-polluted site has not been the topic of many studies. Moreover, degradation rates are underestimated. To explore the possibility of using bio-augmented phytoremediation to improve TNT-extraction and decrease toxicity, we characterised the cultivable soil and plane-tree associated bacteria from a TNT-contaminated site. In soil, gram-negative bacteria dominated the collection of isolates represented by *Enterobacteriaceae*. All these strains demonstrated co-metabolic conversion of TNT to aminodinitrotoluene derivates. Nitroreductase activity was assessed by measuring enzymatic oxidation of NAD(P)H. In the rhizosphere, a variety of bacteria including *Pseudomonas, Burkholderia, Ralstonia and Rhodococcus* were identified by 16S sequencing. Two consortia were compiled, C1 containing *Enterobacteriaceae* and C2 containing plant growth-promoting endophytes. Both consortia effectively transformed TNT co-metabolically. *Nicotiana tabacum* inoculated with single consortium members of C1 show a significant increase in tolerance and growth on TNT. Next, *Arabidopsis thaliana* exposed to TNT and inoculated with C2 demonstrated a marked increase in root length. These results suggest that inoculation of plants with beneficial plant-associated bacteria expressing nitroreductase activity can be an effective way of stimulating phytoremediation.

Keywords: bio-augmented phytoremediation, TNT, nitroreductase, consortia

Bioremediation of Cd and DDTs Co-contaminated Soil by Cd-hyperaccumulator (*Sedum alfredii*) and Inoculation of DDT-Degrading Microbe

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Heavy metals and organic contaminants often coexist in polluted soils. Remediation of co-contaminated soils by heavy metals and persistent organic pollutants is important for securing food safety in agriculture. To date, few studies provide information on remediation of co-contaminated sites with organic and metallic pollutants. We hypothesized that Cd could be phytoextracted by the hyperaccumulator *S. alfredii* and DDTs would be degraded in rhizosphere soil by DDTs-degrading microbes. The objective of this study was to develop a bioremediation strategy for cadmium (Cd) and DDTs co-contaminated soil using hyperaccumulator (*sedum alfredii*) combined with DDT-degrading microbes (DDT-1). The results showed that Inoculation of DDT-1 significantly enhanced rhizodegradation of DDTs, and increased *S. alfredii* biomass, thus improving removal efficiency of DDTs by *S. alfredii* when combined with DDT-1. However, Cd uptake by *S. alfredii* was not significantly influenced by the presence of DDT-1, across both Cd levels (0.895 and 3.225 mg kg^{-1}). Cadmium and DDT residues in co-contaminated soil decreased by 32.1–40.3, and 33.9–37.6%, respectively, in a pot experiment, and the corresponding values were 31.1, and 53.6%, respectively in a field experiment after 18 months of growth. The results from this study indicate that the hyperaccumulator plant-microbe association may be a promising strategy for the remediation of Cd and DDTs co-contaminated soils.

Keywords: Cd-DDT co-contaminated soil, *Sedum alfredii*, Phytoremediation, DDT-degrading microbe, *Pseudomonas* sp. DDT-1

Diversity assessment of endophytic bacteria of the halophytic plant *Tamarix parviflora* and their role in BPA degradation

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Phytoremediation technologies use plants and their associated microorganisms for the degradation of toxic organic contaminants in soil and waters. Specifically, endophytic bacteria may have beneficial effects on their host and appropriate degradation capabilities, leading to the improvement of phytoremediation (Weyens *et al*., 2009).

In the present project (funded by FP-7 project MINOTAURUS), the Rhizodegradation Sequence Batch Reactor (Rhizo SBR) was used. It was artificially polluted with Bisphenol A (BPA, 2,2-bis-(4-hydroxyphenyl)-propane), which is an industrially important compound and an endocrine disruptor, at environmentally relevant initial concentrations of 1 and 10 μg/L, following the plantation of the halophytic plant *Tamarix parviflora*.

The aim of this study was to assess the bacterial endophytic diversity, describe their spatial compartmentalization within the plant and investigate their role in BPA degradation. Cultivable endophytic bacteria were isolated from root, stem and leaf, and their diversity and distribution in those different compartments of the plant were evaluated. They were identified by 16S rRNA gene sequencing and characterized genotypically by amplified rDNA restriction analysis (ARDRA) of their 16S rRNA gene. In order to assess the phenotypic characterization of natural community, the endophytic isolates were screened for BPA tolerance, degradation capacity and heavy metal resistance. Thirty seven morphologically different colonies were distinguished, while most of them exhibited an increased ability to tolerate and degrade BPA. These findings demonstrate that the indigenous endophytic microbial populations of the halophyte *Tamarix parviflora* may play a significant role in a phytoremediation strategy.

Keywords: Endophytic bacteria, *Tamarix parviflora*, BPA degradation, Phytoremediation

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PETROLEUM

Rhizoremediation of Polyaromatic Hydrocarbon (PAH) contaminated soils *Lupinus* species

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Polycyclic aromatic hydrocarbons (PAH) are environmental contaminants of soils of significant concern to the environment and human health. This research aimed to determine the effects of *Lupinus albus* and *L. angustifolius* on the remediation of pyrene an aged creosote contaminated soils.

To assess the effect of lupins on PAH degradation in soil, pyrene and creosote were used as a representative high molecular weight PAH. Sandy loam soil with low nutrient content was amended with PAH to assess the rhizoremediation potential of lupins.Microbial community numbers were assessed using Most Probable Number (MPN) assays and GC-FID for hydrocarbon removal.

Both *L. albus* and *L. angustifolius* were able to significantly increase the degradation of pyrene and creosote in soil comparison withunplanted controls. Overall removal in soils amended with 100 mg kg^{-1} pyrene was 99.0% in *L. albus* or *L. angustifolius* planted systems compared with 78.8% in unplanted controls after 12 weeks. In soil amended with 200 mg kg^{-1} pyrene, 84.7% pyrene removal was attained in the presence of *L. albus*, 83.8% with *L. angustifolius*, and 66.2% in unplanted controls. Average creosote removal after 12 weeks was 89.6% in unplanted controls, compared with 93.8% in the presence of *L. albus* and 99% in the presence of *L. angustifolius*.

The results of this study indicate that lupins show strong potential for application as rhizoremediation candidates in PAH contaminated soils.

Keywords: Rhizoremediation, PAH, soil, Lupins

Engineered Phytoremediation Pilot Study for Petroleum Hydrocarbons in a Deep Confined Aquifer

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A former petroleum refinery in Oklahoma USA has groundwater contaminated with benzene, over a widespread area in excess of 350 acres (140 hectares). A pilot study being implemented at the site is intended to assess the feasibility of using engineered phytoremediation to achieve hydraulic control of groundwater contaminant migration as required by an agreement with the state environmental agency. Light non-aqueous phase liquids (LNAPL) in the form of mobile and residual phases are trapped within the confined aquifer beneath a 5-7 meter thick overlying confining aquitard unit in portions of the site. A total of 96 trees were installed using the *TreeWell*® system, which utilizes drilled boreholes that are lined and backfilled with amended topsoil. Five test plots were constructed and are being evaluated: a background control group, two areas with LNAPL, a main test plot in an area with high dissolved benzene concentrations, and a tree lysimeter test plot (closed system installations that are not open to the aquifer and in which groundwater consumption rates are being measured for future design purposes). Due to possible deleterious effects of the LNAPL on tree vitality, bench-scale soil column testing was performed for the purpose of assessing upward LNAPL migration (driven in part by the buoyancy of the stratigraphically trapped LNAPL) within the *TreeWell*® system soil column. The bench test results and preliminary data from the pilot study will be presented.

Keywords: Engineered Phytoremediation, Benzene, TreeWell, hydraulic control, water-use

Influence of plant root exudates on the mobility of fuel organic compounds in contaminated soils

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Vegetation and its associated microorganisms play an important role in the behavior of soil contaminants. One of the most important elements is root exudation, since it can affect the mobility, and therefore, the bioavailability of soil contaminants.

In this study, we evaluated the influence of root exudates on the mobility of fuel derived compounds in contaminated soils. For this purpose, samples of humic acid, montmorillonite and an A Horizon from an alumi-umbric Cambisol were contaminated with two groups of organic contaminants present in fuel, i.e. oxygenates (MTBE and ETBE) and monoaromatic compounds (benzene, toluene, ethylbenzene and xylene or BTEX). Ten compounds usually found in natural exudates were added (individually and in mixture) in solution concentrations of 1, 10 and 25 mM. Natural root exudates obtained from *Cytisus striatus* were also tested. Samples were analyzed by headspace-gas chromatography-mass spectrometry.

The retention of the contaminants depended both on the sample and the compound properties: xylene was the most retained contaminant (70-80% of xylene added was retained) and MTBE was the least retained (10-50%), and humic acid was the substrate that showed, in general, the highest retention capacity. The addition of exudates caused an increase in the mobility of all the contaminants in the humic acid, whereas the effect was quite the opposite in the A Horizon and the montmorillonite. In these latter substrates, significant correlations ($r>-0.738$) were found between exudate carbon content and the decrease in contaminant mobility.

These results could be of practical use for developing and improving phytoremediation processes of fuel-contaminated soils.

Keywords: plant root exudates, BTEX, fuel oxygenates, contaminant mobility, soil

Rhizoremediation of hydrocarbons in cold climate

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Creosotes, which are mainly a complex mixture of PAHs and phenolic compounds, have commonly been used as wood preservatives for railroad ties/ railway sleepers. In southeastern Finland a site with wood treatment facility for railroad ties was abandoned in the 1960's, and the PAHs have now spread to the groundwater. A total of two hectares of the seven hectare site has to be remediated. Phytoremediation has been suggested for remediation, and implementation of clean up is waiting for permission from the environmental authorities. Hybrid poplars are promising for rhizoremediation of petroleum hydrocarbon-contaminated soils producing much needed biomass that also can be used as renewable energy source.

The environmental pre evaluation of the site is under way and characterisation of PAH levels across the site were done. Mapping of vegetation was performed and soil nutritional and physical parameters were determined. The inherent biodegradation potential was assessed by analysing structural and functional microbial diversity with soil DNA analysis and fingerprinting of 16S rRNA and aromatic ring-cleavage genes. A greenhouse study was initiated in 2009 to select for best hybrid aspen clones for rhizoremediation.

The soil of the creosote contaminated area is mainly fine sand and the dominating vegetation is small Scots pines together with some birches, aspens and willows. Ground vegetation is mainly lichens, and some hay species, indicating low nutrient and moisture levels in soil. Chemical data analysis revealed pollution gradients of PAHs and oil hydrocarbons. Analysis of bacterial community structures showed changing communities across gradients and in different soil strata.

Keywords: *Populus*, phenanthrene, fluoranthene, terminal restriction fragment length polymorphism, extradiol dioxygenase

Effects of Soil Clean - up using *Lemon grass* on the interaction of selected soil minerals with organic pollutants: management implications

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Successful phytoremediation presumes that the target pollutants can be made bioavailable and that a competent flora, community of potential microbial degraders either already exists and can be stimulated or that can be introduced and established. The formation of organic – mineral interfaces in a crude oil inundated ‘’illegal oil pipe tapping soil’’ was studied by monitoring the variables – texture, pH, Organic carbon, total Nitrogen, available P, Dry mass loss, PAHs concentration, and Base (Ca, Mg and K) as well as the 90d resulting clean soil. The clean – up technique employed Lemon grass and introduction of Compost and Humin amendments were undertaken for comparison. After 90d, CO_2 mineralization was significant and N cycling was strongly coupled to the C cycling. Addition of compost indicated co-metabolic degradation. Also, concomitant activity of phytoremediation and the agronomic management practice were more effective. PAHs levels above intervention values was sequestered up to > 50%. The dissipation of the 16 USEPA-listed PAHs was largely enhanced from no significant change to 50.8±12.4% (for humin amended); 62.9±7.1% (for compost amended). The study demonstrated the critical role of Organic matter in determining the fate, mobility, and bioavailability of these priority contaminants

Keywords: Soil interactions, Contaminated Soil, Remediation, Bioavailability, PAHs, Humin

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Impact of metabolites on the phytoremediation of hydrocarbons

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Phytoremediation of petroleum hydrocarbons has proved its efficiency compared to bioremediation. However, treatments usually do not reach a complete removal and some contaminants remain. Several hypothese were proposed to explain this incomplete degradation such as limited bioavailability of hydrocarbons or a production and accumulation of toxic metabolites (Chaillan et al., 2006). The aim of this study was to assess *i)* the effect of plant on residual biodegraded hydrocarbons, and *ii)* the toxicity of the native and partially biodegraded hydrocarbons on plant. Experiments were conducted with maize (*Zea mays*) in hydroponic conditions for 2 months. Three modalities of Hoagland solution growth were tested in four replicates: without contaminant, contaminated with hydrocarbon (fuel, 1 g L^{-1}), contaminated with partially biodegraded hydrocarbon (after 12 weeks of biodegradation). Growth systems consisted in glass bottles filled with 700 mL solution, planted or not with two-weeks maize plant (Chaîneau et al., 2000). Plant development and root system structure, organic compounds nature and concentration, and bacterial community structure were regularly assessed. Results showed that the presence of plant allowed an increase of biodegradation resulting in a higher degradation rate and an absence of some residual organic compounds. However, root architecture was strongly affected by both native and biodegraded hydrocarbons. The decrease in the root length and an increase in the average root diameter were observed. However, the negative effect of biodegraded hydrocarbon on the root system seemed transient and the plant was able to overcome it on the longer term.

Keywords: hydrocarbons, metabolites, rhizodegradation

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ECOSYSTEM SERVICES

Native plants for the phytoremediation of heavy metals and arsenic contaminated soils in an area with high ecological value in La Unión (Spain)

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Regarding plant-based strategies for the large scale remediation of a disturbed soil, such as abandoned mining areas, it should be noted that these tasks require lengthy periods of time to reach a certain level of success (even hundreds of years). Therefore, it is obvious that the plants used will remain in the treated place for more than enough time for their expansion, reproduction, competition with nearby plants, and colonization of the surrounding area. This is of vital importance in the case of high value ecosystems, as in the Natural Park of Sierra Minera in La Unión (Murcia, Spain), where the use of species that may alter the natural habitat is discouraged. For these reasons, with the goal of phytostabilizing the area of El Gorguel (pH=7.49; EC=2.5 dS m^{-1}; $[As]_{total}$=20.6, $[As]_{ext}$=0.7; $[Fe]_{total}$=2210, $[Fe]_{ext}$=0.7, $[Pb]_{total}$=204, $[Pb]_{ext}$=3.3; $[Zn]_{total}$=238, $[Zn]_{ext}$=138; all in mg kg^{-1}), we have studied the potential use of local species, which also show adaptation to the dry-climatic conditions in the area. The three most promising plant species (*Dittrichia viscosa*, *Silybum marianum*, *Nicotiana glauca*) in terms of heavy metals (HMs) and As accumulation and biomass production were selected. Then, a pot assay was established to test the effect of two amendments (compost and a humic fertilizer) and the plant rhizosphere on trace elements availability (studying pore water collected using rhizon-samplers), as well as on plant germination and growth, with respect to an uncontaminated control soil.

Keywords: Phytostabilization, local plants, arsenic, rhizon-samples

In situ amendments allow spontaneous establishment of plants on bare soil near the copper-smelter industry of Lubumbashi (DR Congo)

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Mining activities in the Katanga province (DRC) has created large superficies of bare soils contaminated by trace metals (TMs: Cu, Co, Zn, Pb). Bare soils increase human exposure because of TMs dissemination by water erosion (rain season) and wind (dry season). This study evaluates the effect of amendments on the improvement of soil chemical properties thus permitting spontaneous plant establishment on bare soil contaminated by heavy metals (total Cu concentration: 50000 mg kg^{-1} in bare soil vs. 220 mg kg^{-1} in forest) around the Cu-Smelter of Lubumbashi. Lime (0, 2.5, 5 and 10 t ha^{-1}) and compost (0, 45 and 225 t ha^{-1}) were applied *in situ* in a factorial design. Soil pH, TMs, P and organic matter (OM) were assessed one year after amendment while plant establishment was monitored for 3 years. Cu bioavailability (extractable with NH_4Ac-EDTA pH = 4.65) decreased with either amendment while pH and nutritional status increased (P, OM). Compost had the largest positive effect on species richness and soil cover. Species observed comprise a mixture of true metallophytes (*Bulbostylis pseudoperennis*, *Microchloa altera*) or ruderal weeds (*Celosia trigyna*, *Setaria pallid-fusca*). Most of which are present in the study site as scattered individuals or isolated patches. Plant cover steadily increased in the rainy season. However, only the perennial grass *M. altera* survived in the dry season. Following these results, *M. altera* had been cultivated in the site using combination of lime and compost. Here we report the success of this trial after three years of observation.

Keywords: Katanga, Trace metals, Phytostabilization, Amendments, *Microchloa altera*

Phytotechnologies in intensive agricultural landscapes in Canterbury, New Zealand

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Agricultural landscapes in Canterbury are the most modified and biologically depauperate environments in New Zealand. All of the productive plants used in agricultural and forestry on the plains and down lands are introduced exotic species. There has been a long demise of the unique native vegetation, although there is now substantially increased interest in restoration planting of a diverse array of native trees and shrubs on marginal land, paddock edges, along water races and in storm water swales. This has recognized aesthetic and conservation value, and it is frequently argued that ecological restoration somehow improves the provision of ecosystems services. This paper investigates the benefits of native plants in terms of nutrient and trace element management, and the protection of environmental quality. Our results show that native plant rhizospheres variably modify soil physico-chemistry and regulate pore water quality. Widely different morphologies of plant root systems are found to modify ingress and discharge of water and dissolved substances. This is demonstrated through examples of riparian planting that mitigates pollutant run-off and drainage effluents. Furthermore, different plant species uptake different amounts of nutrients and trace elements from the soil; palatable woody species have the potential to provide a valuable source of trace elements. Plant and soil properties associated with native species offer added value in agricultural systems, in the context of waste management, and also associated with widespread geographical trace element deficiencies (Cu, Co, Zn, Mg, Se, I). These are phytotechnologies that provide economic incentives to increase native species planting on paddock and farm margins.

Keywords: Agriculture, Native plants, Rhizosphere, Pore water, Pollutant management

Floristic composition of the various physiognomies in shasha forest reserve in ile-ife area of South Western Nigeria

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Floristic composition of the standing vegetation and soil seed bank of the various physiognomies in Shasha forest reserve in Ile-Ife, south western Nigeria were examined, with a view to assessing the role of soil seed bank in the restoration and rehabilitation of the degraded forest reserve. Two sample plots, each of 25 m X 25 m were selected in Taungya system, natural regrowth forest and *Gmelina arborea* plantation. All the plants were enumerated and identified to species level. Soil samples were randomly collected and subjected to seedling emergence for six months to determine density and species composition of the seed banks. Results revealed low similarity in species composition in the standing vegetation of the three sites. Similarity index was highest between Regrowth forest and *Gmelina arborea* plantation and lowest between Taungya system and Regrowth forest. *Gmelina arborea* plantation had more woody species in the standing vegetation. Few woody species emerged from soil samples in all the sites. There was low similarity in species composition between the standing vegetation and the seed bank. The seed bank was mainly composed of early successional species and the seeds of primary forest tree species were rare in the soil of the three study sites. The few woody species recorded in the soil seed bank of the sites was an indication that the soil seed bank may not be an important conservation tool for regeneration of the degraded forest. The implication of monitoring the biodiversity of standing vegetation and soil seed bank was discussed.

Keywords: Conservation, seed bank, plantations, floristic composition

Phyto-rehabilitation and economic development of open-pit coal mining residue areas in Quang Ninh province, Vietnam

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After adopting a market-based economy in the mid-1980s, coal mining has intensified in the Northern part of Vietnam, both for domestic use as for export. Especially in the case of open-pit coal-mining, eco-rehabilitation of coal mining waste soil is necessary to recover regional biodiversity, to control pollution spreading (coal dust, leaching and erosion) and to support economic development after mine closure. Regulations are in place to implement such eco-rehabilitation, e.g. through planting of *Acacia* which can be used as *materia prima* for the paper industry.

A consortium of Dutch, Vietnamese and Chinese research institutes carries out a project involving (i) multidisciplinary scientific research (natural and social sciences), (ii) on-site technology demonstration and (iii) knowledge transfer (training courses, university curricula development, feed-back with local farmers). The project aims at testing and evaluating different crop varieties (energy crops, local grass and tree species) which are promising for improving current eco-rehabilitation practices, both regarding phytotechnological aspects and socio-economic aspects. The project started in 2010 and uses results of earlier projects carried out in China. The test site used represents a "worst-case" as the site is located in a windy, erosion-prone area.

The project results indicate that eco-rehabilitation and erosion control at coal mine affected areas is agronomically and technically possible and economically viable for the present local socio-economic conditions. Energy crops (like *Jatropha curcas* and sweet sorghum), high production tree species (*Ailanthus altissima*) and erosion-control species (like *Miscanthus sinensis* and Vetiver grass) perform well. Major threats are the climate conditions and plant diseases especially after storm events.

Keywords: Vietnam, coal mining, eco-rehabilitation, energy crops, erosion control

Organo-zeolitic biofertilizers: A new approach to the promotion of plant growth

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The aim of this proposal is to evaluate the benefits of using an organo-zeolitic fertilizer (biofertilizer) for enhancing plant growth. Apart from the ever increasing cost of chemical fertilizers, their use over the last sixty or more years has had a deleterious effect on soil health. In contrast the biofertilizer, composed of organic waste and crushed zeolitic rock, functions biologically in sponsoring nitrification. Ammonium ions, provided from the degradation of the organic waste, are adsorbed to the zeolite mineral surface thus avoiding loss to the atmosphere by volatilization. Oxidation of the ammonium ions, by soil nitrifying micro-organisms, provides major and trace element nutrients. Analysis of pore water, from substrates amended with the biofertilizer, has shown that its cation concentration is very much higher than that of pore water from un-treated substrates. These cations, which cover a wide range of elements, provide essential major and trace-elements in ionic form that are available for uptake. Without the zeolitic component the degree of nitrification is greatly reduced and the converse applies in that application of zeolitic rock without the organic component has little effect on plant growth. Many countries in the world have extensive deposits of rock containing a high abundance of common zeolite minerals and the organic component, being animal or plant waste, is of general availability which makes the use of the biofertilizer a feasible proposition.

Keywords: Zeolite, Organic waste, Biofertilizer, Ammonium ions

To sustain sustainability in agricultural using industrial by-products

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Sustainability of agriculture depends on environmental circumstances including the available nutrients while there are efforts to minimize the use of non-renewable sources. The produce of fertilizers needs lots of energy and cost. Plenty of harmful gases get out during the production. If we reuse the wastes and by-products – which contain organic matter, micro and macro elements and have got no harmful effect to environment – will be possibility to reduce the use of fertilizers. The aim of our study is to give a brief overview about the effects of sewage sludge and compost on the physiological parameters of plants. Maize (*Zea mays L cvs. Norma SC*) and sunflower (*Helianthus annus* L. Arena) seedlings were used in the experiments. Dry matter accumulation of shoots and roots, relative chlorophyll contents, chlorophyll a, b and carotenes contents were measured. Moreover, we also measured the elements uptake and the leaf area.

The relative chlorophyll contents of maize increased when sewage sludge treatment was applied. The chlorophyll a, b and carotenes contents decreased comprised to the control. This observation was similar in case of sunflower, too. The treatments have advantageous effects on dry matter accumulation of shoots and roots in both experimental plants. The leaf area decreased in all treatments in comparison to the control. The land application of sewage sludge is a common agricultural practice worldwide. It effectively disposes of a waste product while recycling valuable nutrient into the soil - plant system. We have to try to reuse more and more waste and by-products.

Keywords: compost, sewage sludge, sustainable agriculture

CO_2 fixation increase by new effectors of photosynthetic enzymes, ecdysteroids

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Ecdysteroids seem to have a new biological role. These steroid hormones control many physiological processes of insects and crustaceans. They are found also in many plant species, but no other function in plants than antifeeding activity towards herbivorous insects has been ascribed to them until now. Our results show that ecdysteroids and their analogues act as effectors of activity of some plant enzymes and other proteins. As far as some of the identified proteins play very important roles in plant organisms, *e.g*. in photosynthesis, or as pathogenesis-related proteins, the role of ecdysteroids in their regulation has to be clarified. The enzymes include among others ribulose1,5-bisphosphate carboxylase/oxygenase (RuBisCO), PsbP protein of the oxygen evolving complex of FS II or *e.g*. osmotin. The effect of oxysterols on photosynthetic activity of plants was analyzed in several plant species, namely spinach, maize and tetragonia. Photosynthetic activity was examined at the level of oxygen production from leaf discs, photosystem II performance based on the detection of changes in chlorophyll fluorescence kinetics and the content of photosynthetic pigments. The influence of ecdysteroids on RuBisCO was studied using radioactively labelled CO^2. Our results show that different ecdysteroids are able to increase oxygen production by water cleavage and the yield of RuBisCO-mediated reaction in which CO_2 is fixed into organic matter thus allowing fine tuning of the yield of photosynthesis.

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Keywords: CO_2 fixation, RuBisCO, ecdysteroids, oxygen evolving complex

Aboveground biomass, standing floor litter and soils Carbon stock in a 10 year *Tectona grandis* plantation in Ile-Ife, Southwestern, Nigeria

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Reforestation and regeneration activities have the potential to sequester carbon. However, little information on the effects on C budgets in plantation ecosystems is available in Nigeria. This paper attempts to provide information at the micro level in response to the reforestation activities. This study focused on estimation of carbon stock in the above ground biomass, its determination in standing floor litter (leaf and small wood) and soils in a 10 year old *Tectona grandis* and a nearby re-growth at Obafemi Awolowo University, Ile-Ife Nigeria. Four quadrates, each of 25 x 25 m size were established in the plantations and the secondary forest. Soil samples were randomly collected at (0-20 cm) from the study sites, bulk density and total C concentration were analyzed. Total C stocks were calculated by multiplying C concentration with their respective bulk density and sampling depth. Standing floor litter were randomly collected at five points every three months, sorted into different litter components and analyzed for total C concentration and used to calculate total carbon. Total C stock of soil varies from 1.10 in the plantation to 0.99 t ha^{-1} C in the secondary forest. Above ground biomass, standing leaf and wood litter were estimated at 18.26-5.81, 0.49-0.36, 0.06-0.08, t ha^{-1} C, respectively from the plantation to the secondary forest. It is clear that reforestation after 10 years has increased C stocks by 45% in the above ground biomass in the plantations, other parameters determined were also altered.

Keywords: Carbon stock, plantation, reforestation, standing litters

Improving ecosystem health and functioning through phytotechnologies at Freshkills Park, Staten Island, New York, USA

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The 2,200 acre Freshkills Landfill in Staten Island, New York was once the largest landfill in the world, and current reclamation plans will result in the largest landfill-to-park conversion in history. This process involves importing soil to cover the landfill and provide a suitable environment for establishing native vegetation. The imported soil must meet certain regulatory chemical standards before it can be considered suitable for public, non-industrial use. The overall goal of the research is to assess the potential of phytotechnologies to improve the physical, chemical, biological, and agronomic characteristics of soils imported to Freshkills as well as to improve the soil usage classification. To accomplish this, we identified individual parent trees of the *Populus* and *Salix* genera growing on Staten Island, as well as existing seed banks of *Panicum* (switch grass). Scions from 112 total *Populus* and *Salix* genotypes were collected from three Staten Island locations during February 2012 and are currently being propagated for scale-up. Specific genotypes with the greatest ability to remove inorganics that diminish the suitability of the soils will be determined through phyto-recurrent selection, a process consisting of greenhouse studies in Rhinelander and subsequent field trials at Freshkills Park. The long-term goal is to select and establish the most successful genotypes throughout Freshkills Park. The objectives of the current presentation are to highlight the plant selection and propagation phases, as well as key components of the experimental design, in order to stimulate discussion about technical aspects of the project and the potential for future collaborations.

Keywords: ecosystem services, phyto-recurrent selection, soil quality, sustainability

DESIGN

Botanical Biofilters Remove TVOCs from Indoor Air

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We as a culture spend 80 to 90% of our time indoors. It is this indoor environment that has largest impact on our welling being and health. Traditional systems to maintain indoor air quality are very energy intensive. An alternative has been developed which combines the industrial processes, biofiltration and phytoremediation, to form living wall biofilters. These walls are vertical hydroponics where air is actively drawn through the rooting media. In the root zone, beneficial microbes consume organic pollutants such as benzene and formaldehyde and the cleaned air are returned to the occupied spaces. Green plants facilitate the process.

This technology has been extensively tested in the laboratory with very promising results and a small number of case studies are also promising. However, detailed studies of systems in real world settings have been lacking.

The presented study addresses this short coming. Twelve walls ranging in size from less than a square meters to approximately 150 m^2 and from a few weeks old to over six years in age were evaluated for performance.

The air exiting the biofilters was the same quality as outside air in terms of TVOC concentrations. The walls could generate over 100 liters of 'virtual outside air' per second per square meter of living wall. When integrated into a typical building with typical ventilation rates, the biofilter could be expected to reduce TVOCs by 30%.

Keywords: Indoor air quality, biofilter, phytoremediation, living wall, TVOC

Thermal performance of tropical green roofs with different vegetation growth forms

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This study assessed the passive cooling effect of extensive green roofs in humid-tropical Hong Kong. The research site is situated on the top of a university academic building in the built-up part of the city. Three vegetation types (grass, groundcover herb and shrub) with contrasting growth forms, plant height and biomass structure were compared with a bare control plot. Temperature was monitored at seven levels: 200 cm, 60 cm, 20 cm, vegetation surface, soil, Rockwool moisture storage layer, and bottom tile. Thermister temperature probes were used, except surface temperature which was monitored by an infrared sensor. Data were acquired at 15-minute intervals continuously for 12 months, and average diurnal temperature were computed. Variations occurred mainly in daytime; nighttime cooling trends were similar to the control. Planted roofs registered lower maximum and minimum, and except grass the diurnal range did not shrink. The grass roof cooled daytime air temperature effectively to create a suspended temperature inversion. Groundcover and shrub cooled air less effectively than grass. Shrub generated a sub canopy temperature inversion. Control and grass surfaces warmed above ambient temperature, but groundcover and shrub surfaces followed the ambient. A perched thermal discontinuity was detected in groundcover and shrub due to the living-biomass mat. All vegetated plots experienced a subsurface thermal discontinuity due to the air-gap insulation of the plastic drainage layer, which arrested downward heat transmission. The findings provided hints on tropical green roof design and species choice to maximize the thermal performance.

Keywords: Humid-tropical green roof, Thermal performance, Passive cooling, Temperature inversion, Thermal discontinuity

Integrating Phytotechnologies with Design and Landscape Architecture Practice

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Landscape architects are increasingly focused on optimizing natural systems and including plantings that provide ecosystem services in designed environments. Phytotechnologies have the capacity to play a significant role in transforming contaminated urban land, providing a more sustainable choice for land planning. They can also be used to help buffer the non-contaminated site, where the risk of pollution could be possible. However, the science of phytotechnologies is often misunderstood by designers, and currently available documentation on the subject is written by scientists *for* scientists. The scientific language and text-based format is often difficult for landscape architects to understand to create productive, functioning landscapes that incorporate concepts of phytoremediation.

In the Department of Landscape Architecture at Harvard University's Graduate School of Design, case studies have been collected in a two year research seminar to assess opportunities for designers to integrate phytotechnologies. The research not only covers current science, but also older studies that may mislead designers. Suggestions for integration of phytotechnologies into the landscape architecture profession have been assimilated and works of designers who are incorporating phytotechnology concepts are offered. The research is being compiled into a publication that will highlight potential opportunities and conversely limitations of phyto-technology, consider the potential environmental, spatial, cultural and aesthetic qualities of productive vegetation, and summarize recommendations for productive site design utilizing a range of natural cleanup technologies. The work bridges the critical science and engineering associated with phytotechnology site applications and its effective design use in the field.

Keywords: Landscape Architecture, Design, Harvard University

Farming the Pollution
A modular fence for phytoremediation of polluted soil

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This research project focuses on soil, pollution, plants and the opportunities that the relationship between these elements gives to our everyday life. The research starts with an analysis made in collaboration with scientific universities (Wageningen University) and civil services (AbdK Eindhoven). The project will look at the location of The Campine region - in the Netherlands - a border area heavily polluted by industries. In this location heavy metals have contaminated the soil, rivers and buildings thorough the smelting process of ore extraction. People are not completely aware of the fact that a concentration of heavy metals has carcinogenic consequences for humans and are also destructive for the ecosystem. In this sense, soil contamination is a form of invisible pollution. As a designer this observation sparks my curiosity about the processes constituting soil pollution. Accordingly, I propose to use soil pollution as a topic in creating awareness in our society and empower people to act consequently. The result of the research is a manual that shows alternative tools, services and systems for analysis and remediate soil pollution with plants. Also, a case study has been tested in a polluted area for one year and I have reduced contaminants licking using phytostabilization and phytoextraction. The project focuses on the design of the area using fences that reminds the factory that polluted the soil. This design project suggests a method of creating dialogue between man and nature, through the transformation

Keywords: Phytoremediation design, Phytoremediation manual, alternative strategies and services, soil pollution, process installation.

PHYTOFORENSICS

Utilization of trees (willow, poplar) for phytostabilization and biomonitoring of metal fluxes on contaminated land

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More than 30,000,000 ha of soil throughout the world are contaminated with trace elements (TE) and phytostabilisation is one proposed method which aims to reduce environmental risks arising from such soils. This study investigated the biomass production and metal allocation to foliage and wood of willow (*Salix viminalis L.*), poplar (*Populus monviso)* and birch (*Betula pendula*) on five TE contaminated soils, with varying high concentrations of Cu, Zn, Cd and Pb. The results showed that birch could be suitable for phytostabilisation of soils with high Cd and Zn but low Pb concentrations, while poplars and willows could be used to stabilize TE in soils with high Cu and Pb and low Zn and Cd concentrations. Phytostabilisation is however only half of the story. Monitoring of TE fluxes in such areas is essential to determine potential exposure pathways by which the TE may affect humans or ecosystems. Using trees as monitors has the advantage of indicating the concentrations of bioavailable metals over a large volume of soil occupied by the trees root zone. The simultaneous use of trees for phytostabilisation and biomonitoring would be ideal from a management perspective. Independent of the substrates the elemental allocation followed a tree and element specific mathematical function. With the applied mathematical method we were able to determine the total elemental concentration of Zn, Pb, Cd, Fe and Mn from just three leaves of specific positions with an error <20% for 87% of the trees, compared to the XRF measurement of the total foliage.

Keywords: trace elements, monitoring, phytomanagement, pot experiment, soil

Transient Uptake of Chlorinated Solvents by Trees

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Trees' ability to sustainably pump, store and remove contaminants from the subsurface make these plants an attractive supplement to traditional methods for long-term monitoring of chlorinated ethene plumes, a practice termed "phytomonitoring". While phytoscreening has established and qualified links between groundwater and tree contamination, seasonal fluctuations in tree concentrations have been observed, but not explained.

This project developed tools for measuring contaminant concentrations in trees over several years. *In planta* solid-phase microextraction (SPME) coupled with gas chromatography (GC) measured concentrations in four trees at a contaminated site in Rolla, Missouri. A low-profile sampling port was designed to create sealed headspace within the tree. A single measurement could be made in less than twenty minutes, with ten minutes of that time required for transportation to and from the GC. All concentrations were corrected for temperature-dependent partitioning differences. Another method of measuring seasonal variations used solid phase samplers (SPS), developed for equilibrium passive sampling in trees. This method allowed storage of the samples for later analysis by HS-SPME-GC.

The sampling port has allowed more than 70 samples per tree over a two-year period. Concentrations in the tree followed a clear seasonal trend, with highest concentrations observed during the summer months, when translocation of groundwater was highest. Peak concentrations measured in the summer were approximately one order of magnitude higher than minimum concentrations measured in the winter and were correlated with estimates of evapotranspiration. Once seasonal variations are modeled, seasonal tree concentration corrections can be made, making trees a useful long-term monitoring tool.

Keywords: Phytomonitoring, SPME, Chlorinated ethenes, *in planta* sampling

Pollution Investigation by Trees -PIT-

Chris Balouet

Environment International

PIT is an international research programme funded by Ademe. to test phytoscreening and dendrochemical methods on different contaminants: HVOCs, Fossil fuels, PAH, PCBs and dioxins, metals.

27 members from 7 nations have joined PIT, representing a team of highest referenced scientists and organizations, also complementary in their experience and practice.

The program will work on 21 different sites, and allow the analyses of organics for over 500 samples and over 50 cores, by EDXRF, resulting in an estimated total by of 3 millions analytical data.

Analytical methods have been chosen for best detection limits, capacity to discriminate pollutants, repeatability: GC/ECD and GC/FID, GC GC MS (all 3 coupled with SPME), ICP/MS and CVAFS.

Started in 2010, program is halfway, and will end later in 2013.

We will present some of the already acquired data from specific pollutant families (VOCs, semi and non volatiles organics, metals), describing sample preparation, analytical methods and results, some identified limitations and case studies, for both phytoscreening and dendrochemical perspectives.

Keywords: phytoscreening, dendrochemistry, polluted sites

Volatilization of trichloroethylene from trees and soil: measurement and scaling approaches

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The project focus was to develop a simple, low-cost approach for measuring the volatilization of TCE from leaves, truck and soil and to use the measurements to estimate TCE removal from phytoremediation sites at Travis and Fairchild Air Force Bases (AFBs). Tree cores, analyzed by headspace GC/MS, were used to determine the relative TCE concentrations within the plume and estimate the TCE within each tree. Volatilization from leaves was measured by enclosing several leaves inside a glass chamber continuously purged with air to maintain normal transpiration and temperature. Air exiting the chamber was sampled and analyzed for TCE by thermal desorption GC/MS. Humidity probes were used to determine transpiration. Volatilization of TCE from tree trunk and soil surfaces was measured by enclosing a known area within a small stainless steel chamber. Fans mixed the air in the chamber that was recirculated through sorbent tubes to remove TCE. After a measured time, the sorbent tubes were analyzed for TCE. A Thiessen polygon approach was used to estimate the removal of TCE at Travis (839 g/yr) and at Fairchild AFBs sites (18 g/yr) with the majority associated with leaf and soil volatilization. It was also observed that volatilization of TCE from the soil surface was greater inside the planted areas than outside. From estimates of TCE in the groundwater below the sites at Fairchild and Travis AFBs, volatilization removed 5 and 50%, respectively. The lower removal at Fairchild was expected since groundwater concentrations were lower and the growing season shorter.

Keywords: Phytoremediation, groundwater contamination, chlorinated VOCs

Phyto test cells designed to mimic mature rhizosphere effect for regulatory permitting and construction

Licht Louis

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A standard test cell procedure will assist regulatory agencies accept phyto process reactions as an alternative to more conventional mechanical/biological water treatment technology.

The prototypes are a 30 cm x 30 cm x 80 cm well in a box made from water-tight foam is filled with a growing medium containing six populous spp. or Salix spp. trees and irrigated. The PTC can be drained by gravity or pumped to control vadose zone redox conditions.

Typical local soil placed in the PTC is irrigated with effluent to control the dwell time and measure effluent contaminant removal rates. Or contaminated soil is the growing medium blended with available amendments. In all cases, the root expansion allows intimate contact with contaminants either in the water or soil.

Phyto test cells are being used as a standard device that allows statistical evaluation of phyto variables. At three locations, PTC allow treatment potential comparing fixed features like field soil, waste water, and climate while manipulating alternate amendments, plant species, dosing rates and water dwell time.

At another lagoon site, polychlorinated biphenyl's (PCB) in sludge are being phyto treated to determine removal and transformation in this safe and representative way.

The ability to efficiently achieve a valid permit for field-scale phyto treatment is essential for expansion of this technology. This paper will define the technique, evaluation statistics and potential uses based on two years of development.

Keywords: Phyto test cells, standard technique, permit, regulation

METALS: TOXICITY AND UPTAKE (III)

Mechanisms of chromium tolerance, uptake and accumulation in land plants

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Phyto-extraction is a sustainable technology for environmental rehabilitation which uses plants to transfer metal from the soil to the canopy for subsequent treatment. However, the molecular mechanisms involved in metal uptake, translocation, and accumulation are still not completely clear. In this respect, compared to other metals, only few studies have been done regarding chromium behavior despite its extensive industrial use and ensuing environmental abundance. Our research employs complementary strategies to better understand the mechanism underlying uptake, assimilation and accumulation of chromium in plants. These encompass: 1) Screening of potential naturally-selected chromium tolerant and hyperaccumulator plants; 2) Screening of mutagenized *Arabidopsis thaliana* libraries towards identification of genes involved in chromium homeostasis /tolerance; To date, we identified 4 putative mutants with altered response to chromium ions; 3) Assessment of the possible roles of specific transporters in chromium uptake and accumulation, using molecular – genetic approaches. Results from the analysis of transporter mutants will be presented.

Keywords: Phytoextraction, Chromium, Membrane transporters, Heavy metal tolerance, Mutants

Development of practical rice cultivars for Cd-phytoextraction use (2) Characteristics of developing rice lines with high-Cd allele

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Phytoextraction by high-cadmium (Cd)-accumulating rice (*Oryza sativa* L.) cultivars has been proposed as an attractive technique for cleaning up Cd-contaminated paddy field soil. To use rice plant practically for Cd phytoextraction, it is necessary to have several good characters, such as non-shattering, lodging resistance, and high-biomass. We found two high-Cd-accumulating rice varieties 'Jarjan' and 'Anjana Dhan' from the world rice collection (National Insatiate of Agro Biological Sciences Genebank, Japan) (Uraguchi *et al*. 2009). Here we present three studies about developing new type of rice useful for Cd-phytoextraction.

1. Changing of shattering by mutation breeding using gamma-ray
   - We found the non-shattering mutants from both 'Jarjan' and 'Anjana Dhan'.
2. Genetic analysis for grain and straw Cd accumulation
   - QTL (quantitative trait loci) analysis using a backcross inbred lines (Jarjan/Koshihikari//Koshihikari) revealed that a major QTL (*qCdp7*; QTL potentially useful for Cd phytoextraction on chromosome 7) contributed to Cd concentrations in both grains and straw of rice plants.
   - We estimated the phytoextraction-promotion ability of the Jarjan *qCdp7* allele by measuring both the reduction in available Cd in the soil and the decrease in Cd level in rice plants subsequently grown in phyto-extracted soil. The results showed that use of Jarjan *qCdp7* allele was effective at phytoextracting Cd from the soil (Abe *et al*. 2011).
3. Marker-assisted breeding of line with *qCdp7*
   - The Jarjan *qCdp7* allele was introduced into a 'Tachisugata' cultivar resistance to shattering and lodging and producing large biomass through marker-assisted selection. New type rice varieties for Cd-phytoextraction should be produced in near future.

Keywords: Rice, Cadmium, phytoextraction, QTL, Breeding

References:
S. Uraguchi, S. Mori, M. Kuramata *et al*. (2009). Journal for Experimental Botanics, 60: 2677–2688.
T. Abe, F. Taguchi-Shiobara, Y. Kojima *et al*. (2011). Breeding Science, 61: 43-51.

Effect of different P supply on metal and nutrient uptake by co-cropped Ni-hyperaccumulator with *Lupinus albus* grown on two different soils

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A pot experiment was conducted in a growth chamber to co-crop Ni-hyperaccumulator *Alyssum murale* and *Noccaea caerulescens* with *Lupinus albus* in two surface soils (soil1 is Magnesic Eutric Cambisol and soil 2 is Hypermagnesic Hypereutric Cambisol) from a serpentinised harzburgite outcrop in Vosges Mountains (France). Each plant was either co-cropped or mono-cropped. Phosphorus deficiency and P sufficiency were applied to each soil and plant combination. The pots were separated along the diagonal by a 2-layer nylon mesh with a 4-mm distance, the pot space between the two meshes was filled with same soil as in both sides.Both *L.albus* and Ni-hyperaccumulators grew well in the two soils.The results indicated that P supply and co-cropping did various impact on metal and nutrient element uptake by plants.Co-cropped with *L.albus* decreased remarkably Ni uptake by *A. murale* in the two soils especially with P sufficiency and co-cropped with *A.murale* improved significantly Ni uptake by *N. caerulescens* with P sufficiency, on the other hand, co-cropping or P deficiency increased obviously more Mn uptake by *L.albus* in the two soils than corresponding mono-cropping or that with P sufficiency. *A. murale* planted in the soil 2 accumulated far much (5-10 times) more Ni than those planted in the soil1, Ni concentration of *L.albus* in two soils were similar. P dificiency significantly improved Mg uptake by mono-cropping *A. murale*, but P dificiency had no significant impact on Mg concentration of *A. murale* co-cropped with *L.albus.* P dificiency significantly improved Ca uptake by mono-cropping or co-cropping *L.albus.*

Keywords: *Lupinus albus,* Ni-hyperaccumulator, Co-cropping, P deficiency, uptake

Construction and effective expression of the synthetic *merBps* gene in tobacco

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Organomercury, most toxic and biomagnifiable state of mercury in environment, is contributed through anthropogenic and biogenic events. Till date no natural means to detoxify organomercurials could be identified in any eukaryote. Transgenic technology enabled introduction of the *merB* gene encoding organomercury lyase from bacteria into plant species. As optimal expression of genes in living systems rely on codon usage, synthetic *merBps* gene with 143 out of 213 codons distinct from native *merB* gene was constructed in accordance with codon usage in tobacco. The *merBps* gene could be successfully integrated in tobacco through *Agrobacterium* mediated transformation. Although, over 100 independent putative *merBps* transgenic lines could be selected, only ~45% shoots selected using 200 mg/l kanamycin were PCR positive for the *npt*II and *merBps* genes. Based on vigour, few PCR positive transgenic lines were multiplied, rooted, transferred to pots, acclimatized and allowed to flower and fruit. Leaves from three month old *merBps* transgenic plants were used for molecular analysis. Southern analysis indicated presence of single copy of the *merBps* gene in three lines. RT-PCR and Western analysis confirmed successful transcription and translation of the *merBps* gene, respectively. Leaf discs of wild type turned white due to significant decrease in the level of chlorophylls and carotenoids in presence of PMA, but those of transgenic lines remained green, demonstrating that the organomercury lyase encoded by the *merBps* gene is fully functional. These results convincingly demonstrated that the synthetic *merBps* gene can be effectively expressed in plants and exploited for remediation of organomercurial contaminated sites.

Keywords: Organomercurials, synthetic *merB*, Organo mercury lyase, codon usage, tobacco

Ligand Enhanced Heavy Metal Uptake by Fast Growing Trees under Controlled Deficit Irrigation – Results of Batch and Lysimeter Studies

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Phytoremediation of metal polluted soils employs organic ligands to solubilize and enhance plant metal uptake and translocation. The efficient yet recalcitrant EDTA is phasing out in favor of biodegradable TPAs. We tested EDDS, citrate and EDTA efficacy in solubilizing and/or translocating metals into *Eucalyptus camaldulensis* canopy using batch, hydroponic, lysimeter and >50-m^3 constructed basins. The lysimeter and batch used metal polluted biosolids and the basins were packed with Cd-polluted, dredged saline river sediment. Controlled Deficit Irrigation (CDI) of the soil systems was maintained to arrest pollutant migration; the lysimeters used tap or RO water augmented with the 2 mM of either of the ligands + 5 mM ammonium sulfate, and the basins used secondary effluent irrigation. While EDTA and EDDS (and citrate) enhanced metal solubility in batch trials, both strongly retarded Cd plant uptake in the soilless setups. However, in the lysimeter soil system EDDS (and citrate) had no effect what so ever on metal solubility or plant uptake. Yet, EDTA highly increased metal concentrations in the soil solution as well as in *Eucalyptus* leaves. Thus Pb and Cd concentrations were 60 and 200 mg L^{-1} and 10 and 80 mg kg^{-1}, all respectively. Concurrently, the trees tolerated the CDI evolved soil solution salinization at up to 35 dS m^{-1}. The study suggests that under less humid climates, site stabilization and remediation can be achieved with the combination of low dose EDTA augmented CDI of adapted *Eucalyptus*es. RO water widens the scope of the CDI.

Keywords: EDDS, EDTA, Eucalyptus, lysimeter, phytoremediation, sediment

Soil metatranscriptomics for mining eukaryotic heavy metal resistance genes

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Ecosystem contamination by heavy metals is a widespread concern resulting from human industrial and agricultural practices. These contaminations represent a health hazard and result in vast land areas considered unsuitable for agriculture and other activities. Cellular and molecular aspects of heavy metal toxicity, homeostasis, tolerance and resistance have been investigated in a number of metal sensitive model eukaryotic organisms. A bottleneck in the elucidation of the diversity of the cellular responses to heavy metal damages and in the identification of additional resistance genes could be the limited number of biological species investigated so far in this specific context.

We performed a multigenome search for resistance genes by screening soil eukaryotic metatranscriptomes from metal-polluted sites. cDNA libraries made from soil-extracted polyadenylated mRNA were transferred in different cadmium or zinc-sensitive yeast mutants and genes which restored a tolerance phenotype were characterized. This strategy indeed allowed the identification of several gene families, which had not been previously studied in a context of heavy metal resistance. This is the case of a protein family that interfere with iron homeostasis and a novel family of cystein-rich proteins, from unknown, but widespread, organisms. These results highlight the power of functional environmental transcriptomics to explore basic biological processes in eukaryotes.

Keywords: metatranscriptomics, heavy metals, polluted soils, resistance genes

Nitrate nutrition status modulates stress responses of alfalfa plants to mercury

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Mercury (Hg) is one of the most dangerous pollutant heavy metals to the environment. Its accumulation in plants causes several negative effects, among them the induction of oxidative stress. Nitrogen (N) is a fundamental macronutrient in plants, limiting cellular metabolism and overall performance under environmental stress (Vanacker *et al*., 2006). Nitrogen is fundamentally assimilated as NO_3^- after its reduction to NO_2^- by the enzyme nitrate reductase (NR), key step prior the formation of NH_4^+ by nitrite reductase. We studied the physiological effects of Hg (0, 6 and 30 µM) in alfalfa plants grown with low NO_3^- (2 mM; LN) and high (12 mM; HN) concentrations. Several parameters of oxidative stress such as lipid peroxidation, chlorophyll content, biothiol concentration and ascorbate peroxidise (APX), glutathione reductase (GR) enzymatic activities were analysed. Total N accumulation (Kjeldahl) and NR activity were studied also as N-nutrition related indexes. In addition, the expression of APX, GR and NR was examined by Western-blot immunodetection. Plants grown with HN augmented their biomass, and had reduced stress symptoms. For example, GR activity is a very sensitive index of Hg toxicity in plants (Sobrino-Plata *et al*. 2007); and it was clearly less inhibited in HN plants than in LN ones exposed to 6 µM Hg. Therefore, our results showed that plants cultivated in HN were less affected by Hg, highlighting the importance of an appropriate management of the N nutritional status to improve tolerance to heavy metal stress.

Keywords: alfalfa*, Medicago sativa*, mercury, nitrate nutrition, oxidative stress

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Cadmium-Induced Oxidative Stress and Mitochondria: A Kinetic Study in *Arabidopsis thaliana*

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Next to halting further spread of toxic metals into the environment, it is critical to stimulate the development of applied phytoextraction technologies and/or selection of crops with enhanced yield under suboptimal conditions such as cadmium exposure. Cadmium (Cd), a non-essential element, induces oxidative stress-related damage versus acclimation in *Arabidopsis thaliana*. As key players in redox homeostasis and signaling at the crossroads of life versus death, mitochondria could be involved in Cd-induced responses via their enzymatic antioxidative defense system. Also, the mitochondrial alternative oxidase (AOX) is commonly regarded as target and regulator of plant stress responses. Due to its ability to reduce mitochondrial ROS production, we hypothesize that AOX and related processes in the alternative respiratory chain are critical in plant responses to Cd-induced oxidative stress. Transcript levels (qRT-PCR) of genes encoding mitochondrial antioxidative and respiratory enzymes were determined in *Arabidopsis* exposed to environmentally realistic Cd concentrations (5 and 10 μM) in a kinetic setup (0, 2, 24, 48, 72 h). While only slight changes were observed for the antioxidative enzymes, our analysis revealed that roots and shoots both invest in the alternative respiratory chain at transcriptional and protein levels. This supports a role for alternative respiration in bypassing Cd-induced oxidative damage in *Arabidopsis*, which could be important in selecting plants with enhanced yield on Cd-polluted soils.

Keywords: cadmium, oxidative stress, mitochondria, signalling

Diversity of lead hyperaccumulation in *Hirschfeldia incana*: Perspectives for phytoremediation

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Since the early 20th century, Morocco is known for its mining activities. This conducted to several mine sites that are sources of pollution by heavy metals. An exploration of plant species growing on abandoned lead mining sites in the east part of Morocco was considered in the context of phytoremediation program. Among the various plants identified, we selected *Hirshfeldia incana*, a lead hyperaccumulator and a member of the Brassicaceae family, to study mechanisms of Pb tolerance.

Physiological study of *H. incana* in controlled conditions showed a significant difference in lead tolerance and accumulation between a metallicolous population (M) from polluted sites (Oued el Heimer) and non metallicolous population (NM) from unpolluted sites (Tetouan). Results show that NM population is less tolerant but accumulates more lead than the M population.

The close genetic proximity between *H. incana* and *A. thaliana* (89%) allows us to conduct an heterologous hybridization of *H. incana* transcripts on *A. thaliana* microarrays and several genes appear as regulated by lead. Among this list of genes, five genes known to be involved in the molecular responses to heavy metals (metallothionein MT2, Multidrug Resistance-associated Protein MRP14, cooper chaperone CCH, ABC transporters WBC23 and general control non-repressible2 GCN2) were chosen for genetic comparison between M and NM populations of *H. incana*. Sequences analysis didn't show intrapopulation variation whereas the interpopulation variation is significant for the five genes. Gene expression analysis is under investigation to check whether this variability can be associated with the presence of lead in polluted sites.

Keywords: *Hirschfeldia incana*, lead, hyperaccumulation, phytoremediation

Relationships between chemical and biological factors in trace element-contaminated soils managed using Gentle Remediation Options (GRO)

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During the past decades a number of field trials with GRO have been conducted throughout Europe. Each research group adopted its own strategies selecting methods to assess the remediation success. This study aims at selecting methods that describe the bioavailable/bioaccessible trace element (TE) fractions among European case studies (of the EU-FP7 project GREENLAND, No. 266124) and are applicable as indicators for GRO success and sustainability. Two sets of tests were pre-selected based on available literature and previous experience, one for determining TE bioavailability/bioaccessibility in untreated soils and soils treated using GROs (e.g. phytoextraction, phytostabilization) and another for characterizing soil functionality and ecotoxicity, as well as soil microbiological parameters. Soil samples from several case studies representing one of the main GROs (phytoextraction in Belgium, Sweden, Germany, Switzerland and France, and aided phytostabilization in Poland, France and Austria) were collected and analyzed applying the selected tests. Quantifying the bioaccessible TE fractions, the strength of the extractants and extracted Cd, Pb and Zn concentrations were generally in decreasing order: water<$NaNO_3$<NH_4NO_3<EDTA<*aqua regia*. An exception was a very acidic soil where no differences between the water, $NaNO_3$, NH_4NO_3 and EDTA extractions were observed for Cd and Zn. Putting together the preliminary results of the chemical (extractable TE) with the biological parameters (plant growth, worm avoidance, growth and reproduction of nematodes) showed that the best correlation could be observed between worm avoidance and water extractable Cd, Pb and Zn and ammonium nitrate extractable Cd and Pb. Further analyses are pending.

Keywords: bioaccessible trace elements, plant growth, soil extractions, risk assessment, worm avoidance

METALS:
SEARCH FOR NEW POTENTIAL PHYTOEXTRACTORS (I)

Botanical exploration for hyperaccumulating plants in a gold mine

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Phu Thap Fa is a gold mine in northeast Thailand. Along with gold, the company has also extracted copper and iron for exporting. Botanical exploration was conducted at the mine tailing dam and the excavated waste rocks site to address the following points: 1) discover plant species with naturally accumulate high concentration of metals and 2) screen out hyperaccumulator plants which are highly tolerant, having large biomass and can translocate metals from roots to shoots at high rates. The whole plants and the soil at the rhizosphere were sampled and digested with HNO3 and HClO4 before analyzing for the interested metals, i.e., chromium (Cr), cadmium (Cd), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn) and nickel (Ni) with the ICP-OES. The plant samples could accumulate high concentration of 3 metals, i.e. Cu, Fe and Mn. We discovered that 2 plant species in the family Convolvulaceae and Asteraceae were Cu hyperaccumulators according to the criterion set up by Baker et al. (1994) and Brown et al. (1995). These 2 Cu-hyperaccumulating plants have been used as medicinal plants by the local population.

Keywords: hyperaccumulator, copper, manganese, Convolvulaceae, Asteraceae

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Gentle remediation at formerly "Pertusola Sud" zinc smelter: evaluation of native species for phytoremediation purposes

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The master plan for the soil clean-up of the formerly zinc smelter "Pertusola Sud" (Crotone, Italy) considered gentle remediation options for a specific area where in the past were disposed both by-products and industrial wastes. Although the soil is severely contaminated by metals (Cd, Cu, Ge, Hg, In, Pb, Tl and Zn) and metalloids (As and Sb), in this area several plant species grow spontaneously. Plants and rhizosphere soil samples were collected and analyzed for trace element concentrations. The potential of phytoremediation of plants was evaluated considering the concentration of metals and metalloids in the plant tissues and also the bioconcentration factor (BF) and the translocation factor (TF).

The plant requirements for Sb phytoextraction were verified for *Phragmites communis* (1.66 BF, TF 9:02), while *Eucalyptus camaldulensis* (BF 1.11, TF 1.71) and *Galactites tomentosa* (BF 2.30, TF 1.37) were considered for Tl. Potential phytostabilizers species, with high BF and low TF values were: *Acacia saligna* for Sb (BF 1.49, TF 0.46), *Phragmites communis* (BF 1.85, TF 0.55) and *Silene bellidifolia* (BF 1.20, TF 0.71) for Tl. The performances of *Dittrichia viscosa* were assessed, as well. Although less effective than the previous species, the pioneer species *Piptatherum miliaceum* and *Holoschoenus australis* could be useful to restore a green coverage on multi-element polluted soil.

Keywords: Zinc smelter, Gentle remediation, Native species, Bioconcentration factor, Translocation factor

Phytoremediation of lead (Pb) And arsenic (As) by *Melastoma malabathricum L*. from contaminated soil

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This study was conducted to investigate the uptake of lead (Pb) and arsenic (As) from contaminated soil using *Melastoma malabathricum L*. species. The cultivated plants of *Melastoma malabathricum L*. were exposed to As and Pb separately for an observation period of 70 days. From the analysis results, *M. malabathricum* accumulated relatively high range of As concentration in its roots with 6-22000 mg/kg, in stem with 38-580 mg/kg and in leaves with 7–900 mg/kg on a dry plant basis. While arsenic concentrations in the soil were between 2 and 40 mg/kg. The highest concentration of Pb (13,832 mg Pb/kg dry plant) was accumulated in the roots of plants exposed to a concentration of 200 mg/kg Pb in the soil. The accumulation of Pb in stems and leaves differed insignificantly, with a maximum accumulation in stems of 2,600 mg Pb/kg dry plant while maximum accumulation in leaves was 1,100 mg Pb/kg dry plant. Only small amounts of Pb were translocated from roots to overground plant parts (TF<1) for Pb treatment. However, the wider range of TF values (0.02-10) for the plant in As treatment prove that the translocation of As from root to overground part were more effective.

Keywords: phytoremediation, *Melastoma malabathricum L*., lead, arsenic, translocation factor, bioaccumulation factor

Plants growing on mine tailings avoid dispersion and contribute to stabilization of potentially toxic elements

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The aim of this work was to document the potential role of different plants in the remediation of mine tailings containing high concentrations of potentially toxic elements (PTE's). Plants growing on two mine tailings in Zimapan, Mexico were collected and identified. Three possible remediation pathways were addressed: stabilization into roots and rhizosphere, accumulation in aerial plant tissues, and external leaf-deposition of particles containing PTE´s. It has been suggested that plant surfaces represent one of the major pollutant sinks in terrestrial environments, and then comparison between PTE´s-accumulation vs. deposition in plant tissues was quantified in washed and non-washed samples. Maximum concentrations in washed and non-washed samples were as follow: Zn 1419 vs. 2231, for Cd 20 vs. 21, for Pb 195 vs. 419, for Mn 206 vs. 338, for Cu 274 vs. 544, for Ni 44 vs. 52 and for Co 47 vs. 60 mg kg^{-1} dw, respectively, being Zn, Pb and Cu the PTE´s preferentially deposited in leaves. Surface of some plants favored PTE´s deposition: unwashed samples of *Sclerocarpus uniserialis* accumulated 24 times more Mn (56 mg kg^{-1}), 14 times more Zn (2230 mg kg^{-1}), 6 times more of Cu (216 mg kg^{-1}) and 5.7 times more Cd (20 mg kg^{-1}) than washed samples. *Cuphea lanceolata* presented 4.8 times more of Pb (352 mg kg^{-1}). EDAX analysis demonstrated the presence of PTE´s in particles deposited on leaf surfaces, suggesting the role of plants as an outstanding physical barrier against PTE´s dispersion. Plants present several ways to contribute to PTE´s stabilization.

Keywords: particles deposition, metal tolerant plants

METALS: SEARCH FOR NEW POTENTIAL PHYTOEXTRACTORS (II)

Is hybrid larch (*Larix* x *eurolepis* Henry) a good candidate for cadmium phytoaccumulation?

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Tree species are considered as new tools for phytoaccumulation because of their high biomass and deep root system. Gymnosperms are not widely used despite they can be as efficient as Angiosperms (Astier *et al*. 2010). Therefore, we investigated the capacity of a hybrid larch to accumulate cadmium (Cd), because of its fast growth rate and its deciduous needles, what is important to regularly remove Cd.

One-month-old *in vitro* grown plantlets obtained by somatic embryogenesis were exposed for one to four weeks to different Cd concentrations. After one week on 1.5 mM Cd, biomass was similar to control plantlets and shoot Cd concentration was higher than 100 mg. kg^{-1} dry weight, the minimum shoot concentration to qualify a hyperaccumulator. However, a slight decrease of photosynthetic pigment content was registered and the phytochelatin synthesis was not strongly stimulated, suggesting that a longer time of exposure could be toxic. Indeed, growth stopped after two weeks of treatment. Larch plantlets were significantly altered after four weeks on 0.5 mM Cd but they were still healthy on 0.25 mM. Plantlets tolerate this latter Cd concentration for a longer time and store high Cd amount in both shoots and roots. Moreover, our results showed that phytochelatin synthesis pathway would not be the only mechanism involved in Cd tolerance.

Field experiments are necessary to confirm the efficiency of hybrid larch to accumulate Cd. However, *in vitro* culture is an interesting first step to test potential candidates as reported by Capuana (2011).

Keywords: Cadmium, *Larix*, phytochelatin, phytoremediation, somatic embryogenesis

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Astier C, Chaleix V and Faugeron C (2010). Grafting of animated oligogalacturonans onto Douglas fir barks. A new route for the enhancement of their lead (II) binding capacities. Journal of Hazardous Materials, 182: 279-285.

Capuana M (2011). Heavy metals and woody plants - biotechnologies for phytoremediation. iForest, 4: 7-15.

Cadmium and Uranium Phytoextraction potentials of young Douglas fir trees

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Phytoextraction consists in using plants to clean-up contaminated soils. Hyperaccumulator species are able to uptake and accumulate high levels of pollutants, such as heavy metals, but their disadvantage is their relative slow growth and small biomass production. Trees could be good candidates for phytoextraction since they produce large quantities of biomass, and, even they accumulate lower concentrations of metal, their extraction potential could be interesting. Among all tree species, Douglas fir has shown high heavy metal retention capacities, especially barks which can be used as efficient biosorbents (Martin-Dupont *et al.,*2002). Phytoextraction potentials of young Douglas trees were determined for two heavy metals: cadmium and uranium. During two independent studies, Douglas trees were exposed to Cd-contaminated soil (from 0 to 68 ppm) for 9 months or to U-containing soil (from 0 to 2200 ppm) for 4 months. Both Cd and U treatments did not show any significant effect on plant growth during this period, after which metal contents of trees reached 10 $\mu g.g^{-1}$DW for Cd and nearly 200 $\mu g.g^{-1}$DW for U. Transfer to aerial parts led to a strong accumulation into barks which contained the highest concentration of pollutants: about 6 $\mu g.g^{-1}$DW (Cd) and 4.6 $\mu g.g^{-1}$DW (U) for the highest soil content. Furthermore, influence of Cd on cell wall components of barks has been characterized. Quantitative and qualitative modifications in pectin contents were observed, so heavy metal seemed to regulate the biosynthesis and structural modifications of cell wall components.

Keywords: Phytoextraction, cadmium, uranium, Douglas tree, cell wall.

Reference:

Martin-Dupont F, Gloaguen V, Granet R, Guilloton M, Morvan H and Krausz (2002). Heavy metal adsorption by crude coniferous barks: a modeling study, Journal of Environmental Science and Health Part A 37(6): 1063-1073.

The Effects of cadmium on growth and accumulation of *Ipomoea aquatica* Forsk.(Water spinach) and *Brassica campestris* var. chinensis (Pak choy)

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Water spinach (*Ipomoea aquatica* Forsk.) and Pak choy *(Brassica campestris* var. chinensis.*)* are commonly grown vegetables for consumption by local people living in the cadmium (Cd) contaminated area in Mae Sot District, Tak Province, Thailand. The Cd concentration tested at 0.1, 0.3, 0.5, 5 mg L^{-1} exerted no effects on % seed germination of both species. However, Cd at ≥ 0.1mg L^{-1} significantly decreased root elongation of seedlings of both species. The shoot length was significantly decreased at 5 mg L^{-1} Cd at 7 d growth for water spinach and at 14 d growth for pak choy. The Cd at 0.1 mg L^{-1} did not affect dry weight of water spinach at 7 d growth but it was decreased at 14 d growth. At 0.1 mg Cd L^{-1}, dry weight of both 7 and 14 d seedlings of pak choy was decreased. The root of both plant species accumulated more Cd than the shoots in all treatments. At 14 d in the hydroponic experiment, Cd accumulated in roots and shoots of water spinach was 167.21 ± 9.09 and 60.41 ± 8.50 mg kg^{-1}, respectively, and 86.70 ± 1.57 and 504.99 ± 3.75 mg kg^{-1} for pak choy. In this study, Cd in the shoots of pak choy at 5 mg Cd L^{-1} was five times higher than the criterion set for Cd hyperaccumulator (Baker and Brook, 1989; Lui et al., 2007). At 14 d, the translocation factor of pak choy was the highest at 5.82, but was only 0.36 for water spinach.

Keywords: *Ipomoea aquatica* Forsk., *Brassica campestris* var. chinensis., cadmium, accumulation, growth

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Baker AJM and Brooks RR (1989). Terrestrial higher plants which hyperaccumulate metal elements: A review of their distribution, ecology, and phytochemistry. Biorecovery, 1: 81-126.

Wang KS, Huang LC, Lee HS, Chen PY and Chang SH (2008). Phytoextraction of cadmium by Ipomoea aquatica (water spinach) in hydroponic solution: effects of cadmium speciation. Chemosphere, 72, 666–672.

Phytoremediation of a dumpsite at Moniya, Ibadan, in Nigeria using *Chromolaena odorata* and *Portulaca oleraceae* L.

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Heavy metal contaminants in urban dumpsites constitute a major environmental challenge, causing soil and water pollution. Remediation using the appropriate plant species with hyperaccumulation potentials is affordable, ecologically acceptable and environmentally friendly. Therefore, the hyperaccumulation potentials of *Chromolaena odorata* and *Portulaca oleracea* in a heavy metal-contaminated dumpsite at Orisunmibare village, Moniya, Ibadan, Nigeria were investigated.

Fifteen plant species were identified at the dumpsite, the two most common being C. *odorata* and *P. oleracea* with RIV of 31.0% and 20.2% respectively. *Ananas comosus* (L) Merr. had the least RIV (1.2%). Mean concentrations (mg/kg) of Pb, Cd, Al, Zn, Fe, Cu, As and Cr were 0.1±0.01, 0.0±0.01, 0.1±0.01, 0.1±0.01, 2.6±0.01, 1.0±0.01, 0.0±0.01 and 0.0±0.01 respectively for the control soil and 80.31±1.321, 57.87±1.32, 63.8±0.08, 54.2±0.013, 56.47±0.10, 40.3±0.06, 37.5±0.01 and 40.0±0.05 for the polluted soils. Mean concentrations of these heavy metals in *C. odorata* grown on control soils were 2.2±0.14, 10.9±0.26, 8.03±0.70, 5.0±0.16, 4.2±0.28, 5.0±0.15, 10.9±0.27 and 1.8±0.18 respectively, while on polluted soil, their concentrations were 59.2±0.95, 51.8±0.6, 56.0±0.81, 47.7±1.19, 53.7±1.92, 30.7±0.5, 31.5±1.05, 37.2±0.9 respectively. For *P. oleracea,* mean concentrations (mg/kg) of Pb, Cd, AI, Zn, Fe, Cu, As and Cr from control soils were 9.6±0.39, 9.7±0.51, 6.7±0.6, 4.1±0.19, 5.2±1.54, 4.0±0.18, 2.6±0.56 and 6.2±0.42 respectively, while on polluted soils, these were 53.9±1.05, 41.7±0.59, 46.8±0.67, 47.0±1.17, 46.3±0.85, 36.9±0.84, 28.6±0.57 and 34.8±0.89 respectively. Concentrations of heavy metals in the polluted soil at the end of the experiment were significantly reduced by (20-30)%.

Keywords: Heavy metals polluted soil, hyper-accumulators, *Chromolaena odorata*, *Portulaca oleracea*

WETLANDS

Constructed Treatment Wetlands: Industrial Case Studies

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A common legacy of large former petroleum refineries and waste oil recycling facilities is the need for long term treatment of impacted water resources. Furthermore, the presence of petroleum- and chlorinated-based constituents in groundwater systems oftentimes results in geochemical changes in the subsurface that can result in certain naturally occurring inorganics becoming mobilized. This presentation will discuss the efficacy of constructed treatment wetland systems to address common petroleum constituents such as benzene, toluene, ethylbenzene, and xylenes, common chlorinated constituents such as tri- and di-chloroethylene, as well as several inorganic constituents. Several case study examples will be cited where the treatment wetland systems have been in operation for a number of years.

Keywords: Wetlands, Petroleum, Chlorinateds, Inorganics

Potential of a plant commonly used in constructed wetlands to remove veterinary drugs from wastewater

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Intensive animal farming results in overproduction of residues and release of several compounds to the environment, namely veterinary drugs used for therapeutic and prophylactic purposes. Livestock industries effluents untreated and treated can be a significant source of these compounds to the environment since there is limited removal of pharmaceuticals within wastewater treatment plants (WWTPs). Therefore, new approaches are needed to complement the usual wastewater biological treatments. Constructed wetlands constitute an effective technology to reduce/eliminate pharmaceuticals discharge into the environment, with proven results in urban WWTPs.

In this work the potential of *Phragmites australis* was evaluated for the removal of three veterinary pharmaceuticals commonly used in Portugal, enrofloxacin (ENR), ceftiofur (CEF) and tetracycline (TET), from wastewater. Experiments were carried out in controlled laboratory conditions, being the plants exposed for a 7-days period to treated wastewater of a pig farm doped with a known concentration of each of the selected drugs individually.

Results showed that plant promoted the removal from wastewater, of 94% and 75% of ENR and TET, respectively. Microbial abundance estimation revealed that microorganisms were not a major participant in drugs removal. Occurrence of drugs adsorption to plant roots was observed in small extension. Therefore, main mechanisms occurring were drug removal by plant uptake and/or degradation. Present results showed the potential of *P. australis*-planted beds to be used for reducing/removing pharmaceuticals compounds from livestock treated effluents.

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Keywords: veterinary pharmaceuticals, wastewater treatment, constructed wetlands, *Phragmites australis*

Constructed Wetland Pilot Project for FGD Wastewater Treatment

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Constructed wetlands have the potential to remove contaminants from flue gas desulfurization (FGD) wastewater. A pilot constructed wetland has been in operation for more than one year to investigate its applicability for treatment of flue gas desulfurization wastewater. The system consists of a series of different types of wetland cells in parallel treatment trains that include floating, free water surface, vegetated submerged bed, and vertical flow bed wetlands. The 2 acre site is designed to treat about 7 percent of the FGD wastewater. Laboratory column studies have been carried out to investigate the fate of the contaminants in a controlled environment. Significant amounts of selenium have been removed in both the wetland and the column studies. The vegetation in the wetland has been growing well despite the relatively high concentrations of sodium, chloride, and sulfate in the wastewater. Experimental results from 18 months of operation will be presented.

Keywords: Flue gas desulfurization wastewater, constructed wetland, vertical flow bed, vegetated submerged bed, selenium

Evaluation of floating plant root mat for the treatment of groundwater contaminated by benzene and MTBE

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A new variant of constructed wetlands (CWs) – floating plant root mat (FPRM) has been developed, which employs emergent water plants, similar to those used in surface and subsurface flow wetlands, growing as a floating root mat on the water surface. Particularly, this system is forming a dense root mat, whereby a preferential hydraulic flow in the water zone between the root mat and the non-rooted bottom can be expected. FPRM is hybrid of soil matrix-free pond systems and conventional soil matrix based CWs containing macrophytes. Because of their specific structure, floating root mats combine benefits from ponds and CWs, and are therefore used for different types of wastewater such as mine drainage, poultry processing wastewater, piggery effluent, sewage and combined sewer overflow, storm water, eutrophicated lakes and rivers. However, the information about treating volatile organic compounds by FPRM is lacking. In this study, two pilot-scale FPRMs (established in 2008 and 2010, respectively) were investigated for the treatment of groundwater contaminated by benzene and Methyl *tert*-butyl ether (MTBE), with mean concentrations of 13 ± 3 mg L^{-1} and 2.2 ± 0.5 mg L^{-1}, respectively, during the experiment, under temperate climate (East Germany). The plant root mat was formed by common reed (*Phragmites australis*). To better understand the removal pathways, the volatilization of benzene and MTBE was measured by dynamic air chamber. The treatment performance as well as volatilization of benzene and MTBE in the two PRMFs, will be presented.

Keywords: floating plant root mat, volatilization, volatile organic compounds, groundwater

Development of a Constructed Wetland Water Treatment System for Selenium Removal: Use of Mesocosms to Evaluate Design Parameters

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Surface flow wetlands have proved to be successful in removing selenium (Se) from wastewater. Earlier studies from this laboratory have shown that constructed wetlands can remove up to 90% of the inflow Se from oil refinery wastewater, and up to 80% from agricultural irrigation drain water. However, it has proved difficult to lower the Se concentration in the outflow to < 5 µg Se/L. The principal mechanisms of Se removal by wetlands are: 1) the dissimilatory anaerobic reduction of Se oxyanions (selenate and selenite) to insoluble forms (e.g., Se^0, Se^{-2}), and 2) biological volatilization of Se to the atmosphere through assimilatory reduction and methylation by plants and microbes. The goal of the present work is to use greenhouse mesocosms to find ways of enhancing design parameters of constructed wetland water treatment systems so as to reduce outflow Se to < 2 µg Se/L. Here we present data showing the effects of various wetland design options for enhancing Se removal efficiency. These design options include variations in plant species, hydrologic parameters, addition of organic amendments and changes in plant-substrate composition and configurations with and without additional aeration. Our results show that the most rapid and complete removal of Se from the water column was obtained with mesocosms containing cattails planted in cattail litter overlaying sand-peat moss sediment: using this configuration, Se levels were reduced from 15 to 0.1 µg Se/L within 72 hours.

Keywords: Se remediation, constructed wetlands, mesocosms, volatilization, outflow Se

Removal of pathogens and organic contaminants from wastewater using constructed wetlands and UV systems

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The utilization of treated wastewater for agricultural irrigation in arid/desert regions, such as the Middle East (ME), constitutes both a major opportunity and an issue of concern. In view of water shortage and scarcity in ME countries, the advantages of such water reuse is obvious, but also brings up the issue of diseases related to water sanitation. Advanced wastewater treatment plants (WWTPs) are very effective but the high cost of constructing and maintenance as well as the need for highly trained personnel to operate renders them unpractical in rural areas. Constructed wetlands (CW) offer a partial solution to this issue as they are relatively easy and cheap to construct and maintain and are effective for removal of a wide range of pollutants. However, pathogens removal in CW/WWTP systems tends to be not adequate for meeting the current water quality standards. Thus, an additional disinfection stage is needed.

In the current project, the possibility of treating wastewater using CW with UV disinfection system was evaluated. Our data demonstrated that the combined CW-UV systems could offer a good solution for wastewater treatment in small communities. The CW systems lowered COD level down to acceptable levels (i.e. <100 mg/L). Furthermore, although CW *per se* did not offer good enough removal of bacteria, the proper use of low-cost UV systems inactivated the remaining bacteria, even with UV transmission (UVT) of ~40%., resulting in high removal levels of the fecal bacteria and the *E. coli* and the effluents were suitable for unlimited irrigation.

Keywords: Constructed wetlands, UV disinfection, COD removal, effluents

Environmental services and economic opportunities of naturally occurring willows on the Prairies

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Thousands of wetlands are present on the prairies with often willows as the dominant species. These wetlands are therefore also called willow rings. Traditionally farmers have removed the willow rings from their agricultural lands to facilitate farming activities. The removal of the willow rings potentially results in the presence of high salinity levels close to the surface, because the elimination of transpiration by the willows will result in increased shallow groundwater flow. Research indicates that the native willows in the willow rings used to play an important role in the hydrology of the prairies. The willows that are left are heavily degraded due to the lack of disturbance factors such as buffalo herds and wild fires. Replanting the willow rings or rejuvenating old willow rings through regular biomass harvests may restore the historical hydrological cycles, produce an economically sustainable biomass feedstock, and fulfill an environmental service. This study has the following objectives; 1) Assess current health of naturally occurring willow, 2) Identify species in the willow ring and their distribution, 3) Assess yield increase potential due to regular harvests, 4) Evaluate Carbon-sequestration and nutrient uptake of willow rings, 5) Assess the effect of harvesting on evapo-transpiration, ground water fluxes and salt mitigation potential. Previous studies measured up to 70% dead wood in degraded willow rings and field surveys indicated that five willow species are common to willow rings. Initial measurements indicate vigorous resprouting after harvesting and transpiration rates of two native willow species common to willow rings are very promising.

Keywords: biomass, buffer, carbon, salt, wetlands

Biological and sustainable treatment of sulphate loaded water

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Mining residues and excavated material are disposed into tailings and on heaps. They are the origin of highly polluted discharges. To avoid environmental impacts, long-term measures are necessary. Two case studies on biological and sustainable treatment of sulphate loaded water, one in Chile and one in Germany, are developed.

For a Chilean copper mine site a Constructed Wetland for treatment of sulphate and metal loaded water from an active tailing was designed. The pilot plant will be operated by the University Santiago de Chile. Treatment capacity is about 10 to 60 m³ per day. The gained monitoring results on microbial sulphate reduction and metal removal will be used to determine the optimal design and operation parameters for long term treatment.

Numerous coal mine pits in Central Germany were flooded by re-rise of groundwater level after mine closure or controlled feeding using surface water of nearby rivers. The water quality of the created lakes is influenced by interactions with sulphate containing groundwater. BioPlanta has realized a 6 months *on site* water treatment test on sulphate reduction and sulphide immobilization in a biological active filter. The average sulphate content of about 1,200 mg/l could be decreased below the target value of 300 mg/l. In the field test even a higher sulphate reduction rate could be obtained than in previous investigations in technical scale.

Keywords: sulphate, water, biological transformation, Constructed Wetlands, case studies

Organic acid content and copper uptake in two plant species used in a wetland system to treat copper mining effluents

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The effectiveness of using phytotechnologies to treat copper liquid wastes (CLW) depends at a great extent on the adequate choice of the plant species to be used. The ability to uptake copper and to accumulate and mobilize the metal in the plant tissues is relevant. This characteristic has been related to the production of Low Molecular Organic Acids (LMOAs) (Veselý *et al.*, 2011). Therefore, it is possible that the organic acid production by the plant caused by exposure to copper affects the uptake, accumulation and distribution of the metal in the plant tissues.

Polypogon australis and *Phragmites australis* are able to incorporate high concentrations of copper, under laboratory and field conditions (Ye *et al.*, 1997; Ortiz et al, 2008). We studied the accumulation of organic acids in the roots of both species treated with a copper liquid waste (CLW) and a synthetic copper solution; and we measured the amount of copper accumulated in the plant tissues. We found that both copper and CLW treatments provoked an increase in the production of malic, oxalic and acetic acids, but at different times and rates during the treatment. There was an increase in the uptake of copper, being more significant the amount of the metal found in *Phragmites australis*. A differential partition of the metal between the roots and the leaves was observed in the plants.

We suggest that a screening of organic acids production in plants suitable for phytofiltration could be made to determine the efficacy of the species to sequestrate copper.

Keywords: Phitofiltration, organic acids, copper liquid wastes

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SALINITY AND DROUGHT STRESS

Selection of Salt and Boron Tolerant Selenium Hyperaccumulator *Stanleya pinnata* Genotypes and Characterization of Se Phytoremediation from Agricultural Drainage Sediments

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Genetic variation in salt (Na_2SO_4, NaCl) and boron (B) tolerance among four ecotypes of the selenium (Se) hyperaccumulator *Stanleya pinnata* (Pursh) Britton was utilized to select tolerant genotypes capable of phytoremediating Se from salt, B, and Se-laden agricultural drainage sediment. The few individual salt/B tolerant genotypes were successfully selected from among a large population of highly salt/B sensitive seedlings. The distribution, hyperaccumulation, and volatilization of Se were then examined in selected plants capable of tolerating the high salt/B laden drainage sediment. Salt/B tolerant genotypes from each of the four ecotypes had mean Se concentrations ranging from 2510 to 1740 in leaves and 3180 to 2500 in seeds (µg Se g^{-1} DW), while average daily Se volatilization rates ranged from 722 to 1182 (µg Se m^{-2} d^{-1}). After two growing seasons (~18 months), we estimated that hyperaccumulation and volatilization of Se by tolerant *S. pinnata* genotypes and potentially by plant associated microbes can remove approximately 30% of the total soil Se in 0-30 cm of sediment. The salt/B tolerant *S. pinnata* genotypes selected and characterized herein represent promising new tools for the successful phytoremediation of Se from salt/B and Se laden agricultural drainage sediments.

Heavy metal phytoexcretion and phytoextraction using halophytic plants

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Halophytes are naturally present in harsh environments and besides salt they can also tolerate other stresses such as freezing, heat, drought and heavy metals while it has been speculated that they may also be able to accumulate metals. Furthermore, some halophytes use excretion processes through salt glands on their leaf surface as a tolerance mechanism against soil salinity. Recent findings indicate that some of these halophytes have the ability to excrete also metals through these glandular structures as a method of metal detoxification. Based on those findings the term phytoexcretion has been recently introduced to indicate a novel phytoremediation process for sites contaminated with metals bringing new potential for technological developments as the metals can be collected after their excretion (Manousaki *et al.*, 2011; Manousaki *et al.*, 2008).

In this study three halophytic plants *Limonium cornariarum, Halimione portulacoides* and *Tamarix parviflora* are investigated in order to assess their phytoextraction and phytoexcretion potential of lead and cadmium. In order to achieve that goal a six-week experiment was performed with *T. parviflora* and *H. portulacoides* plants grown on soil polluted with 20 ppm Cd and 800 ppm Pb and an eight-week experiment with *L. cornariarum* plants grown on soil polluted with 30 ppm Cd at different soil salinities. The data from these experiments suggest that all three plants are able to tolerate and accumulate the metals and moreover they were found to excrete cadmium and lead in significant amounts on the surface of their leaves confirming the phytoexcretion process and providing new opportunities for phytoextraction and phytoexcretion applications.

Keywords: phytoexcretion, phytoextraction, halophytes, cadmium, lead

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Manousaki E, Kadukova J, Papadantonakis N, Kalogerakis N *et al.* (2008) Phytoextraction and phytoexcretion of Cd by the leaves of Tamarix smyrnensis growing on contaminated non-saline and saline soils. Environmental Research, 106: 326–332.

Salinity effect on Zn uptake by *Brassica juncea*

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In spite of the large number of research papers published about salt tolerance in Brassica, there are contrasting accounts about salinity influence on the uptake of different heavy metals among plants from this genus. Increasing salinity levels affect plant growth, photosynthesis capability, and promote the occurrence of oxidative damage with production of reactive oxygen species and subsequent antioxidant defense. In this study, plants of *Brassica juncea* L. (Czern & Coss) divided through soil sets containing 0, 900 and 1800 mg Zn Kg^{-1}, were treated with solutions containing 0, 60 and 120 mMol L^{-1} of NaCl, with the purpose of observing the effect of salt in Zn uptake, and some physiological responses of this species to the 30 days experiment. Plants growing in 1800 mg Zn Kg^{-1} and treated with 60 mMol L^{-1} of NaCl exhibited higher shoot concentrations of Na^+ (Translocation Factor of 5), and Zn^{2+} (twice than those treated with 120 mMol L^{-1}). Plants located in the 900 mg Zn Kg^{-1} substrate displayed higher shoot levels of Na+ for the 120 mMol L^{-1} treatment (TF of 5), and no significant difference for Zn^{2+}, (although a TF of 4 with the 60 mMol L^{-1} solution was noticed). Production of H_2O_2 and impairment of different physiological parameters related to higher salt content treatment was observed. It was concluded that salinity could play an important role in the uptake of Zn by B. *juncea*. The potential mechanisms behind these results are discussed, as well as the implications for phytoremediation on saline soils.

Keywords: Phytoremediation, Zn, Salinity, Na, Brassica

Cadmium phytoextraction by the halophyte *Limoniastrum monopetalum*

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Preliminary experiments with the halophyte *Limoniastrum monopetalum* (L.) Boiss, have shown that it could be a Cd hyperaccumulator. The goal of this work is to assess the Cd phytoextraction potential of this plant, as halophytes are extremely tolerant plants that grow in harsh environments and can also tolerate other stresses such as chilling, freezing, heat and drought. It has also been speculated that they are heavy metal tolerant and they may be able to significantly accumulate metals. Therefore, halophytes have been suggested to be better adapted to coping with environmental stresses, including metals than salt-sensitive crop plants commonly chosen for phytoextraction studies [1,2]. For this purpose, a six-week experiment with *L. monopetalum* plants grown on soil polluted with 20 ppm Cd at different soil salinities and one year later a second eight weeks experiment with plants grown on soil polluted with 30 ppm Cd at different soil salinities were conducted. The data suggest that *L. monopetalum* is a Cd tolerant plant able to accumulate at least 100 ppm of cadmium in its shoots without showing any significant decrease in terms of biomass production, chlorophyll content or water content suggesting that it could be a Cd hyperaccumulator. These results point to a unique advantage for cadmium phytoextraction purposes. Experimental investigation is continued to examine whether the Cd uptake and translocation rates are maintained on a long-term basis.

Keywords: *Limoniastrum monopetalum*, phytoextraction, phytoexcretion, cadmium

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STAKEHOLDER INVOLVEMENT

Stakeholder engagement and "gentle" remediation options: a critical review

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Gentle remediation options (GRO) such as phytoremediation, *in-situ* immobilisation etc. have been the subject of intensive research and development over a number of years. The application of GRO as practical site solutions however is still in its relative infancy, owing to a number of barriers to their wider adoption, related to perceived uncertainties in achieving effective risk management in the long term. Effective stakeholder involvement is a key principle in the successful application of more sustainable, longer term remediation strategies, and in site regeneration more widely. For GROs this is of particular importance, to ensure that stakeholders understand and agree with how long-term site risks will be managed effectively. This engagement is also necessary so that the full wider economic and other benefits of GRO methods (in terms of financial returns from biomass valorisation or use as a feedstock, CO_2 sequestration, amenity and educational value, ecosystem services, etc.) are realised. This presentation reviews current practice in stakeholder engagement within Europe during land remediation and site regeneration activities, with specific focus on the application of GRO. We present results from the recently completed EU ERA-NET SNOWMAN project SUMATECS and the ongoing EU FP7 KBBE GREENLAND (no. 266124) project on sector perceptions of stakeholder guidance and importance when implementing GRO and other remediation options, and identify current knowledge gaps and strategies to promote more effective stakeholder engagement during GRO application. A range of GRO case studies from across Europe are examined, and comparisons drawn to strategies and practice in North America.

Keywords: Phytoremediation, Stakeholder engagement, gentle remediation, land regeneration, sustainability

Biomass for Bioenergy

Electricity generation with a novel tubular plant microbial fuel cell

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The Plant Microbial Fuel Cell (PMFC) is a technology for the generation of green electricity. The PMFC technology is based on two principles, loss of organic compounds by plant roots i.e. rhizodeposition, and electricity generation by electrochemically active bacteria in a microbial fuel cell (MFC) (Strik *et al.*, 2008). In the PMFC, the plant roots are positioned in the anode compartment of a MFC. The plant roots provide the electrochemically active bacteria with substrate via rhizodeposition and via hydrolysis of dead plant material. Nowadays the power output reaches 0.222 W m^{-2} (2 weeks average) (Helder *et al*. 2011).

The PMFC technology has the potential to avoid competition with food production whereas it can be integrated with agricultural food production (e.g. rice paddy fields), or locations unsuitable for food production such as green roofs and wetlands (Strik *et al.,* 2011). For effective PMFC installation excavation of top soil is not desired. Excavation of top soil has major drawbacks, such as ecosystem destruction, soil erosion, and high rates of fuel consumption. We developed a novel tubular PMFC using less materials as earlier. The average power output was 0.192 ± 0.04 W m^{-3} reactor, and 0.003 ± 0.0009 W m^{-3}reactor for the graphite felt, and graphite granules, respectively. Results show that anode electrode material reduction is possible while achieving comparable power outputs per square meter of membrane. These findings make future applications of the PMFC technology more feasible.

Keywords: Plant Microbial Fuel Cell, Rhizodeposition, Ultrafiltation membrane, *Glyceria maxima*, horizontal drilling

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The fate of trace elements during combustion of woody biomass collected from a phytoremediation field trial

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A poplar field trial consisting of high and very high density plots was implemented in 2007 on a trace elements contaminated agricultural site within the PHYTOPOP project. We measured biomass production and TE accumulation for the 14 cultivars grown on the field trial. The high density plots were harvested in 2010, which allowed us to investigate the fate of trace elements in poplar wood upon conversion of the biomass to heat in a 0.2 MW combustion unit, equipped with bag filter. The combustion technology used in the present experiment allows for an efficient separation of the various ash fractions. The combustion process concentrates copper, chromium and nickel in the bottom ash, heat exchanger ash and cyclone ash fractions. Therefore the impact of the bag filter is negligible for these elements. Conversely, cadmium, lead and zinc are significantly recovered in the emission fraction in the absence of bag filter, above the emission limits. The use of a bag filter will allow concentrating these three TEs in the ashes collected down the fabric filter, thus complying with all regulatory thresholds, i.e. that from the large combustion plant EU directive.

Keywords: Phytoremediation wood, poplar plots, combustion, ash fractions, emission limits

Bacterial Endophytes for Improving sustainability of Bioenergy

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Many endophytic bacteria possess plant growth promoting properties (Ryan *et al.*, 2008). We are investigating the possibility of exploiting these plant growth promotion properties for improving agricultural sustainability of bioenergy crop production (Germaine *et al.*, 2010). Our focus is on two bioenergy crops *Miscanthus* (used for biomass production) and Oilseed rape (used for biodiesel production). An experiment was designed to screen this collection of strains for growth promotion in Oilseed rape which involved inoculating seeds with mixed inocula and a number of treatments were found to have resulted in a significant increase in plant biomass. The strains from these mixed inocula were inoculated individually onto Oilseed rape to assess their potential contributions to plant growth promotion and they were characterized in more detail for phosphate solubilization, indole-3 acetic acid production, ACC deaminase activity, production of volatile compounds, biocontrol ability, resistance to heavy metals and metabolism of organic xenobiotics (Redondo-Nieto *et al.*, 2012). The data indicated that there was a correlation between the growth promotion observed through inoculation of multiple strains and the presence of various plant growth promotion traits carried by these strains. A field trial is planned this year in collaboration with Teagasc (the Irish Food and Agricultural research institute) on Oilseed Rape to evaluate survival of selected strains within the crop under field conditions and to determine their effect on plant growth and seed yield.

Keywords: Pseudomonas, endophyte, plant growth promotion, Miscanthus, Oil seed rape

References:

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Redondo-Nieto R, Barret M, Morrisey J, Germaine K, Martínez-Granero F, Barahona E, Navazo A, Sánchez-Contreras M, Moynihan J, Giddens S, Coppoolse E, Muriel C, Stiekema W, Rainey P, Dowling DN, O'Gara F, Martín M and Rivilla R (2012) Genome sequence of the biocontrol strain *Pseudomonas fluorescens* F113. Journal of Bacteriology, (in press).

Designing a multi-functional sustainable agricultural system at the farm scale using energy crops

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Pressures on agricultural systems have further increased as a result of the focus on bioenergy production to mitigate greenhouse gas emissions and meet energy security demands. The challenge is to grow these energy crops sustainably. A key assumption in most studies focusing on the sustainability of bioenergy crops is that these crops will be grown in a manner similar to traditional agricultural row-crops and hence would similarly impact the environment negatively. Here, we present an alternative strategy where a sustainable agricultural landscape at the farm-scale is designed by growing energy crops in strips adjacent to the traditional row-crops so that nutrients present in the runoff and leachate from the row-crops can be reused by the energy crops. This strategy is being implemented at a 15 acre experimental farm field in Illinois. Baseline studies conducted in 2011 showed that there is significant spatial variability in the productivity of corn within this field as well as areas where nitrate concentrations in the vadose soil water below the rooting depth of corn reached a maximum of 140 ppm. Modeling simulations in combination with baseline field data were used in this study to evaluate and design the optimal placement of energy crops in this landscape such that the nitrate present in the leachate could be used as a subsurface ferti-irrigation for the energy crops. Results indicated that a contour strip consisting of willows placed in the area of low corn yield would best provide environmental services and meet the farmer's economic needs.

Keywords: Bioenergy, agricultural sustainability, nitrate, greenhouse gas emissions

Assessment of the phytoremediation and bioenergy potential in three cadmium-contaminated bioenergy crops: oilseed rape, oat and wheat

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This study investigated the physiological response, Cd accumulation and biogas production of oilseed rape, oat and wheat grown in soil contaminated with 0, 1, 5, 10, 50 and 100 mg kg^{-1} Cd. The experimental results indicated that the growth responses to Cd stress varied with crops type, Cd concentrations in soil and crops growth periods. The aerial biomass yields of oilseed rape, oat and wheat were enhanced when Cd concentration was 5 mg kg^{-1}. The main sites that Cd accumulated in oat and wheat were roots, while the main sites of oilseed rape were leaves when Cd concentration was no more than 50 mg kg^{-1}. Furthermore, an anaerobic experiment was conducted to assess the biogas production of these Cd-polluted crops. The results showed that Cd accumulated in the crops did not significantly inhibit the biogas production of these crops when Cd level in crops was below 56 mg kg^{-1} in this study. Comparing with wheat and oat, oilseed rape contaminated by Cd was no suitable for developing biogas. According to the results mentioned above, oat showed better than oilseed rape and wheat in restoring the Cd-contaminated soil as well as producing bioenergy, which is meaningful to both environmental protection and bioenergy production, especially in cold areas.

Keywords: Bioenergy crop, Morphology, Accumulation, Biogas production, Cadmium contaminated soil

Phytotechnology Opportunities in North Carolina for Producing Woody Biomass for Energy While Accomplishing Phytoremediation

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The need for alternative energy resources in the United States is nearing a critical point. The State of North Carolina has positioned itself to use woody biomass as one of its primary supplies for non-fossil based energy. Yet, it is unclear from where adequate sustainable supplies of woody biomass will come. Gleaning logging residues and harvesting low-value trees from established forests may not supply enough feedstocks for a growing biopower industry, an emerging large wood pellet industry, and an emerging biofuels industry. Short-rotation woody crops (SRWC) will have to be grown to supply the additional feedstocks needed. Agricultural lands are unfavorable as they could have an impact on the food market prices in North Carolina and abroad. Clear-cutting natural stands to establish SRWC is likely to be publicly unacceptable due to perceived ecological consequences including habitat loss for wildlife, biodiversity reduction, and carbon-cycle disturbance. We believe that there are other locations that have yet to be considered. We propose growing bioenergy crops on so-called "liability lands." These lands are ideal for energy production as they do not compete with lands designated for food crops and they may be less likely to negatively affect surrounding ecological communities. Our research aims are to evaluate potential dual benefits of bioenergy crop production and phytoremediation on potential liability lands throughout the State of North Carolina.

Keywords: Phytoremediation, biomass, energy, short rotation woody crops

Valorization of plant biomass harvested at trace element-contaminated sites managed by gentle (phyto)remediation options

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Phytoextraction is a promising technology to remediate soils contaminated with trace elements (TE). One phytoextraction strategy is based on cultivation of high biomass herbaceous species, e.g. sunflower and tobacco, with or without addition of TE mobilizing agents to the soil. The second one is based on cultivation of rapidly growing trees, e.g. willow and poplar, with high TE accumulation ability in short rotation coppice (SRC) and the third one is based on the cultivation of TE-hyperaccumulators. After the cultivation of appropriate plant species on the contaminated soil, TE enriched harvestable plant parts are removed from the site. Problems associated with the treatment and disposal of the metal-enriched biomass produced during phytoextraction limit the development of commercially viable phytoextraction while ensuring that environmental parameters do not infringe current regulation. In the ongoing project GREENLAND (FP7, KBBE-2010-4, 266124) project, one work package aims at testing different various conventional and innovative technologies of biomass valorization, such as combustion, anaerobic digestion, solvolysis and microwave thermal treatment, and determining the fate of the TE in the resulting products of each conversion process. A first round of assays was carried out on a wide range of plant species cultivated at the field trials of the GREENLAND partners. The results will be presented and discussed.

Keywords: bioenergy, phytoremediation, *Salix*, poplar, environmental impact

Application of the Rejuvenate decision support approach

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All across Europe there are areas of land which have been degraded by past use that are not easy candidates for conventional regeneration, or for which conventional regeneration may not be the most sustainable approach. The use of such marginal land for cultivation of non-food biomass is an emerging opportunity that can both address some of the concerns about biomass production on agricultural or virgin / wilderness land and be an incentive to regenerate the areas. It is possible that long term use of marginal land for biomass production may at least offset the costs of its management, and potentially generate profit. In the project Rejuvenate an inclusive decision support approach, referred to as the Rejuvenate DST (decision support tool) including the following key steps (Bardos *et al.*, 2009; Bardos *et al.* 2011):
The identification of crop and use opportunities;
The management and improvement of soil and control of risks;
Understanding and maximising value and sustainability; and
The management of project risks such as technology status, due diligence and stakeholder perceptions.

Currently the practical usefulness of the DST is tested by applying it for a range of ongoing demonstration and potential real sites in cooperation among the Rejuvenate team (Bardos *et al.*, 2009; Andersson-Sköld *et al.*, 2011) and the Greenland, Phytopop and Phytener projects.

The results of the test applications will be provided as appendixes in DST guide and as a basis for a SWOT (strength, weakness, opportunities and threat) analysis of the DST. In this presentation the preliminary results of the test application will be presented.

Keywords: Decision support approach, non-food biomass, test, practical, application

References:

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Enhanced growth of bio-fuel plants on amended coal waste

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In this work further use of a unique biofertilizer, which functions due to the mineral properties of natural zeolite when mixed with an organic waste, has been used to grow bio-fuel plants on coal waste. The growth experiments are reported together with a series of control experiments designed to demonstrate the unique properties of the biofertilizer. The plants grown include: *Salix viminalis, Miscanthus, Beta vulgaris, Zea mays, Brassica napus* and *Linum usitatissimum*. These plants were grown in pots under controlled greenhouse conditions. Coal waste was taken from Calverton colliery, Nottinghamshire and used throughout the work as the plant substrate. It is now becoming clear that coal waste, like many other industrial waste products that have been used in former experiments contain a range of chemical elements that can be ionized by the biological activity of the organo-zeolitic amendment. In this regard many plant nutrient elements are made available for uptake which are both essential and beneficial to plant growth as demonstrated by the large differences in biomass between plants grown in the amended substrate and those grown in the untreated waste.

Keywords: biofertilizer, zeolite, coal waste, ionized, biomass

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Leggo P.J, Ledésert B and Day J. (2010). Organo-zeolitic treatment of mine waste to enhance the growth of vegetation. European Journal of Mineralogy, 22:813-822.

Effect of Pyrolysis Temperature on Chemical and Surface Properties of Biochar of Rapeseed (*Brassica napus* L.)

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Biochar is carbon-rich product generated from biomass through pyrolysis. Biochar (charcoal) can be both used directly as a potential source of solid biofuels and as soil amendments for barren lands. However, chemical composition of the biochar depends on the source of feedstock and pyrolysis conditions. The influence of pyrolysis temperature on the chemical and morphological properties of biochars of Rapeseed (*Brassica napus* L.) was investigated in this study. The final pyrolysis temperature was varied in the range of 400 – 600°C in a fixed-bed reactor at 10 °C.min^{-1} heating rate and under static atmospheric conditions. The ultimate and proximate analyses and calorific values of the biochars were determined, and then the chemical composition of the biochars were investigated using Fourier Transform Infrared Spectroscopy (FTIR). The surface properties of the biochars were also identified by BET surface area and scanning electron microscope (SEM) images. Based upon FTIR results, the hydroxyl groups in the biochars produced at various temperatures were more those of the carbonyl groups. The highest surface area was obtained as 25.38 m^2/g at 550 °C of pyrolysis temperature. The results showed that both chemical and surface properties of the biochars were significantly affected by the pyrolysis temperature.

Keywords: Biomass, Pyrolysis, Biofuel, Biochar, Characterization

Risk Management and Assessment for Public Health (I)

Uptake and distribution of metals in vegetation on metal contaminated dredged sediment derived soils

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Ripened dredged materials are an excellent substrate for vegetation because of their favorable physical properties and chemical fertility. Recycling of elements through litter fall and litter decomposition can be an important pathway for input into the food web. Although general properties of the dredged sediment-derived soils in Flanders favor low mobility and bioavailability of metals, some plants can exhibit elevated metal concentrations in the above ground parts. Willow (Salix sp.) naturally invades dredged sediment landfills, and may accumulate metal levels that are elevated compared to concentrations observed in reference situations. Also poplar tends to exhibit elevated Cd and Zn concentrations. Leaves of maize grown on contaminated dredged sediment-derived soils contained high levels of Zn and Cd. Other plants, such as ash, alder, maple and Robinia, do not accumulate metal levels in excess to these encountered in a not contaminated environment. Metal uptake by vegetation depends on many more factors such stand age, time of the year, and hydrological condition of the site. Appropriate management practices for these lands must aim at minimizing the risk of contaminant dispersal into the environment. Management options for these sites such as wetland conservation, afforestation, short rotation forestry or creation of landscape dikes can only be successful if they are based on a good understanding of the cycling and ecosystem impact of trace metals.

Keywords: dredged materials, metals, vegetation, ecosystem

Phytomanagement in case of a nuclear accident - lessons learnt from Chernobyl and possible application to Fukushima

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The accident at the Fukushima Dai-ichi Nuclear Power Plant has raised questions about the accumulation of radionuclides in soils and the transfer in the food chain. Following a large-scale nuclear accident, the application of countermeasures is a key issue. Numerous countermeasures were developed since the Chernobyl accident and applied on large scale. This presentation discusses plant-based countermeasure strategies and their effectiveness and feasibility against the background of the Fukushima Dai-ichi nuclear accident and the agricultural areas affected.

For optimizing management options, a good knowledge on agricultural practice, soil characteristics, soil contamination levels is required. Planning for agricultural management options requires a holistic approach, considering radioecological, radiological, environmental, economic and socio-cultural and political aspects.

Phytomanagement options such as food crop selection will be briefly discussed. Phytoextraction will be critically examined. Alternative land uses for areas were contamination levels are considered too high to allow for food or fodder production, need careful evaluation. A holistic feasibility assessment for alternative land uses like installing bio-energy crops or fiber crops will be presented.

Views will be confronted with actual remediation activities on agricultural land in Japan.

Keywords: phytomanagement, nuclear accident, phytoextraction, bioenergy crops

Managing the phytoaccumulation of cadmium using lignite

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Soil contamination with cadmium (Cd) occurs worldwide as a result of the application of Cd-rich phosphate fertilizers and sewage sludge as well as industrial emissions. Plants take up relatively high concentrations of Cd from soil, leading to breaches of food safety standards and facilitating the entry of this toxic element into the food chain. A potential solution may be the use of lignite, a low-calorific value coal, as a soil amendment. We tested the capacity of lignite and lignite-soil mixtures to sorb Cd at various pH values. In greenhouse experiments, we tested the effect of lignite on the accumulation of Cd and other elements by perennial ryegrass, *Lolium perenne*. Over a pH range of 4 – 7, the sorbtion of Cd by lignite was 1 -2 orders of magnitude greater than that of a silt loam containing 2% carbon. The addition of 5% w/w lignite to a range of soils revealed that lignite addition was most effective in reducing soluble Cd in soils with a low pH. The addition of just 1% lignite to the aforementioned silt loam reduced plant Cd uptake by 30% while not detrimentally affecting biomass or the uptake of other essential nutrients. The greater effect of lignite reducing plant Cd uptake compared to copper and zinc may be due to the presence of organic sulphur to which Cd, a soft acid, preferentially binds. One unanswered question is the persistence of the Cd-reducing effect of lignite. This is the subject of ongoing research.

Techno-economic assessment of fast pyrolysis for the valorisation of short rotation coppice cultivated for phytoremediation in the Campine

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The Belgian Campine has long been the subject of multidisciplinary research into phytoremediation of a vast area of farmland that has been moderately polluted with heavy metals such as cadmium. On an experimental field in the municipality of Lommel biologists have been investigating the metal extraction potential of energy maize, rapeseed and short rotation coppice amongst others. Especially willow demonstrated some phytoextracting capacity whereas the metal extraction potential of energy maize appeared to be rather limited. From an economic point of view however, energy maize is a feasible option for generating an alternative income for the affected farmers whereas the economic potential of willow has been open for further investigation. Because willow mainly consists of lignin, cellulosis and hemicellulosis, thermochemical technologies are better suited than biological ones for the conversion of willow into valuable products. Fast pyrolysis appears to be more promising than combustion and gasification both from an ecological and an economical point of view. Due to the lower process temperature of fast pyrolysis most of the metals do not volatilize and remain in the residual char. For the small scale of operation expected in the Belgian Campine, fast pyrolysis is also preferred above combustion and gasification in terms of return on investment. However, economic risk analysis showed that even with fast pyrolysis there is a high chance of economic loss. Therefore risk reduction strategies have been identified: combined production of heat and power, fast co-pyrolysis of willow with biopolymers, and activation of the residual char show promising results.

Keywords: cost benefit analysis, risk analysis, fast pyrolysis, phytoremediation, willow

RISK MANAGEMENT AND ASSESSMENT FOR PUBLIC HEALTH (II)

The adoption of gentle groundwater remediation strategies: a health risk based decision analysis

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A broad range of remediation strategies is available to remediate or contain contaminated groundwater. To select a remediation strategy, risk based economic decision analysis is increasingly being applied *(Lemming et al., 2010)*. This study combines remediation costs and avoided health risk costs to evaluate for a specific case study whether phytoremediation is the preferred remediation strategy in comparison with monitored natural attenuation and pump & treat.

To determine the remedial effect of the different remediation strategies, a groundwater flow and transport model is developed. A dose response coefficient is applied to quantify the relationship between a unit of contamination and a unit of impact on the (potentially) affected human health. A decrease in contaminant concentration results in a reduced health risk and hence, reduced health costs. The avoided health costs are considered as the benefit of the remediation strategy. The remediation strategy with the highest net benefit i.e. the difference between avoided health costs and remediation costs should be selected.

For the case study considered monitored natural attenuation is the preferred remediation strategy, followed by phytoremediation and pump & treat. This study further illustrates how the combination of groundwater modeling, health risk assessment and the integration of external benefits results in a valuable decision support tool for contaminated site management.

Keywords: groundwater remediation, health risk assessment, phytoremediation

References:

Lemming G, Friis-Hansen P and Bjerg PJ (2010). Risk-based economic decision analysis of remediation options at a PCE-contaminated site. *Journal of Environmental Management*, 91(5): p. 1169-1182.

Effect of plant root growth on Pb phytostabilization of East Helena, MT, smelter contaminated soils

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Many studies report that adding phosphate to Pb-rich soils can promote formation of pyromorphite and reduce soil Pb bioaccessibility/bioavailability. In addition, some reports show that pyromorphite may form densely within the rhizosphere or within roots such that plant roots phytostabilize soil Pb.

We have been testing methods using mixtures of organic amendments, byproducts, and phosphates to achieve effective remediation of Zn-Pb-Cd-As contaminated sites. At the East Helena, MT, site, only limestone was initially applied to limit Zn phytotoxicity and Cd uptake by forage crops. In the current experiments, we are testing organic, byproduct and phosphate amendments to achieve phytostabilization of all metals. Bulk soils were collected from three areas. Soils were analyzed for total metals, pH, OM, salinity, plant available P, DTPA-extractable metals (using 50 mL/5 g soil), 0.01 M $Sr(NO_3)_2$ extractable metals, and the pH 2.5 bioaccessible metals following the Zia-Codling-Chaney method.

Potential amendments (phosphate and biosolids/manure composts) were thoroughly mixed with the test soils, incubated for 2 weeks, and then pH adjusted to >7.5 (made calcareous) by addition of amorphous $CaCO_3$. Phosphate (superphosphate, fish bones and combinations of organic amendments plus superphosphate) were added (5 moles P per mole Pb). Pots of test mixtures were left without vegetation, or seeded with wheatgrass. After 30 and 90 days soil cores will be evaluated for pH, available P and metals, and for bioaccessible Pb. Changes in bioaccessible Pb will be compared with community goals for site remediation to allow desired reuse.

Keywords: Phytostabilization, lead, cadmium, biosolids, compost

Accumulation of antimicrobials by vegetables

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Through use of biosolids for fertilization and wastewater effluents for irrigation, antimicrobials are introduced into agricultural fields. Our research has demonstrated that the ability of vegetables to accumulate antimicrobials is significant. In hydroponic studies, root concentrations of antimicrobials were 85 to 851 mg triclocarban per kg dry plant and 100 to 815 mg triclosan per kg dry plant. Translocation, while significant, was limited, with only 0.02 to 0.17% of triclocarban and 0.04 to 0.95% triclosan translocated to shoots and leaves in hydroponic studies. Preliminary assessments indicated that the potential exposure to antimicrobials through vegetables is not high enough to prompt concerns with regards to known health effects; however, more research is needed on what concentrations prompt antimicrobial resistance or endocrine disruption. Growth of food crops in soil systems indicated (i) similar or greater shoot concentrations of antimicrobials and (ii) that plant growth reduced leached and soil concentrations of antimicrobials. Ongoing research is investigating fate of antimicrobials in soil systems, including potential plant metabolites of triclosan and triclocarban, utilizing radiolabelled trilcosan and triclocarban. Research has important implications for the use of wastewater products (i.e., biosolids and effluents) for agricultural production of vegetables.

Keywords: uptake, bioaccumulation, antibiotics, food crops, exposure

Phytoremediation uncertainty tackled: a real option approach

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The management of problems related to soil and groundwater contamination involves the search for a balance between costs and benefits, integrating the knowledge from multiple disciplines. A wide range of economic decision tools are available to support the remediation selection process, including Cost Benefit Analysis (CBA), risk-based CBA, and multi-criteria analysis (Bardos *et al.* 2001). However, these tools do not take into account the reversibility of a remediation strategy.

Aim of this study is to examine the value of a phytoremediation project embedding the option to redirect the remediation process once it is proved that the investment would not be economically feasible.

phytoremediation is perceived as a remediation strategy which has considerable strength but also certain limitations. The process of phytoremediation involves complex and uncertain relationships among biomass, contaminant and nutrients which should be included in the economic analysis. This study addresses the unknown efficiency at which the groundwater contamination degrades when phytoremediation is applied. Pindyck (1993) identifies this kind of uncertainty as a technical uncertainty which relates to the physical difficulty to complete the project. This kind of uncertainty can only be resolved by undertaking the project.

A dynamic decision model is developed to determine the critical level of ‘bad’ groundwater samples at which the remediation process should be redirected. It is shown that when remediation strategies are considered as dynamic processes, the value of remediating contaminated soil and groundwater increases.

Keywords: Real options, phytoremediation, uncertainty, decision reversibility

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Pindyck RS (1993). *Investments of uncertain cost.* Journal of Financial Economics, 34(1): 53-76.

Testing combinations of amendments for stabilization of metals in contrasting contaminated soils

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There is a need to reduce negative impacts of trace element polluted soils on human health and the environment. Metals can be stabilized by amendments increasing metal adsorption or altering their chemical form (Basta *et al.*, 2005). Few experiments compare different in situ remediation treatments under similar environmental conditions, or consider whether or not all soil components or properties (microbes, soil fauna, plants, soil retention, colloid stability, etc.) are similarly protected. Within the EU FP7 Greenland project (266124) we compared the impact of novel soil amendments and their combinations with traditional materials on metal solubility and response of plants, soil organisms and microbial activity.

One-year greenhouse pot experiments were established: soil A, less toxic agricultural soil contaminated through long-term Zn/Pb smelter emissions in Poland (pH 7.0); soil B, toxic soil contaminated through smelter dust spill in Poland (pH 6.8); and soil C, toxic Cu-contaminated mine-spoils in Spain (pH 3.6). Amendments were tested individually and in combination in planted and unplanted soils: compost, drinking water residue, iron grit, Ca-phosphate, LD slag, Thomas basic slag, gravel sludge, siderite, Fe nano-sorbent, and cyclonic ash. Rates were optimized in prior batch tests. Soils B and C were planted with grasses, and soil A with lettuce. Plants were periodically harvested, yields recorded and metal content determined. Soil metal extractability and bioaccessibility, pH, EC and enzymatic activity were measured. Soil pore waters were analyzed for trace element/nutrient concentrations. Parallel tests evaluated earthworm behavior and metal accumulation. Amendment effectiveness was assessed in terms of phytoexclusion or phytostabilization.

Keywords: phytostabilization, soil amendments, trace elements, contaminated soil, bioavailability

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Basta, N.T., Ryan J.A., Chaney R.L. (2005). Trace element chemistry in residual-treated soil: Key concepts and metal bioavailability, J. Environ. Qual. 34: 49–63

NANOPARTICLES

Size, shape and surface property based phytotoxicity of gold and silver nanoparticles to plants

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Engineered nanomaterials (ENMs) have been actively explored both as promising materials for a wide variety of electronic, magnetic and optical applications and as contaminants of emerging concern. Previous research has shown that some ENMs exhibit strong toxicity to plants: a key component of the ecosystem. Even though size plays a significant role in the toxicity of ENMs, other important physicochemical properties of ENMs such as their shape and surface decoration also substantially affect their toxicity. The exact correlations between the unique physiochemical properties of ENMs and their toxicity to plants, however, are still unknown. This study aimed to fill the knowledge gap by studying the toxic effects of gold and silver nanoparticles with a broad spectrum of properties on several agricultural species. The experiments were carried out hydroponically in bench scale and multiple toxicity indicators of plants such as their biomass increase, root membrane integrity and photosynthetic efficiency were monitored. Potential accumulation and localization of these NPs in plant tissues were also determined. Preliminary results suggested that NM size, morphology, coating materials and surface charge all play important roles on fate and toxicity of these NPs to plants.

Keywords: silver nanoparticles, gold nanoparticles, surface property, phytotoxicity

Accumulation of engineered nanoparticles in belowground vegetables: Nutritional bioaccessibility and dietary exposure risks

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The release of engineered nanomaterials (ENMs) into the environment has raised serious concerns about their potential risks to food safety and human health. There is a particular need to determine the extent of ENM uptake into plant foods. Belowground vegetables that grow in direct contact with the soil are the foods which will likely accumulate the highest concentration of ENMs and present the most significant risk to human health. However, no such information is currently available in literature. A systematic evaluation of the accumulation of six different ENMs into the tissues of ten common belowground vegetables is underway to identify the plant foods with the greatest potential for ENM accumulation. Additional efforts will use the vegetables produced to examine how basic food preparation steps alter ENM dietary exposure and to estimate the nutritional bioaccessibility of the ENMs from those tissues. Dietary exposure modeling is being planned to quantify dietary risks of ENM exposure. The nutritional bioaccessibility of the ENM will be demonstrated and models projecting age- and gender-specific dietary exposures will be produced to provide a comprehensive picture of the food safety risk posed by these ENMs. Results from this work are expected to help growers, extension agents, and USDA to make sound decisions on choice of crops for particular ENM-impacted soils. The specific accumulation and dietary exposure scenarios associated with particular ENM and plant combinations would allow for recommendations /restrictions concerning which ENM-containing products can be safely applied to human food crops.

Keywords: engineered nanomaterials, engineered nanoparticles, food safety, nutritional bioaccessibility, dietary exposure

Fullerene-enhanced Accumulation of p,p′-DDE in Agricultural Crops

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The effect of C_{60} fullerenes on the uptake and translocation of *p,p′*-DDE was determined in zucchini, soybean, and tomato. The plants were grown in 125-mL jars amended with 12g (100 mL) of dry vermiculite homogenously amended with 0 or 40 mg of C_{60} fullerenes. Prior to planting of seedlings, the jars were amended with 40 mL of Miracle-Gro™ solution containing 100 ng/mL of *p,p′*-DDE with 0 or 100 mg/L humic acid. During 19 days of growth, plants were watered with the same *p,p′*-DDE containing solutions. Plant roots and shoots were extracted and analyzed for *p,p′*-DDE and fullerene content by GC-ECD and LC-UV, respectively. Plant biomass was unaffected by *p,p′*-DDE or fullerene exposure. The total shoot *p,p′*-DDE levels in non-fullerene exposed tomato, soybean and zucchini were 26.9, 131, and 675 ng, respectively; similarly, the total root DDE content for the three plants was 402, 5970, and 5830 ng, respectively. The presence of fullerenes increased the shoot *p,p′*-DDE content of zucchini by 29%; contaminant levels in soybean roots were decreased by 48% in soybean but tomato shoot content was unaffected. The root and total plant *p,p′*-DDE content of all three species was significantly increased by fullerene exposure; enhanced contaminant uptake ranged from 30-65%. Humic acid, regardless of fullerene presence, significantly decreased the uptake *of p,p′*-DDE by all plant species. Fullerenes were detected in the roots of all plants but were not detected in plant shoots. These findings show that the carbon-based nanomaterials may significantly alter the accumulation of co-contaminants in agricultural systems.

Keywords: Nanoparticles, Persistent Organic Pollutants, Fullerenes

Enhanced Level of Glutathione Protects Plants from Silver Nanoparticle Toxicity

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Silver nanoparticles (AgNP) are widely used in agriculture and release of these nanoparticles to the environment has raised serious concerns about their toxicity to environment and human health. However, the biochemical mechanisms by which plants counteract the NP toxicity are largely unknown. Glutathione (GSH) is one of the most important redox buffer in the eukaryotic cell. The importance of GSH synthesis in heavy metals and xenobiotic detoxification is well established. Glutathione synthesis involves two ATP-dependent reactions that are catalyzed by γ-glutamylecysteine synthase (γ-ECS) and GSH synthetase (GS). We engineered *Crambe abyssinica* (a member of Brassicaseae) plants expressing the *E. coli γ-ECS* gene. These plants showed several fold higher levels of GSH in all tissues and further exhibited greater tolerance to heavy metals than wild type (WT) control plants. In order to study if enhanced synthesis of GSH can detoxify AgNP, transgenic lines of *C. abyssinica* expressing γ-ECS were exposed to various concentrations of AgNP and Ag^{2+} ions. Our results showed that transgenic lines were highly tolerant to AgNP and Ag^{2+} ions, attained significantly higher biomass and chlorophyll contents and higher transpiration rate compared to WT plants. Our results further showed that transgenic plants suffered less lipid peroxidation damage as compared to WT controls. Further experiments such as production of total reactive oxygen species (ROS), assays for anti-oxidative enzyme and accumulation of total Ag ions in the plants are currently underway. To our knowledge, this is the first report of the involvement of detoxifying AgNP by GSH in the transgenic plants.

Keywords: Silver nanoparticles, glutathione synthesis, *Crambe abyssinica*, transgenic plants, tolerance

Interaction of Plants and Engineered Nanoparticles

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Commercially-available and specially synthesized nanoparticles of various sizes and coatings have been tested for toxicity, uptake, and translocation in poplar (*Populus deltoides x nigra*, DN-34) grown in hydroponic solution in the laboratory. Nanoparticles of cerium oxide (CeO_2), iron oxide (Fe_2O_3) and silver (Ag^0) have been tested at particle sizes ranging from 5 to 50 nm median diameter.

Silver nanoparticles were quite reactive and underwent partial oxidative dissolution under some experimental conditions. In these cases, the silver ions (Ag^+) were quite toxic to the plants, as reported in the literature. But in general, the three nanoparticles were not significantly toxic to poplar plants as measured by transpiration and growth. In fact, we show some evidence of phytostimulation for all three nanoparticles at low concentration levels (< 1 mg/L) especially for silver nanoparticles. Only at high nanoparticle concentrations ($\geq$ 10 mg/L) was some phytotoxicity observed.

Using ICP/MS analysis of the aerial portions of poplar tissues, it was clear that the nanoparticles were taken up and translocated under normal growing conditions. Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray spectroscopy (EDX) together with TEM and Confocal Laser Scanning Microscopy allowed some interpretations about the mechanism of uptake and the ultimate location of nanoparticles in plant tissues.

Keywords: Nanoparticles, silver ions, phytostimulation

Field Projects

Evapotranspiration (ET) landfill cover in Berlin – field data on ecology and technology of successful pre-successional establishment of conifers

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Berlin's first evapotranspiration (ET) landfill cover caps parts of a former 52-hectare landfill that became the waste site "*Altablagerung Wannsee*" (AAW). Initial planting of 14,000 Scots pines (*Pinus silvestris*) for year round reduction of water percolation failed. In response, field experiment plots were installed and planted with Scots pine, Austrian pine (*Pinus nigra*), and Norway spruce (*Picea abies*) in 2007. Various planting methods, soil amendments, and tree care measures had been applied for attempting successful pre-successional establishment of conifers into the ET landfill cover. Conifer vigor and growth are monitored regularly along with soil parameters, flora, fauna, and weather.

Temperature and precipitation patterns on site seem to confirm global warming effects that affected survival and growth of juvenile conifer trees. Periods of severe drought and heat and increased solar radiation in spring and summer have caused high mortality rates particularly among Norway spruce. Weather-related stress posed upon young conifers was amplified by soil conditions unfavorable to the planted evergreen trees, including soil compaction, basic pH values, and high mineral nitrogen concentrations. Sown grasses and spontaneously emerging forbs seem better adapted to these site conditions. Grasses reached high ground cover abundance values that proved to be fatal for conifer saplings and older but shallowly rooted spruce. Vegetation-driven exhaustion of limited soil water resources seemed particularly critical for young conifers. Irrigation has helped. But watering with local groundwater may have contributed to elevated sodium and boron concentrations in conifer needles. Such side effects can impede intended benefits of tree irrigation.

Keywords: waste site, restoration, vegetation, pine, spruce

Field Demonstration of Energy Plants Production on Heavy Metal Contaminated Farmland

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To explore the possibility of safe and beneficial use of farmland moderately to heavily contaminated by heavy metals, a field demonstration of energy crop production was carried out on a heavy metal contaminated farmland in Zhejiang Province. The results showed the soil pH increased and available heavy metal contents significantly reduced after the addition of 0.1% lime and 0.2% phosphate rock. The biomass of sweet sorghum, sugar cane and vetiver was affected by available heavy metals in the soil. The total sugar and reductive sugar content of the juice from sweet sorghum and sugar cane were not significantly affected by the treatments. Sweet sorghum produced more than twice juice per unit area than sugar cane. The results showed that addition of soil amendments enabled energy crop production on farmland contaminated by heavy metals.

Keywords: Soil amendment, Energy plants, Heavy metal contamination, Farmland

Feasibility of phytoextraction soluble zinc decontamination of topsoil: results of a 5-year field scale experiment in Switzerland

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The phytoextraction with mutant lines of biotechnically improved tobacco and sunflower (non-GMs) can be a sustainable alternative to existing destructive decontamination methods especially for the remediation of soluble zinc of topsoil.

Results of a 5-year time series experiment at field scale at in northeastern Switzerland confirm that the initial soluble (bioaccessible) zinc soil contamination can be lowered by 40-70%, whereas subplots without phyto-extraction treatment remains at initial concentrations within this five year phytoextraction treatment. In 2011 the phytoextraction experiment was enlarged by a factor of 3, and the results show a promising 15-50% reduction of the initial soluble zinc concentration within one harvest, only.

A Mass Balance Analysis MBA confirm the reduction of zinc in soil, and it can be well explained by the plant zinc uptake. Moreover it can be shown, that the phytoremediation plants also partially feed from the pool of the total zinc soil contamination.

Already phytoremediated subplots, that no longer exceed the Swiss trigger value, are now assessed for their stability of the remediation treatment over time.

In contrary to the phytoextraction of the "total amount" of soil contamination that need a long cleaning up time, it can be shown, that the "risk based approach" of phytoextraction of the "bioaccessible amount" of metal soil contamination can be feasible within a few years period.

The Swiss phytoremediation site is part of the GREENLAND network with 14 large field trials (http://www.greenland-project.eu/), where the feasibility of phytoremediation strategies are comparatively assessed for a future sustainable land management in Europe.

Keywords: phytoextraction, phytoremediation, non-GM plants, mutant screening, plant-based feedstock, bioaccessible trace elements

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Performances of gentle (phyto)remediation options at field scale in the GREENLAND network of large trace element-contaminated sites

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Performances of the most promising gentle remediation options (GRO) for trace element-contaminated soils (TECS), i.e. (aided) phytostabilization, phytoextraction, and *in situ* stabilization/phytoexclusion) are assessed in a European network of 14 large field trials, within the framework of the EU FP7 GREENLAND project (http://www.greenland-project.eu/). GRO efficiency is evaluated based on various (a)biotic stresses, climatic conditions, pollutant linkages, (phyto)remediation strategies and sustainable land management. Field sites cover a range of contamination scenarios (e.g. agricultural soils, sludge-amended soils, mine tailings, landfills, dredged sediments, and post-industrial soils).

Harmonized datasets are built up on metal(loid) exposure, plant parameters and yields (notably for plant parts converted into feedstock), mineral and biochemical composition of plant parts, ecological and financial returns, and costs. Matrices are sampled to monitor changes in soil exposure (e.g. labile contaminant pools), transfer to environmental compartments and bioaccessibility, ecotoxicological risks, and soil (multi)functionality and biodiversity. Transfer and bioconcentration factors, shoot metal removal, contaminant fluxes, and tolerance indices are computed. Dose (exposure) – plant response relationships are modelled.

Data are summarized for various plant covers including poplar and willow short rotation coppices, annual crops of secondary metal accumulators (fast-track bred sunflower and tobacco), and metal-excluders (e.g. tolerant grasses, barley and maize cultivars). The long-term efficiency and sustainability of GRO, progresses in remediation objectives (in compliance with national and best procedures), timescale management, maintenance, uncertainty and limitations (including spatial variation of contaminants, water requirements, global changes, etc.), potential flexibility and deployment at other sites are discussed as well as new deployed GRO and cultural practices (e.g. bioaugmentation).

Keywords: phytoextraction, phytostabilization, plant-based feedstock, in situ immobilization, trace element-contaminated soil

Effect of plant cover on long term *in situ* attenuation of a multi-contaminated soil

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Due to man-made activities, large areas of soils are contaminated with multiple pollutants, of high concern due to their toxicity and environmental impact. Plant-microorganisms-based technologies could be a strategy for soil remediation and for the restoration of soil functioning after treatment. A field trial was set up in 2005, to study plant-microorganisms-assisted attenuation of a multi-contaminated soil (PAHs (polycyclic aromatic hydrocarbons) and heavy metals) (Ouvrard *et al.*, 2010). Four treatments were included: unplanted, planted with alfalfa with or without mycorrhizal inoculation, naturally colonized by indigenous plants, and thermally treated soil planted with mycorrhizal alfalfa. Soil, soil leachate and plants were sampled twice a year for pollutant analysis. In parallel, plant growth and diversity, soil fauna and microbial diversity and activity, soil and solution ecotoxicity were determined. A slow decrease over time of PAH concentrations was observed, without significant difference between planted and unplanted plots, which could be related to the low availability of the PAHs in aged contaminated soil. However, the dynamics of bacterial and fungal communities were mainly driven by plants (Caupert et al, under revision). The presence of plants had an influence on the abundance and activity of all organisms examined in the study, favouring the whole trophic chain development (Cébron et al, 2011). Finally, the chronic toxicity of leachates was lower in the vegetated treatments in comparison to unplanted plots. Although plant cover did not directly affect the pollutant attenuation, it decreased water leaching and was the main driver of biological diversity and activity.

Keywords: Multi-contaminated soil, PAH, field trial, phytoremediation, biological activity

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WASTEWATER

Field-Scale Phyto Waste Water Treatment Considering Soil Complexity

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Phyto processes must create a predictable rhizosphere reactor when used for waste water treatment instead of conventional mechanical and biological treatment.

Port of Morrow at Boardman OR generates 5 million gallons every day of high nitrogen waste water from food processors. During the growing season, effluent is irrigated on 5000 acres of food crops at agronomic rates to provide both water and nutrients. Agronomic rate is the core concept regulating nitrogen applied to any agricultural crop; for example alfalfa is about the highest applied dose at 450 lb nitrogen/acre/year. Winter nitrogen application is currently a challenge; effluent must be either stored in large ponds or mechanically treated at great cost for both energy and capital.

Phyto treatment using dense poplar roots is being tested for both crop uptake and year-round rhizosphere denitrification with 'clean water' discharge to the aquifer.

In 2011, 15 phyto test cells (PTC) have shown 90+% nitrogen removal in less than a 30-day dwell time through an 80 cm (30 in) rhizosphere during tree dormancy. In 2012, an 8.3 acre field demonstration plot planted with 7,000 trees irrigated with effluent is mimicking the PTC to create this deep rooting and winter nitrogen treatment.

Full scale requires 500+ acres w that vary widely due to soil heterogeneity and non-uniformity of applied water. Irrigation operation focuses on statistically explicit water management to minimize the nitrate-nitrogen breakthrough frequency by ensuring adequate root zone dwell time. Statistic modeling data correlates effluent dosage rate to treatment based on dwell time in root zone with average soil conditions.

Keywords: wastewater, rhizosphere, soil variability, bolean**,** phyto test cells

Recent advances in Phytoremediation Technologies involving Aquatic plants for the Removal of Toxic Heavy Metals from polluted wastes, soil and sludge

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We are developing technologies for biosorption of toxic heavy metals by biomass of aquatic plants, and for the active removal of toxic heavy metals from soil and sludge by the water lily Nymphaea.

A. biofilters made of *Azolla, Salvinia* and *Pistia* biomass were found most efficient in hyper filtration of polluted waste water in model system and industrial wastes. Removal of chromate and arsenate was demonstrated to be most effective, reading few ppb at the column outlet. Treatment of aquifer polluted with lead, yielded detoxified water containing less than 5 ppb Pb^{+2}, and a solution containing 4 ppm Pb_{+2} was decontaminated to outlet solution containing 10 ppb of Pb^{+2}. the 10 kg Azolla biomass was found to bind 50 g Pb^{+2}, 200 g Cd^{+2} and 200 g Cr^{+6}.

B. sludge produced by waste treatment of Cd-Ni batteries was most effectively polished by *Nymphaea*, removing Cd+2 from 60 mg/kg in the sludge to 20 mg/kg after active removal by *Nymphaea* plants. The metal was stored in the blades , petioles and roots of the *Nymphaea* plants. The largest scale demonstration of the *Nymphaea* removal, was conducted with 100 ton of Pb^{+2} polluted soil, produced by car battery industry. 1400 *Nymphaea* plants were planted in the polluted soil containing 1150 ppm Pb^{+2}, the containers was flooded, and the *Nymphaea* plants removed 50 kg of Pb^{+2} throughout one year of active removal. The *Nymphaea* was found to remove toxic metals ions from heterogeneous mixture and from soil and sludge of different contents.

Keywords: Azolla, Salviniva, Biosorption, Nymphaea, Phytoremediation.

Safe disposal of dairy/domestic wastewater via irrigation of reconstructed mixed forest - forage formation

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The Golan Heights is a significant segment of the watershed of the Sea of Galilee, which comprises ca. 30% of the Israel's water supply. The alternatives for safe disposal of dairy farm sewage effluent are limited and sewage often overflows into local streams ending in the lake. For the last 10 years we examined disposal of dairy/domestic lagooned effluents by irrigation of a reconstructed pasture–*Eucalyptus* formation, hypothesizing that under deficit irrigation (220 mm +600 mm winter rains) most of the wastewater constituents will be retained within the soil-plant system. These constituents were quantitatively detected in the soil (twice yearly), soil solution and runoff (throughout the winters). In comparison, another 20-ha not-irrigated basin was similarly examined. Following, are average (2006-2011; 133-603 samples/parameter) concentrations in the effluent, overall loads, and net recoveries in winter runoff of some constituents: OC: 482 mg/l, 5400 kg/ha, of-which 6% recovered in runoff; N: 177, 2000, 27; P: 43, 500, 6; K: 287, 3200, 20; Cl^-: 221, 2000, >100; Na^+: 106, 2900, 86; B: 0.22, 2.4, 10; Fe: 1.4, 16, 10; Zn: 0.28 mg/l, 3 kg/ha, and 6% of the load was recovered in the runoff (all respective units). Fecal bacteria occurrence in runoff was typical of fresh water streams. Veterinary antibiotics (Fine *et al.*, 2011) were either missing altogether or occurred in 2-10% of the runoff samples from the irrigated site and at the low concentration range of 0.5-35 µg/L. Hence, we showed that disposing dairy wastewater in such a systems posed little threat to pollutants emission into water resources.

Keywords: Eucalyptus, pollutants, nutrients, N-P-K, salinity, trace elements

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Performance comparison between free surface and sub-surface flow systems in phytoremediation study for hydrocarbon removal by *Scirpus grossus*

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Two types of flow, free surface (FSF) and sub-surface flow (SSF) systems were examined to remove Total Petroleum Hydrocarbon (TPH) in phytotoxicity of *Scirpus grossus* to diesel as a hydrocarbon model. The removal efficiency of TPH with different flow systems was compared. Several wastewater parameters including *T*, pH, DO, and ORP were recorded during the experimental runs. In addition, plant overall length, wet weight, and dry weight were also monitored. The average wastewater parameters for FSF and SSF systems respectively were as follows: T (°C): 25-30°C, 24-28°C; pH: 4.1-8.2, 5.9-7.6; DO (mg/L): 3.3-7.2, 3.3-7.2; and ORP (mV): -70-+173, -33.3-+64.83. The phytotoxicity test using bulrush plant of *Scirpus grossus* was run for 72 days with different diesel concentrations (1%, 2%, and 3%). Comparison between the two flow systems showed that SSF was more efficiency than FSF system to remove TPH from the synthetic wastewater with average removal efficiencies of 93.27 and 80.2 % respectively. In addition, the results also showed that the removal efficiency was statistically significant different in sampling time and systems used, but there is no significant with diesel concentrations.

Keywords: Phtotoxocity, Scirpus grocuss, free surface flow, sub-surface flow, hydrocarbon.

POSTERS

A: Plant-Microbe Interactions: Metals

A1

Contribution of the plant rhizosphere system to the phytoremediation of metals in estuarine areas

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Some salt marsh plants have already shown to have potential for metal phytoremediation in estuarine areas (Almeida *et al*., 2011) ,being important to study and test strategies to enhance that potential. The aim of this work was to evaluate how the rhizosphere of *Juncus maritimus* and *Phragmites australis* plants influenced Cd removal from sediments favoring environmental decontamination.

For this study, plants of both species, collected in Rio Lima estuary together with the sediment involving their roots, were placed in vessels and maintained in greenhouses, exposed to natural environmental and light conditions. A nutritive saline solution was added to all vessels through an automated irrigation system (2 daily cycles of flood / draught) to mimic the tides and maintain plants at optimum nutritional conditions. After 2 weeks of acclimation, all vessels were spiked with a saline $CdCl_2$ solution (20 mg L^{-1} of Cd). Solution was in contact with the plant rhizosphere for about 6h. Afterwards a solution containing an autochthonous enriched consortium of microorganisms resistant to Cd (prepared in the laboratory) was added to half of the vessels. Vessels were maintained for 2 months in the abovementioned conditions, being afterwards disassemble. For that, plants aboveground tissues were separated from belowground structures, which in turn were carefully separated from the sediment. Cadmium was determined as before (Almeida *et al*., 2011).

Analyses are still in course but obtained results will allow evaluating the role of the microorganisms present in the rhizosphere of both plants for Cd phytoextraction.

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Keywords: plant rhizosphere, cadmium, estuarine areas, autochthonous microorganisms

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A2

Occurrence of symbiosis in roots of wild plant species on fluoride polluted soils in Southern Tunisia

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The phosphate fertilizer industry has developed in some Tunisian cities along the Southern coast. A main source of environmental pollution is due to this industry of phosphates rock mining and processing, with particularly fluorine and trace metals dispersion. Rain and storage water can seep through the stockpiled phosphogypsum, carrying the contaminants to the soil and groundwater; air pollution also occurs due to wind-dispersed waste products. At the vicinity of the industrial sites, autochthonous vegetation however develops. Although floristic studies on these areas were carried out, there is a lack of knowledge on rhizosphere action and biotic interactions on the mechanisms of pollution tolerance of local plant species. The site of Gabes submitted to a high fluorine pollution was compared to a reference site. The aim of this study was part of a work aiming at understanding how local vegetation can tolerate the high pollution level in soils and air and, may be used as vegetative barrier. Three perennial plant species i.e. *Erodium glaucophyllum*, *Rhanterium suaveolens* and *Atractylis serratuloides* were selected to investigate their root symbiosis. Soils and root samples were collected on five locations for each plant species in both sites. Soil samples were analysed using a specific electrode and ICP-AES for fluorine and trace elements, respectively.

All three species were colonized by arbuscular mycorrhizal fungi and, in a lower level, by dark septate endophytes on both polluted and reference sites. Root symbioses may play a role in the ability of these plant species to constitute a vegetative barrier.

Keywords: Fluoride pollution, dark septate fungi, arbuscular mycorrhizae, trace elements

A3

Role of plant growth promoting bacteria and soil amendments in phytoremediation of heavy metal contaminated sites

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On a global basis, soil contamination with cadmium, lead, and zinc is one of the most pervasive environmental problems. In the surroundings of a former Pb/Zn smelter in Arnoldstein (Austria) heavy metal concentrations exceed thresholds for food and feedstuffs. Phytoremediation (in our case the combination of immobilization and phytoexclusion) could be a cost-effective system for improvement of the use of contaminated areas. The aim was to study the effects of plant growth-promoting bacteria (PGPB) and immobilizing soil amendments on heavy metal tolerance of plant and uptake. Pot experiments were performed whereby two maize cultivars were cultivated in varying contaminated soil and treatments (*Burkholderia phytofirmans* strain PsJN with and without amendment). Inoculation with strain PsJN significantly improved the root and shoot biomass of maize. Rhizosphere and leaves were analyzed for heavy metal content. Results indicated that immobilizing amendments had significant effects on the reduction of ammonium nitrate extractable Zn (< 80%) and Pb (<50%) compared to the controls. Concentration of Zn and Pb in plants was reduced by combined immobilizer and PGPB up to 65% and 40%, respectively. Three different media allowed the selection of 500 isolates based on colony morphology from contaminated soil and plants. For characterization of bacteria, 16S rDNA genes were sequenced from the isolates and the plant growth-promoting potential was analysed by screening for the production of 1-aminocyclopropane-1-carboxylic acid deaminase, siderophores and indole acetic acid. Selected strains will be further tested for heavy metal mobilization and plant growth-promoting effects in interaction with the plant.

Keywords: Immobilization, Phytoremediation, Treatment, Heavy metals, 16S rDNA

A4

The inoculation of phenotypically interesting bacteria, selected from representative bacterial communities associated with *Brassica napus*, to enhance Cd phytoextraction

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Cultivable bacterial strains associated with field-grown *Brassica napus* (soil, rhizosphere and roots) from a site contaminated with trace elements (Cd, Zn and Pb) and a non-contaminated control site were isolated and characterized.

The phenotypic information of the isolated bacteria suggested that a contaminated environment creates a selective pressure in favor of plant growth promoting bacteria tolerant to specific trace element concentrations, especially in the rhizosphere and roots.

At both field sites the bacterial rhizosphere community was correlated with the bulk soil community, which indicates that site-specific soil factors play a more significant role than root exudates in influencing the composition of rhizosphere communities. On the contrary, endophytic root communities from both sites were similar. We hypothesize that common seed endophytes as well as plant-driven microbe selection are responsible for these similarities.

Plants were inoculated with the best performing bacteria that were selected based on *in vitro* tests. Inoculated and non-inoculated *B. napus* seeds were grown on Cd-containing vertical agar plates and after 10 days the plants were analyzed for their Cd uptake, primary root length and plant biomass. The bacterial *in planta* potential to improve Cd phytoextraction was further investigated during pot experiments using 5 mg Cd per kg sand. Phytotoxicity was evaluated based on growth parameters and the oxidative degradation of lipids.

The next step is to exploit the most appropriate plant-associated bacteria in the field, turning *B. napus* into a Cd phytoextractor that at the same time can be economically valorized.

Keywords: Cadmium, Phytoextraction, *Brassica napus*, Inoculation, Plant-associated bacteria

A5

Phytoprotective effect of Arbuscular Mycorrhizal Fungi on uptake and arsenic accumulation in the Tropical Leguminous species

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The remediation of arsenic (As)-contaminated areas has been a considerable challenge to the scientific community worldwide, especially in mining areas producing large quantities of waste that dramatically affect plant cover. We assessed the effects of inoculation with the arbuscular mycorrhizal fungi (AMF) *Glomus etunicatum*, *Acaulospora morrowiae*, *Gigaspora gigantea*, and *Acaulospora* sp. on four leguminous species, *Acacia mangium*, *Crotalaria juncea*, *Enterolobium contortisiliquum*, and *Stizolobium aterrimum*, as well as determining the effect of AMF on the phytoremediation potential of leguminous plants in As-contaminated soil. The experiment included a control that did not receive AMF inoculation. We used an entirely randomized design with four replicates. The phytoremediation potential of leguminous plants was assessed by the translocation index (TI) and bioaccumulation factor (BF). The protective effect of AMF was assessed by the phosphorus (P)/As ratio, and plant antioxidant enzymes were also examined. The colonization of leguminous plants by mycorrhizal fungi ranged from 24 to 28%, and AMF inoculation led to a significant increase in the dry-matter production of all plant shoots tested. The leguminous plants had low As TI values. AMF inoculation significantly improved the P/As ratio and decreased both the As BF and the activity of antioxidant enzymes, highlighting the phytoprotective effect and potential use of *C. juncea* and *S. aterrimum* species in the remediation of As-contaminated soils. Future studies are necessary to examine both plant and AMF tolerance strategies.

Research supported by CAPES, FAPEMIG and CNPq

Keywords: contaminated soil, trace element, phytoremediation, bio-accumulation, antioxidant enzymes

A6

Improving biomass production of willow for phytoremediation of metal-contaminated soils using plant growth promoting bacteria and fertilization strategies

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Cleaning up metal-contaminated soils using short rotation coppice of willow is a very promising technique. Besides a gradually decontamination of the soil by extraction of metals, willow clones also have an economic advantage for farmers since the harvested biomass can be used for bioenergy purposes. This work aims to increase biomass production of willow by (a) selecting the best performing clones, (b) inoculating these clones with selected plant growth promoting bacteria and (c) testing different fertilization strategies.

A greenhouse experiment was set up using metal-contaminated soil. Two experimental willow clones, *Salix viminalis* (Clone1) and *Salix alba x alba* (Clone2), were used and inoculated with 5 selected PGPB: *Rahnella sp.* (Rh1), *Bosea sp.* (Ro1), *Caulobacter sp.* (Ro2), *Curtobacterium sp.* (St1) and *Pseudomonas sp.* (St2). Half of the plants was fertilized using 2 different techniques, (i) a commercial slow release fertilizer mixed with the soil and (ii) weekly pouring of the plants with a solution of N, K and Mg. The willow cuttings were planted the 17th of February and will be harvested the 17th of May.

Follow up revealed already that bacteria Rh1 and St2 and bacteria Ro2 and St2 increase biomass production of respectively Clone1 and Clone2. Fractionated fertilization with N, K and Mg had no significant effect until now. However, the commercial fertilizer decreased biomass production significantly. Measuring more biomass parameters at harvest will certainly demonstrate more effects of the PGPB and the fertilization strategies. The best performing combinations (clone-bacteria-fertilization) will be *tested in situ* on a contaminated area.

Keywords: Phytoextraction, willow, biomass, PGPB, fertilization

A7

Role of Cu-resistant bacteria in promotion of Cu accumulation and growth, reduction of Cu toxicity in *Commelina communis*

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The effects of heavy metal-resistant bacterial strain USTB-H on Cu accumulation, plant growth and reduction of Cu ion toxicity in hyperaccumulator *Commelina communis* were investigated. The bacterial strain was isolated from Cu-contaminated soils and identified as belonging to *Stenotrophomonas* species. The strain was showed a specific tolerance to Cu through binding the Cu ions to the cell walls to reduce their entry into the cells. The inoculation with USTB-H significantly enhanced citric acid level in plants, which could be an important reason of the increase of Cu accumulation in *C. communis*. The results also showed USTB-H secreted indole-3-acetic acid (IAA) and therefore promoted plant growth. Moreover, the bacteria effectively improved the antioxidant enzyme defence system to alleviate the oxidative damage induced by Cu, including Superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT), and Ascorbate peroxidase (APX). The above results suggested the usefulness of *C. communi* inoculated with Cu resistant bacteria USTB-H as a method for the phytoremediation of Cu-contaminated soils.

Keywords: Cu-resistant bacteria, Cu accumulation, plant growth, antioxidative enzyme defense

A8

Screening of potentially beneficial plant-associated bacteria for use in phytoremediation

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Phytoextraction aims to remove trace metals from the soil through their uptake and accumulation by plants; whereas phytostabilisation aims to establish a vegetation cover and promote *in situ* inactivation of metals. Plant-associated bacteria can improve both processes: inoculating plants with plant growth promoting (PGP) bacteria enhances plant metal tolerance, growth and survival; some microorganisms can reduce metal mobility and improve phytostabilization, while metal-mobilizing microbial strains can increase metal bioavailability, plant metal uptake and phytoextraction efficiency.

Within the framework of the EU FP7 "Greenland" project (266124) this study was aimed at selecting efficient plant-microbial combinations. Bacterial strains were isolated from the rhizosphere of either trace metal-tolerating or –accumulating plants. Seventy-four Cd/Zn-tolerant bacterial strains were associated with pseudometallophytes (*Betula* sp., *Cytisus* sp. or *Festuca* sp.) growing in Pb/Zn mine tailings, and 350 Ni-tolerant bacterial strains were isolated from the rhizosphere of the Ni-hyperaccumulators *Alyssum serpyllifolium* subsp. *lusitanicum* and *A. serpyllifolium* subsp. *malacitanum* growing in ultramafic soils. Isolates were characterised genotypically (BOX-PCR, 16S rDNA) and phenotypically (metal-tolerance, metal-solubilising capacity, antibiotic resistance, biosurfactant production and PGP traits). From these bacterial collections 30 isolates were selected (on the basis of phenotype) for re-inoculation trials. Inoculated plants were grown in 2:1 perlite:sand substrate for 8 weeks with metal (Cd, Cu, Ni or Zn)-enriched Hoagland solution. Four plant species were used: *Alyssum pintodasilvae*, *Brassica juncea*, *Festuca pratensis* and *Salix viminalis*. Some isolate-plant combinations showed potential application in phytostabilisation or phytoextraction techniques although further studies in soil conditions are required.

Keywords: hyperaccumulators, metal-tolerant gramineae, rhizobacteria, woody crops, PGP bacteria

A9

Exploitation of PGPB strains within phytoremediation protocols for the reclamation of soils contaminated by roasted arsenopyrite residues

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This study aimed at evaluating the effect of the bioaugmentation of PGPB strains, highly resistant to arsenic, on the efficiency of *Pteris vittata* - grown on an arsenopyrite contaminated soil - in phytoextracting this metalloid. The research was carried out in the frame of the R.E.P.E.T. (Rhyzosphere Enhanced Phyto-Extraction Technology) Project, granted by the Tuscan Regional Government, for the reclamation of a former dump site highly polluted with As. Initially, a characterization of the bacterial community acclimated to As was carried out starting from enrichment cultures. 109 bacterial strains were isolated in axenic culture and phylogenetically identified through 16S rRNA sequencing analysis. The bacterial isolates were then analyzed for the occurrence of PGP traits such as ACC-deaminase activity, IAA release, and production of siderophores as well as for the presence of As-resistance genotypes such as *ars* and *aox.* On the basis of the evidences gained, three bacterial strains - *Ochrobactrum* sp. E, *Pseudomonas putida* O and *Achromobacter xylosoxidans* P - were chosen for the bioaugmentation trials. Tests were performed in mesocosms under glasshouse conditions for six months. At the end of the experiment, both plant biomass production and total As content in plant tissues were measured. The persistence of bacterial inoculawas assessed by means of PCR-DGGE analyses. Results revealed that the bacterial inocula exerted positive effects on plant biomass production, with an increase up to 20% when strain O and P were added. Nevertheless, the total phytoextraction efficiency seemed not to be highly influenced by the inoculation of PGPB strains.

Keywords: *Pteris vittata*, PGPB, arsenic, phytoremediation, bioaugmentation

A10

Phytoremediation abilities of maize (*Zea mays* L.) inoculated with plant growth promoting rhizobacteria in zinc and cadmium contaminated soils

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Heavy metal contaminated soils are a worldwide problem. Efforts to reduce their high impact and diminishing their concentration by using sustainable and low-cost strategies such as phytoremediation may be a promising path in remediation techniques and may lead to positive impact towards the resolution of this problem. Maize is one of the most cultivated crops worldwide and beyond its food energy value it is also considered as an accumulator and a metal tolerant plant especially for Cd and Zn suitable for biomass production. It has been reported that crop yield can be increased by plant growth promoting rhizobacteria which induce mechanisms that can increase absorption of heavy metals from soil.

In this work, *Zea mays* was grown in Cd and Zn contaminated soils at four different concentrations of each metal in greenhouse conditions. After germination, pots were inoculated with two plant growth promoting rhizobacteria - *Ralstonia eutropha* and *Chryseobacterium humi.* At the end of the experiment plants were harvested and levels of Zinc and Cadmium in their roots and shoots were determined. Dry biomass, nitrogen and phosphorus levels were also assessed. The bacterial dynamics were evaluated by using molecular tools such as denaturing gradient gel electrophoresis.

Bacterial inoculation was significant in *Zea mays* growth, providing a better settlement of these plants in contaminated soils. Hence, this study is a contribution to recognize the importance of the interaction of several factors, such as bacteria, soil and heavy metal levels, with plant growth in order to develop phytoremediation applications.

Keywords: Phytoremediation, soil pollution, *Zea mays*, heavy metal, plant growth promoting rhizobacteria.

A11

Anatomy and ultrastructure alterations of *Leucaena leucocephala* (Lam.) inoculated with mycorrhizal fungi in response to arsenic-contaminated soil

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Many studies demonstrate the potential application of mycorrhizal fungi (AMF) for remediation purposes, but little is known on AMF potential to enhance plant tolerance to As and the mechanisms involved in this process. We carried anatomical and ultrastructural studies to examine this symbiotic association and the characteristics of shoots and roots of *Leucaena leucocephala* in As-amended soils (35 and 75 mg As dm^{-3}). The experiment used 3 AMF isolates from uncontaminated soils: *Acaulospora morrowiae*, *Glomus clarum*, and *Gigaspora albida*; a mixed inoculum derived from combining these 3 isolates (named Mix AMF); and, 3 AMF isolates from As-contaminated areas: *Glomus clarum, Paraglomus occultum*, and *Acaulospora morrowiae*. Phytotoxicity symptoms due to arsenic contamination appeared during plant growth, especially in treatments without AMF application. Inoculation with *Glomus clarum* and the mixture of species (*Acaulospora morrowiae, Gigaspora albida*, and *Glomus clarum*) resulted in better growth of *L. leucocephala* in soils with high As concentrations, as well as significant As removal from the soil, showing a potential for using AMF in phytoextraction. Light and scanning electron microscopy studies showed damage in all treatments, with ultrastructural changes being observed in leaves and roots of *L. leucocephala*, especially with the addition of 75 mg dm^{-3} of As.

Keywords: Phytoprotection, Mycorrhizae, Light microscopy, Electron Microscopy

A12

Effects of Cadmium on growth and accumulation of some commonly grown vegetables

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Cadmium (Cd) contamination is a major environmental and public health problem in Mae Sot District, Tak Province, Thailand. The Cd polluted areas are downstream from a canal that passes through zinc mines. The local inhabitants grow vegetables for their own consumption and sale in the local market. In this study, we selected three commonly grown vegetables in the Brassicaceae family i.e., Pak choy (*Brassica campestris* var. chinensis), Chinese kale (*Brassica alboglabra* Bailey) and Chinese cabbage (*Brassica pekinensis* Rupr.), as our test plants. In the hydroponic experiment, we studied the effects of Cd (as $CdNO_3$) at various concentrations (0-9 mg L^{-1}) on % seed germination, inhibition of root and shoot length of the three tested plant species. In the field experiment, arbuscular mycorrhizal fungi (AMF) and Cd-tolerant fungi (CTF) isolated from the Cd polluted soil were separately applied to three vegetable seedlings before planting to determine if the microorganisms could reduce the uptake of Cd into the edible parts of the tested vegetables. The experimental plots were conducted for 8 wks in the high Cd contaminated area with three replicates. All plant materials and soils at the rhizosphere were sampled and digested with HNO_3 and $HClO_4$ before analyzing with ICP-OES. The results will be discussed.

Keywords: Cadmium, Phytoextraction, Arbuscular mycorrhizal fungi, Cadmium tolerant fungi, Vegetables

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B: Metals: Toxicity and Uptake

B1

The effect of chemical additives on availability of heavy metals (Pb, Cd and Zn) of soil

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In this research, the availability of heavy metals of Pb,Cd, and Zn has been studied by the use of different additives on the soil. Extraction process of heavy metals from soil have done by distilled water, calcium chloride, potassium nitrate, ammonium citrate and EDTA at concentration of 0.25, 0.5, 1, 2 and 4 Molar and by the use of a rotary shaker with 75 rpm rotation for 10 hours; and then the concentration of each element have been measured by Atomic Absorption Spectrometer(A.A.S). Concentration of Pb, Cd and Zn in studied soils were obtained as 50±12.5127±16.13510 and 3000±180 mic/gr respectively. The availability of Zn by different additives and also in distilled water was higher than Pb and Cd. Mean extraction of Pb by EDTA, Ammonium Citrate and Potassium Nitrate was 2110,1025 and 195 mic/gr respectively. And also the mean Cadmium by these compounds were 83,71,and 56 and for Zn were 7894,4982 and 2744 mic/gr respectively. Best concentration of Zn and Cd was extracted by potassium nitrate at morality of 2 and by EDTA and ammonium citrate at morality 1.And also the best amount of Pb was extracted from soil by EDTA and ammonium citrate at morality 1 and by potassium nitrate on concentrations above one. Application of chemical additives such as EDTA and ammonium citrate plays an important role in dissolution of heavy metals and thus in increasing their availability in soil.

Keywords: Availability, Heavy metals, Soil, Additives

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B2

Eriophorum sp. and *Lolium perenne* L. metabolic adaptations to metals- and metalloids-induced anomalies in an industrial area

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The current study aimed to investigate the role of glutathione (GSH) and its associated enzymes in the metabolic adaptation of two grass species namely *Eriophorum* sp. and *Lolium perenne* L. to metals and metalloids stress in the vicinity of a chemical industrial complex (Estarreja, Portugal). Both soils and plants from the industrial area exhibited differential concentrations of major metals and metalloids including As, Cu, Hg, Pb and Zn. In particular, *L. perenne* shoot displayed significantly higher and lower concentrations of Pb and As respectively at contaminated site (vs. *Eriophorum* sp.). Irrespective of sites, *L. perenne* shoot exhibited significantly higher total GSH pool, oxidized glutathione (GSSG) and oxidized protein (vs. *Eriophorum* sp.). Additionally, severe damages to photosynthetic pigments, proteins, cellular membrane integrity (in terms of electrolyte leakage) and lipid peroxidation were also perceptible in *L. perenne* shoot. Contrarily, irrespective of the sites, activities of catalase and GSH-regenerating enzyme, GSH-reductase and GSH-metabolizing enzymes such as GSH-peroxidase and GSH-sulfo-transferase were significantly higher in shoot of *Eriophorum* sp. The study revealed that despite the higher content of GSH pool, *L. perenne* suffered higher (in terms of high GSSG and oxidized protein) with multi-metals stress due to its higher exhibition of shoot Pb, low reactive oxygen radical-processing potential (exhibited in terms of low catalase activity) and poor GSH-pool-utilization efficiency depicted by significantly lower activities of GSH-associated enzymes (vs. *Eriophorum* sp.). The outcome of the present study may be significant for understanding vital GSH-mediated metals and metalloids tolerance mechanisms in plants under environmentally realistic conditions.

Keywords: Antioxidants, Glutathione, Grass species, Oxidative stress, Metals-metalloids pollution, Soils, Tolerance

B3

Cr(VI) phytoremediation strategy of *callitriche cophocarpa*

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The present work focused on the qualitative and quantitative investigation of Cr(VI) phytoremediation strategy of aquatic cosmopolitan plant *Callitriche cophocarpa*. The research relayed on the analysis of the redox reaction of Cr(VI) to Cr(III) that is considered to be important detoxification mechanism of highly reactive and mobile Cr(VI) ions. The plant species was chosen according to its unusual accumulating potential of Cr (Augustynowicz *et al*., 2010). Plants were immersed for 5 days in 1 mM (52 mg l^{-1}) Cr(VI) (potassium dichromate) solutions in *semi*-natural conditions, *i.e.* in water of environmentally-relevant composition and appropriate light intensity. Total amount of Cr was removed from the solution up to the final value of 54%. No plant-induced Cr(VI) reduction attending Cr accumulation was observed in Cr(VI) solutions besides from the fourth day of incubation. Contrary to these results, according to the method of electron paramagnetic resonance spectroscopy (*L-band EPR*), biphasic signal of Cr(V) attending Cr(VI) to Cr(III) reduction was detected inside the plant tissue every day of investigations. Our results show that *C. cophocarpa* does not induce Cr(III) precipitation in aquatic environment, although the plant exhibits effective mechanism of Cr(VI) reduction inside the cells. Therefore, we assume that phytoextraction but not phytostabilization is the main strategy of Cr(VI) phytoremediation by *C. cophocarpa* in aquatic systems.

Keywords: *Callitriche*, chromium, EPR, phytoremediation, aquatic macrophytes

Reference:

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B4

Variability of cadmium tolerance and accumulation in the model species *Arabidopsis halleri*

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The Brassicaceae *Arabidopsis halleri,* which is able to grow on both metal contaminated and non-contaminated environments, is a model species to study metal tolerance and accumulation. This species shows exceptional ability to tolerate and accumulate Zn and Cd and is closely related to the model plant *Arabidopsis thaliana*. In contrast to Zn , knowledge on Cd tolerance and accumulation in *A. halleri* remains sparse. Cd tolerance seems to be constitutive within the species (while Cd hyperaccumulation is not) but only two metallicolous populations were studied in controlled conditions (Bert *et al.*, 2003; Zhao *et al.*, 2006). To understand the evolution of these complex naturally-selected traits in *A. halleri*, an important task is to characterize their natural variation within the species.

We have phenotyped Cd tolerance in eight populations of *A. halleri* from metal contaminated and non-contaminated sites. Cd accumulation and mineral nutrient profile was also estimated in these populations after contamination at different Cd concentrations. In particular the interaction between Cd and Ca has caught our attention. Cadmium shares many physical similarities with Ca, notably charges and ionic radius and competition between these two elements is well known in plants. In non-hyperaccumulator species, Cd was shown to be transported through Ca transport systems and Ca addition has a protective effect against Cd toxicity. Nevertheless, interaction between Ca and Cd was poorly investigated in hyperaccumulators. Possible changes in Ca homeostasis to adapt to high cellular Cd levels are studied.

Keywords: hyperaccumulator, cadmium, natural variation, calcium, *Arabidopsis halleri*

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B5

Effect of Poultry Compost on Bioavailability of Fe, Cu and Pb and their Uptake by Maize in Spent Engine Oil Contaminated Soils

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The study investigated the bioavailability of Fe, Cu and Pb and their uptake by maize cultivated on spent-engine-oil (SEO) contaminated soils when poultry compost (PC) was used as soil enhancer. This was a greenhouse experiment. It was a factorial combination of three SEO treatments (0%, 1% and 2%) with six levels (0, 2, 4, 6, 8 and 10 t ha^{-1}) of PC applications in a completely randomized design. The increased soil pH and percent soil organic carbon favored metals' solubility and bioavailability. As metals bioavailability increased with increase in PC, their uptake by maize were enhanced. Metals availability in the soil and uptake by maize was in the order Fe > Pb > Cu. Approximately 10% of metal uptake in the root was found in the shoot of the test crop. There was evidence of nutritional quality reduction of maize when cultivated on SEO contaminated soils with or without poultry compost fertilization.

Keywords: metal uptake, nutritional quality, poultry compost, soil health, spent engine oil

B6

2D DiGE coupled with dual thiol-staining reveals proteins targeted by Cd induced oxidative stress in chloroplasts of *Arabidopsis thaliana*

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Reactive oxygen species (ROS), play a major role in signaling and regulation. Especially in chloroplasts, where most of the plant cell reducing potential is produced by the electron transport chain, enzyme activity can be controlled by oxidation or reduction of cysteine thiols. Furthermore, the natural formation of ROS during photosynthesis renders mechanisms of protection against excess ROS vitally important.

The accumulation of ROS during cadmium (Cd) exposure can exhaust protective measures and create an oxidative stress by induce a shift in redox homeostasis. The increase of the oxidative state in the cell can lead to the oxidation of regulatory cysteines and have a major impact on enzyme activity in plant cell metabolism.

Differential in Gel Electrophoresis (DiGE) allows the quantification of protein abundance on 2D electrophoresis gels. Coupled to a dual thiol staining, the redox state of protein thiols can also be measured. The reduced and oxidized thiols are labeled in the same sample with different molecules and after separation by 2D electrophoresis the ratio between oxidized and reduced forms of proteins can be calculated. With this technique it is possible to detect key proteins that undergo redox changes during Cd induced oxidative stress.

Keywords: Arabidopsis, Cadmium, Cysteine oxidation, Oxidative Stress, Proteomics

B7

Effects of cultivation systems on poplar proteome under control and cadmium exposed conditions

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In order to map the effects of one specific actor, ideally all other actors should remain the same. Due to high variation in field situations, cultivation methods providing high controllable conditions and rendering as low variation as possible are mostly chosen to work with for molecular research. Although gained insights in molecular systems only apply for highly controlled lab conditions, they often serve as basis to formulate hypothesis for future field experiments.

In order to get more insights in the basic molecular mechanisms of phytoextraction on cadmium contaminated fields, a first study was performed to reveal the effect of cadmium exposure on the proteome of *Populus deltoides x (trichocarpa x deltoides)*. Therefore, a hydroponic cultivation system was chosen to minimize all additional variations. However, focusing on future field experiments, extrapolation of the gained insights was questioned: using hydroponic cultivation systems involves a risk at anaerobic conditions, prevents the establishment of a rhizosphere, forms roots that are incomparable to roots formed in soils and supplies a steady and constant availability of nutrients. Moreover, it is known that plants in hydroponic cultivation systems accumulate more cadmium than those grown in soil systems (Grant et al, 1998). Therefore, with the prospect to field experiments, the effect of cadmium on the proteome of *Populus deltoides x (trichocarpa x deltoides)* cultivated in soils was studied. Obtained results were used to reveal the effect of cultivation methods and to give an idea of the possibility to extrapolate data gained by controlled lab conditions to field situations.

Keywords: Phytoextraction, Poplar, cadmium, DIGE, cultivation systems

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B8

Effect of elevated manganese levels on *Canna* × *generalis* growth and Mn uptake

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Manganese is a naturally occurring element in rock, soil and water, concentration of which is constantly increasing in the environment. Despite manganese is an essential microelement, in high concentrations it may be harmful for plants, animals and human beings.

The aim of this study was to assess potential use of *Canna* × *generalis* in manganese phytoextraction and possible negative impact of elevated levels of manganese on selected morphological and physiological parameters in *Canna* × *generalis* plants. Plants were cultivated in nutrient solution (Hoagland) for 5 weeks from supplying various doses of manganese (between 0.5 and 1600 mg dm^{-3}). Non-destructive physiological measurements were conducted in one week intervals. Manganese content, relative water content (RWC) and biomass accumulation were assayed at the end of the experiment.

Obtained results show that *Canna* × *generalis* is able to grow and accumulate large quantities of Mn, up to 25200, 1500 and 24900 mg kg^{-1} DW for roots, shoots and leaves respectively. *Canna* × *generalis* turned out to be very tolerant to increased manganese concentrations in hydroponic culture. Negative effect on growth, development and biomass production was recorded only in the highest concentration. Those data correspond well with decreased total chlorophyll content, values of selected chlorophyll *a* fluorescence parameters and reduced RWC. Lower manganese concentrations did not cause any symptoms of toxicity. These results supports the conclusion that *Canna* × *generalis* can be used in phytoremediation of manganese.

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Keywords: manganese uptake, stress tolerance, phytoextraction, *Canna* × *generalis*

B9

Extraction of Some Economic Elements Using Phytomining from Soil Deposits at Um-Bogma Area, South Western Sinai, Egypt

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The present study aimed to evaluate the ability of some metal scavenging plants, viz, Sunflower (Helianthus annus) and Kenaf (Hibscus cannbinus)to uptake naturally occurring metals of economic value i.e. U, Co, Cu, Cd, Pb, Zn and rare earth elements. And to evaluate the effect of EDTA in enhancing uptake of U, Co, Cu, Cd, Pb and Zn.

Under green house conditions Sunflower and Kenaf plants were grown for different periods (20, 40 and 60 days) for each plant in pots (12 Kg) of clay soils were collected from Um-Bogma area which contains 300, 550, 1550, 200, 1400, 12000 and 650 mg/kg of U, Co, Cu, Cd, Pb, Zn and rare earth elements, respectively.

Results revealed that Sunflower accumulated a concentration of 1098.5, 15.9, 30.4, 80.6, 0.06464 and 528.9 for Zn, Cd, Cu, Co, U, Pb in roots, respectively.

Keywords: phytomining, Sunflower, Kenaf, U, Cd, Pb, Co, Cu, Zn, EDTA

B10

Diversity of lead hyperaccumulation in *Hirschfeldia incana*: Perspectives for phytoremediation

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Since the early 20th century, Morocco is known for its mining activities. This conducted to several mine sites that are sources of pollution by heavy metals. An exploration of plant species growing on abandoned lead mining sites in the east part of Morocco was considered in the context of phytoremediation program. Among the various plants identified, we selected *Hirshfeldia incana*, a lead hyperaccumulator and a member of the Brassicaceae family, to study mechanisms of Pb tolerance.

Physiological study of *H. incana* in controlled conditions showed a significant difference in lead tolerance and accumulation between a metallicolous population (M) from polluted sites (Oued el Heimer) and non metallicolous population (NM) from unpolluted sites (Tetouan). Results show that NM population is less tolerant but accumulates more lead than the M population.

The close genetic proximity between *H. incana* and *A. thaliana* (89%) allows us to conduct an heterologous hybridization of *H. incana* transcripts on *A. thaliana* microarrays and several genes appear as regulated by lead. Among this list of genes, five genes known to be involved in the molecular responses to heavy metals (metallothionein MT2, Multidrug Resistance-associated Protein MRP14, cooper chaperone CCH, ABC transporters WBC23 and general control non-repressible2 GCN2) were chosen for genetic comparison between M and NM populations of *H. incana*. Sequences analysis didn't show intrapopulation variation whereas the interpopulation variation is significant for the five genes. Gene expression analysis is under investigation to check whether this variability can be associated with the presence of lead in polluted sites.

Keywords: *Hirschfeldia incana*, lead, hyperaccumulation, phytoremediation

B11

Photoprotective and antioxidant responses of *Populus* clones I-214 and Eridano submitted to elevated Zn concentrations

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Rooted cuttings of two poplar clones (*Populus* x *canadiensis* Mönch.- clone I-214 and *P. deltoides* x *maximowiczii* – clone Eridano) were treated for three weeks in hydroponic culture with high levels of Zn (1mM and 5mM). In plants, excessive Zn accumulation can contribute to the formation of Reactive Oxygen Species (ROS) whose accumulation can directly affect growth and photosynthetic activity. Here we characterized photoprotective and antioxidant responses in leaves to determine the ability of both clones to cope with stress conditions induced by Zn treatments.

Chloroplast pigments neoxanthin, lutein, violaxanthin (V), antheraxanthin (A), zeaxanthin (Z), chlorophylls a, and b, β -carotene, α-tocopherol and (β+ γ) tocopherol were analyzed by HPLC while ascorbate (ASA), glutathione (GSH) and phenolic content were determined by spectrophotometric techniques. Furthermore, gas-exchange and chlorophyll fluorescence were characterized with portable systems (Li-COR 6400 and Mini-Pam Walz, respectively).

Increasing [Zn] markedly declined photosynthesis, stomatal conductance and growth especially in I-214, which showed signs of very severe damage (*i.e.* wilted and necrotic leaves).

The elevated ASA, α- and (β+ γ) tocopherol content and de-epoxidation index (A+Z/VAZ), which is related with the dissipation of excess irradiance energy as heat, shown by Eridano clone under increasing [Zn], maintained the PSII functionality as observed from the higher values of maximum PSII quantum.

Eridano showed a higher tolerance to Zn with a greater capability to accumulate Zn in roots. Lower Zn accumulation in Eridano leaves was associated to the observed higher photo – and antioxidant protection and improved gas-exchange rates and growth with respect to I-214.

Keywords: Antioxidants, heavy metals, phytoextraction, phytoremediation, poplar

B12

Antioxidant response to Cd of two populations of *Dittrichia viscosa*

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Dittrichia viscosa (L.) Greuter grows spontaneously in metal-polluted sites of Asturias (Spain). Because in phytoremediation the application of plant species adapted to local climatic and soil conditions is often favored we have tested its Cd accumulation and tolerance.

Furthermore, *D. viscosa* is a pseudo-metallophyte, so in order to elucidate if its metal tolerance is a constitutive trait we compared two clones from contrasting populations: DV-A (metallicolous) and DV-W (non-metallicolous). Plants of both clones were grown in hydroponics with a culture solution supplemented with 0, 5, 10 and 15 mg Cd L^{-1}. After 10 days of culture, cadmium accumulation and plant growth were measured, as well as other stress markers such as photosynthetic pigments and efficiency, lipid peroxidation, phenols, H_2O_2 and proline. We also analyzed the activity of the antioxidant enzymes guaiacol and ascorbate peroxidases, catalase, superoxide dismutase and gluthatione reductase and their isozyme pattern.

Our results confirmed a high Cd tolerance and accumulation of *D. viscosa* in both clones, which suggests that these traits are constitutive in this species. However, when Cd concentration in solution exceeded 10 mg Cd L^{-1}, DV-A was more tolerant than DV-W. The physiological mechanisms involved in Cd tolerance also differed between them, although phenols and guaiacol peroxidase played an important role in both clones. The effective Cd detoxification of DV-A consisted mainly in a promoted ascorbate peroxidase activity and a better efficiency of enzymes catalase and glutathione reductase.

Keywords: antioxidant, cadmium, *Dittrichia viscosa*, population, tolerance

B13

Eichhornia crassipes (Mart.) Solms., a hyperaccumulator: a potential candidate for studying heavy metals stress

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Water hyacinth (*Eichhornia crassipes*) has been well-known for their capacities to hyper accumulate heavy metals from contaminated water. The X-Ray Diffraction (XRD) and Energy Dispersive X-ray Elemental Spectrometry (EDX) analysis of the soil samples revealed the presence of Fe, Mn, Cu, Zn, Hg, Cr, Pb, Ni, Co, As and Cd. However, the Atomic Absorption Spectrometry (AAS) study of soil and water has ascertained the contamination of heavy metals that were in Pb>Cr>Cu>Ni>Cd and Pb>Cr>Ni>Cu>Cd order in soil and water respectively. The AAS analysis of plant samples quantized that heavy metals Pb, Cr, Ni, Cu and Cd accumulated to higher extent. The discrete variation in protein profile during stress may suggest the synthesis of specific stress responsive proteins playing crucial role in the stress response. Furthermore, high expression of alcohol dehydrogenase (ADH), peroxidase (POX) and altered regulation of esterase (EST) were observed in root tissue during stress. The enhanced accumulation of metals in Eichhornia and altered regulation in proteins and enzymes strengthen its candidature to be used as an organic indicator of heavy metal contamination. However, this demands thorough specific gene expression and regulation study at the molecular level.

Keywords: *Eichhornia,* Heavy metals, isozymes, phytoremediation

B14

Phytochelatins, organic acids and cell walls in relation to Cd accumulation of *Dittrichia viscosa*

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Plants may chelate the absorbed metal through glutathione, phytochelatins and organic acids among others, and they can also limit the entrance of Cd by binding it to the cell wall. *Dittrichia viscosa* accumulates high Cd concentrations in its tissues, but the mechanisms involved in this capacity are still unknown. The aim of this study was to evaluate the Cd detoxification strategies used by this species.

We have compared the clones DV-A (metallicolous) and DV-W (non-metallicolous) grown in hydroponics with 0, 5 10 and 15 mg Cd L^{-1} for 10 days and afterwards we measured glutathione, phytochelatins and organic acids concentrations and the fraction of the total Cd bound to cell-wall components.

Plants of DV-A and DV-W accumulated Cd over the hyperaccumulation value and most of it was kept in the cell wall. In both clones, phytochelatins rose with increasing Cd concentration. Plants of DV-W synthesized much more glutathione and phytochelatins than DV-A in their shoots, and, at the lowest Cd concentration, also in roots. As regards organic acids, we can highlight that in contrast to DV-A, in DV-W the synthesis of malonic and malic acids increased greatly with Cd exposure. On the contrary, citric acid concentration in shoots decreased with Cd treatment in both clones whereas in roots it increased.

Thus, retention of Cd in the cell wall seems to be an important mechanism involved in Cd tolerance and accumulation of *D. viscosa*. Phytochelatins and organic acids might also have a role in Cd detoxification, especially in non-metallicolous populations.

Keywords: organic acids, cadmium, cell wall, *Dittrichia viscosa*, phytochelatins

B15

Phytoremediation using alder species: physiological and molecular analyses reveal evidences of inter- and intra-specific variability

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Since two decades, the potential of herbaceous plants to be used in phytoremediative techniques has been widely studied but research activities on tree species, especially turned toward the molecular scale, remain more scant. Although the extraction and accumulation of trace elements by trees appears very limited, Mertens *et al.* (2004) pointed out that some species, and notably those from the genus *Alnus*, might be putative phytostabilisators. Nonetheless, research programs on the response of trees to metallic stress have rarely focussed on the molecular mechanisms occurring within the plant.

To assess the potential of alder species to remediate trace elements and to describe their responses at the molecular scale, 2 *A. glutinosa* (L.) and 1 *A. incana* (L.) vegetatively propagated clones were grown in liquid medium supplemented with a mixture of Cd, Ni and Zn. By combining physiological measurements with the quantities of metals accumulated in the saplings, evidence of intra- and inter-specie variability to face the constraint were observed. The comparison of the root and leaf carbohydrates, particularly those involved in the primary metabolism, between plants exposed or not to the metal constraint indicates that the response of *A.incana* (L.) is much more important than that of *A. glutinosa (L.)*. The proteome analysis confirmed this observation. Furthermore, the most pronounced and numerous proteome changes were observed in roots whereas the leaf proteome was less affected. Finally, not only plant but also bacterial proteins where found differentially expressed in roots, indicating that the actinorhizal symbiosis is disturbed/modulated by the polymetallic constraint.

Keywords: phytoremediation, heavy metal, tree, proteomics, carbohydrate

Reference:

Mertens J, Vervaeke P, Schrijver AD, Luyssaert S (2004). Metal uptake by young trees from dredged brackish sediment: limitations and possibilities for phytoextraction and phytostabilisation. Science of The Total Environment, 326: 209-215.

B16

Nitrate nutrition status modulates stress responses of alfalfa plants to mercury

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Mercury (Hg) is one of the most dangerous pollutant heavy metals to the environment. Its accumulation in plants causes several negative effects, among them the induction of oxidative stress. Nitrogen (N) is a fundamental macronutrient in plants, limiting cellular metabolism and overall performance under environmental stress (Vanacker *et al*., 2006). Nitrogen is fundamentally assimilated as NO_3^- after its reduction to NO_2^- by the enzyme nitrate reductase (NR), key step prior the formation of NH_4^+ by nitrite reductase. We studied the physiological effects of Hg (0, 6 and 30 µM) in alfalfa plants grown with low NO_3^- (2 mM; LN) and high (12 mM; HN) concentrations. Several parameters of oxidative stress such as lipid peroxidation, chlorophyll content, biothiol concentration and ascorbate peroxidise (APX), glutathione reductase (GR) enzymatic activities were analysed. Total N accumulation (Kjeldahl) and NR activity were studied also as N-nutrition related indexes. In addition, the expression of APX, GR and NR was examined by Western-blot immunodetection. Plants grown with HN augmented their biomass, and had reduced stress symptoms. For example, GR activity is a very sensitive index of Hg toxicity in plants (Sobrino-Plata *et al*. 2007); and it was clearly less inhibited in HN plants than in LN ones exposed to 6 µM Hg. Therefore, our results showed that plants cultivated in HN were less affected by Hg, highlighting the importance of an appropriate management of the N nutritional status to improve tolerance to heavy metal stress.

Keywords: alfalfa*, Medicago sativa*, mercury, nitrate nutrition, oxidative stress

References:

Vanacker H, Sandalio LM and Jiménez A (2006). Roles for redox regulation in leaf senescence of pea plants grown on different sources of nitrogen nutrition. Journal of Experimental Botany,57:1735-1745.

Sobrino-Plata J, Ortega-Villasante C, Flores-Caceres ML, Escobar C, Del Campo FF, Hernandez LE (2009). Differential alterations of antioxidant defenses as bioindicators of mercury and cadmium toxicity in alfalfa. Chemosphere, 77:946–954.

B17

Cadmium-Induced Oxidative Stress and Mitochondria: A Kinetic Study in *Arabidopsis thaliana*

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Next to halting further spread of toxic metals into the environment, it is critical to stimulate the development of applied phytoextraction technologies and/or selection of crops with enhanced yield under suboptimal conditions such as cadmium exposure. Cadmium (Cd), a non-essential element, induces oxidative stress-related damage versus acclimation in *Arabidopsis thaliana*. As key players in redox homeostasis and signaling at the crossroads of life versus death, mitochondria could be involved in Cd-induced responses via their enzymatic antioxidative defense system. Also, the mitochondrial alternative oxidase (AOX) is commonly regarded as target and regulator of plant stress responses. Due to its ability to reduce mitochondrial ROS production, we hypothesize that AOX and related processes in the alternative respiratory chain are critical in plant responses to Cd-induced oxidative stress. Transcript levels (qRT-PCR) of genes encoding mitochondrial antioxidative and respiratory enzymes were determined in *Arabidopsis* exposed to environmentally realistic Cd concentrations (5 and 10 μM) in a kinetic setup (0, 2, 24, 48, 72 h). While only slight changes were observed for the antioxidative enzymes, our analysis revealed that roots and shoots both invest in the alternative respiratory chain at transcriptional and protein levels. This supports a role for alternative respiration in bypassing Cd-induced oxidative damage in *Arabidopsis*, which could be important in selecting plants with enhanced yield on Cd-polluted soils.

Keywords: cadmium, oxidative stress, mitochondria, signalling

B18

Structural and functional responses of sunflower and tobacco grown on a Cu-contaminated soil series

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A sunflower mutant line and a tobacco motherline, selected for their high shoot Cu, Zn and Cd concentrations, were grown on a series of Cu-contaminated soils (13-1020 mg Cu kg^{-1}) to assess their potential uses for biomonitoring in a routine way and phytoremediation. Morphological and biochemical plant responses and shoot composition were measured upon harvest. Modeling of dose-effect relationship (DER) was carried out for relevant plant parameters and indicators of Cu exposure.

Morphological parameters of sunflower, i.e. hypocotyl length, shoot and root yields, and leaf area, decreased as Cu exposure rose and their DER well fitted a sigmoid curve. The EC_{50} of these parameters ranged between 200 and 400 mg Cu kg^{-1} soil, corresponding to 290-430 µg Cu L^{-1} in soil pore water, and 30-40 mg Cu kg^{-1} DW in shoots. Biochemical parameters were early endpoints, with relatively high signal intensity, but often displayed a DER with a hormesis effect. Correlation between total antioxidant capacity and photosynthetic pigment density suggested their influence, especially carotenoids to prevent oxidative stress. Copper excess in roots and shoots led to imbalance in nutrient homeostasis. Tobacco showed higher Cu-tolerance and phytoextraction capacity due to its antioxidant potential. The EC_{50} for biochemical parameters was in the 600-700 mg Cu kg^{-1} soil range, corresponding to 480-500 µg Cu L^{-1} in pore water. The antioxidant status in tobacco was not provided by increase in carotenoids, but by other metabolites. The usefulness of this sunflower mutant line and tobacco mother line for biomonitoring and phytoremediation of Cu-contaminated soils was discussed.

Keywords: antioxidant capacity, mutant line, phenotypic trait, phytoextraction, phytotoxicity

B19

Relationships between chemical and biological factors in trace element-contaminated soils managed using Gentle Remediation Options (GRO)

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During the past decades a number of field trials with GRO have been conducted throughout Europe. Each research group adopted its own strategies selecting methods to assess the remediation success. This study aims at selecting methods that describe the bioavailable/bioaccessible trace element (TE) fractions among European case studies (of the EU-FP7 project GREENLAND, No. 266124) and are applicable as indicators for GRO success and sustainability. Two sets of tests were pre-selected based on available literature and previous experience, one for determining TE bioavailability/bioaccessibility in untreated soils and soils treated using GROs (e.g. phytoextraction, phytostabilization) and another for characterizing soil functionality and ecotoxicity, as well as soil microbiological parameters. Soil samples from several case studies representing one of the main GROs (phytoextraction in Belgium, Sweden, Germany, Switzerland and France, and aided phytostabilization in Poland, France and Austria) were collected and analyzed applying the selected tests. Quantifying the bioaccessible TE fractions, the strength of the extractants and extracted Cd, Pb and Zn concentrations were generally in decreasing order: water<$NaNO_3$<NH_4NO_3<EDTA<*aqua regia*. An exception was a very acidic soil where no differences between the water, $NaNO_3$, NH_4NO_3 and EDTA extractions were observed for Cd and Zn. Putting together the preliminary results of the chemical (extractable TE) with the biological parameters (plant growth, worm avoidance, growth and reproduction of nematodes) showed that the best correlation could be observed between worm avoidance and water extractable Cd, Pb and Zn and ammonium nitrate extractable Cd and Pb. Further analyses are pending.

Keywords: bioaccessible trace elements, plant growth, soil extractions, risk assessment, worm avoidance

B20

Sandy soil amended" with Bentonite clay" enhance accumulation of zinc and chrome by Mediterranean halophyte species *Atriplex halimus* L.

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In this work, absorption of zinc (Zn) and chromium (Cr) by *Atriplex halimus* cultured on sandy soil and sandy soil amended with natural Bentonite were studied. Two metal concentrations (62,50 mg Kg^{-1} and 125 mg Kg^{-1}) were used.

The parameters measured were plant growth, biomass production and plant metal content in different plant organs. Results showed in first, that plant growth is very affected on sandy soil than in soil amended with bentonite. The stem growth of *Atriples halimus* is more affected than roots by metals in sandy soil. Cr is more toxic than Zn. The biomass decrease proportionally with increase metal dose applied in soil. However plant biomass yield is very high on soil amended with bentonite than in sandy soil.

Atriplex halimus Mediterranean plant is saltbushes that have a high potentiality to absorb quantities of Zn and Cr. The yield of metals absorbed must be enhanced when use 10% of bentonite as amendment in sandy soil.

Keywords: *Atriples halimus*, Bentonite Amendment, sandy soil, Zinc, Chromium, Heavy metal.

B21

Genetic architecture of cadmium tolerance in the metal hyperaccumulator *Arabidopsis halleri*

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The so-called metal hyperaccumulators are plants that can live on soils heavily polluted by metals and possess the ability to accumulate extraordinary high concentration of these metals in above-ground tissues (*e.g.* >1% shoot DW for Zn or 0.01% for Cd). These plant species attract interest as they constitute powerful models for studies on metal tolerance and accumulation and for identification of potential target genes for genetic engineering.

The Brassicaceae *Arabidopsis halleri* exhibits hypertolerance and hyperaccumulation to Zn and Cd and is considered as the best model because it is closely related to the model plant *Arabidopsis thaliana*. Nevertheless, the mechanisms underlying Cd tolerance in *A. halleri* is still poorly understood. Recently, the genetic architecture of this trait in *A. halleri* was investigated using interspecific crosses with *A. lyrata petraea* (non-tolerant and non-accumulator relative). A major QTL region was identified, co-localizing with *HMA4*, a gene encoding a metal-transporting ATPase (Courbot *et al.*, 2007). In addition, two other large QTLs were highlighted, covering about 15 and 30 cM.

In order to pinpoint novel candidate genes for Cd tolerance we have conducted the fine-mapping of these two unexplored QTLs. To increase the mapping resolution we have screened a large progeny from the cross used in Courbot *et al.* (2007). The recombinants individuals in the regions of interest were phenotyped for Cd tolerance and genotyped for additional markers. This strategy allowed us to narrow down the QTL intervals to few cM and to identity promising candidates.

This work contributes to the understanding of naturally evolved hypertolerance to Cd in plants.

Keywords: hyperaccumulator, cadmium tolerance, genetic architecture, *Arabidopsis halleri*

Reference:

Courbot M, Willems G, Motte P, Arvidsson S, Saumitou-Laprade P and Verbrug.gen N (2007) A major QTL for Cd tolerance in Arabidopsis halleri co-localizes with HMA4, a gene encoding a heavy metal ATPase. Plant Physiology, 144: 1052-1065.

B22

Capability of heavy metals absorption by corn, alfalfa and sunflower intercropping date palm

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Environmental pollution with heavy elements is one of the most problems of human societies. date palm is one of the most important product in Khoozestan province. This study was conducted in order to the possibility of using plants for refining heavy elements from date palm orchard. this pot experiment study was conducted in greenhouse in a completely randomized design. Treatments were: control (without plant cultivation), planting date palm alone, planting date palm with alfalfa, corn or Sunflower in three replicates. Lead and cadmium concentrations in shoot and root crops and the date palm and in the soil before the treatments and the total weight of harvested plants and seedlings in different treatments were measured. Percent reduction in pollution associated with each element in the soil of each treatment was determined. Analyzed by SPSS 16 software and mean comparison by Duncan test was performed. Results showed that corn planting date palm the most influence in reducing cadmium and alfalfa planting date palm the most influence on reducing lead.

Keywords: Corn, alfalfa, sunflower, lead, cadmium

B23

Growth and Heavy Metal Uptake by Date Palm Grown in Mono and Dual Cultures in Heavy Metal Contaminated Soil

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Plants are able to influence the availability of heavy metals in the rhizosphere due to root exudates and other mechanisms resulting in a change in their phytoextraction capability. The modern technology of co-planting techniques has therefore been introduced to phytoextract heavy metals from polluted agricultural soil. Separate tested were carried out with (monoculture) and inter cropping (dual culture) cultivation systems of date palm (*Phoenix dactylifera* L.) and sole crops of maize, alfalfa and sunflower in soil polluted with heavy metals. Corn inter cropped with date palm recorded significantly higher fresh and dry weights in the pot. Fresh and dry weights of date palm intercropped with corn were significantly higher ($P<0.05$) than the date palm intercropped with alfalfa. Sole corn recorded the highest copper and manganese uptake index and sole sunflower recorded significantly higher amounts of cadmium and lead uptake than the other treatments. Intercropping did not significantly affect the accumulation and removal of heavy metals in contaminated soil.

Keywords: intercropping, phytoremediation, corn, alfalfa, sunflower

B24

Antioxidant Defense Mechanisms in Response to Arsenic in Two Tropical Fern Species

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Arsenic toxicity in plants has been attributed to arsenic-induced reactive oxygen species (ROS), which causes oxidative damage to biomolecules and eventual cell death. In response, plants have developed mechanisms to protect cell and sub-cellular components from the effects of ROS by using enzymatic and non-enzymatic antioxidants like superoxide dismutase, catalase, and ascorbate peroxidase. This work aims to examine (i) arsenic accumulation and (ii) the role of anti-oxidative metabolism in arsenic tolerance for two tropical fern species (*Dicranopteris flexuosa* and *Thelypteris salzamannii*), which were exposed to 0, 10, 25, 40, and 50 mg L^{-1} of arsenic ($Na_2HAsO_4.7H_2O$). Arsenic accumulation in fronds and roots increased with an increase in arsenic concentration in the growth medium. In addition, arsenic accumulation was highest in the roots as compared to its detection in the fronds in both species. Moreover, both ferns responded differently to arsenic exposure in terms of anti-oxidative defense. Higher levels of superoxide dismutase and ascorbate peroxidase were observed in *Thelypteris salzamannii* than in *Dicranopteris flexuosa*, showing their active involvement in the arsenic detoxification mechanism. However, in both ferns, the activity of catalase increased in response to lower concentrations (10 and 25 mg L^{-1}) of arsenic, but decreased when plants were exposed to higher arsenic concentrations (40 and 50 mg L^{-1}). These results indicate that arsenate toxicity causes oxidative stress in the fronds of *Dicranopteris flexuosa* and *Thelypteris salzamannii*.

Keywords: *Dicranopteris flexuosa, Thelypteris salzamannii,* antioxidative enzymes, arsenic

B25

Heredity of zinc tolerance in *Suillus luteus*

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Metal contamination in the natural environment has a destructive influence on biodiversity and even on human health. The strong selective pressure exerted by exposure to toxic concentrations of metal ions allows only the survival of organisms that can sufficiently alter their metabolism or that have developed adaptive metal tolerance. Adaptive metal tolerance has been observed in animals, plants, bacteria and fungi, but seems to be species specific or even ecotype specific. For some species of ectomycorrhizal basidiomycetes, such as *Suillus luteus* and *S. bovinus*, the presence of adaptive metal tolerance has been shown in previous research.

In the current study, we investigate the heredity of metal tolerance in *S. luteus*. We studied three different populations of *S. luteus* in the northern Campine region in Belgium. Two populations grow on sites contaminated with zinc, cadmium and lead and one population grows on non-contaminated soil. We tested the zinc tolerance of ten diploid genotype per field and of 20 haploid cultures that resulted from single spores of each genotype. For all haploid and diploid strains we calculated the EC-50 value as a measure of zinc tolerance. Furthermore, we also tested the zinc tolerance of diploid strains obtained from crossings between monokaryotic strains.

We show that in most cases 0%, 50% or 100% of monokaryotic strains resulting from one basidiocarp are zinc tolerant. This suggests the presence of one gene determining zinc tolerance in *S. luteus*. The crossings experiments furthermore suggest that the gene responsible for zinc tolerance is inherited co-dominantly within this species.

B26

Mercury and organic acids in roots of *Medicago sativa* and *Silene vulgaris*

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Organic acids produced by plants are likely to enhance the transport of colloid associated Hg from vegetated Hg mine tailings to enable colloid release (Slowey *et al.,* 2005). This low molecular weight compounds can act as sinks in which to store large quantities of the toxic element (Meagher *et al*., 2005). The aim of this work was to study whether the concentration of Hg in soils might affect to organic acid composition in roots of *Silene vulgaris* and *Medicago sativa* grown in soils artificially contaminated with Hg. A greenhouse experiment was carried out in an agricultural soil (pH=8.55, O.M. 0.63%), spiked by Hg (as $HgCl_2$) at two dosage (0.6 and 6 mg Hg $\cdot Kg^{-1}$ soil). Mercury concentration increased both in roots and aerial part according to the dosage, without biomass decreasing. Main values for the water extractable organic acids measured in roots of *Silene vulgaris* were oxalic and citric. In roots of *Medicago sativa* were malic>citric> > oxalic. The Hg concentration in root did not seem to affect the solubility of the main organic acids.The differences found are mainly due to physiological differences between the two species studied, more than the Hg concentration supplied to soil. High mercury retention by roots with low transfer of shoots is desirable in case of *Medicago sativa,* where the shoots are foodstuff. *S. vulgaris* retains more mercury in root than in shoot (Pérez-Sanz *et al.*, 2012), that makes this species a good candidate to phytostabilization technologies where, the influence of organic acid to microbial activity in rhizosphere might also be relevant.

Keywords: mercury, organic acids, poluted soil

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B27

Growth and trace element accumulation of *Salix smithiana* on seven different trace element-contaminated soils

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Risk assessment and phytomanagement of metal-polluted soils requires information on plant responses to metal availability in soil but the predictability of metal accumulation in plants may be limited by metal toxicity and inherent shortfalls of the bioavailability assays. The aims of this work were to: 1. evaluate the relation between soil properties and traditional metal availability assays (soil extraction, soil solution concentration, K_d) versus DGT in polluted soils with widely varying properties; 2. establishing relations between metal availability assays in soil and metal concentrations and accumulation in a metal-accumulating Salix smithiana clone; 3. evaluating as to whether soil properties, metal toxicity and competition with other ions for uptake affect these relations and may thus obscure the predictive power of the soil-based availability assays. Therefore we measured the uptake of Cd, Pb and Zn in a Cd/Zn- accumulating *Salix smithiana* clone grown on soils with differential characteristics and metal availabilities, determined by classical soil extraction (0.05 M Na_2EDTA, 1 M NH_4NO_3), soil solution obtained by centrifugation and diffusive gradients in thin films (DGT). While growth reductions were observed on soils with high chemical metal availability, metal toxicity apparently distorted correlation between DGT-measured concentrations (C_DGT) and metal concentrations in willow shoot biomass. The weaker correlation for Cd may be related to competitive Zn uptake. Except for Cd, metal uptake in willow was generally better predicted by C_DGT than by soil solution concentrations or extractable fractions.

Keywords: Salix, phytoextraction, trace element, bioavailability, DGT

B28

Impact of iron plaque on arsenic uptake and speciation in rice (*Oryza sativa* L.) along plant development

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Rice (*Oryza sativa*) is a staple crop for over half of the world's population. In numerous areas of South and South-east Asia, soils and aquifers are arsenic contaminated leading to a potential health risk to populations due to As accumulation in rice. In most As-affected areas of South and South-east Asia, groundwater is rich in iron. Excess of both elements are serious threat to sustainable agriculture, in relation to a decrease of rice yield and a contamination of rice grains by toxic As. Nevertheless, the ability of rice species to carry oxygen from the air down its stem through aerenchyma and discharge it through its roots, creates an oxidized zone around individual roots in which Fe is oxidized to the Fe(III) state and forms a coating called "iron plaque". This Fe plaque may retain As and reduces its availability for the plant. We aimed to investigate the impact of combined As and Fe stresses on rice growth and yield and to study the influence of Fe plaque on As uptake and speciation. Twenty five days old rice seedlings were exposed to As (V) (50mM and 100mM), Fe(II) (125 ppm) or combined treatments until plant maturation. The presence of Fe allowed the development of a Fe plaque on the rice roots that limited the As accumulation and modified its speciation in the different plant organs. Combined stresses also significantly reduced the plant yield and affected plant nutrition.

Keywords: rice, iron plaque, arsenic, heavy metals, plant yield

B29

Synchrotron based correlative FTIR and LEXRF analyses of metal hyperaccumulating *Noccea praecox* leaves

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Metal hyperaccumulating plants have unique ability to accumulate and withstand extraordinary high metal levels in above-ground parts, a trait highly appreciated in phytoremediation efforts. Simultaneous application of Synchrotron Radiation Fourier-Transform Infrared Microspectroscopy (SR-FTIR) and Synchrotron Radiation Low-Energy X-Ray Fluorescence (LEXRF) enables a deeper understanding of the importance of plant structural biochemistry for the processes involved.

On their way to the shoots, metals pass diverse biomolecules that may act as their potential binding sites. Metal ligands involved in metal chelation for their transport and storage primarily include organic acids (e.g. malic, citric and oxalic acids) stored in the vacuole, histidine, nicotianamine, phytic acid, metallothioneins, phytochelatins, low-molecular-weight thiols, such as glutathione. Metal sequestration at the tissue and cellular levels further contribute to successful metal detoxification and the significance of the activity and abundance of metal transporters in hyperaccumulation is well resolved (Krämer 2010). The potential role of biomolecules present in the apoplast to metal tolerance, however, is much less understood.

We aimed to investigate the involvement of biomolecular composition of leaves in metal tolerance of hyperaccumulating *Noccea praecox* (formerly *Thlaspi praecox*) (Koch and Mummenhof 2001; Likar et al. 2010) at the cellular and (sub)cellular levels. Spatial distribution of biological macromolecules (e.g. carbohydrate, lipid, ester, pectin and lignin) of the metal-enriched epidermal cells of leaves acquired using SR-FTIR was therefore complemented with their elemental composition revealed by LEXRF at Elettra Sincrotrone Trieste. The results point to a functional significance of the spatial distribution of specific biological macromolecules within metal accumulating cells and tissues.

Keywords: FTIR; hyperaccumulation; leaves biochemistry; LEXRF; *Noccea praecox (Thlaspi)*

References:

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B30

Ecological understanding and molecular mechanisms of plant root growth responses to excess metals, in support of growth improvement for safe biomass production or phytoremediation strategies

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Diffuse contamination by excess metals affects large areas worldwide. Bringing these lands back into sustainable use can reduce detrimental environmental and socio-economic impacts. There are opportunities to use these contaminated lands for the production of renewable energy biomass and industrial feedstock applications.

Economically interesting, high biomass producing plants are subject to the toxic effects of the metals, which generally cause growth inhibition, so plant growth under these conditions needs to be optimized. Although the physiology and molecular mechanisms of metal uptake, sequestration and detoxification are under intense investigation, root system development under these circumstances is poorly understood. Yet, placement of roots is an important factor in phytoremediation (in which growth inhibition should be reduced to allow placement in contaminated soil patches), and in safe biomass production (in which root growth should be directed to less- or non-contaminated soil-patches).

Using root growth of *Arabidopsis thaliana* in vertical agar plates as a model, we had previously observed that excess of three different metals (cadmium, copper and zinc) caused distinct effects on lateral roots (LRs). These metal-specific morphological responses indicate the existence of underlying metal-specific sensing and signaling pathways. Using split-root systems, we also found distinct local and/or systemic responses that influence root growth distribution under metal stress. In a reverse genetics approach, mutants in genes related to root growth and hormonal signaling have been studied to identify underlying molecular components. Knowledge is generated that will support strategies for plant growth optimization for the purpose of phytoremediation or safe biomass production.

Keywords: roots, metal pollution, biomass, phytoremediation, growth response

B31

Development of practical rice cultivars for Cd-phytoextraction use (1) Genetic and physiological characteristics of high-Cd accumulating rice cultivars

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Phytoextraction has drawn much recent attention as a promising technique because it is environmentally friendly and cost-effective. A high–Cd–accumulating rice cultivar seems to be a good material for extracting Cd from the Cd-polluted paddy fields. In this study, we selected several rice cultivars carrying high-Cd allele from diverse rice germplasms and genetically and physiologically characterized the Cd transport system in rice to produce a desirable phytoremediator in Cd-polluted paddy fields.

We found three *indica* rice cultivars with markedly high Cd concentrations in their grains and shoots from the world rice core collection (WRC), consisting of 69 rice accessions which covers the genetic diversity of almost 32,000 accessions of cultivated rice (Uraguchi *et al.* 2009). To characterize the differences in Cd dynamics between the high- and low-Cd accumulating rice cultivars, we used positron-emitting ^{107}Cd tracer and an innovative imaging technique, the positron-emitting tracer imaging system (PETIS). The apparent differences were clearly shown as Cd retention in the roots, the rates of Cd translocation from the roots to the shoots, and the long-distance Cd transport to the panicles (Ishikawa *et al.* 2011). To identify the gene loci for Cd accumulation in rice, we conducted the QTL genetic analysis and found the major QTL gene (*qCdp7*) for increasing Cd accumulation(Abe *et al.* 2011). This gene derived from the high-Cd accumulating cultivars would be useful for developing practical rice cultivars for Cd-phytoextraction use.

Keywords: cadmium, phytoextraction, rice, QTL, PETIS

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S. Ishikawa, N. Suzui, S. Ito-Tanabata *et al.* (2011) BMC Plant Biol., 11:172.

B32

Cadmium-induced effects on the ethylene pathway and the link with oxidative stress in *Arabidopsis thaliana*

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Anthropogenic activities introduced toxic metals in all ecosystem compartments worldwide. Plants can absorb and accumulate these metals, inducing stress as a result of excess metal concentrations inside the plant. Phytohormones are often affected by stress conditions and play important roles in the response to environmental stimuli. The gaseous plant hormone ethylene is considered as the 'stress hormone', involved in multiple processes during the entire lifecycle of the plant. Previous studies indicate an increasing ethylene production after exposure to toxic metals. This can inhibit plant development and accelerate senescence processes.

In polluted areas, agriculture has to re-orientate to non-food crops. These crops are selected for their metal resistance and accumulation capacity to phytostabilize or -remediate the soil. A better understanding of the effects of toxic metals on the ethylene pathway in plants can help improving their metal resistance.

Although the effect of toxic metals on ethylene production and signalling in combination with oxidative stress has up till now only been scarcely investigated, there are various indications for a link between both. In this study we investigated the effects of cadmium (Cd) exposure on wildtype *Arabidopsis thaliana* plants as well as different ethylene signaling mutants. By measuring (1) the ethylene production, (2) the 1-aminocyclopropane-1-carboxylic acid content (ethylene precursor) and (3) the expression of genes involved in the production and signal transduction of ethylene together with oxidative stress-related genes, we confirmed the involvement of ethylene in Cd stress responses.

Keywords: Cadmium, Ethylene, Oxidative stress, *Arabidopsis thaliana*, Mutants

B33

Assessment on Cobalt and Copper speciation in metalliferous soils from Katanga (DR Congo): a tool to improve phytoremediation efficiency.

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The abundance of anthropic Cu-Co contaminated sites is a major environmental and health issue in Katanga (DR Congo). Indeed, the risk of metal transfer is high because of the less vegetation cover. Phytoremediation appears as solution of rehabilitation. However, the variability of metalliferous soils (mine deposits, tailings and bare soils around ore-smelters) could express variation of soil conditions, thus influence difference of Cu and Co availability. Phytoremediation strategy could be influence by the metal availability. The objective of this study was to assess Cu and Co speciation in metalliferous soils from Katanga to improve phytoremediation. Eight metalliferous sites were studied (3-4 samples of soil per site). Total concentrations of trace metals, pH and organic matter were measured. Cu and Co available concentrations were furthermore modelled using Windermere Humic Aqueous Model (WHAM 6). Results showed high variability of Cu and Co speciation. Highest percentages of Co were free in soil solution or bounded to Mn-oxides. For Cu, highest percentages were bounded to Fe-oxides and humic acid. As Fe is more ubiquitous than Mn in Katangese soils, Co were more available than Cu in metalliferous soils. Otherwise, results showed lower concentrations of available Cu and Co in some bare metalliferous sites compared to sites covered by vegetation. In these cases, absence of vegetation could be explained by extreme physical conditions rather than chemical. These results have practical implications in phytoremediation such as species selection, choice of amendments for specific situations. Analysis of TM speciation could provide interesting information to guide phytoremediation project.

Keywords: Katanga, Copper, Cobalt, Speciation, Phytoremediation

B34

Effect of selenium speciation in soil on selenium accumulation, growth and physiology of Kenaf (Hibiscus cannabinus)

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Kenaf (Hibiscus cannabinus) is cultivated in several countries for cooking, cattle grazing and paper production. However, it was previously also identified as a potential Se-accumulating plant that may be used for phytoremediation.Therefore, we aimed to assess how Se speciation in soil affects its uptake by the plant, biomass production and plant physiology. Therefore, kenaf was grown in a greenhouse on a sandy-loam soil treated with two different Se species (selenite and selenate) at two different levels of Se (0 to 4 mg kg-1 soil). Biomass production and Se concentrations in the plants were analysed at harvest, whereas chlorophyll contents and photosynthesis were monitored during growth. The extent of Se accumulation in the plant was found to strongly depend on the form in which Se is applied to soil. Using selenate instead of selenite resulted in a Se uptake that is more than 100 times higher. However, using selenate also resulted in decreased plant productivity at a dose of 4 mg Se kg-1 soil, coinciding with a concentration of1019±136 mg Se kg-1 DW in the plants. This coincided with the observation that net photosynthesis and chlorophyll content were also affected in plants grown on soils treated with selenate at 4 mg Se kg-1 soil. Depending on the Se speciation and availability in the soil, kenaf may be used for phytoextraction of Se. Moreover, depending on the obtained concentrations, produced Se-enriched kenaf may be used as feed additive or fodder crop to deal with Se deficiency in Se-deficient regions.

Keywords: chlorophyll content, photosynthesis, plant growth, selenium

B35

Heavy metal-induced oxidative stress in Miscanthus giganteus grown on a highly contaminated site

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Revegetation of polluted soils by means of energy crops is an innovative strategy to maintain and improve economic viability of the phytomanagement. Among the numerous plant species tested, Miscanthus sp. has emerged as a promising renewable energy source suitable for remediation of heavy metal-contaminated soils. Miscanthus sp. has a high biomass yield production in temperate climate and has, in addition, the capacity to germinate and grow on a metal-rich environment. However, little information is available on the tolerance of this plant to the toxicity of metals in a field situation, while the adaptation of plants to environmental conditions is an important factor in determining the success of phytoremediation. The aim of the present work was to evaluate, in a field experiment, the physiological responses to a metal-contaminated soil of two Miscanthus giganteus differing by their origins (Bical and Autrichienne). The experimental site consisted of an agricultural field loc ated near the Metaleurop Nord smelter (Noyelles-Godault, North of France). Shoots were planted in 2007 on high metal contaminated and uncontaminated experimental fields. Four years after the beginning of the experiment, the metal toxicity in plants was determined by measuring oxidative stress parameters including malondialdehyde production, glutathione status, 8-hydroxydeoxyguanosine formation, superoxide dismutase and peroxidase activities. Comparison of oxidative stress parameters and distribution patterns of metals in roots and leaves will allow us to estimate the effectiveness of Miscanthus sp. for sustainable long-term management of heavy metal-contaminated soils. Additionally, the approach will lead us to evaluate benefits of mycorrhizal inoculation on plants metal tolerance.

Keywords: phytomanagement, energy crops, Miscanthus giganteus, heavy metals, oxidative stress

C: Persistent Organic Contaminants

C1

Phytoremediation of organochlorines. Lindane phytotoxicity *vs* uptake on *Zea mays*

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Organochlorines (OCs) are known to be persistent 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 OCs, lindane, was experimented. Screening of plant species adapted 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 lindane (0.07 – 7 mg/l) to determine toxicity featuringand then, uptake rate (1 month exposure). Using biomass, oxidative stress, apoptosis and cell death indicators, the lindane effects on root tissues were characterized. Compared to controls treatment with lindane caused swelling of root tips. Root cell viability was strongly dependent on the lindane concentrations: apoptosis and cell death exponentially increased with lindane concentrations. Plants exposed to lindane (0.7 mg/l) did not show extreme phytotoxic symptoms and were able to absorb lindane. Root uptake seemed to be correlated to lipophilicity (strong correlation between *RCF* and LogK_{ow}) and at 28 days of exposure, plants bioconcentrated 80% of the total ^{14}C-lindane. Therefore, *Zea mays* could be a good plant candidate to remediate area contaminated by lindane, in a pollution range comprised between 0 to 30 mg kg^{-1} (San Miguel *et al.*, 2012).

Funding for this project was provided by a grant from la Région Rhône-Alpes.

Keywords: *Zea mays*, Organochlorines, Apoptosis, Uptake, Phytoremediation

Reference:

San Miguel A, Faure M, Ravanel P, Raveton M (2012). Concentration responses to organochlorines in Phragmites australis. Ecotoxicology, 21: 315-324.

C2

Effect of presence of Sorbitol in culture medium on growth of *Lemna minor* and uptake of phenol

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Limited research showed that presence of some carbohydrates in the culture medium will stimulate frond regeneration in Lemna minor, and it is expected that presence of this carbohydrates will affect the growth and uptake of organics by this plant. According to literature review, it was not found any study on uptake of organic pollution by Lemna –minor in the presence of carbohydrate in growth medium. The aim of this study was to determine the effect of presence of Sorbitol in culture medium on growth and uptake of phenol by Lemna- minor.

Experiments were carried out in the presence and absence of Sorbitol in the growth solution. The growth solution was Hoagland's medium according to Standard Methods and phenol and Sorbitol were added to it separately. Two sets of vessels containing culture medium namely containing 100 ppm of Sorbitol and without Sorbitol were examined. Each of these sets was placed in two categories including containing phenol and without phenol. The initial concentrations of phenol were 5.0, 10.0, 25.0 and 50.0 mg/L and fresh weight of Lemna minor were 0.2, 0.4 and 0.6 grams. The plants were cultured in a multiple series of vessels containing 100 mL of culture medium of the above mentioned two sets of vessels for 4 weeks. Control vessels containing growth medium and phenol but without Lemna-minor were examined with the same manner in order to determine the phenol lost by evaporation. All vessels were placed in a water bath at 30°C near window in the laboratory at natural daytime photo period condition for more than four weeks. Every three days water samples were taken from vessels. Before and after sampling, the weights of vessels were determined by analytical balance and the evaporated water was replaced by adding distilled water in the vessels. The remained phenol concentration was determined by spectrophotometer. The fresh weight of Lemna was determined by direct weighting of vessels by analytical balance.

Uptake of phenol by lemna-minor increased with increasing the contact time and decreased slightly by increasing phenol concentration. For 5 and 10 ppm of phenol in solutions, presence of Sorbitol in culture medium had a little enhance on plant growth, but in the vessels containing 25 and 50 ppm phenol, presence of sorbitol didn’t any effect on growth of Lemna-minor. When the growth of plant was enhanced slightly, the uptake of phenol was increased. Phenol uptake was not dependent to initial weight of Lemna minor. By increasing the phenol concentration, the growth of Lemna-minor was decreased.

Presence of Sorbitol in the culture medium had enhancing effect on growth of lemna-minor in the vessels containing low concentrations.

C3

Engineered Phytoremediation of Chlorinated VOCs in Fractured Rock

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Over a 20 year period, the Baker Hughes Inc. Centrilift oilfield pump manufacturing facility in Claremore, Oklahoma USA has used a several remediation methods (soil excavation and off-site disposal, air sparging/soil vapor extraction, groundwater circulation wells, high vacuum multiphase extraction, carbohydrate injection and the current pump-and-treat system) in its efforts to clean up groundwater contaminated with trichloroethene; 1,1,1 trichloroethane; and associated degradation products. Due to challenging hydrogeologic conditions (fractured rock 3-10 meters below ground surface) and the nature of the contaminants, the result from these past and ongoing significant expenditures for capital and operations and maintenance has been modest improvement in groundwater quality but with no foreseeable end point in an area with sensitive off-site receptors. Baker Hughes' objective is to reduce the contaminant concentrations and to assure that no off-site contaminant migration can occur. In March 2012, as part of a planned site-wide revision of the remediation program, the facility utilized an engineered phytoremediation whereby 52 trees were installed within large diameter boreholes using the *TreeWell®* system for groundwater hydraulic control and in situ treatment. This paper presents the challenges and solutions of drilling and completing the tree installations in highly variable fractured shale and sandstone within the constraints of very tight spaces at a busy operating plant. Preliminary data concerning tree health and growth and hydrologic effects will be presented.

Keywords: Phytoremediation, fractured rock, TreeWell, trichloroethylene, operating facility

C4

DDX Accumulation in Grafted Cucurbitaceae Grown under Greenhouse and Field Conditions

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Large pot and field experiments were conducted to assess the effect of grafting on accumulation of weathered DDX (the sum of *p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE) from soils to plant systems. In two-year study, intact squash (*Cucurbita maxima x moschata*) and watermelon (*Citrullus lanatus*), their homografts, and compatible heterografts were grown in the soil contaminated with weathered DDX at 150-1760 ng/g. DDX concentrations in plant compartments were measured and compared in the grafted and nongrafted plants.

In the pot and field study, the highest stem DDX concentrations measured in heterografted watermelon plants were 140 and 19 times greater than contaminant concentrations in the intact watermelon, respectively. Grafting watermelon onto squash rootstock significantly increased DDX uptake into the watermelon shoot system. Similar to stem DDX content, the highest DDX concentration was measured as 6.10 µg/L in xylem sap of heterografted watermelon plants grown in the pots. The DDX concentrations were 0.5-9 ng/g in fruit of the cultivars. Data show that the DDX concentrations in the xylem sap, stems, leaves, and fruit of watermelon plants were significantly increased by squash rootstocks.

Keywords: grafted watermelon, homografted, heterografted, *p,p′*-DDE, DDX

C5

Phytoremediation of Chlorpyrifos Using Poplar and Willow Plants

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Chlorpyrifos (CPS) is an organophosphorus insecticide that is implicated in environmental and human health problems. These problems may be solved by the phytoremediation technology. Here we introduce three approaches to phytoremediation of CPS. First of all, seven species of poplar (*Populus* sp.) and willow (*Salix* sp.) were investigated to evaluate plant potential for CPS uptake. Analysis of the CPS removal from hydroponic solution showed that CPS can be taken up by all seven species. Significant amounts of CPS accumulated in plant tissues, but did not persist, suggesting further metabolism of CPS in tissues. To our knowledge, this work represents the first report for phytoremediation of CPS using poplar and willows (Lee *et al*., 2012). The second approach is phytoremediation of CPS by transgenic poplars. The hypothesis is that the transgenic expression of genes, cytochrome P450 2B6 (CYP2B6) and paraoxonase 1 (PON1), involved in CPS metabolism is expected to increase removal of CPS. A poplar clone has been successfully transformed with two genes. However, there was no significant difference between wild type and transgenic plants, when they were investigated for CPS uptake and degradation. The further study of transgenic poplars is in progress. The third approach is the use of plant-derived PON1 protein for rapid on-site remediation of spills as a soil amendment. In order to express the PON1 protein at high levels in plants, instead of the nuclear DNA, the DNA in the tobacco's chloroplasts was modified. The investigation of the chloroplast transgenic tobacco for further analysis is ongoing.

Keywords: chlorpyrifos, organophosphorus insecticide, cytochrome P450 2B6, paraoxonase 1, chloroplast transformation

Reference:

Lee KY, Strand SE and Doty SL (2012). Phytoremediation of chlorpyrifos by *Populus* and *Salix*. International Journal of Phytoremediation, 14: 48-61.

C6

Atrazine phytoremediation in Humid Pampa plots (Argentina) under intensive agriculture management

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Humid Pampa, the main agricultural region in Argentina, covers about 600.000 Km^2 largely dedicated to intensive agricultural practices. As one of the main world atrazine consumers (7000 tons/year), developing of biotechnological strategies to minimize the environmental impact associated to this herbicide application is necessary. In order to achieve this aim, a pilot scale open field experiment was carried out to assess the phytoremediation performance of the recently reported atrazine tolerant ryegrass species (*Lolium multiflorum*) in a corn field. Accordingly, a 16 hectares field was selected in Ramallo city vicinity (30°40’35,2’’ S – 60°09’21,0’’ W), as representative of the intensive agricultural practices of this region. Soil was sampled before sowing and 36 plots of 100 m^2 were delimited in order to representatively cover the plot. Then, corn crop was conventionally sowed and 2 $L.ha^{-1}$ of atrazine (Atrazine500-Dow) applied postemergence. After corn harvest, ryegrass was sowed at 22 $Kg.ha^{-1}$ rate in a paired plots fashion. Composite core soil samples were monthly collected from every plot and separated in 5, 15 and 25 centimeters deep in order to assess either the horizontal or the vertical atrazine movement. Soil samples were extracted and atrazine and metabolites content analyzed by HPLC-UV. Parallel, soil agronomical properties were assessed. Results indicate that ryegrass implantation significantly improves atrazine degradation, soil agronomical properties and reduce the herbicide movement caused by rainfall. Concluding, the especially designed ryegrass intercropping strategy fits as biotechnological strategy for atrazine environmental impact associated to intensive agronomical practices of the region.

Keywords: Atrazine, Phytoremediation, Field scale, Ryegrass

C7

Study the effect of fertilization phytoremediation potential in the field

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Stockpiles of obsolete pesticides, their storage containers, and environmental contamination resulting from uncontrolled release of these materials pose dangerous mutagenic and ecological risks in Kazakhstan. Results of research have shown, that the soil sample analysis around the former warehouses showed pesticide contamination in which included metabolites of DDT (2,4 DDD; 4,4 DDD; 4,4 DDT; 4,4 DDE) and isomers of HCH (a HCH; b HCH; g HCH), where the concentration of POPs exceed the Kazakhstan MAC (maximum allowable concentration) for soil contaminated by 10 to hundreds of times. This situation is a serious environmental problem resulting in potential ecological and health risks for nearby settlements. Genetic heterogeneity of populations of wild and weedy species growing on pesticide-contaminated soil provides a source of plant species tolerant to these conditions. These plant species may be useful for phytoremediation applications. Low phytoextraction percentage is in part connected to slow growth of plants and limited biomass production. We were conducted using mineral fertilizers to increase plant biomass and monitor its effect on phytoextraction potential in the territory of the former warehouses of obsolete pesticides. Objects were the pesticide-tolerant species: *Artemisia annua, Ambrosia artemisifolia, Amaranthus retroflexus, Xanthium strumarium and Helianthus annuus.* Additions of fertilizer appeared to increase plant biomass production and increase the amount of pesticide accumulated in plant tissue**:** *Xanthium strumarium* - 500 μg (without fertilizer 262 μg), *Helianthus annuus* - 1505 μg (without fertilizer 460 μg).This work can be required for the development of pesticide remediation technologies for obsolete pesticide-contaminated sites.

Keywords: phytoremediation, metabolites, DDT, HCH, wild plant

C8

Clean up of a PCBs contaminated site trough an assisted anaerobic/aerobic process, combining phyto- and bio-remediation.

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Polychlorinatred biphenyls (PCBs) are a class of persistent pollutants found in the environment as mixtures of some of the 130 congeners (molecules differing for number and position of chlorine atoms on the biphenyl rings). Micro-organism populating contaminated soils are known to degrade PCBs through different metabolic pathways. Anaerobic reductive dechlorination is normally necessary to degrade higher chlorinated PCBs, while aerobic oxidative degradation is suitable for lower chlorinated congeners.

The objective of this PhD thesis is to develop an *in situ* treatment, effective in cleaning up a long-term PCBs-contaminated soil. The test site is a previously industrialized area, localized in Papigno (Umbria region), it is contaminated by PCBs (0,14-3,42 mg/Kg), heavy metals and hydrocarbons; as such, defined by Italian Ministry of Environment as Site of National Interest (SIN). We will exploit the micro-organism potential both in anaerobic and aerobic conditions, without and with plants (*Populus* spp.) respectively. Plants in fact, aid micro-organism to degrade chlorinated compounds by enhancing the oxygen supply in the rhyzosphere, by releasing root exudates containing sugars, amino acids and carboxylic acids readily available for bacterial metabolism and by producing some pollutant analogue inducing PCBs cometabolism (e.g., salicylic acid or carvone).

Our project envisages laboratory tests on microcosms filled with long-term PCBs-contaminated soil. Shifts in bacterial community and identification of the main microbial components will be examined through FISH technique (Fluorescence In Situ Hybridization), in conjunction with analysing of the changes in content of the different PCB congeners in soil, while monitoring plant response to PCBs.

Keywords: PCBs, polychlorinated biphenyl, phytoremediation, bioremediation, degradation

C9

The differences of flax cultivars in response to nitrocompounds contamination

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For phytoremediation technologies the selection of appropriate plant species is the key factor. Different cultivars of crop plants are good candidates for phytoremediation due to known optimized agricultural technologies. Many different crop species such as maize (Aggarwal and Goyal, 2007), hemp (Tlustoš *et al.*, 2006) or sunflower (Vamerali *et al.*, 2010) were tested for accumulation of heavy metals and forthe tolerance to their high soil concentrations (Cheng, 2003).Only few authors interested in phytoremediation of organic compounds by crop plant.

In our experiments we tested the toxicity of trinitrotoluene, dinitrotoluene and nitroglycerine on germinating seedlings of four representative flax cultivars (*Linum usitatissimum:* Vénika, Viola, Tábor, Flanders). These nitroaromatic compounds are known as effective explosives contaminated areas surrounding of today's and former plants and areas of their utilization. The highest differentiations between the cultivars were observed in response to low concentrations of the explosives. For the next experiments the *in vitro* cell cultures of cultivars mentioned above were initialized. The results of our investigation confirmed also the possibility of flax cell suspension to degrade the nitrocompounds.

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Keywords: flax, explosives, toxicity

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C10

Difference in uptake and translocation of POPs by several plant families

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Uptake ability of persistent organic pollutants (POPs) from soil by several plant families was compared. Maize, sunflower, soybean, tomato, broccoli, cucumber, pumpkin and zucchini were grown for 21d in soils amended with HCHs (α-HCH, β-HCH, γ-HCH, δ-HCH), chlorinated cyclodienes (dieldrin, endrin, heptachlor epoxide) and DDTs (p,p'-DDE, p,p'-DDD), and then POPs contents in the shoots and roots were measured. The uptake ability of POPs from soil was evaluated using the amount of POPs in whole plant per root weight, and the translocation ability of POPs from root to shoot was evaluated using the amount of POPs in shoot per transpiration rate.

The uptake ability of chlorinated cyclodienes and DDTs was higher in cucurbits (cucumber, pumpkin and zucchini) and sunflower. The higher uptake ability might be derived from much lipid (n-hexane soluble component) contents in root of sunflower and cucurbits. The translocation ability of chlorinated cyclodienes and DDTs was hiher in cucurbits than that in non-cucurbits, and that of DDTs, with higher $\log K_{OW}$ in POPs was much higer only for zucchini. On the other hand, the uptake ability from soil to plant and translocation ability from root to shoot of HCHs, with lower $\log K_{OW}$ in POPs was nearly the same in all the tested plants. It was suggested that as $\log K_{OW}$ increased, interspecific difference was observed in uptake and translocation of POPs in plants.

Keywords: HCHs, chlorinated cyclodienes, DDTs, cucurbits

C11

Plant uptake and translocation of dieldrin: Role of protein-like materials in xylem sap of Cucurbitaceae

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Cucurbitaceae has been known to take up a large amount of hydrophobic organic chemicals (HOCs) like persistent organic pollutants. To understand the HOCs uptake mechanisms of Cucurbitaceae, we compared dieldrin uptake potentials of several plant families. Corn, sunflower, soybean, tomato, broccoli, cucumber and zucchini were grown in a dieldrin contaminated soil, and the amount of dieldrin in their shoots and roots was measured. Dieldrin was similarly detected in all roots, but high concentrations of dieldrin were detected in the shoots of Cucurbitaceae (zucchini and cucumber) only. The dieldrin distribution ratios in the shoots of zucchini and cucumber were 47.5 and 30.2%, respectively, while that in non-Cucurbitaceae plants was <1%. Thus, Cucurbitaceae have unique HOCs translocation mechanisms that differ from those in non-Cucurbitaceae, and the amounts of HOCs in the shoots are affected by the root-to-shoot translocation ability. Xylem saps of the tested plants were collected, passed through the column packed with C8 granules adsorbed with dieldrin, and the amount of dieldrin in the leachate was measured. Xylem saps of zucchini and cucumber leached dieldrin adsorbed on C8 granules, but those of the other plants did not. Xylem saps of zucchini and cucumber eluted high amounts of dieldrin from the size-exclusion chromatography (SEC) column before the fractions of Apotinin (6.5 kDa). The enhancement of dieldrin mobility by xylem sap was reduced by proteinase and heating. It was suspected that the protein-like materials in xylem sap of Cucurbitaceae have a role to translocate dieldrin from roots to shoots.

Keywords: Cucurbitaceae, dieldrin, translocation, uptake, xylem sap

C12

Oxidative Stress in Environmental Toxicity: Effects of Herbicide Use on *Brassica napus*

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With the growing world population, the agricultural sector is increasingly relying on pesticide-use to ensure food production. Though, the short- and long-term effects of herbicides on crop quality are not yet fully understood. Herbicides contain active ingredients, which act specifically on plant physiology such as photosynthesis and major biosynthetic pathways. The effects of selected herbicides are tested on life history traits, metabolic detoxification processes and the cellular redox status of *Brassica napus* (rapeseed) and its relative *Arabidopsis thaliana*. Growth experiments in the laboratory and the field will determine if the herbicides have an impact on plant yield, root and leave growth. The level of oxidative stress is determined by investigating membrane damage and behavior of redox balance components.

Preliminary range finding experiments on *B. napus* showed reduced root growth and biomass production and augmented membrane damage at increasing herbicide concentrations. The results of this study can contribute to select a set of early response plant parameters, which reflect long-term effects on crop quality and which are consequently useful for herbicide development and selection of crop varieties.

Keywords: herbicides, oxidative stress, crop quality, *Brassica napus, Arabidopsis thaliana*

C13

Uptake and Translocation of Weathered Chlordane and *p,p′*-DDTs by *Cucurbita pepo* Hybrids

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Cucurbita pepo ssp *pepo* (zucchini) accumulates significant levels of persistent organic pollutants in its roots, followed by high contaminant translocation to the stems. Most other plant species, including *C. pepo* ssp *ovifera* (squash), do not have this ability. To investigate the mechanism of contaminant accumulation, two cultivars each of parental zucchini and squash, as well as first filial (F1) hybrids and F1 backcrosses (BC) of those cultivars, were grown under field conditions in a soil contaminated with weathered chlordane (2.29 µg/g) and DDX residues (0.30 µg/g). The parental zucchini had stem-to-soil bioconcentration factors (BCF) for chlordane and DDX of 6.23 and 3.10; these values were 2.2 and 3.7 times greater than the squash, respectively. Chlordane and DDX translocation factors were 2.1 and 3.2 times greater for zucchini than for squash. The parental zucchini and squash also differed significantly in chlordane component ratios (relative amounts of trans-nonachlor [TN], cis-chlordane [CC], trans-chlordane [TC]) and enantiomer fractions for the chiral CC and TC. Hybridization of the parental squash and zucchini resulted in significant differences in contaminant uptake. For the three component ratios (CR) and the enantiomer fraction (EF) values, subspecies specific differences in the parental generation became statistically equivalent in the F1 hybrid plants. When backcrossed (BC) with the original parents, the zucchini and squash F1 BC cultivars reverted to the statistically distinct CR and EF patterns. This pattern of trait segregation upon hybridization suggests single gene or single locus control for persistent organic pollutant (POP) uptake ability by *C. pepo* ssp *pepo*.

Keywords: Persistent Organic Pollutants, Chlordane, Zucchini, Cucubita pepo

D: Plant-Microbe Interactions: Organics

D1

Burkholderia fungorum strain DBT1 in microbe-assisted phytoremediation of a soil polluted by PAHs

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Assisted phytoremediation is a technology in which plant-associated microorganisms are used to enhance the ability of plant to tolerate and remediate pollutants. *Burkholderia fungorum* DBT1 is a bacterial strain isolated from a wastewater drain of an oil refinery capable to transform dibenzothiophene, phenanthrene, naphthalene and fluorene (Andreolli *et al.*, 2011). Recently, *B. fungorum* has been found to be a widely diffused endophytic species in hybrid poplar (Yrjälä *et al.,* 2010).

In order to evaluate the effects of a PAHs-transforming non-endophytic strain on the phytoremediation of organic contaminants, *B. fungorum* DBT1 was inoculated in hybrid poplar (*P. deltoides* x *P. nigra*). The plants were grown for 18-weeks in presence or absence of naphthalene, phenanthrene, fluorene and dibenzothiophene (488 mg kg^{-1} soil each).

Results showed that *B. fungorum* DBT1 was present in high concentration in poplar roots (2.9-9.5 x 10^3 CFU g^{-1}), although the strain was not detected in stem, leaves and rhizosphere. When poplars are planted in uncontaminated soil, the infection evidence negative effects on biomass index, leaves and stem dry weight, without show signs of sickness. On the other hand, plants inoculated with DBT1 strain provide protection against the phytotoxic effects of PAHs in term of roots dry weight. Even if the presence of plants were the main effective treatment for PAHs dissipation (82.41%-86.74%), poplar inoculated with the strain DBT1, showed that plant-bacteria system removed more PAHs than plants alone (up 99%).

This work demonstrates that a non-endophyte strain with proper metabolic features could be suitable for improving phytoremediation in PAHs contaminated soils.

Keywords: *Burkholderia fungorum* DBT1, assisted phytoremediation, PAHs

D2

Optimization of DDE-phytoremediation by exploiting plant-associated bacteria and nanoparticles

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Researchers at The Connecticut Agricultural Experiment Station observed a remarkable difference in the accumulation of 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene (DDE) at the subspecies level of *Cucurbita pepo. C. pepo ssp pepo* (zucchini Raven) accumulates DDE, while *C. pepo ssp ovifera* (squash Zephyr) did not possess this capacity.

In a first part, this study investigates whether the differences in DDE-accumulation can be related to differences in the plant-associated bacterial populations. Bacteria associated with DDE-exposed (100 µg L^{-1} DDE) plants of the accumulating and non-accumulating subspecies were isolated and characterized. The results revealed that bacteria associated with the DDE-accumulator zucchini Raven were more numerous and diverse and show more plant growth promoting capacity and potential DDE-degradation capacity than bacteria associated with the non-DDE-accumulating squash Zephyr.

The second part of this project concentrates on silver nanoparticles (AgNPs) and their capability to increase the DDE-uptake by plants. Here, the possibility to optimize DDE-phytoremediation by exploiting both plant-associated bacteria and AgNPs was investigated. Plants were exposed to 100 µg L^{-1} DDE, 500 mg L^{-1} AgNPs or both. The effects of DDE and AgNPs on plant growth and on the plant-associated bacterial populations were investigated.

DDE proved to have a toxic effect on plant growth, while AgNPs did not cause a decrease in biomass. On the contrary, DDE increased the number of endophytes in zucchini Raven, while exposure to AgNPs caused a severe decrease. Bacteria that were isolated from plants that were exposed to DDE or AgNPs experienced less toxic effects when again exposed to these products.

Although AgNPs can be used to increase the DDE-uptake of Cucurbita pepo, it also has severe toxic effects on the plant-associated bacteria. Therefore, a good balance has to be discovered between the advantages and disadvantages of AgNPs for bacteria-enhanced phytoremediation of DDE using Cucurbita pepo ssp. pepo.

Keywords: DDE, *Cucurbita pepo*, nanoparticles, phytoremediation

D3

Plant growth promoting bacterial strains isolated from poplar growing on a diesel contaminated soil

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During phytoremediation, plants are attracting contaminants towards their rhizosphere, where a strongly increased bacterial activity is occurring due to the exudates released by the roots. In turn the bacteria can stimulate plant growth directly or indirectly within the frame of a mutual beneficial partnership. Consequently, phytoremediation of diesel-contaminated sites can be very promising when diesel-degrading bacteria possessing plant growth promoting traits are present in the rhizosphere or inside the plant. Furthermore, to improve phytoremediation of diesel-contaminated soils, these bacteria can be enriched by inoculation. That work has two overriding objectives: (1) development of a robust system for remediation of diesel-contaminated sites by means of Plant – Bacteria partnerships and (2) sustainable production of useful biomass.

Bulk soil, roots, stems, leaves and rhizosphere of poplar trees growing on a diesel contaminated soil were sampled to isolate and characterize the bacterial strains. Sampling was performed at the site of the Ford Motor Company in Genk (Belgium). At this site, hybrid poplar [*Populus deltoides* x (*trichocarpa* x *deltoides*) cv Grimminge] was planted to contain a diesel plume. After purification, total genomic DNA of all morphologically different bacteria was extracted and aliquots of the extracted DNA were used directly for PCR without further purification. PCR products of the 16S rDNA amplification were directly used for 16S rDNA restriction analysis (ARDRA) and strains with representative patterns were selected for sequencing.

The isolated bacteria were screened for their ability to grow and produce biosurfactants in the presence of diesel as well as their plant growth promoting characteristics namely: inorganic P solubilization, phytate production, siderophores, IAA, ACC deaminase, organic acids, acetoin and butanediol dehydrogenase. The results revealed 15 promising strains that will be further tested for their biodegradation ability. The selected strains will grow in flasks with liquid MSM medium containing 1000 mg kg^{-1} diesel as a benchmark concentration. The flasks will be agitated at 150 rpm at 28°C constantly up to 4 weeks and at 1week intervals 20 ml will be extracted and subjected to GC-FID analysis.

Based on the results, the 2 strains with the highest degradation rates will be selected for an inoculation experiment. In this experiment, diesel-exposed poplar cuttings will be inoculated by the selected strains and their phytoremediation capacity will be evaluated by measuring changes in diesel concentration in the soil.

D4

Environmental genomics of willows and microorganisms applied to the decontamination of abandoned industrial sites

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A research project involving scientists from various disciplines has been undertaken to develop an innovative phytoremediation approach for decontaminating industrial sites polluted by complex mixtures of toxic inorganic and organic compounds such as trace metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), etc. This approach integrates field work and experiments in a controlled environment, and draws upon the latest life science technologies: genomics, proteomics, metabolomics and bioinformatics. The project aims to identify the best willow cultivars for soil decontamination, describe plant physiological responses to contaminated soils, as well as characterize the microbial community structures and functions that are directly and indirectly involved in decontamination processes. Our investigation will shed light on how plants, together with microbial communities, respond to natural environments that contain a broad variety of toxic inorganic and organic compounds. The results of this project will provide the basis for improving the detoxifying capacities of willows as well as of a selected set of microorganisms (bacteria, mycorrhizal and other fungi), and allow the development of green technologies for decontaminating soil from both inorganic and organic pollutants.

Keywords: Willows, genomic, microorganisms, phytoremediation, pollutants

D5

Rhizoremediation as the sustainable solution for the treatment of soil pollutants, may we design universal biological system for remediation?

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Remediation processes based on plants and plant–microbe interactions have been proposed to clean up sites contaminated with organic xenobiotics. However, a critical criterion is to find species and conditions to support the metabolism and survival of the degrading rhizobacteria. PCB and chlorobenzoate (CBA) degradation in contaminated soil vegetated with three plant species was analyzed after 6 months of cultivation. To monitor plant-microbial interactions we used several molecular methods with the aim to follow different aspects of PCB and CBA degradation. Cultivable bacteria were identified by MS MALDI-TOF, their degradation abilities analyzed by GC or HPLC. Metagenomic approach based on isolation of soil DNA and sequencing of 16S rDNA showed bacterial populations in rhizosphere. SIP method (stable isotope probing) combined with 454 technology (pyrosequencing) determined active bacteria and their degradative genes in horseradish, black nightshade and tobacco rhizosphere. Members of *Achromobacter*, *Variovorax*, *Methylovorus*, *Methylophilus*, *Hydrogenophaga* or some unclassified bacteria were detected to have derived carbon from biphenyl or chlorobenzoate. The relative abundance of taxa was determined. The HPLC analysis of plant exudates determined root compounds, potential stimulators of bacterial degradation (vanillic, ferulic, cinnamic, caffeic acid, limonen, naringin etc). The addition of these compounds to contaminated soil, as bacterial degradation inducers, showed their crucial potential stimulating also final PCB or chlorobenzoate removal.

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Keywords: rhizoremediation, rhizosphere microbes, metagenomics, PCBs, chlorobenzoates

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D6

Oyster mushroom (*Pleurotus ostreatus* L.) improve degradation of polycyclic aromatic hydrocarbons during composting litter from urban greenery

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Plants accumulate in leaves heavy metals (HM) and polycyclic aromatic hydrocarbons (PAH) and in urban areas amounts of these pollutants can be very high. Therefore litter from municipal greenness should be utilized in controlled conditions. Composting belongs to most environmental friendly method, however content of HM and some PAHs often increases. In this study we aimed at estimation of effect of using of oyster mushroom (*Pleurotus ostreatus* L.) on degradation of PAHs in plant litter. Leaves collected from eight small-leaved lime (*Tilia cordata* L.) trees growing in the neighborhood of road with heavy car traffic were composted in plastic bags with or without fungal mycelium. Mycelium was applied as: (1) cereal seeds overgrown by fungi, (2) spent mushroom substrate (SMS) and sawdust was added to improve fungi growth. After eight weeks of composting samples were air dried and homogenized. Concentration of 16 PAHs was measured using GC/MS or HPLC and, HM using XRF spectrometer (INNOVOX). Concentration of PAHs in compost was lower than in leaves, hoverer decrease of five and six rings PAHs was very small. Addition of oyster mushroom mycelium increase degradation of all PAHs between 10 to 30%, including those of high rings and combination of grain mycelium was most effective. HM content in compost slightly increased but below permissible levels. We conclude that addition of oyster mushroom improves compost quality.

Keywords: composting, oyster mushroom, aromatic hydrocarbons

D7

Shift in Naphthalene-Degrading Bacterial Communities Associated with Rhizoremediation of Diesel

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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 to identify and compare ^{13}C-naphthalene-utilizing bacteria in the willow-planted versus unplanted (control) treatments. Sequencing of 16S rRNA genes from SIP incubations was conducted to identify bacterial populations associated with naphthalene biodegradation and inputs of fertilizer. Terminal restriction fragment length polymorphism (T-RFLP) of ^{13}C-DNA was also performed. In the pot study, growth of *S. alaxensis* resulted in the greatest loss of DROs, while treatments amended with fertilizer contributed to a significant increase in the MPN of culturable diesel degrading microorganisms. A variety of bacteria active in naphthalene utilization were identified using 16S rRNA sequencing of ^{13}C-DNA. T-RFLP profiles of naphthalene-utilizing bacterial populations suggest that the presence of willow and/or fertilizer may have enhanced the growth of a particular bacterial taxon. 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.

Keywords: Rhizoremediation, Diesel, Willows, MPN, SIP, 16S rRNA

D8

Comparative analysis of high and low erucic acid rapeseed response to Diesel oil and petroleum-degrading microorganisms

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Plant-assisted bioremediation (rhizoremediation) seems to be a potential tool to uptake, inactivate or completely remove organic xenobiotics from the polluted environment. Therefore, it is of key importance to find an adequate combination of plant species and microbial consortia that together enhance the clean-up process.
To reveal the potential of rapeseed in rhizoremediation, the seedlings, as well as adult plants of high and low erucic acid rapeseed (HEAR and LEAR, respectively) were investigated upon their exposition to different Diesel oil concentrations and soil microbes that are capable of degrading petroleum hydrocarbons [1]. The oxidative stress in the HEAR/LEAR shoots and roots is reflected by varying activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), as well as Reactive Oxygen Species (ROS). Moreover, glutathione transferase (GST), as an exemplary enzyme of the cellular detoxification system, was also tested. The impact of bacteria consortia on the physiology of HEAR and LEAR plants was estimated by measuring their photosynthetic capacity upon stress conditions. Interestingly, we found that petroleum hydrocarbons were more degraded in the soil which was not inoculated with bacteria and only influenced by the rapeseed rhizosphere. Whether it is the matter of phytotoxicity of bacterial exudates, such as biosurfactants, it remains to be clarified.

This project is supported by the Foundation for Polish Science, from the HOMING PLUS Programme, co-financed from EU structural funds under Action 1.2 'Strengthening the human resources potential of science' of the Innovative Economy Operational Programme 2007–2013.

Keywords: Plant-assisted bioremediation, rhizoremediation, petroleum hydrocarbons, petroleum-degrading microorganisms

Reference:

Owsianiak M, Szulc A, Chrzanowski Ł, Cyplik P, Bogacki M, Olejnik-Schmidt AK, and Heipieper HJ (2009). Biodegradation and surfactant-mediated biodegradation of diesel fuel by 218 microbial consortia are not correlated to cell surface hydrophobicity, Applied Microbiology and Biotechnology 84:545–553.

E: PETROLEUM

E1

Biologically-assisted treatment of soils both contaminated with Heavy Metals and Polycyclic Aromatic Hydrocarbons

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This PhD research plan hypothesizes that the phytoremediation of contaminated soils with both heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs) could be improved through the chemical treatment with biodegradable compounds such as low molecular weight organic acids (LMWOAs) and surfactants added as amendments. LMWOAs and surfactants are proposed to increase the bioavailability of HMs and PAHs to plants and microorganisms, in order to enhance the phytoremediation efficiency of contaminated soils in an environmentally friendly approach. The main general objective is to study the effect of LMWOAs and surfactants on the phytoremediation of soils contaminated with HMs and PAHs. Specific objectives include: (1) studying the effects of LMWOAs and surfactants on the HM and PAH bioavailability, (2) analyzing the phytoremediation efficiency of LMWOAs and surfactants, (3) comparing the phytoremediation efficiency of LMWOAs and surfactants and (4) proposing a combined amendment treatment with LMWOAs and surfactants suitable for the phytoremediation of soils contaminated with both HMs and PAHs. To accomplish the mentioned objectives the following methodologies are intended. The bioavailability of contaminants will be studied by *in situ* soil solution extraction studies. The rate of HM phytoextraction will be assessed by the determination of plant biomass, quantification of HMs in plant parts and soils and calculation of phytoextraction parameters. The study of the performance of PAH rhizodegradation will be achieved by the quantification of PAHs in soil, calculation of PAHs removal rate and measurement of soil microbial biomass and activity.

Keywords: Phytoremediation, heavy metals, polycyclic aromatic hydrocarbons, low molecular weight organic acids, surfactants

E2

Successful rhizoremediation of polyaromatic hydrocarbons using Lupins

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In this project we assessed the effect of lupins on microbial degradation of pyrene and creosote contaminated soils. Sandy loam soil with low nutrient content was amended with pyrene at a concentration of 100mg kg^{-1}. Creosote amended soil (5000 mg kg^{-1}) was allowed to age for 2 years prior to commencement of growth trials. Growth trials were conducted under greenhouse conditions for a period of 12 weeks. Two species of lupin (*L. albus* and *L. angustifolius*) showed high germination and plant growth under most conditions, with increased root growth seen in some contaminated treatments. Nodulation was seen on both lupin species, and proteoid roots developed on *L. albus* plants during the course of growth trials.

Both *L. albus* and *L. angustifolius* were able to significantly increase the degradation of pyrene and creosote in soil comparative to unplanted controls. Overall removal in soils amended with pyrene was 99.0% in planted systems compared with 78.8% in unplanted controls after 12 weeks. Mean creosote removal after 12 weeks was 89.6% in unplanted controls, compared with 93.8% in the presence of *L. albus* and 99% in the presence of *L. angustifolius*. No significant difference was seen between either lupin species for their ability to degrade the tested PAH. No bioaccumulation of the pollutant was detected. There exists the potential for Lupins as a croppable species for remediation of PAH contaminated soils.

Keywords: Rhizoremediation, PAH, Lupins

E3

Identification of plant species around oil spilled environments in delta state Nigeria

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This study was carried out to identify plants that survive in oil polluted sites in locations where oil exploration and exploitation take place in six Local Government Areas of Delta state Nigeria. The locations included oil locations, flare stations, flare knock down drum stations and mustering points which had experienced oil spill at recent times. It involved the collection and the identification of the plant species and studying their abundance and distributions. Sixty different plants belonging eighteen families were identified. Some plants were found in only one location each while some were found in two or more locations each. *Andropogon gayamus, Heteropogon contortus, Eleucine indica and Panicum repens* were the most prevalent plants encountered in the study as they were found in most of the sites. Plants in the poaceae family are the most abundant with 22 plants encountered in the study. Erhioke flare station had the highest diversity of plants with 19 plant species while Oweh mustering point had the least diversity of plants with 4 plant species. The results obtained from this study show that some native Nigerian plants are resistant to petroleum contamination. It is recommended here that such plants can be evaluated for their potentials to clean up petroleum polluted soils. This can help in the reclamation of the oil polluted soils in the Niger Delta region of Nigeria in particular and to solve the problems of oil pollution generally.

Keywords: Pollution, Plant collection, Exploitation, Survival, Remediation

E4

Chrysanthemum leucanthemum L. potential to Crude oil phytoremediation

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Chrysanthemum leucanthemum L. is az prennial plant which is able to grow under stress environment. Its ability to tolerate though situation made it as a suitable plant to examine its potential to grow under crude oil contamination. in current study C. leucanthemum plants sown in pots under 4 different concentration of crude oil which were 2.5%, 5%, 7.5% and 10% (w/w), control pots had no contamination. Soil TPH of each treatment examined after 2 and 4 months. Results showed significance decreases in soil TPH contamination, microbial counts increased during 4 months. plants physiological parameters include chlorophyle, proline and peroxidase activity indicated that C. leucanthemum is able to tolerate crude oil pollution and grow safety. Current survey showed that C. leucanthemum is a suitable plant to phytoremediate crude oil contamination. Statistical Analysis has done by SPSS 19.

Keywords: *Chrysanthemum leucanthemum*, Crude oil, Phytoremediation, TPH

E5

Effects of Soil Clean - up using *Lemon grass* on the interaction of selected soil minerals with organic pollutants: management implications

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Successful phytoremediation presumes that the target pollutants can be made bioavailable and that a competent flora, community of potential microbial degraders either already exists and can be stimulated or that can be introduced and established. The formation of organic – mineral interfaces in a crude oil inundated ''illegal oil pipe tapping soil'' was studied by monitoring the variables – texture, pH, Organic carbon, total Nitrogen, available P, Dry mass loss, PAHs concentration, and Base (Ca, Mg and K) as well as the 90d resulting clean soil. The clean – up technique employed Lemon grass and introduction of Compost and Humin amendments were undertaken for comparison. After 90d, CO_2 mineralization was significant and N cycling was strongly coupled to the C cycling. Addition of compost indicated co-metabolic degradation. Also, concomitant activity of phytoremediation and the agronomic management practice were more effective. PAHs levels above intervention values was sequestered up to > 50%. The dissipation of the 16 USEPA-listed PAHs was largely enhanced from no significant change to 50.8±12.4% (for humin amended); 62.9±7.1% (for compost amended). The study demonstrated the critical role of Organic matter in determining the fate, mobility, and bioavailability of these priority contaminants

Keywords: Soil interactions, Contaminated Soil, Remediation, Bioavailability, PAHs, Humin

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E6

Biodegradation of BTEX with Tomatoes (*Lypersicon esculentum*) and Lemon grass (*Cymbopogon citratus*) in petroleum hydrocarbon - contaminated soils

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Soils contaminated with hydrocarbons are a serious problem in Nigeria and 70% of such soils are distributed in the Niger Delta. This pot experiment was conducted to determine the efficiency of Tomato and Bambara beans on reduction of hydrocarbon load at crude oil contaminated soil. The plant's potential for stimulating the biodegradation processes of petroleum hydrocarbons was tested under the application of two fertilizer types. The rhizospheres and phyllospheres of Tomatoes and Bambara beans raised in an oil – stressed soil (10%, w/v) were rich in oil-utilizing bacteria and accommodated large numbers of free-living diazotrophic bacteria, with potential for hydrocarbon utilization. The specific root surface area was reduced under the effects of petroleum. Phytoextraction of potentially toxic BTEX was investigated and results indicated uptake values up to 4.48 mg/kg into the shoot tissues of Bambara beans grown in crude oil contaminated soil. The manure application significantly increased the shoot BTEX concentration for Bambara beans. There was no evidence of Tomato enhancing the biodegradation of crude oil in soil under the conditions of this trial. These results indicated that Bambara beans can be useful for phytoremediation of hydrocarbons – contaminated soil.

Keywords: Phytoextraction, Environment, Biostimulation, BTEX, Free-living diazotrophic bacteria

E7

The comparative effect of fresh and spent engine oil on the anatomy and morphology of *Zea mays*.

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The exposure of plants to spent and fresh engine oil has been of great concern to man because they create unsuitable conditions for agricultural and recreational activities. The comparative effects of spent and fresh engine oil on the anatomy and morphology of *Zea mays* were investigated in this study. Soil samples were contaminated with spent and fresh engine oil in the following percentage concentrations; 0%, 2%, 4%, 6%, 8%, and 10% w/w. Seeds of *Zea mays* were sown in the soil samples and germination and some growth parameters were monitored. These include rate of germination, percentage germination, percentage survival, total number of leaves present per plants, leaf area, leaf area index, shoot length and chlorophyll content. Results obtained revealed that seeds sown in soil contaminated with fresh engine oil had delayed rate of germination compared to seeds sown in soil contaminated with spent engine oil. Spent engine oil had adverse effect on the leaf area compared to fresh engine oil. Although fresh engine oil had a greater devastating effect on the germination and growth performance of *Zea mays*, the effect of spent engine oil on the anatomy of the roots and leaves of *Zea mays* was more devastating. A significant difference was observed at 2% and 4% concentrations ($P<0.0001$). From the results obtained, indiscriminate and unhealthy disposal of spent engine oil on land has significant effect on early growth of plants; therefore recycling of spent engine oil to more useful substance should be encouraged.

Keywords: *Zea mays,* Anatomy, morphology, spent engine oil, fresh engine oil

E8

Assessment of the Potential of *Cynodon dactylon* linn. for Phytoremediation of Crude oil Contaminated Soil

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The growth of *Cynodon dactylon* under different concentrations of crude oil was assessed to determine its phytoremediating potential in a greenhouse experiment. The crude oil was applied at the rate of 0.0, 2.5, 5.0, 7.5, 10.0 and 12.5 ml bowl^{-1} to sixty bowls (each measuring 30 cm x 12 cm) arranged in a completely randomized design. Five 2 cm long rhizomes of *C. dactylon* were planted in each bowl one week after soil contamination. Growth parameters were measured weekly after four weeks of establishment for another eight weeks. The results showed that all the crude oil treatments, except at 5.0 ml bowl^{-1} were significantly ($p < 0.05$) different with regard to the leaf length, leaf breadth and number of leaves at 5 WAP and 12 WAP. The percent cover of *C. dactylon* at different crude oil contamination was: 0.0, 70.0%; 2.5, 67.0%; 5.0, 60.0%; 7.5, 67.0%; 10.0, 52.0%; 12.5, 64.0%. The higher the crude oil, the higher was the organic carbon content, cation exchange capacity of the soils and total hydrocarbon of the leaf. The test plant performed optimally at high concentration of the contaminant. The study therefore concluded that *C. dactylon* could be effectively used in the phytoremediation of crude oil contaminated soil.

Keywords: Crude oil, *Cynodon dactylon*, phytoremediation, soil contamination, soil health

E9

Impact of metabolites on the phytoremediation of hydrocarbons

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Phytoremediation of petroleum hydrocarbons has proved its efficiency compared to bioremediation. However, treatments usually do not reach a complete removal and some contaminants remain. Several hypothese were proposed to explain this incomplete degradation such as limited bioavailability of hydrocarbons or a production and accumulation of toxic metabolites (Chaillan et al., 2006). The aim of this study was to assess *i)* the effect of plant on residual biodegraded hydrocarbons, and *ii)* the toxicity of the native and partially biodegraded hydrocarbons on plant. Experiments were conducted with maize (*Zea mays*) in hydroponic conditions for 2 months. Three modalities of Hoagland solution growth were tested in four replicates: without contaminant, contaminated with hydrocarbon (fuel, 1 g L^{-1}), contaminated with partially biodegraded hydrocarbon (after 12 weeks of biodegradation). Growth systems consisted in glass bottles filled with 700 mL solution, planted or not with two-weeks maize plant (Chaîneau et al., 2000). Plant development and root system structure, organic compounds nature and concentration, and bacterial community structure were regularly assessed. Results showed that the presence of plant allowed an increase of biodegradation resulting in a higher degradation rate and an absence of some residual organic compounds. However, root architecture was strongly affected by both native and biodegraded hydrocarbons. The decrease in the root length and an increase in the average root diameter were observed. However, the negative effect of biodegraded hydrocarbon on the root system seemed transient and the plant was able to overcome it on the longer term.

Keywords: hydrocarbons, metabolites, rhizodegradation

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E10

The effect of rapeseed oil on the rhizodegradation of a PAH-contaminated soil

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Plants have the ability to promote degradation of polycyclic aromatic hydrocarbons (PAHs) in contaminated soil by supporting PAH degrading microorganisms in the rhizosphere (rhizodegradation). The aim of this study was to evaluate, if rapeseed oil can increase rhizodegradation, because various studies have proven that vegetable oils (e.g. sunflower, rapeseed, soybean oil) are able to act as an extracting agent for PAHs in soil for remediation purposes and might therefore also increase bioavailability of PAHs for microbial degradation. In this study different grasses and leguminous plants were tested. The results suggested a significant impact of the oil on plant growth (decrease of plant height and biomass). The rapeseed oil affected the detected concentrations of different PAHs in soil from a former wood impregnation factory in Kühnsdorf (Austria) in opposite ways. In soil treated with rapeseed oil the content of some PAHs appeared to be increased compared to untreated soil, while other PAHs showed a decrease after three months. The results of the pot experiment did not show an increased degradation of the sum of 16 EPA-PAHs degradation by plants within three months. However, a rhizobox experiment showed a significant reduction of PAH concentration in the rhizosphere of alfalfa (*Medicago sativa*) cv. Europe. Our investigations also showed significant differences in the degradation behavior of the 16 individually analyzed PAHs.

Keywords: rhizodegradation, rhizosphere, 16 EPA-PAH, rapeseed oil, rhizoboxes

F: Ecosystem Services

F1

Jatropha Perfect feedstock for biodiesel and rural electrification

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A decentralized power generation plant fuelled by straight jatropha oil is implementing in various villages in Ghana. The project aims to improve socio-economic conditions of small rural populations in Africa through small-scale electrification ,demonstrate the potential of CHP generators as a means of bringing electrification to small, off-grid villages in Africa, demonstrate the potential of jatropha oil and other Straight Vegetable Oils (SVOs) from oilbearing energy crops as a means of running a small CHP generator (40kW),identify and demonstrate potential communal or small-holder ownership models for the successful sustainable management of the generator/extractor and jatropha seed production and disseminate the model for replication in other areas. In summary, the jatropha based electrification in Ghana will reduces greenhouse gas emissions. However, environmental benefits can only be achieved if jatropha is cultivated not to compete with food. Under this condition, jatropha-based electricity generation might be a useful alternative to other renewable electrification options, as the technology is very sturdy and can be maintained even in remote and highly under-developed regions

Keywords: electrification, jatropha, greenhouse gas emission, environment

F2

Attributes of ecological relevance for the monitoring of soil quality in a field chemostabilization study

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During chemostabilization programs, the incorporation of organic amendments to heavy metal polluted soils, together with the establishment of a vegetation cover, contribute to a reduction in metal bioavailability and the recovery of soil quality. Nevertheless, the establishment of follow-up monitoring programs is essential to ensure the long-term effectiveness of chemostabilization programs. In this respect, although soil microbial properties are being increasingly used as indicators of soil quality, we frequently feel that there is still an unsolved gap between the measurements soil microbial properties and the concept of soil quality. With the objective of reducing this gap, we recently proposed to link the concept of soil quality to that of ecosystem health through the grouping of microbial properties within a set of ecosystem attributes of ecological relevance (vigor, organization, stability, suppressiveness, redundancy). In order to validate this approach, these attributes were measured in a field study where increasing doses of lime-treated sewage sludge were applied to a metalliferous soil from an abandoned mine heavily polluted with Cd, Pb and Zn. The evolution through time of metal bioavailability, soil pH, soil nutrient contents and vegetation growth was also monitored. The assessment of soil quality at the attribute level might facilitate the communication between scientists and non-scientists.

Keywords: Chemostabilization, ecosystem health, heavy metals, resilience, soil quality

F3

Microbial Activities in the Rehabilitated Landfill

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A landfill is a constructed space on the ground to store waste, as it gradually breaks down into chemically inactive material. The landfill is filled with garbage and these garbage was covered with soil or quarry. The landfill remediation operation is responsible for the restoration of the site and creates a low hill planted with native plants which are indigenous to the area. The aim of the study was searching ways to use of leachate water from solid waste landfill sites for irrigation of plant species which normally grow in the wild. The study focus on the plant species which were Althea rosea (hollyhock), Cynodon dactylon (Bermuda grass) and Melilotus officinalis (Yellow melilot). During the two years of the study the plants were irrigated with tap and leachate water under drought conditions. After the experiment populations of Escheria coli, total coliforms and fecal coliform bacterias in the soil samples were analyzed and it was seen that using leachate water to cultivate different kinds of plants increased the populations of fecal coliforms and total coliform in the soil. Another result was show that Yellow melilot decreased the coliform amount in the soil.

Keywords: Landfill, leachate, remediation, soil, microbial activity

F4

Phytoextraction and Ecological Catalysis: a symbiosis for future

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The main goal of this presentation is to prove the great interest of metal hyperaccumulator in industrial biotechnologies and chemocatalytic processes. Phytoextraction can constitute the starting point of an original and efficient approach to modern heterogeneous catalysis (Grison et al. 2011)

Ni and Zn hyperaccumulator extracts allow the preparation of original polymetallic catalysts, which are multi-component chemical systems. Specific interactions and cooperative effects modulate the overall chemical behavior of these catalysts; they lead to synergetic effect, which improves the catalytic performance.

Different illustrative examples are proposed; the synthesis of platform molecules and natural compounds is presented. This approach is a green solution with chemical benefits: high yields, excellent regio- and stereoselectivity, small amounts of catalysts and concrete perspectives towards the exhaustion of mineral resources.

From these studies, it may be concluded that phytoextraction and metal hyperaccumulating plants are a great interest for cutting-edge Green Chemistry.

Keywords: Metal hyper accumulating plants, phytoextraction, ecocatalytic approach, biobased organic synthesis

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F5

The Systematic Ecosystem Change

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Virtually all of Earth's ecosystems have been significantly transformed through human actions. Changes have been especially rapid in the last 50 years and today the fastest changes are taking place in developing countries. Ecosystems are particularly affected by large-scale fishing, freshwaters use, and agriculture.

Ecosystem depend on fundamental environmental cycles such as the continuous circulation of water, carbon , and other nutrients. Human activities have modified these cycles, especially during the last 50 years, through increases in freshwater use, carbon dioxide emissions, and fertilizer use. This in turn has affected the ability of ecosystems to provide benefits to humans.

Many animal and plant populations have declined in numbers, geographical spread, or both. For instance, a quarter of mammal species are currently threatened by extinction. Human activity has caused between 50 and 1000 times more extinctions in the last 100 years than would have happened due to natural processes. Increasingly, the same species are found at different locations on the planet and the overall biodiversity is decreasing, because some rare species are lost and common ones spread to new areas. Overall, the range of genetic differences within species has declined, particularly for crops and livestock.

Keywords: ecosystems, freshwaters, environmental cycles ,nutrients, carbon dioxide emissions ,populations, biodiversity, genetic, species

F6

Aboveground biomass, standing floor litter and soils Carbon stock in a 10 year *Tectona grandis* plantation in Ile-Ife, Southwestern Nigeria

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Reforestation and regeneration activities have the potential to sequester carbon. However, little information on the effects on C budgets in plantation ecosystems is available in Nigeria. This paper attempts to provide information at the micro level in response to the reforestation activities. This study focused on estimation of carbon stock in the above ground biomass, its determination in standing floor litter (leaf and small wood) and soils in a 10 year old *Tectona grandis* and a nearby re-growth at Obafemi Awolowo University, Ile-Ife Nigeria. Four quadrates, each of 25 x 25 m size were established in the plantations and the secondary forest. Soil samples were randomly collected at (0-20 cm) from the study sites, bulk density and total C concentration were analyzed. Total C stocks were calculated by multiplying C concentration with their respective bulk density and sampling depth. Standing floor litter were randomly collected at five points every three months, sorted into different litter components and analyzed for total C concentration and used to calculate total carbon. Total C stock of soil varies from 1.10 in the plantation to 0.99 t ha^{-1} C in the secondary forest. Above ground biomass, standing leaf and wood litter were estimated at 18.26-5.81, 0.49-0.36, 0.06-0.08, t ha^{-1} C, respectively from the plantation to the secondary forest. It is clear that reforestation after 10 years has increased C stocks by 45% in the above ground biomass in the plantations, other parameters determined were also altered.

Keywords: Carbon stock, plantation, reforestation, standing litters

F7

Organo-zeolitic biofertilizers: A new approach to the promotion of plant growth

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The aim of this proposal is to evaluate the benefits of using an organo-zeolitic fertilizer (biofertilizer) for enhancing plant growth. Apart from the ever increasing cost of chemical fertilizers, their use over the last sixty or more years has had a deleterious effect on soil health. In contrast the biofertilizer, composed of organic waste and crushed zeolitic rock, functions biologically in sponsoring nitrification. Ammonium ions, provided from the degradation of the organic waste, are adsorbed to the zeolite mineral surface thus avoiding loss to the atmosphere by volatilization. Oxidation of the ammonium ions, by soil nitrifying micro-organisms, provides major and trace element nutrients. Analysis of pore water, from substrates amended with the biofertilizer, has shown that its cation concentration is very much higher than that of pore water from un-treated substrates. These cations, which cover a wide range of elements, provide essential major and trace-elements in ionic form that are available for uptake. Without the zeolitic component the degree of nitrification is greatly reduced and the converse applies in that application of zeolitic rock without the organic component has little effect on plant growth. Many countries in the world have extensive deposits of rock containing a high abundance of common zeolite minerals and the organic component, being animal or plant waste, is of general availability which makes the use of the biofertilizer a feasible proposition.

Keywords: Zeolite, Organic waste, Biofertilizer, Ammonium ions

F8

CO_2 fixation increase by new effectors of photosynthetic enzymes, ecdysteroids

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Ecdysteroids seem to have a new biological role. These steroid hormones control many physiological processes of insects and crustaceans. They are found also in many plant species, but no other function in plants than antifeeding activity towards herbivorous insects has been ascribed to them until now. Our results show that ecdysteroids and their analogues act as effectors of activity of some plant enzymes and other proteins. As far as some of the identified proteins play very important roles in plant organisms, *e.g.* in photosynthesis, or as pathogenesis-related proteins, the role of ecdysteroids in their regulation has to be clarified. The enzymes include among others ribulose1,5-bisphosphate carboxylase/oxygenase (RuBisCO), PsbP protein of the oxygen evolving complex of FS II or *e.g.* osmotin. The effect of oxysterols on photosynthetic activity of plants was analyzed in several plant species, namely spinach, maize and tetragonia. Photosynthetic activity was examined at the level of oxygen production from leaf discs, photosystem II performance based on the detection of changes in chlorophyll fluorescence kinetics and the content of photosynthetic pigments. The influence of ecdysteroids on RuBisCO was studied using radioactively labelled CO^2. Our results show that different ecdysteroids are able to increase oxygen production by water cleavage and the yield of RuBisCO-mediated reaction in which CO_2 is fixed into organic matter thus allowing fine tuning of the yield of photosynthesis.

Acknowledgements**:** The research was supported by grant P501/11/1650 of the Czech Science Foundation.

Keywords: CO_2 fixation, RuBisCO, ecdysteroids, oxygen evolving complex

F9

Ecological sustainability of Mediterranean landscapes. Benefits from phytotechnology: the case of Villena Municipality (Alicante, SE. Spain)

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Conventional Water Treatment Plants (CWTPs) represented since early 90's the paradigm in water pollution control in many Mediterranean semiarid landscapes in Spain. Following EU water directives implementation CWTPs were built with two main goals: a) reducing sanitary risks via fresh vegetables or water consumption and b) improving water quality affecting water public domain (rivers, ponds, ramblas or aquifers).

This contribution analyzes the case of Villena municipality in the province of Alicante (SE. Spain). The use of CWTPs as the only sewage water depuration process (WDP) since 1992 led to the disappearance of the habitats that sustained populations of fartet (*Aphanius iberus*) a little fish included in the red list of vertebrates in risk of extinction and many other species present in these wetland ecosystems. We analyze the potential of phytodepuration in this scenario from a perspective of landscape sustainability. Using GIS tools, available data for water quantity and quality in and out of the CWTP, aerial photo analysis of land use changes, and climatic and surface hydrological information we suggest alternative scenarios in which phytotechnology would have substantially improve the ecological status of this landscape.

Water Framework Directive (WFD) implementation is discussed, pointing out the importance of adapting the concept of ecological flow at different spatial scales for these type of semiarid Mediterranean ecosystems. Using phytotechnologies as a way to obtain and manage micro-ecological flows must be a priority to the sustain small but very relevant wetlands and their associated biodiversity.

Keywords: water pollution, *Aphanius iberus*, WFD, GIS, land use changes

F10

Relevant ecosystem services of Vinalopo River (SE. Spain). Why WFD implementation forgets Phytotechnologies in semiarid medditerranean landscapes?

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Vinalopo river basin extends over 1979 square kilometers, most of them within the limits of Alicante province (SE. Spain). Its main course, 90 kilometers in length, connects inland cool sub humid with coastal warm semiarid Mediterranean ecosystems, reaching the sea in Santa Pola Salines Natural Park. The quality and quantity of its natural water flow defines clear sectors. Just the upper course (one fifth of its total length) has a regular water flow regime. Inflows from Conventional Water Treatment Plants (CWTPs) determine habitat heterogeneity, and ecological effects on riparian vegetation productivity and in biodiversity. Passive phytodepuration occurs, ameliorating water quality reaching surface aquifers, but surprisingly, no reference is done to phytotechnologies among the "important topics (IT)" identification generated as deliverables from the Water Framework Directive protocols implemented by the Watershed Authority (Confederacion Hidrografica del Jucar).

This contribution analyzed the priorities of IT 03.03 "Pollution control and improvement of physico-chemical quality of Vinalopo river waters" in relation to ecosystem services. This goal was addressed at different spatial scales pointing out the importance that appropriate scaling of management decisions has. Solutions implementing available phytotechnologies in the four main municipalities scenarios (Villena, Elda, Novelda and Elche) were examined leading to more integrated ecosystem management alternatives than the conventional ones. Habitat homogenization and water reutilization by agriculture appeared as driving forces in the regression of wetland ecosystems linked to the Vinalopo river. Environmental conditionality subsidies must be addressed in relation to these high water demand agro ecosystems to avoid very negative ecological impacts.

Keywords: ecosystem management, WFD, Wastewater impacts, scaling management, land use changes

F11

Comparisons of different plant species and soil additions for the vegetalization of highly disturbed soils

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The establishment of a productive vegetation is a prerequisite to rehabilitate or phytoremediate a disturbed land. Often, industrial sites have no proper soil and do not contain organic matter. This lack of biological properties impairs the capacity of plants to grow. This project aims at evaluating the potential of eight plant species and four soils amendments to promote plant growth on a simulated industrial soil.

The plant species tested were: perennial ray grass (*Lolium perenne*), alfalfa (*Medicago sativa*), tall fescue (*Festuca arundinacea*), barley (*Hordeum vulgare*), indian mustard (*Brassica juncea*), pumpkin (*Cucurbita pepo*), alder (*Alnus crispa*) and willow (*Salix miyabeana*). To simulate the soil conditions found on industrial sites, four soil mixtures were prepared: T1, Control only with potting soil; T2 gravel with a 2 cm layer of potting soil; T3 gravel with a 2 cm layer of potting soil and Biochar; T4 gravel with a 2 cm layer of potting soil and mineral fertilizer. The experimental design comprised six blocks for a total of 192 pots.

After three months of growth, all the species tested grew well in the simulated soils, irrespective of the treatment applied. A significant reduction was observed in the above and below ground biomasses when phosphorus-rich mineral fertilizer was applied. The plant species that displayed the more below ground biomass was alder, willow and alfalfa, independent of the fertilization treatment applied In conclusion, this project illustrated that the plants tested were able to produce a root system in the mineral soil used simulating an industrial environment.

Keywords: Vegetalization, Phytoremediation, Roots, Biochar

F12

To sustain sustainability in agricultural using industrial by-products

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Sustainability of agriculture depends on environmental circumstances including the available nutrients while there are efforts to minimize the use of non-renewable sources. The produce of fertilizers needs lots of energy and cost. Plenty of harmful gases get out during the production. If we reuse the wastes and by-products – which contain organic matter, micro and macro elements and have got no harmful effect to environment – will be possibility to reduce the use of fertilizers. The aim of our study is to give a brief overview about the effects of sewage sludge and compost on the physiological parameters of plants. Maize (*Zea mays L cvs. Norma SC*) and sunflower (*Helianthus annus* L. Arena) seedlings were used in the experiments. Dry matter accumulation of shoots and roots, relative chlorophyll contents, chlorophyll a, b and carotenes contents were measured. Moreover, we also measured the elements uptake and the leaf area.

The relative chlorophyll contents of maize increased when sewage sludge treatment was applied. The chlorophyll a, b and carotenes contents decreased comprised to the control. This observation was similar in case of sunflower, too. The treatments have advantageous effects on dry matter accumulation of shoots and roots in both experimental plants. The leaf area decreased in all treatments in comparison to the control. The land application of sewage sludge is a common agricultural practice worldwide. It effectively disposes of a waste product while recycling valuable nutrient into the soil - plant system. We have to try to reuse more and more waste and by-products.

Keywords: compost, sewage sludge, sustainable agriculture

F13

Soil formation in reclaimed loess in the South-Eastern Kazakhstan

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In the article questions relating to the primary soil-forming process after remediation on the exposed loess for the periods from 1991 to 2011.

The studies found that under the influence of soil-forming factors of phytocoenosis reaches a quasi-climax for 15 - 16 years. As a result of syngenetic rare species (rare ruderal) formed grass - Multiplant (heterograminae - heteroherbae ass) quasi-climax phytocoenosis. In the process of syngenetic soil formation on loess rocks observed changes in morphological characteristics of the soil forming.

In the process of syngenetic soil formation on loess soil rocks revealed the following processes: secondary argillization, humus accumulation, illimerization (lessivage) and leaching.

Keywords: soil formation, soil process, phytocoenosis succession, loess

F14

Improving ecosystem health and functioning through phytotechnologies at Freshkills Park, Staten Island, New York, USA

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The 2,200 acre Freshkills Landfill in Staten Island, New York was once the largest landfill in the world, and current reclamation plans will result in the largest landfill-to-park conversion in history. This process involves importing soil to cover the landfill and provide a suitable environment for establishing native vegetation. The imported soil must meet certain regulatory chemical standards before it can be considered suitable for public, non-industrial use. The overall goal of the research is to assess the potential of phytotechnologies to improve the physical, chemical, biological, and agronomic characteristics of soils imported to Freshkills as well as to improve the soil usage classification. To accomplish this, we identified individual parent trees of the *Populus* and *Salix* genera growing on Staten Island, as well as existing seed banks of *Panicum* (switch grass). Scions from 112 total *Populus* and *Salix* genotypes were collected from three Staten Island locations during February 2012 and are currently being propagated for scale-up. Specific genotypes with the greatest ability to remove inorganics that diminish the suitability of the soils will be determined through phyto-recurrent selection, a process consisting of greenhouse studies in Rhinelander and subsequent field trials at Freshkills Park. The long-term goal is to select and establish the most successful genotypes throughout Freshkills Park. The objectives of the current presentation are to highlight the plant selection and propagation phases, as well as key components of the experimental design, in order to stimulate discussion about technical aspects of the project and the potential for future collaborations.

Keywords: ecosystem services, phyto-recurrent selection, soil quality, sustainability

F15

Ecosystem services associated with purpose-grown *Populus* and *Pinus* in North America

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Forest biomass constitutes ~30% of the total biomass that can be produced in the United States, making adequate woody feedstock availability necessary for environmental and economic sustainability. In addition to natural stands, improved woody biomass production and management systems are needed to: maintain healthy forests and ecosystems, create high paying manufacturing jobs, and meet energy demands. Short rotation *Populus* species and hybrids are renewable energy feedstocks for biofuels, bioenergy, and bioproducts that are strategically placed in the landscape to conserve soil and water, recycle nutrients, and sequester carbon. Similarly, plantations of *Pinus* have been established throughout North America as key components of woody feedstock portfolios. The objective of the current presentation is to integrate results from two region-wide studies of these genera to assess the impacts of varying climatic and soil conditions on the growth, productivity, and wood properties of these purpose-grown trees, with special emphasis on carbon sequestration the provision of additional ecosystem services. Results will be presented from two *Populus* regional testing networks throughout the north-central United States that were ten and twenty years old at the time of sampling, along with a unique range-wide network of white pine (*Pinus strobus* L.) provenance trials established in the early 1960's in the eastern United States and Canada. In total, we tested twenty three plantations: four ten-year-old and eleven twenty-year-old of *Populus* and eight of white pine. Information will be presented about key linkages among climate and carbon issues associated with growing these dedicated feedstocks for multiple uses, including phytotechnologies.

Keywords: energy security, feedstock production, hybrid poplar, white pine, sustainability

G: DESIGN

G1

Floating Phytoremediation Practice in Yundang Lagoon, Xiamen, China

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Eco-floating-bed (EFB) phytotechnology is recognized as an economic, efficient, environment-friendly and biosecure novel technology in the field of aquatic bioremediation. In practical application, combine with the soilless-culture of industrial crops, EFB may be used to cultivate crops, as well as to purify water quality in aquatic environments. Here, concerning with either the ecological restoration or the seawater-tolerant vegetable production in eutrophic saline waters, the results of our study and the practical demonstration of EFB technique in the Yundang Lagoon (a coast lagoon in Xiamen, south east China) were introduced. Further, the application prospects and technological problems of this novel technology in practice are discussed.

Keywords: Floating phytoremediation, Eco-floating-bed, Practical application, Yundang Lagoon

G2

Charcas de Los Cabezos landscape project. Habitat creation and ecological restoration of some ecosystems from the old Laguna De Villena. (Alicante, SE. Spain)

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Biodiversity is site and habitat dependent and species extinctions are related to habitat lost. Species restoration is strongly affected by stakeholders perspective and by decision makers will. Habitats regression is a direct effect of stakeholders demands. Sustainability of ecological restoration projects depends on the conflicts they generate. Realistic restoration must recreate an habitat and facilitate natural species flow. The spatial and temporal scale of every project step is very relevant to its success.

This contribution analyzes a specific landscape project “Charcas de los Cabezos” from a restoration ecology perspective. The main goal of it nowadays, is to redesign a portion of land, owned by the municipality of Villena (Alicante, SE, Spain), to obtain a set of wetlands with different water regimes. Public benefits would be the creation of an environmental educational infraestructure and the first active project to recreate some of the ecosystems and associated biodiversity of the “old” Laguna de Villena. Dried out in early 1800’s constitute nowadays the SIC “Laguna y Saleros de Villena” and illustrates the natural history associated to freshwater demands in semiarid landscapes. After analyzing the characteristics of this Laguna from historical information, potential habitats to be restored were analyzed. The second goal was the discussion of the characteristics of a constructed wetland that using outflow from Villena Water Depuration Plant would allow a daily input of a maximum of 200 cubic meters to the Public Water Domain. Spatial design of phytotechnologies, efficiency and sustainability of the CW were discussed.

Keywords: Constructed wetlands, wastewater reutilization, restoration ecology, Eco-development, *Aphanius iberus*

H: PHYTOFORENSICS

H1

Rapid Phytoforensics for Energetics

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Military munitions and propellants are common contaminants from years of production, distribution and use. Fugitive contaminants are difficult to detect in groundwater and the potential remediation efficacy relies on accurate delineation of the contaminated areas. As vascular plants extract water and nutrients from the subsurface, they also accumulate trace contaminants from their surroundings. Novel sampling and analytical approaches can access this data which exists *in planta*.

To analyze plant samples, LC-MS-MS analytic methods for Perchlorate, PETN, HMX, RDX, TNT, 2A-DNT, nitroguanadine, and DNAN were developed with a total run time of < 6 minutes and method detection limits (MDLs) are the lowest yet reported for many compounds, down to 20 ng/l. Novel centrifugation techniques extract the transpiration tissues stream fluids mechanically without any solvents and extracts are filtered injected directly, compared to traditional solvent extractions and condensing.

The advantages and disadvantages of the rapid plant sampling methods will be discussed. Plant tissue - subsurface concentration relationships of Perchlorate, PETN, HMX, RDX have been shown to be essentially linear for the rapid centrifugation methods, revealing potential for using a variety of plant species for phytoforensic analysis. Different plant species had widely varying subsurface: plant relationshps. By developing novel plant sampling methods and advancing analytical methods, subsurface contaminant delineations may be conducted without ever breaking the surface, thereby minimizing concerns of UXOs in preliminary site investigations. These methods are also very rapid, inexpensive and minimally invasive to property or to the ecosystems we are working to protect.

Keywords: Energetics, Explosives, Phytoforensics, phytomonitoring, uptake

H2

Phyto test cells designed to mimic mature rhizosphere effect for regulatory permiting and construction

Licht Louis

Ecolotree Inc. 3017 Valley View Ln, NE. North Liberty IA 52317

A standard test cell procedure will assist regulatory agencies accept phyto process reactions as an alternative to more conventional mechanical/biological water treatment technology.

The prototypes are a 30 cm x 30 cm x 80 cm well in a box made from water-tight foam is filled with a growing medium containing six populous spp. or Salix spp. trees and irrigated. The PTC can be drained by gravity or pumped to control vadose zone redox conditions.

Typical local soil placed in the PTC is irrigated with effluent to control the dwell time and measure effluent contaminant removal rates. Or contaminated soil is the growing medium blended with available amendments. In all cases, the root expansion allows intimate contact with contaminants either in the water or soil.

Phyto test cells are being used as a standard device that allows statistical evaluation of phyto variables. At three locations, PTC allow treatment potential comparing fixed features like field soil, waste water, and climate while manipulating alternate amendments, plant species, dosing rates and water dwell time.

At another lagoon site, polychlorinated biphenyl's (PCB) in sludge are being phyto treated to determine removal and transformation in this safe and representative way.

The ability to efficiently achieve a valid permit for field-scale phyto treatment is essential for expansion of this technology. This paper will define the technique, evaluation statistics and potential uses based on two years of development.

Keywords: Phyto test cells, standard technique, permit, regulation

H3

The TSCF: Modeling beyond log K_{ow}

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For decades, researchers have used the transpiration stream concentration factor (TSCF) to measure the likelihood a plant will uptake a neutral organic compound, relying on octanol-water partitioning to predict the TSCF. Researchers have observed bell-shaped distributions or sigmoidal relationships, with disagreement occurring at low log K_{ow} values, where other physico-chemical interactions may dominate hydrophobic interactions.

An alternative modeling method used in this study is a poly-parameter linear free energy relationship (PP-LFER), which use solute parameters to characterize sorption to another phase. These solute parameters describe fundamental properties such as hydrogen bonding and dipole interactions, allowing for broad, meaningful predictions. In this work, using a PP-LFER model to explain TSCF resulted in an R^2 of 0.5, a substantial improvement over fitting a K_{ow} model.

Another predictive tool uses the methodology of Lipinski (Lipinski et al. 1996), where a compound's ability to permeate membranes was predicted using molecular weight, octanol-water partitioning, and hydrogen bond donors/acceptors. Lipinski-like filters have been applied to insecticides and foliar herbicides (Tice 2001). When applied to TSCF's greater than 0.1, we find most compounds have a log K_{ow} less than 5, a molecular weight less than 500, fewer than 5 hydrogen bond donors, and fewer than 10 hydrogen bond acceptors, a finding which agrees with Lipinski's rule for pharmaceuticals.

Collectively, these models have helpful explanatory and predictive power. The physico-chemical mechanisms that affect passive uptake of compounds by plants can be better understood, allowing the fate of yet developed compounds to be predicted with fewer laboratory tests and resources.

Keywords: Quantitative structure-activity relationship (QSAR), Poly-parameter linear free energy relationship (PP-LFER), Lipinski's rule of five

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H4

Atriplex atacamensis: a xero-halophyte species of interest for phytostabilization on arsenic-affected soils

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Arsenic is of environmental and health concern due to its high toxicity even at trace levels.

Atriplex atacamensis is a xero-halophyte species endemic from Atacama's desert (North Chile). Arsenic content in soil and in water at Quillagua (Antofagasta's area, province de Tocopilla) was 198µg total As g^{-1} soil and 2511 µg/l respectively.

Plants growing on these area contained 8.6 µg g^{-1} of arsenic in leaves, 4.5µg g^{-1} in stem and 35.2 µg g^{-1} in fruits. Young seedlings issued from seeds collected on these plants were grown in nutrient solution under controlled environmental conditions and exposed for 14 and 28 days to 0 or 1000 µM arsenate. Total arsenic was measured by ICP-OES and speciation was quantified by HPLC-HGAFS.

More than 75% of plants exposed to the highest As dose remained alive until the end of the treatment. Plant growth was inhibited in stress condition. Arsenic accumulated up to 400 µg g^{-1} DW in the shoots and 3500 µg g^{-1} DW in the roots. Arsenate drastically impaired P nutrition Although an increase in total non-protein thiols suggested that arsenite fixation on sulfhydryl groups could occur in stressed tissues, the majority of soluble arsenic remained in its oxidized state As(V).

Since it is resistant to As and stores high amounts As in roots, this plant should be considered for phytostabilization purposes in arid areas.

Keywords: Arsenic, Atriplex atacamensis

I: Metals: Search for New Potential Phytoextractors

I1

Cleaning up contaminated soil with Pb and Cd by native plants of Iran

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This study was investigated remediation ability of native plants of Iran in contaminated soils with Pb and Cd. Three native plants of north Iran(Abutilon theophrasti, Amaranthus retoflexus, Zea maize) was studied. Extraction of soil and plant samples was performed by HNO3 ,HCl and HNO3, HClO4 and H2SO4 based on ASTM. Lead and Cadmium concentration in soils and plants was determined by Atomic Absorption Spectrophotometer Perkin-Elmer 603 of analysis lab of Tehran university. Lead concentration in roots of plants(Basic soils) was more than shoots. In Acidic soils(exception of zea maize), Lead amount in the shoots was more than roots of plants. Lead concentration in roots and shoots of Velvet leaf, Amaranths and Zea maize(Group 3) in basic soils were (54.5 and 25.5 mg/kg), (37.6 and 69 mg/kg) and(32 and 47 mg/kg) and in acidic soils were (92.5 and 71.4 mg/kg), (120.5 and 89 mg/kg) and(66.7 and 85 mg/kg) respectively. Cadmium concentration in basic soils were (4 and 6.5 mg/kg), (6.2 and 8 mg/kg) and (3.4 and 5.6 mg/kg) and in acidic soils were (4.5 and 5.8 mg/kg), (5.4 and 7.6 mg/ kg) and (4.8 and 6 mg/kg) respectively. Biomass of these plants in group 3 were less than control treatment. Considering to ncreasing of Lead and Cadmium accumulation by plants in acidic soils, using of phytoremediation for cleaning up of contaminated soils with Lead and Cadmium is appropriate. In the other hand, application of minerals and salts in acidic soils is one useful method of heavy metls stabilization in soils.

Keywords: Phytoremediation, Soils, Lead, Cadmium, Iran

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I2

Effect of Chemical Compounds on Removal and Stabilization of Heavy Metals (Pb, Cd, Zn) in Soil and Contamination of Water Resources

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Nature of heavy metals and its dissolving in soil has so important in the transmition and entering of these hazardous materials into plants and water resources(1,2). This study, has been performed stabilization and dissolving of Pb, Cd and Zn in contaminated soils by several chemicals. This cross- sectional descriptive study, soil samples(36) have been collected from Amol industrial places. Concentration of Pb, Cd and Zn as mg/kg have been calculated by Atomic Absorbtion Spectrometr 603 Perkin-Elmer. Dissolving of these heavy metals was investigated by Potassium chloride, Ammunium phosphate, Ammonium acetate and DTPA. Pb, Cd and Zn concentrations of soil samples were 206±59.8, 11.6±1.8 and 1148±465 mg/kg. Mean dissolving concentration of Pb, Cd and Zn with Ammonium phosphate were 11(5.3%), 1.5(12.9%) and 114.5(9.97%) mg/kg and with Ammonium acetate and DTPA{115(92.7%), 191(55.8%), 6.2(61.2%), 7.1(53.4%) and 476(56.5%), 648.5(41.5%)} respectively. Based on this study, Ammonium phosphate and EDTA carry out main effect on heavy metals studied stabilization and dissolving in soils. Therefore, using of Ammonium phosphate will useful for prevention of heavy metals entrance to underground water resources.

Keywords: Heavy metals, Stabilization, Dissolving, Removal, Remediation, Contaminated Soil

I3

Phytoremediation Potentials of *Commelina spp,* in the Semi-arid Region of Bagwai Local Government Area, Kano-Nigeria

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Levels of heavy metal concentrations in Commelina spp and soil were assessed using atomic absorption spectrophotometry. The concentration of iron (10.69 µg/g), copper (5.5484 µg/g), cobalt (4.9778µg/g), manganese (2.8308µg/g), nickel (2.6409µg/g) and lead (1.7053µg/g) were higher in the plant than in their respective soils. This shows that iron, copper, cobalt, manganese nickel and lead were most bioaccumulated by the plant. The highest concentration factor was observed for iron (CF≈12), copper (CF≈8), nickel (CF≈6), manganese (CF≈5), chromium (CF≈4), cobalt (CF≈4), zinc (CF≈3). The plant can therefore be considered to be an endemic indicator species with potential for use in bioaccumulation, phytoremediation / phytoextraction as interrelationships between these metal concentrations in the soil and their tissues were significant ($p<0.05$).

Keywords: Heavy metals*, Commelina spp,* bioaccumulation, phytoremediation, semi-arid, Bagwai

I4

Use of Short Rotation Coppice (SRC) Willow for the bioremediation of landfill leachates

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With a high percentage of global waste being placed in landfills and landfilling producing large amounts of highly hazardous contaminants, landfill leachate is becoming a serious issue. Leachate is toxic and costs tens of thousands of pounds to contain or to treat. The use of SRC willows (*Salix spp*.) to bioremedate landfill leachate has been demonstrated to work relatively well (Aronsson *et al.* 2010) as willows are known for their ability to uptake contaminants like heavy metals uptake such as Cd (Dickson and Pulford 2005), Zn and Cr (Landberg and Greger 1996). Willow is a diverse genus with many forms and varieties, so that genotype selection may aid site capture and improve specific contaminant phytoremediation. Over 100 willow genotypes have been screening for their reaction to landfill leachate. The impact on genotypes was measured using growth parameters and chlorophyll content. Preliminary results have indicated that particular genotypes are well suited for the phytoremediation of landfill leachate*. S. dasyclados* 'SV1', *Salix spp.*'970416' and *S. Burjatica* 'Germany' have been shown to be particularly promising clones with better growth and higher chlorophyll levels. The results from these screening studies will be used to select the most effective genotypes to enable further investigations addressing issues that affect the practicalities of using SRC willow as a bioremediation tool. This ANSWER (Agricultural Need for Sustainable Willow Effluent Recycling) project is part funded by the European Union's European Regional Development Fund (ERDF) through the INTERREG IVA Cross-border Programme, managed by the Special EU Programmes Body (SEUPB).

Keywords: Bioremediation, willow, landfill leachate, genotype

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I5

Uptake of As, Cu, Pb, U and Zn by aquatic plants in contaminated stream waters: potential for bio indication and phytoremediation

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This work is part of a wider study, which is being developed across the central region of Portugal, including the uraniferous province of Beiras and constitutes a general description of the results obtained in aquatic species to use for bioindication of contamination and to the application of phytoremediation techniques. Several plants have evolved heavy metal tolerance strategies and detoxification mechanisms that enable them to survive, grow and reproduce in metal contaminated sites. Some of these aquatic plants have been reported to accumulate significant amounts of specific trace elements. This strategy provides a great interest in t he identification of areas contaminated with both natural contaminants, originating from rocks or minerals deposits with large amounts of toxic elements, or urban, industrial or generated by the mining industry effluents.

We found that the species *Callitriche lusitanica*, *Ranunculus tripartitus*, *Callitriche brutia* and *Lemna minor*, accumulate large amounts of arsenic, from 430 to 2346 mg/kg (DW). The species *Callitriche stagnalis*, *Fontinalis antipyretica*, *Callitriche hamulata* and *Potamogeton pusillus* accumulate uranium at concentrations from 365 to 1949mg/kg (DW). The species *C. lusitanica*, *C. brutia*, *Fontinalis squamosa* and *C. stagnalis* accumulate zinc to a range of 1744 to 2313 mg/kg (DW). *Myriophyllum spicatum*, *Spirodella polyrhiza*, *L. minor* and *R. trichophyllus* accumulate lead to a values of 91 to 1104 mg/kg (DW). Finally *C. stagnalis*, *R. trichophyllus*, *C. hamulata* and *C. brutia* accumulate copper from 82 to 161 mg/kg (DW). We conclude that some of these species shows great potential for phytoremediation of contaminated effluents by these elements.

Keywords: Aquatic macrophytes, Bioaccumulation, Phytofiltration, Phytotechnology, Contaminated water

I6

Arsenic tolerance and accumulation in *Isatis cappadocica* Desv. and its potential for phytoremediation of As-contaminated soil

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Phytoremediation using hyperaccumulators is a promising technique of removing soil pollutants. In this study, effect of arsenic (As) on growth responses, leaf photosynthetic rate and As accumulation capability of *Isatis cappadocica* (a brassica collected from Iranian arsenic-contaminated mine spoils and control populations), was investigated. Both populations of *I. cappadocica* were considerably more tolerant than the reference Brassica species (*Descurenia sofia*). The 1000µM arsenate exposure inhibited root growth completely in the *D. sofia,* but only by about 50% and 40% in the nonmine and mine populations of *I. cappadocica* respectively. Furthermore, the chlorophyll content in both population of *I. cappadocica*, did not show statistical differences, especially when plants were exposed to 5 to 800 µM As. The chlorophyll a fluorescence kinetics (Fv/Fm) and electron transfer rate (ETR) values of treated *I. cappadocica* populations remained unaffected, indicating normal photosynthetic efficiency and strength of plants in the presence of As. After 28 days exposure to 1300µM As, shoot As concentrations of mine and nonmine populations were reached 310 and 345 mg kg^{-1}, respectively. The high As tolerance, and great capacity to accumulate As under hydroponic conditions suggest that *I. cappadocica* could be useful for the remediation of sites contaminated with As. According to these results, it was shown *I. cappadocica* had strong tolerance and accumulation capability to As, therefore it is a potential As-hyperaccumulator.

Keywords: *Isatis cappadocica,* Arsenic, tolerance, Phytoremediation

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I7

The potential of lead absorption by corn, alfalfa and sunflower

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Lead is the most important environmental pollutants that has created many problems in recent years. Efforts toward refining efficiency of the phytoremediation in removing contamination from soil systems have been conducted. In the Khoozestan province due to there are oil wells and other industries, heavy metal contamination of soil is environmental problems. In order to investigate the potential absorption of lead from soil by various plants, soil with high concentrations of lead was selected in this research and a pot experiment as a completely randomized design in a greenhouse was conducted. Treatments were: control (no planting plants), alfalfa, sunflower, corn. Then, plants harvested from the soil and lead concentrations in soil, root and shoot measured and then, data analyzed with SPSS 16 software and mean comparison was performed with Duncan test. Results showed that sunflower to remove lead from soil than corn And alfalfa is of higher ability.

Keywords: Corn, alfalfa, sunflower, lead, phytoremediation

I8

Phytoremediation of cadmium by corn, alfalfa and sunflower

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Soil contamination can be accelerated with industrial activities, agriculture (pesticides and fertilizers used). Cadmium is a toxic element that is saused human metabolic disorders. According to published reports, the border areas of South, South West and West are widely exposed to these pollutants. So many types of cancers in Khuzestan and Fars had increased in recent years. Concentration of cadmium in some agricultural land more than 1mg /kg soil have been reported. In order to the potential of cadmium uptake from soil by various plants was investigate, this pot experiment study was conducted in greenhouse in a completely randomized design as. Treatments were: control (no planting plants), alfalfa, sunflower, corn.Then *plants* harvested from the soil and cadmium concentration in soil, root and shoot were measured, and then translocation Factor (TF), uptake index (UI) and bio- concentration factor (BCF) was calculated. Analysis of variance was done with SPSS 16 software. Results showed that sunflower higher remove cadmium from the soil than corn and alfalfa.

Keywords: Corn, alfalfa, sunflower, cadmium, phytoremediation

I9

A Potential Method for Remediation of Cadmium pollution in Aquatic Medium by hydrophyte, *Ceratophyllum demersum* L.

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Cadmium is a trace element. This heavy metal in low concentration is harmful to life, and considered as a dangerous pollutant. With increasing of population and increasing of the valuable water resource pollutions, a demand for new and inexpensive methods for remediation and improving of water quality has felt. Cadmium leads to pollution and reduction of water quality; sometimes even toxicity through contaminated sources such as wastewater (Agricultural, municipal and industrial). Phytoremediation with aquatic macrophytes is an effective and inexpensive method for improving water quality and wastewater. In this study, a potential method for remediation of cadmium pollution in aquatic medium was reviewed within 14 days cultivation of *Ceratophyllum demersum* in contaminated synthetic wastewater (made by Hoagland nutrient solution*)*, at the four different concentration of Cadmium (0, 1, 2, 4, and 6 mg L^{-1}). Maximum bio-concentration factor and maximum uptake index calculated from 2 mg L^{-1} and 6 mg L^{-1} metal concentration were 707.9 and 3.92 mg per pot, respectively. Maximum (3.6 g/day) and minimum (1.62 g/day) biomass production caused from 0 mg L^{-1} and 6 mg L^{-1} of pollutant concentrations. The plant accumulated cadmium efficiently, so that the remediation efficiency was near to 82%. However, the pollutant removal was not completed in a short time, but phytoremediation of Cadmium and other pollutants from wastewater and other aqueous solutions by *Ceratophyllum demersum* as a native aquatic plant of most of Iran's rivers could be an efficient and appropriatemethod.

Keywords: Cadmium, Wastewater Phytoremediation, *Ceratophyllum demersum* L., Aquatic macrophyte, Heavy metals.

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I10

Phytoremediation of a dumpsite at Moniya, Ibadan, in Nigeria using *Chromolaena odorata* and *Portulaca oleraceae* L.

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Heavy metal contaminants in urban dumpsites constitute a major environmental challenge, causing soil and water pollution. Remediation using the appropriate plant species with hyperaccumulation potentials is affordable, ecologically acceptable and environmentally friendly. Therefore, the hyperaccumulation potentials of *Chromolaena odorata* and *Portulaca oleracea* in a heavy metal-contaminated dumpsite at Orisunmibare village, Moniya, Ibadan, Nigeria were investigated.

Fifteen plant species were identified at the dumpsite, the two most common being C. *odorata* and *P. oleracea* with RIV of 31.0% and 20.2% respectively. *Ananas comosus* (L) Merr. had the least RIV (1.2%). Mean concentrations (mg/kg) of Pb, Cd, Al, Zn, Fe, Cu, As and Cr were 0.1±0.01, 0.0±0.01, 0.1±0.01, 0.1±0.01, 2.6±0.01, 1.0±0.01, 0.0±0.01 and 0.0±0.01 respectively for the control soil and 80.31±1.321, 57.87±1.32, 63.8±0.08, 54.2±0.013, 56.47±0.10, 40.3±0.06, 37.5±0.01 and 40.0±0.05 for the polluted soils. Mean concentrations of these heavy metals in *C. odorata* grown on control soils were 2.2±0.14, 10.9±0.26, 8.03±0.70, 5.0±0.16, 4.2±0.28, 5.0±0.15, 10.9±0.27 and 1.8±0.18 respectively, while on polluted soil, their concentrations were 59.2±0.95, 51.8±0.6, 56.0±0.81, 47.7±1.19, 53.7±1.92, 30.7±0.5, 31.5±1.05, 37.2±0.9 respectively. For *P. oleracea,* mean concentrations (mg/kg) of Pb, Cd, AI, Zn, Fe, Cu, As and Cr from control soils were 9.6±0.39, 9.7±0.51, 6.7±0.6, 4.1±0.19, 5.2±1.54, 4.0±0.18, 2.6±0.56 and 6.2±0.42 respectively, while on polluted soils, these were 53.9±1.05, 41.7±0.59, 46.8±0.67, 47.0±1.17, 46.3±0.85, 36.9±0.84, 28.6±0.57 and 34.8±0.89 respectively. Concentrations of heavy metals in the polluted soil at the end of the experiment were significantly reduced by (20-30)%.

Keywords: Heavy metals polluted soil, hyper-accumulators, *Chromolaena odorata*, *Portulaca oleracea*

I11

Evaluation of different species from *Rumex* genus to phytotechnologies

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Biodiversity prospecting would lead the discovery of wild plants that could clean polluted ecosystem (Prasad and Freitas, 2003), and avoid the introduction of non-native and potentially invasive plants (Mench *et al*., 2009). *Rumex* represent a genus of wild species widely distributed in the Mediterranean area. Species of the gender *Rumex* such as *R. acetosa* L. or *R. crispus* L. have been proved to accumulate heavy metals. *R. Pulcher L.* has also been found in polluted lands as the Aznalcollar mine (Southern Spain). The objective of the work was to get metalliferous populations of *Rumex* genus in old mining areas from Comunidad de Madrid (Spain) to be compared with non-metalliferous populations from agricutural areas. Some of them have been traditionally used as edible plants, and in general, they show high concentration of organic acids and bioactives compunds in their composition (Sanchez-Mata *et al*., 2012). Four old mines areas from Madrid were prospected. Populations of *R. Crispus L.* and *R. Pulcher L.* were found in the four prospected areas; *R. induratus* L. in two of them, and *R. Conglomeratus L.* and *R. Papilaris L.* in only one of the sites. Germination and early development of *R. Pulcher L.* and *R. Crispus L.* growth in agar medium with increasing concentrations of Cr, Cd or Zn were evaluated as a first approach to determine the tolerance levels.

Keywords: Rumex, mining areas, germination test, metal, phytoremediation

References:

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I12

Early response of willows to high silver concentration

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Silver (Ag) is considered a potential pollutant for the environment. Most Ag is released as a byproduct of copper, gold, lead, and zinc refining. It is also produced by other industrial activities such as photographic film, medicinal chemicals, tableware, electroplating, mirror, and jewellery. Very little information is currently available on silver tolerance by high plants. Some authors reported that sprays containing 9.8 ppm dissolved Ag kill maize, and sprays containing 100-1,000 ppm dissolved Ag kill young tomato and bean. Others observed hyperaccumulation of silver in *Brassica juncea* and *Medicago sativa* supplied with silver concentration as high as 1,000-10,000 ppm.

This study investigated the potential of willow (*Salix* spp.) to phytoremediate silver-contaminated soil. In a preliminary hydroponic trial, we tested the responses of 20 willow varieties to high Ag concentration. Ten-day old rooted willow cuttings were grown in hydroponic at a concentration 50 ppm of $AgNO_3$. After two weeks of growth we evaluated plant response to this treatment. We found that in all cultivars the growth of roots and shoots was negatively affected by this dose, showing that very much lower concentration should be addressed when willows have to be used for phytoremediation of silver.

Keywords: phytotechnology, phytoremediation, *Salix*, heavy metals

J: Wetlands

J1

Nutrient recovery and pathogen reduction in duckweed based constructed wetlands

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Direct application of manure slurry in cropped-land causes two basic problems. First, large number of pathogens present in manure can contaminate crops. Second, excess and unbalanced amount of nutrient contained in manure slurry can leach to water bodies, causing eutrophication. Experiments were conducted to address above issues by using a combination of surface flow (SF) and subsurface flow (SSF) constructed wetlands. The wetland system was subjected to diluted animal slurry (after solid-liquid separation). Three concentration of diluted slurry, low, medium and high strength (about 250, 500 and 1000 mg/L COD, respectively) were used. Fifty percent of duckweed was harvested from SF wetlands every week. Influent and effluent total nitrogen (TN), total phosphorus (TP), chemical oxygen demand (COD) and E. coli along with duckweed production and nitrogen and phosphorus content in duckweed were monitored. Results showed that substantial amount of nutrients were removed from manure using constructed wetlands. TN and TP in duckweed were proportional to the TN and TP content in the media. E. coli were completely eliminated during low loading, but persisted during high loading. Results demonstrated that constructed wetlands are sustainable ecological approach to address nutrients and pathogen issues related to animal manure.

Keywords: constructed wetland, nutrient recovery, *E. coli*, duckweed, slurry

J2

Biological and sustainable treatment of sulphate loaded water

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Mining residues and excavated material are disposed into tailings and on heaps. They are the origin of highly polluted discharges. To avoid environmental impacts, long-term measures are necessary. Two case studies on biological and sustainable treatment of sulphate loaded water, one in Chile and one in Germany, are developed.

For a Chilean copper mine site a Constructed Wetland for treatment of sulphate and metal loaded water from an active tailing was designed. The pilot plant will be operated by the University Santiago de Chile. Treatment capacity is about 10 to 60 m³ per day. The gained monitoring results on microbial sulphate reduction and metal removal will be used to determine the optimal design and operation parameters for long term treatment.

Numerous coal mine pits in Central Germany were flooded by re-rise of groundwater level after mine closure or controlled feeding using surface water of nearby rivers. The water quality of the created lakes is influenced by interactions with sulphate containing groundwater. BioPlanta has realized a 6 months *on site* water treatment test on sulphate reduction and sulphide immobilization in a biological active filter. The average sulphate content of about 1,200 mg/l could be decreased below the target value of 300 mg/l. In the field test even a higher sulphate reduction rate could be obtained than in previous investigations in technical scale.

Keywords: sulphate, water, biological transformation, Constructed Wetlands, case studies

J3

Nutrient release from biomass decay of submerged aquatic vegetation (SAV) in wetland — a laboratory simulation study

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SAV enhances assimilation of nutrients in wetland, but may serve as internal sources when its biomass residues are decomposed. A laboratory incubation study was conducted to investigate release characteristics of nitrogen (N) and phosphorus (P) from decaying biomass residues of four common SAV species: hydrilla (*Hydrilla verticillata*), naiad (*Najas guadalupensis*), potamogeton (*P. illinoensis*), and chara (*Chara spp*). Plant biomass samples were collected from the storm water treatment areas in south Florida, USA and incubated in water at 40°C for 126 days. At the end of incubation, the mixtures were passed through a 1-mm sieve, the plant debris above the screen were rinsed, oven-dried, and weighed. Subsamples of the suspension (representing floc) was analyzed for pH, electrical conductivity (EC), turbidity, total solids, total N and P, and the remaining suspension was centrifuged (representing pore water-PW) and analyzed for dissolved nutrients. The results showed that hydrilla had the highest (0.007930 d^{-1}), whereas chara had the lowest decay rate (0.002798 d^{-1}). pH, EC, turbidity, and total solids in the floc varied with SAV species. The concentrations of NH_4-N, NO_3-N, and PO_4-P in PW were similar. However, higher total N was measured with chara and hydrilla, whereas a larger amount of total P was released from naiad. The decay rate of SAV biomass was negatively correlated with C/N or C/P ratio in the plant. Chara and potamogeton appear to be better candidates than hydrilla or naiad for application in wetland due to their lower decay rate and smaller nutrient release.

Keywords: Biomass decay, nutrient release, submerged aquatic vegetation, water quality, wetland

J4

Effect of wastewater salinity on treatment performance and growth of Phragmites and Populus in constructed wetland and sand filter

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The utilization of alternative water sources like reclaimed municipal and/or industrial wastewaters appears to be a promising options. In this context "Era-SME CLEARH2O" project has been carried out, where we focused on industrial wastewater with high salinity. The recommended solution was a phytoremediation, combining wastewater treatment and water-reuse for plants growth. A pilot experiment has been set-up, combining vertical constructed treatment wetlands (CWs) and vertical sand filters (VFs). The Phragmites australis have been planted in CWs and cuttings of four Populus clones (Populus deltoides Bart. cl. I-69/55 (syn. Lux), Populus × canadensis Moench cl. I-214, Populus × canadensis Moench cl. Guardi, Populus alba L. cl. Villa Franca) have been planted in VFs. The objective was to compare treatment performance in terms of COD and BOD_5 reduction and plant growth response in conditions with high salinity. The water salinity had been gradually increased, up to to 11 g/L in VFs and up to 30 g/L of NaCl in CWs. The COD reduction efficiency was lower in experimental CWs and VFs compared to control units, while this was not reflecting in BOD_5 reduction efficiency. The growth of biomass was reduced in all experimental units. The Phragmites produced more biomass compared to Populus in all units. Populus × canadensis Moench cl. I-214 exhibited the greatest mean aboveground woody dry mass in control treatment, but was the least productive in experimental treatments. The greatest mean aboveground dry mass in VF experimental units was produced by Populus alba L. cl. Villa Franca.

Keywords: industrial wastewater, salts, vertical constructed treatment filter, planted sand filter, wastewater treatment

J5

Copper tolerance in macrophyte populations: Innate tolerance and/or phenotypic plasticity?

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Macrophytes are recognized for their innate tolerance to PTTE (potentially toxic trace elements) and are widely used to remove these PTTE from wastewater and sediments in constructed wetlands (CWs). In this study, Cu-tolerance levels of rooted and emergent macrophyte populations (n = 50) of *Phragmites australis, Phalaris arundinacea, Typha latifolia, Menta aquatica, Iris pseudacorus* and *Juncus effusu*s were compared based on dry root biomass produced during a 21-day growth period with increasing Cu exposures (0, 2.5, 5, 15, and 25 µM Cu). Effective concentrations-EC_{50} and RTEI (Relative Treatment Efficiency Index) were determined. All macrophytes survived at high Cu concentrations. Based on root production, *J. effusus* presented the lowest Cu-tolerance (EC_{50} between 4.8 and 12.4 µM Cu), whereas *I. pseudacorus* and *T. latifolia* generally displayed the highest Cu-tolerance (EC_{50} respectively 14 - >25 µM and >25 µM Cu). These data confirm innate tolerance of macrophytes to Cu. However, Cu-tolerance levels differ across populations of *P. arundinacea* and *P. australis,* with respectively one and two populations being more Cu-tolerant than the other ones*.* This suggests a phenotypic plasticity of these plant species depending on Cu concentrations in their habitat. Therefore both innate tolerance and phenotypic plasticity would explain the performance of some macrophyte populations exposed to high Cu exposures.

Keywords: Constructed wetland**,** *Juncus effuses, Phalaris arundinacea, Phragmites australis, Typha latifolia*

J6

Organic acid content and copper uptake in two plant species used in a wetland system to treat copper mining effluents

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The effectiveness of using phytotechnologies to treat copper liquid wastes (CLW) depends at a great extent on the adequate choice of the plant species to be used. The ability to uptake copper and to accumulate and mobilize the metal in the plant tissues is relevant. This characteristic has been related to the production of Low Molecular Organic Acids (LMOAs) (Veselý *et al.*, 2011). Therefore, it is possible that the organic acid production by the plant caused by exposure to copper affects the uptake, accumulation and distribution of the metal in the plant tissues.

Polypogon australis and *Phragmites australis* are able to incorporate high concentrations of copper, under laboratory and field conditions (Ye *et al.*, 1997; Ortiz et al, 2008). We studied the accumulation of organic acids in the roots of both species treated with a copper liquid waste (CLW) and a synthetic copper solution; and we measured the amount of copper accumulated in the plant tissues. We found that both copper and CLW treatments provoked an increase in the production of malic, oxalic and acetic acids, but at different times and rates during the treatment. There was an increase in the uptake of copper, being more significant the amount of the metal found in *Phragmites australis*. A differential partition of the metal between the roots and the leaves was observed in the plants.

We suggest that a screening of organic acids production in plants suitable for phytofiltration could be made to determine the efficacy of the species to sequestrate copper.

Keywords: Phitofiltration, organic acids, copper liquid wastes

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J7

Changes in productivity and nutrient contents induced by photosynthetic biomass removal in the middle of the growing season in macrophytes from Mediterranean semiarid climatic conditions

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Maximizing primary productivity and nutrient uptake of macrophytes is one of the main goals in constructed wetlands to avoid large accumulations of organic matter in the soil. Encouraging decomposition by rotation of the aerobic-anaerobic conditions of these organic matter stocks has proved useful. But active uptake of nutrients by vegetation and exporting biomass out of the system would be a faster and effective strategy. Optimization of these practices requires the development of vegetation management protocols adapted to specific species, macroclimatic and microclimatic scenarios.

This contribution analyzed the effects of photosynthetic biomass harvesting in the middle of the growth period on the production of aboveground biomass and its nutrient contents. It also reviews the results in similar macroclimatic conditions in which summer season is hot and dry. *Typha angustifolia, Phragmites australis, Scirpus holoschoenus and Scirpus maritimus* were the species studied. Artificial water pools about half square meter in surface included 10 individual plants of the same species in polyethylene bags containers. Soils with red clays with gypsum from Keuper geological formations were used. The gradient of organic matter accumulation in the soil was artificially reproduced by the addition of sludge to the soil. Sludge was applied in every container after planting and in no direct contact with the plant. Sludge was covered with soil after application. The amount of sludge applied represented: 0, 10 and 20% in volume of the soil per container. The research was conducted during two growing periods 2005 and 2006.

Keywords: Constructed wetlands, sludge, plant production, phytoremediation, biomass

J8

Sulphide toxicity to wetland plants: experimental approaches for toxicity quantification

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Compared to the research conducted on the toxicity of different phytotoxins (e.g. heavy metals), the work carried out on sulphide toxicity to different wetland plants is very limited. The knowledge of the toxicity mechanisms and concentrations is essential to depict the functioning of the wetland plants in different environments, and to better understand the related processes in treatment wetlands. Methods are currently being developed using visualization techniques, sulphur isotopic ratios, and nutrient uptake quantification; in laboratory-scale systems under identified environmental conditions. Both hydroponic and soil-based systems will be employed, to assess the toxicity concentrations and the possible detoxification mechanisms in both scenarios. Depending on the PhD work progress, first results will be shown. It is expected that toxicity level to plants is influenced by the soil as a diffusion barrier. Therefore, in hydroponic systems higher toxicities at same concentrations are expected compared to soil-based systems.

Keywords: sulphide toxicity, wetland helophytes

J9

Phytoremediation of organic pollutants in hybrid constructed wetland by P. australis

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Hybrid Subsurface Constructed Wetlands (HSCWs) are a combination of engineered systems that have been modified and constructed to utilize the natural processes involving wetland vegetation, media (Gravel, …), and the associated microbial biofilms to assist in treating wastewaters. Usually, due to use of plants to remediation of pollutants, all wetlands classified as a subset of Phytoremediation. In this research 4 rectangular bed subsurface constructed wetland with combination of the common reed (P. australis) and gravel (12-25 mm sizing) used for the research goals. Objectives of this research were organic pollutants removal, by the common reed (P. australis). The University wastewaters are fed in at a storage basin for pumping to the inlet pipes. Different valves arranged for feed control and also change the flow regime to the beds. The weak wastewater of the University enriched with organic materials to provide suitable concentrations of organic materials. Different flow regime (horizontal-vertical and downflow-upflow path) evaluated. All samples at the inlet and outlet zone analyzed for the organic pollutants. Results of the system indicated, phytoremediation technology for reduction of organic pollutants succeed to reach to limits of the secondary treatment.

Keywords: Phytoremediation*, P. australis* , organic pollutants, Iran

J10

Phytoremediation of 4 toxic metals in hybrid constructed wetland

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Phytoremediation is a technology that usually occurs in every treatment system with presence of plants. The different wetlands are also included. Hybrid constructed wetland is a combination of subsurface constructed wetland with different pattern, plants, media and flow regime sometimes conflicting of hydraulic flow. To evaluation of phytoremediation of wastewater for different pollutants including heavy metals, a research pilot constructed for hybrid wetland at Mazandaran University of medical sciences in Sari city, northern Iran. Location of this pilot is in adjacent of the wastewater treatment plant of the University. The design typically consisted of 3 rectangular bed planted with the common reed (P. australis) and 1 rectangular bed as blank. All of them are impermeable. Mechanically pre-treated wastewater is fed in at a storage basin for pumping to the inlet pipes. Different valves arranged for feed control and also change the flow regime to the beds. The weak wastewater of the University enriched with different concentrations of 4 toxic metals (Pb, Hg, Cr^{+6}, Cd) and passes slowly through the filtration medium under the different flow regime (horizontal-vertical and downflow-upflow path). All samples at the inlet and outlet zone analyzed for the heavy metals. Results of the system as a phytoremediation technology for heavy metals evaluated and had promising results that were discussed in detail in this article.

Keywords: phytoremediation, heavy metals, Iran

K: Salinity and Drought Stress

K1

Transformation of dredged sediments into usable soil applying non-conventional techniques

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A phyto-technique was tested to transform slightly polluted and saline sediments into a techno-soil for agronomic and land restoration uses. The non-conventional technique was applied to the sediments dredged from Livorno port, Italy, and from the Kishon River and Ashdod port in Israel. The experimental facilities consisted of constructed basins containing about 100 m^3 sediment (Italy) and three 60 m^3 sediment (Israel), which were sealed with a HDPE liner. We will report data on leachate, pore water, plant and sediment compositions which were monitored on a weekly to twice-yearly from 5/2010 through 5/2012. In Italy, the basin was planted with mixtures of *Paspalum vaginatum*, *Tamarix gallica* and *Spartium junceum*, and rain-fed. In Israel, sediment salinity was partially leached, and deep-rooting, fast-growing, salt resistant tree species (*Eucalyptus*es and *Casuarina*) were planted. Summer irrigation in Israel used wastewater effluent at controlled deficit irrigation (CDI) regime (2% cumulative leaching fraction) which raised pore water salinity from ≈10 to ≈20 dS m^{-1}. During the second summer (May-Sept., 2011), the irrigation water was augmented with Na_4EDTA at 7.5 mM. We hypothesize that little if any solubilized metal will leach under the CDI, and that following cessation of ligand application and ensuing degradation, released metals will be plant absorbed and/or re-adsorbed to the sediment, thus escaping non-prescribed winter leaching. The obtained results partially confirm our hypotheses in terms of choice of trees, metal solubilization, EDTA degradation, and control of leachate volume and composition. It seems however that most all solubilized metals ended in the trees roots.

Keywords: EDTA, metals, phyto-treatment, salinity, sediment, techno-soil

K2

Evaluation of salt tolerance in *Myrtus communis* L.: an integrative approach for the recovery and recolonization of salt affected soil

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Salinity is one of the major abiotic stresses that affects crop productivity and quality. Today more than 800 million hectares of land throughout the world are salt affected. Of the 1500 million hectares of land farmed by dry land agriculture, 32 million are affected by secondary salinity to varying degrees. The understanding of the mechanism of salinity tolerance allows the selection of the tolerant species or genotypes to be used in the recovery of salt affected soils.

The present work describes the use of *in vitro* shoot cultures to determine salt stress effects in the Mediterranean species *Myrtus communis* L.

To detect the effect of salt, micropropagated and rooted shoots were grown in liquid culture media added with 0, 125 or 250 mM NaCl. Sampling for the analyses was made after 15 and 30 days. Shoots and roots length, pigments content, osmolality, lipid peroxidation determined by malondialdehyde (MDA) assay, guaiacol peroxidase (G-POD) activity and DNA fragmentation were determined.

High values of shoots osmolality were found only at 250 mM NaCl, while G-POD activity enhanced according to the treatments. Shoots and roots length significantly decreased in relation to the exposure time and NaCl concentrations. Salt stress markedly reduced chlorophylls content, while carotenoids amount and lipid peroxidation were not significantly affected. DNA damage was not detected.

The results obtained suggest that *M. communis* is a species tolerant to salt and *in vivo* studies are in progress to evaluate the possibility to use myrtle as pioneer species to recover salt affected soils.

Keywords: *Myrtus communis*, *in vitro* cultures, salt tolerance, abiotic stress

K3

Is tall fescue (*F. arundinacea* Schreb.) a good tool to recover soils affected by salinity?

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Salinity is considered a limiting factor to crop production. Understanding how plants respond to salt stress can play a major role in the stabilization of crop performance and the recovery salt-affected soils. *F. arundinacea* Schreb. is a well-adapted cool season forage grass for agriculture in unfavorable environments. This beneficial feature is also the result of a mutualistic symbiotic relationship with fungi belonging to the *Neotyphodium* genus. This endophyte can contribute to an array of host fitness enhancements, including protection against herbivores and improvement of abiotic stress tolerance.

The aim of this study was to evaluate the protective effects of the fungus against salt stress in tall fescue at different developmental stages, i.e. from seed germination to seedling stage (42 days). Salt stress effects in fescue were evaluated by detecting the concentrations of pigments, phenols and proline and by determining the activity of guaiacol peroxidase (G-POD) in infected and endophyte-free plants, treated with different concentrations of NaCl: 25, 50, 100 and 200 mM.

The salt stress induced an enhancement of Chlb, proline and phenols contents and G-POD enzyme activity. Tall fescue is a species tolerant to salinity and the presence of the endophyte improved the biomass and the agronomic quality of its host plant by increasing the plant natural defence barriers against salt stress. Moreover, the presence of fungus enhanced germination of seeds. Therefore the infected fescue is an excellent forage crop for high salinity soils in which other grasses may not be cultivated.

Keywords: tall fescue, *Neotyphodium*, salt stress, soil recovery

K4

Evaluation of *Pteris vittata* for the use of arsenic contamination derived from tsunami sediments and its salt tolerance

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Due to the Great Tohoku earthquake on 11th March, 2011, tsunami attacked the Pacific coast of Tohoku district, and resultantly, a wide range of arsenic (As) contamination in soil has occurred by tsunami sediments. For the remediation of huge-contaminated area, the application of phytoremediation by *Pteris vittata*, As-hyperaccumulator, has expected. To evaluate the application of phytoremediation by *P. vittata* for the area, salt tolerance of *P. vittata* and phytoextraction of As from the soil affected by tsunami sediments was investigated.

The germination efficiency of *P. vittata* spores under salt stress was examined on 1/2 MS media containing various concentrations of salt (0-600 mM NaCl). The germination efficiency was considerably decreased more than 100 mM NaCl. At 200 mM, the gametophytes exhibited morphological defect. The growth inhibition of *P. vittata* was observed with the salinity corresponds to 66 mS/m of electric conductivity (EC). In As-contaminated soil by tsunami, the growth inhibition was observed with 79.5 mS/m of EC. Thus, it was considered inappropriate to utilize *P. vittata* for phytoremediation of seriously salt-damaged soil. Laboratory phytoremediation experiment was conducted using two types of As-contaminated soils for 166 days. *P. vittata* grew and accumulated As at 264 mg/kg-DW into their shoots. Water soluble As in soil was apparently decreased by the accumulation in the plant body. With all these results, it is concluded that *P. vittata* is applicable to the phytoremediation of As contaminated soil with low salinity by tsunami.

Keywords: Arsenic, Tsunami, Salt stress, *Pteris vittata*, Phytoremediation

K5

The study of effects different integrated irrigation methods with salin water and well water on sunflower yield

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In order to study of effects different integrated irrigation methods with saline water and well water on sunflower yield, experimental research in the field of Islamic Azad University Shahr-e-Qods Branch in 2011 was performed. This trial randomized complete block design with 3 replications and 4 treatments included four integrated irrigation levels of as a treatment 1: (25% salin water and 75% well water), treatment 2: (50% salin water and 50% well water), treatment 3: (75% salin water and 25% well water) and treatment 4 (100% welll water as a control) was performed. The results showed that between treatments there are significant difference at one percent and the treatment 4 ie conventional water with 3461 kg/ ha won the highest yield. Also with increasing salinity of water irrigation, yield treatments is redused, So that treatment 1 with 3170 kg/ ha, treatment 2 with 1280 kg/ ha and treatments 3 with 551 kg/ ha, respectively, faced with declining yield. The percent yield reduction in treatment 1 was lower than treatments 2 and 3.

Keywords: integrated irrigation, saline water, yield, sunflower

L: Biomass for Bioenergy

L1

Application of the Rejuvenate decision support approach

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All across Europe there are areas of land which have been degraded by past use that are not easy candidates for conventional regeneration, or for which conventional regeneration may not be the most sustainable approach. The use of such marginal land for cultivation of non-food biomass is an emerging opportunity that can both address some of the concerns about biomass production on agricultural or virgin / wilderness land and be an incentive to regenerate the areas. It is possible that long term use of marginal land for biomass production may at least offset the costs of its management, and potentially generate profit. In the project Rejuvenate an inclusive decision support approach, referred to as the Rejuvenate DST (decision support tool) including the following key steps (Bardos *et al.*, 2009; Bardos *et al.* 2011):
The identification of crop and use opportunities;
The management and improvement of soil and control of risks;
Understanding and maximising value and sustainability; and
The management of project risks such as technology status, due diligence and stakeholder perceptions.

Currently the practical usefulness of the DST is tested by applying it for a range of ongoing demonstration and potential real sites in cooperation among the Rejuvenate team (Bardos *et al.*, 2009; Andersson-Sköld *et al.*, 2011) and the Greenland, Phytopop and Phytener projects.

The results of the test applications will be provided as appendixes in DST guide and as a basis for a SWOT (strength, weakness, opportunities and threat) analysis of the DST. In this presentation the preliminary results of the test application will be presented.

Keywords: Decision support approach, non-food biomass, test, practical, application

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L2

Effect of Pyrolysis Temperature on Chemical and Surface Properties of Biochar of Rapeseed (*Brassica napus* L.)

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Biochar is carbon-rich product generated from biomass through pyrolysis. Biochar (charcoal) can be both used directly as a potential source of solid biofuels and as soil amendments for barren lands. However, chemical composition of the biochar depends on the source of feedstock and pyrolysis conditions. The influence of pyrolysis temperature on the chemical and morphological properties of biochars of Rapeseed (*Brassica napus* L.) was investigated in this study. The final pyrolysis temperature was varied in the range of 400 – 600°C in a fixed-bed reactor at 10 °C.min^{-1} heating rate and under static atmospheric conditions. The ultimate and proximate analyses and calorific values of the biochars were determined, and then the chemical composition of the biochars were investigated using Fourier Transform Infrared Spectroscopy (FTIR). The surface properties of the biochars were also identified by BET surface area and scanning electron microscope (SEM) images. Based upon FTIR results, the hydroxyl groups in the biochars produced at various temperatures were more those of the carbonyl groups. The highest surface area was obtained as 25.38 m^2/g at 550 °C of pyrolysis temperature. The results showed that both chemical and surface properties of the biochars were significantly affected by the pyrolysis temperature.

Keywords: Biomass, Pyrolysis, Biofuel, Biochar, Characterization

L3

The influence of *CCR*-downregulation (reducing lignin levels) on the bacterial communities associated with field grown *Populus tremula x alba.*

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Genetically modified (GM) plants, despite being heavily debated for their potential risks, could hold several advantages including enhanced pest resistance and improved wood quality. However, the overall impact of GM plants on the structural and functional composition of plant-associated microbial communities is poorly understood. Genetic modification of lignocellulosic plant-biomass aims the reduction of lignin levels to improve wood quality and processing efficiency. Transgenic downregulation of genes (*e.g.* cinnamoyl-CoA reductase, *CCR*) in the lignin biosynthesis of poplar reduces lignin levels but also results in changes in the soluble phenolic pools (xylem), elevated stress levels and reduced biomass production (Leple JC *et al.*, 2007).

Despite the wealth of data available on the consequences of lignin engineering on cell wall structure, (a) the effect on the plant-associated bacterial communities, especially under field conditions and (b) the potential to exploit these bacteria to improve the biomass production of *CCR*-deficient poplar trees have not yet been addressed. Poplar trees (wildtype and *CCR*-deficient) were sampled in October 2010 and 2011 and cultivable bacteria were selectively isolated from the rhizosphere, roots and stems of wildtype and *CCR*-deficient poplar trees using phenolic carbon sources (*e.g.* ferulic acid) linked to *CCR*-downregulation. Further, the poplar-associated bacteria were screened (a) semi-quantitatively for their carbon source use (Biolog Microarrays) and (b) for plant growth promoting characteristics to select ideal endophytes for inoculation experiments.

The selective isolation revealed significant higher amounts of bacteria capable of degrading the selected carbon sources in the *CCR*-downregulated poplars and higher bacterial diversity (Shannon-Wiener Index) as compared to the wildtype poplars.

Keywords: Poplar, lignin, plant-associated bacteria

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L4

Valorization of plant biomass harvested at trace element-contaminated sites managed by gentle (phyto)remediation options.

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Phytoextraction is a promising technology to remediate soils contaminated with trace elements (TE). One phytoextraction strategy is based on cultivation of high biomass herbaceous species, e.g. sunflower and tobacco, with or without addition of TE mobilizing agents to the soil. The second one is based on cultivation of rapidly growing trees, e.g. willow and poplar, with high TE accumulation ability in short rotation coppice (SRC) and the third one is based on the cultivation of TE-hyperaccumulators. After the cultivation of appropriate plant species on the contaminated soil, TE enriched harvestable plant parts are removed from the site. Problems associated with the treatment and disposal of the metal-enriched biomass produced during phytoextraction limit the development of commercially viable phytoextraction while ensuring that environmental parameters do not infringe current regulation. In the ongoing project GREENLAND (FP7, KBBE-2010-4, 266124) project, one work package aims at testing different various conventional and innovative technologies of biomass valorization, such as combustion, anaerobic digestion, solvolysis and microwave thermal treatment, and determining the fate of the TE in the resulting products of each conversion process. A first round of assays was carried out on a wide range of plant species cultivated at the field trials of the GREENLAND partners. The results will be presented and discussed.

Keywords: bioenergy, phytoremediation, *Salix*, poplar, environmental impact

L5

Comparison of *Arabidopsis thaliana* with normal and reduced lignin: differences in growth, bacterial population and cadmium responses

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Lignin forms a major obstacle during the processing of lignocelluloses biomass to biofuel. Lowering the lignin-content through genetic manipulation is a promising strategy to increase the process-efficiency but alters the composition of the soluble phenolics inside the xylem and influences the growth and development of the plant.

An even better situation can be created if the biomass can be cultivated on marginal lands, which cannot be used for foodcrop-production (e.g. Cd-contamination). Furthermore, cleaning the soil through phytoremediation would provide an additional benefit. From this point of view, also the influence of Cd-exposure on the plants and their associated bacteria will be investigated.

In this project the effects of a modification in the lignin-synthesis of *Arabidopsis thaliana*, namely the downregulation of the caffeoyl-CoA-O-methyltransferase (*CCoAOMT*) or the cinnamoyl-CoA-reductase (*CCR*) gene, on the plant-associated bacteria and on the plants growth were investigated.

In the first part, the genotypic and phenotypic characteristics were studied of all bacteria isolated from the leaves of Cd-exposed and non-exposed *A. thaliana*. The composition of the bacterial population was influenced by both the modification and Cd-exposure. In the second part, the effect on plants level was examined. Here the *ccr*-mutant showed significantly less root-inhibition and leave growth-inhibition after Cd exposure in comparison with WT and *ccoaomt.* Although the *ccr*-mutant had a significantly higher Cd-content.

It can be concluded that both the modification and Cd-exposure had an effect on plants and bacterial level. Further investigation is needed to unravel the processes that are associated with these observations.

Keywords: endophytes, lignin, cadmium, phytoremediation, cinnamoyl coenzym A reductase

L6

Ricinus communis L.*:* an alternative for oil production and phytoremediation

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The objective of this research was to know the use of *Ricinus communis* L., a non-food crop, as energetic and vegetal plant species to phytoremediate metal polluted sites. The study was followed in the arid region of Zimapan (Hidalgo State, Mexico). Three barren mine tailings heaps: San Francisco (SF); Santa Maria (SM) and Gomez (G), and an abandoned cultivated area (SE) were selected for this study, where 18 *R. communis* plants were naturally established and no remediation attempts have been done. pH of the mine tailings was neutral; except two sites from SF tailings had acidic pH (3-5); with also acidic leachates (2). Very high total concentrations of Zn, Mn, Pb and Cd were quantified in some rhizospheres, and few of them presented high DTPA- and water-available concentrations of Zn, Pb and Cd. *Ricinus* behaved as a Ni, Zn, Mn and Cd shoot excluder plant, but almost all plants had Pb shoot concentrations above phytotoxicity levels. Seed-extracted oil content was high (41-64%). Our results appear to be the first showing oil content in seeds of *Ricinus* harvested in metal mine tailings. Hence, these polluted sites should be economically valorized because oil production and phytostablization of mine tailings may occur at the same time. Relevant traits to produce in very marginal soils make Castor bean an interesting plant to use for biofuels; which may contribute to produce seeds as raw material and biomass; however, its agronomical management and genetic improvement are relevant aspects to consider to optimal yields in polluted sites.

L7

Enhanced growth of bio-fuel plants on amended coal waste

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In this work further use of a unique biofertilizer, which functions due to the mineral properties of natural zeolite when mixed with an organic waste, has been used to grow bio-fuel plants on coal waste. The growth experiments are reported together with a series of control experiments designed to demonstrate the unique properties of the biofertilizer. The plants grown include: *Salix viminalis, Miscanthus, Beta vulgaris, Zea mays, Brassica napus* and *Linum usitatissimum*. These plants were grown in pots under controlled greenhouse conditions. Coal waste was taken from Calverton colliery, Nottinghamshire and used throughout the work as the plant substrate. It is now becoming clear that coal waste, like many other industrial waste products that have been used in former experiments contain a range of chemical elements that can be ionized by the biological activity of the organo-zeolitic amendment. In this regard many plant nutrient elements are made available for uptake which are both essential and beneficial to plant growth as demonstrated by the large differences in biomass between plants grown in the amended substrate and those grown in the untreated waste.

Keywords: biofertilizer, zeolite, coal waste, ionized, biomass

References:

Leggo P.J, Ledésert B and Day J. (2010). Organo-zeolitic treatment of mine waste to enhance the growth of vegetation. European Journal of Mineralogy, 22: 813-822.

L8

Obtaining natural yeast strains capable of fermenting solutions with high levels of glucose

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This paper describes the technical characteristics of a set of yeast strains with resistance to high glucose content. These strains were obtained as a result of a long process of utilization of Biodynamic Agriculture, and using culture and fermentation processes extremely friendly with the environment. The result is the isolation and characterization of some wine yeasts, which produce natural wines with an alcoholic strength unbecoming a natural wine: 18-20 degrees of ethanol. Therefore, these yeasts open the door to a commercial production process of ethanol for fuel uses much more efficient compared to the processes described to date.

Keywords: yeast, ethanol, high glucose

L9

Genetic characterization of *Jatropha curcas* L. accessions from different countries

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"JatroMed" is a EU supported 4-year project with the aim of covering the energy needs of small farmers and rural communities of Egypt, Morocco and Algeria by using the bio-oil of *Jatropha curcas* L. (www.jatromed.aua.gr). Selection and characterization of jatropha genotypes are crucial aspects for the success of the project. Eleven accessions were collected from Mexico (Michoacán and Veracruz), Brazil (GHS-B), Dominican Republic (GHN-D), India (JCLMax3.0, QVP3014 and JAT106), Vietnam (JAT072), Thailand (JAT083), Ghana (GHA1) and Egypt (EJAT). Additional accessions (3 wild-types and 4 varieties) were included in the analysis for comparison. Genetic characterization was performed on 5 samples/accession using 14 molecular markers (SSR, EST-SSR and SCAR) spread across the *J. curcas* genome. Markers amplified a total of 29 different alleles that were used to estimate the genetic diversity by cluster and principal components analyses. The analyses revealed the presence of two main genetic groups separated by a coefficient of similarity (c.s.) of 0.72. Within the first group all 4 varieties and 9 of the JatroMed accessions (GHA1, QVP3014, GHN-D, GHS-B, JCLMAX3.0, JAT072, JAT083, JAT106 and EJAT) grouped with a c.s. of 0.87, indicating high similarity of these accessions with the cultivated gene-pool. In the second group the two Mexican lines (Michoacán and Veracruz) and one wild-type clustered with a c.s. of 0.83, indicating a wider genetic base of these two lines. Within-line c.s. ranged from 0.83 (Michoacán) to 0.96 (EGYPT) and was usually higher in the 9 JatroMed accessions that clustered in the cultivated group.

Keywords: JatroMed, *Jatropha curcas*, Genetic diversity, Biofuel, Energy crops

L10

Biofuel crops for multipurpose benefits: Prospects and perspectives

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It has been widely accepted that increasing carbon dioxide concentration is a serious threat to the homeostasis of various ecosystems and is primarily responsible for global warming and the subsequent climatic changes. In this context, using bioenergy crops for soil carbon sequestration in degraded or marginal land is a triple term environmental benefits since growing plants will act as a sink of carbon and can successfully improve the fertility of soil. *Jatropha curcas* L. has gained considerable attention as a potential feedstock of biodiesel and many Jatropha plantations have been established in tropical and subtropical regions worldwide. We have done trials in different types of soil (sodic, clay etc.) with integrated nutrient and microbial amendments. The studies provide useful data to establish relationship between nutrient availability, soil carbon stock, glomalin content and soil aggregates as well as soil carbon sequestration, wasteland reclamation and local sustainable development of biofuel crops. Above all, biofuel cultivation has attracted increasing research and policy support aiming to reduce green house gas emissions and the dependence on fossil fuels. The present paper briefly present the multipurpose benefits of bioenergy cultivation in degraded soils and the way forward.

Keywords: biofuel, soil carbon sequestration, climate change, jatropha

L11

Energy crops production at low contaminated site

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Low contaminated sites near the industrial plants are not suitable for food crops cultivation. Increased concentrations of toxic metals in agricultural soils can cause death of plants or reduce their production. Contaminants accumulated in food crop may also cause danger to human health. Though, low contaminated sites seem to be applicable for other useful plants. Nowadays, there is a huge energy demand and those fields can be planted with energy crops. Therefore, the aim of our work was to observe the vitality and metal accumulation in a variety of energy crop plants that grew in contaminated field. For our research we chose low contaminated site near Bratkovice in Czech Republic. The site is located next to an old industry zone in an often flooded area and it is polluted with heavy metals such as cadmium, lead, and zinc. A variety of energy crops were sown on the plot and detection of heavy metals contents in roots and shoots of 17 plant species was performed during three growing seasons. Our results showed that most of the plant species accumulated a small amount of metals in their shoots; therefore, they may be applicable for bioenergy. On the other hand, metals contents in shoots of some species were much higher. For that reason these plants may be more appropriate for phytoextraction.

Acknowledgements**:** This work was supported by project LH11048.

Keywords: bioenergy, crop production, heavy metal, soil pollution

L12

Towards the sustainable cultivation of microalgae to produce renewable biofuels and added-value chemicals

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Microalgal cultures represent a promising alternative source of biomass for biofuel and chemicals production, since they can reach higher energy yields per area than conventional biomass crops and can be grown on marginal land using waste, saline, or brackish water. Their use can thus avoid the food-fuel competition for land, resulting in major economic and social benefits. At the present stage however, major developments are needed for making microalgae-to-biofuels process technologically and economically feasible. With respect to a sustainable algal biomass production, the following parameters are thought to be of utmost importance: CO_2 concentration; light intensity and quality, photoperiod; photobioreactor design; batch, fed-batch or continuous culture conditions; possible use of wastewater streams. The purpose of the ongoing research is thus to clearly assess the potential and real influence of these parameters on the quantitative and qualitative production of biomass, lipids and added-value chemicals by several microalgal species; and on their respective productivity, taking into account the probable antagonism between biomass and lipid production, often depending on the nitrogen supply. Several species of microalgae are grown at laboratory scale to compare and contrast biomass yield and lipid productivity. In addition to the potential use of oil for biodiesel production, we are currently working towards demonstrating the technical and economical feasibility of an innovative process for syngas production by hydrothermal processing of microalgae. The process is envisioned as a closed-cycle with respect to nutrients, water and CO_2, that are separated and reused for microalgae growth, potentially resulting in a sustainable process.

Keywords: Biofuels, Bioenergy, Microalgae, CO_2 mitigation, wastewater treatment

L13

Immobilization of heavy metals and metalloids by using of energy crops

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Phytostabilization is a method that exploits plants to immobilize contaminants in soil or ground water using roots absorption, adsorption onto the surface of the roots, or formation of insoluble compounds as a result of interactions of contaminants with plant exudates in rhizosphere. This method reduces the mobility of contaminants and thus prevents their migration into the groundwater or into the air. Therefore, phytostabilization can be used for restoration of the vegetative cover at sites where the original vegetation disappeared due to a high content of metals in the soil. Plant species that are tolerant to high concentrations of metals can reduce migration of contaminants by wind erosion of exposed soil surfaces and at the same time they can reduce leaching of contaminants into the groundwater.

This Czech-Chinese project is focused on utilization of charcoal made from bamboo or other plant residues to increase the production of energy crops and also to immobilize heavy metals in soils contaminated by industrial activities. The knowledge about energy crops cultivation on contaminated soils enriched by charcoal should provide new possibilities for solving problems with contamination of water and soil, which we are facing around the world.

Acknowledgements**:** This work was supported by project Kontakt No. LH 12162 (Ministry of Education, Youth and Sports).

Keywords: phytostabilization, energy crops

L14

Increasing Oil Yield and Quality of Biofuels Production in Camelina sativa via Manipulating Triacylglycerols Synthesis Pathway

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Liquid transportation fuels based on plant seed oils (e.g. biodiesel and green diesel) have tremendous potential as environmentally, economically and technologically feasible alternatives to petroleum-derived fuels. Plant oils can be directly converted to biodiesel with existing technologies, and therefore could replace a significant proportion of the petroleum-based fuels. Camelina sativa, a non-food crop with high seed yields and oil content, has been proposed as an ideal crop for biofuel production. Further increase in seed oil yield by increasing the flux of carbon from increased photosynthesis to triacylglycerol (TAG) synthesis will make this crop more profitable. In seeds, TAG is synthesized from glycerol-3-P and fatty acids through the sequential activities of the glycerol-3-P acyltransferase, lysophosphatidic acid acyl transferase and diacylglycerol acyltransferase. The overexpression of enzymes that catalyze synthesis of the glycerol backbone and conjugation of fatty acids to the glycerol backbone appear to be far more promising targets for increasing TAG accumulation. Therefore, we aim to target the expression of genes in two critical steps in TAG synthesis. The first target is the production of glycerol-3-phosphate for use as the backbone for TAG synthesis. Our second target is the overexpression of genes involved in acylation of fatty acids in the downstream process for TAG synthesis. Further, we will be combining the overexpression of genes in these two steps, which will help us to achieve a synergistic effect on the flux through the TAG synthesis pathway, and thereby further increase the oil yield.

Keywords: *Camelina sativa*, triacylglycerols, biofuels, transgenic plants, carbon flux

L15

The good and bad of biofuels

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With the global increase in biofuel production, transport, storage, and distribution, the frequency of releases to the environment may be dramatically increasing as well. These fuel constituents (e.g. ethanol, biodiesel) contain different physical, chemical, and biological properties than their petroleum counterparts which ultimately can result in different environmental fate and transport behaviors. The good news is biofuels are highly biodegradable...the bad news is biofuels are highly biodegradable. The introduction of biofuels into the subsurface can rapidly consume all available electron acceptors, rapidly driving the system into methanogenesis. At large scale biofuel release sites such as train car derailments, methane has been measured at super-saturation levels in water, in high (50%+) percentage levels in soil gas, and directly fluxing out of the ground at levels similar to municipal landfill systems. Given the explosivity of methane, these subsurface vapor plumes represent significant safety concerns.
This presentation will discuss the current state of understanding of biofuel fate and transport behaviors, provide case study examples, and discuss potential mitigation measures, including phytotechnologies, for preventing methane risks.

Keywords: Biofuels, methane, methanogenesis, fate, transport

L16

Economic viability of long-term phytoremediation using fodder maize on marginally contaminated land

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Biomass is considered as one of the main sources for sustainable energy in the future. Maize cultivated on contaminated agricultural land can be used simultaneously as a safe feed and biomass resource for energy conversion processes. Nowadays, the corn is used as feed for animals since metal accumulation in this part of the plant is limited and lies far below legal threshold values for use as feed. The plant stem itself accumulates more metals, and is left on the field, which allows for organic material to be reintroduced into the soil, but also has the unintended consequence that the metals are reintroduced into the soil. By collecting these plant residues from the field (separately or as silage), a double target could be fulfilled. Farmland could envisage long term remediation, whereas the additional harvest of biomass could be used for renewable energy production in a biomass conversion plant. The economic viability of the harvest of this residual biomass stream is examined for a case study in the Campine region (Belgium) and builds further on existing economic analyses within this region. Three scenarios are compared: (i) no harvest of residual, (ii) separate harvest of residual, and (iii) combined harvest of grains. The scenarios will be compared based on their different impacts on (a) long-term potential use of the land (*i.e.* potential remediation), (b) energy use in general, and (c) farmer income.

Keywords: Long-term phytoremediation, Biomass conversion, Economic viability, Fodder maize

L17

The use of energy maize (*Zea mays*) for phytoattenuation of metal-enriched soils in the Campine region (Flanders, Belgium)

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In the 19th century up to 1970, historic atmospheric deposition of trace metals from metal refinery activities has caused elevated concentrations in agricultural soils in the Campine region. 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. Cd concentration is exceeding legal threshold values for fodder crops. Therefore, safe applications must be considered, while preserving the income of affected farmers. Both goals might be achieved using phytoattenuation with energy maize. To study the potential and constraints, 4 ha were planted with six commercially available *Z. mays* cultivars on a moderately contaminated soil (3 mg Cd kg^{-1} soil and 430 mg Zn kg^{-1}on average). Biomass production of energy maize (20 ton DM ha^{-1}) showed no significant difference in yield in comparison with energy maize grown on non-contaminated soils. Batch (14 days) and continuous tests (435 days) for anaerobic digestion showed a biogas production potential of the silage (215±23 Nm^3 ton^{-1} FM) similar to reference material. The economic outlook for farmers growing energy maize on contaminated soils seems favourable. Model calculations indicated no losses in their average yearly income given base case assumptions. Because of its easy applicability and positive economic feasibility, growing energy maize on metal enriched soils may constitute a valuable and sustainable alternative use of these contaminated soils.

Keywords: Campine region, *Zea mays*, metal distribution, bio-energy, cadmium

L18

A review of North American *Populus* phytotechnologies research published from 1989 to 2011

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Largely because of their broad genetic variability and associated high gains from selection during tree improvement programs, *Populus* species and hybrids are among the most-studied trees in the world. In addition, *Populus* is an ideal genus for research because of the direct connection to multiple end uses that benefit society, regardless of specific geographic location. In addition to energy and fiber applications, the success of using *Populus* for phytotechnologies is mainly due to their ability to grow fast, transpire high volumes of water, and tolerate heavily contaminated sites. The objective of the current presentation is to synthesize the results of *Populus* phytotechnologies research conducted in North America and published from 1989 to 2011. The presentation will be organized according to categories such as whether the research was conducted in the field or under controlled conditions or whether the contaminants were organic or inorganic. The review builds upon the recent development of the database of North American *Populus* research conducted during this time period (Zalesny and Coyle, 2012), which contains a total of 125 unique phytotechnology-related studies reported in peer-reviewed literature. In addition to the information listed above, metadata will be analyzed to provide matrices and other summary outputs highlighting specific genomic groups, genotypes, and uptake potential into leaf, woody, and root tissues.

Keywords: ecosystem services, hybrid poplar, inorganics, organics, phyto-recurrent selection

References:

Zalesny RS Jr. and Coyle DR (2012). Short rotation *Populus*: a bibliography of North American literature, 1989-2011. U.S. Forest Service, Northern Research Station, General Technical Report.

M: Risk Management and Assessment for Public Health

M1

Uptake, translocation and metabolism of diclofenac in *Typha* and in *Brassica* plants

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The uptake of human painkiller diclofenac from hydroponic culture medium was investigated by Cattail (*Typha latifolia* L.) and Indian mustard (*Brassica juncea* L. Czern.) plants; the transport and the distribution of metabolites in the plant tissues were determined to explain the possible risk of entering these agents via plant accumulation into the food chain. The plants were treated with 1 mg/mL diclofenac for one week. Root and shoot samples were collected after 24, 72 and 168 hours of treatment from both species and after 30 days of treatment from cattail. Diclofenac and its metabolites were detected with LC-MS analysis after extraction and clear up with SPE.

In both species, diclofenac was already detected after 24 hours of exposure in the plant roots, followed by its transport to the arial parts of the plants. We identified 4´-OH diclofenac, OH-diclofenac glutathione and diclofenac glucopyranoside as metabolites. They were found mainly in root tissues, suggesting the main site of metabolism is centered on the roots.

Based on our results we can confirm the ability of the plants to take up human medicine residues from the environment. The determinate uptake and transport rates indicates a theoretically low contamination hazard of the food chain due to pharmaceutical accumulation in plant shoots. However, in practical phytoremediation processes plant density and root surface might compensate for it. In addition we should note that this investigation does not provide information on the effect of long-term exposure to these substances, which is more related to natural conditions.

Keywords: diclofenac, uptake, metabolism, *Typha, Brassica*

M2

X-ray micro-analysis from root, stem and leaf parts of *Atractylis serratuloides (Asteraceae)* under fluoride pollution

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Since the sixties, Tunisia, has developed phosphates rock processing causing various air emissions, more particularly fluorine and SO_2, which are the main local source of environmental pollution. Besides being submitted to the climatic constraints of this Mediterranean area, the vegetation of Southern Tunisia is strongly disturbed, by fluoride atmospheric pollution that threaten crops and autochthonous vegetation. The site of Gabes submitted to a high fluorine pollution was compared to a reference site, and the vegetation cover, determined.

Amongst all plant species listed in both sites, *Atractylis serratuloides*, belonging to the *Asteraceae* family, was selected as a potential vegetative barrier to lower the impact of the fluoride atmospheric pollution. A first step was to detect and localize into the plant tissues the elements such as F and S due to the pollution. Fine cross sections of root, stem and leaf parts were prepared and observed by scanning electronic microscopy coupled with EDX micro-analysis. No F was detected by this method in all plant organs despites the high concentrations of F measured in soil and plant parts in Gabes. However, S and Ca concentrations were higher in the polluted samples. The occurrence of calcium oxalates, notably in the peripheral tissues of stem and leaf parts, may play a role in lowering fluorine toxic effect. Since *A. serratuloides* is widely distributed along the gradient of pollution, even at the vicinity of industrial source of pollution, this halotolerant species may be of interest as a barrier for crops protection against this wind-dispersed pollution.

Keywords: *Atractylis serratuloides*, Fluoride pollution, SEM coupled with EDX

M3

The role of sewage sludge treatments on the establishment of different species on a mine tailing from Riotinto (SW Spain)

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Spain is one of the main municipal sewage sludge-producing countries in Europe generating an important disposal problem. The use of sewage sludge in agriculture to improve crop production efficiency is related to the stabilizing process before spreading. This research aims to evaluate the efficiency of the application of differently treated urban sewage sludge to a Riotinto mine soil on the establishment of various species (ray grass, tomato and ahipa). Three sewage sludge treatments were considered: air-dried stabilized (SS), composted (CS), and composted with olive prune wastes (CLV). Pot experiments were performed amending the soil with the three sludges each at 2 and 10% under greenhouse conditions. Due to the low soil pH (2.3), the soil was limed up to pH 6-7 before amendment (control). Germination rate, photosynthetic pigments, growth and main soil properties were determined. All treatments increased the biomass when compared with the control. Sludge treatments affected biomass production in different ways, though SS addition at 10% hampered the vegetal development of the species. An enhancement of chlorophyll a, b and carotenoid content occurred with sludge addition and with the dose applied. Moreover, soil shoot water content was not significantly modified by sludge application. In general, the best plants growth was achieved after addition of CLV, even if differences were found according to the different species.

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Keywords: mining, amendment, ray grass, tomato, ahipa

M4

Restoration of acidic mine soils with native (*Cistus ladanifer* L.) and non-native (*Medicago sativa* L.) plants using soil conditioners

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Revegetation of soils from mining sites in Mediterranean areas is difficult not only because of the unfavorable conditions such as acid pH, high metal load and unbalanced nutrients, but also because of the climatic stresses. This research aims to investigate the possibility of reclaiming an area from Riotinto district (Huelva, Spain) with the application of soil improvers (composted sewage sludge (CS), commercial organic fertilizer (F) and lime (Lm)) and planted with two different species: Cistus ladanifer, a naturally occurring shrub and *Medicago sativa,* a grass considered as a nurse crop for an early vegetation purpose. This research included soil incubation experiment and pot culture to evaluate the plant response in the different growth media. CS was applied at two rates, Lm was provided to reach a physiological pH and F was used at agronomic rate. Addition of CS altered the CO_2 soil production as a function of the dose giving respiration rates up to 6 times that of the native soil. No plants grew in native soil (pH 2.5). Although the combined addition of Lm+CS (pH 7) showed the best *C. ladanifer* development, the addition of F and CS increased the soil pH up to 5 providing a favourable media for the species growth. For the grass, the addition of the two organic amendments also increased soil pH (3.5 for F and 5 for CS) allowing plants to grow adequately for the first two weeks, although after this period only plants in treatments CS+Lm survived. Thanks to Junta de Andalucía-P08-RNM3526.

Keywords: compost, fertilizer, mining, revegetation

M5

Impact of biochar on arsenic uptake in tomato plants (*Solanum lycopersicum* L.)

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Arsenic is a metalloid occurring worldwide and is toxic and carcinogenic for humans. Soil contamination with As dangerous because plants can uptake and translocate As to their edible parts. Tomato is one of the most consumed vegetable in Europe and in Italy it represents an important aspect of economical prosperity and food capital. Tomato can uptake As from contaminated soils and irrigation water at quite high concentrations compared to other crops, thus it is of great importance to evaluate and prevent risk of arsenic intake by humans from the consumption of this vegetable.

Biochar is made by the pyrolysis of biological residues and is characterized by a porous, low density carbon rich material. Its application to soil to improve cation exchange capacity, moisture and pH are proven, and an emerging immobilization tendency has been found for some contaminant. In this study we evaluate arsenic uptake by three tomato cultivars (*Solanum lycopersicum* L.) grown on As contaminated soil amended with biochar (30% by volume) and maintained in controlled conditions (23°C, 30% humidity and 16h photoperiod). We evaluated contaminant leaching by pore water extraction and analysis with ICP-OES, together with ICP-OES analysis of contaminant concentration in roots, stems and leaves. Physiological analyses were conducted to determine effects of biochar on biomass production, photosynthesis activity and cellular respiration, with the overall aim to determine whether biochar may reduce As uptake, and thus transfer, in tomato grown on a contaminated soil.

Keywords: Biochar, arsenic, food safety, tomato, inorganic contamination

Reference:

Beesley L, Marmiroli M (2011). The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environmental Pollution, 159: 474-480.

M6

Evergreen plant species are useful for phytoremediation of particulate matter and heavy metals from the air

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Particulate matter (PM), when inhaled, is damaging for human health. To reduce its concentration in air, plants could be used as biological filters, accumulating PM on their foliage.

In this work, usefulness of following evergreen species for PM and heavy metals accumulation from ambient air in urban areas was investigated: *Taxus baccata* L., *Hedera helix* L. and *Pinus sylvestris* L. Also effect of rain on PM deposited on foliage was studied. An experiment was carried out in three locations at two pollution levels and at different exposures to precipitation: (i) near a busy road (polluted site), (ii) near the same road, under a viaduct (polluted site, protected from rain) and (iii) in an agricultural field (clean site). Plants were established in spring and leaf samples were analyzed for deposited PM after several time intervals.

Obtained results show that foliage accumulated an increasing quantity of PM in successive months after plant establishment. Amount of PM and heavy metals accumulated on foliage differed considerably among species and between locations. Out of all species greatest amount of air pollutants was found on foliage of plants grown at location protected from rain and smallest accumulation was noted at clean site. Among analyzed species greatest mass of deposited PM and heavy metals was recorded for *P. sylvestris*. Comparison of polluted locations showed that precipitation removes a considerable proportion of deposited PM from foliage during season.

Acknowledgements: This study were supported by Polish-Norwegian Research Fund granted to S.W. Gawroński and A. Sæbø, grant # PNRF-193-AI-1/07.

Keywords: particulate matter, heavy metals, precipitation, phytoremediation, evergreen species

M7

Assessment of plant toxicity in artificial soils based on organic and mine wastes

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Rehabilitation of degraded landscapes to restore ecosystem health constitutes a main research goal worldwide. Artificial soils derived from low-cost wastes can improve soil quality while providing a suitable strategy for wastes disposal. However, such strategy requires the assessment of potential environmental hazards. In this context, plant toxicity test were carried out. Technosols (or artificial soils) were produced from sewage sludge (SVC, 19% organic carbon), Fe-mine waste (FeM, 44% Fe oxides) and biodiesel waste (DRS, 62.6% organic carbon). The formulated soils were obtained by combination of such components at several proportions and incubation under saturation at 28 ºC for 30 d. Additionally, technosols pH was lowered with acid solution. Pregerminated seeds of barley (*Hordeum vulgare*) were planted on cylindrical pots filled with sand and each technosol was applied as a top dressing (2% w/w). The pots were moistened to field capacity, fertilized with KH_2PO_4 and KNO_3 and grown under controlled conditions. After 10 days the longest root per seedling was measured. In general, the addition of different ratios of FeM and DRS resulted in a significant positive effect on barley root elongation (Duncan's test, $p<0.05$). Otherwise, the strongest positive effect was obtained for FeM used as single amendment, while for DRS 50% inhibition dose was determined at 7% w/w. Results have also revealed a synergistic effect on root elongation for the combination of DRS and FeM. Furthermore, plant toxicity was related to pore-water chemistry, e.g. dissolved organic carbon.

Research was partially supported by Junta de Andalucía-P08-RNM3526 (Spain) and PDMK/10/080 (Belgium).

Keywords: mine wastes, revegetation, technosol, toxicity, pore water

M8

Phytoremediation technology development for contaminated soil in the zone of former airport

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The problem of soil remediation and recultivation in the zone of a former airport is currently the object of considerable attention in the present research. The first stage of investigation was determination of the agrochemical characteristics and level of topsoil contamination with heavy metals by Atomic Absorption Spectrophotometry (AAS). The results obtained show that soil on the territory of former airport is defined as moderately contaminated with Cd, Cu and Pb (exceeding the MPC by 2-4 times) and uncontaminated with Zn, Mn and Co. Pollution has distinct zonal character and was observed near runway, taxiway and department of repairing.

Moreover insignificant environmental pollution with oil products was defined on the territory not far from both former oil products storage and department of aircrafts repairing.

Appling the soil environment indexes allowed to take into consideration the synergetic action of chemical pollutants that are present in the investigated environment.

The risk of repeated airborne contamination of soils in the zone of former airport was assessed due to biotesting (*Lactuca sativa L.* and *Allium cepa* as biotesters) and chemical analysis of atmospheric precipitation (by ASS).

In accordance with agrochemical analysis soil belongs to typical chernozem with acceptable characteristics that conditions necessary of the soil treatment and profitability of the territory reuse.

Based on received results the phytoremediation technology for contaminated soil in the zone of former airport was developed with taking into account climatic peculiarities of the region.

Keywords: remediation, soil, heavy metals, former airport

M9

Testing combinations of amendments for stabilization of metals in contrasting contaminated soils

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There is a need to reduce negative impacts of trace element polluted soils on human health and the environment. Metals can be stabilized by amendments increasing metal adsorption or altering their chemical form (Basta *et al.*, 2005). Few experiments compare different in situ remediation treatments under similar environmental conditions, or consider whether or not all soil components or properties (microbes, soil fauna, plants, soil retention, colloid stability, etc.) are similarly protected. Within the EU FP7 Greenland project (266124) we compared the impact of novel soil amendments and their combinations with traditional materials on metal solubility and response of plants, soil organisms and microbial activity.

One-year greenhouse pot experiments were established: soil A, less toxic agricultural soil contaminated through long-term Zn/Pb smelter emissions in Poland (pH 7.0); soil B, toxic soil contaminated through smelter dust spill in Poland (pH 6.8); and soil C, toxic Cu-contaminated mine-spoils in Spain (pH 3.6). Amendments were tested individually and in combination in planted and unplanted soils: compost, drinking water residue, iron grit, Ca-phosphate, LD slag, Thomas basic slag, gravel sludge, siderite, Fe nano-sorbent, and cyclonic ash. Rates were optimized in prior batch tests. Soils B and C were planted with grasses, and soil A with lettuce. Plants were periodically harvested, yields recorded and metal content determined. Soil metal extractability and bioaccessibility, pH, EC and enzymatic activity were measured. Soil pore waters were analyzed for trace element/nutrient concentrations. Parallel tests evaluated earthworm behavior and metal accumulation. Amendment effectiveness was assessed in terms of phytoexclusion or phytostabilization.

Keywords: phytostabilization, soil amendments, trace elements, contaminated soil, bioavailability

M10

Development and use of plant test system for evaluation of biological quality of metal contaminated soils in Bulgaria

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Plant test system for evaluation of toxicity of metal contaminated soils has been developed and applied in several industrially contaminated regions inBulgaria. It is based on both morphological (leaf area and plant fresh biomass) and physiological (photosynthetic performance and root peroxidase activity) endpoints of young plants grown in excess heavy metals in the root media at controlled environment. Cucumber was chosen within the tested plant species (bean, cucumber, maize, lettuce) as the most suitable species based on both high sensitivity to metal stress and fast growth. The plant test system allows classifying phytotoxicity of metal-contaminated media into five toxicity classes: nontoxic (I), slightly toxic (II), moderately toxic (III), strongly toxic (IV) and lethal (V). In a comparative study was established that the sensitivity of the new test is similar to that of known test (bean test – Vangronsveld and Clijsters, 1992). The plant test system has been applied to evaluate phytotoxicity of soil samples taken from several industrially polluted regions in Bulgaria. The results are disscused in term of the proper choice of phytotechnologies for sustainable land use in the contaminated regions.

Keywords: heavy metals, plant test system, phytotoxicity, growth, photosynthesis

Reference:

Vangronsveld J and Clijsters H (1992). *A biological test system for the evaluation of metal phytotoxicity and immobilisation by additives in metal contaminated soils.* – In: E. Merian and W. Haedi (Editors), Metal compounds in environment and life, 4. Special supplement to Chemical Speciation and Bioavailability., Wilmington: Science Reviews Inc., 1992: 117-125.

N: NANOPARTICLES

N1

Risks associated with the accumulation of engineered nanomaterials in root and tuber crops

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Food security and safety are issues of growing global concern. The rapid increase in the use of engineered nanomaterials (ENMs) is expected to result in the release of those ENMs into the environment and their accumulation in biosolids. There is also a pressing need to understand the accumulation of ENMs in food crops. Several current efforts underway to examining the accumulation of ENMs in edible leaves, fruits, and seeds, yet there has been little study of ENM accumulation in belowground crops. Root and tuber crops grow in direct contact with the soil substrate and accumulated higher concentrations of ENMs compared to aboveground tissues. The risks of ENM accumulation in such food crops has yet to be examined. Our research team has recently received a grant from the USDA Agricultural and Food Research Initiative to examine the accumulation of six metallic ENMs in the tissues of ten common belowground vegetables. In addition to examining the extent of accumulation in these plant foods, the project will also examine the spatial distribution of the ENMs within each tissue, assess the nutritional bioaccessibility of the ENMs using a physiologically-based extraction test, and will develop age- and gender-specific dietary exposure models. Results from this work will allow growers, extension agents, and USDA to make sound decisions on choice of crops for particular ENP-impacted soils. The specific accumulation and dietary exposure scenarios associated with particular ENP and plant combinations would allow for recommendations /restrictions concerning which ENP-containing products can be safely applied to human food crops.

Keywords: engineered nanomaterials, engineered nanoparticles, food safety, dietary exposure

N2

Preparation of nanocapsuls from extracts of *Matricaria chamomilla* and *Calendulla officinalis* for application in hygienic-cosmetic products

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There are many medicinal plants such as: Thym, Aloe, Maricaria, Calendulla in Iran that their extracts are used in medicinal or hygienic-cosmetic products. Today, these extracts are added in special dose to other drug constituents, traditionally. The main aim of this study was preparation of nanocapsuls from two extracts of *Matricaria chamomilla* and *Calendulla officinalis* and application in cream formulation. So, the flowers of these plants were prepared. After separation and drying the petals, their extracts were prepared by maceration with different solvents. After evaporation the solvents and concentration the extracts the nanoparticles were prepared by two methods. In the first method, two organic and aqueous phases were prepared. Aqueous phase consisted starch, plant extract and ethanol and organic phase was combination of cyclohexane and chloroform. Aqueous phase injected to organic phase by ultrasound with flow rate of 80 degree/min. After homogenization of both phases the mixture was centrifuged at 3500 rpm for 20 minutes. The residue was freeze dried and some of the samples were spray dried. The SEM photos were prepared from samples. The results showed with controlling some parameters like extract amount, time of stirring, centrifuge rpm and so on preparation of nanocapsules was possible. Of course the most amounts of particles were microcapsules. The particles were used in cream formulation. In second method nanocapsules were prepared using poly lactic-glycolic acid that all of the particles had nano dimensions.

Keywords: *Matricaria chamomilla, Calendulla officinalis*, nanocapsule

N3

Whole-genome expression analysis of two *Arabidopsis* mutants resistant to CdS NPs

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Nanotechnologies are a rapidly growing industry and it is expected to reach a market size of 2.6 trillion dollars by 2015. Manufactured NPs are currently used in different areas such as electronics, biomedicine, pharmaceuticals, cosmetics. Little is known about mechanisms of biological uptake and interaction with cells, biological compartmentalization and chemical behavior in the environment. The aim of our research was to gain information about the potential toxicity of CdS NPs using *Arabidopsis thaliana* (L.) Heynh as a model system for an *in vivo* study.

During the last year we isolated two mutant lines (*atnp01* and *atnp02)* tolerant to lethal concentrations of CdS NPs, mutagenized with Ac/Ds maize transposon. The tolerant phenotypes were characterized at physiological and molecular level. Now the expression profile analysis was performed using Affymetrix GeneChip Arabidopsis ATH1 Genome Array. Microarray analysis showed two different expression profiles for the two mutants, results suggest two different behaviors in the CdS NPs tolerance. To exclude the possibility that Cd^{2+} and CdS NPs could use the same tolerance mechanism we analyzed the genes mainly involved in the Cd^{2+} response finding only one gene in common. Microarray analyses confirmed that our mutant lines can be an instrument for the comprehension of the CdS NPs tolerance/resistance mechanisms. Through our approach we tried to merge the two classical Top-down and Bottom-up approaches to find the genes directly involved in the CdS NPs tolerance and define pathways and networks of genes interactions relating the CdS NPs tolerance mechanisms.

Keywords: *Arabidopsis thaliana*, CdS nanoparticles, microarray

Reference:

Bancroft I, Bhatt A, Sjodin C, Scofield S, Jones J and Dean C (1992). Development of an efficient two-element transposon tagging system in Arabidopsis thaliana. Mololecular Genetics, 233: 449-461.

N4

Bio-magnetic monitoring of particulates through roadside plant leaves

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Global air quality is greatly being altered through the problem of particulate matter (PM) pollution which is inextricably linked with human health. Moreover, vehicle derived pollutants as well as industrial emissions simultaneously release deleterious fine-grained PM and magnetic particles into the atmosphere. Since conventional technologies for monitoring of PMs are not feasible in totality, they paved the way to bio-magnetic monitoring approach. Magnetic properties of PM or dust may act as a valuable tool in assessing the burning phenomenon of climate change, as demonstrated through the glacial and interglacial characterization of Chinese Loess Plateau on one hand, while on other hand it act as a proxy for particulate pollutants through roadside plant leaves. Present paper provides an overview on the problem of PM pollution, its environmental geomagnetic studies with special reference to bio-magnetic monitoring through roadside plant leaves. Further, the paper describes the author's research work on magnetic properties of *Ficus infectoria* in a coalmine area and preliminary studies on magnetic properties of dust loaded roadside plant leaves, Aizawl, Mizoram, North East India. Results revealed that bio-magnetic monitoring of particulate pollution may biomonitor the extent of dust/particulate pollution in an effective and systematic way. More focused research works are required in the multifaceted environmental dimensions of magnetic monitoring particularly bio-magnetic monitoring of particulate pollution with roadside plant leaves and hence possess the potential to become a new frontier in the field of feasible phyto-technologies.

Keywords: Roadside plant leaves, bio-magnetic monitoring, particulates, human health, phyto-technologies

N5

Trans-generational Effect of Cerium Oxide Nanoparticles on Tomato

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Increased applications of cerium oxide nanoparticles in consumer products have caused growing concern about the ecotoxic effects of these nanoparticles. Even though the acute toxicity of engineered nanoparticles (ENPs) to plants has been widely scrutinized, information is severely lacking on the chronic toxicity of ENPs, especially the long term generational effects. In this study, we collected tomato seeds from control tomato plants (control F1 seeds) and plants exposed to 10 mg/L of cerium oxide nanoparticles (treated F1 seeds) and re-germinated them in the presence of 0 and 10 mg/L of cerium oxide nanoparticles. Even though the germination rate was similar, treated F1 seeds required longer time to germinate than control F1 seeds in the presence of 10 mg/L of cerium oxide nanoparticles. Both seeds germinated similarly in the absence of cerium oxide. The treated F1 seedlings transpired less water and gained less biomass than control Fl seedlings during the two week exposure both 0 and 10 mg/L of cerium oxide nanoparticles. To further gauge the differential impacts of cerium oxide nanoparticles on Fl seedlings, the root membrane integrity, chlorophyll concentration and composition of the F1 seedlings as well as the uptake and accumulation of cerium oxide nanoparticles by the F1 seedlings were measured. The results suggest that treated F1 seedlings responded differently to cerium oxide nanoparticles from the control F1 seedlings.

Keywords: trans-generational effect, cerium oxide nanoparticles, phytotoxicity

N6

Nanomaterial contamination of agricultural plants

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Although the use of nanomaterials (NM) has increased dramatically, the risks posed to humans and the environment have been investigated only recently. Previous investigations from our laboratory have shown that seed germination and root elongation assays are inappropriate when evaluating NM phytotoxicity. Zucchini was exposed hydroponically to bulk, ion, and nanoparticle Ag, Cu, Si, Au, as well as carbon-based particles (fullerenes, single/multi-walled nanotubes[S/MWCNT]); biomass, transpiration, and NM accumulation were determined. Nanoparticle Ag, Si, and MWCNTs reduced plant biomass and transpiration by 30-91% compared to equivalent bulk materials; element accumulation was inversely related to particle size. Fullerenes, Au, and SWCNTs had no impact on plant growth. Current investigations are focused on a screening study evaluating the acute toxicity of 12 different nanoparticles (including bulk/ion controls) to 12 agricultural crops under hydroponic and soil conditions. Results to date show numerous instances of particle size-specific phytotoxicity, as well as concentration-dependent and species-specific response to NM exposure. Separately, the effect of fullerenes on the accumulation of secondary organic pollutants (*p,p'*-DDE) by zucchini, tomato, pumpkin, and soybean was determined. For select species, fullerenes promoted *p,p'*-DDE accumulation in the roots and shoots, suggesting enhanced uptake through a fullerene-pollutant complex. Our data clearly demonstrates differential toxicity, accumulation, and fate of several nanomaterials relative to respective bulk particles and ions. The implications of these findings for accurately determining the fate and transport of nanomaterials in agricultural systems, as well as for exposure and risk posed to humans, will be discussed.

Keywords: Nanoparticles, nanomaterials, phytotoxicity, nanotoxicology, fullerenes

O: Field Projects

O1

Potential of Safflower (*Carthamus tinctorius* L.) for Phytoremediation of Soils Contaminated With Heavy Metals

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A field study was conducted to evaluate the efficacy of safflower plant for phytoremediation of contaminated soils in the absence and presence of organic soil amendments (compost and vermicompost).

The experiment was performed on an agricultural field contaminated by the Non-Ferrous-Metal Works near Plovdiv and the region of Lead and Zinc Complex near Kardjali, Bulgaria. The treatments consisted of a control (no organic amendments), compost amendments (added at 5 and 10%), and vermicompost amendments (added at 5 and 10%). The concentrations of Pb, Cu, Zn and Cd in safflower (roots, stems, leaves and seeds), safflower oil and meal were determined.

The application of soil amendments favors plant growth and development. Organic amendment application led to an effective immobilization of Pb, Zn and Cd mobile forms in soil. A correlation was found between the quantity of the mobile forms and the uptake of Pb, Zn and Cd by the safflower seeds. Tested organic amendments significantly influenced the uptake of Pb, Cu, Zn and Cd by safflower plant. Oil content and fatty acids composition were affected by compost and vermicompost amendment treatments.

The 10% compost and 10% vermicompost treatment led to decreased heavy metal contents in safflower oil bellow the regulated limits. The possibility of further industrial processing will make safflower economically interesting crops for farmers of phytoremediation technology.

Keywords: phytoremediation, safflower, heavy metals, organic amendments

O2

Effect of experimental and environmental conditions on As phytoextraction by *Pteris vittata*: first-year results of a field study

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The fern *Pteris vittata* is a known arsenic hyper accumulating plant. Its potential for phytoremediation of contaminated soils has been assessed in a number of studies, mainly at laboratory or glasshouse scale. The few field surveys reported in the literature indicate that the characteristics of the site (As levels, climate, soil properties etc.) and growth conditions (plant density, fertilization, growth/harvest cycles, etc.) may strongly affect As uptake as well as biomass production.

Here we report the first-year results of a small-scale field study with *P. vittata* carried out in an experimental plot with a moderate As contamination, aimed at evaluating the feasibility of a phytoremediation intervention on the wider area that includes the plot, and on similar sites. The study area is located in north-eastern Italy.

The experimental design was planned to compare and evaluate the effect of different conditions on plant growth and As uptake. Ferns, pre-inoculated with arbuscular mycorrhizal (AM) fungi in order to enhance biomass production, were planted in early summer 2011 in nine experimental plots about 240x240 cm wide, with different plant density (about 6, 8 and 11 plants/m^2) and subjected to different fertilizer additions. After the first growth cycle, ended in December 2011, fronds were harvested and the plants were covered for the winter period, before a second growth cycle.

Arsenic bioaccumulation in fronds and biomass yield were measured, in order to assess As removal efficiency, to determine the optimal conditions for As phytoextraction and to plan the experimental activities for the second year.

Keywords: Phytoremediation, Phytoextraction, Arsenic, Pteris vittata, Arbuscular mycorrhizae

O3

Results of field project on restoration of a dumping site consisting of arsenopyrite roasting residues

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One of the main objectives of the project Rhizosphere Enhanced Phyto Extraction Technology (R.E.P.E.T.) supported by Regional Government of Tuscany (Italy) was to identify a suitable and effective phytoremediation pilot plant for the *in-situ* restoration of dumping site contaminated by residues from the roasting process of arsenopyrite. The contaminated site is located in the South Tuscany, inside the industrial area of Solmine s.p.a., that produced sulphuric acid by roasted pyrite for more than thirty years, in the last century . The present study refers to the experimental tests carried out either *ex-situ* in pots and *in-situ* in dumping site during the years 2010 and 2011. The plant species used for phytoextraction are: 1. poplar, hybrid ORION (*Populus deltoides* x *Populus nigra*), to clean up heavy metal; 2. the arsenic-hyper-accumulating Chinese brake fern (*Pteris vittata*) for the metalloid-As. Both *ex situ* experiments in pots (laboratory-scale) and *in situ* in plots were set in order to assess the plant survival, arsenic accumulation, physiological and biomass parameters, as well as enzymatic stress responses through monitoring of catalase, and –SH groups activities in leaves and roots. The study of the parameters above listed, together with improvements in the site using agronomic techniques (such as additions to the site of plant compost and pot soil) allowed the construction of a pilot-plant with an area of 4.500 m^2.

Keywords: arsenic, phytoremediation, *Pteris vittata*, Poplar hybrids, roasted arsenopyrite

O4

Phytotreatment of industrial landfill leachate: a full-scale realization

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Phytotechnologies could be useful to reduce or cancel the transfer of landfill leachate to external treatment systems, reducing their impact on the environment. For this purpose, the plants should consume the leachate directly *in-situ*. The water fraction of the leachate is consumed by the soil-plant evapotranspiration, while the degradation of organic compounds and the immobilization of the different elements occurs on root-soil system (Zalesny, 2008).

We present an application recently realized in central Italy, where the leachate produced by an exploited industrial landfill was used as input of water for woody plantations realized on the top of already closed landfills. The low phytotoxicity of the industrial waste (stable in composition during the time) and the availability of additional water as output of the industrial process, allowed the phytotechnological approach.

Three different evergreen woody species were selected, according to the local climate conditions, and tested comparatively to evaluate the best compromise between the leachate consumptions and management costs. As additional constraints for the plant selection, we considered the limited soil depth and the seasonality of leachate production.

The leachate was provided to the plant by an automated irrigation system, based on the continuous record of the soil water status. The algorithm was defined to maintain the soil water content between the lower and upper limits, identified as optimal for both soil and plant functionality and at the same time avoiding any possible run-off of the leachate.

The layout of the phytotreatment system and the performances during the first year of functioning will be presented.

Keywords: Industrial landfill leachate, automated irrigation, evergreen woody plants

Reference:

Zalesny JA, Zalesny RS, Wiese AH, Sexton BT and Hall RB (2008). Uptake of macro- and micro-nutrients into leaf, woody, and root tissue of populus after irrigation with landfill leachate. Journal of Sustainable Forestry, 27(3): 303-327.

O5

Biological indicators for monitoring mine soil health recovery during amendment assisted phytostabilization – a small-scale field study

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Amendment assisted phytostabilization, i.e. the use of plants in combination with amendments to reduce the bioavailability of contaminants, is an *in situ* cost-effective and environmentally friendly technology to deal with heavy metal soil contamination. A small-scale field study was carried out for 6 months in an abandoned Pb/Zn mine area in northern Spain in order to assess the effectiveness of biological indicators in monitoring phytostabilization processes and soil health recovery. To that end, four organic amendments (poultry and sheep manures, cow slurry and paper mill sludge mixed with poultry manure) were applied on vegetated and non-vegetated sites. Amendment application decreased Pb and Zn bioavailability resulting in lower shoot metals concentration in native vegetation. Components from amendments and metal bioavailability decrease stimulated soil basal respiration and microbial functional diversity (measured by Biolog EcoPlates™). *Lactuca sativa* L. root elongation from bioassay was an excellent biomarker to monitor metal bioavailability. The benefits observed in metal bioavailability and biological parameters due to amendments application were more outstanding in non-vegetated areas than in vegetated ones. Plant biomass and colonization of bare soils by plants improved after amendments application. As a result of mine tailing heterogeneity, amendments affected in different ways soil microbial properties, metal bioavailability and plant biomass depending on the site. In terms of metal immobilization, basal respiration and plant biomass improvement, cow slurry was the amendment showing better results among vegetated sites. In non-vegetated areas, paper mill sludge mixed with poultry manure was the most convenient treatment.

Keywords: Amendments, Bioindicators, Heavy metals, Metals bioavailability, Phytostabilization

O6

Suitability of compost and mycorrhizal-based biofertilizer for vegetation establishment on a mine tailing

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Plant cover establishment on a Fe mine tailing (Alquife, Spain) is hampered by adverse chemical and physical properties, such as deficient and unbalanced nutrients, low organic matter content, lack of microbiota and physical impediments for root development. Successful revegetation of this harsh mine waste can be achieved through amelioration of the substrate properties. Sewage sludge composted with pruned olive wastes and/or a commercial biofertilizer (GLOMYGEL®, MYCOVITRO SL, Granada Spain), containing a special formulation of eco-compatible mycorrhizal fungi, have been applied to restore the biogeochemical cycles. Small field plots were amended with compost at 66 t/ha and/or mycorrhizal biofertilizer at the producer's recommended doses. Several plant species (pistachio, rosemary, thyme, caper and juniper) of varied economical or environmental interest and suited to the Mediterranean climate were selected for the revegetation plan. Plant survival rate was above 80% with general healthy plant growth, except for pistachio which showed visual symptoms of nutrient deficiency or toxicity. Soil productivity was assessed through chemical, biochemical and microbiological parameters. Values of C/N indicated that all treatments (C/N 15-34) improved the plant growth conditions compared to the non-amended plot (C/N 60). The most suitable conditions for plant growth, establishment of microbial activity and improvement of soil biochemical properties were achieved for plots treated with compost in combination with GLOMYGEL®, demonstrating a significant potential to overcome adverse soil and environmental conditions.

Research was partially supported by Junta de Andalucía-P08-RNM3526 (Spain)

Keywords: sewage sludge, revegetation, nutrients, plant survival

P: WASTEWATER

P1

Comparative analysis of free surface and sub-surface flow system performance in phytoremediation of hydrocarbons using *Scirpus grossus*

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Two types of flow, free surface flow (FSF) and sub-surface flow (SSF) systems were examined to remove Total Petroleum Hydrocarbon (TPH) in phytotoxicity of *Scirpus grossus* to diesel as a hydrocarbon model. The main objective of this study was to compare the performance of the two systems to select a better performance for future planning of pilot scale wastewater treatment system. The removal efficiencies of TPH with two flow systems were compared. Several wastewater parameters including temperature, T (°C), dissolved oxygen, DO (mg/L), Oxidation Reduction Potential, ORP (mV), and pH were recorded during the experimental runs. In addition, plant overall length, wet weight, and dry weight were also monitored. The phytotoxicity test using bulrush plant of *Scirpus grossus* was run for 72 days with different diesel concentrations ($V_{\text{diesel}}/V_{\text{water}}$) 1, 2, and 3%. In general, the two flow systems have performance efficiency in hydrocarbon removal but the difference with the pollutant concentration, SSF system able to tolerate at higher concentration than FSF. Comparison between the two flow systems showed that SSF system is more efficient than FSF system to remove TPH from the synthetic wastewater with average removal efficiencies of 91.5 and 80.2 % respectively. The results also showed that the removal efficiency was statistically significant different in systems used.

Keywords: Phytoremediation, phtotoxocity, Scirpus grocuss, free surface flow, sub-surface flow, hydrocarbon.

P2

Poplars and food processing wastewater treatment

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Land application of high-strength, high flow food processing wastewater may result in anoxic and anaerobic soil environments. Metals like manganese, iron, and arsenic can leach under such conditions to pollute ground water, adversely affecting a major source for drinking water. This research aims to develop poplar plantations as a low cost, sustainable approach to treating food processing wastewater that prevent metal leaching to groundwater. The experiments are conducted in field, pilot, and laboratory scale.

In field scale, about an acre of land that is applied with actual food processing wastewater is divided to four sub plots with alternating planted and unplanted plots. Each plot is instrumented to measure rainfall, temperature, infiltration, soil moisture and to collect soil water samples. Additionally, fifteen 5 ft tall, 3 ft diameter HDPE columns, instrumented with soil oxidation reduction potential sensors, soil moisture sensors and temperature sensors, are being used to study the fundamental processes and effects of soil and plants on treatment of food processing wastewater by applying synthetic food processing wastewater. At laboratory scale, 15 columns, each 1.5 ft high and 0.5 ft diameter, are used to study the effects of food processing wastewater on plant growth and metal mobilization by applying factory produced wastewater. Leachates from all experiments are analyzed for COD, pH, transition metals, and anions. By combining the results from above experiments, the research will develop site specific and processor specific design recommendations for treating food processing wastewater.

Keywords: Poplar, Food wastewater, metal mobility, phytoremediation, toxicity

P3

Cyperus longus L. as biological purifier of wastewater for irrigation purposes. Removal efficiency and Zn, Cd, Cu, Fe and Mn

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The common and cosmopolitan distributed wastewater macrophyte, namely; *Cyperus longus* L. was tested as a biological wastewater purifier. Indoor experiments were mainly based on conventional wastewater treatment processes, besides specified design of sand filtration pots, implanted by *C. longus* L. Untreated and treated wastewater samples were analyzed for their key physico-chemical properties and some heavy metals (only removal efficiency of heavy metals Zn, Cd, Cu, Fe and Mn by *C. longus* L. is highlighted in this paper). After 129 days (including 45 days, the time needed for growth/stabilization of *C. longus* L. in experiment pots) the *C. longus* L. was harvested and heavy metals were analyzed in root and shoot systems. The removal efficiency (i.e. uptake/ bioaccumulation rate) then was followed up. Results showed that; the accumulation rate in the plant roots was much higher than the shoots. Higher metal bioaccumulation per cent was noted in roots; Zn (0.522%), Cu (0.821%), Fe (80.480%), Mn (1.886%) and Cd (0.659%) compared with control (irrigated with clean water); Zn (0.147%), Cu (0.167%), Fe (12.590%), Mn (0.331%) and Cd (0.124%). On the other hand, metal bioaccumulation per cent in shoot system was; Zn (0.412%), Cu (0.458%), Fe (4.540%), Mn (1.719%) and Cd (0.567%) compared with control. Always, the more replicated sand filtration pots the highest removal efficiency of heavy metals was achieved.

Keywords: Phytoremediation, *Cyperus longus* L., Removal Efficiency, Heavy Metals

P4

Evaluation of the potential of native aquatic plants for phytoremediation of uranium-contaminated waters: from field into the laboratory

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The observations made within the 200 field sampling sites (uraniferous province of Central Portugal) showed that U was detected in the surficial waters in a wide range of concentrations (0.23 – 1220 µg/L). The natural background for U in superficial waters in the studied area was defined as being 1.8 µg/L. 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 species 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 DW), in *Potamogeton natans* L. (94.50 mg/kg DW), in *Potamogeton pectinatus* L. (364.84 mg/kg DW), and in the free-floating *Lemna minor* L. (42.46 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 3.4×10^3 (average), confirming its high potential for uranium phytoremediation. 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).

Keywords: Phytofiltration, Uranium, *Callitriche stagnalis*, Mine waters

P5

Effects on plants, soil and water quality of irrigating short rotation willow coppice with municipal effluent

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There is a growing interest in using Short Rotation Coppice (SRC) willows as a means of treating municipal wastewater. This may represent an ideal solution to clean-up polluted water and reduce emission of greenhouse gases (Perttu, K. & Kowalik, P., 1997). Willows have many advantages over other plants included: longer growing season, reducing soil losses, large root systems, wide genetic diversity (Mirck J *et al.*, 2005; Kuzovkina Y and Volk T, 2009). There have been a number of studies in SRC willow to treat landfill leachates, sewage sludge, municipal and industrial effluents. The main purpose of this screening study was to select the most promising genotypes to be used for bioremediation. The effect of prolonged use of wastewater on willow growth was assessed using shoot and root biomass. 132 willow genotypes were tested using a hydroponic solution. Chlorophyll content, plant height and changes in pH and conductivity were measured in order to evaluate the level of stress. Genotypes: *Salix spp.* \`RR05196\`, \`RR05039\`, \`RR06070\`, \`00279\`, \`00187\` and *Salix viminalis x S. viminalis* "Jorr" showed the best response in increased biomass. This ANSWER (Agricultural Need for Sustainable Willow Effluent Recycling) project is part funded by the European Union's European Regional Development Fund (ERDF) through the INTERREG IVA Cross-border Programme, managed by the Special EU Programmes Body (SEUPB).

Keywords: Wastewater, hydroponic solution, bioremediation, willow, genotype

References:

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Mirck J, Isebrands JG, Verwijst T, Ledin S (2005). Development of short-rotation willow coppice systems for environmental purposes in Sweden. Biomass and Bioenergy, 28/2:219-228.
Perttu K and Kowalik P (1997). Salix vegetation filters for purification of waters and soils. Biomass and Bioenergy, 12: 9-19.

P6

Nutrient mass balance in the phytotreatment of landfill leachate using oleaginous crops

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The search for sustainable treatment methods represents one of the major challenges in the waste management field. In this context, a research in lab scale at the University of Padua was conducted, to verify the phytoremediation of leachate using Brassica napus (rapeseed), Glycine max (soybean) and Helianthus annuus (sunflower) that are oleaginous plants suitable for biodiesel production. The phytotreatment was conducted in pots, 20 l of volume each; in order to reproduce different growing conditions, the pots were filled with two different types of substrates: sand and soil. Each species was planted twice in the two different substrates and irrigated with increasing leachate concentration: the nitrogen load varied from 200 mgN/m2·day at the beginning of the research period, to 2000 mgN/m2·day at the end of the experiment.

The results obtained confirm the feasibility of using oleaginous species in the phytotreatment of old leachate, particularly in view of the good removal efficiencies of pollutants obtained. The nutrient mass balances underline the role of the different substrates in the plants growing and in N and P removal from leachate (h > 70% for total Nitrogen and h>95% for Phosphorus, and h > 80% for COD). As confirmed by nutrient mass balances, soil filled pots, generally ensured higher pollutants removal than sand filled ones. The growth plants irrigated with the old landfill leachate reacted positively by the characteristics of feed water. To define the value of the project parameters for the phytotreatment plant, it is important also to consider the interaction between soil and wastewater.

Keywords: Leachate, Phytoremediation, biodiesel, energy crops

P7

Leachate Treatment via irrigation on landfill surface planted with poplar and willow trees

Licht Louis

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Landfill leachate is being drip-irrigated year-round on EBuffers planted on landfill surfaces. Leachate that was formerly hauled to waste water treatment plants is taken from existing facilities that normally includes pumping, tank storage and truck-loading facilities. Phyto processes use the water and nutrients in the leachate while sequestering metals and sorbing/mineralizing petrochemical organics.

Techniques for system operation will be reviewed. Soluble iron oxidation has been part of two installations to reduce plugging potential. The leachate is filtered and metered to subsections of the field irrigation system. The leachate is applied through drip irrigation either on the surface or subsurface. They operate year-round to eliminate leachate hauling in winter as well as the growing season.

ETCaps have been installed in Chicago IL., St. Louis MO., and Atlanta GA. In 2011, the combined leachate irrigation removed 6.5 million ton miles of leachate hauling on heavily used highways. The leachate irrigation back on to the landfill cover removed sufficient load from the waste water treatment plants to free capacity for 400 homes.

Keywords: landfill, leachate, irrigation, phyto, ETCap

P8

Species Trials of Short Rotation Woody Crops on a Wastewater Application Fields in North Carolina, USA

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The woody biomass industry in North Carolina is rapidly developing, and there is substantial interest to evaluate woody biomass potential from forest and managed lands in N.C. Municipal wastewater treatment plants are one example of managed lands available for woody biomass production. Currently, there are 86 municipal systems in N.C. accounting for 3541 hectares of wastewater application fields. This project will compare survival and biomass potential of native and non-native tree species at two municipal wastewater treatment systems in Gibson and Jacksonville, North Carolina, USA. Nutrient concentrations in groundwater and soil were monitored concurrent with woody biomass production. The Gibson WWTP is 2.8 hectare facility that currently irrigates sycamore trees and services about 600 citizens. Jacksonville is a much larger municipality that irrigates about 2,000 hectares of primarily loblolly pine forest. Trial studies were started at both sites in 2012 with preliminary trial study initiated at Gibson, NC in 2011. At both sites, a randomized block design was used to evaluate the performance of 6 hybrid poplar clones on wastewater-irrigated land. Early summer drought conditions in 2011 resulted in high mortality for most hybrid poplar species at the Gibson site. Surviving trees did grow well with the onset of rain. Total biomass productivity ranged from 3.7 to 21.8 dm^3 for surviving hybrid poplar clones. The site was replanted in 2012 as part of a larger study with ArborGen. 2012 survival and growth will be discussed at both sites.

Keywords: Wastewater application, Hybrid Poplars, Biomass