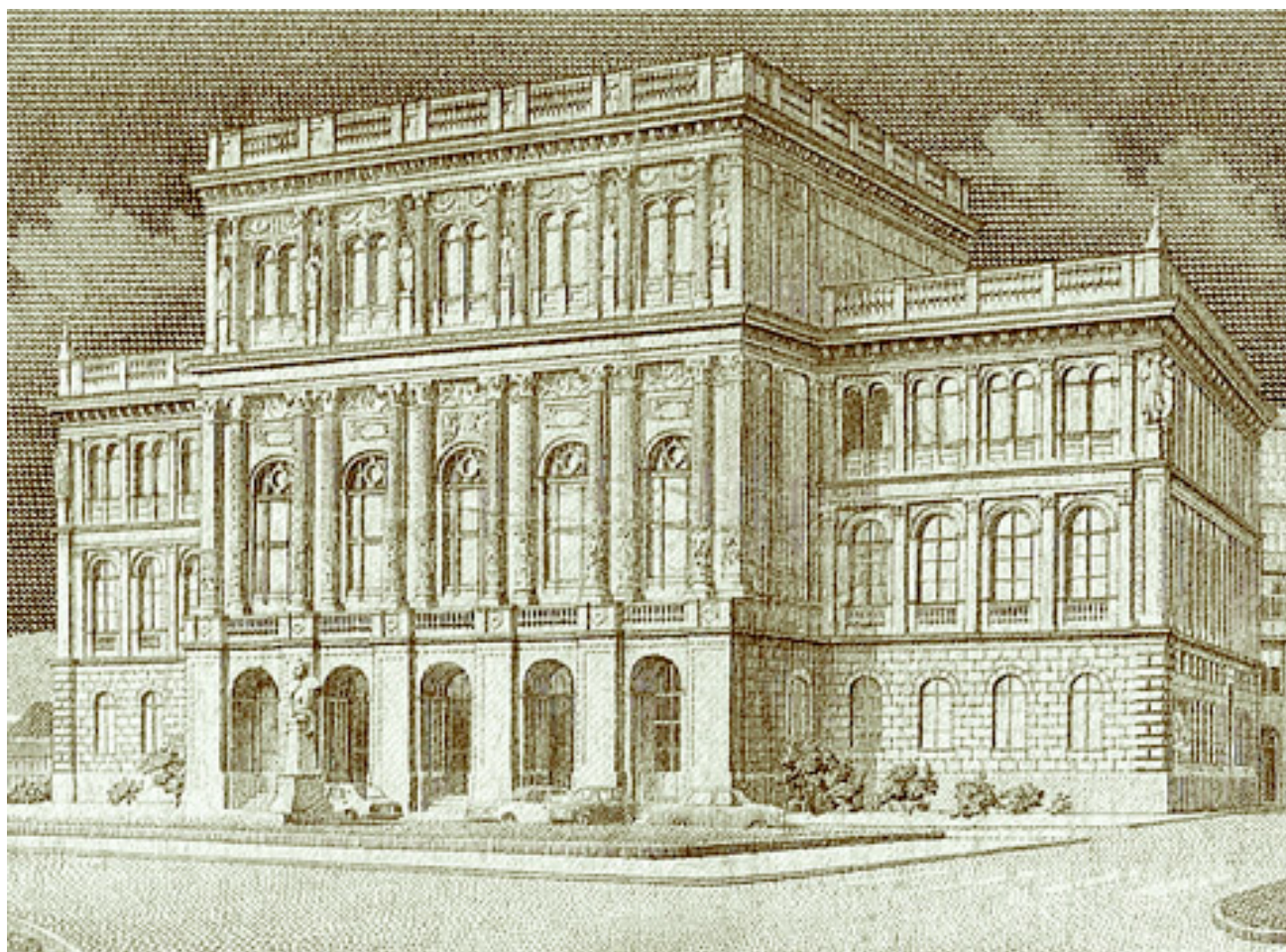


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# International Conference on Agri-Environmental Chemistry and Toxicology

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September 20-22, 2011, Budapest, Hungary

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# Scientific Program

**Tuesday, September 20, 2011**

## Welcoming Address

8:00–9:00 *Registration*

9:00–9:15 **Tamás Németh** (Secretary General of the Hungarian Academy of Sciences [HAS])

Welcome remarks, general comments on behalf of the HAS

## Morning Session

### *Session 1. Overview of Current Agri-Environmental Issues*

Chair: **Tamas Komives** (Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary)

09:15–9:45 **Peter Schröder** (German Research Center for Environmental Health, Germany)

Agriculture: best practices to reduce environmental impact

09:45–10:00 **Márton Jolánkai** (St. István University, Gödöllő, Hungary)

Climatic aspects of agri-environmental pollution

10:00–10:15 **Lukas Y. Wick** (Helmholtz Centre for Environmental Research UFZ, Germany)

Surfing the 'fungal web': fungal mycelia as effective dispersal networks of bacteria and chemicals for biodegradation of soil contaminants

10:15–10:30 **Tibor Bíró** (Károly Róbert College, Gyöngyös, Hungary)

Application of remote sensing in the red sludge environmental disaster in Hungary

10:30–10:45 *Coffee break, poster viewing*

### *Session 2. Pesticides and Fertilizers*

Chair: **Márton Jolánkai** (St. Stephan University, Gödöllő, Hungary)

10:45–11:15 **John E. Casida** (University of California at Berkeley, USA)

The greening of pesticide-environment interactions

11:15–11:30 **Alexandra ter Halle** (Université Blaise Pascal, France)

Photodegradation of pesticides on crops: a dissipation path often overlooked

11:30–11:45 **Gábor Gullner** (Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary)

Sulfur supply of susceptible and resistant tobacco plants influences their defense reactions against Tobacco mosaic virus inoculation

11:45–12:00 **Monilal Chatterjee** (Bidhan Chandra Agriculture University, India)

Spinetogram: A new microbial insecticide for effective management of chili and cabbage pests

12:00–12:15 **Swapan K. Mandal** (Department of Agril. Entomology, Bidhan Chandra Krishi

Viswavidyalaya, India) Bioefficacy of cyazypyr 10% OD, a new anthranilic diamide insecticide, against the insect pests of tomato and its impact on natural enemies and crop health

12:15–14:00 *Lunch, poster viewing*

## Afternoon session

### *Session 3. Phytoremediation*

Chair: **Alexandra ter Halle** (Université Blaise Pascal, France)

14:00–14:30 **Qing X. Li** (University of Hawaii at Manoa, USA)

Biomass production of five biofuel crops and phytotoxicity to seed germination and early growth of nine plants grown in polycyclic aromatic hydrocarbons heavily contaminated soil

14:30–15:00 **Jean-Paul Schwitzguébel** (Ecole Polytechnique Federale de Lausanne, Switzerland)

Accumulation of pollutants and plant wellness: bottleneck or key to successful phytoremediation?

15:00–15:15 **Naser A. Anjum** (University of Aveiro, Portugal)

Assessment of strategies adopted by *Phragmites australis* for its dominance in mercury-contaminated Ria de Aveiro coastal lagoon, Portugal

15:15–15:30 **László Simon** (College of Nyíregyháza, Hungary)

Passive phytoextraction of toxic elements from sewage sludge compost by *Salix viminalis* energy plants

15:30–16:30    *Poster viewing*

## Wednesday, September 21, 2011

### Morning session

#### *Session 4. Genetically Modified Crops*

Chair: **Zoltán Király** (Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary)

- 09:00–09:30    **John L. Huppatz** (Commonwealth Scientific and Industrial Research Organization, Canberra, Australia)  
Genetically modified crops - science, politics and perception
- 09:30–10:00    **Balázs Barna** (Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary)  
Abiotic and biotic stress tolerant plants with elevated antioxidant capacity
- 10:00–10:15    **Gábor Bakonyi** (St. István University, Gödöllő, Hungary)  
Preference of *Folsomia candida* (Collembola) fed on *Bt* and non-*Bt* food sources of various genetical events
- 10:15–10:30    **András Takács-Sánta** (Eötvös Loránd University of Sciences, Budapest, Hungary)  
Does the world need genetically modified plants?
- 10:30–10:45    **András Székács** (Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary)  
Reception of genetically modified plants in the European Union – the scientific background of the Hungarian moratorium on *MON 810*
- 10:45–11:00    *Coffee break, poster viewing*

#### *Session 5. Ragweed Pollen Allergy*

Chair: **Zsuzsa Basky** (PPI, Hungary)

- 11:00–11:30    **Michel Thibaudon** (RNSA, France)  
Different ways to measure health impact due to exposure to ragweed pollens
- 11:30–12:00    **Kristóf Nékám** (National Institute of Rheumatology and Physiotherapy, Hungary)  
Allergenic response to airborne ragweed pollens
- 12:00–12:15    **Tamás Kőmíves** (Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary)  
Precision management of ragweed
- 12:15–12:30    **Zsuzsa Basky** (PPI, Hungary)  
Non-herbicidal control of ragweed
- 12:30–14:00    *Lunch, poster viewing*

### Afternoon session

#### *Session 6. Industrial chemicals, persistent organic pollutants*

Chair: **Qing X. Li** (University of Hawaii at Manoa, USA)

- 14:00–14:30    **More Nandkishor** (B B A Central University, Lucknow, India) Determination of heavy metals in a stretch of river Gomati (UP, INDIA) using aquatic plants as a model system
- 14:30–15:00    **Rahmiana Zein** (Andalas University, Indonesia) The use of snake fruit (*Salacca edulis*) shell as sorption material of cadmium, chromium, zinc and copper ions present in wastewater
- 15:00–15:30    **Emese Bertáné Szabó** (Institute of Food Processing, Quality Assurance and Microbiology, Debrecen, Hungary)  
Recent results to the evaluation of the long term effects of metal pollution in Tisza river
- 15:30–15:45    **Sándor Némethy** (Károly Róbert College, Gyöngyös, Hungary)  
Sources, assessment, remediation and prevention of persistent organic pollutants. The Ecocycle-model as a solution. Swedish case studies, environmental monitoring and legislation

#### *Session 7. Summary session*

Chair: **John E. Casida** (University of California at Berkeley, USA)

- 15:45–16:30    Summary discussions on current agri-environmental issues



## Thursday, September 22, 2011

### Visiting a Polluted Site under Remediation

**András Bittsánszky** (Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary)

08:30                      Departure by bus  
12:00–14:00            *Lunch*  
20:00 (approx.)        Return to Budapest

## Poster Sessions

### Session 1. Overview of Current Agri-Environmental Issues

- 1. Response of glutathione conjugation system to soil borne *Rhizoctonia* infection of okra**  
**A. Bittsánszky, G. Oros** (Plant Protection Institute, Hung. Academy of Sciences, Budapest, Hungary)  
**V. Ravishankar Rai** (Department of Studies in Microbiology, University of Mysore, India)
- 2. Diversity of arbuscular mycorrhizal fungi (AMF) in a Hungarian vineyard (*Vitis vinifera* L.)**  
**G. Csima, K. Posta** (Szent István University, Plant Protection Institute, Gödöllő, Hungary)
- 3. Use of natural zeolite as support of pendimethalin-degrading bacteria in bioremediation assay**  
**P. Pinilla, M.J. Martínez-Íñigo, M.C. Lobo** (IMIDRA, Alcalá de Henares (Madrid), Spain)
- 4. Some effects of etofenprox and lead on the chicken embryo**  
**O. Pálfi, L. Várnagy, K. Balogh, M. Erdélyi, M. Mézes** (Szent István University, Gödöllő, Hungary)
- 5. Reducing environmental risk by breeding mlo resistant spring barley (*Hordeum vulgare* L.) lines**  
**A. Bakó** (Fleischmann Rudolf Research Institute, Kompolt, Hungary)  
**M. Hajós-Novák** (Szent István University, Gödöllő, Hungary)  
**K. Manninger** (Institute for Plant Protection, Budapest, Hungary)
- 6. Comparison of the legal regulations of chemicals and pesticides in the European Union**  
**Gy. Németh, A. Székács** (Plant Protection Institute, Hungary)
- 7. Screening kernels of maize (*Zea mays* L.) inbred lines and hybrids for mycotoxin content after natural and artificial infection**  
**B. Nagypál** (Breeding Station of KWS)

### Session 2. Pesticides and Fertilizers

- 8. Agricultural application of bio-ash – toxicological aspects**  
**Á.D. Anton** (Budapest University of Technology and Economics, Hungary)  
**M. Rékási, N. Uzing, A. Anton** (Research Institute for Soil Science and Agricultural Chemistry, Budapest, Hungary)
- 9. In silico design of pesticide molecules**  
**B. Bordás, I. Bélai, T. Kőmíves** (Plant Protection Institute, Hung. Academy of Sciences, Budapest, Hungary)
- 10. Risk of agricultural use of anaerobic digestate and compost materials**  
**M. Gulyás, G. Füleky** (Szent István University, Department of Soil Science and Agricultural Chemistry, Gödöllő, Hungary)  
**A. Tomocsik, V. Orosz, M. Makádi** (University of Debrecen Centre for Agricultural and Applied Economic Sciences Research Institutes and Study Farm Research Institute of Nyíregyháza, Hungary)
- 11. Effects of root exudates originated from mycorrhizal plants on pathogenic *Fusarium* sp**  
**I. Hernádi, K. Posta** (Plant Protection Institute, Szent István University, 2100, Gödöllő, Hungary)

12. **Bio-insecticidal toxin crystals of *Bacillus thuringiensis* type strains: microscopic observations and protein patterns**  
J. Kutasi (BioFil Ltd., Budapest, Hungary)  
I. Puspán, R. Kovács, É. Kárpáti (Eötvös Loránd University of Sciences, Budapest, Hungary)  
J. Makk, (Saniplant Ltd., Budapest, Hungary)
13. **Screening of pesticide residues in three potentially suitable amendments for agricultural soils**  
R.A. Pérez, C. Sánchez-Brunete, B. Albero, E. Miguel, J.L.Tadeo, (Departamento de Medio Ambiente, INIA, Madrid, Spain)  
J. Alonso, M.C. Lobo (IMIDRA, Alcalá de Henares (Madrid), Spain)
14. **Assessment of the effects of herbicide application on target and non-target organisms**  
J. Žaltauskaitė, V. Brazaitytė (Vytautas Magnus University, Kaunas, Lithuania)

### Session 3. Phytoremediation

15. **Chemical background of the amendment materials used in the remediation process of a heavy metal polluted area**  
K. Antal, L. Blaskó, J. Budai (University of Debrecen, Karcag Research Institute, Karcag, Hungary)
16. **Overproduction of GSH (glutathione) by 35S-gshI poplar (*Populus x canescens*)**  
A. Bittsánszky, G. Gullner, T. Kőmíves (Plant Protection Institute, Hung. Academy of Sciences, Budapest, Hungary)  
G. Gyulai, G. Heltai, (Szent István University, Gödöllő, Hungary)
17. **Lead content of the vegetables and of agricultural soils located in the vicinity of tailing deposits from Baia Mare area**  
G. Oprea, C. Mihali (North University of Baia Mare, Baia Mare, Romania)  
A. Michnea (Environmental Protection Agency Maramureş, Baia Mare, Romania)  
M. Şenilă, C. Roman (INCDO-INOE 2000-Research Institute for Analytical Instrumentation, Cluj-Napoca, Romania)  
A. Pop (University of Agricultural Sciences and Veterinary Medicine Cluj Napoca, Romania)  
I. Gogoaşă (Banat University of Agricultural Sciences and Veterinary Medicine Timisoara, Romania)

### Session 4. Genetically Modified Crops

18. **Inter-laboratory comparison of Cry1Ab toxin quantification in *MON 810* maize by enzyme-immunoassay**  
E. Takács, A. Székács, B. Darvas (Plant Protection Institute, Hungary)  
G. Weiss (Ecostrat GmbH, Germany)  
D. Quist (Genok Centre for Biosafety, Norway)  
A. Hilbeck (Institute of Integrative, Switzerland)

### Session 5. Ragweed Pollen Allergy

19. **Relationship of soil contamination and microorganisms of ragweed (*Ambrosia artemisiifolia* L.) pollen**  
M. Dobróné Tóth (College of Nyíregyháza, Institute of Environmental, Nyíregyháza, Hungary)  
R. Rohr (Claude Bernard Université, Lyon, France)

### Session 6. Industrial Chemicals, Persistent Organic Pollutants

20. **Ameliorative effect of *Moringa oleifera*, activated charcoal and charcoal on lead toxicity in Wistar rats**  
F.E. Ekwain (University of Buea, Cameroon)  
T.M. Ahmed (Laboratory of Forensic Toxicology, Egypt)  
M.D. Matey (Kwame Ninsin University, Ghana)  
I.S. Idoko (Amado Bello University, Nigeria)  
A.T. Peter (University of Ibadan, Nigeria)  
N Ozele (National Veterinary Research Institute, Vom Nigeria)

- 21. Exposure to acetamide-generating compounds is a risk factor of acute hepatic inflammation**  
**M.Y. Liu, D.Z. Hsu** (National Cheng Kung University Medical College, Tainan, Taiwan)  
**S.P. Chien** (Institute of Living Sciences, Tainan University of Technology, Tainan, Taiwan)
- 22. Germination and early development of *Brassica napus* L. and *Brachypodium distachyon* (L.) Beauv. growth with Zn, Cr(VI), As(V) or Cd**  
**B. Montalbán, A. Pérez-Sanz, A.E. Pradas del Real, P. Gonzalo, A. Plaza, M.C. Lobo** (IMIDRA, Alcalá de Henares [Madrid], Spain)

## AGRICULTURE: BEST PRACTICES TO REDUCE ENVIRONMENTAL IMPACT

Peter Schröder

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Department Microbe-Plant Interactions, Neuherberg, Germany

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Agricultural practices have undergone intense changes over the last decades which are evidenced by significant advances in technology and mechanisation, specialisation of agricultural farms, discontinuation of labour-intensive farming branches, increased field size, drainage of wetlands and the removal of hedges and boundary strips. In addition, more and more yield-raising production means (fertiliser, pesticides and fuel) have been used, tillage treatment has intensified and the import of animal fodder and fertilisers has increased. Further, an increase in livestock per hectare of land has resulted in higher amounts of manure per hectare, thus polluting the groundwater. Parallel to this development, there is a decrease in available work force, and farmers and their families are increasingly employed in areas outside of agriculture. The urbanisation of former farming villages, as well as the sealing of surfaces, has increased; flowering meadows are now hardly found, whereas 'standard' green areas are expanding. In addition, agricultural research and advisory services have been for a long time principally oriented at enhancing production and lowering costs and expenditure of human labour. The negative consequences of these developments are known: increased erosion, occurrence of fertilisers and pesticides in groundwater, soil compaction from heavy machinery, depletion of fauna and flora due to large crop fields and destroyed habitats, and an inappropriate mass livestock husbandry. Animal production concentrates in certain regions, with the consequence of an uncoupling of the production of feed and the generation of high amounts of waste and pollution of soils and groundwater. The fact that most of the livestock is heavily dependent on medication causes further threats to end users and the production basis. Agriculture has to nourish the population and provide plant and animal resources for secondary industry processing. Farming assures the economic existence of individual farms and the rural community by utilising land for effective agricultural production. This provokes conflicts with other land use demands such as industrial, transportation and housing development, the production of drinking water or preservation of natural resources for recreation and wildlife refuges.

It is logical that all forms of agriculture cause changes in the balances and fluxes of the pre-existing ecosystem, thereby limiting self-regulatory ecosystem (resiliency) functions. The intensive agriculture of the past, with its strong reduction of landscape structures and vast decoupling of energy and matter cycles, has caused stress and degradation of the production basis which has already led to a loss of these resiliency functions in many regions; massive influence has been also exerted on neighbouring compartments.

Agriculture can best reduce environmental impact, when land is cultivated according to the principles of sustainability and ecological compatibility. When adjoining systems will not be exposed to excessive quantities of carbon, nitrogen compounds and xenobiotics, land use can conserve or re-establish ecosystem control functions. When the diversity of plant, animal and microbial organisms and their communities will increase and rare species will be able to establish sustainable populations, land use can conserve or re-establish habitat functions. And finally, when the economical efficiency of resources required for food production will be

increased, and the quality of products will be maintained at a high level, land use can conserve or re-establish the economical and ecological productivity.

The *FAM Munich Research Network on Agroecosystems* investigated the hypotheses listed above in a cooperation between the Center of Life and Food Sciences, formerly known as the Agricultural Faculty of the Technical University of Munich in Freising-Weihenstephan, and the GSF – National Research Center for Environment and Health in Munich-Neuherberg. Approximately 30 scientific groups collaborated on this project. The German Federal Ministry for Education and Research provided funding for the FAM project, as a centre of ecosystem research. The Bavarian State Ministry for Research and the Arts paid the overhead costs and for the agrarian management of Research Station Scheyern. The GSF – National Research Center for Environment and Health in Munich-Neuherberg and the Technical University of Munich in Freising-Weihenstephan participated with their own financial resources. FAM was part of the international ecological research program ‘Man and the Biosphere’ (MaB) of the UNESCO (United Nations Educational, Scientific and Cultural Organization).



## **CLIMATIC ASPECTS OF AGRI-ENVIRONMENTAL POLLUTION**

*Márton Jolánkai*

Szent István Egyetem, Gödöllő

Agriculture in general and crop production in particular are highly affected by climate change impacts. Results of recent climate change researches have highlighted, that climate change impacts may influence almost all fields of agricultural activities; production efficiency, quantitative and qualitative deterioration of crop yields produced for alimentary purposes, and determine post harvest manifestation of agricultural products inducing hazard in the field of food safety, transport, storage and distribution. Agriculture has a special place in human activities since it is closely linked to nature and wildlife. For many centuries pollution was considered solely as the presence of unfavourable alien matter in the environment, but in reality pollution is far more than that.

A basic characteristic of agri-environmental pollution is that, unlike industrial or urban pollution, it is largely independent of mankind, since many pollution or degradation processes may begin with no direct relationship to human activities. Examples of this are soil erosion, or irreversible damage to natural ecosystems by climatic factors (drought, flood, water logging, salinity induced by irrigation, etc.). Biological pollution, like weed infestation in agricultural fields, epidemics and gradations from abandoned or set-aside areas, which may also be a source of human ailments like pollen allergy, the poisonous effect of mycotoxins on farm animals and humans, new pests and diseases, the emission of greenhouse gases by soils and ruminants, and biological factors which cause quality deterioration represent an increasing pressure on agri-environment.

The problems of any of the above are manifold:

- High variability of yield performance in accordance with weather extremities.
- Economic losses in agricultural and food production.
- Quantitative and qualitative deterioration of food and feed products.
- Lack of sustainable long term vertical and horizontal technology structures.
- Limited chances for forecast and prevention, as well as for technological implementation.
- Environmental hazards affecting agro-ecology as a whole.

The present study is intended to give an overview of research results in relation with climatic aspects of agri-environmental pollution in Hungary.

## **SURFING THE ‘FUNGAL WEB’: FUNGAL MYCELIA AS EFFECTIVE DISPERSAL NETWORKS OF BACTERIA AND CHEMICALS FOR BIODEGRADATION OF SOIL CONTAMINANTS**

*Lukas Y. Wick<sup>1</sup>, Shoko Furuno, Thomas Banitz, Hauke Harms*

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Contaminant biodegradation is often limited by their low availability to degrading microorganisms. This limitation is caused by the coactions of restricted mobility of soil bacteria and retarded transfer of organic contaminants. To improve biodegradation, it is crucial to homogenize bacteria and contaminants at the microscale and thereby decrease their interspaces. In order to cope with heterogeneous environments mycelial fungi have developed a unique network-based growth form. Unlike bacteria the mycelia of fungi spread ubiquitously in the soil and penetrate air-water interfaces and thus cross over air-filled pores between the bacteria and contaminants in the vadose<sup>1</sup>. In air-filled soil, enhanced homogenization of bacteria and contaminants may be achieved by bridging physical air gaps with fungal hyphae thus enabling substrate-directed mobilization of bacteria along chemical gradients. To date, studies have described the influence of the translocation of plant-relevant nutrients within mycorrhizal networks only. In this contribution we present the biodegradation-enhancing influence of fungal dispersal networks on the transport of contaminant-degrading bacteria and the translocation of hydrophobic organic contaminants (HOC), respectively. Our data show that fungal networks (i) act as dispersal networks for both undirected and targeted (chemotactic) mobilization of contaminant degrading bacteria (‘fungal highways’) <sup>2,3</sup>, (ii) increase the mobility of a wide range of aliphatic and aromatic petroleum hydrocarbons due to HOC-translocation in their cytoplasmic streaming (‘fungal pipelines’), and hence (iii) improve the accessibility of bacteria to soil contaminants and, concomitantly, their bioavailability and biodegradation <sup>4,5</sup>. Given their ubiquity and length of up to 100 m g<sup>-1</sup><sub>dry soil</sub> fungal networks hence appear to play a significant role in homogenization and translocation of immobile HOC in soil. Our data further suggest that dispersal contribute to the successful attenuation of soil-HOC, seeing that biodegradation as crucial microbial ecosystem service depends on the contact between indigenous microbes and contaminants.

### **References**

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2. Kohlmeier S, Smits THM, Ford R, Keel C, Harms H, Wick LY. *Environ Sci Technol* 2005, 39, 4640-4646.
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5. Banitz T, Wick LY, Fetzner I, Frank K, Harms H, Johst K. *Environ Poll* 2011, (in press).

## **APPLICATION OF REMOTE SENSING IN THE RED SLUDGE ENVIRONMENTAL DISASTER IN HUNGARY**

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One of the largest industrial disasters in Europe took place in the village of Kolontár (Hungary) on October 4, 2010. Due to a ruptured dam, more than 1 million m<sup>3</sup> of red sludge flooded the nearby towns via the Torna creek. The spilled material contained a highly alkaline solution (>12 pH) had resulted in a complex environmental disaster, requiring a multi-disciplinary approach regarding the assessment and the remediation of the situation.

The research and surveying was coordinated by the Hungarian Academy of Sciences with the primary task on determining the spatial extent of the red-mud sludge spill. This study focuses on the results of a multisensory survey (the horizontal integration of the Lidar technology, the hyperspectral and thermal infrared imaging).

The Károly Róbert College has developed a remote sensing protocol, which greatly assists both the domestic and international disaster management (forecast, damage surveying and control). The aerial multi-sensor monitoring system applied by the College is able to reach any part of Europe within a few hours.

In case of the Hungarian red sludge disaster the primary objective of the hyperspectral remote sensing mission was to estimate the environmental damage, the precise size of the polluted area, the rating of substance concentration in the sludge. The secondary objective was to provide geodetic data necessary for the high-resolution visual information from the data of an additional Lidar survey, and for the coherent modeling of the event. For quick assessment and remediation purposes, it was deemed important to estimate the thickness of the red mud, particularly the areas where it was deposited in a thick layer. The results showed that some of the existing tools can be easily modified and implemented to get the most out of the available advanced remote sensing data.

## THE GREENING OF PESTICIDE-ENVIRONMENT INTERACTIONS

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Pesticides are an essential component of current crop protection systems. They have also been the actual or purported cause of much environmental damage. This review first describes some of the eco-problems and then attempts to evaluate progress in the greening of pesticides relative to purity, persistence, photoproducts, metabolites and ecotoxicology. Interestingly, the first “green” insecticide was an arsenical introduced in 1867 with the name “Paris Green” for its natural color; at that time persistence and the environment were not even considered. Pesticide-induced environmental damage became of special concern only with the introduction of liposoluble and persistent chlorinated hydrocarbon insecticides in the 1940s and 1950s leading to fish and bird kills and bioaccumulation through food chains. DDT was everywhere and its dangers were popularized in the book “Silent Spring”. The insecticide lindane was a minor component in a mixture of persistent but otherwise ineffective hexachlorocyclohexane isomers used in massive amounts. A billion pounds of toxaphene was used before the minor insecticidal component and fish toxicant was identified. About 70 pounds of the chlorinated cyclodiene insecticide endosulfan spilled into the Rhine River with widespread fish kills. Organophosphate and methycarbamate insecticides, although less persistent, had high acute toxicity with evidence in some cases of teratogenesis and cannabinoid and delayed neurotoxic effects in model systems. Toxic photoproducts (e.g. photodieldrin and desulfinylfipronil) and metabolites (e.g. sulfones of anticholinesterase insecticides with thioether substituents) required special studies. In California, derris resin with rotenone was added to a lake as a piscicide and metam sodium spilled into a river with major fish kills. The potential was shown for atrazine herbicide to produce hermaphrodite frogs and of methyl bromide to help deplete the ozone layer. Tetrachlorodibenzodioxin was found to be an exquisitely toxic impurity in 2,4,5-T herbicide and is a continuing toxicological and environmental problem. But it is not all bad. Pesticide-environment interactions are improving as greater emphasis is placed on selectivity and biodegradability. Two major classes of synthetic insecticides (pyrethroids and neonicotinoids) were initially too photolabile but became broadly practical when photostabilized by substituent changes. Pyrethroids with low fish toxicity were discovered by further structural changes. Neonicotinoids overcame most of the prolonged persistence and bird and fish toxicity problems except for honeybee mortality. Eco-friendly juvenoids, ecdysonoids and diamides with high specificity for insects entered major use. Many of the new herbicides inhibited photosynthesis and amino acid biosynthesis and fungicides blocked ergosterol biosynthesis at targets not present in animals. Herbicide safeners were introduced to enhance detoxification in crops but not weeds and inducers of host plant defense systems helped control pathogens. Genetically-modified crops revolutionized pest management in some but not all parts of the world. *Bacillus thuringiensis* (BT) delta-endotoxin expressed in crops protected them against lepidopterous larvae thereby reducing the need for multiple treatments with synthetic organics. Herbicide-detoxifying enzymes were expressed in crops but not weeds so broad spectrum glyphosate and glufosinate could replace a large set of synthetic herbicides. The evolution of pesticides is clearly moving towards lower environmental persistence and

impact. The increasingly restrictive and complex laws relative to pesticide safety, although differing between countries and regions, have led to better environmental studies prior to registration and more careful use in pest control. Thus, there is hope and evidence for the greening of pesticide-environment interactions.

Last updated: 2/16/11



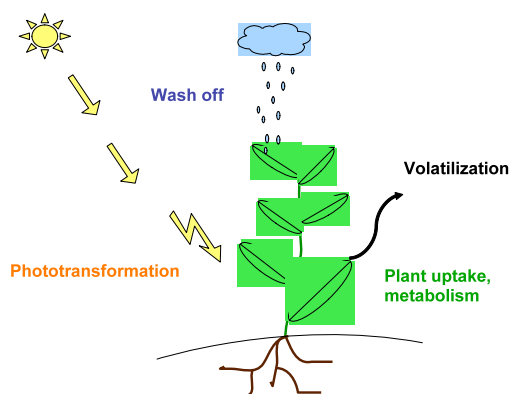
## PHOTODEGRADATION OF PESTICIDES ON CROPS: A DISSIPATION PATH OFTEN OVERLOOKED

*A. ter Halle<sup>1</sup>, S. Monadjemi<sup>1</sup>, G. Vyard<sup>1</sup>, J. Chastain<sup>1</sup>, C. Richard<sup>1</sup>*

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Concerns about the environmental impact of pesticides has prompted research on their transport from treated fields to the air, other land and water bodies, together with ecotoxicological studies about effects on ecosystems. As a consequence, pesticides are already some of the most thoroughly evaluated molecules on the market. The effect of sunlight on pesticide degradation is systematically studied in water and on soil. However, photodissipation on leaves, first recipients of sprayed phytochemicals, is scarcely considered (main paths reported in the following scheme).



We designed a protocol to assess photostability of pesticides on leaves <sup>1</sup>. To mimic the hydrophobic surface of plants a test based on thin wax films was developed. The thin film model has proven valid by field monitoring.

For example, the fungicide chlorothalonil presents an extrapolated half-life of photolysis (DT<sub>50</sub>) in the field on leaves of about 25 days when tested pure. With commercial formulation DT<sub>50</sub> drastically drops between 4 to 7 days. We have demonstrated that upon light irradiation chlorothalonil reacts with oxygen to form the oxidant specie singlet oxygen. Finally chlorothalonil undergoes reductive dechlorination.

In total twelve pesticides from very distinct chemical families have been tested. Most of the molecules present a high photoreactivity on leaves. The results indicate that pesticide photolysis on crops after application is a significant phenomenon and this evaluation should be systematic. Photodegradation on crops could be a limiting factor for pesticide effectiveness; it could be overcome with formulation adjustments.

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## **SULFUR SUPPLY OF SUSCEPTIBLE AND RESISTANT TOBACCO PLANTS INFLUENCES THEIR DEFENSE REACTIONS AGAINST *TOBACCO MOSAIC VIRUS* INOCULATION**

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Atmospheric sulfur depositions were strongly reduced in the 1980s so that soil sulfur deficiency became a nutrient disorder and some fungal infections became increasingly obvious. In agronomic field experiments a new form of disease resistance, the sulfur-induced resistance (SIR) was described. Sulfur is essential for the biosynthesis of cysteine, methionine and the tripeptide glutathione (GSH) in plants. GSH and other sulfur compounds play important roles in plant defense reactions against microbial pathogens. However, very little is known about the importance of soil sulfur supply in the resistance of plants against virus infections.

The molecular mechanisms underlying SIR were investigated in genetically susceptible and resistant tobacco plants in response to *Tobacco mosaic virus* (TMV) inoculation. To evaluate the influence of sulfur nutritional status on virus infection, tobacco plants were treated with nutrient solutions containing either sufficient sulfate (+S) or no sulfate (–S). Sufficient sulfur supply resulted in reduced levels of TMV coat protein mRNA in both susceptible and resistant plants. In resistant tobacco plants grown with sufficient sulfate (+S plants) substantially less necrotic lesions developed during a hypersensitive response (HR) when compared to plants grown without sulfate (–S plants). Enhanced virus resistance correlated with elevated levels of cysteine and glutathione and an early induction of a stress-related and antioxidant genes. In susceptible tobacco leaves the expression of genes encoding PR-1a, adenosine 5-phosphosulfate reductase and gamma-glutamylcysteine synthetase was markedly up-regulated by TMV inoculation<sup>1</sup>. Sufficient sulfur supply resulted in stronger gene expression levels. In resistant leaves the up-regulation of genes encoding a glutathione S-transferase and a salicylic acid-binding catalase was observed. The expression of several pathogenesis-related genes was also markedly induced in TMV-infected resistant plants under +S conditions. These results demonstrated the strong influence of sulfur supply on TMV resistance in tobacco plants.

<sup>1</sup> Höller K, Király L, Künstler A, Müller M, Gullner G, Fattinger M, Zechmann B (2010) Enhanced glutathione metabolism is correlated with sulfur induced resistance in *Tobacco mosaic virus*-infected genetically susceptible *Nicotiana tabacum* plants. *Molecular Plant-Microbe Interactions* 23: 1448-1459.

The financial support of the Hungarian Scientific Research Fund (OTKA K 77641) is gratefully acknowledged.

## **SPINETORAM: A NEW MICROBIAL INSECTICIDE FOR EFFECTIVE MANAGEMENT OF CHILLI AND CABBAGE PESTS**

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A few numbers of microbial insecticides have been developed recently. Such insecticides have novel mode of action, exhibit high levels of toxicity to insect without any cross resistance and resurgence outcome and at the same time spare the non-target organisms. The new insecticide Spinetoram (XDE-175) is a naturally derived insecticide produced by fermentation of the bacterium *Saccharopolyspora spinosa* a rare actinomycete collected from soil that has been synthetically modified. It is a mixture of spinosyn J and L and acts as contact and stomach poison. It offers excellent selectivity to beneficial organisms with low environmental risk to human beings. Spinosyns act through a novel site in the nicotinic receptor that is distinct from neonicotinoids or any other nicotinic actives.<sup>1</sup>

In field evaluation, Spinetoram 12% SC performs exceedingly well against all the major pests of chilli and cabbage. Chili thrips (*Scirtothrips dorsalis*.) tobacco caterpillar (*Spodoptera litura*.), fruit borer (*Helicoverpa armigera*) and Diamond Back Moth (*Plutella xylostella*) were successfully checked at 50 g a.i. /ha with increase of yield as compared with spinosad, indoxacarb, fipronil and emamectin.

In another observation Spinetoram showed fewer hazards to natural enemies' viz. *Menochillus* sp., *Syrphus* sp. and *Chrysoperla* sp. at different doses compared to untreated control. The compatibility of Spinetoram with other insecticides and fungicide was well and no any phytotoxicity was noted at any stages of the growth of crop.

It has novel mode of action, disrupts the functions of GABA receptors of small neurons in the central nervous system and is considered to be 'fast-acting' insecticide provides 7 to 14 days of control and degrades rapidly in the environment. The chemical Spinoteram is highly vulnerable to both lepidopteran and thysanopteran pests, offers excellent selectivity to beneficial organisms with low environmental risk to human beings and have potential use in Integrated Pest Management (IPM) systems.

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**BIOEFFICACY OF CYAZYPYR 10% OD, A NEW ANTHRANILIC DIAMIDE  
INSECTICIDE, AGAINST THE INSECT PESTS OF TOMATO AND ITS IMPACT  
ON NATURAL ENEMIES AND CROP HEALTH**

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Field studies were undertaken for two consecutive seasons to evaluate the bioefficacy of cyazypyr 10% OD, a new molecule belonging to anthranilic diamide group of insecticides, along with imidacloprid 17.8% SL and fipronil 5% SC as standard checks, against the insect pest complex of tomato vis-à-vis its impact on natural enemies and crop health. The toxicity of cyazypyr 10% OD and four other molecules, each at two doses (diluted in 600l water), against the pupal stage of *Trichogramma pretiosum*, an important egg parasitoid of *Helicoverpa armigera*, was evaluated in the laboratory. Cyazypyr 10% OD @ 90 and 105g a.i. / ha was highly effective in controlling the fruit borer, *Helicoverpa armigera*, aphid, *Aphis gossypii* and white fly, *Bemisia tabaci*, and increasing the yield of marketable fruits. This insecticide @ 60 – 105g a.i. / ha effectively controlled the leaf miner, *Liriomyza trifolii*. Imidacloprid 17.8% SL @ 22.5g a.i. / ha and fipronil 5% Sc @ 60g a.i. / ha were also highly effective in controlling the pests. Considering the bioefficacy and yield, cyazypyr 10% OD @ 90g a.i. / ha may be recommended for effective control of pest complex of tomato. Cyazypyr 10% OD @ 45 – 105g a.i. / ha did not significantly reduce the field population of natural enemies and was safe to the crop even @ 360g a.i. / ha. In laboratory test, cyazypyr @ 90 and 60g a.i. / ha found to be safe to *T. pretiosum* pupae, causing only 23.17 and 20.73% mortality, respectively and was on a par with flubendiamide 39.35% SC @ 40g a.i. / ha. Acetamiprid 20% SP @ 25 and 20g a.i. / ha, rynaxypyr 20% SC @ 40 and 30g a.i. / ha and flubendiamide 39.35% SC @ 30g a.i. / ha were relatively less toxic, Whereas chlorpyrifos 20% EC @ 500 and 300g a.i. / ha was extremely toxic to the parasitoid.

# **BIOMASS PRODUCTION OF FIVE BIOFUEL CROPS AND PHYTOTOXICITY TO SEED GERMINATION AND EARLY GROWTH OF NINE PLANTS GROWN IN POLYCYCLIC AROMATIC HYDROCARBONS HEAVILY CONTAMINATED SOIL**

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Phytoremediation is a proven technology for environmental cleanup, particularly in tropical and sub-tropical environments. There are advantages in that multiple growing seasons and increased soil temperature accelerate the clean-up processes. There are millions of acres of chemically contaminated lands in the US on which biofuel crops can be planted for dual purposes of biomass production and land reclamation. Seeds of 13 tropical and temperate plant species were germinated and grown for 10 days in petroleum contaminated soil containing 3148 µg/g of polycyclic aromatic hydrocarbons (PAHs). The results indicate that the presence of PAHs enhanced both emergence and early seedling growth with some of the species tested. Kiawe tree germination rate was 7-fold higher in PAH soils than that in the control media. The potential biofuel grasses sugarcane, banagrass, switch grass, vetiver and miscanthus showed degradation of PAHs in at least one of the amended PAH-contaminated soils in 35 days of growth. Banagrass biomass production in all the treatments was far greater than the other four species. Vetiver degraded all PAHs when planted in the PAH soil amended with 1/3 of the Promix soil (a 2/3 PAH soil volume). Among five biofuel crops tested, banagrass produced a tripled amount or more of biomass than all the other species in the LF-14 soil or its amended soils. The dry weight biomass of banagrass averaged ~3 g/day in all PAH soils and 6 g/day in Promix as harvested at the ground level. Banagrass in 90-cm spacing could produce approximately 30 tons/ha/yr of dry matter in a 70-day crop season. The results warrant further investigation of biofuel crops for phytoremediation and biomass production purposes. The results of this work suggests that future plantings be considered using these and other crops in combination with applicable contaminants to help clean up the contaminated environment and reduce petroleum dependency.



## **ACCUMULATION OF POLLUTANTS AND PLANT WELLNESS: BOTTLENECK OR KEY TO SUCCESSFUL PHYTOREMEDIATION ?**

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Because plants are static and live in a competitive and sometimes hostile environment, they have evolved mechanisms that protect them from abiotic and biotic stress. These mechanisms include detoxification and sequestration of xenobiotic compounds and of heavy metals, exploited in any phytoremediation process. However there must be a limit on the amount of pollutants that can be accumulated and detoxified without disrupting the normal plant metabolism and wellness.

Enzymes involved in xenobiotics detoxification are often linked to the redox chemistry of the cell. The activities of cytochrome P450 monooxygenase, peroxidase and glutathione transferase have implications on the regulation of cellular redox status, closely related to mitochondrial respiration, also involved in maintaining the energy balance. Factors that disturb plant redox or energy status can thus affect both primary and secondary metabolism. For example, overloading a plant with high concentrations of xenobiotics requiring oxidation by P450 may compete with the normal functions of these enzymes. An increase in their activity may impose a major demand on both O<sub>2</sub> and NAD(P)H pools. This could have significant effects on the overall redox and energy balance, thus compromising the primary and secondary metabolic processes in the plant and its wellness and survival.

Molecules involved in the conjugation of xenobiotics, like glutathione, also play a major role in normal plant metabolism. The presence of xenobiotic compounds induces the biosynthesis of glutathione transferases and thus an increased use of glutathione. Plant glutathione level and redox status are thus affected under such conditions. On the other hand, phytochelatins are derived from glutathione and involved in the detoxification of several heavy metals with their –SH groups. In either case, implications on sulphur supply and metabolism are expected.

Links do thus exist between the degree of accumulation and tolerance of metals and xenobiotics, the redox status and the antioxidant capacity of a plant. The lecture will highlight some well known or still unknown features, of utmost importance for a successful implementation of phytoremediation.

# ASSESSMENT OF STRATEGIES ADOPTED BY *PHRAGMITES AUSTRALIS* FOR ITS DOMINANCE IN MERCURY-CONTAMINATED RIA DE AVEIRO COASTAL LAGOON, PORTUGAL

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*Phragmites australis* (Cav.) Trin. ex Steudel. is a dominant salt marsh plant species in Ria de Aveiro coastal lagoon where several tons of mercury (Hg) has been deposited mainly in the sediments due to discharges from a chlor-alkali industry over the last fifty years. The present study assesses the strategies adopted by *P. australis* for its dominance in Hg contaminated area through evaluating the uptake, partitioning and transfer of Hg in its growing season (spring, summer, autumn and winter) at Hg-free (reference) and Hg-contaminated sites. Irrespective of the sampling times/seasons the vegetation of *P. australis* significantly influenced the sediment's pH, redox potential (Eh), organic carbon content (expressed as percent loss on ignition, LOI) and its Hg concentration as well. Compared to the reference site, pH and Eh were found maximum at contaminated sites during autumn; whereas the LOI, dry weight, Hg in root, rhizomes and leaves gradually increased from spring till autumn (the reproductive phase of *P. australis*) and decreased thereafter in winter. Additionally, irrespective of the sampling times/seasons and sites roots accumulated the highest Hg followed by rhizome and leaves. Moreover, the bioaccumulation and translocation factors were observed differentially influenced by seasonal changes. Taking together the results, *P. australis* withstand the highly Hg-contaminated Ria de Aveiro due to its extraordinary ability to pool the maximum Hg in its below ground parts (roots and rhizomes) (high bioaccumulation factor) and to protect its above ground parts (low translocation factor) against Hg-toxicity by the Hg-exclusion and/or -stabilization strategy; and hence this may be the reason of the abundance and/or dominance of *P. australis* in the area with high Hg contamination. Although, *P. australis* may not be suitable for Hg phytoextraction but this plant species has enough potential to be used for Hg stabilization and restoration of sediments/soils rich in Hg as well.

## PASSIVE PHYTOEXTRACTION OF TOXIC ELEMENTS FROM SEWAGE SLUDGE COMPOST BY *SALIX VIMINALIS* ENERGY PLANTS

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Basket willow (*Salix viminalis* L. var. *gigantea*) energy plants were grown in open-field experiment. The brown forest soil (sand texture, pH<sub>KCl</sub> 7.7, humus 1.6%, CEC 11.5 cmol<sub>c</sub>/kg; Cd-0.11, Cu-7.1, Pb-8.1, Zn-25.0 mg/kg in aqua regia extract) was treated with municipal sewage sludge compost (MSSC) (50 and 100 t/ha wet weight which equals to 25.4 t/ha and 50.8 t/ha dry weight in 2008; 25 t/ha wet weight which equals to 12.8 t/ha dry weight in 2009). The MSSC was moderately contaminated with toxic metals (Cd-<0.5, Cu-140, Pb-19, Zn-440 mg/kg in aqua regia extract in 2008, and Cd-<0.5, Cu-299, Pb-69, Zn-865 mg/kg in aqua regia extract in 2009). From 50 or 100 t/ha MSSC the Zn (max. 133 mg/kg d.w.), Cd (max. 1.15 mg/kg d.w.) and Cu (max. 7.73 mg/kg d.w.) accumulation in leaves or twig tips of basket willow was low 15 months after MSSC application. Pb accumulation in the above ground organs of willow was negligible. Accumulation of Zn in treated willow leaves was doubled 4 months after 25 t/ha MSSC application, while in treated twig tips 30% less Zn was found than in control (Figure 1). 20 months after MSSC application in the harvested twigs (shoots without leaves) of 50 or 100 t/ha MSSC-treated cultures the Zn concentrations were only 9.8-14.3% higher than in controls. We can therefore suppose that is not a direct danger of considerable Zn accumulation in the bioash formed after incineration of MSSC-treated willow twigs in power plants.

This work was sponsored by the Scientific Council of the College of Nyíregyháza (2010), by Nitrogénművek Vegyipari zRt. (Pétfürdő) fertilizer producing company (2008, 2010), and by European Union FP-7-regions-2009-01 No. 245449 „Biomass Mobilization” project (2010, 2011).

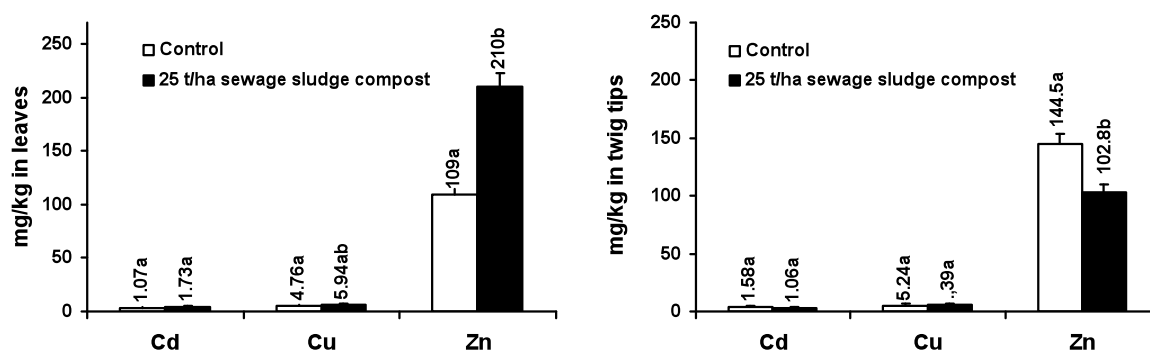


Figure 1. Toxic elements in the leaves or twig tips of basket willow 4 months after 25 t/ha municipal sewage sludge compost application (Nyíregyháza, Hungary) (October 2009). ANOVA Tukey's b-test. Data are means of 4 replications. Values in columns followed by the same letter are not statistically significant at  $P < 0.05$ .

## GENETICALLY MODIFIED CROPS – SCIENCE, POLITICS AND PERCEPTION

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Since the first commercial plantings of genetically modified (GM) crops in 1996, the area planted to these crops has grown exponentially and currently covers nearly 10% of the total arable land on the planet. The major GM crops have been soybean, maize canola and cotton. Gene technology offers a powerful new tool to assist plant breeders to introduce resistance to insects and diseases, as well as traits for higher quality and nutritive value. The technology holds the tantalizing possibility of producing higher yields of more nutritious foods grown in more environmentally sustainable ways. However, the first introduced traits have been agronomic, and, while benefiting the farmer, have had little impact on the consumer. As a result the technology is still controversial with concerns over food safety remaining, despite strict regulatory guidelines in place in many countries. Perception and acceptance remains an issue as consumers seek reassurance about the safety of the food they eat and it is therefore essential that regulation has a sound scientific basis.

GM crops, globally and in Australia, have, up until now, provided direct benefits to the farmer and to the environment. For example, GM cotton in Australia has resulted in a reduction of more than 90% in the use of agrochemicals. The only other GM crop currently grown in Australia is herbicide resistant canola. Although widely grown in North America for many years, its introduction to Australia was marred by controversy and delayed by State government moratoria.

Significant research towards GM crops with health benefits is currently underway in CSIRO laboratories. For example, the addition of genes into canola to produce the health promoting omega-3 fatty acids, DHA and EPA, has resulted in a modified canola plant where nutritional value is enhanced by the change in the composition of the seed oils. Equally important is the use of GM technology in conventional plant breeding. This is well illustrated by a variety of barley known as BarleyMax, which was developed in CSIRO laboratories. Marketed as a breakfast cereal with a low glycemic index and a high fibre content, this barley has been shown in clinical trials to have significant health benefits.

## **ABIOTIC AND BIOTIC STRESS TOLERANT PLANTS WITH ELEVATED ANTIOXIDANT CAPACITY**

*Balázs Barna and Zoltán Király*

Both abiotic environmental stresses (air pollutants, heavy metals, cold, heat, drought etc.) and pathogen infections induce rapid accumulation of harmful reactive oxygen species (ROS), which can be neutralized by enzymatic and non-enzymatic antioxidants. ROS are involved not only in limiting pathogen ingress but also in induction of host cell death and in signal transduction. Consequently, elevation of antioxidant capacity of plants could improve their tolerance to necrotrophic pathogens and abiotic stresses.

Paraquat, as a ROS producing agent was used for *in vitro* selection of different plants tolerant to ROS. Indeed, *in vitro* selected paraquat tolerant (PT) *Nicotiana tabacum* cv. Petit Havana plants showed enhanced resistance to fungal toxin-, heavy metal-, freezing- and heat-stresses, as well as to necrotrophs like *Alternaria alternata*, *Botrytis cinerea*, or virus lesions, as compared to paraquat sensitive (PS) control tobacco plants. Senescence of PT plants was slower and protein and chlorophyll contents, as well as levels of phospho- and galactolipids were higher in PT tobacco, as compared to the PS control plants. PT tobacco leaves had elevated superoxide dismutase (SOD) and catalase (CAT) activities than the PS plants, indicating an increased antioxidant capacity.

Transgenic plants (overproducing cytokinins, ferritin, or SOD and CAT genes) with high antioxidant capacity have elevated stress tolerance. Thus, *in vitro* selection of plants tolerant to reactive oxygen species or incorporation of antioxidant genes seem to be effective tools to get crops with elevated tolerance to abiotic and biotic stresses.



## PREFERENCE OF *FOLSOMIA CANDIDA* (COLLEMBOLA) FED ON *Bt* AND NON-*Bt* FOOD SOURCES OF VARIOUS GENETICAL EVENTS

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Considerable debates are current about the environmental effect of *Bt*-maize *MON 810*. The results referring to collembolans are based on standard laboratory or field tests. So far little attention has been paid to food preference of soil animals which, however, is a key factor in several integrating variables such as – for example – decomposition and nutrient cycling. The main questions of this series of experiments were whether collembolan show feeding preference to one of the maize types involved in the studies. Paired-choice assays were conducted in Petri-dishes. The number of fecal pellets nearby the food plant served as representative of the food consumption. Tests were performed with single animals per arena and with animals in groups. Maize leaves were tested in all cases. *F. candida* fed less *Bt*-toxin producing *MON 810* maize than its isogenic counterpart. No such difference in food choice was observed in the case of the other two collembolan species (*Heteromurus nitidus*, *Sinella coeca*). In opposition to these results *Bt*-maize *DAS-59122-7* was preferred by *F. candida* to its isogenic counterpart. In another series of experiments *F. candida* populations were fed continuously on *Bt*-maize for different periods to a maximum of 31 months. Collembolan food consumption, reproduction and food preference did not correlate with the duration of *Bt*-maize consumption. Nevertheless, differences of these parameters between populations were significant. Populations which preferred isogenic to *Bt*-maize fed more food and reproduced better than those showing no preference. These results may give insight into the reason for the feeding activity of soil animals in *Bt*-toxin containing soil being lower than that in control soil. Our results show that food preference depends on (i) *Bt*-maize variety and (ii) the collembolan species involved in the test. The above detected phenomena may lead to an altered maize residue decomposition or nutrient cycling rate.

## **DOES THE WORLD NEED GENETICALLY MODIFIED PLANTS?**

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Agriculture, at a global level, produces tremendous amounts of food at tremendous environmental costs. Moreover, even though the food produced would be sufficient for the nearly 7 billion human inhabitants of the world, about 1 billion people suffer from hunger due to unequal food distribution. Would genetically modified (GM) plants be able to reduce environmental problems and hunger? The answer of the agrobiotech industry is usually a definite 'yes' to this question, but reality is much more complicated. GM plants produced nowadays do not (really) alleviate environmental problems, and it is totally uncertain, which types of GM plants will be chosen for cultivation in the future. Furthermore, it is very likely that increasing yields by the help of GM plants would not reduce hunger, since it is not a yield-related issue. All in all, although in certain cases GM plants would seem to be a good choice, there are virtually no signs of this technology increasing human well-being, while it is burdened with great risks.

## RECEPTION OF GENETICALLY MODIFIED PLANTS IN THE EUROPEAN UNION – THE SCIENTIFIC BACKGROUND OF THE HUNGARIAN MORATORIUM ON *MON 810*

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The advantages of genetically modified (GM) crops are yet far from the promises envisioned during the biotech boom in the eighties-nineties of the previous century. In fact, the vast majority of the first generation GM crops are biological devices for pesticide applications. In turn, acceptance of GM crops is rather hesitating within the heavily segmented agricultural setup of the European Union (EU). The sole GM crop submitted for cultivation authorization in Hungary is *MON 810*, the insect-resistant GM maize by Monsanto. As a result, the majority of the domestic environmental studies on GM crops have been focused on, and a national moratorium is being in force against cultivation of this genetic event. Fundamental or philosophical aspects behind the moratorium include the role of the crop in supporting monoculture-based production and consequent effects on biodiversity; as well as the lack in compliance with integrated pest management. Issues in environmental chemistry include the actual toxin load *MON 810* maize implements, and observed toxin persistence between vegetation periods. Severe limitations in terms of analytical (bio)chemistry are the lack of proper analytical standards of the target analyte, plant-expressed Cry1Ab toxin, and sources of systematic error in its determination. Problems in ecotoxicology include the effects on protected species through pollen, and resistance development against the toxin. And last, not least, essential problems in registration/legislation of the technology include the facts that *MON 810* is technically based on an unregistered active ingredient; that co-existence of this GM technology with ecological agriculture seems impossible; and that several erroneous legislative concepts are being represented at EU level e.g., substantial equivalence, attempts to avoid tier testing and erroneous statistical approach to the ‘natural background’ of GM crops.

## DIFFERENT WAYS TO MEASURE HEALTH IMPACT DUE TO EXPOSURE TO RAGWEED POLLENS

*Michel Thibaudon<sup>1</sup>, Gilles Oliver<sup>1</sup>*

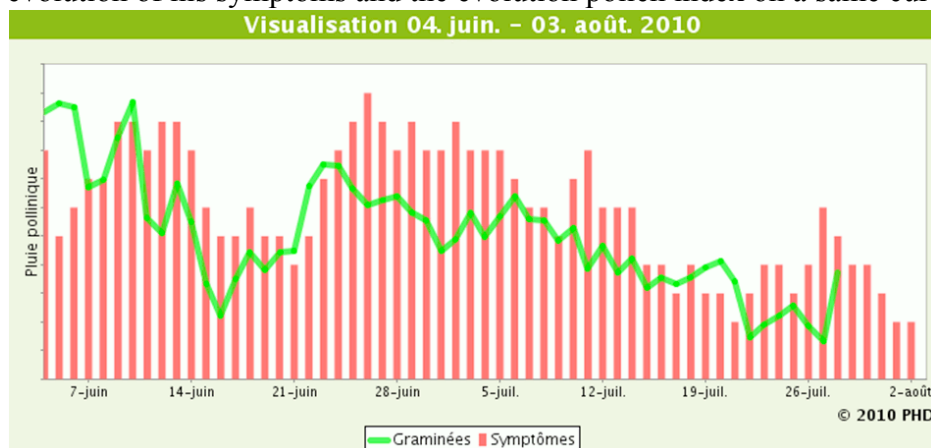
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To measure population exposure to ragweed pollen, the R.N.S.A (National Aerobiological Monitoring Network, a French association) has a pollen trap network located in urban areas which record continuously airborne pollen. The light microscope analysis (with a bi-hourly time step) gives information about the daily concentrations and the circadian rhythm of ragweed grains. It is thus possible to follow the evolution of pollination during each day of each season and to compare seasons and years at each station.

For the health impact measurement, RNSA set up a clinician network representing all the French territory. Physicians pass clinical data on to RNSA via an electronic clinical report which sums up the evolution and the intensity of symptoms noticed on patients (conjunctivitis, rhinitis, cough, asthma, cutaneous signs). By assigning coefficients to each of these symptoms, it is possible to establish a clinical index for the patients of each physician, each area and even for different periods.

On the other hand, since 2010, an electronic pollen diary has been set up by EAN team on the following website <https://www.pollendiary.com/Phd/en/start>. With this pollen diary, it is the patient himself who gives information about his symptoms, the way he feels, etc... He can follow the evolution of his symptoms and the evolution pollen index on a same curve.



The data collected via this system allow determining the reality of the sensitivity and the thresholds concerning for instance the amount of pollens necessary to get first symptoms.

## ALLERGIC RESPONSE TO AIRBORNE RAGWEED POLLEN IN HUNGARY

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**Aim:** To investigate whether there is a link between, or a facilitating effect of Ragweed (*Ambrosia artemisiifolia*, Rw) sensitization and sensitization to various other pollens in regions of high and low Ragweed pollen counts among patients with respiratory allergy (allergic asthma and/or allergic rhinitis) in Hungary.

**Methods:** Blood samples of 50 – 50 adult patients with respiratory allergy for at least two pollen seasons (symptoms at least during the Rw part of the pollen season: August to October) were taken in three cities. High annual total Rw counts were characteristic for two cities (Kecskemet and Nyiregyhaza) in SE-Hungary, and low for one (Eger, N-Hungary). Samples were screened for specific IgE antibodies to 31 allergens, including all typical in- and outdoor allergens for Hungary, and 13 food allergens. For statistical analysis Kruskal – Wallis and Mann-Whitney, and chi square tests were used.

**Results:** While Hungary is the top Rw-polluted country in the world, large differences exist within the country. Eger, considered as low-Rw area was with the lowest ever Rw total pollen count of any season (in 2005 : 390 pollen). Kecskemet and Nyiregyhaza had for more than one year of the last decade total counts close to 20,000, and daily maximum of 1200 and 1100, respectively. While there was no significant difference among the number of Rw sensitized patients in the three cities (Eger-52% , Kecskemet 56% , Nyiregyhaza 54% of all investigated) highly significant differences existed among median Rw-specific IgE values: Eger 5.4, Kecskemet 5.8 , and Nyiregyhaza 24.2 kU/L. (Here, 1/3 of all patients: over 54 kU/L.). Similar sensitization patterns existed for plane tree (2.6 ; 5.0 or 10.9 kU/L) or plantain ( 1.7 ; 3.5 or 9.6 kU/L). No significant differences were observed e.g. for latex (1.2 ; 2.6 ; 3.6) or mustard (1.1 ; 0.6 ; 0.5). Risk of polysensitization ( three or more sensitizations) in Rw allergic patients was : OR 5.8 (95% CI 2.85-11.7).

**Conclusion :** there is a clear link between Rw sensitization and sensitization to other pollen allergens. Whether it is a cause or a consequence remains to be elucidated as we do not have data (serial investigations of sensitization patterns) on the natural course of Ragweed allergy.



## PRECISION MANAGEMENT OF RAGWEED

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The main cause of allergy and pollen asthma in Hungary is pollen from ragweed (*Ambrosia*) a genus in the Asteraceae. Despite continuous efforts by the Hungarian government during the last ten years to eradicate ragweed, levels of its pollen in the air did not diminish. Ragweed infestation is heaviest in sunflower (*Helianthus annuus* L., the third most important crop in Hungary) fields, producing the overwhelming majority of allergenic pollen in the air (in the end of the summer pollen counts reach 1000 grains m<sup>-3</sup>) even in urban areas. In the presentation we show the current situation in Hungary, the most recent measures, and the strategic program based on the remote sensing and precision weed management we developed for controlling ragweed and suppressing its pollen production.

In recent years a Hungarian private farm in Zimany (Southern Hungary) significantly improved its spatial information infrastructure and enabled us to carry out the research and development of site-specific weed management methods. Over the past four years, we developed off-line, map-based methods for a) pre-emergent precision herbicide treatments to control weeds in maize and sunflower (the application algorithm is based on humus and sand content of the soil), and b) a post-emergent precision herbicide treatment in row crops, which uses a two-component herbicide combination with variable composition (the dose and the herbicide ratio is based on the weed composition and density). In on-line precision post-emergent treatments in maize we used Weed Seeker (NTech Industries, USA) sprayers to apply the herbicide under the leaf canopy, while in row-treatments the Weed Seeker sprayers were mounted on a precision cultivator (Garford Farm Machinery, UK). During the experiments special attention was paid for the efficient control of ragweed.

## COMMON RAGWEED (*AMBROSIA ARTEMISIIFOLIA* L.): ABILITY OF NATIVE INSECTS IN HUNGARY TO CONTROL ITS SPREAD

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Common ragweed (*Ambrosia artemisiifolia* L.) was first detected in Hungary in the early 1920s. Under favourable climatic and environmental conditions during the past 60 years it became the most frequent weed species, covering 5.3% of the arable crop area. Now it is present on 5 million of the 6.5 million hectares of arable crop area of Hungary. Surveys were conducted for indigenous insects associated with common ragweed in Hungary. The most frequently occurring insects were Cynipids (25%), followed by plant bugs (Heteroptera) (22%). Proportions of flies (Diptera), Hymenoptera, and spiders (Araneae) were equally *ca.* 9 percent. Beetles (Coleoptera) made up 8 percent of the total catch, followed by thrips (Thysanoptera) 5%, psyllids (Psyllidae) 4%, butterflies (Lepidoptera) and aphids (Aphididae) 2-2 %, and Collembola made up 4% and others 1%. The majority of the collected species were polyphagous. Apart from plant bugs, psyllids and aphids most species were univoltine, thereby their feeding damage was not sufficient to fulfil the requirements for biological control agent candidates. In addition, three aphid species were found feeding on common ragweed. Among these, *Brachycaudus helichrysi* (Kaltenbach) caused chlorotic spots and leaf distortion on infested plants. On rare occasions, *Aphis fabae* (Scopoli) formed dense colonies on ragweed stems and *Myzus persicae* (Schulzer) was found on the lower side of the fully developed leaves of the without causing any visible symptoms.

## **DETERMINATION OF HEAVY METALS IN A STRETCH OF RIVER GOMATI (UP, INDIA) USING AQUATIC PLANTS AS A MODEL SYSTEM**

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Pollution of water bodies has become very acute these days due to rapid industrialization urbanization and other developmental activities. Among the pollutants heavy metals enter water bodies through variety of sources. Interestingly aquatic plants have ability to remove or accumulate heavy metals such as Copper, Nickel, Iron, Arsenic, Cadmium etc from water bodies. Aquatic plant species are important mainly because they form a substantial component of primary production in water bodied. Moreover aquatic plants are also used in water quality studies to monitor heavy metals and other pollutants in aquatic ecosystem.

The main objective of this work was to determine the levels of heavy metals namely Cadmium (Cd) Copper (Cu) Arsenic (As) and Iron (Fe) in five aquatic plants namely *Ranunculus sceleratus*, *Hydrilla verticillata*, *Botumus umbelatus*, *Sagitaria japonica* and *Mimulus glaberatus* at 15 sampled sites of a stretch of Gomati River one of the tributaries of River Ganges at Lucknow, India.

Our studies reveal that heavy metals accumulate in all the aquatic plant studied. However the heavy metals concentration detected in the descending order of  $Fe > Cu > Cd > As$ . One of the sites sampled was highly polluted having higher concentration of metals in the water as well as in the plants species. Moreover the concentration of heavy metals are not alarmingly high except Cadmium (Cd). Species dependent accumulation of metals were observed like *Hydrilla verticillata* tend to accumulate maximum Arsenic (As) and Iron (Fe) *Mimulus glaberatus* tend to accumulate maximum Copper (Cu) *Sagitaria japonica* tend to accumulate maximum Cadmium (Cd) Thus provide a compelling evidence for the prospect of remediation of metal contaminated water bodies.

**The use of Snake fruit (*Salacca edulis*) shell as sorption material of cadmium, chromium, zinc and copper ions present in wastewater**

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**ABSTRACT**

Pollution of the natural water environment by heavy metals has become a serious problem in some industrialized countries. The release of large quantities of heavy metals from industries into the environment has resulted in number of environmental problems. Locally available waste of snake fruit (*Salacca edulis*) shell, was found to be a low cost and promising adsorbent for heavy metals adsorption, i.e., cadmium, chromium, zinc and copper ions present in waste water. It was found that the biosorption capacities were significantly affected by solution of pH, contact time and initial metal ions concentrations.

Under the optimal experimental conditions, snake fruit shell could remove 93.84% of cadmium ion from waste water, followed by zinc, copper and chromium ion with removal efficiency are 82.8%, 74.3% and 63.10%, respectively.

Snake fruit shell showed the highest potential for the removal of heavy metals present in waste water.



Figure. Snake fruit

## RECENT RESULTS TO THE EVALUATION OF THE LONG-TERM EFFECTS OF METAL POLLUTION IN TISZA RIVER

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In January and March 2000 two tailings dam failures occurred in the upper Tisza catchment in Bozanta Mare and Baia Borsa (Romania). These resulted metal pollution in the Lápos-Szamos-Tisza and Visó-Tisza river systems. The mining accidents were followed by a flood event. Many studies reported that elevated Cu, Zn, Pb and Cd concentrations were observable in water, sediment and flooded areas as well<sup>1, 2, 4, 5</sup>. Most of the metals were released in sulfide form, associated with fine-grained sediments, which has a low mobility in river sediment, but the oxidation of these in floodplain soils may cause metal mobilization<sup>1, 5</sup>. The aim of this study was to determine the Lakanen-Erviö (LE) extractable<sup>3</sup> metal contents of floodplains in four sampling sites (Tivadar, Vásárosnamény, Rakamaz, Tiszacsege) along the Tisza, and to compare them to our earlier results<sup>2</sup>. Cu, Zn contents were measured by an Optima 3300 DV ICP-OES (Perkin-Elmer). The measurement of Pb and Cd was conducted by a QZ 939 GF-AAS (Unicam) in 2000 and by an X7 ICP-MS (Thermo Fisher) in 2011.

According to the statistical analyses (Wilcoxon test; SPSS 13.) of the data it can be established that during the 11 year period the easily available Cd, Cu, Zn and Pb contents of Tiszacsege floodplain and Cd, Cu, Zn contents of Vásárosnamény floodplain increased significantly (Table 1.). This may be caused by the periodical flood events and the mobilization of the pollutants. To find the explanation of this increase we need further investigations (solid state partitioning of metals by sequential extraction).

Table 1: LE extractable (easily available) Cu, Zn, Cd and Pb contents of floodplain soils in two sampling sites

Site (year)	Vásárosnamény (2000)				Tiszacsege (2000)				Vásárosnamény (2011)				Tiszacsege (2011)			
Depth (cm)	Cu	Zn	Cd	Pb	Cu	Zn	Cd	Pb	Cu	Zn	Cd	Pb	Cu	Zn	Cd	Pb
0-10	32	60	0.75	42	36	70	1.09	22	56	106	1.79	47	32	105	1.16	19
10-30	20	37	0.58	30	17	38	0.56	14	39	72	1.56	42	15	29	0.46	9
30-50	16	34	0.48	28	19	40	0.74	16	30	68	1.38	44	39	79	1.50	23
50-70	12	31	0.45	30	19	39	0.69	18	23	53	1.13	36	36	89	1.40	31
70-90	14	41	0.59	41	22	44	0.71	22	22	68	1.32	51	32	76	1.34	24
90-110	13	40	0.53	38	17	39	0.54	20	24	80	1.42	61	25	62	1.02	26
110-140	10	34	0.39	26	10	21	0.23	14	20	71	1.31	48	31	80	1.10	34
140-170	6	26	0.22	15	8	18	0.19	13	8	43	0.66	12	27	77	0.89	32
170-200	3	19	0.04	7	11	23	0.29	15	7	35	0.48	10	20	54	0.70	26
200-230	6	26	0.20	15	13	26	0.32	16	7	42	0.53	11	27	73	0.91	42
230-260	7	28	0.25	18	10	28	0.30	21	17	80	1.36	38	23	53	0.79	30
260-300	4	17	0.08	11	10	20	0.22	14	8	30	0.56	14	17	35	0.43	24

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## **SOURCES, ASSESSMENT, REMEDIATION AND PREVENTION OF PERSISTENT ORGANIC POLLUTANTS. THE ECOCYCLE-MODEL AS A SOLUTION. SWEDISH CASE STUDIES, ENVIRONMENTAL MONITORING AND LEGISLATION**

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Persistent organic pollutants are either deliberately produced substances by the industry for a wide range of applications (i.e. pesticides, polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs) or byproducts from various activities, such as industrial or combustion processes (i.e., polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polycyclic aromatic hydrocarbons (PAHs)<sup>1</sup>. Despite a considerable decrease in POP levels during the last decades<sup>3</sup>, unintentional production and accumulation of these substances still occur, mostly due to uncontrolled combustion processes, the lack of life cycle assessment and long-range transboundary air pollution. Remediation methods (mostly phytoremediation) are being developed in order to reduce POPs in the environment, but there are some legal obstacles of certain methods involving the application of transgenic plants<sup>2</sup>, which might offer a fairly fast solution, provided that the precautionary principle is not compromised. The proper life-cycle analysis of industrial products, the combination of bio-energy crop production<sup>5</sup>, constructed wetlands, reed-beds and phytoremediation resulting in complete agro-ecocycles, may constitute the basis of a sustainable, poison-free society. Sweden has the strictest and most comprehensive environmental legislation in Europe (the Swedish Environmental Code and environmental strategies based on the sixteen Environmental Quality Objectives). The Swedish Government commissioned the Swedish Chemicals Inspectorate jointly with the Swedish Environment Protection Agency to prepare a national implementation plan for the Stockholm Convention on Persistent Organic Pollutants<sup>4</sup>. The ecocycle-model should provide the most suitable solution for reducing the level of POPs or eliminating these substances from the environment, but the application of transgenic plants (keeping in mind to prevent harmful environmental effects, such as unwanted gene-flow to natural ecosystems) should be considered as a viable and sustainable possibility.

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## RESPONSE OF GLUTATHION CONJUGATION SYSTEM TO SOIL BORNE RHIZOCTONIA INFECTION OF OKRA

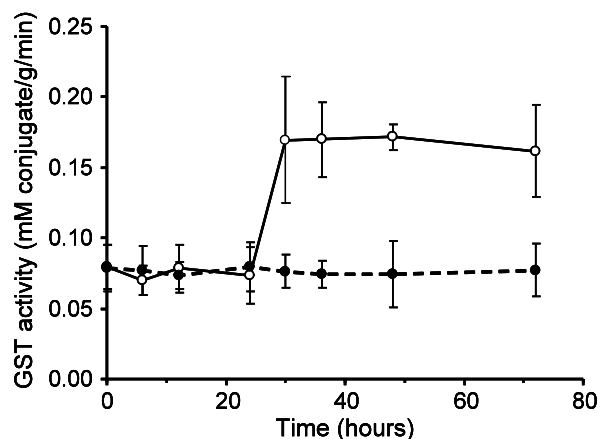
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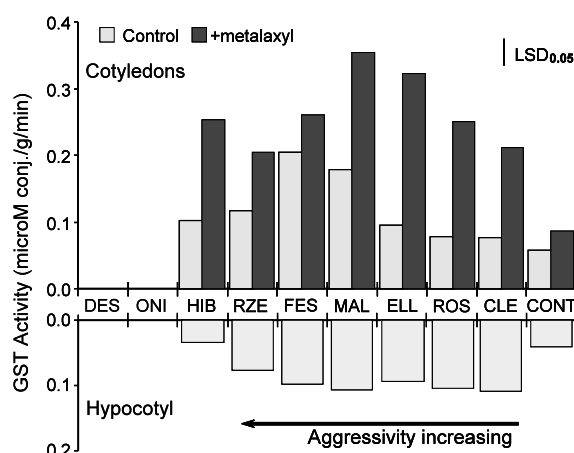
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Okra seedlings tolerated soil-borne *Rhizoctonia* infection in strain dependent manner. There was no connection revealed between pathogenicity of strains and their taxonomic position or origin, however, the okra proved to be susceptible to strains highly pathogenic to other host plants as well. *R. zeae*, a species new for European flora, was as aggressive to okra as the most potent *R. solani* strains. The effect of *Rhizoctonia* infection on mass accumulation of hypocotyls was more prominent than that of cotyledons. The protein content and Glutathione S-transferase (GST) activity increased in parallel with evolution of disease syndrome. Metalaxyl, an acetanilide type systemic antioomycete fungicide induced GST activity in cotyledons with 24 hours a phase, and this induction was more outstanding in symptomless seedlings grown up in *Rhizoctonia* infested soil. It might be concluded, that the stress response of plants in tolerant host/parasite pair takes effect at higher level than in susceptible relationships.



Inducibility of GST activity in cotyledons.

One of the fully opened cotyledons of okra seedling was treated with metalaxyl 5 nM per leaf, while the untreated serve as control.



The effect of soil-borne *Rhizoctonia* infection on the inducibility of GST activity in tissues of okra seedlings.

The seeds germinated in soil infested pre-sowing with *Rhizoctonia* strains of various origin (*R. solani* strains ELL, CLE, DES of potato, FES of grass-land, HIB of *Hibiscus rosa-sinensis* L., MAL of *Malus domestica* Bork., ONI of *Allium cepa* L. and RZE = *R. zeae* of grass-land ).

The joint research work was supported by Indo -Hungarian Education Exchange program and Indo-Hungarian S&T programme.

## DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) IN A HUNGARIAN VINEYARD (*Vitis vinifera* L.)

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Arbuscular mycorrhizal fungi (AMF) have a close association with roots of huge variety of plants, including natural and agricultural plants. AMF have been shown to increase nutrient uptake, enhance resistance to plant pathogens, stabilize soil structures and make nutrient chain between plants. While natural ecosystems have been estimated to contain up to 25-30 AMF species levels, diversity of these organisms often decrease in agricultural system. Understanding the differences in diversity of AMF with respect to different agricultural systems which could help to show at least one reason for inefficient mycorrhizal inoculation in high-input system and give opportunity for better utilization of the symbiosis in agro-systems.

Vineyard is an important economic plant in Hungary with over 550 thousand tons being harvested from 204524 acres in the year 2009 but there is no information is available about the AMF community of Hungarian vineyard. The aim of this work was to study the community structure of AMF both in the rhizosphere and in the roots of grapevine.

Based on the differences in small subunit (SSU)/18S ribosomal genes nested-PCR procedure was used to identify groups of AM fungi that are active in the colonization of grapevine roots<sup>1</sup> and maize trap culture was settled for analysis AMF living in the rhizosphere.

All of sequence types we found here belonging to the *Glomus* clade. Besides *Glomus* A fungi, only the members of the *Glomus* B group occurred, however at significantly lower frequency. The reason for colonization only by one specific group of mycorrhiza could be, that in a seasonally stable plant culture like vineyards the time allows specification in community structures. In the future we could analyse the community structures of arbuscular mycorrhizal fungi in different regions of the country, in case of different kind of grapevines regarding to different soil structures, the age of the vineyard and environmental effects as well as the effects of agricultural practices. It would be a great success to make a diversity map about all these results and compare the quality and quantity of the different vine products from different vine regions of Hungary.

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## USE OF NATURAL ZEOLITE AS SUPPORT OF PENDIMETHALIN-DEGRADING BACTERIA IN BIOREMEDIATION ASSAYS.

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Pendimethalin (N-(1ethylpropil)-2,6-dinitro-3,4-xylidine) is a herbicide often utilized for pre-emergence or early post-emergence control of weeds in various crops. The strong adsorption of pendimethalin to most soils can build up the concentration of the herbicide which needs to be removed<sup>1</sup>.

The aim of this study was to prove the use of zeolite (mordenite) for immobilizing pendimethalin-degrading bacteria in order to render longer viability and higher density of the inoculum in soil bioremediation processes. Natural zeolites are inexpensive material with high porosity and a large surface area, suitable for the immobilization of microorganisms<sup>2</sup>.

*Pseudomonas fluorescens* strain VSE capable of metabolizing pendimethalin was used for bioaugmentation assays and, immobilized cell carriers were obtained and added to 500-g plastic trays of contaminated soil (5 µg pendimethalin g<sup>-1</sup> of soil). In parallel, natural attenuation, biostimulation with a non-ionic surfactant and, combined biostimulation-bioaugmentation with free cells were assayed in soil microcosms. After 35 days of incubation in a climatic chamber at 25 °C and 65% air humidity, half life (DT<sub>50</sub>) of pendimethalin in the different soil microcosms was compared.

In natural attenuation assays pendimethalin showed a DT<sub>50</sub> of 78 days whereas, biostimulation with the non-ionic surfactant decreased DT<sub>50</sub> value to 24 days. As already reported in the case of other bioaugmentation processes<sup>3</sup>, the survival of free bacterial cells for just a short period of time after inoculation could explain the minor decrease of DT<sub>50</sub> to 18 days. The removals of pendimethalin degradation in microcosms receiving repeated free cell inoculum and those receiving bacterial cells immobilized on zeolite were similar, resulting in DT<sub>50</sub> of 13 and 15 days, respectively. However, the dose of cell carriers applied to soil accounted for a lower charge of inoculum (4 10<sup>6</sup> CFU g<sup>-1</sup> of soil). This fact suggested the enhanced survival of the immobilized inoculum and its adaptation to the nutritional conditions of the contaminated soil.

### Acknowledgements

Financial support from Comunidad de Madrid. (EIADES Project, S2009/AMB-1478).

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## SOME EFFECTS OF ETOFENPROX AND LEAD ON THE CHICKEN EMBRYO

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Embryonic viability and some biochemical factors of oxidative stress were examined in hatchling chickens (*Gallus gallus*) following air cell administration of etofenprox (Trebon® 10 F, contains 10% etofenprox) and lead (lead acetate). The concentrations were calculated from the suggested doses of land application (Trebon® 10 F: 1 ml/L) and the current Hungarian legislation of the surface water quality standard<sup>1</sup> (lead: 100 µg/L). As an advanced pirethroide insecticide, etofenprox is used widespread because it's low toxicity in homoeothermic animals. Its land application time consists with the breeding season of several bird species living in agricultural areas, therefore the contact between the pesticide and the eggs of the birds could be a potential danger for the birds reproduction. The effects of lead are well-studied in birds as an oxidative stress promoter. It can evoke the release of iron from ferritin and thus enhance the formation of reactive oxygen species<sup>2</sup>, but there are no data available about the free radical generation effect of etofenprox. In our experiment 0.1 ml test solutions (etofenprox, lead acetate or distilled water) was injected to the air cell on day 12 of incubation. This stage is the time of development of musculoskeletal system which is a frequent target of many xenobiotics<sup>3</sup>. Chicken embryos were euthanatized by cold shock and evaluation of development as well as liver samples were collected on day 19 of incubation. No lethal effect of the studied solutions was found, and also no significant malformations were observed in the embryos from the treatment groups. Both of the studied solutions (etofenprox or lead acetate) had significant effect on the reduced glutathione content of the liver as compared to the control, but only the lead acetate resulted significant changes in the activity of glutathione peroxidase or the level of malondialdehyde. Amount of conjugated dienes of the liver homogenate did not increase as effect of the test solutions. These results suggest that etofenprox such as lead was generated reactive oxygen species formation, but in the case of etofenprox the antioxidant, namely glutathione redox, system defended the biomolecules, in particular lipids, from oxidative damage. However, lead increased the rate of lipid peroxidation, which was supported by the higher malondialdehyde, a meta-stable end-product of that process, content. These results regarding the effects of etofenprox requires further research for better understanding of the mechanism, because it could associate with some ecotoxicological and ecological aspects in free living birds.

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## REDUCING ENVIRONMENTAL RISK BY BREEDING *mlo* RESISTANT SPRING BARLEY (*Hordeum vulgare* L.) LINES

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Barley (*Hordeum vulgare* L.) is an important crop in Hungary, grown on about 300 000 hectares. Within this total area of land, the spring barley growing area comprises about 110 000 ha. Its grain is mainly used for malt, as human food and as seed. Powdery mildew is the most important pathogen of spring barley in Hungary affecting the foliage and the heads. The windborne, biotrophic, fungal pathogen of cultivated barley and wild barley is particularly prevalent under cool conditions when the maximum daily temperature does not exceed 25 °C (Dreiseitl et al., 2006). Severe infection not only reduces yield but also lowers the quality and grade of the grain produced. Systematic foliar fungicides are registered to control powdery mildew. However, breeding resistant cultivars is an economical and environmentally safe way of protecting barley against powdery mildew, and they have benefit under organic growing conditions. Resistance provided by *Mlo* genes is a unique form of monogenic resistance that is not classically race specific and is conferred by a series of recessive alleles (Jørgensen, 1992).

In the Fleischmann Rudolf Research Institute, Kompolt, Hungary the spring barley powdery mildew resistance breeding program started in 2003. Within this program high yielding good quality spring barley lines/varieties were crossed with powdery mildew resistant lines/varieties. Leaves of 1040 F<sub>2</sub> plants were artificially inoculated in the greenhouse with Hungarian *B. graminis* f.sp. *hordei* populations to reveal resistant genotypes. By 2009, 12 *mlo* resistant barley lines with a high yielding ability and good quality were produced. The objective of this study was to screen our 12 *mlo* resistant barley lines for yield and the most important agronomic characteristics in field trials at one location and for two years using the cultivars 'Pasadena' and 'Scarlett' as susceptible controls.

In 2009, the growing season was dry, and the yield ranged between 4130 kg ha<sup>-1</sup> and 5800 kg ha<sup>-1</sup> with an average of 4920 kg ha<sup>-1</sup>. Three resistant barley lines significantly over yielded both susceptible standards. In 2010, the spring was extremely rainy, and there was water stress. The yields were by 72.8% lower than in the previous year. The average yield was only 1340 kg ha<sup>-1</sup>. In this wet year the seed production of six resistant barley lines was significantly higher than that of the susceptible varieties. Spring barley line M-03/96-2 has shown significantly higher yield potential in both seasons when compared to the susceptible cultivars. Its hectoliter weight and thousand grain weight were also favourable.

In the near future we have to determine the virulence of the *B. graminis* f.sp. *hordei* populations collected in Hungary. It will be important to test the resistance of our spring barley lines with isolates having virulence to the major resistance genes currently found in Hungary and Europe, as well.

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## **COMPARISON OF THE LEGAL REGULATIONS OF CHEMICALS AND PESTICIDES IN THE EUROPEAN UNION**

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Pesticide producers are subject to possible obligations under the REACH regulation controlling registration, evaluation and authorization of hazardous chemicals. Although active substances have been exempted from the directive, but only to the extent of those quantities that are used for plant protection purposes. Moreover, only the active ingredients and not other formulation composites received the exempt status. REACH registration requirements show similarities with the pesticide authorization process, moreover, the required ecotoxicological tests are of the same extent as in the case of an active substance manufactured/imported in at least 100 tonnes/year quantity. A further common characteristic is the higher legal level and more centralized feature of the regulations, leading to the establishment of different authorization zones within the Union. Although this is considered a favorable trend in case of chemical substances as the free movement of goods is considered, its benefit regarding pesticides is rather questionable due to the significant differences in ecological characteristics among different biogeographic areas within the European Union.

## SCREENING KERNELS OF MAIZE (*Zea mays* L.) INBRED LINES AND HYBRIDS FOR MYCOTOXIN CONTENT AFTER NATURAL AND ARTIFICIAL INFECTION

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Maize (*Zea mays* L.) is an important crop in Hungary grown on about 1.4 million hectares. During the past few years, due to global warming, the attack of three *Fusarium* species has become more frequent in Hungary. The climate in Murony, in the southern part of Hungary is favourable for *F. verticillioides* infection. This pathogen prefers warm and dry weather at silking and during the grain-filling period. It reduces maize yield and contaminates the grain with micotoxins, such as fumonisins (FUM), thus lowering grain quality. Fumonisins are environmental toxins that can be found both in raw and processed corn products. Their levels in the raw corn are influenced by environmental factors, by storage conditions and by the European corn borer damage. The EU released limits for *Fusarium* mycotoxins. For animal feeding the suggested limiting FUM concentration is between 2.000 and 8.000  $\mu\text{g kg}^{-1}$ , and 1  $\mu\text{g kg}^{-1}$  for food. Losses of yield and grain quality might be reduced by growing resistant hybrids rather than applying chemical control.

The aim of this study was to screen in vivo DH inbred lines and their hybrids for FER and FUM content after inoculation and natural infection with *F. verticillioides*.

A set of 150 maize inbred lines belonging to a mid-late maturity group was produced by in vivo dihaploid technique in the Breeding Station of KWS, Hungary. The resistance of inbred lines and their hybrids against FER was tested after artificial inoculation with one isolate of *Fusarium verticillioides*, FV234/1 as well as after natural infection at one location and during a two-year period. Lines and hybrids were tested in a randomized complete block design with two replications. Each single-row plot consisted of 20 plants. The FUM producer FV234/1 isolate was provided by Prof. M. Lemmens (IFA, Tulln, Austria) and the inoculum was produced by the group of Prof. T. Miedaner (University of Hohenheim, Germany). Artificial inoculation, ear rot rating and toxin analyses were performed according to Nagypál (2010). Data analyses were done by PLABSTAT (2005).

Natural infection was low in both 2008 and 2009. In 2008, the mean mycotoxin concentration of hybrids was 6.00 ppm. Three hybrids were found having FUM concentrations in grain beyond the legal EU limit and a low severity of FER. In 2009, at more lines after natural infection the toxin content of the seeds exceeded the EU limit. In hybrids after natural infection only one possessed high toxin content. In 2009, after inoculation the average FUM content of the lines was 36.2 and 21.6 ppm of the hybrids, and FUM concentrations of 19 hybrids were below the EU limit. After inoculation phenotypic correlation coefficients for FUM concentration and Fusarium ear rot both in lines and hybrids were high and significant in both years ( $r=0.54^{**}$  and  $0.89^{***}$  in 2008;  $0.91^{***}$  and  $0.87^{***}$  in 2009). After natural infection the correlation coefficient was significant only at lines ( $r=0.77^{***}$ ) in 2009. By breeding hybrids with low FUM content in the seeds we can reduce environmental risk factors.

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## AGRICULTURAL APPLICATION OF BIO-ASH - TOXICOLOGICAL ASPECTS

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The biomass as a renewable energy source has become more important in recent years. But the combustion of biomass infers the problem of the increasing volume of bio ash. In a newly developed processing method the ash can be used as a stock for K fertilizer. Depending on the K content of the ash potassium sulphate and potassium nitrate can be produced that can be used alone or in mixture with other fertilizers for plant nutrition in agriculture. According to the planned method the ash residue without high alkaline K compounds can be used as a soil amending material. The effect of the ash residue on soil properties was investigated in a pot experiment. Two soils were used in the experiment: an acidic sandy and a neutral loamy soil. The ash residue was mixed in 0, 1, 3, 5 m/m% to the soils in three replications. Since the ash residue contained potentially hazardous elements, toxicity measurements were performed. The toxicity of the ash residue was investigated in a *Sinapis alba* germination test, *Vibrio fischeri* bioluminescence inhibition assay and by the determination of heterotroph total count.

On the loamy soil 1 % ash residue had a positive effect on the root growth of the test plant but the 5 % treatment decreased the root length by 40 %. On acidic sandy soil the decrement was 70% in this treatment. The inhibition on shoot growth provides more objective results on toxicity. 1 % ash residue had positive effect on shoot elongation on both soil. On higher loads the ash residue interfered the shoot growth. On sandy soil the ash residue inhibited the total plant growth while on loamy soil the in 1 and 3 % ash treatment had positive effect on the total plant length. But in case of 5 % ash residue treatment the effect was negative.

The ash residue did not have any unambiguous negative effect on the *Vibrio fischeri* test organism. However a weak (20 %) inhibition could be seen on the sandy soil in the 3 and 5 % ash residue treatments.

The results of heterotroph total count showed that after ash residue addition the number of cells increased significantly on each soil as a result of the high nutrient content of the ash residue. The ash residue in itself was so toxic that only a negligible number of microorganisms live in it.

Based on these results up to 1 % load the as residue did not have toxic effects on the investigated soils thus it can be used as a soil amending material.

### Acknowledgement

This work was funded by NKTH (BIO\_HAM2, TECH\_08-A4/2-2008-0175).

**IN SILICO DESIGN OF PESTICIDE MOLECULES.**Bordás, B.; Bélai, I.; Kőmíves, T.

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At the Organic Chemistry Research Unit quantitative structure-activity relationships (QSAR) studies and computer-aided molecular modeling experiments are carried out in order to implement an *in silico* design protocol to generate pesticide lead compounds.

**Compound library – Docking - Receptor**

Compound libraries are bought, downloaded, designed, built and/or compiled in silico. Target receptors are downloaded from various databases on the internet. *In silico* screening of virtual compounds is performed by employing various docking programs for the estimation of the binding energy between receptor and ligand. Studies along this line included structure-activity correlations derived for a novel type of benzoylbiuret insect chitin synthesis inhibitors<sup>1</sup>; comparative three-dimensional quantitative structure-activity relationship study of safeners and herbicides<sup>2</sup>; review of the CoMFA and CoMSIA methodologies employed for ligand-based computer-aided pesticide design<sup>3</sup>; interpretation of scoring functions using 3D molecular fields exemplified by mapping the diacyl-hydrazine-binding pocket of an insect ecdysone receptor<sup>4</sup>; QSAR study for the identification of theoretical molecular descriptors relevant to the uptake of persistent organic pollutants (POPs) from soil by zucchini<sup>5</sup>.

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## RISK OF AGRICULTURAL USE OF ANAEROBIC DIGESTATE AND COMPOST MATERIALS

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The quality required of a compost depends on its final use. All the requirements must be focussed on obtaining a product with an acceptable aspect and smell, which is hygienic and free from (or with only traces of) impurities and contaminants. The concept of compost quality especially relevant when the compost is used directly, as a substrate for seedling production or in pots; these applications need high-quality compost. The quality of compost based on concentrations of heavy metals and organic pollutants and on the absence of pathogens (human and animal indicator species).

The results of a field experiment using sewage sludge compost will be demonstrated on a poster. The aim of the experiment was to prove the absence of the risk using this material in cereal production. The results of the last years suggest that there was no toxic element accumulation in the grain yield of triticale plants with respect to control treatment.

Anaerobic treatment is mainly performed as mesophilic treatment, at temperatures between 30 °C and 40 °C, but some systems are designed for thermophilic treatment at 45-55 °C. Monitoring of pathogen reduction in different stages of treatment of biodegradable waste including wastewater products and manure shows that conventional treatment by mesophilic digestion gives a limited reduction in the content of pathogens. Using thermophilic digestion of treated sewage sludge improves the final hygiene quality. The effects of anaerobic treatment on pharmaceutical residues in wastewater are similar to those pathogens.

In a biotest with ryegrass plants the potential toxic effect of anaerobic digestate was measured. The rapid biotest produced toxic reduction of plant growth and root development at high rate application of digestate during the 2 weeks period. The reason of the reduction of root system could be explained with the high ammonium concentration of digestate.



## EFFECTS OF ROOT EXUDATES ORIGINATED FROM MYCORRHIZAL PLANTS ON PATHOGENIC *FUSARIUM* SP.

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It is well known that arbuscular mycorrhizal (AM) fungi could enhance the uptake of phosphorus, but they also contribute to the absorption of other immobile ions, such as zinc and copper. Furthermore, they can protect the plants against pathogenic microorganisms<sup>1</sup>, improve soil structure and confer heavy metal resistance to plants.

Alleviation of damage caused by soil-borne pathogens has been widely reported in mycorrhizal plants, however the mechanisms taking part in that are mostly unknown. The experimental results presented here represent one step towards such an understanding. Our aim was to study the influence of root exudates originated from mycorrhizal(AM+) and non-mycorrhizal(AM-) plants on the growth and virulence of *Fusarium* sp.

Pepper plants (AM+, AM-) were growing for one month in a sterile sand culture at controlled condition than root exudate of both treatments were collected using water system<sup>2</sup>. Pathogenic *Fusarium* sp. was maintained on tomato agar containing sterilized mycorrhizal or non-mycorrhizal root exudates and after one week of growth their infection-ability was tested by pepper crops.

Microscopic examination of sporulation of *Fusarium* sp. showed clear differences between the treatments: root exudates of mycorrhizal plants decreased the quantity and the quality of conidium. Inoculation of pepper crops with *Fusarium* sp. growing on mycorrhizal root exudate caused less damaged than control(AM-) suggesting the indirect role of mycorrhizal fungi on plant protection system.

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**BIO-INSECTICIDAL TOXIN CRYSTALS OF  
BACILLUS THURINGIENSIS TYPE STRAINS:  
MICROSCOPIC OBSERVATIONS AND PROTEIN PATTERNS**

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*Bacillus thuringiensis* is Gram-positive spore-forming soil bacterium with entomopathogenic properties. It produces insecticidal proteins during the sporulation phase as parasporal crystals. These crystals are predominantly comprised of one or more proteins, crystal (Cry) and cytolytic (Cyt) toxins, also called delta-endotoxins.

These toxins are highly specific to their target insect, are innocuous to humans, vertebrates and plants, and are completely biodegradable. Therefore, *B. thuringiensis* is a viable alternative for the control of insect pests in agriculture and of important human disease vectors.

Cry proteins are specifically toxic to insect orders *Lepidoptera*, *Coleoptera*, *Hymenoptera* and *Diptera*. Cyt toxins are mostly active against *Diptera*. Additionally, *B. thuringiensis* produces a number of extracellular compounds, such as phospholipases, proteases and chitinases, and other toxins, such as beta-exotoxin and vegetative insecticidal proteins (Vip), that may contribute to its virulence.

In this study we compared several well-known *B. thuringiensis* type strains, regarding their sporulated cultures, parasporal crystals and protein patterns of the spore-crystal preparations.

Type strains were *B. thuringiensis* ssp. *kurstaki* NCAIM B.01262, *B. thuringiensis* ssp. *israelensis* B.01289, *B. thuringiensis* ssp. *kumamotoensis* DSMZ 6070 and *B. thuringiensis* NCAIM B.1292. Sporulated cultures were observed by phase contrast and scanning electron microscopy. Adequate methods were developed for the separation of spore-crystal fractions and differential solubilization of crystal proteins. Polypeptide components of spore-crystal suspensions were revealed by SDS-PAGE.

Our results showed that in sporulated cultures cells with endospores, free spores, crystals and vegetative cells in various amounts could be observed under phase contrast microscope. Scanning electron microscopy observations confirmed the presence of distinctively shaped parasporal crystals in some sporulated cultures.

SDS-PAGE patterns of the spore-crystal preparations and the corresponding vegetative cell suspensions were essentially different, regarding the number and size of detected polypeptide bands. In the spore-crystal patterns polypeptides resembling Cry and Cyt proteins in molecular weight were detected. However, correlation between the presence of crystals and detection of Cry/Cyt protein resembling polypeptides varied in the type strains.

On the basis of microscopic observations and SDS-PAGE patterns potential target insects of the *B. thuringiensis* type strains were predicted.

This work was supported by EU co-financed KMOP-1.1.1-08/1-2008-0042 grant of National Development Agency, Hungary

## SCREENING OF PESTICIDE RESIDUES IN THREE POTENTIALLY SUITABLE AMENDMENTS FOR AGRICULTURAL SOILS

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Pesticides play an important role in agriculture but many of them have been classified as organic pollutants and have been banned by regulatory organizations. Due to the wide use of diverse classes of pesticides, it is important to use multiresidue methods that allow the determination of these compounds at very low concentration in complex environmental matrices. In recent years, there has been increasing attention toward the use of wastes from different origins as amendment in agriculture because it is a way of recycling these residues and improving soil characteristics simultaneously. Nevertheless, the potential amendment could contain a wide range of contaminants, such as metals, pathogens and organic pollutants. Nowadays, the EU regulates the use of sewage sludge in agriculture to protect the environment<sup>1</sup> setting the limits of concentration of heavy metals in sludge and in soils, but organic contaminants are not yet considered. Although the presence of pesticides and other organic contaminants have not been regulated in products for soil amendment, it is important to know their level and behavior when agricultural soil is amended with them.

The main objective of this study was to carry out the simultaneous determination of 45 pesticides of various classes in three different potential amendments for agricultural soils: recycled de-inking paper sludge, composted sewage sludge and thermally dried sewage sludge. Previously to the evaluation of pesticides in these samples, their physico-chemical characterizations and the analysis of heavy metals were done to establish that their limits comply with the EU legislation. The presence of pesticides was evaluated using a multiresidue extractive method based in a methodology previously developed in our group<sup>2</sup>. Extractions were carried out by matrix soil-phase dispersion and analyses were performed by gas chromatography with electron impact mass spectrometric detection in selected ion monitoring mode (GC-MS-SIM) using external standards. The results showed the presence of a pesticide in thermally dried sewage sludge and de-inking paper sludge. Triadimefon was found in the first one (1058 ng/g) and dichlofluanid in the latter (474 ng/g). These results demonstrate the potential occurrence of pesticides in products which could be use as soil amendment.

### Acknowledgements

Financial support from Comunidad de Madrid. (EIADES Project, S2009/AMB-1478).

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## ASSESSMENT OF THE EFFECTS OF HERBICIDE APPLICATION ON TARGET AND NON-TARGET ORGANISMS

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Pesticides are ubiquitous in the environment and have significant environmental and public health impact. This study examined the target and non-target effects of herbicide Sekator OD 375 (amidosulfuron (9%), iodosulfuron methyl, sodium salt (2.2%), and mephenpyr diethyl (22%)) to target weed species *Chenopodium album* L., to two non-target plant species – barley *Hordeum vulgare* L.; duckweed *Lemna minor* L. and one aquatic microinvertebrates *Daphnia magna*. The terrestrial plants were sprayed directly with five various dilutions of herbicide sprays (solutions equivalent to 0.1-0.001 of recommended field application rate), the aquatic plants and microinvertebrates were affected by the same concentrations of herbicide in their growth medium. The measured endpoints in plants bioassays were: survival, height of the shoots, the dry biomass, content of photosynthetic pigments and malondialdehydes (MDA), the growth rate of *L. minor*. The mortality of *D.magna* was observed.

All plant species had a strong phytotoxic response to herbicide application, though the response of target species was more pronounced. The growth of shoots of *Chenopodium album* and *Hordeum vulgare* was inhibited by 37.1% and by 18.4% respectively. The dry biomass of barley was by 33 %, of *Chenopodium album* – by 71.5% lower than in control. Herbicide application caused significant reduction in photosynthetic pigment concentrations and the response of *Ch. album* was more pronounced. The highest concentration of herbicide resulted in decrease of chl a content by 24 and 45% in *H. vulgare* and *Ch. album* respectively; the content of chl b decreased by 11 and 70 % respectively. The content of carotenoids was by 24-30 % lower than in control plants and there was no species specific difference. The aquatic plants *Lemna minor* was more susceptible to herbicide and showed extremely strong response: in the highest concentrations of herbicide the breakdown of all colonies was observed. The biomass of *Lemna minor* in lowest concentrations of herbicide was approximately by 31 % lower than in control. Application of herbicide provoked an oxidative stress; the concentrations of MDA in *Ch. album* and *H. vulgare* were, respectively, by 8.4, and 1.2-fold higher than in control.

The microinvertebrates *D. magna* were very sensitive and application of herbicide caused 50-100 % mortality of *D.magna*. The results of logistic regression show that the risk of death of tests organisms increases with the concentration of herbicide ( $\chi^2=35.16$ ,  $p<0.05$ ).

The study revealed that application of herbicides causes the adverse effects not only in target vegetation, but in non-target organisms as well. The results suggest that herbicides runoff in drainage and rivers could have a significant adverse effect on the growth of aquatic plants and invertebrates.

## CHEMICAL BACKGROUND OF THE AMENDMENT MATERIALS USED IN THE REMEDIATION PROCESS OF A HEAVY METAL POLLUTED AREA

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The investigated area is a slag site near Gyöngyösoroszi that is a polluted mining region in Hungary. The sludge accumulated in this area has extremely low pH and is contaminated with a high quantity of toxic heavy metals (eg. Zn, Cu, Mn, Fe). The aim of our work was to study the efficiency of compost and lime amendments on this sludge by pot experiments and analytical measurements. Compost can increase the soil pH and contains a high proportion of humic-like materials that decrease the bioavailability of heavy metals in soil. Lime is also a wide-spread amendment material which is used as chemical immobilizer. We studied the effect of different compost and lime doses (0, 0.5, 1, 2, 4, 6, 10 m/m %) on the growth and heavy metal uptake of Italian ryegrass (*Lolium multiflorum L.*) and we carried out *pH* measurements in the amended sludge.

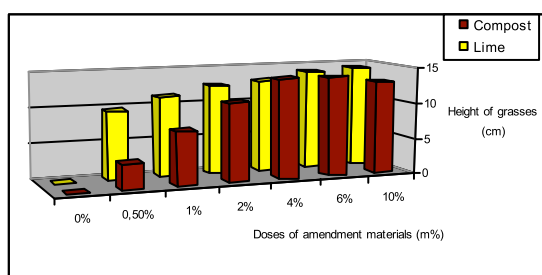


Fig. 1. The efficiency of amendment materials on the height of plant

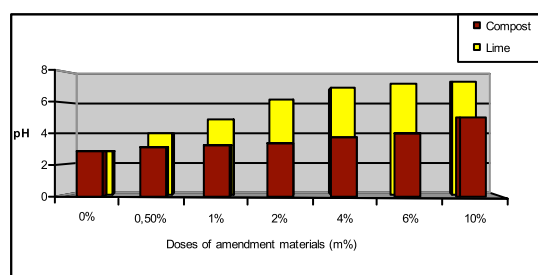


Fig. 2. The efficiency of amendment materials on the pH

In the range of 0-4 m/m% the lime had more effect on the growth of the plants than the compost while above 4 m/m% the efficiency of the two materials was nearly equal (Fig. 1.). However the pH values showed that lime is more active in decreasing the acidic character of the soil (Fig. 2.), the grass did not grow higher applying lime amendment than using compost.

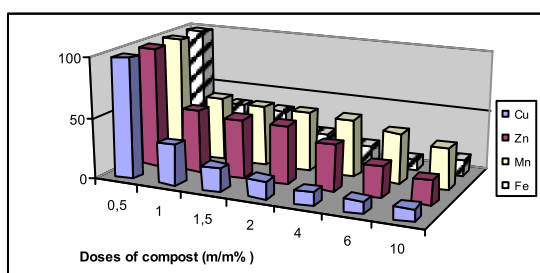


Fig. 3. Relative decrease of heavy metal uptake of plants at different compost doses

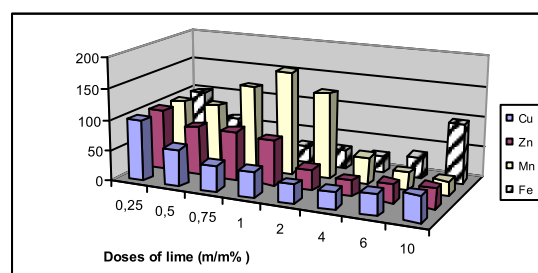


Fig. 4. Relative decrease of heavy metal uptake of plants at different lime doses

Figure 3-4. show that the compost clearly decreased the heavy metal uptake of grass while the lime did not. It can be explained with the compost organic matter content which is a good complex-forming agent and a good source of nutrients for plants, while the lime does not have such beneficial features.

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## OVERPRODUCTION OF GSH (GLUTATHIONE) BY 35S-*gshI* POPLAR (*Populus x canescens*)

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Transgenic clones of 35S-*gshI* poplar (*Populus x canescens*) was applied for overproduction of GSH (glutathione). Gene expression of 35S-*gshI* transgene, cloned from *E. coli* (X03954) (Arisi et al., 1997 *Plant Physiology* 117: 565-574; Noctor et al., 1998 *Plant Physiology* 118: 471-482) were analyzed in RT-qPCR (reverse transcriptase quantitative PCR) by determining the level of *gshI*-mRNA ( $\gamma$ -glutamylcysteine synthetase-mRNA) and by measuring (HPLC) the production of GSH and precursor cysteine in the fresh leaves. For internal control of RT-qPCR, the constitutively expressed housekeeping poplar genes  $\alpha$ -tubulin, and the endogenous poplar gene *gshI* were used. The  $2^{-\Delta\Delta Ct}$  method (Livak and Schmittgen 2001) was applied for data analysis. The transgene expression level of 35S-*gshI* was 13.5 times higher in the TR-*lgI6* clone than in TR-*ggs11* clone. The endogenous poplar gene *gshI* showed elevated co-expression with transgene 35S-*gshI* with about 1.3 - (TR-*lgI6*) and 3.2 (TR-*ggs11*) times increment. Due to the double gene expressions of endogenous *gshI* and transgene 35S-*gshI* in transgenic lines the GSH production of TR clones showed 200% (TR-*ggs11*; 331 nmol x g<sup>-1</sup> FW) and 300% increment (TR-*lgI6*; 504 nmol x g<sup>-1</sup> FW) compared to *gshI* production of WT (174 nmol x g<sup>-1</sup> FW). Under paraquat stress TR(*ggs11*) clone showed a further extreme GSH production (1932 mmol) (Fig. 1) These results underline the importance of transgenic poplars for molecular pharming of GSH and the application for phytoremediation.

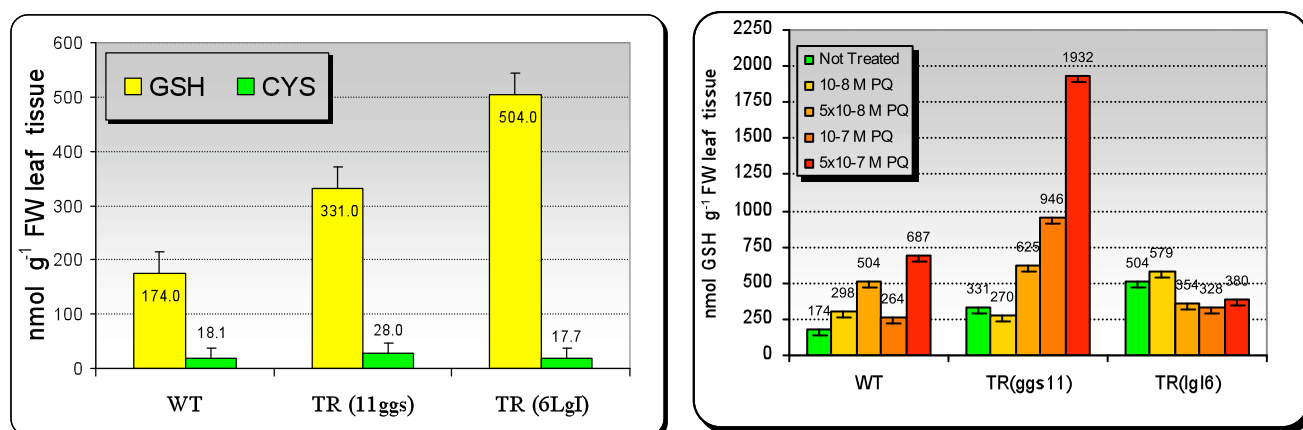


Figure 1. Overproduction of GSH and precursor amino acid cysteine (CYS) without (left) and under paraquat (PQ) stress (right) by the WT poplar (*Populus x canescens*) and two lines of 35S-*gshI* transgenic (TR) lines TR(*ggs11*) and TR(*lgI6*).

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## LEAD CONTENT OF THE VEGETABLES AND OF AGRICULTURES SOILS LOCATED IN THE VICINITY OF TAILING DEPOSITS FROM BAIJA MARE AREA

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Baia Mare is a mining and nonferrous metallurgical center located in the north west of Romania. Large quantities of wastes resulted from the ore processing activities and were stored in the tailing deposits located in the western area of the city. Near the village Bozanta there are three such as tailing deposits characterized by a high content of lead. Lead is well known as a high toxic element. The lead contamination of the cultivated soils is of major concern because it could be uptaken by plants entering in the food chain and affecting human health.

The objective of this study was to investigate the levels of Pb contaminations in the soils and vegetables from Bozanta area. Soil and plant samples had been collected during August 2010 from the private gardens of the residents of the village. Soils and plant samples had been collected also from a reference area, Berbesti, far from the pollution sources. The determination of Pb in soils and plant samples was done using the inductively coupled plasma atomic emission spectrometer, ICP-AES. The results of the study are presented in Table 1 and 2. In Bozanta area Pb content of the soil samples exceeded in all cases the alert threshold (50 mg/kg) and in 90 % of the cases the intervention threshold (100 mg/kg) for sensitive soils while Pb in plants exceeded the maximum level of 0.30 according to European Commission Regulation No 1881/2006 in 74%.

Table 1. Lead in soil in Bozanta and reference area

Sampling area	Average (N=10)	Median	Minimum	Maxim	Standard deviation
Bozanta	198	178	72	376	90.7
Reference area	39.4	25	12	91	30

Table 2. Lead in plants in Bozanta and reference area

Sampling area	Average (N=10)	Mediane	Minimum	Maxim	Standard deviation
Bozanta	1.39	1.10	0.13	10.7	1.83
Reference area	0.67	0.46	0.04	3.98	0.76

The authors acknowledge funding support grant no. 52157/1.10.2008 (CISPPA) from the Romanian Ministry of Education, Research and Innovation, within the 4 th Program of PNCDI II.

## INTER-LABORATORY COMPARISON OF CRY1AB TOXIN QUANTIFICATION IN *MON 810* MAIZE BY ENZYME-IMMUNOASSAY

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A laboratory ring trial was performed in four laboratories for determination of Cry1Ab toxin in leaf material of MON 810 maize using a standardized enzyme-linked immunoassay protocol. Statistical analysis was carried out by the ISO 5725-2 guidelines, sample standard deviation and standard error (SD, SE), within-laboratory and inter-laboratory SD and SE were calculated. Measured inter-laboratory average (IA) values were  $12.5 \pm 4.0$ ,  $15.3 \pm 4.6$  and  $72.6 \pm 17.8$  µg/g for three lyophilized samples, and  $27.8 \pm 4.3$  µg/g for a frozen sample, yet, Cry1Ab concentrations ranged 66.5–160.1% of the corresponding IA. Determined concentrations by in-house protocols were statistically not different in one laboratory and different in two laboratories from the corresponding values by the joint protocol. Results emphasize the importance of a standardized protocol among laboratories for comparable quantitative Cry1Ab toxin determination. However, even when using a standardized protocol, significant differences still occur among toxin concentrations detected in different laboratories, although with a smaller range of variation.

## RELATIONSHIP BETWEEN THE SOIL CONTAMINATION AND MICROORGANISMS OF RAGWEED (*AMBROSIA ARTEMISIIFOLIA* L.) POLLEN

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Toxic metal contamination is a major environmental and human health problem. A prominent concern is a reduction in biodiversity. Ragweed possesses all 'weapons' of aggressive weeds such as high ecological tolerance and ability of rapid regeneration and colonisation which enable them to become the pioneer plant species of secondary succession. This plant prefers slightly acidic and sandy soils, where it may cover 90-100 percent of the area. The danger of ragweed is reflected by the allergic symptoms which are caused by the pollens (one plant may release as much as 8 billion pollens). In Hungary, appearance of ragweed (*Ambrosia artemisiifolia*, L.) was firstly observed at the beginning of the 20th century and was followed by the rapid spread of this plant species. Presently, it can be found on the whole area of the country. Besides producing billions of pollens, ragweed also has an additional property according to which growing concentrations of cadmium, copper, nickel, and zinc in the soil increase the cadmium, copper, nickel and zinc content of roots, leaves and staminate inflorescence. Metal accumulation in plants also modifies the composition of epiphytic microorganisms. Structure and proportions of pollen promote the attachment of environmental pollutants.

The studies focused on „total” cadmium, copper, nickel and zinc content of the soil on three ecologically distinct areas, which were different in species composition, metal content and evolution.

At the communal landfill site and at the industrial galvanic-sludge disposal site in *Ambrosia artemisiifolia* L. the average contents of heavy metals are: cadmium 0.25 – 0.43 mg·kg<sup>-1</sup>, copper 8.68 – 11.50 mg·kg<sup>-1</sup>, nickel 2.18 – 9.10 mg·kg<sup>-1</sup> and zinc 37.00 – 133.45 mg·kg<sup>-1</sup>. The zinc content of staminate inflorescence in Site II. and the cadmium, copper, nickel and zinc content of staminate inflorescence in Site III. are significantly increases as opposed to that non-ruderal sites (Site I.) considered as a control site. Parallel with the increase in the cadmium, copper, nickel, and zinc contents of the staminate inflorescence, the number of culturable microorganisms was increased. The number of microorganisms increases in the ruderal sites as opposed to that in non-ruderal site.

In our study, the pollen surface of ragweed was dominantly colonized by the species of genera *Pseudomonas*, *Enterobacter* and *Alternaria*. Increased number of deformed pollen could be observed on the ragweed of ruderal sites. There is a positive correlation between the number of pollen microorganisms and the amount of deformed pollen.

## AMELIORATIVE EFFECT OF *MORINGA OLEIFERA*, ACTIVATED CHARCOAL AND CHARCOAL ON LEAD TOXICITY IN WISTAR RATS

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Conventional treatment of lead poisoning has been based on chelating agents which are relatively expensive for poor communities. In this present study, we evaluated the efficacy of *Moringa oleifera* aqueous leaf extract, activated charcoal and charcoal in treating lead induced toxicity in wistar rats. The rats were divided into 5 groups of 10 rats. An oral daily dosage of 1000 mg/kg body weight of lead acetate was administered to rats in 4 groups for 7 days. The positive control group received distilled water. After 7 days of lead acetate administration, 10 rats were sacrificed from a lead acetate group and control group. From day 8 to day 21, an oral daily dosage of 1000 mg/ kg body weight of *M. oleifera*, activated charcoal and charcoal were administered separately to 3 of the lead acetate treated groups while one of the groups was allowed untreated. On day 21, all the remaining rats were sacrificed. During and after the administration period, rats were examined for clinical signs, body weight changes, serum biochemistry, haematological parameters and histopathological lesions. Lead acetate significantly decreases body weight, aspartate aminotransferase, alanine aminotransferase, and red blood cells count. Gamma glutamyl aminotransferase, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were increased. Histopathology examination revealed damages in the liver, brain, muscle and kidneys. Activated charcoal and *M. oleifera* showed ameliorative effects in the haematology, serum biochemistry and histopathological analysis. Our results suggest that *M. oleifera* aqueous leaf extract and activated charcoal can ameliorate lead induced toxicities.

## EXPOSURE TO ACETAMIDE-GENERATING COMPOUNDS IS A RISK FACTOR OF ACUTE HEPATIC INFLAMMATION

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Acetamide-based compounds widely used in industries and their toxic effect have not been studied. Thioacetamide (TAA) is widely used in industry and is known to be a hepatotoxicants in experimental animals. However, the mechanism underlying TAA-induced acute inflammation is still unclear. We investigated the mechanisms and the involvement of main TAA metabolites in acute hepatic inflammation induced by TAA in rats. Acute hepatic inflammation was induced by TAA (0, 10, 30, and 100 mg/kg, ip), while the inflammatory indicators including cytokines and nitric oxide were determined 0, 1, 3, 6, and 12 h after TAA injection. Hepatic pro-inflammatory cytokines were measured quantitatively using enzyme-linked immunosorbent assay. Rabbit anti-neutrophil serum and SKF525A (cytochrome P450 2E1 [CYP 2E1] inhibitor) were used to examine the roles of neutrophil and cytochrome in the initiation of TAA-induced acute hepatic inflammation, respectively. In addition, TAA-S-oxide and acetamide were also used to examine the involvement of TAA metabolites in the early stage of TAA-induced hepatic inflammation. TAA increased, within 6 h, hepatic tumor necrosis factor- $\alpha$  production, interleukin-1 $\beta$ , nitrite levels, inducible nitric oxide synthase expression, and myeloperoxidase activity. Rabbit anti-neutrophil serum inhibited all TAA-increased parameters. CYP 2E1 inhibitors showed significant inhibition of tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$ , nitrite, and myeloperoxidase activity after TAA was given. In addition, acetamide, but not TAA-S-oxide, increased myeloperoxidase activity and all tested proinflammatory mediators' generation. We hypothesize that acetamide-associated neutrophil activation is involved in TAA-induced hepatic inflammation, at least partially. We conclude that exposure to acetamide-generating compounds is a risk factor of acute hepatic inflammation.

# GERMINATION AND EARLY DEVELOPMENT OF *Brassica napus* L. AND *Brachypodium distachyon* (L.) Beauv. GROWTH WITH Zn, Cr(VI), As(V) OR Cd

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Growing energy crops on polluted soils might lead to accumulation of heavy metals in plant tissues and reemission of contaminants into the atmosphere during combustion. Phytostabilization could reduce heavy metals bioavailability in soils and obtain renewable energy from lands useless (1). Some of these fast-growing, high biomass crop plant species are known to display a significant heavy metal tolerance, particularly those from the genus *Brassica* (2). *Brachypodium distachyon* (L.) Beauv. has also been recently proposed as a model specie to development of bio-energy (3). The present work reports data concerning the ability of *Brachypodium distachyon* and *Brassica napus* L. seeds to germinate and grow in media containing different doses of Cd, Cr, As and Zn in order to evaluate their use as energy crops in polluted sites. Four jars per treatment were spiked separately with the following metal doses (i) 0, 5, 10, 30 mg·l<sup>-1</sup> of Cd; (ii) 0, 5, 10, 30 mg·l<sup>-1</sup> of As (V); (iii) 0, 20, 60, 100 mg·l<sup>-1</sup> of Zn and (iv) 0, 40, 80, 100 mg·l<sup>-1</sup> of Cr (VI) in a growing chamber at 25 °C, 12 h of photoperiod. The seedlings were harvested after two weeks. Germination rate, and length of roots and shoots were recorded. The plants were then separated into roots and shoots, dried in an oven at 70 °C for 72 h, and the dry weight was determined. In general, biomass reduction and length decreasing was observed as consequence on increasing metal dosage in both species, but the effect was different attending to metal and specie. The highest toxicity level was found in plants treated with Cr(VI), specially in root developed. In this case, roots were drastically reduced from the lowest dosage in both *B. napus* as *B. distachyon*. Exposures of 30 mg·l<sup>-1</sup> of Cd and As (V) reduced the shoot elongation by 50 % in both species, while root was affected by lower dosages than 30 ppm. Related to *B. distachyon* and *B. napus* growth with Zn, this metal content did not affect shoot elongation, root length or biomass of the first one, but shoot and root elongation of *B. napus* were reduced from the lowest dose of Zn. From this preliminary germination test, *B. distachyon* seems to tolerate medium and high metal content exception to Cr (VI). Previous studies (2) support the feasibility of *B. napus* to be used in phyremediation of polluted soils and to obtain biomass energy, from the results obtained here *B. napus* could be suitable to tolerate moderate concentration of Zn, As and Cd.

**Acknowledgements:** EIADES PROGRAM S2009/AMB-1478, RTA000150-00-00-INIA; Contratación de Doctores INIA-CCAA” and FPI-INIA to support the predoctoral granted Blanca Montalbán.

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