



WG2 and WG3 2<sup>nd</sup> Scientific Workshop  
31 August – 02 September 2006

Phytotechnologies  
to promote  
sustainable land use and improve food safety

Saint-Etienne Workshop

-omics approaches and agricultural management:  
driving forces to improve food quality and safety?

EUROPEAN COOPERATION IN THE FIELD OF SCIENTIFIC AND TECHNICAL RESEARCH



# COST 859

## PHYTOTECHNOLOGIES

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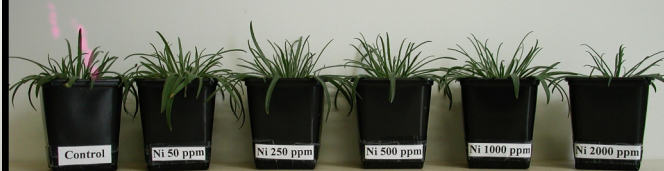
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UNIVERSITE JEAN MONNET ET ECOLE NATIONALE SUPERIEURE DES MINES  
SAINT-ETIENNE, FRANCE



## **COST Action 859**

### ***Phytotechnologies to Promote Sustainable Land Use and Improve Food Safety***



### ***WG2 & WG3 second Scientific Workshop***

### ***"-omics approaches and agricultural management: driving forces to improve food quality and safety?"***

***31 August - 2 September 2006  
Saint-Etienne, France***

**Ecole Nationale  
Supérieure des Mines**  
SAINT-ETIENNE





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Université Blaise Pascal de Clermont-Ferrand, Ville de Saint-Etienne, Région Rhône-  
Alpes

And also many thanks to

Jacqueline Joly and Stéphanie Javelle for their invaluable help in preparing the meeting.





## **PROGRAMME**



## Day 1 - Thursday 31 August 2006

### 8h30 - 9h30

Registration / Delivery of access card to the workshop area  
Installation of posters and breakfast

### 9h30: Opening session

Chair: Jean-Claude Leclerc (FR) Reporter: Kim Yrjälä (FI)

09h30 - 10h15	Welcome and goals of the Workshop
10h15 - 11h00	Mark G. M. Aarts (NL) Omics of the removal of heavy metals from the environment – mechanisms, tools and plant selection (Keynote lecture)
11h00 - 11h45	Steve P. Mc Grath (UK) Food quality and safety issues concerning elements that are essential or contaminants in crops (Keynote lecture)
11h45 - 12h30	Define actions by the co-ordinators of the Working Groups
12h30 - 14h00	Lunch

### 14h00: Second session “Omics uses to characterise the responses of plants exposed to inorganic contaminants”

Chair: Bal Ram Singh (NO) Reporter: Marie-Theres Hauser (AT)

14h00 - 14h20	Ann Cuypers (BE) Oxidative stress as a modulator in cadmium induced alterations on transcriptome and proteome in <i>Arabidopsis thaliana</i>
14h20 - 14h40	Barbara Borcz (PL) Identification of tobacco genes regulated by cadmium using suppression subtractive hybridization method
14h40 - 15h00	Isabelle Duquesnoy (FR) Genetic variability of <i>Agrostis tenuis</i> proteome expression after arsenate and arsenite treatments
15h00 - 15h20	Andrea Pirondini (IT) Analysis of genetic variation in the Monte Prinzera population of <i>Thlaspi caerulescens</i>
15h20 - 15h35	Directives
15h35 - 16h05	Poster session and Coffee-break

**16h05: Third session** “Physiological mechanisms involved in detoxification and tolerance in plants exposed to inorganic contaminants”

Chair: Nathalie Verbruggen (BE) Reporter: Jean-Paul Schwitzguébel (CH)

16h05 - 16h25	Peter Schröder (DE) Influence of heavy metals on glutathione conjugation – implications for phytoremediation under multiple pollution
16h25 - 16h45	Philippe Vernay (FR) Changes in free amino acids in hyperaccumulator and tolerant plants during nickel stress
16h45 - 17h05	Cristina Ortega (ES) Characterization of early oxidative stress defences to Cd and Hg in alfalfa seedlings
17h05 - 17h25	Abdul Razaque Memon (TR) Cu tolerance and accumulation in Brassica nigra and development of in vitro regeneration system for phytoremediation
17h25 - 17h40	Directives
17h40 - 18h30	Poster session

Gala dinner: Le Bougainvillier (bus departure from the Ecole des Mines at 19h30)

## Day 2 - Friday 1<sup>st</sup> September 2006

8h30 - 9h00

Welcome and breakfast

09h00: Fourth session “Advances in omics to investigate the behaviour of organic xenobiotics in plants”

Chair: Peter Schröder (DE)

Reporter: Alexander Lux (SK)

- |               |   |
|---------------|---|
| 09h00 - 09h20 | Melissa Brazier-Hicks (UK)<br>Functional importance of the family 1 glucosyltransferase UGT72B1 in the metabolism of xenobiotics in <i>Arabidopsis thaliana</i> |
| 09h20 - 09h40 | Cyrille Forestier (FR)<br>Transport of GS-X conjugates by <i>Arabidopsis thaliana</i> as a strategy for phytoremediation  |
| 09h40 - 10h00 | Luísa Davies (PT)<br>Identification of genes responsible for <i>Phragmites</i> phytoremediation: Possible application in agricultural soils decontamination     |
| 10h00 - 10h20 | Juliane Neustifter (DE)<br>Phytoremediation and detoxification of organic compounds in <i>Phragmites australis</i>  |
| 10h20 - 10h30 | Directives  |
| 10h30 - 11h00 | Poster session and Coffee-break   |

11h00: Fifth session “Beneficial or detrimental effects of organics in food”

Chair: Patricia Harvey (UK)

Reporter: Umit Baris Kutman (TR)

- |               |  |
|---------------|--|
| 11h00 - 11h30 | Jenny Gee (UK)<br>Genomics as a tool to assess the health benefits of dietary phytochemicals   |
| 11h30 - 11h50 | André Gerth (DE)<br>Genomics-assisted production of plant biopharmaceuticals   |
| 11h50 - 12h10 | Isabel Hilber (CH)<br>Organochlorine pesticide residues assessed in a greenhouse survey in 2005 and a concept of bioavailability experiments |
| 12h10 - 12h30 | Muriel Raveton (FR)<br>Relations between pollen or seed quality and soil pest control in sunflower cultures                                  |
| 12h30 - 12h40 | Directives   |

12h40 - 14h10

Lunch

### 14h10: Sixth session “Food safety and food chain contamination”

Chair: Juan Navarro (ES)

Reporter: Pascale Goupil (FR)

- |               |  |
|---------------|--|
| 14h10 - 14h30 | Artak Ghandilyan (NL)<br>Genetic studies and QTL mapping for mineral homeostasis in a RIL population of <i>Arabidopsis thaliana</i>                    |
| 14h30 - 14h50 | Bal Ram Singh (NO)<br>Effects of chloride, sulphate and nitrate on cadmium in rhizosphere soil and its uptake by ryegrass                              |
| 14h50 - 15h10 | Antonio de Haro-Bailon (ES)<br>Arsenic, lead and cadmium uptake and distribution in broccoli, cauliflower and radish plants grown on contaminated soil |
| 15h10 - 15h30 | Agnes Piquet-Pissaloux (FR)<br>Transfer soil/grassland on Ni-Cr rich soils and derogation of French regulation of sewage sludge spreading              |
| 15h30 - 15h40 | Directives   |
| 15h40 - 16h10 | Poster session and Coffee-break  |

### 16h10: Seventh session “Omics and xenobiotics: practical agronomic issues”

Chair: Michel Mench (FR)

Reporter: Henrique Guedes-Pinto (PT)

- |               |   |
|---------------|---|
| 16h10 - 16h30 | Elena Strijakova (RU)<br>Influence of activated carbon on soil fertility and quality of crops grown in contaminated soil: a Russian experience          |
| 16h30 - 17h00 | Frank Laternus (SE)<br>Organic contaminants from sewage sludge applied to agricultural soils – false alarm regarding possible problems for food safety? |
| 17h00 - 17h30 | Elizabeth Chanliaud (FR)<br>Improving the mineral content of wheat: A breeder point of view   |
| 17h30 - 18h00 | Discussion and directives   |
| 18h00 - 18h30 | Outcomes from the meeting and next steps  |

## Day 3 - Saturday 2 September 2006 (optional)

<u>09h00: field trip</u>	Departure from the “Ecole des Mines”
10h00 - 11h00	Suc de Clava: "a high Cr-Ni-rich serpentine natural environment"
11h30 - 12h30	Visit of a wine-cave (Côtes du Rhône)
13h00 - 14h00	Lunch
15h30	Lyon Saint-Exupéry airport
16h15	Lyon Part-Dieu railway station
17h15	Saint-Etienne Ecole des Mines





## **OPENING SESSION**

Chair: Jean-Claude LECLERC (FR)

Reporter: Kim YRJÄLÄ (FI)



## Omics of the removal of heavy metals from the environment - mechanisms, tools and plant selection

Mark G.M. AARTS<sup>1</sup>, Judith E. VAN DE MORTEL<sup>1</sup>, Sangita TALUKDAR<sup>1</sup>, Ana ASSUNÇÃO<sup>1</sup>, Andrea PIRONDINI<sup>1,2</sup>, Jian WU<sup>1,3</sup> and Henk SCHAT<sup>4</sup>

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<sup>4</sup>Ecology and Physiology of Plants, Vrije Universiteit, Amsterdam, The Netherlands

The past ten years have brought tremendous progress in the molecular understanding of plant mineral homeostasis, especially for the plant reference species *Arabidopsis thaliana*. This knowledge has also been instrumental to investigate, on a molecular level, the rare phenomenon of heavy metal hyperaccumulation as found in several other Brassicaceae species such as *Arabidopsis halleri* and *Thlaspi caerulescens*. These species are an example for future phytoremediation “crops” in terms of their exceptional metal tolerance and root-to-shoot metal translocation properties, but not in terms of biomass production. The use of “omics” technologies like transcriptomics and comparative genomics already revealed many genes that are supposed to play a crucial role in maintaining metal homeostasis in the hyperaccumulating species. How to use this information to further unravel the metal hyperaccumulation trait and to apply it for the development of efficient phytoremediation crops is one of the scientific challenges we face in the near future. This presentation will aim to provide an overview of the current understanding of plant heavy metal uptake, translocation and storage, and will try to illustrate and speculate on the possibilities to improve metal hyperaccumulation in crop species by genetic engineering.

## Food quality and safety issues concerning elements that are essential or contaminants in crops

Steve P. McGRATH, Mingsheng FAN, Colin W. GRAY and Fangjie ZHAO

Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK.

Environmental factors such as fertiliser additions, yields and variety can have effects on the concentrations of trace elements in the edible parts of crops and therefore in food. There is currently considerable public concern about two quite divergent issues: 1. deficiencies of essential elements such as Fe, Zn, Mn, Cu and Se and 2. excesses of elements that are potentially toxic or cause diseases (e.g. Cd, Pb, Hg). The two important main processes are depletion of trace element resources versus the deposition of excess elements due to such practices as the re-cycling of wastes include sewage sludge, farm manures or urban composts.

Therefore, first on the science agenda is that we know what changes are taking place. Mass balances can help us to study these, but very few are performed and few “soil monitoring” studies/sites have begun in relatively recent years. The problem is that trace element concentrations change only slowly, so it takes time to see differences. Modelling approaches, resulting in ‘projections’ are usually the only indicators that we have. Although even these are rare, we have some knowledge about which essential or potentially toxic elements are building up. However, there is little information about where and under what conditions the amounts of trace essential elements are decreasing. Both of these approaches normally deal with the “total” concentrations of trace elements present. Perhaps a bigger issue is the bioavailability of those elements in soils.

Another approach is to look only at the quality of crops themselves, and whether these are changing over time. We now have European Food Regulations for Hg, Cd and Pb (plus some organic contaminants) in many different foodstuffs. These set different maximum allowed concentrations in various foodstuffs to be marketed (EU, 2001). From the field to raw foodstuff produced at the farm gate, by far the biggest concern is over Cd, but this is mainly due to build up in soils due to past use of Cd-contaminated P fertilisers and sewage sludges. Knowledge and regulations now exist to control this, but many fields are still contaminated, and may need management changes or remediation.

What can be done about deficiency or excess of trace elements? First of all we need to know whether we have any problems of lack of essential elements or what are the acceptable (non-toxic) levels of elements present is excess. For example, cereals are an important component of human Se intake, and we know that the concentrations of Se in UK wheat grain are low (c. 25 µg/kg; Adams et al, 2002). Soil or crop strategies can be used, depending on the trace element in question. Research is under way to biofortify various crop with Se either by adding it to fertilisers or by selection of lines which take up and translocate more Se to the grain and also in the processed flour. So, location and the bioavailability of trace elements in crops and food is also very important, because if they are not transported to the edible parts or if changes due to selection of genotypes inadvertently increase the concentration of anti-nutritional factors such as phytates and

phenolics in the final products, we will miss the target. Finally, various environmental interactions can alter the bioavailability of trace elements to crops and again if we ignore these we will fail.

#### References

- Adams ML, Lombi E, Zhao FJ and McGrath SP (2002). Evidence of low selenium concentrations in U.K. breadmaking wheat grain. *J Sci Food Agric*, 82: 1160-1165.
- European Commission (2001). Regulation (EC) No. 466/2001 of 8 March 2001.



## **SECOND SESSION**

### **“Omics uses to characterise the responses of plants exposed to inorganic contaminants”**

Chair: Bal Ram SINGH (NO)

Reporter: Marie-Theres HAUSER (AT)



## Oxidative stress as a modulator in cadmium induced alterations on transcriptome and proteome in *Arabidopsis thaliana*

Ann CUYPERS, Karen SMEETS, Brahim SEMANE, Joske RUYTINX, Frank VAN BELLEGHEM, Nathalie VANHOUDT & Jaco VANGRONSVELD

Hasselt University, Environmental Biology, Centre for Environmental Sciences, Agoralaan Building-D, B-3590 Diepenbeek, Belgium

Increased levels of heavy metals in the environment affect a variety of responses in plants. Oxidative stress can act as a modulator in their stress-action, leading to damage of cellular components at high exposure levels or triggering a defence network and hence an adaptive response after exposure to low metal concentrations.

In this study three weeks-old *A. thaliana* seedlings were exposed to 10  $\mu\text{M}$  Cd during 24h/72h. With regard to the cellular redox balans, focus of the analyses was on 3 different processes: (1) nature of the reactive oxygen species (ROS) induced using EPR measurements, (2) origin of the ROS by analysing TBArm and gene expression analysis (real-time PCR) of lipoxygenases and NADPH-oxidases and (3) the antioxidative defence network (antioxidative enzymes and metabolites). Furthermore the impact on transcriptome and proteome was investigated in order to retrieve information concerning cellular responses. In this study environmental realistic exposure concentrations were used, therefore the outcome is highly relevant for further research on heavy metal contamination. Elevated metal concentrations in the environment cause great losses in crop production worldwide. Although the overall response is similar, it is important to gain more knowledge on the underlying molecular mechanisms, which can deliver more information in the development of new strategies for growing non-food crops on metal contaminated agricultural soils, whether or not aiming phytoremediation.

A striking difference in ROS production confirmed the importance of oxidative stress as a modulator in cellular cadmium responses under environmental conditions. Moreover, study at different cellular levels (transcriptome and proteome) revealed that oxidative stress-related components are probably a crucial factor in regulating a further adaptive response. Looking more into detail, root cadmium application resulted in severe stress intensity, which suggested to be ‘sensed’ by the plasma membrane leading to strong increases in lipid peroxidation and a high induction of the cytoplasmic lipoxygenase gene. Furthermore NADPH oxidases play an important role in case of Cd stress. In leaves, metal concentrations were much lower and hence the stress intensity mild. Plasma membranes and chloroplasts seemed to be the ‘sensing sites’. Obviously, the interplay between production and scavenging of ROS was balanced, and a major emphasis is attributed to the role of the cellular redox state in intracellular signaling.

## Identification of tobacco genes regulated by cadmium using suppression subtractive hybridization method

Barbara BORCZ, Jolanta KAMINSKA, Małgorzata LEWANDOWSKA, Marta PIECHO & Agnieszka SIRKO

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Cadmium decreases growth rate of plants by affecting various aspects of metabolism. Binding of cadmium to sulfhydryl groups of proteins leads to inhibition of their activities and disruption of structure. Toxic cadmium levels inhibit the normal uptake and utilization of macro- and micronutrients, result in perturbations in the intracellular calcium level and disturbance of the cellular redox control. The precise knowledge of the changes in gene expression in response to cadmium exposure is crucial for a successful manipulation of the mechanisms governing distribution of this metal within plant tissues as well as tolerance to its toxicity. Transcriptional responses of various plants, mostly hyperaccumulators, to the toxic levels of cadmium have been studied by several research groups. The steps involved in metal hyperaccumulation are essentially the same as in non-hyperaccumulating plants. The basic difference is made by the ability of hyperaccumulators to accumulate very high concentration of metal in aerial parts without showing toxicity symptoms. However, such plants are usually slow-growing, low-biomass-producers.

*Nicotiana tabacum* is a non-edible, large-biomass-producing plant. In addition, tobacco can be relatively easy genetically modified and analyzed. The above features would make it a good candidate for phytoremediation purposes provided that the metals can be efficiently accumulated in the harvestable above-ground parts of the plants. Our previous results indicated that in tobacco exposed for three weeks to the toxic levels of cadmium (i) concentrations of this metal are about two-fold lower in shoots than in roots, (ii) mechanisms of long distance cadmium translocation are limiting cadmium accumulation in shoots and (iii) cadmium toxicity measured by inhibition of plant performance is positively correlated to cadmium contents in the plant tissues. Cadmium accumulation in aerial parts and plants ability to tolerate cadmium toxicity could be improved by identification and a subsequent modification of the identified bottle-neck activities.

In this work, tobacco was used as a model plant to investigate transcriptional responses of non-hyperaccumulators to cadmium exposure. Tobacco plants were treated with cadmium for a short and a long period of time in order to identify genes whose products might be responsible for “a fast response to the presence of cadmium in the medium” and “a long-time dealing with cadmium toxicity”, respectively. Suppression subtractive hybridization (SSH) method was chosen to create the libraries enriched in cDNAs up- and down-regulated by cadmium in each case. The SSH libraries were made using plants treated with cadmium and plants grown in control conditions and screened for regulated genes using fluorescent differential display technique. The selected cDNA inserts were sequenced and regulation of the identified genes was confirmed by Northern-blot or reverse transcription PCR.

## Genetic variability of *Agrostis tenuis* proteome expression after arsenate and arsenite treatments

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Quantitative proteomics, the technology for high-throughput measurement of protein concentrations from 2-D electrophoresis, often reveals high levels of genetic variability of proteome expression in the species studied. A majority of proteins, including enzymes, display quantitative and qualitative variation, the extent of which may exceed an order of magnitude.

To gain insight into plant responses to arsenic, the effect of arsenic exposure on *agrostis* (*Agrostis tenuis*) leaf proteome has been examined. *Agrostis* plants were fed hydroponically with different concentrations arsenate or arsenite (134 and 668 $\mu$ M) for 8 days and changes in differentially displayed proteins were studied by two-dimensional electrophoresis and digital image analysis. *Agrostis* leaf proteins were up- or down-regulated by arsenate and arsenite treatments, among which some were selected as being quite reproducibly affected by the metalloid.

## Analysis of genetic variation in the Monte Prinzera population of *Thlaspi caerulescens*

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The natural Zn/Cd/Ni hyperaccumulating and hypertolerant species *Thlaspi caerulescens* (J&C. Presl.) is widely distributed over Europe, found in dispersed metalicolous and non-metallicolous populations from Scandinavia to Spain and from the UK to the Czech Republic. Among these populations there are considerable differences in visible phenotypes as well as metal accumulation and tolerance characteristics. Most metalicolous populations show strong tolerance to the most abundant metals at their site of origin and most non-metallicolous populations are not particularly tolerant. In general all populations studied so far (hyper)accumulate zinc.

We are interested in studying the natural variation within and between different *T. caerulescens* populations. In this study we focus on the population collected at Monte Prinzera (MP), a serpentine hill in the close vicinity of Parma, Italy. The particularity of MP ophiolitic rocks rich in Ni, Fe and Co and the multiplicity of its microenvironments allow for the growth of an exceptionally rich diversity of flora, that includes rare species peculiar to these environments, such as the hyperaccumulators *T. caerulescens*.

Seeds were collected from individual plants at five different sites on the hill in the early summer of 2005. Seeds were germinated in the laboratory and grown on 0.5 Hoagland's solution to maturity. Evident differences in morphology of leaves and rosette were observed between *T. caerulescens* growing on different sites. Leaves were sampled for mineral analysis and for DNA isolation. Ten sets of PCR primers corresponding to random ESTs and promoter or coding regions of selected putative metal homeostasis genes were used to amplify genomic DNA fragments. Their DNA sequences were analyzed to determine polymorphisms between individual plant and to establish the level of variation within the populations. Sequences from *T. caerulescens* populations from elsewhere in Europe and from related species were used as comparison.

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## **THIRD SESSION**

“Physiological mechanisms involved in detoxification  
and tolerance in plants exposed to inorganic  
contaminants”

Chair: Nathalie VERBRUGGEN (BE)  
Reporter: Jean-Paul Schwitzguébel (CH)



## Influence of heavy metals on glutathione conjugation – implications for phytoremediation under multiple pollution

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Keywords: multiple pollution, organic xenobiotics, detoxification, inducible metabolism

Environmental pollution by heavy metals is in many cases accompanied by pollution with organic foreign compounds and vice versa. Phytoremediation but also phytostabilization in such areas of multiple pollution is complicated, and only few plant species have been shown to survive under such adverse conditions. Heavy metals are well known for their ability to interfere with the plant's metabolism and to induce the formation of reactive oxygen species (ROS), albeit through different reactions. Especially arsenic, cadmium, lead and copper have been investigated with respect to these effects.

On the other hand, it has been shown that ROS may serve as signalling molecules for a number of defense reactions in plants, including alterations in the sulphur metabolism. Tightly connected to the sulphur metabolism in plants is the predominant detoxification pathway of halogenated organic pollutants and herbicides, i.e. the glutathione S-transferase dependent detoxication. Glutathione S-transferases (GST) catalyze the conjugation of reduced glutathione to the electrophilic centers of such xenobiotics. They comprise a very heterogeneous family of enzymes that can be subdivided into distinct classes with tasks in plant metabolism and defence. More than 50 gst genes have been identified in model plants. To date their functions are not known in too much detail, but it is clear, that their expression and activity is strongly modulated by oxidative stress and ROS. In order to obtain information on the physiological background of the ROS formation and the influence on the detoxification of organic pollutants, we have investigated the reaction of plants used in phytoremediation, e.g. Typha and Phragmites, but also a plant cell culture that has been used as a biomonitor of stress. The general answer of these plants toward heavy metals in concentrations from 10 to 250  $\mu$ M is an increase in GST activity and several other enzymes like peroxidase, ascorbate peroxidase and glutathione reductase, connected to strong alterations in the glutathione pool.

Interestingly, and different from the general opinion, we found that even mild heavy metal stress might lead to the inhibition of detoxification reactions in plants. Glutathione S-transferase activity for specific organic substrates was lacking after 6 to 24 hrs of incubation with heavy metals, whereas other substrates were conjugated at even higher rates. This development depends heavily on the heavy metal and its concentration. Mixtures of heavy metals show a tendency towards synergistic effects.

Under real life conditions and multiple pollution scenarios this might mean that the combination of heavy metals and organic pollutants (a) can be tackled by the plant, if the correct enzyme activity is induced, or (b) leads to rapid development of stronger stress due to the additional action of the undetoxified organic xenobiotic. Whether this effect is

connected to a depletion of GSH, or an overload of storage pools of the plants, is critically discussed.

### Changes in free amino acids in hyperaccumulator and tolerant plants during nickel stress.

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Heavy metals are important environmental pollutants and many of them are toxic even at very low concentration. Remediation of sites contaminated with toxic metals is particularly challenging. Unlike organic compounds, metals cannot be degraded, and the cleanup usually requires their removal. The commonly used treatment methods for metal polluted sites are still extremely costly. Phytoremediation is the use of plants to extract, sequester and/or detoxify pollutants. Plants are ideal agents for soil and water remediation because of their genetic, biochemical and physiological properties. Plants that take up relatively large amounts of heavy metals from the soil and sequester them in their tissues are called hyperaccumulators. For instance, nickel (Ni) hyperaccumulators, which comprise more taxa than any other type of metal hyperaccumulator are able to contain more than 1000  $\mu\text{g Ni.g}^{-1}$  dry tissue (Brooks et al., 1977). Because metals are complexed with common metabolic products such as citrate, malate, oxalate or amino acids (Kramer et al., 1996), translocation and compartmentalization may represent the main direct metabolic pathway for metal-based defenses. Our work investigate the quantification of 17 amino acids by HPLC (Waters AccQ Tag) in order to evaluate their influence on the presence of Ni in the plant and its ability to tolerate this metal. So, various types of plants among wich *Datura innoxia* (tolerant), and *Lolium perenne*. (agronomical interest), have been grown in hydroponic conditions. Amino acids are quantified in roots, root exudates and in leaves. Simultaneously, Ni is quantified by SAAF in order to determine its repartition in the various plants.

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## Characterization of early oxidative stress defences to Cd and Hg in alfalfa seedlings

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Our studies are focused to the understanding of plant cell responses to cadmium and mercury. Alterations of glutathione (GSH) cellular content and increased peroxidation in root epidermal cells were found (Ortega-Villasante et al, 2005). We observed that redox homeostasis was compromised shortly after exposure to 30  $\mu$ M Cd and 30  $\mu$ M Hg. The redox cellular balance mainly depends on glutathione-ascorbate cycle, which might be relevant for plant tolerance to heavy metals. Therefore, we try to complement the characterisation of early plant responses to Cd and Hg by analysing the expression of genes coding for enzymes involved in this cycle and in GSH metabolism. A semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) technique was used in conjunction with heterologous primers derived from *M. truncatula* sequences. Changes in several genes such as GSH synthetase I (GSHI), GSH synthetase II, GSH reductase, etc., were studied. In the STSM-COST859 carried out in the Laboratoire de Physiologie et de Génétique Moléculaire des Plantes, Brussels, the RT-PCR technique was used to study gene expression patterns in *Arabidopsis thaliana* plants treated with 10  $\mu$ M Cd up to 7 days. No significant differences were found in the expression of different isoforms of APX. This stay served us to implement the techniques in our lab in Madrid. Thus, the same approach revealed differences in gene expression of GSHI, among other genes studied, in *M. sativa* seedlings exposed to several concentrations (3, 10 and 30  $\mu$ M) and times (up to 24 h) of Hg. The tolerance to Cd of *Nicotiana tabacum* plants that overexpress MnSOD (Bowler et al, 1991), an enzyme key in the detoxification of oxygen reactive species was tested. The transgenic line evaluated showed higher SOD activity as visualised by in gel staining. Apparently, although able to eliminate higher proportion of ion superoxide, little effect was found in the tolerance of those plants to Cd. Results of different stress indexes and responses will be further discussed.

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damage mediated by oxygen radicals in transgenic plants. *The EMBO Journal* 10: 1723-32.

## Cu tolerance and accumulation in *Brassica nigra* and development of in vitro regeneration system for phytoremediation

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Key words: Phytoremediation, *Brassica nigra*, Metal Uptake, Tissue Culture.

Phytoremediation technology uses plants to remove pollutants from the environment. The use of metal accumulator plants to clean-up soil and water contaminated with toxic metals is the most rapidly developing, environmental friendly and cost-effective technology. Several metal accumulator plants which can accumulate large amounts of Ni, Cu, and Cd have been identified. In present study, we surveyed Southeastern Turkish (Anatolian) flora in order to find out endemic heavy metal accumulator plants. Several metal accumulator plants were found and *Brassica nigra* was found to be accumulator of Cu and Cd. Previously we screened 15 cotton genotypes for metal resistance and two of them were selected as Cd, Cu and Ni resistant and we developed a suitable cost effective protocol for plant regeneration for these genotypes (Biçakçi and Memon, 2005). In present investigation, our main aim is to use *Brassica nigra* for cleaning-up metal contaminated areas of Anatolian and Thrace plateaus of Turkey and have initiated this research project to generate these plants for mass production. For this purpose, firstly we determined the accumulation capacity of *Brassica nigra* cells and whole plants grown in suspension culture and MS media, respectively. In addition, we developed a simple and cost effective regeneration system for *Brassica nigra*, which could be used for genetic transformation. *Brassica nigra* seeds were sterilized and germinated on Murashige and Skoog's (MS) medium without hormones. Thirty days old plants were divided into shoot parts, apex, and hypocotyls. These explants were cultured in MS media containing 20 g l<sup>-1</sup> sucrose, 1 ml l<sup>-1</sup> MS vitamin solution and different plant growth regulators with various combinations. After shoot formation from explants, 15-20 days old shoots were sub-cultured to five different MS media for root formation (Fig 1). After root formation, plants were transferred to the soil culture (Fig 1).

The suspension cell culture was derived from callus generated from hypocotyls of *B. nigra*. Parts of callus was sub-cultured in sterile liquid MS medium and after 15 days these cells were transferred to 250 ml fresh media and final concentration of the cells were maintained as 2 x 10<sup>6</sup> cell ml<sup>-1</sup>. After 24 hours growth, cells were exposed to CuSO<sub>4</sub> for 72 hours and following concentrations were maintained: 0, 50, 100, 200, 500, 1000 µM. For determination of the metal accumulation capacity of whole plant, *B. nigra* seeds were sterilized and germinated on MS medium without hormones. After germination, thirty days old plants were transferred to solution culture and were exposed to 0, 50, 100, 200, 500, 1000 µM CuSO<sub>4</sub> for 72 hours. Plant material was wet digested with HNO<sub>3</sub> and HClO<sub>4</sub> acid mixture (5:2) and metal accumulation capacity of the cells and whole plants were determined by atomic absorption spectrophotometer.

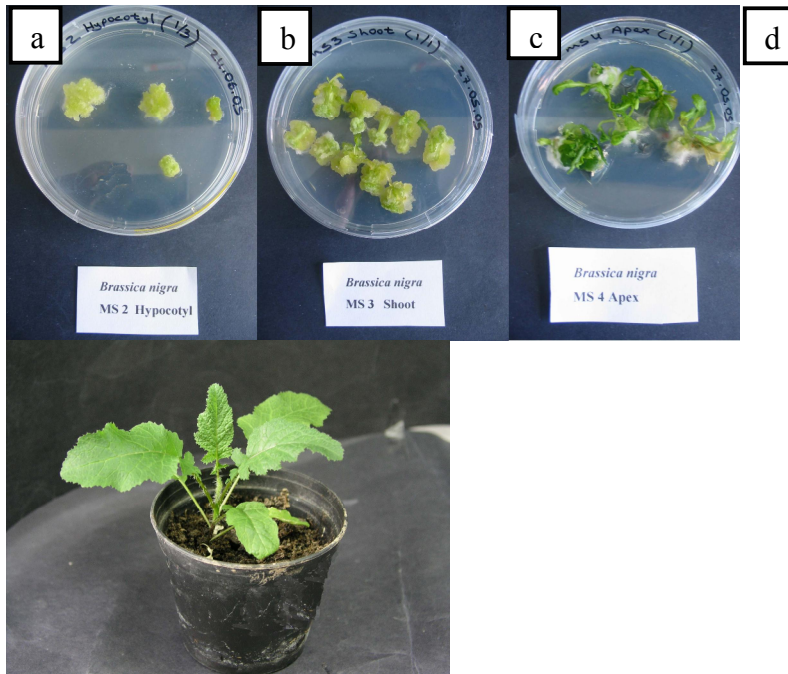


Figure 1. Callus developed from *Brassica nigra*: a) hypocotyls; b) shoot; c) shoot regeneration from apex; d) *Brassica nigra* regenerated from apex

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## **FOURTH SESSION**

### **“Advances in -omics to investigate the behaviour of organic xenobiotics in plants”**

Chair: Peter SCHRÖDER (DE)  
Reporter: Alexander LUX (SK)



## Functional importance of the family 1 glucosyltransferase UGT72B1 in the metabolism of xenobiotics in *Arabidopsis thaliana*

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The conjugation of synthetic compounds with sugars in plants by glycosyltransferases is a major route of detoxification of both pollutants and pesticide metabolites. Using *Arabidopsis thaliana* as a model plant species, we have demonstrated that both plants and cell cultures rapidly detoxify the recalcitrant pollutant 3,4-dichloroaniline (DCA) by N-glycosylation (Lao et al., 2003). The enzyme responsible for the greater part of DCA conjugation in *Arabidopsis* cell cultures has been purified and identified using proteomics as the family 1 glucosyltransferase UGT72B1 (Loutre et al., 2003). The recombinant UGT72B1 was highly active in conjugating DCA, as well as the chlorinated phenol 2,4,5-trichlorophenol, thus demonstrating both N-glucosyltransferase and O-glucosyltransferase activity. The importance of UGT72B1 in the detoxification of DCA in *Arabidopsis* was examined in plants with modified expression of UGT72B1. *Arabidopsis* root cultures over-expressing UGT72B1 (72B1-OE) showed enhanced activity toward DCA in vitro while activity toward DCA was significantly reduced in a T-DNA knockout mutant designated *ugt72B1* (Brazier-Hicks and Edwards, 2005). The metabolism of DCA in planta was found to be impaired considerably in the *ugt72B1* plants while over-expression of UGT72B1 appeared to have little affect on DCA metabolism. Surprisingly, phytotoxicity studies revealed that the knockout plants were able to tolerate DCA much better than wild-type plants, while the 72B1-OE plants were more susceptible. Analysis of the bound residues revealed that significantly more DCA was co-polymerised with lignin in the *ugt72B1* plants than the over-expressors suggesting this route of metabolism resulted in more efficient removal and detoxification of DCA than glucosylation by UGT72B1.

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## Transport of GS-X conjugates by *Arabidopsis thaliana* as a strategy for phytoremediation

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In the last years, major scientific progress has been made in understanding the mechanisms of metal transport in different organisms. This understanding is of particular interest in the objective of using plants for phytoremediation of heavy metal contaminated soils. Our objective is the investigation of the role of ABC proteins involved in heavy metal(oid)s detoxification and/or transport either after conjugation to glutathione (ScYCF1, hMRP1) or phytochelatins (SpHMT1).

Since glutathione is able to complex a large variety of metal(oid)s, we focused our attention on the possibility to over-express exogenous GS-X (MRPs) transporters in plants. We examined a pleiotropic and well-known protein, the human Multidrug Resistance Protein 1 (HsMRP1) as well as its yeast counterpart, YCF1 (Song et al., 2003). HsMRP1-GFP under the control of CaMV35S promoter was introduced by agrotransformation in *Arabidopsis thaliana* using a pGREEN vector. In transgenic plants, the expression of HsMRP1-GFP is restricted to the plasma membrane and this has been confirmed in purified mesophyll cell protoplasts and their vacuoles. Unfortunately, no phenotype was observable in HsMRP1-GFP transformed plants. To evaluate the functionality of the protein in these plants, we are currently characterizing the transport of different well-known glutathione conjugates in mesophyll cell protoplasts.

In plants, a major role for phytochelatins (PCs) in heavy metal sequestration and/or transport has been proposed. In this context, we focused our attention on the only PCs ABC-transporter known till now, HMT-1 from *Schizosaccharomyces pombe*. This transporter has been localized in the yeast tonoplast and is responsible of PCs-Cd complexes transport into the vacuole (Ortiz et al., 1995). Determination of the mechanism of transport of this protein is under investigation in various expression systems.

*Arabidopsis* plants overexpressing YCF1 clearly demonstrated that metals can be safely accumulated via GS-X pumps in the vacuole (Song et al., 2003). This open new strategy for phytoremediation that we are currently investigating by using other exogenous pleiotropic ABC transporters with the final goal to use these plants on multi-contaminated soils.

## Identification of genes responsible for *Phragmites* phytoremediation: Possible application in agricultural soils decontamination

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The common reed (*Phragmites australis*) is widely used as a phytoremediation agent for the treatment of effluents rich in organic matter and nutrients. However, this plant can be used in the treatment of soils contaminated with organics enabling their agricultural use improving food quality and safety. The application of phytoremediation presents many advantages over other methods, as it is a more environment-friendly approach with lower costs.

Very little is known about the biochemical processes behind phytoremediation. In fact, very few *Phragmites australis* enzymes or their genes are known and no study has ever managed to correlate gene expression of plant enzymes with the capacity of degrading wastewater pollutants. We are currently trying to identify some of the enzymes responsible for phytoremediation by means of transcriptomics. For that purpose, we are extracting and purifying total RNA from several parts of the plant, such as leaves and roots. Using designed primers for enzymes such as peroxidases, catalases and superoxide dismutases we are checking for respective gene expression under different stress conditions invoked by an organic pollutant like an azo dye.

After the *Phragmites australis* RNA extraction in non and high stress conditions, RT-PCR is carried out and the cDNA synthesized from mRNA is detected by agarose gel electrophoresis. The appearance and intensity of the bands are indicative of the genes that are being expressed or inhibited, where the detoxification is occurring and the time response taken by the plants.

A parallel approach is also carried out by the measurement of the catalytic activities of the enzymes corresponding to the cDNA that is being synthesized. In our studies it has been already observed an increase in peroxidase, catalase and superoxide dismutases activity after contact with the pollutant. It was already determined that the response to the oxidative stress is very quick, within a couple of days.

Although the *Phragmites australis* used in this study are being fed with the pollutant within a constructed wetland, it is possible to expand their application to contaminated soils and monitor its degree of pollutant phytotoxicity. On the other hand, the fact that *Phragmites australis* belongs to the Poaceae (gramineae) family like rice, wheat, maize, barley and rye makes this genomic approach a valuable new tool in the monitoring of the food safety.

## Phytoremediation and detoxification of organic compounds in *Phragmites australis*

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*Phragmites australis* is one of the most frequently used plant species for the phytoremediation of organic pollutants and removal of nutrients from waste waters. It possesses excellent salt tolerance, high removal capacity for nitrogen and a remarkable stress defence capacity. Current studies show a high potential of these plants for waste water cleanup. Our aim in the COST Action 859 STSM “Phytoremediation and detoxification of organic compounds in *P. australis*” was to identify key glutathione transferases in this plant with phyto-protective roles relating to the detoxification of major organic xenobiotics in waste water and determine their regulation by chemicals.

Studies in this field have demonstrated seasonal variations in GST activities in the foliage and the rhizomes. In addition, GST activities in *P. australis* were enhanced by following exposure to herbicides and the maize safener benoxacor. To determine which classes of GSTs were enhanced, protein-extracts from benoxacor - treated and untreated plants were analysed by Western blotting using antisera raised against phi (F) and tau (U) class GSTs known to be involved in herbicide metabolism in maize. Based on the recognition of GSTF and GSTU like polypeptides the types and regulation of these enzymes has then been studied using a combination of proteomic and genomic approaches.

## **FIFTH SESSION**

### **“Beneficial or detrimental effects of organics in food”**

Chair: Patricia HARVEY (UK)

Reporter: Umit Baris KUTMAN (TR)



## Genomics as a tool to assess the health benefits of dietary phytochemicals.

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The potential health benefits of consuming a diet rich in fruits and vegetables have been much publicised in recent years. Various associations have been made between particular groups of phytochemicals and protection against major illnesses such as cancers and cardiovascular disease. An understanding of the mechanisms involved and obtaining biomarkers of effect has so far proved extremely difficult. The introduction of ‘omic’ technologies has, however, opened up an alternative means of assessing protective potential in vivo. Whereas past studies were generally focussed on individual compounds or foods, and established biomarkers, this new approach offers a far broader search tool and the possibility of convincing evidence to support consumer advice, and encourage compliance with guidelines.

At the beginning of 2004 COST Action 926 was funded with the title ‘Impact of new technologies on the health benefits and safety of bioactive plant compounds’ and acronym Bioprofit. It’s primary objective is to ensure that maximum benefit is gained from the application of new technologies in cellular and molecular biology to the study of bioactive components in fruits and vegetables, with particular emphasis on their protective effects against chronic diseases. Benefits are seen as improved public health and the increased competitiveness of the food and related industries.

By a variety of dissemination methods it aims to promote scientific collaboration, integrate results, involve the food industry and consumer organisations, evaluate plant-derived food/health supplements and ensure processing methods are optimal and safe. The inaugural meeting was held in February 2004, followed by a major conference in Budapest in the autumn of the same year. A second major conference, entitled ‘Improving the health value of plant foods - phytochemical optimisation’, was held at Egmond aan Zee in The Netherlands between 12-14<sup>th</sup> October 2005. Workshops with integrated Working/Focus group meetings have been held in Prague and Karlsruhe, targeted at specific aspects of the Action. In collaboration with COST 927, a third conference will take place in Vienna between 12<sup>th</sup>-14<sup>th</sup> October this year, to address ‘Molecular and physiological effects of bioactive food compounds’. Particular emphasis will be placed on dietary phytochemicals and human health, from molecular targets to biomarkers of efficacy’.

Eighteen countries are currently involved in the Action and further information on the project and it’s activities can be obtained by visiting the COST 926 website at [http://www.uochb.cas.cz/Zpravy/COST\\_926/](http://www.uochb.cas.cz/Zpravy/COST_926/).

## Genomics-assisted production of plant biopharmaceuticals

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Plants have always been an important source for the discovery of novel pharmaceuticals. New advances in biotechnology make it possible to turn plants into “factories” that produce therapeutically active proteins and industrial chemicals, a technology known as plant molecular farming or biopharming. Today, the production of biopharmaceuticals in genetically modified (GM) plants can lead the way to new drugs without the ethical and technical drawbacks of traditionally expression platforms like mammalian cell lines or the yeast-based fermentation system.

In Germany, research into application of plant biotechnology has largely been concentrated on plant genomics and proteomics to increase the knowledge on plant-related processes, including the development of modern biopharmaceuticals that fulfil future demands. Numerous plant genes encoding enzymes of secondary metabolism have been identified allowing the biosynthesis of medicinally and economically important phytochemicals in transgenic plants. Furthermore, plants and plant cell cultures are used as a general platform for the large-scale production of edible vaccines, antibodies, and therapeutic proteins. However, the commercialization of plant biopharmaceuticals is overshadowed by the uncertain regulatory terrain, particularly with regard to the adaptation of good manufacturing practise regulations to field-grown plants.

BioPlanta has developed a platform technology for the cultivation of in vitro plants based on the temporary immersion system. In bioreactors with fully automated nutrient and gas exchange roots, micro-tubers, leaves or whole plants can be cultured for the production of plant pharmaceuticals following the European GMP guidelines. The efficient usage of the BioPlant system for the production of biopharmaceuticals has been successfully tested in transgenic tobacco. Based on these results, a production capacity per year of 18 tons of plant biomass and 800 grams of recombinant protein, respectively, can be calculated by numbering up the bioreactor modules to a total volume of 20 m<sup>3</sup>. In addition to advances of using plants as expression system the in vitro production of plant-derived biopharmaceuticals is free of contamination with microorganisms, pesticides, and herbicides. With regard to the continued debate over the “safety” of genetically modified plants in Germany, the BioPlant system enables the cultivation of GM plants in a closed environment. No deliberate release of genetically modified organisms is necessary, which yields a high public acceptance.

## Organochlorine pesticide residues assessed in a greenhouse survey in 2005 and a concept of bioavailability experiments

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Organochlorine pesticides (OCP's), such as dieldrin or DDT, were applied world wide against pests in horticulture, fruit and arable crops. Although OCP's were banned 20 years ago, they still persist in soils and are taken up by the plants (Mandl and Lindner 1999). OCP residues in soil, for instance dieldrin, has been detected in high economic value crops (Wyss et al., 2004) such as cucumber and pumpkin (Cucurbitaceae), which strongly accumulate OCP in their fruits. Consumers generally expect that food is safe and uncontaminated, but those who buy organically grown products are particularly concerned about health and thus sensitive to food quality. Therefore, OCP residues in organic food stuff have become a major issue not only for food control authorities, but also for organic farming labels.

In 2005, we performed a survey of OCP contamination in Swiss greenhouse fields on which cucumbers were grown by organic farming. We analyzed the OCP contents of soil and plant samples from 27 sites (6 × 15 m sub-plot each, 20 soil cores bulked to a joined sample, 20 cucumber fruits per sub-plot) from the most important organic producers. For comparison some samples were also collected from conventional Swiss cucumber growers. Samples were analyzed by GC-MS. Fifteen out of the 27 soils were contaminated by OCP's in the range of 0.02 to 1.3 mg kg<sup>-1</sup> (sum of all OCP's analysed). On two of these sites, also the cucumbers contained OCP's at concentrations of 0.02 and 0.04 mg kg<sup>-1</sup>. Dieldrin was detected in eight out of the 15 OCP contaminated soils at concentrations ranging from 0.01 to 0.14 mg kg<sup>-1</sup> (Swiss trigger value for dioxins and furanes<sup>3</sup>: 20 ng I-TEQ (International Toxicity Equivalents) per kg dry substance of topsoil - Verordnung über Belastungen des Bodens (VBBo), vom 1. Juli 1998, 814.12). The data indicated an increased dieldrin uptake by cucumbers if soil pH was < 7 and C<sub>org</sub> was < 5 %. However the sample size of contaminated cucumbers was too small to test for statistical significance. Correlation between sub-plots and bulked whole-field samples indicated that the sub-plots represented the whole greenhouse field very well (R<sup>2</sup> = 0.84, n = 26, p = 0.0001).

In a pot experiment we are currently investigating if dieldrin uptake by cucumbers from contaminated soil (0.07 mg kg<sup>-1</sup>) can be prevented by mixing activated charcoal into the soil prior to planting cucumbers in order to immobilise the contaminant (Mandl and Lindner 1999) and thus to reduce its bioavailability. In order to characterize the bioavailability of dieldrin in soil we are testing the use of Tenax® beads as surrogates of roots in absorbing dieldrin. OCP concentrations will also be measured at various growth stages and after harvest in cucumber samples.

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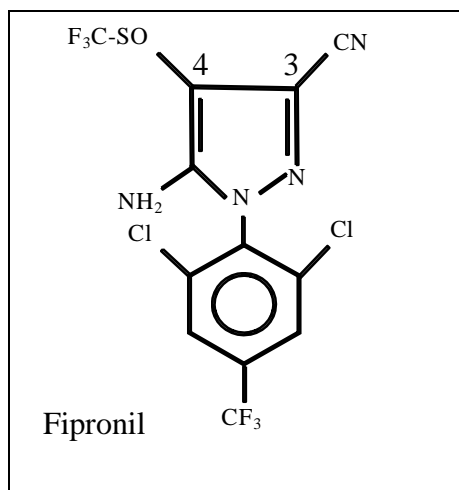
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## Relations between pollen or seed quality and soil pest control in sunflower cultures

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Cedex 09

Several cultivated plants (wheat, corn, sunflower, beets, potatoes) require to be protected against the larvae of insects such as *Agriotes*, which live inside the soil.



Fipronil (5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethyl-sulfinyl-pyrazole) is a powerful phenyl-pyrazole insecticide. This molecule acts on the chloride channel of neurones, inhibiting the GABA control. Fipronil is currently used as a seed-coated treatment (Regent TS) against *Agriotes* sp larvae in maize and sunflower cultures.

Our work demonstrated that non-negligible amounts of the pesticide used as a seed coating treatment penetrate inside the cultivated sunflower plants and is transported into leaves. Afterwards, a lower percent of the active ingredient or of its main lipophilic metabolites can enter the phloem and be transported into flower and reach either the pollen or the developing seeds. Although the amounts of xenobiotics reaching these parts were very low, they might contribute to decrease their dietary quality either for pollinator insects or to mankind.

In order to avoid this, we studied the use of the treated seeds by *Agriotes* larvae and demonstrated that these larvae were strictly depending from the cultivated seeds and seedlings for their feeding which was very selective. As a consequence, we showed that the insecticide-coated seeds could be replaced by granules composed of seed flow mixed with the insecticide, independently from the from the culture time suppressing the possible danger for the pollinators and consumers.



## **SIXTH SESSION**

### **“Food safety and food chain contamination”**

Chair: Juan NAVARRO (ES)  
Reporter: Pascale GOUPIL (FR)



## Genetic studies and QTL mapping for mineral homeostasis in a RIL population of *Arabidopsis thaliana*.

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Large amount of people in the world faces malnutrition, caused by the lack of the micronutrients in diet. Knowledge on the genes controlling the micro- and macro element homeostasis in plants will facilitate the improvement of crops' nutritional value and safety with potentially beneficial effects on human and/or animal health. The orthologues of the identified *Arabidopsis thaliana* genes additionally could be important function in hyperaccumulation in hyperaccumulator plants, which might be used for phytoremediation purposes. As far as minerals are concerned, the iron and zinc deficiency in humans are probably among the largest micronutrient deficiencies.

*Arabidopsis thaliana* is a well-characterised plant species, very amenable for large-scale genetic analysis. The Quantitative Trait Loci (QTL) analysis is an unbiased investigation of genes affecting a certain trait, meaning that genes corresponding to structural as well as regulatory aspects of the process under investigation can be identified.

For this reason, immortal segregating population derived from inter-accession cross of Landsberg erecta (Ler) and Kondara (Kond) has been grown on soil and on hydroponics. Root, rosette and seed mineral content have been analyzed. Large variations for content were observed depending on growth conditions. The QTL indicating the presence of genes with differential effects on cationic micronutrient homeostasis have been identified. The QTL identified in different growth conditions mainly did not collocate, which opened very exciting future research challenges.

## Effects of chloride, sulphate and nitrate on cadmium in rhizosphere soil and its uptake by ryegrass

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Anions ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$ ) form a series of complexes with cadmium depending upon their concentration in soils and thereby they affect Cd availability to plants. A greenhouse study was conducted to investigate the effects of the three anions on Cd uptake by ryegrass grown in a loam soil with a pH value of 4.8 and organic carbon and Cd contents of 3% and  $0.25 \text{ mg kg}^{-1}$ , respectively. Each anion was applied at two levels i.e.  $\text{Cl}^-$  (0 and  $250 \text{ mg kg}^{-1}$ ),  $\text{SO}_4^{2-}$  (0 and  $338 \text{ mg kg}^{-1}$ ) and  $\text{NO}_3^-$  (99 and  $148 \text{ mg kg}^{-1}$ ).

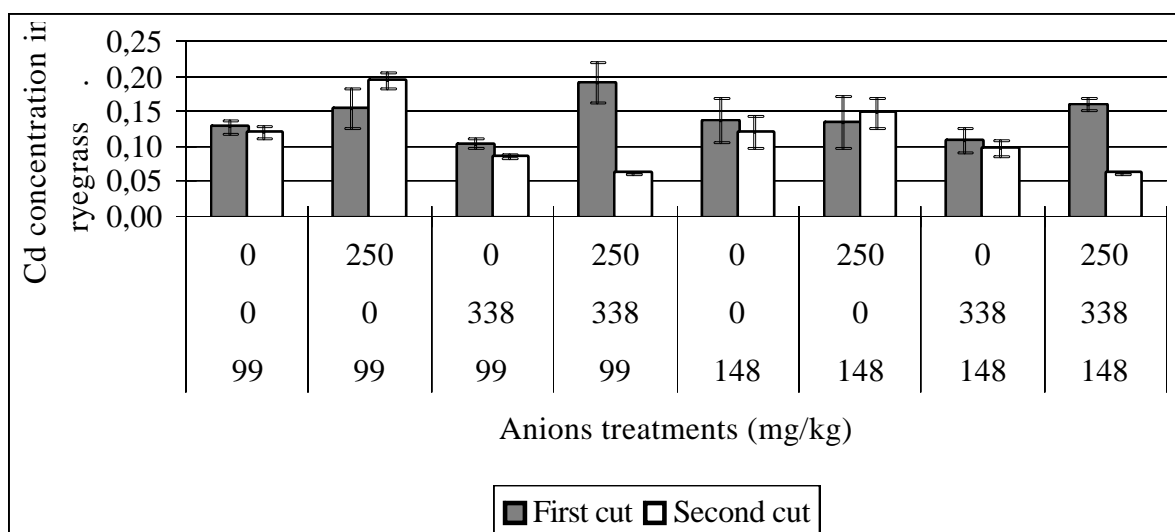


Figure 1: Effect of  $\text{Cl}^-$  (first row),  $\text{SO}_4^{2-}$  (second row) and  $\text{NO}_3^-$  (third row) on the concentration of Cd in ryegrass ( $\text{mg kg}^{-1} \text{ DW}$ ). Mean values of three replicates. Bars denote standard deviation.

The application of  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  resulted in acidification of both bulk and rhizosphere soils but that of  $\text{NO}_3^-$  tended to increase the soil pH. The effect of any single anion was more pronounced at lower concentration of another anion. For example, reduction in soil pH caused by  $\text{Cl}^-$  was greater at lower levels of  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$  application. The three anions affected the  $\text{NH}_4\text{NO}_3$  extractable Cd in bulk and rhizosphere soils differently. The highest Cd concentration in rhizosphere soil was observed at higher level of  $\text{SO}_4^{2-}$  and lower levels of  $\text{Cl}^-$  and  $\text{NO}_3^-$  application. Cadmium concentration decreased with increased application of  $\text{Cl}^-$ , which is quite contrary to results reported previously by other investigators (Boekhold et al, 1999). They found increased Cd concentration in soil in the presence of  $\text{Cl}^-$  because  $\text{Cl}^-$  forms complexes with Cd. At higher level of  $\text{Cl}^-$ ,  $\text{NO}_3^-$  application tended to increase Cd concentration in soils.

Cadmium uptake by ryegrass was increased significantly with  $\text{Cl}^-$  application but it decreased with increased levels of  $\text{SO}_4^{2-}$  and was little affected by  $\text{NO}_3^-$  application. Increased plant

uptake and decreased extractable Cd in soils by  $\text{Cl}^-$  application suggest that plant are able to take up different Cd/  $\text{Cl}^-$  complexes because either these complexes are more diffusible in soils or more plant available (Smolders and McLaughlin, 1996).

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## Arsenic, lead and cadmium uptake and distribution in broccoli, cauliflower and radish plants grown on contaminated soil

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Arsenic, lead and cadmium uptake by cauliflower (*Brassica oleracea* L. var. botrytis L.), broccoli (*Brassica oleracea* L. var. italica Plenck) and radish (*Raphanus sativus* L.) plants grown in contaminated soil was studied. The main objectives of this work were to study the distribution of the accumulated As, Pb and Cd in root, shoots and edible parts of the plants, and also, to establish whether these element concentrations in edible parts of these vegetables are potentially dangerous to human health. The results presented demonstrate that there is not a risk associated with consumption of cauliflower, broccoli and radish contaminated with As and Cd, grown on the conditions of this work. In contrast, the levels of Pb found in the edible parts of broccoli varieties were above the limits considered as safe for human consumption.

## Transfer soil/grassland on Ni-Cr rich soils and derogation of French regulation of sewage sludge spreading

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The region of Auvergne is characterised by a wide geological diversity leaving it in a high content of metallic trace element (MTE) of agricultural land. Particularly, large areas on volcanic soils are enriched in Ni and Cr (8000 Km<sup>2</sup> of cattle pasture soils) superior to 50 and 150 ppm from French regulations of sewage sludge. However, a derogation of interdiction of sewage sludge spreading is possible with the environment study showing that the MTE aren't mobile and bioavailable. The project managed from 2002 to 2005 has characterized the transfer of MTE from soil to native and spreading grassland. So, a MTE of content base has been established from different species of grassland in function of development stages, of environment conditions (soil, climate) and of agricultural practices (number of cuttings, nitrogen fertilization) located on seven sites. In the second time, the results have permitted to propose the approach of derogation demand and the reference table of MTE contents beyond of theses the spreading isn't authorized. The studied sites will be in the future the observatory of the derogation approach which require the plant monitoring during ten spreadings according to French “precautionary principle”.



## **SEVENTH SESSION**

### **“Omics and xenobiotics: practical agronomic issues”**

Chair: Michel MENCH (FR)

Reporter: Henrique GUENDES-PINTO (PT)



## Influence of activated carbon on soil fertility and quality of crops grown in contaminated soil

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Previously we demonstrated that the use of activated carbon could substantially extend application of bio- and phytoremediation for soils contaminated with organic chemicals [Vasilyeva et al., 1996; Vasilyeva et al., 2004]. The adsorbent decreases soil toxicity for degrading microorganisms and plants that creates favorable conditions for mineralization of degradable contaminants (e.g. herbicide propanil and chloroanilines) or strong binding of some persistent contaminants (e.g. 2,4,6-trinitrotoluene – TNT, and polychlorinated biphenyls - PCB). Simultaneously the activated carbon restricts migration of the contaminants in the environment that permits to carry out in situ soil remediation. The activated carbon is highly porous adsorbent having high capacity to adsorb many organic contaminants. Having highly microporous structure the activated carbon can influence on soil fertility. The main purpose of this presentation is to outline the influence of the activated carbon on soil properties and quality of crops grown in contaminated soils to determine the best forms and doses of the adsorbent which do not decrease soil fertility.

The experiments were conducted under the laboratory and microfield conditions with three types of soils as well as with granulated activated carbon (GAC) and powdered activated carbon (PAC) “Agrosobrb” (Russia). It was demonstrated that soil amendment with the GAC (up to 0,1 or 10% w/w) decreased crops contamination and practically did not reduce soil fertility. It increased total soil porosity and water holding capacity to some extent. On the contrary, the PAC contains a substantial fraction of very fine particles ( $<1 \mu\text{m}$ ) to soil organic matter. At high doses of PAC (more then 1 or 10% depending on soil type) the surface of soil microparticles becomes highly hydrophobic, and metal bridges between soil organic matter are destroyed, thus destructing soil aggregates. As a result the volume of water carrying pores, as well as soil bulk density and water permeability are reduced, that creates unfavorable conditions for plant growth.

It was also indicated that introduction of activated carbon progressively increased soil pH (up to pH 6,5-7,5 depending on dose) and the amount of exchangeable phosphorous. At the same time the introduced adsorbent almost did not change the amount of exchangeable ammonium, nitrate and potassium. Thus, the activated carbon did not influence negatively on the availability of macro fertilizers to plants.

Finally it was concluded that amendment of the contaminated soils with the GAC (up to 5 or 10%) reduced bio- and phytotoxicity as well as contamination of crops and simultaneously it did not decrease soil fertility while the increased doses of PAC (1 or 5%) might substantially decrease soil quality. Therefore GAC is more preferable adsorbent in compared to PAC for the purposes of bio- and phytoremediation of highly contaminated soils.

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## Organic contaminants from sewage sludge applied to agricultural soils – False alarm regarding possible problems for food safety?

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The large amounts of sludge produced during wastewater treatment have to be taken care of by the society. Previously, sea dumping was a common fate of sludge. As sea dumping of sewage sludge became more and more banned other methods for disposal had to be found, and, as a result, increased in their use. At present, there are mainly three disposal methods for processed sludge: agricultural application as fertiliser, land filling and incineration (Figure 1).

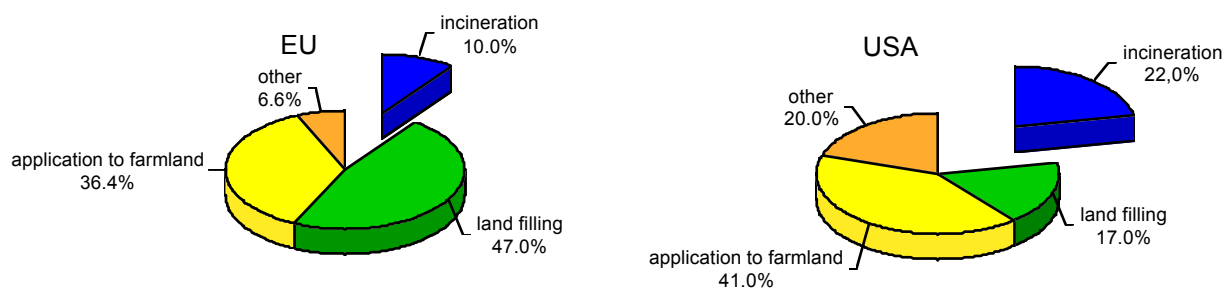


Figure 1: Disposal and application of sewage sludge in Europe and the US (Renner 2000)

Sewage sludge produced in wastewater treatment contains large amounts of organic matter and nutrients and could, therefore, be suitable as fertiliser. However, with the sludge, besides heavy metals and pathogenic bacteria, a variety of organic contaminants can be added to agricultural fields. Whether the organic contaminants from the sludge can have adverse effects on human health and wildlife if these compounds enter the food chain or groundwater still remains a point of controversial discussion. The amount of sewage sludge produced will be increasing in Europe in the future. Application of sewage sludge to agricultural soils is sustainable and economical. However, this solution also involves risks with respect to the occurrence of organic contaminants and other potentially harmful contents such as pathogens and heavy metal present in the sludge. There have been concerns that organic contaminants may accumulate in the soil, be taken up by plants and thereby transferred to humans via the food chain, but studies hitherto have revealed no immediate risks. However, conclusions have to be drawn carefully, as the studies done until now have been limited. This presentation provides a brief overview on the present situation in Europe and a summary of some recent results on the possible uptake of organic contaminants by crops after addition to agricultural fields by sewage sludge. There are environmental, political as well as economical incentives to increase the agricultural application of sludge. However, such usage should be performed with care as there are also

ways in which sludge fertilisation could harm the environment and human health. Further studies are required, including a larger variety of common crops and additional organic contaminants before final statements can be done.

## Improving the mineral content of wheat: A breeder point of view

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The improvement of the nutritional value of bread or cereal product is a complex task requiring the optimisation of the micronutrient contents of wheat and their bioavailability in derived food products. The aim of the present project was to explore and valorise the intrinsic nutritional qualities of wheat, especially mineral contents. An integrated program was initiated to increase the overall mineral content of wheat by a synergy between plant breeding and production schemes.

In order to estimate the improvement potential of wheat mineral content it was necessary to quantify the biodiversity of the targeted traits. The study of an international core-collection confirmed a large difference in mineral composition within varieties of wheat. No segregation of the mineral contents based on the geographical origin of the seeds was revealed. The variability observed in the French adapted cultivars was only slightly narrower than that in international accessions. For a breeding objective this variability (up to 5-fold variation) is highly significant.

No competition was observed between the different nutrient contents. An improvement in the wheat nutritional quality could then be envisaged without compromising any essential mineral targets. However a strong negative correlations were observed between most of the minerals and yield. An encouraging consequence of this observation is that mineral contents were significantly correlated with protein content and so with technological value. But this is a critical point for the economical viability of nutritional innovation.

The determinants of mineral content variability were of differing nature. More specifically breeding for higher grain magnesium content should be successful considering the wide biodiversity for this criterion and the small Genotype-Environment interaction measured. Improving zinc content could be more difficult because of the weaker genotype influence and the stronger GxE interaction. Traditional breeding for iron seed content appears almost impossible due to the strong GxE interaction and consequently the low genotype effect on this criterion.

Traditional breeding should then be able to produce varieties of nutritional interest while maintaining good agronomic and technological value. Some crosses have already been initiated between genotypes of interest and performing varieties. Rare alleles have been identified by systematic study of germplasm diversity. They are now used in the genetic identification of relevant molecular markers, necessary for efficient introduction of nutritionally correlated chromosome segments in a genetic base of good agronomic and end-use criteria.



## **POSTER SESSION**

*(Posters are listed according to author's alphabetical order)*





## Effect of cadmium on polyamine content in *Brassica juncea* seedlings

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Heavy metal contamination is one of the major environmental stresses that can affect plant metabolism and their toxic effects in soils are the results of mining, industrial and agricultural activities. Cadmium is one of the most toxic metals to both animals and plants. Polyamine metabolism is altered under several environmental stress conditions such as osmotic stress, salinity, heat, chilling and UV radiations, but there is scarce knowledge about the effect of heavy metals on this metabolism.

The purpose of this work is to establish the relationship of Cd accumulation to polyamine content and to describe some of the metabolic changes in plants caused by Cd administration.

Indian mustard (*Brassica juncea*) seedlings were raised in vermiculite with Hoagland nutrient solution, under a 16h photoperiod, at 22°C. After 2 weeks, seedlings were transferred to a liquid medium and treated with CdCl<sub>2</sub> (0, 100, 250 and 500µM) added with MES buffer (1mM) for 7 days.

Growth: after 3 weeks, the fresh and dry weights of the different organs were measured.

Pigments: chlorophylls a, b and carotenoids were determined following the method of Lichtenthaler (1987).

Polyamines (PA): free PA were extracted in HClO<sub>4</sub>, and dansylated. Dansylpolyamines were determined by HPLC according to the procedure of Flores and Galston (1982), slightly modified (Le Guen-Le Saos, 2001).

Exposure for 7d of Indian mustard seedlings to 500 µM CdCl<sub>2</sub> caused a 57% decrease of the fresh weight and a 20% increase of the dry weight of the different parts of the plants. Growth parameters were not affected by 100 or 250 µM CdCl<sub>2</sub>. The Cd<sup>2+</sup>-exposed plants showed no significant differences in chlorophyll a, chlorophyll b and carotenoid contents neither was the chlorophyll a/chlorophyll b ratio (2.85) affected. Analysis of polyamines showed that spermidine was the predominant polyamine in all parts of the plants and Cd<sup>2+</sup> treatment induced a large increase in putrescine content, dependent on the Cd<sup>2+</sup> concentration. Plants exposed to 500 µM Cd<sup>2+</sup> contained 3 to 4 times more putrescine in their leaves and stems than did controls. Putrescine accumulation was accompanied by lower increases of spermidine and spermine.

The data obtained under our experimental system are indicating that PA might be implicated in the protection response to Cd<sup>2+</sup>-induced stress.

## Status of microsomal detoxification enzymes (Cytochrome P450 and Glutathione-S-Transferase) in yam bean (*Pachyrhizus erosus* (L.) Urban)

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Food safety depends highly on the possible metabolization of pesticides in crop plants. The present study investigates the status of detoxification enzymes in the membrane fraction of the so far underexploited crop, *Pachyrhizus* (Yam bean). This plant, originating from Southern America, conquers numerous food production areas because of its rapid growth, high yields and low fertilizer demands. However, information about pesticide metabolism in this species is lacking.

Cytochrome P450s and glutathione-S-transferases (GSTs) constitute two of the largest groups of enzyme families that are responsible for detoxification of exogenous molecules in plants. Their activities differ from plant to plant with respect to metabolism and substrate specificity which is one of the reasons for herbicide selectivity. In the tuber forming yam bean, the legume *Pachyrhizus erosus*, their activities at the microsomal level were investigated to determine the detoxification status of the plant. The breakdown of the herbicide, isoproturon to two distinct metabolites, 1-OH-IPU and Monodesmethyl-IPU was demonstrated.

Furthermore, microsomal GST activity was determined with model substrates, but also by the catalysed formation of the fluorescent glutathione bimine conjugate. This study demonstrates for the first time microsomal detoxification activity in *Pachyrhizus* and the first fluorescence image description of microsomal GST catalysed reaction in a legume.

## Morphology and chlorophyll content of *Brassica napus* regenerated in vitro from tTCLs in the presence of zinc

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Pollution by metals such as zinc is very common. Zinc toxicity is associated with both the blockage of xylem elements and the inhibition of photosynthesis. Among different methods used to remediate polluted soils, phytoremediation appears cost effective to address recalcitrant environmental contaminants [1]. Ideally, hyperaccumulator plants should be cultivated for that purpose but only few of them yield a high biomass. The objective of this work is to produce variants or mutants, regenerated from tissue culture, modified for main factors controlling the uptake, transport and accumulation of pollutants in crop plants [2]. The genotypes also modified for heavy metal (HM) content (hyper or hypo accumulators) will also provide a useful tool to achieve a better understanding of the mechanisms involved in this complex phenomenon.

In this study, rapeseed var. Drakkar was selected for its high yield in biomass and its appartenance to the Brassicaceae family. Furthermore, various genotypes are easily available and this crop belongs to the same genus of several hyperaccumulator species. The plant was putatively modified for Zn tolerance and accumulation through in vitro selection: a selective pressure was applied during a fast neoformation process developed from transversal Thin Cell Layers (tTCLs) to select tolerant cells and tissues. The explants were cultivated directly in the presence of Zn at different concentrations (up to 500  $\mu$ M). The regenerated shoots were acclimatised; the survival plants were transferred in pots containing sterile peat and cultured in greenhouse. The measurement of growth parameters (fresh and dry weights) and pigment content were carried out after one month.

The concentration of Zn applied during the neoformation process influenced significantly both morphological and physico-chemical characteristics of plants cultivated in greenhouse. Thus, plants regenerated in the presence of Zn at 100  $\mu$ M exhibited greater size and higher biomass combined with a great precocity in flowering. The very first analyses show modification in chlorophyll contents.

The outcome of the present study encourages further researches in the use of in vitro regenerated plants, in combination with mutagenesis, to create innovative lines of hyperaccumulator plants. Their aptitude, to accumulate Zn will be compared with that of normal plants cultivated in the presence of different Zn concentrations. Afterwards, the present investigations should be extended to other heavy metal-plant combinations.

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## Poplar under ozone stress: a proteomic approach

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In the last century, tropospheric ozone concentration has largely increased. In the northern hemisphere, mean ozone concentrations average around 60 ppb ( $\sim 120 \mu\text{g}/\text{m}^3$ ), against only 10 ppb in the year 1900. On hot summer days, concentrations can even increase to 160 ppb or more, as has been observed in past summers. The cause of this increased ozone pollution is the advancement of industrialization, which increased NO<sub>x</sub> levels in the atmosphere. Tropospheric ozone is created by the reaction between oxygen and UV activated NO<sub>x</sub>. These increased ozone concentrations have negative effects on human health, but also on crop and forest plants.

Up until now, research on ozone stress on plants was mainly based on morphological or physiological aspects, or on the resemblance of ozone stress to other stresses. Indeed ozone seems to activate similar pathways than pathogen attack. But some mechanisms are specifically activated by ozone, like the anaplerotic pathway of PEPc.

In this work ozone stress was studied on hybrid poplar in growth chambers. Plants were fumigated at 120 ppb ozone, a high concentration that is not yet considered as peak, but chronic. Samples were collected after 3, 14 and 35 days, followed by a recovery period of 10 days. Two leaf levels were considered according to their developmental status at the beginning of the treatment, namely adult leaves and young leaves.

Proteins were extracted with TCA/acetone, preceded by a solubilization step, then labeled with CyDyes DIGE<sup>®</sup> fluorochromes by GE Healthcare. First dimension was carried out on 24cm pre-cast IPG strips by GE Healthcare with a pH gradient of 4-7. Two samples (one ozone, one control) and an internal standard (mix of all the samples) were loaded per strip. Second dimension was run on SDS-PAGE.

The analysis has been done by comparing treatment, leaf level and harvest date.

Gel analysis with DeCyder<sup>®</sup> software by GE Healthcare has shown that differences in protein expression are present. Protein identification is, at this date, still in progress.

## The genomics of detoxification in *Phragmites australis*

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Although widely used in phytoremediation, very little is known about the molecular mechanisms underlying *Phragmites australis* detoxification of xenobiotics. This Poaceae reed is currently being studied in our lab to elucidate its role in the removal of Acid Orange 7 (AO7), a model soil and effluent contaminant. For this purpose, several known genes related to oxidative stress response enzymes in the Poaceae family were aligned, as no genes for *Phragmites* have been sequenced or described. This was used to design primers that could identify and follow up the expression genes such as those of peroxidase, catalase and superoxide dismutase in *Phragmites* plants subjected to AO7. Preliminary results show that there is indeed a difference in gene expression when subjected to xenobiotics stress, at either the leaf or root level, of some of the above enzymes, and sequencing of the DNA already yielded positive identification for at least one catalase involved in the process. These studies will enable not only the understanding of oxidative stress response mechanisms in *Phragmites* but will also allow the prediction of soil decontamination using other Poaceae, as well as the assessment of safety in crop effluent contamination through the efficiency of detoxification in plants belonging to this family. As the Poaceae account for about 70% of the calories ingested either directly or indirectly by humans, these studies will be of major importance for the understanding and management of food and crop contamination and their mechanisms of detoxification, thus leading to an improvement in food safety.

## Effects of AtPCS1 overexpression on cadmium tolerance and accumulation in tobacco and Arabidopsis plants

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Keywords: PCS1overexpression, tobacco, arabidopsis, cadmium tolerance, cadmium accumulation

Phytochelatins (PCs) are thiol peptides involved in heavy metal tolerance and detoxification in plants. PCs are synthesized enzymatically from reduced glutathione (GSH) and the reaction is catalyzed by a transpeptidase, phytochelatin synthase (PCS). It was reported that in Arabidopsis AtPCS1 overexpression confers Cd hypersensitivity but increases Cd transport from roots to shoot (1, 2).

We overexpressed the Arabidopsis phytochelatin synthase gene (AtPCS1) in the non-accumulator plant *Nicotiana tabacum*. We transformed wild-type plants and plants harbouring the oncogene *rolB*, that induces an expansion of the root system (3). We verified cadmium tolerance and accumulation in relation to the level of PCs and glutathione. We demonstrated that overexpression of AtPCS1 increased PC content and enhanced Cd tolerance of *rolB* roots and of *rolB* and wild-type seedlings. This effect was greatly enhanced when reduced glutathione was added to the culture medium. An increased Cd accumulation was also observed in roots and shoots of seedlings and adult plants, matched by a higher production of PCs in both organs and also dependent on GSH supply. However plants overexpressing AtPCS1 show the same ratio of Cd between roots and shoots as in wild-type plants. We conclude that overexpression of AtPCS1 in tobacco plants causes an increase in Cd tolerance and accumulation directly related to the availability of GSH. In contrast Cd translocation seems to be independent of AtPCS1 overexpression (4). Currently we are performing tolerance and accumulation experiments with arabidopsis plants overexpressing AtPCS1, at the same experimental conditions used for tobacco plants.

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## Preliminary results of OMC use for elimination of organic/inorganic soil contaminants

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The soil compartment has a great potential to a considerable reduction of bioavailability of organic/inorganic pollutants. To improve natural capability of soil, to decrease availability contents of contaminants and to prevent their input into plant production is possible by using organo-mineral complex (OMC). OMC should combine in synergic effect of natural materials, clay mineral (zeolite-clinoptilolite) and organic matter (humic acids (HA)), both natural products with excellent sorption properties. Organo-mineral complex containing ca 5 mg HA/ g of zeolite possessed the best retention ability towards heavy metals (Cd, Cu) and some organic pollutants (Barančíková et al., 2004).

OMC was used to increase retention capacity of soil to organic pollutant pentachlorophenol (PCP). Sorption experiments were carried out on three soil types: Calcaro-Haplic Chernozem, Gleyic Fluvisol and Arenic Regosol. The best results – the highest adsorption and retention - were obtained at soil with addition of OMC (ca. 75 % adsorption and retention). Soil with addition of pure zeolite showed values of desorption comparable to or slightly higher than that observed with the pure soil. Desorption of PCP (calculated from the adsorbed amount) reached in all types of soil with the addition of OMC lower values than in soil alone and in soil with zeolite, thus indicating that OMC had a better adsorption/desorption ratio.

The results obtained in our study indicate that OMC applied into soil (mixed with soil) has better retention abilities in comparison to the clay minerals or soil alone.

In our previous work was found, that organo-mineral complex containing 5 mg HA/ 1 g zeolit has also suitable retention capacity to heavy metals (Makovníková et al., 2004). In this paper preliminary results of OMC application in a green spot experiment are showed. The efficiency of OMC was investigated under control conditions on two soil types, Fluvisol and Cambisol, cropped with Spring Barley (*Hordeum vulgare*). Preliminary results of soil samples after harvest show decrease of Cd and Pb mobile forms in both soil types in treatment with OMC in comparison to soil alone. The highest decrease of Pb mobile content in treatment with CaCO<sub>3</sub> and OMC addition on Cambisol was found. Obtained results can provide reasonable base of successful application of organo-mineral complex in in-situ remediation technologies.

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## Physiological and genotoxical responses to arsenate and arsenite stress of two species (*Vicia faba* and *Zea maize*)

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The element As is often found in the natural and polluted sites of French Massif Central. The aim of present study is to determinate the physiological and genotoxical responses of *Vicia faba* and *Zea maize* exposure to environmental arsenic. The effects of two inorganic forms of As (AsV: As<sub>2</sub>O<sub>5</sub> and AsIII: AsO<sub>3</sub>) are studied with the increased concentration As ( from 134 to 668 µM) at three pH (4, 7 and 9). The As exposure involves the physiological modifications of the *Vicia faba* root system. After 42 hours of exhibition, arsenic treatments lead to a change of colouring of the roots of *Vicia faba* and also an inhibition of the roots growth. Also, the roots are more dark with increasing arsenic concentrations. At the same concentration, AsIII is more toxic on the physiological parameter than AsV. The genotoxical response is studied by the micronuclei frequency test. The two forms of As are different by the valence and the toxicity, AsIII is more cytotoxic and less carcinogenic than AsV. However, the two forms of As treatment involve chromosomic aberrations and genotoxic effects on the roots of two species. If a relation exist between As concentration and the number of micronuclei, the test of micronuclei can't use as an indicator of soil As concentration.

## Use of visible and near-infrared spectroscopy as a rapid, clean and low cost technique to screen metals(oïds) in plant matrices

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Near-infrared spectroscopy (NIRS) has been applied for decades to the analysis of agro-food products, and in recent years its use has been extended to the determination of mineral species and trace elements in organic and inorganic matrices. The NIR spectrum contains physical and chemical information of the product being analyzed. The spectral information has its origin in the different vibrational modes of the molecules caused by their interaction with the electromagnetic radiation absorbed at wavelengths between 750 and 2500 nm. The use of chemometrics allows the relevant information contained in the NIR spectra to be extracted, to develop calibration models that permit the prediction of the composition of unknown samples. This technique is rapid and, in contrast to the standard techniques of analysis, can be performed at a low analytical cost and without using chemicals. In addition, those error sources related with the laboratory analysis are avoided. The control of those sources of error specific of the NIR analysis leads to equations of high accuracy and precision. The application of NIRS to the determination of arsenic, lead, copper and zinc in wild (*Amaranthus blitoides*) and cultivated (*Brassica juncea*, *Oriza sativa*) plant species has revealed its potential in the screening of these elements in plant matrices.

## Biomass, soil properties and the metallome of green plants: a network which can be analyzed for food quality and phytoremediation

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Like other organisms (e.g. fungi), green plants use organic compounds which behave as chelating ligands to extract essential metal ions such as Mg, Fe or Zn from soil or groundwater; other kinds of ligands are used for transport within the plant and fixation in sprout or photosynthetic organs. Though all of these ligands display some selectivity (even simple ones like citrate), there is sometimes substantial co-extraction of other metals which are either non-essential or even outright toxic to either plant or man. In the last case, this is a precondition for phytoremediation given the plants can be grown and harvested efficiently on polluted soils. Hitherto, hyperaccumulators were identified mainly by analysing plant samples, neglecting the information which can be obtained from the ligands directly, be the latter secreted into soil water or contained in the plant. For this purpose, an empirical relationship was determined which links stabilities of complexes – and thus potential for extraction or bioconcentration, respectively – to a property which in turn describes the principal ligand sites, whether bound to simple molecules or to polymers (proteins etc.). The relationship is:

$$-\log k_{\text{diss}} = x \times E_L(L) + c \quad [1]$$

where  $E_L(L)$  means the electrochemical ligand parameter (LEVER 1990),  $-\log k_{\text{diss}}$  refers to complex stability and  $c$  and  $x$ , respectively, denote intercept and slope of the regression equation. Fungi, with metal demands different from green plants, produce other ligands, having substantially higher  $E_L(L)$  than green plants and thus another selectivity. Alkaloids in hyperaccumulator “milk” and phytochelatinines on the other hand differ from both, shifting accumulation behaviour into different directions. These directions (trends) can be seen in the diagram (fig. 1) produced from eq. [1] and a list of  $c$ - and  $x$ -values calculated (abridged version: table 1). Going beyond the cases where  $E_L(L)$  is known because the ligands involved are well-defined (oxalate, amino acids, hydroxamates [bacteria, soil fungi]), an effective value of  $E_L(L)$  can be determined by comparing bioconcentration ratios of several different metals to the  $c$  and  $x$  values. The bandwidth of the corresponding values is large but of course smaller than the difference between extremely donating vs.  $\pi$ -backbonding ligands. Eventually, ligand properties of the soil can be determined along the same method. Together they control the efficiency of extraction. Phytoremediation of a certain metal can then be planned, knowing  $c$  and  $x$ , by selecting some plant (or fungus) which grows on the corresponding substrate and provides optimum affinity for the metal to be removed.

Work on effects of ligand transformation (derived from decomposition of leaf litter etc.) on shaping of plant societies (succession) along specific demands is in progress. This work includes direct determination of  $E_L(L)$  for ligands involved in soil/plant metal transfer by electrochemical methods (cyclic voltammetry of their Ru(III) complexes).

## Expression of $\gamma$ -ECS and Ycf1 in tobacco influences metal uptake

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Phytoremediation can decrease the availability of toxic compounds in moderately heavy metal contaminated soils. Plants that hyper accumulate metals in their above ground parts are often small in size. Combining the characteristic of high biomass production of tobacco with specific genes, responsible for high uptake of metals in hyper accumulators (e.g. *Thlaspi caerulescens*), can improve phytoremediation of moderately contaminated soils. In the present study we expressed the *Escherichia coli*  $\gamma$ -ECS enzyme (Noctor et al., 1996) and the YCF1 gene of *Saccharomyces cerevisiae*, an MgATP-energized vacuolar glutathione S-conjugate transporter (Ze-Sheng Li et al., 1996), under the 35 S cauliflower mosaic virus promoter, in *Nicotiana tabacum* (SR1) and double mutants are being established. The transgenic single and double mutants will be compared to WT tobacco with respect to their metal accumulation and tolerance.

Tobacco has been transformed using leaf disc transformation. Expression levels are confirmed by RT-PCR. WT and transformed plants will be phenotyped for metal tolerance and accumulation using hydroponics supplemented with heavy metals (Cd, Zn, Cu and Fe). We expect to find a higher amount of accumulated metals in the single over-expressing mutant lines. We hypothesize that the double mutants will show an even higher metal uptake compared to the single mutant lines.

We thank Dr. Lise Jouanin for providing us the construct containing the *E. coli* gene *gsh1* and Professor Youngsook Lee for providing us with the construct containing the *S. cerevisiae* gene YCF1. Furthermore we thank ‘Project bureau ABdK’, Eindhoven, The Netherlands for their funding.

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## Integrated Data Base of countermeasures implemented on radioactively contaminated lands and areas of its application

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The data base “Countermeasures” has been developed and completed on the base of experience of the elimination of Chernobyl consequences in agriculture. The main goal of our investigation is the collection and processing of the accumulated after the accident information for compiling a database (DB) on countermeasures in agriculture. The assessment of efficiency of the countermeasures applied on natural and arable lands, has been done. The reduction of radioactivity in final product is the main evaluative criterion of countermeasures efficiency.

DB represents a storage of data with possibility to search the information, to make queries and reports. Suitable forms are created for input, edition and preview of the information. DB contents 1328 records on the “Countermeasures in plant production” and 1220 records on the “Countermeasures at meadow ecosystems”. Separate record of DB includes the information about experimental application of countermeasure directed to decrease the accumulation of radionuclides in agricultural crops and has been collected from 1987 year to present day.

DB integrates 10 denominations of countermeasures, mainly soil ameliorations (different doses and combinations of mineral and organic fertilizers, lime, clayey minerals, and radical improvement); 24 denominations of agricultural crops (cereals, vegetables, potato, forage crops and natural grasses); 4 prevalent types of soil (Podzoluvisol, Arenosol, Fluvisol, Histosol of different texture, moisture and physical-chemical properties).

Thus, presented DB is potentially applicable in the wide fields. Such DB serves the foundation to provide support in making practical decisions in the field of radiation safety of the population in the situation of radioactive contamination of the environment. The DB can be used for choosing of the most effective and acceptable protective measure considering the specific conditions of contaminated areas such as soil type and level of contamination. As well as the DB can be useful for researches and education.

The evaluation of countermeasures efficiency, comparative analysis and prediction of the radionuclide content in plant and animal products after countermeasures application is possible to do on the base of DB components.

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## Overexpression of a *Thlaspi caerulescens* metallothionein modifies the tolerance and accumulation capacities of heavy metals in plants

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A functional screening in yeast allowed to identify various cDNAs from the Cd/Zn hyperaccumulator *Thlaspi caerulescens*, that were able to improve growth on toxic Cd concentrations. The most isolated cDNAs were those encoding metallothioneins, which are cysteine rich small proteins (for review: Cobbett and Golsbrough, 2002). Among these, MT3 was the most represented. A first characterisation of the TcMT<sub>3</sub> cDNA was published (Roosens et al., 2004; Roosens et al., 2005) and showed a variation in the amino acid sequence compared with its closest homologue in *A. thaliana*, in particular in Cys residues positions. Expression in the yeast support a capacity for TcMT<sub>3</sub> to bind Cu, Cd and to lesser extend Zn. Moreover, MT3 was highly overexpressed in the shoot of *T. caerulescens*, compared to *A. thaliana* and induced by copper.

Further characterisation was achieved by expressing TcMT3 in *N. plumbaginifolia*. Transgenic lines were obtained but none of them had a high level of TcMT3 expression, comparable to the one in *Thlaspi caerulescens*.

35S::TcMT3 plants showed a delayed growth in normal conditions, a decrease in fertility suggesting that the overexpression of TcMT3 may be prejudicial in normal condition. In strong support to this observation, lipid peroxidation in plants in control growth conditions was higher in 35S::TcMT3 plants compared to transgenic controls or wild type. Furthermore 35S::TcMT3 tobacco plants showed an improved growth (higher biomass and higher root length) with the addition of concentrations of Cu or Cd that were highly toxic for the wild type, compared to control conditions. Actually plants look really healthier in Cd or Cu contaminated media. Accumulation of heavy metals seemed to be also affected. Interestingly, a similar phenotype was also observed in 35S::TcMT3 *Arabidopsis thaliana* plants.

The basis of this phenotype is currently under investigation and will be presented.

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## The Arabidopsis protein AtOSA1 localized in the chloroplast responds to cadmium and oxidative stress

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Analysis of gene expression in *Arabidopsis thaliana* using cDNA-microarrays showed that AtOSA1 (*A*rabidopsis *O*xidative *S*tress related *A*bc1 like protein) transcripts are affected by Cd<sup>2+</sup> treatments. The protein sequence of AtOSA1 reveals the presence of an Abc1 domain. Proteins exhibiting such a domain are not related to ABC transporters. Abc1-like proteins have been identified in prokaryotes and eukaryotes. In eukaryotes they have only been identified in mitochondria. AtOSA1 is the first member of this family to be localized in the chloroplasts. Compared to the wild type a AtOSA1 T-DNA-insertion mutant has an increased sensitivity towards oxidative, light and cadmium stress, and exhibits an elevated overall superoxide dismutase activity. AtOSA1 was not able to complement *Saccharomyces cerevisiae* strains with a non-functional mitochondrial ABC1 gene indicating that it mediates different function(s). Indeed when expressed in a cadmium-sensitive yeast strain, AtOSA1 was able to restore cadmium tolerance. Our data suggest that the chloroplast AtOSA1 protein is involved in response to reactive oxygen species as well as cadmium treatments. A possible mechanism of action will be discussed.

## Variation in zinc deficiency tolerance and zinc accumulation in seeds in a RIL population of *Arabidopsis thaliana*

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Zinc (Zn) deficiency is a worldwide problem which results in significant losses in crop yield and has detrimental effects on quality. It is also a well-documented nutritional problem in human beings, especially in the developing world, leading to severe health complications such as mental and growth retardation, delayed sexual maturation, immune disorders, dermal problems, impaired wound healing and anorexia. Any effort aimed at improving Zn concentration in grain will, therefore, greatly contribute to human health and also crop production globally. Significant genotypic variations in Zn deficiency tolerance and Zn accumulation in seeds are known to exist among different cultivars of major crop plants. These natural variations are being utilized in conventional and modern breeding programs, but there is a lack of knowledge on molecular mechanisms affecting Zn accumulation in grain and tolerance to Zn deficiency. In this research, we initiated a large screening study by using an *A. thaliana* recombinant inbred line (RIL) population, which was developed from a cross between “Landsberg erecta (Ler)” and “Cape Verde Islands (Cvi)”, to evaluate the genotypic variation of Zn accumulation in seeds and Zn deficiency tolerance on a Zn-deficient calcareous soil from Central Anatolia in the greenhouse. Preliminary results indicated that there was a significant variation among the parents and RILs. This variation will be used for the identification of region(s) (QTLs) responsible for zinc deficiency tolerance. Physiological data, being collected, are based on dry weights and Zn concentrations of whole plants during the flowering stage and in mature seeds. The impact of Zn-deficient soil conditions on the growth and development as well as the seed Zn accumulation of each RIL will be presented at the meeting.

## Alleviating effect of silicon on cadmium toxicity in hydroponically cultivated maize

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Silicon is not considered as essential element for plants, but for many species including some important crops it is beneficial. Silicon can benefit plant growth through greater yields and it also can improve the resistance of plants to various abiotic and biotic stresses. The increased tolerance of several plant species was found to toxic elements as Mn, Al and Fe. More recently an alleviating effect of silicon was shown to cadmium in some species. In the present study the effect of silicon on Cd toxicity was studied in hydroponically cultivated maize seedlings.

Maize (*Zea mays* cv. Jozefina) was grown in Hoagland solution without Si (control), with addition of Si (in the form of sodium silicate solution) (Si+), with addition of cadmium in the form of  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  in concentration 5  $\mu\text{M}$  (Cd+) and with addition of Cd and Si in the same concentration as in the previous treatments (Cd+Si+). Solutions were changed every other day and pH was adjusted to 6.2. Plants were grown till the stage of second fully developed leaf in the growth chamber.

The effect of the treatments was compared on growth parameters, fresh and dry weight. In Si+ treatment several growth parameters were positively influenced comparing with the control. The Cd treatment resulted in reduced growth of shoot and root. The alleviating effect of silicon on cadmium toxicity in Cd+Si+ treatment was evident in both root and shoot. The content of cadmium was determined in plant parts and compared between the treatments. Additionally structural comparison of roots between the treatments was realized focused on proportion of individual tissues and development of apoplastic barriers. Activities of some antioxidative enzymes were measured in roots and leaves of treated maize plants to see how these plants defended against oxidative stress caused by Cd.

The positive effect of silicon against stresses remains still largely unclear. However, the present study shows, that silicon can improve the resistance of economically important crop to one of the most toxic elements present in the environment and perhaps also improve the food safety.

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## GST detoxification activity in M3 sunflower mutants - new perspectives for phytoremediation?

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Sunflowers (*Helianthus annuus* L.) are very important crop plants with high biomass and high nutrient uptake, but their capacity for phytoremediation has only scarcely been addressed.

Recently, sunflower mutants had been exposed to elevated metal concentrations in the field in Switzerland, which gave us the opportunity to investigate the detoxification capacity for organic xenobiotics of these plants under the influence of heavy metals.

Two groups of sunflower mutants were investigated. The M<sub>2</sub> mutants showed increased metal accumulation and biomass production. Some mutants showed also enhanced metal accumulation.

The M<sub>3</sub> mutants were studied for biomass production, metal accumulation and GST detoxification activity. The most promising M<sub>3</sub> sunflower mutants exhibited an improvement in biomass production as compared to the control inbred lines.

When measuring the sunflower's GST activity it became clear that the best model substrate is not CDNB (1-chloro-2,4-dinitrobenzene), as reported from most other plant and animal species, but NBoC, a closely related compound (nitrobenz(o)ylchloride).

Sunflowers pregrown in the greenhouse were found to be better accumulators for heavy metals and they tolerate higher stress (POX and APOX).

Interestingly, glutathione reductase activity is depressed in plants exhibiting higher metal concentration. The potential of sunflower plants for phytoremediation is critically discussed with view to food quality and safety aspects.

## A novel role for plant defensins: a defensin from the zinc hyperaccumulating plant *Arabidopsis halleri* confers zinc tolerance

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Metal tolerance of hyperaccumulating plants is a poorly understood mechanism and yet promising both for the better understanding of plant metal physiology and biotechnological applications. In order to unravel the molecular basis of zinc (Zn) tolerance in the Zn hyperaccumulating plant *Arabidopsis halleri*, we carried out a functional screening of an *A. halleri* cDNA library in the yeast *Saccharomyces cerevisiae* to search for genes conferring a Zn tolerance in yeast cells. We identified four *A. halleri* defensin genes (AhPDF). Defensins, found in a very large number of organisms, are known to be involved in the innate immune system but have never been found to play any role in metal physiology. The expression of AhPDF1.1 in *A. thaliana* Col-0 made the transgenic plants more tolerant to Zn than the wild type ones. Thus, AhPDF1.1 is able to confer Zn tolerance in yeast and in plant. In *A. halleri*, defensins are constitutively accumulated at a high level in shoots compared with *A. thaliana*. Both mRNA and protein steady state levels were shown to increase upon a Zn treatment showing that *A. halleri* defensin pools are Zn responsive. We thus assume that defensins could be involved in *A. halleri* Zn tolerance and that a wider role of plant defensins in metal physiology should be considered.

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## French bread wheat cultivars differ in grain Cd concentrations.

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Cadmium concentration in bread wheat grains from French agricultural soils at trace element background levels most frequently ranged from 0.02 (1<sup>st</sup> decile) to 0.11 (9<sup>th</sup> decile) mg kg DW<sup>-1</sup> with 0.05 and 0.5 as median and maximum values (QUASAR programme, Mench and Baize, 2004). It generally depends on various factors including wheat cultivars. Therefore grain Cd concentration of French bread wheat cultivars (*Triticum aestivum* L.) was studied over a 3-year period at two experimental sites. At site A (Indre county, France) the soil is stony, medium-depth, calcareous and clayey; at site B (Yonne county, France) the soil is stony and very shallow. Both are developed over Oxfordian hard limestones. Grains were manually dehusked, washed (3 times) in deionised water, rinsed (4 times) in bidistilled water, milled (< 0.5 mm) in a tungsten covered grinder with a Ti grid, wet digested in 14 M HNO<sub>3</sub>:H<sub>2</sub>O<sub>2</sub> (30%) solution under reflux, and concentration analysed with GFAAS, a certified reference material (BCR wheat flour) being included in all analytical series. All values are expressed in mg kg DW<sup>-1</sup>. At site A, 35, 36, and 33 cultivars were successively grown in year 1, year 2 and year 3. Median values were 0.042, 0.029, and 0.053. Differences between the minimal and maximum values varied by a factor 2 (year 3) to 4.5 (year 1). At site B, 29, 37, and 33 cultivars were successively cultivated depending on year. Median values were 0.048, 0.081 and 0.042. Minimal and maximum values ranged from 0.024 to 0.142. Difference between minimal and maximum grain Cd concentrations varied by a factor 2 (year 2) to 4 (year 1). Median values were slightly lower compared with the QUASAR one due to lower 9<sup>th</sup> decile and maximum values, except in year 2 at site B. European maximum permissible Cd concentration in wheat grain was never exceeded. The assumption of mean equality for grain Cd concentration across the cultivars was tested by a variance analysis on a dataset including all cultivars (n=9) cultivated every year at both sites. Only one 1<sup>st</sup> order interaction, i.e. site x year, was significant. (Cultivar x year) and (cultivar x site) interactions were not relevant, thus grain Cd concentration was explained by cultivars. According to Student-Newman-Keul test, Tremie and Soissons displayed the highest grain Cd concentrations (means 0.072 and 0.068 respectively) compared with other cultivars (mean 0.045). Site effect on grain Cd concentration depended on year, year 2 data being lower at site A and higher at site B. However these neighbour sites had similar climatic conditions and their soil types were relatively close. Modelling was developed on 11 cultivars cultivated in both year 1 and 3, data being transformed in ln([grain Cd]). Mean of residues was nul, variance was constant (Golffed and Quandt test), and residue distribution was normal. All interactions were insignificant. Unparametrical Wilcoxon test and variance analysis indicated that French bread wheat cultivars differed in grain Cd concentration. Mean value comparison (Student-

Newmann-Keuls) resulted in 3 cultivar groups. Mean value of grain Cd concentration at site A (0.052)

differed significantly ( $t=-2.139$ ;  $p=0.04$ ) from that at site B (0.046), whereas year did not affect grain Cd concentration in this model. It is concluded that modelling Cd in French bread wheat grain should account for cultivar effect. Selecting low Cd-accumulating cultivars provides alternatives to reduce Cd intake in human diet and it needs omic's researches.

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## An alternative metabolic pathway of nitrogen in plants

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According to the Kjeldahl method, nitrogen in biological systems (including plants) is classified into two forms: inorganic nitrogen (excluding ammonia) that is almost exclusively nitrate (and nitrite), and organic nitrogen (and ammonia) that is stoichiometrically recoverable by the Kjeldahl method (and therefore called Kjeldahl nitrogen). The century-old Kjeldahl method is still the sole method available for the analysis of organic nitrogen in biological systems. In our attempt to study the mechanism of nitrogen dioxide (NO<sub>2</sub>) metabolism in the plant-mediated decontamination of this major air pollutant we unexpectedly discovered that about one-third of the total nitrogen derived from NO<sub>2</sub> taken up in the leaves of *Arabidopsis thaliana* was converted to neither inorganic nor Kjeldahl nitrogen, but instead to an as yet unknown nitrogen (Morikawa et al., 2004; 2005). We hereafter designate this nitrogen unidentified nitrogen (UN).

All of the 12 plant species tested were found to form UN derived from NO<sub>2</sub> (about 10–30% of the total nitrogen derived from NO<sub>2</sub>). The UN was formed also from nitrate nitrogen in various plant species (Morikawa et al., 2004; 2005). The leaves of naturally fed vegetables, grass and roadside trees were found to possess the UN.

In theory, candidate UN-bearing compounds include those that possess a nitrogen atom linked to an oxygen atom or atoms, e.g. nitro, nitroso, and oxime groups, or to a second nitrogen atom, e.g. azo compounds and hydrazines (Morikawa et al., 2004). The UN-bearing compounds identified to date in the extracts of the leaves of *Arabidopsis thaliana* fumigated with NO<sub>2</sub> include a  $\Delta^2$ -1, 2, 3-thiadiazoline derivative (Miyawaki et al., 2004) and 4-nitro- $\beta$ -carotene as well as S-nitroso compounds and nitrotyrosines (Morikawa et al., 2004; 2005). Thus, there is an alternative pathway of nitrogen to form UN-bearing compounds in plants. We have proposed a mechanism for the formation of UN-bearing compounds, in which reactive nitrogen species such as nitric oxide and peroxyxynitrite are involved as intermediates (Morikawa et al., 2004).

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## Expression of pea (*Pisum sativum* L.) seed ferritin cDNA in *Brassica juncea* for improvement of nutrition through fortification of iron

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Iron is an essential element for metabolism of all forms of life. The bioavailability of iron is fairly low in the vegetable foods, causing iron deficiency mostly in developing countries where vegetable diets are consumed. Over three billion people all over the world suffer from iron deficiency. Through genetic engineering, it is feasible to increase iron concentration substantially in edible plant parts (fortified foods rich mineral nutrients). Iron deficiency reportedly causes decreased immunity, neurocognitive function, oxidative stress and development finally causing anemia, contributing factor in over 20% of maternal deaths in Asia and Africa.

*Brassica juncea* (Indian mustard) is used as vegetable, salad, condiment, fodder oilseed, widely consumed as a leafy vegetable in northern parts of India and is amenable to genetic transformation. Hence, chosen for the present study using 35S constitutive overexpression of pea seed ferritin cDNA (gifted by Professor J.F. Briat to whom the authors of this work are grateful).

The methodology includes construction of vector, transfer of binary vector to *Agrobacterium tumefaciens*, plant transformation and confirmation of integration and expression of the transgene. Putative transgenics obtained in T<sub>0</sub> and T<sub>1</sub> have been confirmed by Polymerase chain reaction (PCR) with ferritin and hptII primers. Progeny test on the seeds of the T<sub>0</sub> plants indicated the Mendelian pattern of inheritance of the marker gene. The integration was confirmed by southern hybridization of digested genomic DNA of T<sub>0</sub> and T<sub>1</sub> generations with ferritin and hygromycin probes. The expression of transgene in T<sub>1</sub> generation was confirmed by RT-PCR. Ferritin subunits expression in T<sub>1</sub> generation transformants was further confirmed by immunoblot using anti ferritin antibody and other established procedures.

The iron content in transgenic plants (T<sub>1</sub>) varied between 273 and 533 µg/g dry weight (DW) and iron levels in the leaves of negative controls ranged from 180 to 251 µg/g DW indicating a two-fold increase in the iron content in leaves from the transgenic plants.

Importance of

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## Cellular redox homeostasis in alfalfa seedlings is altered upon exposure to cadmium and mercury

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Alfalfa (*Medicago sativa*) plantlets were exposed to Cd or Hg to study the kinetics of diverse stress indexes. In the so-called beaker-size hydroponic system, plantlets were grown in 30  $\mu\text{M}$  of Cd or Hg for 7 days. Oxidative stress took place and increased over time, being observed a linear response with Cd but not with Hg. To improve the sensitivity of the stress assays used, we developed a microassay hydroponic system, where seedlings were exposed for 24 h. Although a clear growth inhibition was observed, there were neither significant changes in non-protein thiol tissue concentration nor alterations in conventional oxidative stress indexes. To trace early responses to Cd and Hg, we used a microscopic analysis with novel fluorescent dyes, that allowed to visualise in alfalfa seedling roots minute cellular responses to 0, 3, 10 and 30  $\mu\text{M}$  of both metals: i) 2',7'-dichlorofluorescein diacetate ( $\text{H}_2\text{DCFDA}$ ), that labels peroxides; ii) Amplex Red, which is oxidised in situ by  $\text{H}_2\text{O}_2$  to resorufin in the presence of peroxidases, iii) monochlorobimane (MCB), that stains reduced glutathione/homoglutathione ( $\text{GSH}|\text{hGSH}$ ); and iv) propidium iodide (PI), that marks nuclei of dead cells.

Oxidative stress and cell death increased in the range of exposure of 6-24 h to Cd and Hg, whereas labelling of  $\text{GSH}|\text{hGSH}$  decreased acutely. Therefore, both Cd and Hg not only compromised severely the cellular redox homeostasis, but also caused cell necrosis. In plants treated with 1 mM L-buthionine sulfoximine, a potent inhibitor of  $\text{GSH}|\text{hGSH}$  synthesis, only the oxidative stress symptoms appeared, indicating that the depletion of the  $\text{GSH}|\text{hGSH}$  pool was not sufficient to promote cell death, and that other phytotoxic mechanisms might be involved.

To clarify this later aspect, alfalfa seedlings were studied also in exposure intervals ranging from few minutes until 24 h as maximum. Physiological parameters related to oxidative stress were also assessed: superoxide dismutase, ascorbate peroxidase, accumulation of redox metabolites (ascorbate, GSH and hGSH) and concentration of metals. 30  $\mu\text{M}$  Hg caused a surge of fluorescence due to oxidation of  $\text{H}_2\text{DCFDA}$  and Amplex Red few minutes after metal application, whereas Cd did not caused so quick response. At prolonged exposure, the oxidative stress derived fluorescence quenched in Hg-treated seedlings, as phytotoxicity increased and led to cell death. Higher grow inhibition, greater proportion of oxidised homoglutathione (hGSSGh), and transient activation of ascorbate peroxidase correlated well with acute stress induced by Hg, that

ultimately derived in metabolic failure. In seedlings grown in the presence of Cd a milder phytotoxic effect was observed.

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## Phytoferritins: functions in plants and significance in fortified foods

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"Ferritin", a metalloprotein is rich in iron, and ubiquitous in all organisms. Plant ferritins play a pivotal role in many important redox reactions. Iron is present in cytochromes, FeS proteins like ferredoxin. It is a constituent of catalytic enzymes viz., catalase, peroxidase, nitrogenase etc. The function of ferritin in plants is storage of Fe for short or long periods to protect the cell against the toxic effects of free Fe, thus serving as a primary antioxidant. Ferritin readily meets the demand for iron of the developing chloroplast.

Anemia caused by iron deficiency is a serious disease afflicting a large human population in developed and developing countries. Free ionic iron is extremely toxic to humans and plant cells as well catalyzing the production of reactive oxygen species and free radicals which act as mediators of cellular damage. Ferritins sequester free iron, thereby induce iron homeostasis in cellular system.

Iron forms insoluble complexes in the presence of oxygen (hydrous ferric oxide). Free ionic iron can be extremely toxic, catalyzing the production of highly reactive free radical species, leading to cellular damage. Ferritins sequester free iron, thereby preventing the cellular damage from toxic free radicals. Ferritin is found to protect the in vitro translational system from the harmful effects of heavy metal ions. Ferritin thus plays a crucial role in protecting the biosynthetic machinery at the translational level. Apart from sequestering iron, ferritin plays a major role in chelating other divalent cations, namely  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$  etc. in vitro and thus defend the cellular system against heavy metal toxicity.

Iron being an essential nutrient, can be supplemented to human diet in bioavailable non-toxic form rather than elemental/ionic form in tablets/capsules which is toxic. Over expression of ferritin, iron rich metalloprotein in edible plant parts would sequester excess iron and contributes to iron-homeostasis making available iron in a non-toxic, bioavailable and soluble form to the consumer.

Phytoferritins are usually abundant in non-photosynthesizing tissues such as roots, root nodules, senescing cells and seeds, but present in smaller amounts in photosynthesizing tissues. Several of the legume seeds are a rich source of ferritin. The functions of ferritin in plants are discussed in this presentation. Importance of iron fortified foods for human health are also discussed.

## Uranium, thorium and strontium in conifers grown on uranium mine dumps (the east carpathians, Romania)

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A study concerning the U, Th and Sr transfer to conifers grown within a uranium ore deposit was conducted on the Crucea mine (the East Carpathians, Romania). The bioaccumulation of U, Th and Sr in the conifers resulted in the removal of these metals from the soil and reduces the risk to the environment. Phytostabilization is attractive to reduce bioavailability and offsite migration of contaminants. Unlike other phytoremediative techniques, the goal of phytostabilization is not to remove metal contaminants from a site, but rather to stabilize them and reduce the risk to human health and the environment.

The increased concentration of uranium and thorium in the surfaced soils is related to dispersion of those elements from wastes. The bioavailability of the radioactive metals from the soil (EDTA extraction) decrease in the following order: Th(21,91%) > U(6,20%) > Sr(3,01%). Increase of uranium, thorium and strontium in soil may lead to enhanced uptake of U, Th and Sr by plants growing in the contaminated soils.

The fir *Abies alba* and the spruce *Picea excelsa* were found to have a high uptake capacities of uranium. The highest concentration was achieved in *Abies alba* roots and twigs. We found that uranium concentration in plant tissues follow the order: roots >> stems > twigs > needles. Apparently, uranium is absorbed through the roots system and limited translocation to other plant parts occurs; most uranium taken up by the firs remained in the roots.

For the behavior of Th and Sr, we noticed that the first concentrate mainly in the roots, that is just like in the case of U. The levels of thorium concentration in conifers are usually low. We suggested that due to ability of solid phase of soil to absorb Th<sup>4+</sup> ions, the bioavailability of Th in soils might be rather low. On the other hand, it is known that tetravalent thorium is able to form complexes with organic molecules that roots produce into the rhizosphere; this complexes seem to be more soluble and mobile than the ions themselves. Therefore, the Th-organic complexes may be easily absorbed by roots and translocated to other parts of conifers. Strontium has a different behavior, its distribution in the vegetal organs of the conifers being almost equal.

There appears to be competition from calcium at high concentrations, but not from phosphate. This suggests that the uptake and translocation mechanism of uranium may be similar to those of calcium. Since the plants do not need U and Th neither for their metabolism, nor for their structure, it follows that the assimilation of these elements is being done through passive processes (non-metabolic ones). The passive absorption implies the diffusion of uranyl ions and organically bound Th<sup>4+</sup> from the soils in the endodermis of the roots, do to their imperfect selectivity and increased permeability of cell membranes.

We consider *Abies alba* and *Picea excelsa* behaviors very interesting because they can reach and accumulate U, Th and Sr over very long periods of time. Those plants are tolerant to the radionuclide contamination. It apparently blocked and/or “stored” efficiently the heavy metals particularly in root cells and diminishing the quantity of mobile natural heavy radionuclides. It could be used for the regreening of sites with moderate heavy / radioactive metal pollution.

## Safe gaseous treatment for the control of quality, fungal decay and expression of defense-related genes in table grape

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Postharvest deterioration of table grapes normally results from desiccation and fungal decay, largely caused by *Botrytis cinerea*. This pathogen, which has a considerable economic impact in horticulture, is usually controlled by means of fungicides, but this can lead to multiple resistance in the pathogen population. Moreover, because postharvest chemical treatments are restricted in most countries, safe alternative control technologies need to be developed to assure high quality fruits and control fungal attack. Commercial alternatives to the use of SO<sub>2</sub> generators have been proposed to maintain the quality of table grapes over the short term. However, SO<sub>2</sub> treatment may cause damage to the grape berries if used excessively and some consumers develop allergic reactions to sulphite residues. A promising alternative strategy that could replace the use of fungicides to control grape decay is to stimulate the natural defense capabilities of fruits using non-chemical treatment. Some defense molecules regulate different aspects of ripening process and also improve levels of health promoting substances. Our work was aimed to analyze the effectiveness of gaseous treatments (short-term high CO<sub>2</sub> levels) for improving appearance of bunches, maintaining the quality of the berries and controlling fungal decay in table grapes (*Vitis vinifera* cv. Cardinal) stored at low temperature. Furthermore, we determine how this relates to the accumulation of pathogenesis-related (PR) protein genes coding for chitinase and  $\beta$ -1,3-glucanase and to the pattern of stilbene synthase (STS) gene expression and trans-resveratrol levels. cDNAs encoding a class I chitinase (*Vcchit1b*) and a class I  $\beta$ -1,3-glucanase (*Vcgns1*) were isolated from the skin of grapes using RT-PCR and RACE-PCR strategies. Pre-treatment with 20% CO<sub>2</sub> consistently delayed the expression of *Vcchit1b* and *Vcgns1* as well as the increase in chitinase activity in the skin. The pattern of STS gene expression and trans-resveratrol content in CO<sub>2</sub>-treated grapes was consistent with the reduction of natural total decay produced by this pretreatment. These technological and molecular results point out to new aspects of research aimed to improve agronomic performance in the pre and post-harvest stages in a sustainable manner and fruit nutritional quality.

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## Growth of sorghum plants on contaminated soil

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Keywords: lead, cadmium, Sorghum bicolor, accumulation, autochthonous microorganisms, pot experiment.

In previous studies we showed that the contamination of the soils in south of the city of Plovdiv (South Bulgaria), had been strongly influenced by the Non-Ferrous-Metal Smelter, NFMS (Shilev & Naydenov, 2005). The Pb concentration in these soils reaches values of about  $1 \text{ g kg}^{-1}$ , while the Cd one is almost  $26 \text{ mg kg}^{-1}$  in the most contaminated sites. Even the heavy metals are mainly in insoluble state, plant tissue, in general, and the edible parts, in particular, accumulate dangerous levels of them.

In our study, sorghum plants (*Sorghum bicolor* (L.) Moench var. Keller) were grown in green house conditions in pots with contaminated and uncontaminated soil (control). Contaminated soil was used without mixing or was mixed with uncontaminated one in two different ratios – 1:1 (v/v, 50 %) and 1:3 (25 %), contaminated: uncontaminated soil. As we showed in other studies (Shilev et al., 2006, 2003), the autochthonous soil microorganisms play an important role in plant rhizosphere. In this sense, developed mixture of nutrients was added to the soil of each treatment, and mixed.

The addition of nutrients for activation of microbial growth resulted in increased fresh weight of plants in the control treatment and in the treatment with 50 % of contamination. In other treatments the results were in range of standard error. In all treatments with nutrients (with exception of 25 % contamination) the soil pH was lower, than in those without nutrients. The higher difference was observed when the contamination was 50 %. In the treatments with nutrients, decreasing soil contamination, the shoot tissue content of Pb increases. The same effect was found in previous studies with maize (Shilev & Naydenov, 2005). In contrast, cadmium content in shoots had increased together with increment of the contamination. We found the higher shoot concentration of Cd in the treatment with addition of nutrients and no dilution of contamination –  $71,14 \text{ } \mu\text{g g}^{-1}$ , which corresponds to 30 % enhancement compared with that without nutrients.

In conclusion, the sorghum plant can be suitable to replace traditional crops (vegetables, corn, etc.) in regions with contaminated soil, although its posterior use should be appraised very carefully.

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## Microbiota of birch and aspen for phytoremediation of polyaromatics

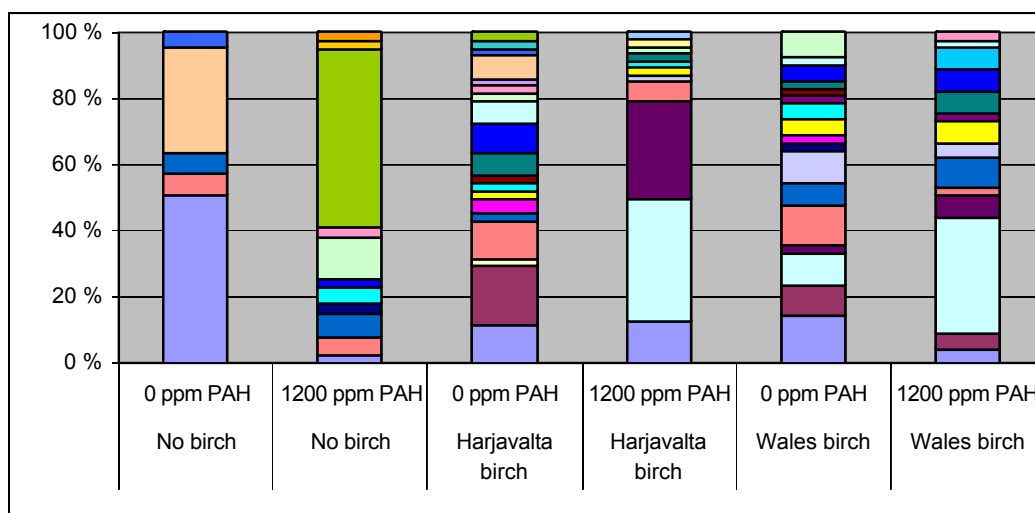
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Petroleum hydrocarbons containing polyaromatic compounds (PAHs) are worldwide pollutants in soil, and the problem is pronounced in Northern countries where the biological activity is low due to lower temperatures. Birch (*Betula pendula*) and aspen (*Populus tremula*), which are important trees in the boreal vegetation zone, are used in development of phytoremediation in northern climates. The woody plant microflora is of central importance in phytoremediation, but have not been studied to a greater extent (1). For bioremediation of organic compounds bacteria with effective catabolic pathways are needed. The extradiol upper pathway dioxygenase gene was used as marker to study the catabolic bacterial community in phytoremediation (2). The gene product catalyses extradiol ring cleavage of biphenyls, naphthalenes and also polyaromatics like dibenzothiophenes. Catabolic bacterial communities were assessed by DNA-RFLP fingerprinting (Fig. 1)



**Figure 1: Restriction fragment length polymorphism (RFLP) of catabolic marker gene.**

The catabolic bacterial diversity was higher in rhizosphere-associated soil than in bulk soil and addition of PAHs changed the bacterial communities in all cases. Our study showed the birch rhizosphere induces an impressive catabolic bacterial diversity both in clean and polluted soil underlining the thought that phytoremediation can be used to elevate the bacterial potential in soil for degradation of polyaromatics.

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## Plants as useful tool for removal of physiologically active contaminants from water systems

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Municipal wastewaters contain many organic compounds which originate from the wide use of pharmaceuticals, fragrances, and domestic cleaners. These products are often called Pharmaceutical&Personal Care Products. Pharmaceuticals after intake into organisms are partly metabolized and metabolites with unchanged molecules are excreted via urine or feces. Domestic cleaners, washing powders and cosmetics contain various fragrances and some of them are considered as endocrine disrupting chemicals. The presence of chemicals from both groups in the wastewaters is a good prerequisite for the use of phytotechnology (algae, water plants, or cultivated terrestrial plants and their root cultures respectively).

In the centre of our investigations is ethynylestradiol from the group of pharmaceuticals and musk ketone from musk compounds. Ethynylestradiol is a common part of anti-baby pills. Although the amounts of this compound in one pill is very small, the wide use and biological activities of parent compound and/or metabolites could cause ecological problem. Musk compounds (musk ketone, galaxolide, tonalide) are usual components of washing powder perfumes and their intake into wastewaters is very high. The efficiency of the removal in sewage treatment plant at the compounds discussed is relatively small and these compounds can contaminate surface waters.

In the study the interaction between discussed compounds and higher plants was investigated as the basis for next use of algae, water plants and root cultures. The determination of low-level concentrations of studied substances in the water solutions is essential and it was solved by solid phase extraction with subsequent measurement by HPLC. The developed method is based on common SPE materials and reverse phase chromatography and could be used at routinely determination of these compounds in commercial analytical laboratories.

The interactions with plants were tested by the use of in vitro cultivated *Zea mays*, *Amaranthus* sp., *Sinapis alba*, *Brassica napus* plants. The concentrations of musk compounds decreased during cultivation period, and the plant metabolism was evidently influenced by the xenobiotic compound, as can be documented by the analysis of plant extracts. At ethynylestradiol the influence is not so dramatical, but the decrease of hormone concentration was also observed.

The obtained results show, that tested plants are able to extract studied compound from the water solutions and probably accumulate these compound and their metabolites in their tissues. It can be very important at the production of these plants in the neighbourhood of the rivers as well as at the use of tested plants for the phytodecontamination..

## Genotoxicity and cytotoxicity of cadmium and copper on plants

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Heavy metals, such as Cd and Cu, inhibit the growth of plant roots. With the aim of determining the genotoxic effect of two heavy metals, a micronucleus test was carried out on the root tips of some plants.

The addition of Cu SO<sub>4</sub> or Cd Cl<sub>2</sub> to sprouts of *Vicia Faba* and *Pisum sativum* induce the formation of micronuclei, which is observed since 2.5 mM for *Vicia Faba*. *Pisum sativum* is shown to be less susceptible to the genotoxic effect of these heavy metals.

The amount of micronuclei was shown to be higher with Cd than with Cu, for the same concentrations.

In addition to these nuclear entities, other types of anomalies which affected the structure of the chromosomes and their number, were detected. Abnormal chromosomes and a clastogenic effect were also observed. Some steckinesis, chromosome fragmentation, anaphase bridges, are induced by the treatments. Sprouts of maize have shown a few pictures of clastogenicity.

The cytotoxicity and genotoxicity of Cd and Cu are related to the production of reactive oxygen species (ROS) and hydrogen peroxide the main ROS involved, which can induce DNA injuries and gene mutations.

The study of the expression of stress-related genes is investigated in order to understand the molecular response of our plant models to these heavy metal treatments.

## Atmospheric nitrogen oxides are a plant vitalization signal

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Atmospheric nitrogen dioxide (NO<sub>2</sub>) has been regarded as an air pollutant since the substantial part of urban NO<sub>2</sub> is reported to originate from the exhaust gas of the vehicles. However, we have recently reported (Morikawa et al., 2004) that about one-third of NO<sub>2</sub>-derived N (NO<sub>2</sub>-N) taken up into plants are converted into a previously unknown Kjeldahl-unrecoverable organic nitrogen (designated unidentified nitrogen or UN), and that the UN-bearing compounds include a novel heterocyclic Δ<sup>2</sup>1,2,3 thiadiazoline derivative and nitroso- and nitro-organic compounds (Miyawaki et al., 2004; Morikawa et al., 2005). These results strongly suggest that the exogenously supplied or atmospheric NO<sub>2</sub> is not a simple supplemental N source or a pollutant, but instead that it functions as an air-borne signal of reactive nitrogen species (Morikawa et al., 2004; 2005). Consistent with this is the reports that endogenously produced nitrogen oxides (NO<sub>x</sub>) such as nitric oxide (NO) functions as a vital plant signal. However, whether exogenously supplied atmospheric NO<sub>x</sub> plays a role as a plant signal has never been addressed.

We have been investigating plant-mediated mitigation of atmospheric NO<sub>2</sub> and the use of NO<sub>2</sub> as an alternative N fertilizer. In our attempt to produce NO<sub>2</sub>-philic plants that can grow with NO<sub>2</sub> as the sole nitrogen source, we unexpectedly discovered (Takahashi et al., 2005) that although NO<sub>2</sub>-N taken up by plants (*Nicotiana plumbaginifolia*) during the growth for 10 weeks in the air containing 150 ppb <sup>15</sup>NO<sub>2</sub>, which corresponds to a heavily polluted urban air, exogenously-supplied NO<sub>2</sub> almost doubled biomass and total leaf area, the contents of C, N, S, P, K, Ca and Mg, and the contents of free amino acids and crude proteins.

Stable isotope mass spectrometry of <sup>15</sup>N/<sup>14</sup>N ratio has shown that the nitrogen derived from NO<sub>2</sub> was less than 3%. Thus atmospheric NO<sub>x</sub> played only a negligible contribution in the total nitrogen of plants. Our findings indicate that the atmospheric NO<sub>x</sub> at an ambient concentration is a plant vitalization signal. More recently, *Arabidopsis thaliana*, sunflower, and pumpkin have been shown to be vitalized in response to atmospheric NO<sub>x</sub>.

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## Heavy metal accumulation and gene expression of *Salix caprea*

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Heavy metal polluted soils are a major problem for plant growth and a threat to animal and human health. Therefore several approaches have been proposed to decontaminate sites and among the biobased methods phytoextraction, i.e. the use of plants to clean up contaminated soils, is one of the most promising technique. Phytoextraction is a low cost technology that leaves the soil fertile and is able to support subsequent vegetations. However phytoextraction is slow and thus perennial plants are needed that exhibit a high biomass production, tolerate and accumulate heavy metals and are easy to harvest.

*Salix caprea* (goat willow) is a fast growing shrub with a broad geographic distribution. This species can be found as pioneer plant at metalicolous site. That *S. caprea* is a promising phytoextractor has been shown in hydroponic experiments with metalicolous and non-metallicolous isolates that accumulate up to 250 mg Cadmium and 1300 mg Zinc per kg dry weight in leaves. In order to reveal the molecular basis and to identify genes responsible for heavy metal tolerance we analyzed the genetic difference between excellent and poor *S. caprea* Zn/Cd hyperaccumulators. We further correlated the activity of candidate genes of particular *S. caprea* isolates with their ability to accumulate these heavy metals during a vegetation period of three months. Among the candidate genes are metal chelators as the putative *S. caprea* orthologs of phytochelatin synthases, O-acetylserine lyase, metallo-thioneins and metal transporters as P-type ATPases and ABC-, Zn- and Ca-transporters. Results of these association studies will be presented and discussed.

## Effect of $\text{CdCl}_2$ and $\text{Cd}(\text{NO}_3)_2$ on the growth of poplar callus culture in the presence of $\text{ZnSO}_4$

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The contamination of the environment by Cd is caused mainly by human activity. This dangerous metal can be transported in living cells by identical mechanisms like Zn and can substitute this essential metal in organic compounds. Plants have developed different ways of tolerance to pollutants from the environment. These properties of plants can be used for the decontamination of the environment. The use of selected metal-accumulating plants in the environmental clean-up technology is phytoremediation. Trees represent a suitable model for phytoremediation. They are not pretentious on soil and watering because of a huge root system. They are long-life plants accumulating xenobiotics for several decades. Our experiments were aimed at the effect of cadmium in the presence of  $\text{ZnSO}_4$  in poplar callus. Poplar callus (*Populus alba* L. *pyramidalis*) was cultivated on modified DIAZ-COLON medium (1972, DC) supplemented with  $\text{CdCl}_2$  ( $10^{-4}$ ,  $10^{-5}$  a  $10^{-6}$  M), or in combination with  $\text{ZnSO}_4$  in  $3 \cdot 10^{-5}$  M,  $10^{-3}$  M, or  $1,03 \cdot 10^{-3}$  M concentrations. In parallel experiments  $\text{CdCl}_2$  was replaced by  $\text{Cd}(\text{NO}_3)_2$ . Poplar calli were cultivated under 16h photoperiod,  $45\text{--}60 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  light intensity, and  $23 \pm 1$  °C. The growth parameters of the callus culture were expressed as growth curves of fresh and dry masses, and growth dynamics has been determined. The content of accumulated Cd and Zn in callus cells was determined by the AAS.

Comparison of callus culture growth dynamics on media supplemented with  $\text{CdCl}_2$  or  $\text{Cd}(\text{NO}_3)_2$  showed variations in dependence on their concentration ( $10^{-4}$ ,  $10^{-5}$  or  $10^{-6}$  M) and combination with  $\text{ZnSO}_4$  (in concentrations  $3 \cdot 10^{-5}$ ,  $10^{-3}$ , alebo  $1,03 \cdot 10^{-3}$  M). From the results it is evident that the culture growth on media enriched with  $\text{CdCl}_2$  (mainly in concentrations  $10^{-5}$  and  $10^{-6}$  M) was better compared with  $\text{Cd}(\text{NO}_3)_2$ . The growth dynamics on media supplemented with  $\text{Cd}(\text{NO}_3)_2$  was more even-tempered and the growth was positively influenced mainly between the 7<sup>th</sup> and 21<sup>st</sup> day of cultivation. Both higher concentrations of  $\text{ZnSO}_4$  in combination with  $\text{CdCl}_2$  were responsible for a slower growth, in some cases for growth inhibition, compared with  $\text{Cd}(\text{NO}_3)_2$ . In the presence of  $\text{Cd}(\text{NO}_3)_2$  the growth dynamics was the highest between the 7<sup>th</sup> and 21<sup>st</sup> day of cultivation, similar as at the lowest concentration of  $\text{ZnSO}_4$ . The variations in the growth intensity were dependent on  $\text{Cd}(\text{NO}_3)_2$  concentration and presence of  $\text{ZnSO}_4$ . Cd accumulation was determined in callus cells after 28 days of culture on all media used.

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## Toxicity screening of sulcotrione and its photochemical by-products: bacterial, algal and higher plants bioassays

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Sulcotrione is the active ingredient of Mikado<sup>®</sup>, a commercially available plant protection product which is applied for the control of wide range of annual and perennial broadleaf weeds. Sulcotrione (2-[2-chloro-4-(methylsulfonyl)benzoyl]-1,3-cyclohexanedione) is one of the relatively new triketone herbicide which replaces atrazine in maize treatment [1]. While the main hydrolysed metabolites (1,3-cyclohexanedione and 2-chloro-4-methylsulfonylbenzoic acid) of sulcotrione in soil have been already characterized [1,2], no product of photochemical reaction resulting from solar radiation has been reported in the literature.

Only recently chromone derivative was determined as a main phototransformation by-product of sulcotrione in the cuticular wax coating the leaves [3]. The same product of sulcotrione phototransformation appeared in aquatic medium. This photoproduct was isolated and fully characterized.

Firstly, the toxicity of sulcotrione and chromone were determined using aquatic organisms: luminescent bacterium *V. Fischeri* in Microtox<sup>®</sup> standard test and growth inhibition of alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) tests. The effect of Sulcotrione on growth of algae can be ascribed to the inhibition of p-Hydroxyphenyl pyruvate dioxygenase (HPPD), the enzyme which plays a crucial role for photosynthesis organism. The 14-fold decrease in toxicity (higher value of EC<sub>50</sub> mg/L) value was noted for chromone derivative. However, the chromone derivative showed two-fold higher toxicity than sulcotrione towards *V. Fischeri*. In addition, the toxicity of irradiated sulcotrione solutions was determined by Microtox<sup>®</sup> at two different irradiation times, corresponding to 38% and 65% of sulcotrione conversion extent. The toxicity of the irradiated mixtures was 10-fold greater than that of pure aqueous sulcotrione.

In the further studies the short-term effect (within 8 days) of the new chromone derivative (using various dosages 1, 3, 9 mg per plant) on physiological status of maize (*Zea mays*) and white mustard (*Sinapis alba*) was evaluated at controlled condition in growth chamber. The physiological response of plants was followed by Chlorophyll a fluorescence (in vivo) as well as chlorophyll and carotenoid contents (after the harvest) analysis [4].

The parallel studies with sulcotrione and Mikado product (equivalent to sulcotrione content) showed high potential to act as an phytosanitary product. For maize at lowest dosage of sulcotrione/mikado (amounting 1 mg per plant) did not affected the

photosynthesis extend examined by the maximum quantum efficiency of PSII photochemistry ( $F_v/F_m$ ) nor the

quantum yield of electron transfer at PSII ( $\Phi_{PSII}$ ). However, greater dosage of molecule (amounting 9 mg per plant) inhibited photosynthesis of maize as high as 70% on the young leaves within 8 d. A decrease in chlorophyll and carotenoid contents are detected, which is rationalized by visible blanching symptom.

The white mustard is a weed which competes with maize in the culture, proved to be very sensitive to sulcotrione/Mikado. In the plants that were sprayed with the lowest dosage, a decrease of 80% and 100% was noted for  $F_v/F_m$  and  $\Phi_{PSII}$  respectively within 5 days after the treatment.

By contrast, chromone derivative, irrespective of the dosage, did not affect the photosynthetic capacity neither on the maize nor on the white mustard. The results demonstrated that photochemical transformation of sulcotrione makes this molecules phytosanitary inactive. Consequently, it could trigger off higher expenses of herbicide application; thereby higher amount of herbicide is introduced into the environment in order to ensure the plant protective activity. (Less sustainable use of chemicals!).

On the other hand, an effect of product resulted from photochemical degradation of sulcotrione in the aquatic medium on health and environment needs to be clarified.

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## **FIELD TRIP**

*The “Suc de Clava”: a metal-rich serpentine area*



## Serpentine areas of Massif Central

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Serpentine rocks come from hydrothermal or metamorphism alteration of olivine or pyroxene contained in deep basic magmatic rocks. Serpentine is rich in magnesium and iron and poor in calcium and silicium. Its global composition is:  $\text{Mg}_6 [\text{Si}_4\text{O}_{10}(\text{OH})_2](\text{OH})_6$ . However variations come from the exact formation and alteration processes. Serpentine is bad for plants because of a very low calcium/magnesium ratio, and also from high Cr, Ni and Fe contents.

In the Massif Central, serpentine outcrops occur in granitic areas raised at the end of hercynian orogenese, 300 millions years ago: serpentine areas are rather small, some main areas are included in the Natura 2000 network, such as “Puy de Wolf” near Decazeville which is 124 ha large, and “Suc de Clava” near Annonay, that we will visit, which is only 13 ha. Other, non registered areas, exist near St-Etienne such as “la Roche” at 1100m altitude, covering about 2 ha, and “Roche Noire”, covering 50m<sup>2</sup> only.

Serpentine areas are not cultivated, are rocky, with only grasses, little shrubs, and rare trees. The “Puy de Wolf” is mainly exposed toward south, and has no tree. The “Suc de Clava” has pine woods in the places facing north.

Serpentine soils are thin and strange, with brown or red colour due to high iron content. Few organic matter and few clay are due to low content of calcium and aluminium.

In our lab we studied mainly the “Suc de Clava”. The soil pH is around 5,7 to 5,9, Ni content is around 1500 mg/kg, Fe is about 100 000 mg/kg, Cr is more variable : between 700 and 1250 mg/kg, Cu vary from 21 to 78 mg/kg.

At the “Suc de Clava” some species are absolute serpentinophytes such as the very rare fern *Asplenium cuneifolium*, others are local serpentinophytes such as *Noccaea montana* (Brassicaceae). We will also see less strict serpentinophyte such as the rare fern *Notholaena marantae*, which lives only on a dozen of places in south France, and mainly on serpentine, and sometimes on other rocks poor in calcium and not acidic.

The Poaceae *Festuca caesia* gives to the suc its characteristic grey-blue color



The “Suc de Clava”: a serpentine area in Massif Central

A general view of the top of the “Suc de Clava”



*Asplenium cuneifolium*, an absolute serpentinophyte.

Crustose lichens on serpentine rock



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