Phytoremediation in 2005: the European initiatives between research, application and training

Prof. Nelson Marmiroli
Department of Environmental Sciences
University of Parma, Italy

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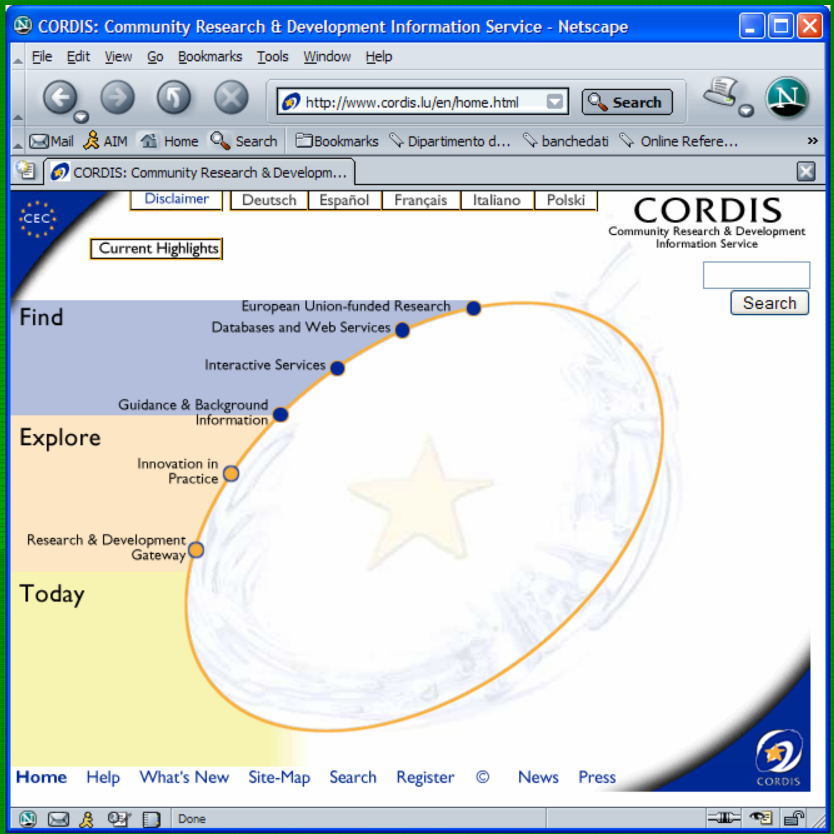
The European scene

- A recent survey based on publications and conferences showed that participation to phyto-oriented research involves
  - 29 countries
  - About 350 research groups
  - 60% academic, 30% research institutes, 10% private companies

It is worth mentioning that EU with the recent enlargement is composed of 25 countries, and that 20 countries do not take part into it

Research on phytoremediation in Europe

- The CORDIS WWW page, collecting information on EU-funded research, lists 24 main research initiatives in the last five years
- In particular, 9 out of these referred to RTD projects with funding ranging from 600000 to 1671430 Euro



- PHYTODEC
- Coordination: The Netherlands
- 9 partners
- A decision support system devoted to quantification of cost/benefit relationships in the use of vegetation for the management of heavy metal polluted soils and dredged sediments

- ENDEGRADE
- Coordination: Denmark
- 7 partners
- Application of endophytic degrader bacteria in improving phytoremediation of organic xenobiotics

- MYCOREM
- Coordination: Germany
- 9 partners
- Use of mycorrhizal fungi in phytoremediation of metals and PAHs

- PHYTAC
- Coordination: Finland
- 9 partners
- Development of systems to improve phytoremediation of metal contaminated soils through improved phytoaccumulation

- MYRRH
- Coordination: Belgium
- 4 partners
- Use of mycorrhizal fungi for the phytostabilisation of radio-contaminated environments

- METALLOPHYTES
- Coordination: Denmark
- 10 partners
- An integrated approach for the removal of toxic metals from polluted soils with the help of plants

- CEMBA
- Coordination: United Kingdom
- 5 partners
- Capillary Electrophoresis based instrument using Microsystem and Biosensor technologies for bioremediation monitoring (metals and organics)

- METALHOME
- Coordination: United Kingdom
- 9 partners
- Molecular mechanisms underlying metal homeostasis in higher plants

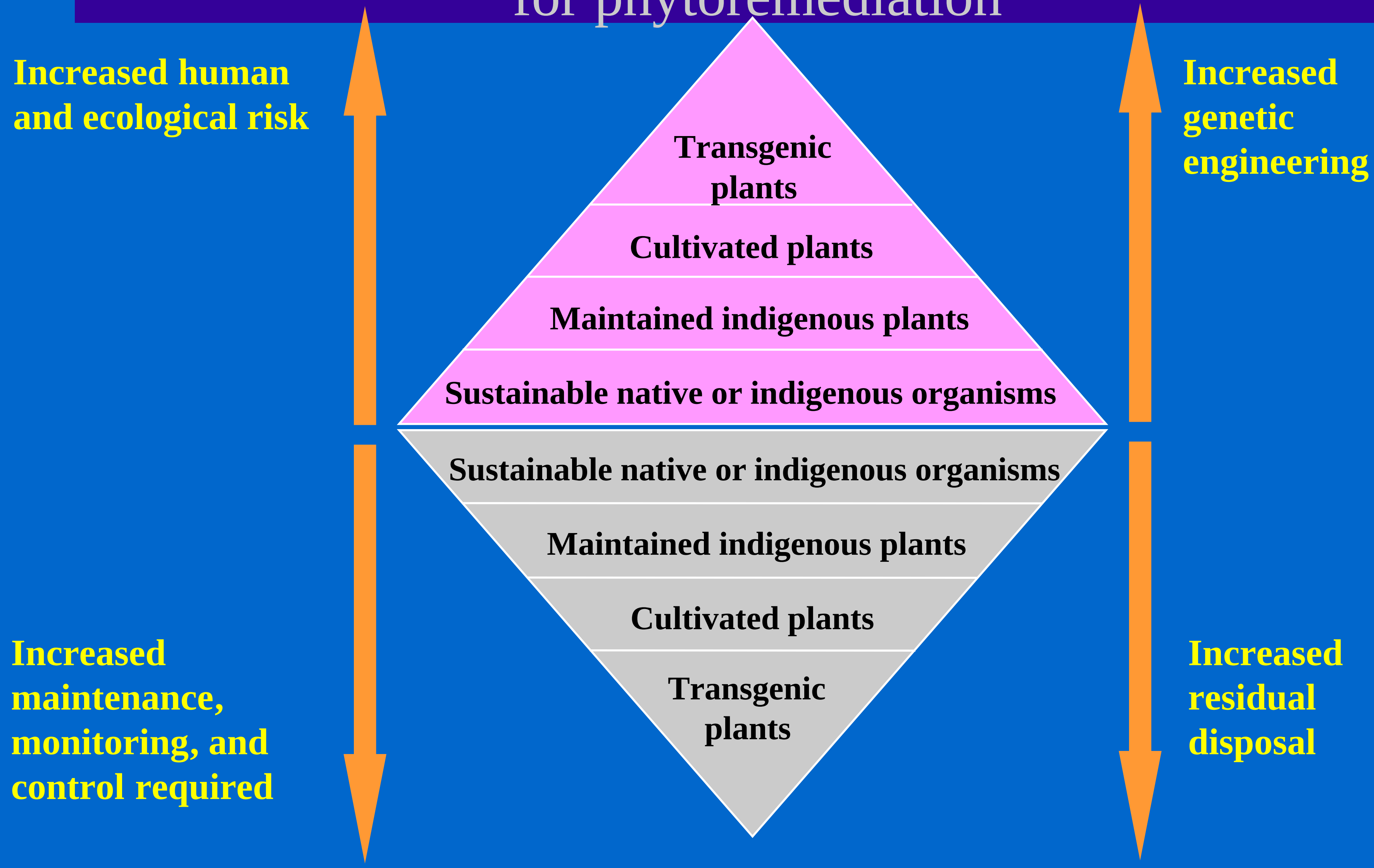
- MERCURY
- Coordination: United Kingdom
- 6 partners
- Development of enabling technologies and monitoring systems for the remediation/detection of mercury in southamerican waters. Design of chelators with therapeutical properties

Trends in research

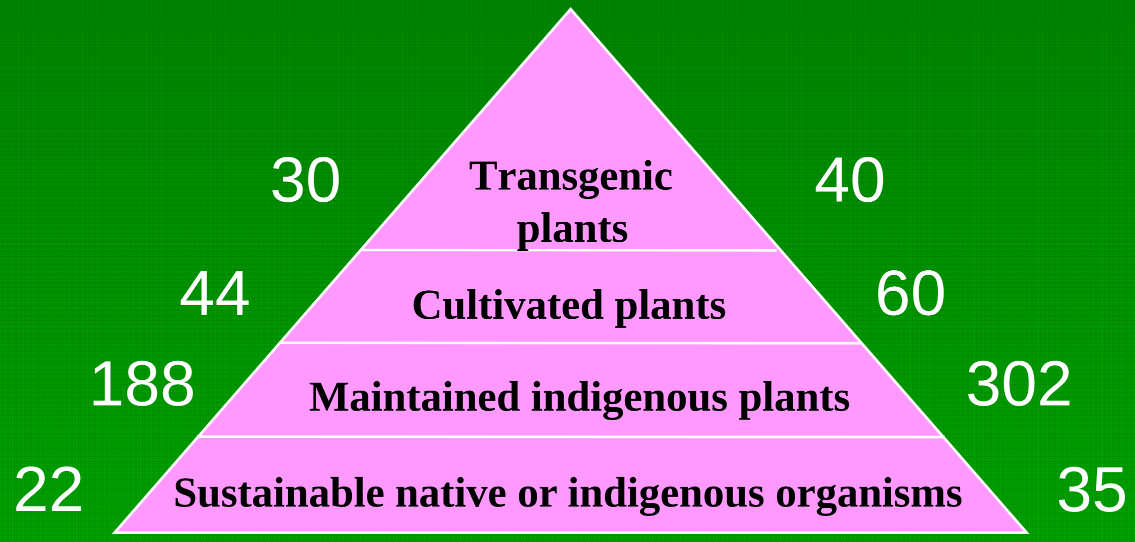
- A survey in the literature production for the period 2002-2005 has been reviewed and data compared with trends and needs forecast some 10 years ago for phytotechnologies

- A prevision made 10 years ago and published in Marmiroli and McCutcheon, 2003 (in McCutcheon and Schnoor (eds), Wiley Interscience).

Plant types relationships with advantages/disadvantages for phytoremediation



Plant utilisation from the literature



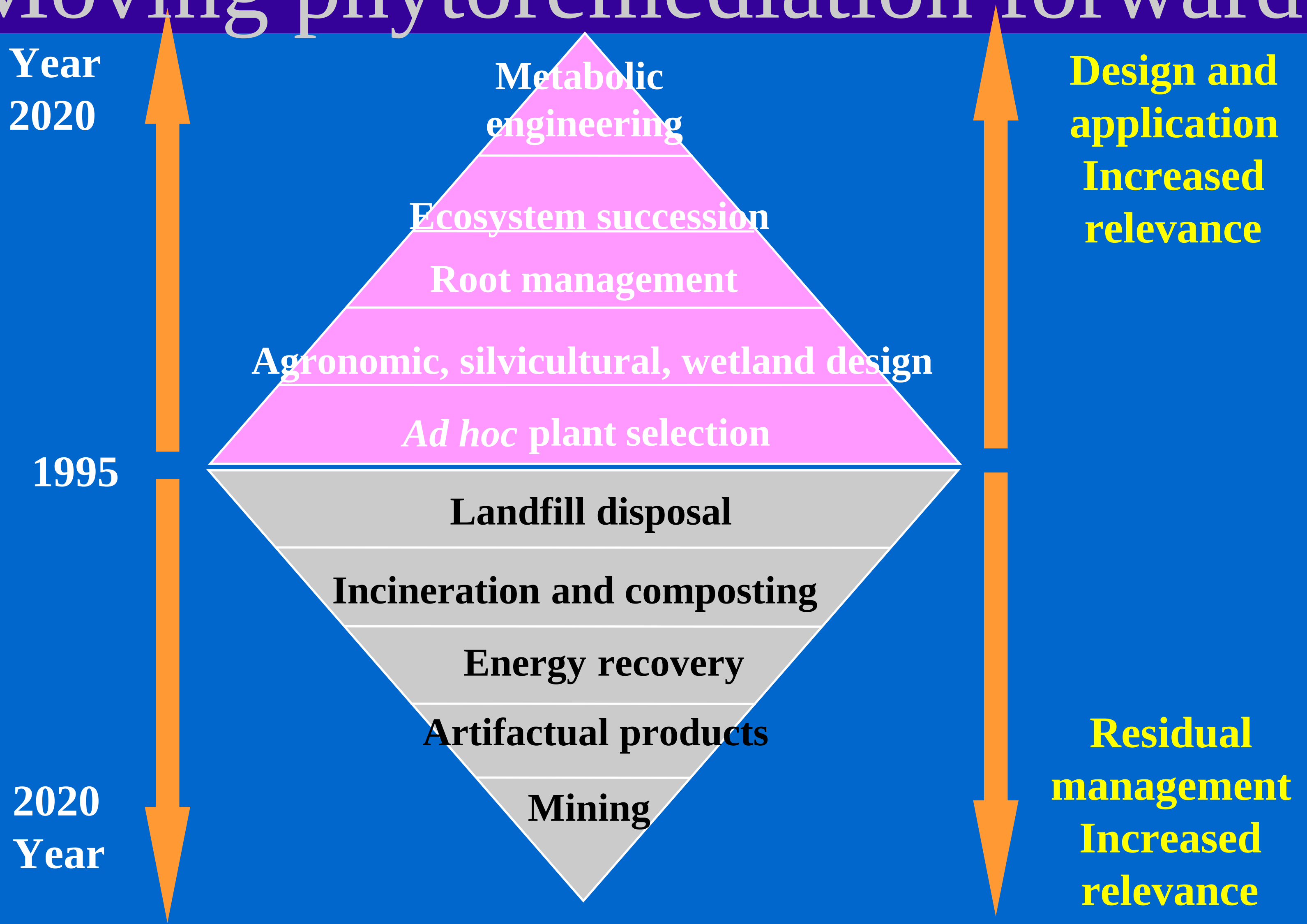
European situation

Non-European situation

The situation is similar in Europe and elsewhere. Transgenic plants are becoming more popular, whereas native plants lose ground as compared to “maintained” situations.

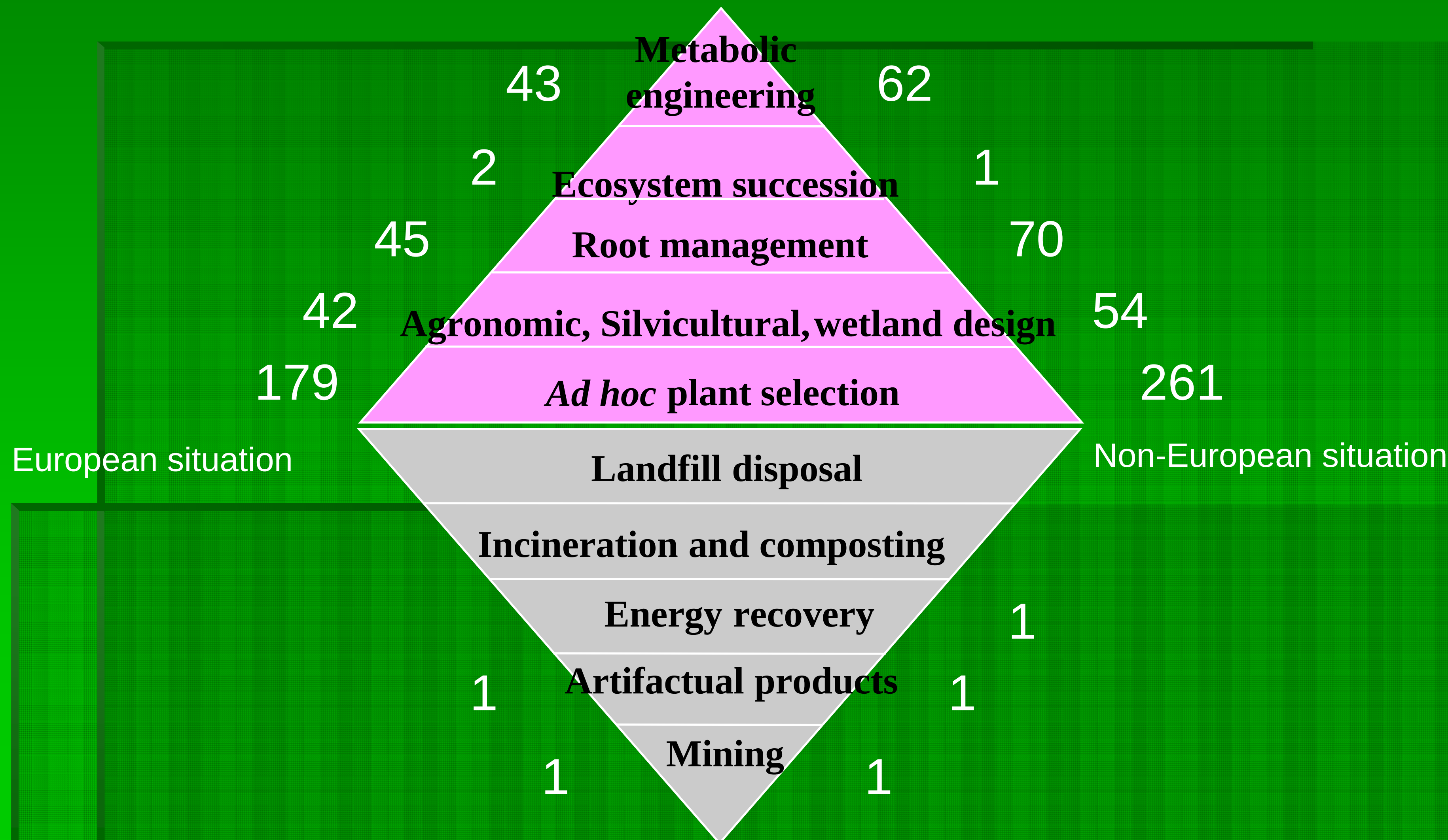
A prevision on the needs for the future made 10 years ago and published in Marmiroli and McCutcheon, 2003 (in McCutcheon and Schnoor (eds), Wiley Interscience)…

Moving phytoremediation forward



… and the situation now.

Research topics

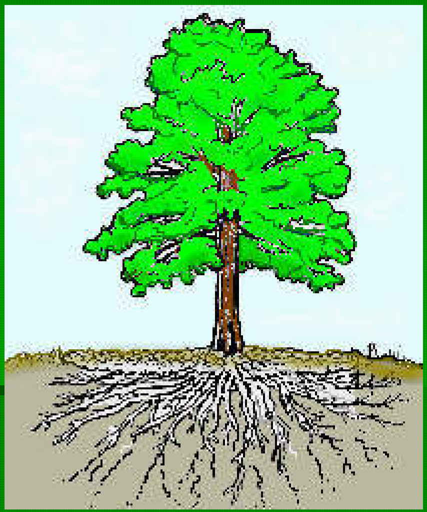


Comments

- Research interests are proceeding quite similarly if we compare Europe and the rest of the world
- Most efforts are focused on understanding the basic mechanisms in order to select the best plant species
- On the contrary, natural attenuation solutions are scarcely taken into consideration (at least in the scientific literature)
- Root environment and interactions with microorganisms are also attracting considerable interest
- As for disposal of the phytoremediation material, it exerts small attention: only 5 papers in the last 4 years
- A new line of interest concerns management tools for phytoremediation, such as Decision Support Systems, regulatory and economic aspects, with about 13 papers in the last 4 years

Main topics of research in Europe

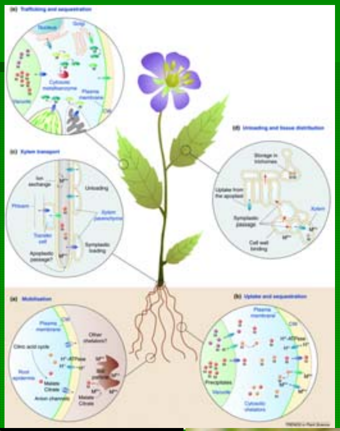
Understanding the basic mechanisms

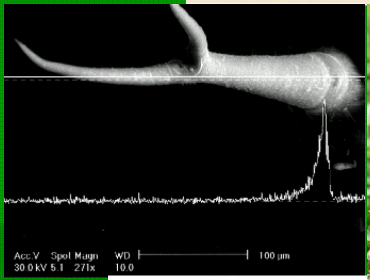


Implementing knowledge for application

Basic mechanisms: heavy metals

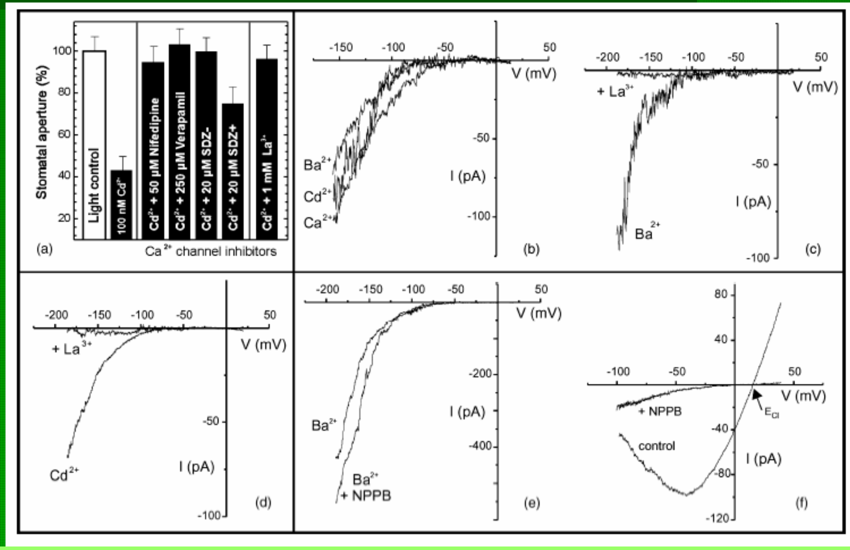
- Analysis of basic genetic, molecular and cellular mechanisms accompanying transport, accumulation, and tolerance of plants to heavy metals
- Information about synteny and genomic knowledge
- Plants of interest: Brassicaceae, *Thlaspi caerulescens*, *Arabidopsis halleri*, *Brassica juncea*, woody plants
- Interaction between plant roots and radionuclides, transfer to stems and leaves and any stabilisation within plant structures. Most elements are studied in their non radioactive forms, to establish mechanisms and strategies to be used in phytoremediation. Autoradiography of radionuclides distribution in total plants is also performed





Metals: transport

- Cadmium enters into the cells via Calcium channels in *Arabidopsis thaliana* (Perfus-Barbeoch et al. 2002). In hyperaccumulating species the channels vary according to the ecotype (Cosio et al. 2004)



Calcium-channel inhibitors suppress the Cd2+-induced stomatal closure and Ca2+ channels at the plasma membrane of guard cells are permeant to Cd2+.

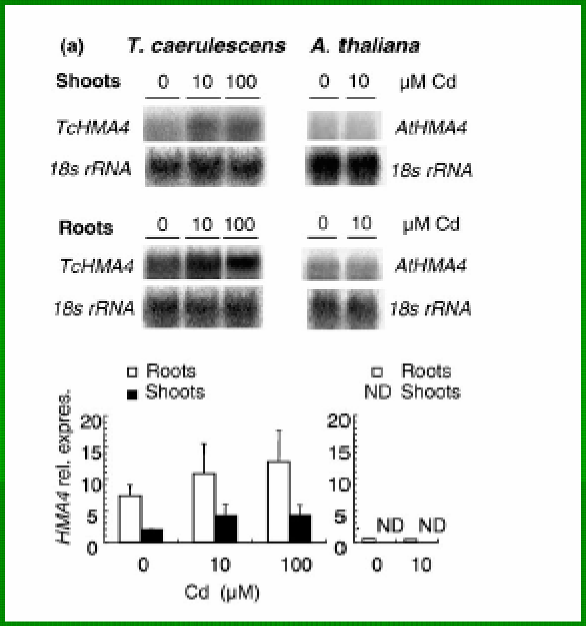
Metals: transport

- For radionuclides, transport and distribution can be studied through autoradiography, allowing an evaluation of the potential for phytoremediation in each plant (Soudek et al. 2004)

Distribution of Cs in reeds (left, shoot and root) and poplar (right), exposed to 0.5 mM Cs and traced with ^{137}Cs

Metals: transport and accumulation

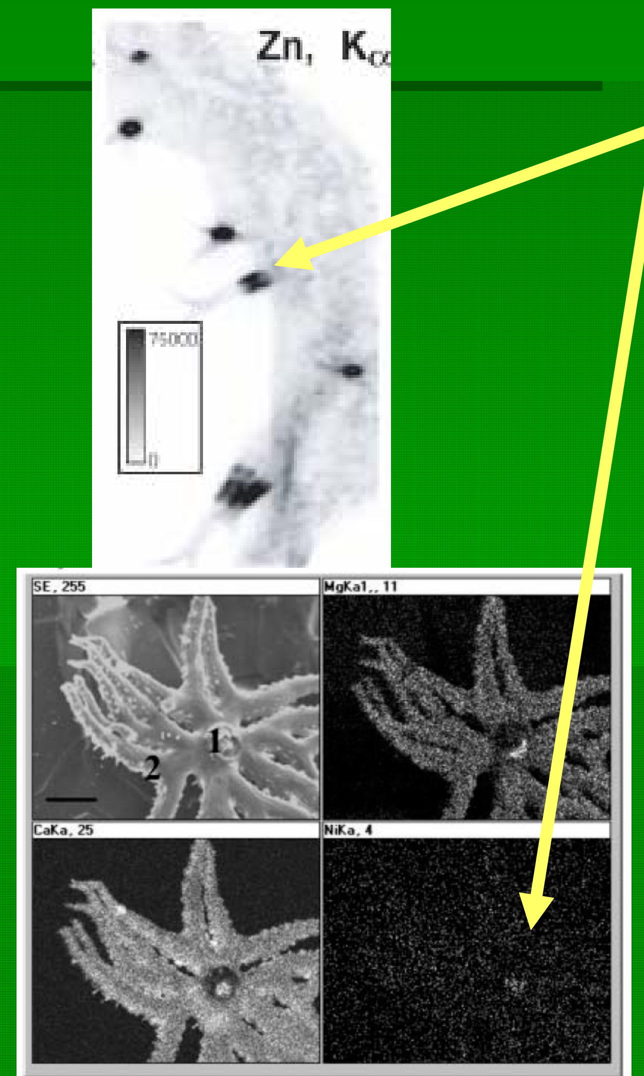
- A novel CPx-ATPase involved in Cd transport and hyperaccumulation in *Thlaspi caerulescens* has been identified (Bernard et al. 2004)



In *Thlaspi*, the mRNA for the new gene is 3 times more abundant in roots than in shoots and it can be induced by Cd treatment. In *Arabidopsis* it is absent from shoots, it cannot be induced and it is 20 times less abundant.

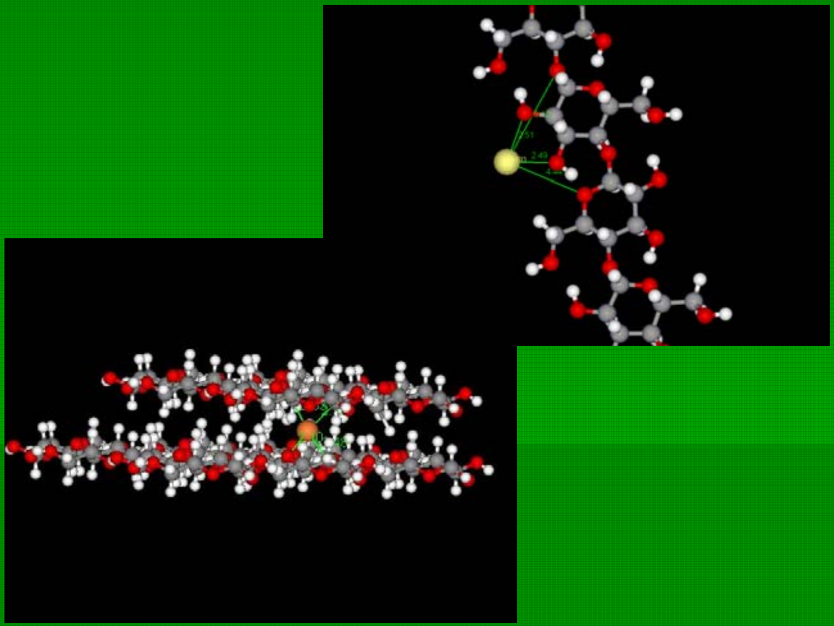
Metals: accumulation

- In *Arabidopsis halleri*, Zn accumulates in vacuoles of trichomes mainly complexed with organic acids (Sarret et al. 2002)
- Also in *Alyssum bertolonii* Ni accumulates to large extent in vacuoles of trichomes (Marmiroli et al. 2004)



Metals: sequestration

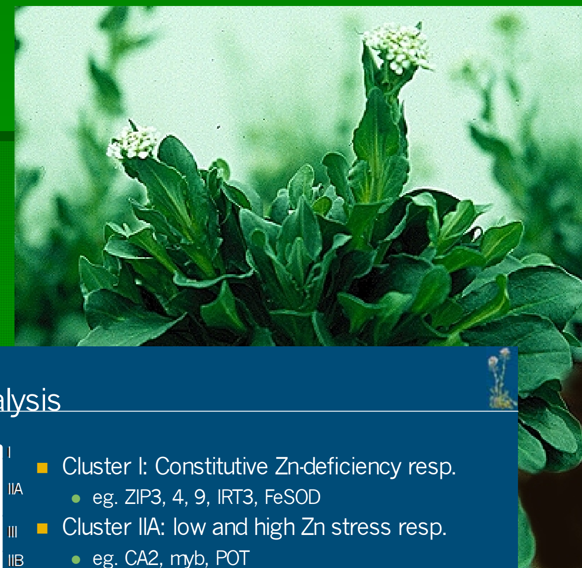
- Metals can combine with plant molecules and macromolecules for effective sequestration
- In walnut evidence obtained with EXAFS suggest an interaction between Pb atoms and 4 oxygen atoms in lignocellulose of the root periderm (Marmiroli et al. 2005)

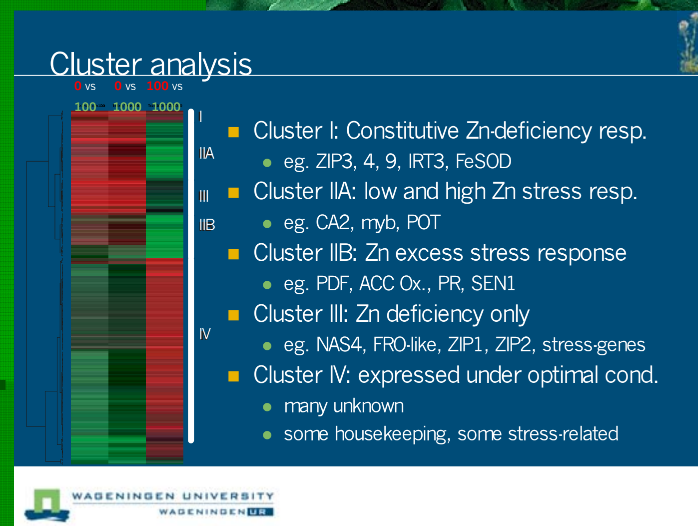


Molecular models of Pb interaction with cellulose

Metals: gene identification

- Comparative genomics (EST libraries, microarrays) of Zn accumulation in *Thlaspi caerulescens* and *Arabidopsis thaliana* leads to the identification of orthologs, novel sequences, and molecular markers (Aarts, 2004)

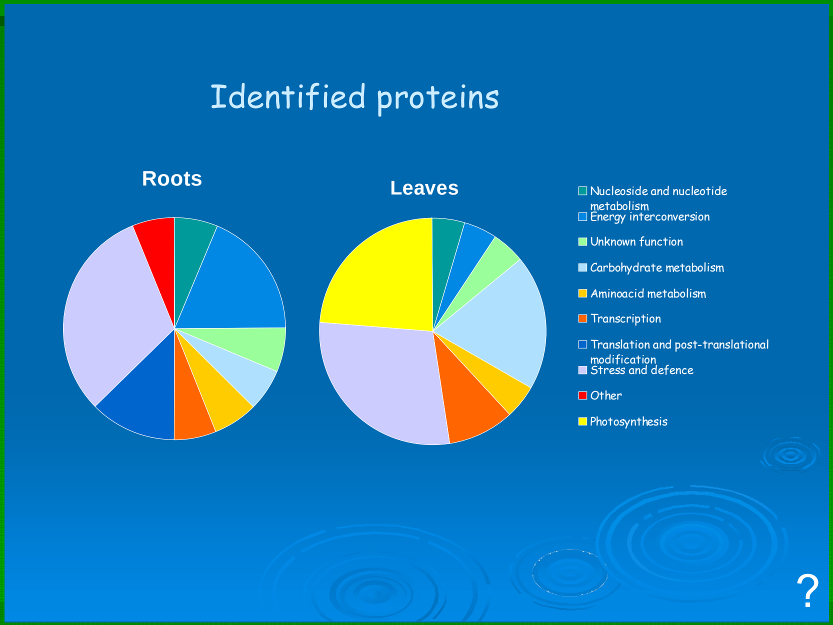




Microarray analysis of gene expression in Thlaspi when exposed to different Zn concentration brings to identification of differentially expressed genes and interesting new functions

Metals: protein identification

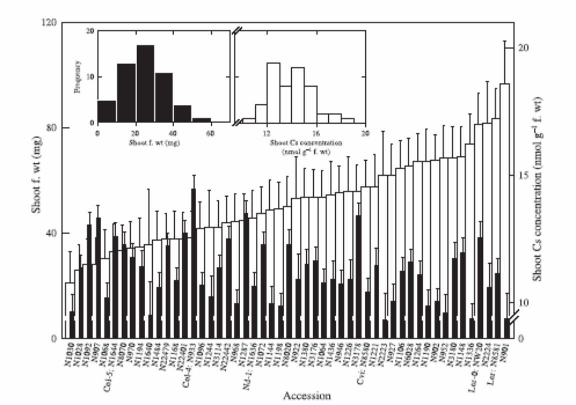
- Proteomics (2D gel and statistical analysis) of *Thlaspi caerulescens* in conditions of exposure to different Zn concentrations lead to identification of specific root and leaf proteins involved in tolerance and accumulation (Kärenlampi 2004)



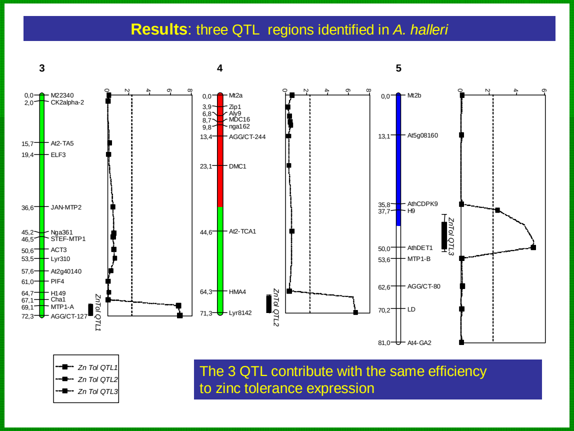
Many differentially expressed proteins were related to photosynthesis, carbohydrate and energy metabolism and stress. Identification of proteins specific to hyperaccumulator ecotypes is still in progress.

Metals: genetic mapping

- Quantitative Trait Loci affecting Cs concentration in shoots have been identified in *Arabidopsis thaliana* (Payne et al. 2004)
- Maps are in progress for *Thlaspi caerulescens* (Denieau 2004) and *Arabidopsis halleri* (Saumitou-Laprade 2004) to show location of tolerance genes so far identified



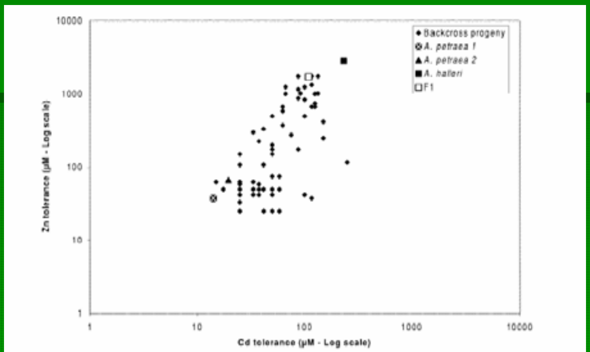
Shoot Cs concentration of 51 accessions of *Arabidopsis thaliana* and frequency distribution of the trait



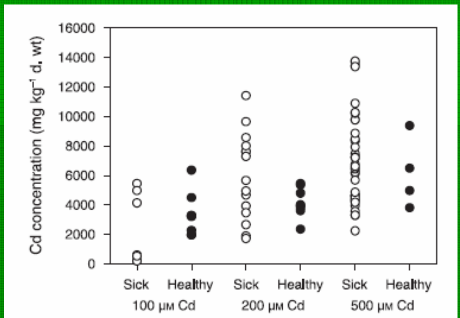
Three QTLs for Zn tolerance in a genetic map obtained for *Arabidopsis halleri* x *A. lyrata* cross

Metals: genetic bases of tolerance

- Genetic bases of tolerance to heavy metals in segregating populations: co-segregation studies show that Cd and Zn tolerance are affected by the same genetic elements in *Arabidopsis halleri*, but tolerance was independent of accumulation (Bert et al. 2003)
- Tolerance and accumulation are genetically distinct phenomena also in *Thlaspi caerulescens* (Zha et al. 2004)



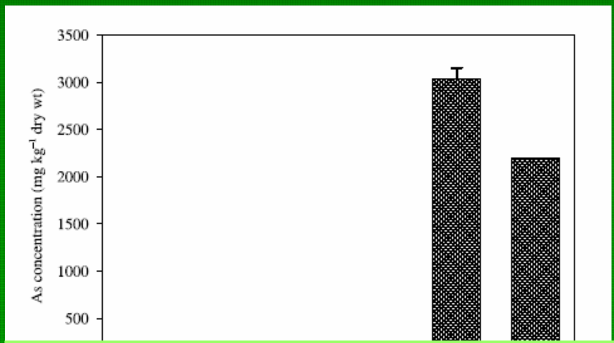
Plants from the backcross progeny (A. halleri x lyrata) were characterised for Zn tolerance and Cd tolerance: interestingly, the result of the correlation analysis showed a positive and significant relationship between the two characters.



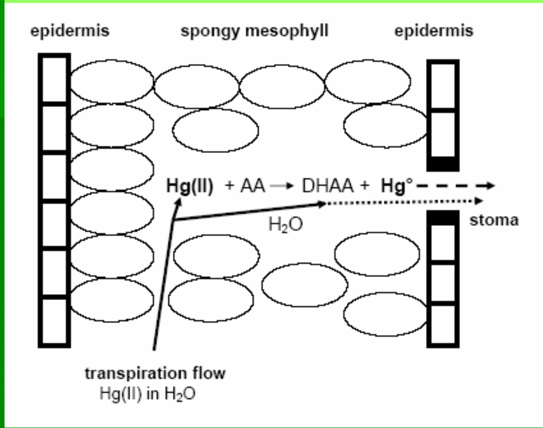
Shoot Cd concentrations in individual F2 plants from the G × P cross (accumulator vs non accumulator) showing healthy or toxicity (sick) symptoms showed no correlation with the tolerance phenotype.

Metals: new contaminants

- Recently, plants have been investigated for their ability to take up and accumulate Arsenic (Zhao et al, 2002)
- Also, the ability of plants in reducing Hg2+ into Hg° has been analysed (Ernst, 2004)



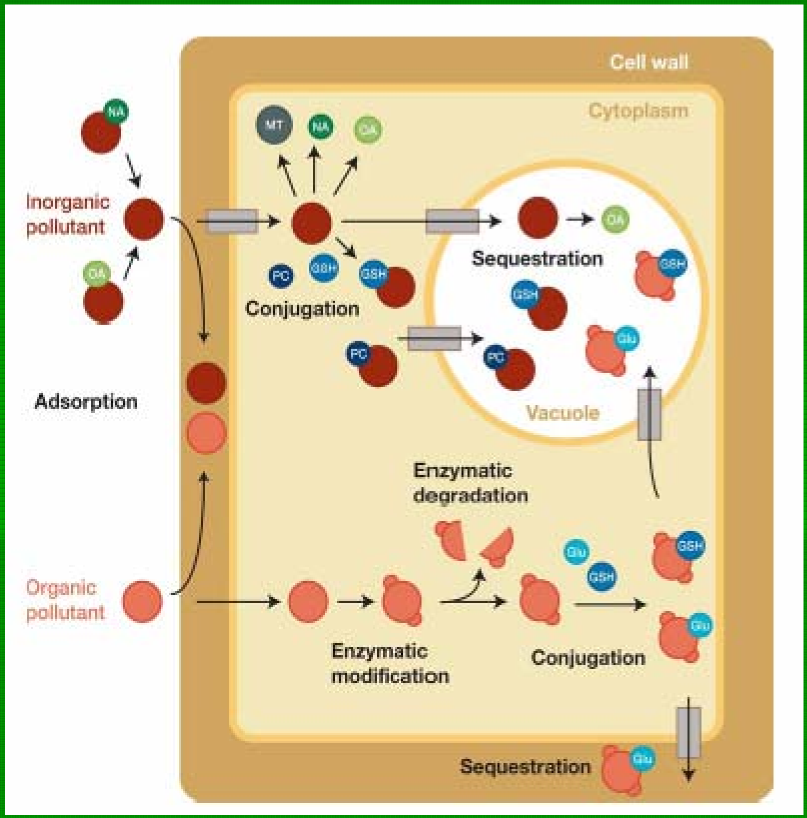
Concentrations of As in fronds of different ferns



Part of a leaf's cross section showing transpiration flow of Hg(II) to the site of reduction in the apoplastic space and Hg° emission through the stomata.

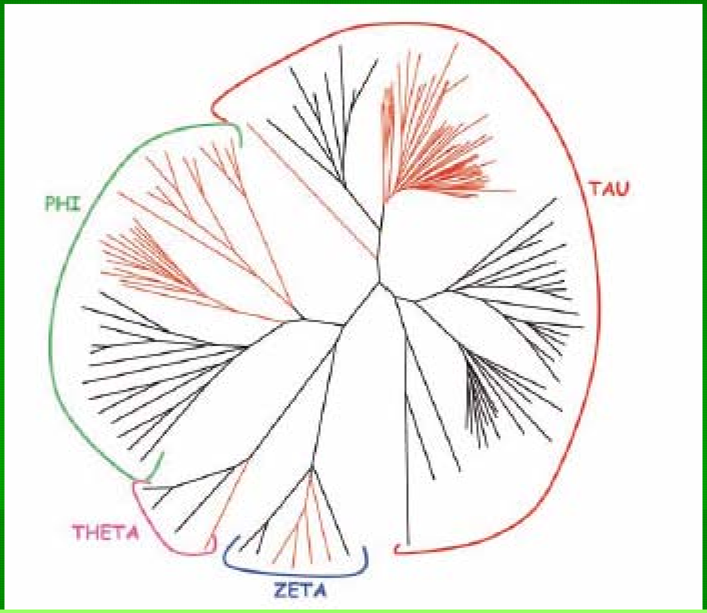
Basic mechanisms: organic pollutants

- Basic enzymology of phytodegradation of PAHs, TNT, dyes, herbicides
- In vitro cultures or whole plants are utilised for this purpose
- Recombinant DNA approach to improve the capabilities of selected plant species



Genetic control mechanisms in phytoremediation of organic pollutants

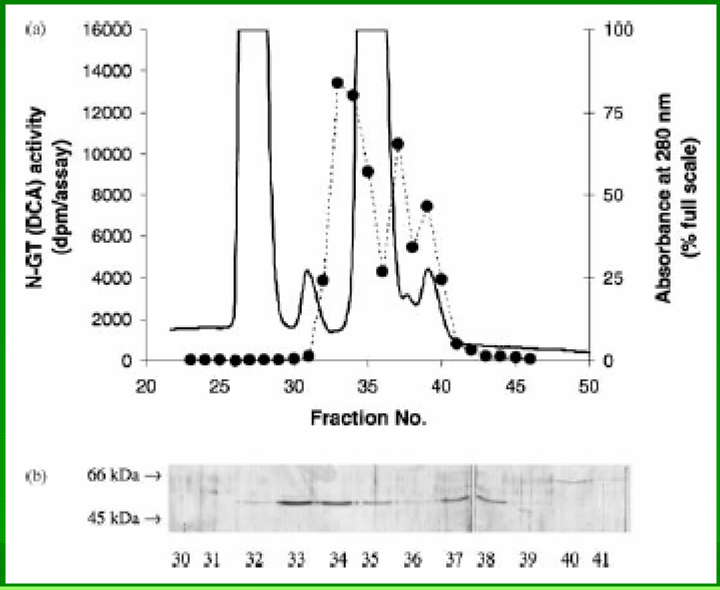
- A comprehensive analysis of glutathione-S-transferases has been carried out in a model species, rice, with identification of classes and phylogenetic relatioships (Soranzo et al. 2004)



Phylogenetic tree, based on the coding sequences of cytosolic GSTs, 86 from the monocots rice and maize (in red) and 60 from the dicots soybean and Arabidopsis (in black).

Analysis and identification of metabolic enzymes involved in assimilation

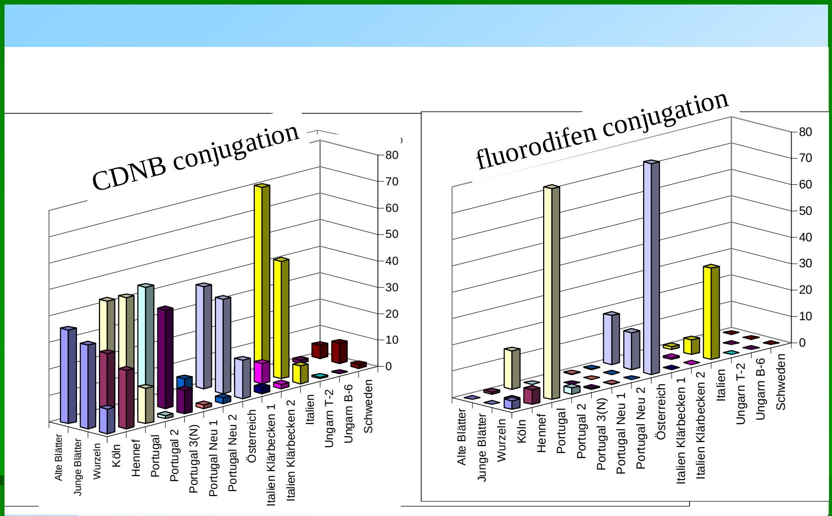
- Purification and analysis with MALDI-TOF-MS of a glucosyltransferase from *Arabidopsis thaliana* involved mainly in detoxification by conjugation of 3,4-dichloroaniline lead to discovery of an enzyme which is inducible by pollutants (herbicide safeners) (Loutre et al. 2003).



Anion exchange chromatography of the affinity purified N-GT. Fractions sampled across the activity peaks were analysed by silver staining after SDS–PAGE and the 50 kDa polypeptide in fraction 33 subjected to peptide mass fingerprinting analysis.

Organics: protein diversity

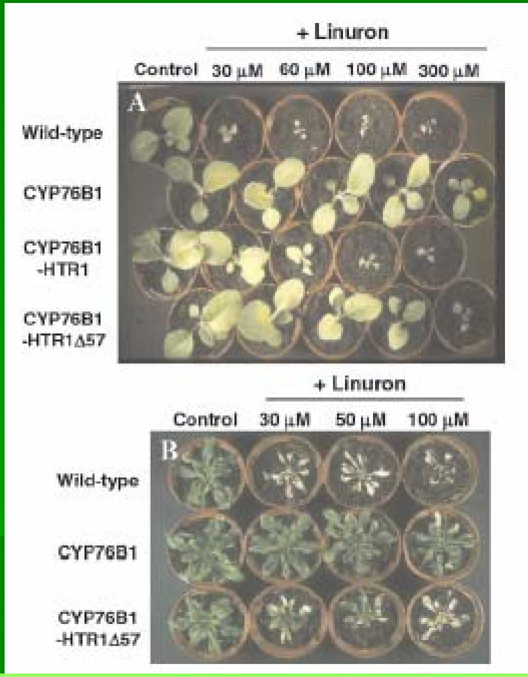
- Collecting *Phragmites* plants from several constructed wetlands across Europe and comparing enzyme activity for GST brought to the conclusion that a correlation exists among contaminants concentration and enzyme activity in the plants (Schroeder 2004)



- high CDNB GST in expanded leaves, lowest in rhizomes
- plants from waste water treatment possess higher activity
- best herbicide detoxification in waste water plant rhizomes

Organics: transgenic approach

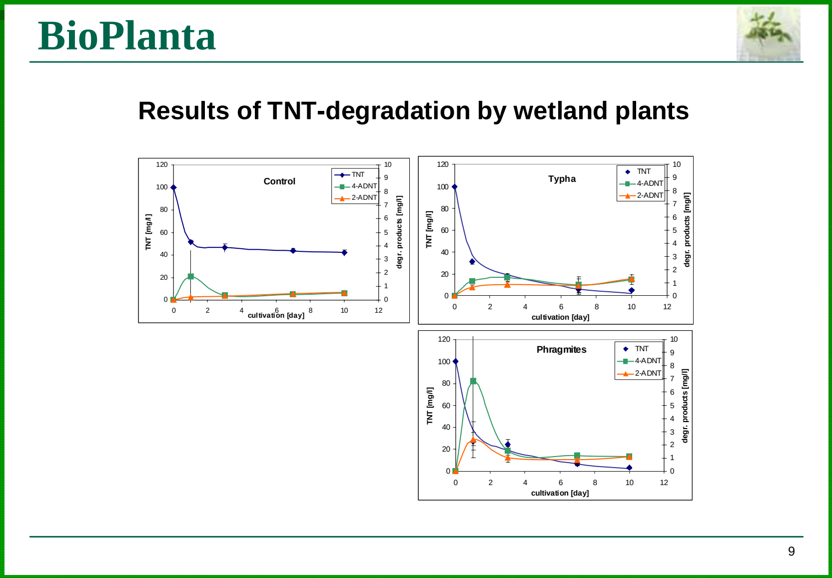
- Transgenic approach has been prospected to improve the performance of plants in phytoremediation. Jerusalem artichoke cytochrome P450 was cloned into *Arabidopsis* and tobacco conferring increased herbicide tolerance (Didierjean et al. 2002).



Increase in phenylurea tolerance in several transgenic constructs of tobacco (upper panel) and *Arabidopsis* (lower panel)

Organics: in vitro cultures for bioassessment studies

- In vitro cultures of wetland plants were utilised for bioassessment study in order to determine their capacity to assimilate and degrade TNT in a typical wetland (Gerth 2004). Bioassessment studies are pivotal to the technological implementation of wetlands.



In *Carex* almost 100% of the applied TNT was detected as TNT or as the first reduction products 2- and 4-ADNT in the medium. An efficient exclusion mechanism is suggested for this plant. In *Juncus*, *Phragmites* and *Typha* lower final TNT-concentrations are reached and the metabolite concentrations are only about 40% of the initial TNT-concentration. In these species uptake and internal metabolization seem to play a role.

Implementation of knowledge for application

- Topics of interest
  - Constructed wetlands
  - Phytostabilization
  - Phytoextraction
  - Short rotation forestry
  - Crop plants (maize, rice, linseed, flax, sunflower)
  - Biofortification

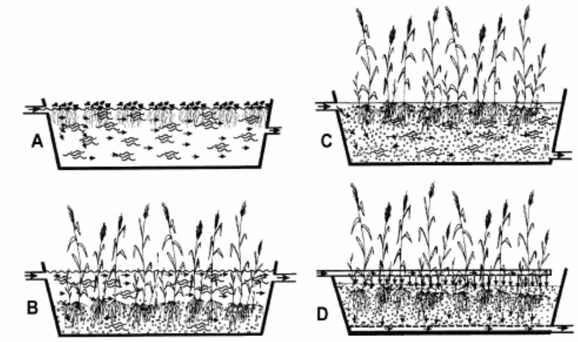
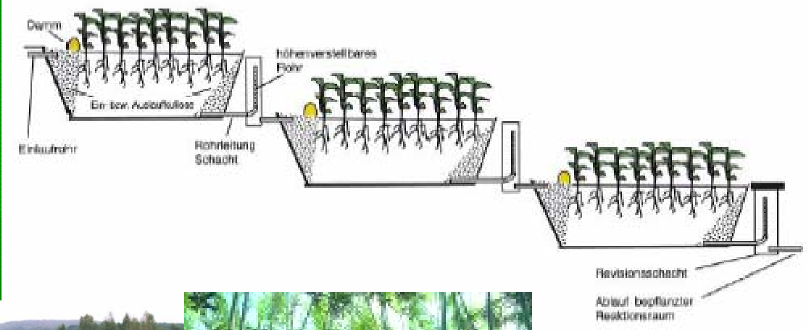
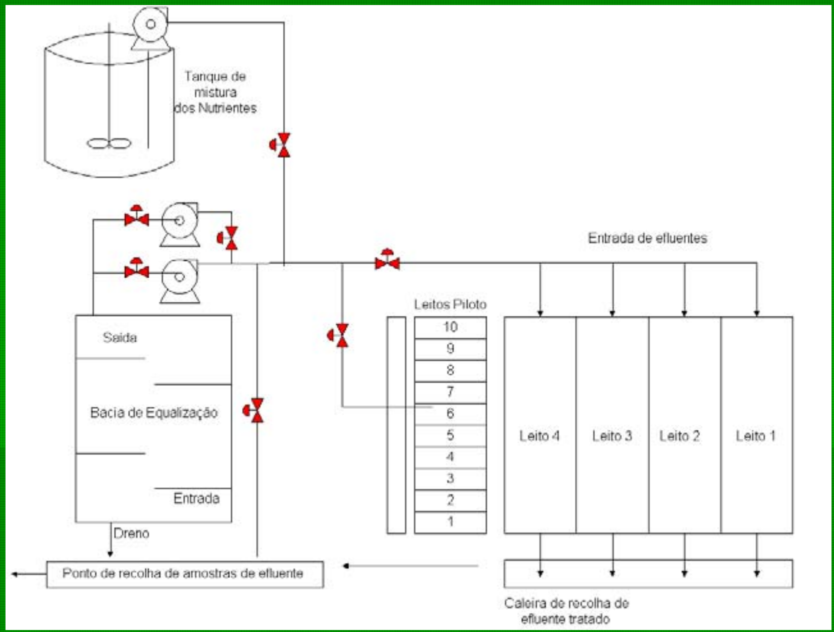


Fig. 2. Pond/wetland systems for wastewater treatment (A, pond with free-floating plants; B, horizontal surface flow wetland or pond with emergent water plants; C, horizontal subsurface flow wetland; D, vertical flow wetland).



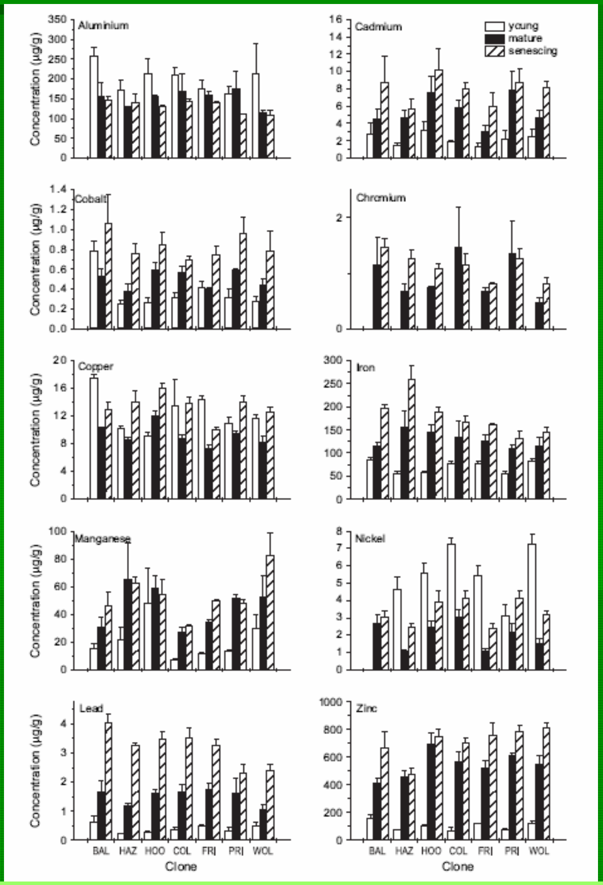
Constructed wetlands

- Constructed wetlands for the treatment of industrial effluents (aniline, nitrobenzene, sulphanilic acid) planted with *Phragmites* extended over 10000 m^2 (Martins-Dias, 2004) representing an innovative technological approach in the treament of this type of effluents.



Applications: short rotation coppice forestry

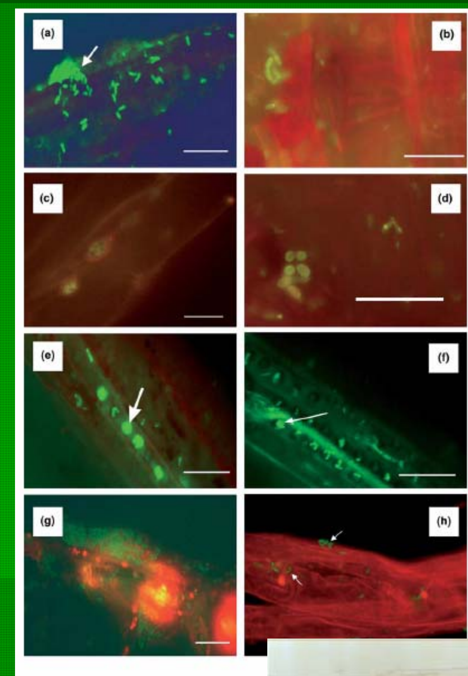
- SRC gives economic advantages, for wood, paper and pulp production, energy recovery. Clones of willow and poplar have been selected for this purpose to increase biomass and provide treament.
- Trees can also uptake heavy metals; clonal variation is being explored (Laureysens et al, 2004)



Variation in mean metal concentration in young, mature and senescing poplar leaves for clones Balsam Spire (BAL), Hazendans (HAZ), Hoogvorst (HOO), Columbia River (COL), Fritzi Pauley (FRI), Primo (PRI) and Wolterson (WOL).

Application: interaction with microorganisms

- Endophytic bacteria improve tolerance and increase phytoremediating capacity for organic contaminants (Barac et al. 2004; Germaine et al. 2004)
- Also wetland plants like *Phragmites* can have endomycorrhizae (Mantovi et al. 2003)



New application: atmosphere

- The Kyoto protocol concerning reduction of pollutants in the atmosphere, especially CO2, brings to three possible solutions:
  - Trading of shares
  - Decreasing emissions
  - Increasing assimilation, i.e. Kyoto forests

New application: natural remediation

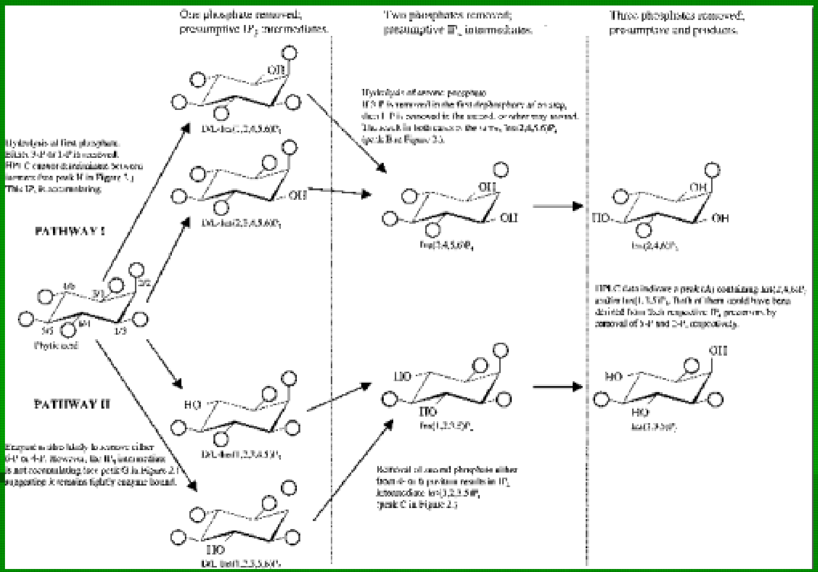
- When dealing with metal-contaminated soils, natural remediation processes can be enhanced by manipulating certain biogeochemical processes that promote reduction in the bioavailable fraction of the metals
- This assisted NR can be achieved adding particular amendants to the soil (lime, phosphate, biosolids)

New application: explosive detection

- The Danish Aresa Biodetection company has patented a transgenic *Arabidopsis thaliana* capable of detecting anti-personnel mines.
- This plant is able to change its green colouring for an intense red colour when its roots come into contact with the nitrogen dioxide (NO2) gas given off by the explosives.

New application: biofortification

- Crops can be `biofortified' with micronutrients using plant breeding and/or transgenic strategies, increasing micronutrient levels in these foods without negatively impacting crop productivity
- Phytic acid in the tissues decreases availability of mineral nutrients, and therefore improvement or genetic engineering can produce plants with lower contents of phytic acid, or expressing the phytase enzyme



New application: biofumigation

- *Brassica juncea* is a well-known phytoremediating plant, but it is explored also for its capacity to produce glucosinolates. These secondary metabolites have a fumigant action, combating pests or pathogens. The plant can be used as “green manure” or in crop rotation
- Correlation between glucosinolate production and metal accumulation have not yet been explored

Networking

- Until 2004, COST Action 837 “Plant biotechnology for the removal of organic pollutants and toxic metals from wastewaters and contaminated sites”
  - Selection of appropriate plants for the uptake and metabolism of organic pollutants.
  - Delineation of metabolic pathways and enzymes involved.
  - Identification of metabolites produced and study of their (eco)toxicological behaviour.
  - Selection of plants able to hyperaccumulate toxic metals.
  - Understanding the physiological and biochemical mechanisms leading to the uptake, translocation and accumulation of metals.
  - Production of a databank of genes/enzymes that will improve the rate and extent of detoxification of organic pollutants and toxic metals.
  - Evaluation of the prospect of using metabolic engineering tools to improve the capacity of higher plants for phytoremediation and clean-up of industrial effluents.
  - Generation, cultivation and evaluation of plants adapted to decontamination of specifically polluted sites or wastewaters.
  - Execution of pilot studies in the scale-up of selected plants with an increased capacity for the biodegradation of xenobiotics and accumulation of toxic metals.

Networking

- From 2004, COST Action 859 “Phytotechnologies to promote sustainable land use management and improve food safety”
  - WG1: Plant uptake/exclusion and translocation of nutrients and contaminants
  - WG2: Exploiting 'genomics, proteomics and metabolomics‘ approaches in phytotechnologies
  - WG3: Improving nutritional quality and safety of food crops
  - WG4: Integration and application of phytotechnologies

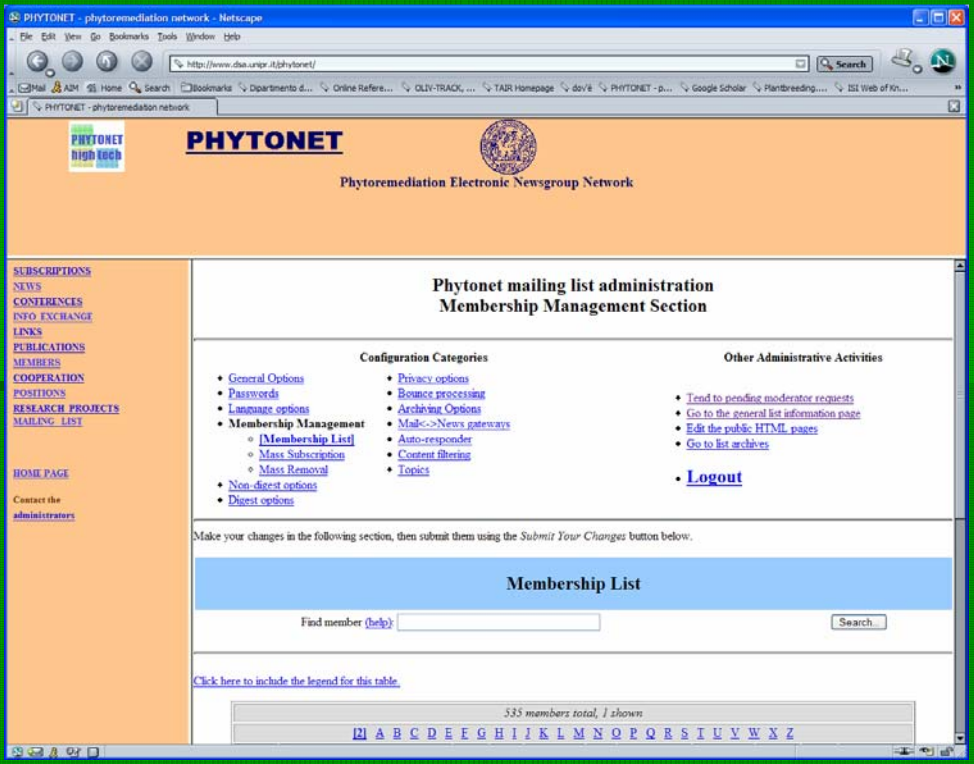
www.gre.ac.uk/cost859

Thematic networks

- PHYTONET (www.dsa.unipr.it/phytonet)
  - The Phytonet Newsgroup was developed to allow easy worldwide communications between scientists who work on problems related with Phytoremediation and Application of Plant Systems to Environmental Control.
- ESSENCE (essence.vsnu.nl/)
  - ESSENCE is a European network for people engaged in environmental sciences and sustainable development in higher education throughout Europe.

Mailing list

- 535 members
- Discussion on several scientific aspects, job offers, books and publications, conferences, etc.



Education and training

- University degrees in
  - Environmental Sciences
  - Biotechnology
- Master Courses
  - E.g. International University Master on Science and technology for sustainable development in contaminated sites (Parma)
- PhD Courses

NATO ASI School, 2005

Travel grants from NSF are available for US applicants!

Conclusions

- A survey on the scientific literature was made of the last 4 years by using broad range keywords in the field of phytoremediation. The survey showed that research in US and Europe proceed on similar tracks with comparable priorities (i.e. basic mechanisms, plant selection).
- As compared with a forecast made about ten years ago, the priorities were conserved, with the exception that natural attenuation and ecological successions were not investigated deeply.
- Particularly suffering is research on transgenic plants for environmental applications, probably because of the negative impact from agricultural applications. Apparently, public opinion and end-users are not sufficiently prepared to understand subtle scientific differences.

Conclusions (continued)

- What seems to differ mostly in US and Europe is the extent of cases to which phytoremediation was successfully applied. Whereas in US legislation and normative favoured the application of phytotechnologies (SUPERFUND), in Europe contradictions and limitations in current normative rather prevents exploitation of phytoremediation.
- Normative constraints, associated with a higher value of the land (due to its limited availability) and limitations in time, are still favouring the use of conventional remediation technologies.
- Education and training of both scientists and decision-makers to a new scientific and environmental vision can help to expand the application of phytotechnologies in the future.

Thanks
for your
attention!