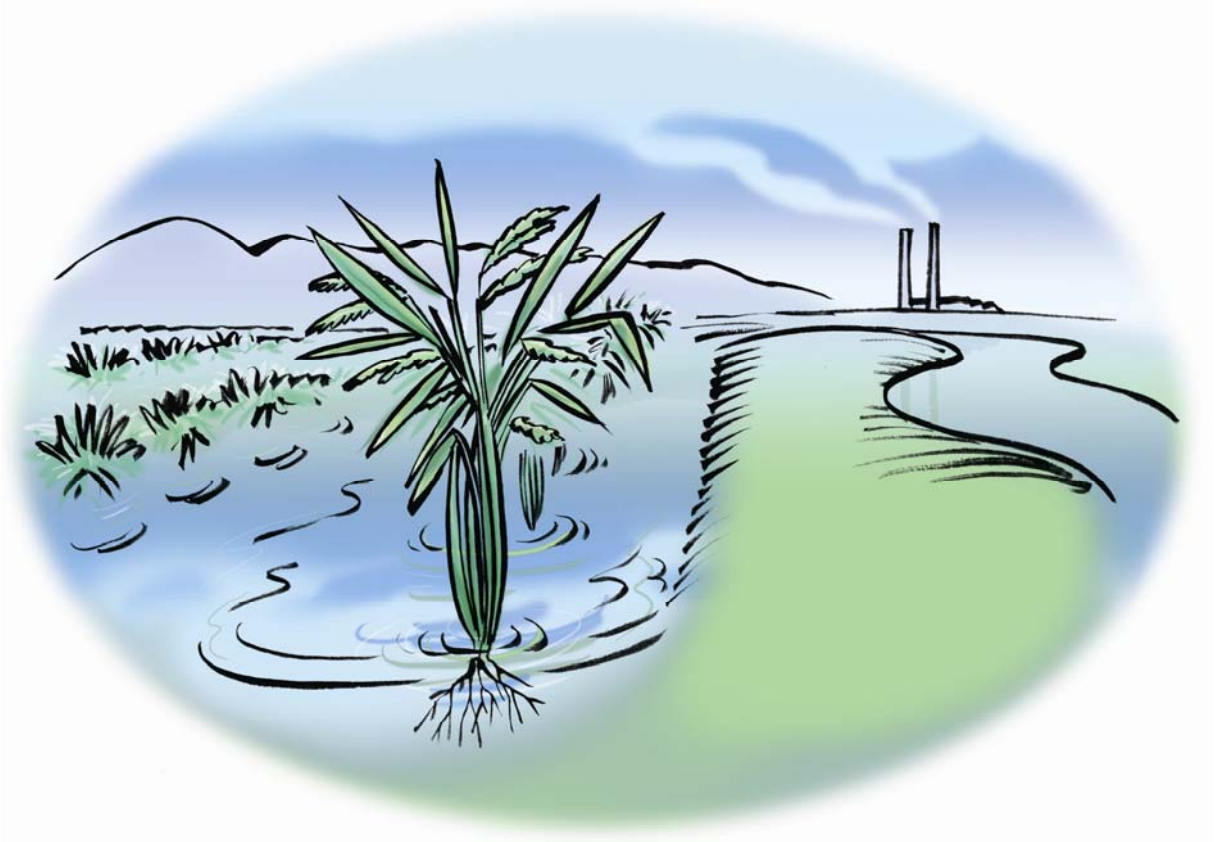
20th New Phytologist Symposium

Arsenic: unravelling its metabolism and speciation in plants

Douglas Hotel, Aberdeen, Scotland, UK

26–27 June 2008



Programme, abstracts and participants

New Phytologist

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Arsenic: unravelling its metabolism and speciation in plants

Douglas Hotel, Aberdeen, Scotland, UK

Organizing committee

Henk Schat (*Amsterdam, The Netherlands*)

Andy Meharg (*Aberdeen, Scotland*)

Steve McGrath (*Rothamsted, UK*)

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Programme, abstracts and participant list compiled by Jill Brooke.
Arsenic illustration by Sam Day www.samday.com

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Session 1: Environment 1
Chair Henk Schat (Amsterdam, The Netherlands)

1.1 Arsenic dynamics in the rhizosphere of wetland plants: Redox gradients and microbial processes

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Wetland plants are capable of delivering oxygen below ground, thus leading to high oxygenation of the rhizosphere, while the bulk soil remains largely anaerobic creating a redox gradient between the rhizosphere and bulk soil. As a result of this oxygenation in the rhizosphere, iron plaque (deposition of iron oxides) is commonly formed on the surfaces of wetland plant roots. The structure of iron plaque is characterized as amorphous or crystalline iron (oxyhydr)oxides. Iron plaque is thought to have a strong affinity for arsenate, therefore influencing the dynamics of arsenic in soil-plant systems. In this presentation the role of iron plaque mediated arsenic sequestration and speciation change in the rhizosphere of wetland plants will be discussed. In addition, the microbial processes likely influencing iron cycling and arsenic biogeochemistry will also be considered in detail. These include the nitrate-dependent anaerobic iron oxidizing bacteria and ammonia oxidizing bacteria and archeae in the rhizosphere of wetland plants. Finally this talk will try to present a picture of the possible coupling between nitrogen, iron and arsenic through microbial processes.

1.2 Arsenic in aerobic rhizospheres

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There are still only a small number of publications that explicitly deal with the fate of arsenic in aerobic rhizospheres. Most of the information available is conceptual and has been inferred from soil and plant science.
At high redox potential, arsenic in soil solutions is known to be present as arsenate ($HAsO_4^{2-}$ and $H_2AsO_4^-$, depending on pH). Typically, arsenic solubility is controlled by sorption on oxides/hydroxides of iron, aluminium and manganese unless highly soluble primary minerals such as scorodite are present in the soil solid phase. Due to ion competition, arsenic solubility is typically enhanced in soils containing large amounts of dissolved organic compounds such as organic acids, and at high phosphate levels.
Plant root activities are thought to modify arsenic solubility, the rate of replenishment from the soil solid phase and bioavailability in the rhizosphere through organic acid exudation, change in redox potential and excretion of protons. Plants can regulate arsenic uptake by switching between low- and high-affinity uptake systems and thus control toxicity. Only few plants are able to hyperaccumulate arsenic whereas most plants are excluders. Interactions between plants and rhizosphere microorganism can further modify phytoavailability. There is evidence that the resistance of both plants and mycorrhizal fungi to high arsenic concentrations can determine the uptake behaviour of the plant – mycorrhizal associations.
The largely unexplored fate of arsenic in aerobic rhizospheres requires further experimental and modelling work to understand the complex interactions controlling arsenic bioavailability and to enable effective management of arsenic-contaminated soils by phytoextraction or to minimise the transfer into other environmental compartments and the food chain.

1.3 The role of arbuscular mycorrhizal fungi in arsenic uptake and assimilation by plants

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Competition between phosphate (Pi) and arsenate (As(V)) for uptake by plant roots has been demonstrated in solution culture. This has led to suggestions that arbuscular mycorrhizas (AM), which play a major role in P nutrition, may also modify uptake of As(V) or compensate for reduced Pi uptake under As stress. Several investigations have shown that both P nutrition and AM provide 'protective effects' against As in soil-grown plants, but the mechanisms are unclear. We found no evidence for competition between As(V) and Pi for direct uptake from soil. New molecular and physiological research on the integration of AM and direct root uptake of Pi has established that the AM pathway contributes a high proportion of total P uptake, with the direct uptake pathway frequently playing a reduced role. We will discuss the implications of these changes in uptake pathway for As(V) uptake and toxicity in AM plants.

1.4 Arsenic reduction and transport in the arsenic hyperaccumulating fern *Pteris vittata*

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Pteris vittata sporophytes hyperaccumulate arsenic to 1–2% of their dry weight. Like the sporophyte, the gametophyte also hyperaccumulates arsenic, reducing arsenate (As[V]) to arsenite (As[III]), and storing arsenic as free As[III] (Gumaelius *et al*., 2004; Pickering *et al*., 2006). Here we report the isolation and characterization of both an arsenate reductase (PvACR2) (Ellis *et al*., 2006) and an arsenite transporter (PvACR3) from *P. vittata* that can suppress the arsenate sensitivity and arsenic hyper-accumulation phenotypes of *Saccharomyces cerevisiae* (yeast) lacking the arsenate reductase ScACR2 or arsenite transporter ScACR3, respectively. Recombinant PvACR2 protein has *in vitro* arsenate reductase activity, and lacks phosphatase activity, similar to the canonical yeast arsenate reductase ScACR2. Further, expression of *PvACR3* in yeast was observed to decrease the rate of arsenic accumulation, suggesting that PvACR3 can act to efflux arsenite from cells. The steady-state level of *PvACR2* expression in *P. vittata* was found to be similar in the absence and presence of arsenate, and this correlates with total arsenate reductase activity. However, the steady-state level of *PvACR3* expression in *P. vittata* was rapidly increased within 24hr after exposure to arsenate. Further work is underway to determine the role of both PvACR2 and PvACR3 in arsenic hyperaccumulation in *P. vittata*.

1.5 Phytoremediation of arsenic contaminated soils

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Phytoremediation is often said to be an option for remediation of polluted soils. This presentation will discuss which terrestrial plants are known to hyperaccumulate arsenic, what characteristics are typical of this phenomenon, and attempt to give an indication of the adaptive significance.

It is emphasised that the ability to hyperaccumulate arsenic should be demonstrated on real field contaminated soils. Bioconcentration factors obtained from studies using hydroponic culture, sand culture, or even soils spiked with soluble arsenic, do not give a realistic measure of how the plants will perform on field contaminated soils, where arsenic is usually much less bioavailable. Hydroponic culture or spiking experiments are useful for investigating mechanisms of arsenic uptake and tolerance, but often the results cannot be extrapolated to the field. Arsenic tolerance is also an important trait, because arsenic-sensitive plants are not likely to establish and efficiently produce large biomass on contaminated soils. The efficiency of phytoextraction is determined by two key factors: biomass production and the arsenic bioconcentration factor. The latter, defined as the ratio of arsenic concentration in plant shoots to arsenic concentration in soil, is a measure of the ability of a plant to take up and transport arsenic to the shoots, which are the easily harvestable parts. With rare exceptions, most plants have a bioconcentration factor for arsenic of much smaller than 1. For these plants, phytoextraction is not feasible regardless how large their potential biomass production is.

1.6 Arsenic efflux

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Reduction of arsenate followed by efflux of arsenite is an important mechanism of arsenic tolerance employed by microorganisms. We have recently shown that arsenate in the medium was rapidly transformed to arsenite in the presence of plant roots. Further experiments showed that this was a result of arsenate uptake and reduction by roots, followed by efflux of arsenite to the medium. This process is common in many plant species, including *Arabidopsis thaliana*, tomato, rice, barley, wheat, maize and *Holcus lanatus*. An exception is the hyperaccumulator *Pteris vittata*, which takes up and transports As to shoots extremely efficiently and releases little arsenite to the external medium. Arsenite efflux was inhibited by the metabolic inhibitor carbonylcyanide m-chlorophenylhydrazone, suggesting that it is an active process. The role of arsenite efflux in arsenic tolerance in higher plants remains unclear. In *Holcus lanatus*, an As-tolerant ecotype took up less arsenate, and also released less arsenite to the medium, than an As-sensitive ecotype. Arsenite efflux as a proportion of the arsenate uptake appeared to be similar in the two contrasting ecotypes, suggesting that there is no adaptive enhancement of arsenite efflux in the tolerant ecotype. Delivery of As to xylem also involves arsenite efflux. We have found that arsenite is the predominant species of As in the xylem sap of a range of plant species, including *Pteris vittata*, tomato, rice, barley and *Holcus lanatus*. Possible mechanisms of arsenite efflux to xylem will be discussed.

1.7 The arsenic biogeocycle

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Arsenic is ubiquitous in the environment. Volcanic activity and hydrothermal sources are major geochemical sources of human exposure to arsenic. The Yellowstone Caldera, the largest volcanic system in North America, discharges inorganic arsenic from hot springs, where soluble arsenic can be 4 mM, one of the highest concentrations in the world, and abundant microbial life grows there. Recently we found that the thermophilic, acidophilic alga *Cyanidoschyzon merolae* methylates arsenic to trimethylarsine gas. This primitive plant, which grows at ~40 - 57˚C and pH 0.2 - 4.0, forms the major biomass in these hot springs. We cloned *C. merolae* arsenic methylation (*arsM*) genes and showed that they confer arsenite resistance in *E. coli*. Purified algal ArsM is a thermophilic enzyme that methylates As(III) to a variety of products, including TMA gas.

Anthropogenic sources of arsenic include herbicides such as monomethylarsenate (MMA) and dimethylarsenate (DMA). During the last century, Atochem/Elf Aquitaine Desiccant L-10 arsenic acid (H_3AsO_4), euphemistically called “harvest aid for cotton”, was used to defoliate cotton crops. In the southern United States both inorganic and organic arsenic are found in rice that is now grown in those fields. We propose to use transgenic *Oryza sativa* expressing *arsM* genes for arsenic phytoremediation.

In addition, MMA and DMA, used as herbicides in Florida, endanger the water supply of Florida municipalities. These herbicides are demethylated to inorganic arsenic in soil, an important and not widely understood step in the arsenic biogeocycle. We show that demethylation of MMA requires reduction of MMA(V) to MMA(III) followed by demethylation to As(III). We have isolated organisms that reduce MMA(V) to MMA(III) or demethylate MMA(III) to As(III). The organisms responsible for transformation of this organoarsenical herbicide are currently being characterized, and the genes/enzymes responsible will be identified.
Supported by NIH grants R37GM55425, R01GM52216 and R01AI45428.

1.8 The risk from arsenic in the food-chain

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Arsenic in rice grain is speciated, in the main, by inorganic arsenic and DMA. Recent findings show that rice is the dominant exposure route to most of the world's population to inorganic arsenic, a chronic carcinogen. In particular, regions of the globe that suffer from elevated arsenic in the soils (from pesticide application, irrigation with contaminated tubewater and through contamination with base and precious mining, etc.) have particularly high grain inorganic arsenic concentrations. Rice is unusual with respect to grain crops in that it is grown anaerobically and it is this soil management that leads to greatly enhanced accumulation of arsenic. Under reduce conditions arsenic is mobilized as the reduced oxyanion arsenite. Arsenite is readily assimilated by rice roots, probably via aquaglycerin porins. Once in the roots both arsenate and arsenite induce PC production, and As-PCs are thought to be sequestered in vacuoles. However inorganic arsenic makes its way to shoots and grains, potentially through repeated association disassociation cycles with PCs, plus loading/unloading into xylem and phloem (for grain). A lot less is known about DMA metabolism and transport in plants, even with respect to whether it is produced *de novo* in *planta* or not. However, DMA has a poor affinity for –SH groups, potentially meaning that it has a less hindered transport path to shoots and grain.

This talk will set out to characterise why rice arsenic physiology is problematic with respect to human food consumption. Examples of key risks arising from arsenic in rice grain around the globe will be highlighted. Strategies for altering rice arsenic metabolism for reducing grain arsenic concentrations will be discussed.

1.9 Membrane transporters involved in the movement of arsenic

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Arsenic mainly enters plants as AsV or AsIII[1]. Intracellular AsV is reduced to AsIII which is either chelated by organic compounds and deposited in vacuoles in this form or it enters vacuoles in inorganic form. Long distance transport from root to shoot occurs in most plants and in this case too, both complexed and inorganic forms may contribute.
Although there is some good evidence that AsV uptake is mediated by phosphate transporters, no specific proteins have been identified that participate in any of the other processes described above. We used various approaches to gain insight into Arabidopsis membrane transporters that potentially play a role in arsenic transport.
Our data show that KO-mutants in all tested vacuolar ABC transporters (MRP1, 2, 4 and 10) had no or little effect on seedling growth in the presence of As. In a KO mutant in one member of the MATE family (DTX32) we found an increased sensitivity to AsV but not AsIII. Though moderate, the effect was recorded in three independent alleles of DTX32 and may be related to higher shoot As levels. Testing of KO mutants in various aquaglyceroporins (NIP5;1, NIP6;1 and NIP7;1) showed the most prominent phenotype in *nip7;1* plants. The latter showed increased tolerance to AsIII but not AsV. Heterologous expression of AtNIP7;1 in yeast showed it is capable of affecting AsIII tolerance in yeast and of transporting AsIII, suggesting that AtNIP7;1 is a main contributor to AsIII uptake in Arabidopsis roots[2].

[1]Tripathi RD, Srivastava S, Mishra S, Singh N, Tuli R, Gupta DK, Maathuis FJM (2007) Arsenic hazards: Strategies for tolerance and remediation by plants. Trends Biotechnology 25: 158–165
[2]Stanislav V Isayenkov, Maathuis FJM (2008) The Arabidopsis thaliana aquaglyceroporin AtNIP7;1 is a pathway for arsenite uptake FEBS Lett, 582: 1625–1628

Session 2: Environment 2
Chair Steve McGrath (Rothamsted, UK)

2.1 The role of arsenate reductase in arsenate toxicity and tolerance in plants

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Arsenite [As(III)] is often considered to be more toxic than arsenate [As(V)] and it has been suggested that the toxicity of As(V) results in fact from its reduction to As(III) within cells. Yet plants posses effective As(V)-inducible As(V) reductases and sequester arsenic, even when taken up as As(V), predominantly as As(III). As(III) is sequestered in vacuoles, complexed with phytochelatins (PCs) and glutathione. Inhibition of PC synthesis resulted in hypersensitivity to both As(V) and As(III). The rate of PC accumulation in As(V) exposed roots appeared to be limited by the activity of arsenate reductase (ASR), rather than that of phytochelatin synthase (PCS) itself. Deletion or ectopic over-expression of *ASR* in Arabidopsis yielded inconsistent tolerance phenotypes, dependent on the level of As(V) exposure and, particularly, the phosphate (P) nutritional status of the plant. When grown under P limitation (1-5 µM P), the *asr* knock-out mutants were consistently hypersensitive to As(V), whereas the 35S::*ASR* plants were consistently As(V)-hypertolerant, as compared to wild type. When grown under luxurious P supply (1 mM P), *asr* mutants were consistently hypertolerant to As(V) and 35S::*ASR* plants were hypersenstive, though only at higher As(V) exposure levels. When grown under intermediate P supply levels (10-100 µM P), the *asr* mutants were slightly As(V)-hypersensitive, though only at lower As(V) exposure levels, and the 35S::*ASR* plants were hypertolerant at lower exposure levels, but hypersenstive at higher ones. We conclude that under conditions of P limitation As(V) tolerance is apparently limited by the As(V) reductase activity, suggesting that cellular toxicity is caused by As(V) as such. Under luxurious P supply, even wild type-level ASR activity is apparently toxifying rather than detoxifying, suggesting that As(V) is relatively non-toxic in P-rich cells.

2.2 The ecological genetics of arsenic tolerance in *Holcus lanatus*

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Previous work has shown that arsenic tolerance in *Holcus lanatus* is governed by a single major gene, which achieves tolerance by suppressing the high affinity phosphate uptake system. In populations from uncontaminated soils, this gene is always polymorphic. The widespread polymorphism suggests that some form of balancing selection must be acting on this system. No correlation has been found between the proportion of tolerant individuals in a population and the phosphorus status of the site, or other edaphic characteristics. Tolerant and non-tolerant individuals were collected from six sites in Devon, and grown in a common garden experiment. Great differences were found between individuals in characters including flowering time, flower number and total plant dry weight, but no systematic differences associated with tolerance phenotype were detected. Seeds from five populations, each with approximately 50% tolerant individuals, were sown at two different densities on rich and poor soils. After 4 months, substantial “self-thinning” had taken place, and the survivors were tested for tolerance. The poor soils had a lower proportion of tolerant individuals than the rich ones, suggesting that non-tolerance, and the presence of a high affinity phosphate uptake system, may be important in seedling establishment on low nutrient soils. It is still not known, however, what process maintains this polymorphism.

2.3 Ecological factors affecting arsenic assimilation by plants

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North West England has large areas of former industrial land that are contaminated with arsenic and heavy metals. Several sites have clearly defined sources of elevated soil As, a known timeline of pollution input and support developing or fully developed plant communities. We have investigated the soil-plant transfer of As in several such sites and have demonstrated a minimal transfer to lower plants, and shoot systems of both herbaceous and woody species. No species that has colonised these sites appears to accumulate As to an extent that presents a potential risk. Sequential analysis of soils indicates that As is largely confined to less labile fractions. However, new management strategies for rehabilitation of brownfield soils may enhance the mobility of As from these less labile fractions and potentially increase plant As uptake. We have investigated the consequences of application of greenwaste compost to urban brownfield soils and found that this treatment promotes the appearance of As in soil pore water, measured 'in situ'. This in turn has been shown to affect plant As uptake from compost-amended soil. The use of greenwaste compost to treat historic As-polluted soils should be approached with caution, as this may disturb the equilibrium that currently exists between soil and plant As where vegetation has naturally regenerated.

Session 3: Technologies
Chair Andy Meharg (Aberdeen, UK)

3.1 Application of synchrotron techniques to investigate in-situ arsenic speciation

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The speciation, or chemical form, of elements governs their fate, toxicity, mobility, and bioavailability in contaminated soils, sediments and water as well as food chain transfer mechanisms. To assess these chemical properties and to accurately gauge contaminant impact on human health and the environment we need to characterize metals at the atomic level to explain macroscopic observations. One can employ an array of techniques to address speciation; however, true in-situ analysis is limited to a few options such as advanced synchrotron radiation methods to elucidate metal speciation and distribution.

Our ability to determine contaminant speciation in the natural environment enhances efforts to understand the mobility, bioavailability, and fate of contaminants in environmental systems, to assess health risks posed by them, and to develop methods to remediate contaminated sites. To attain in-situ atomic level information on the speciation of contaminants we utilize high-energy synchrotron X-rays to probe the chemical environment. At the Advanced Photon Source (APS) of Argonne National Laboratory (Argonne, IL), we incorporate X-ray absorption (XAS), X-ray fluorescence (XRF), and micro-tomography spectroscopies to analyze environmental samples to determine the true, in situ speciation of contaminants. XAS determines the speciation of arsenic while XRF illustrates the two-dimensional distribution of arsenic relative to other elements of interest. Micro-tomography can be utilized to produce three-dimensional images of arsenic distribution. These methods are not limited by sample composition and have been successful in examining soils, plants, and microorganisms. These innovative research tools are expanding our ability to directly identify the role of contaminant speciation on many dynamic processes that influence risk.

This presentation will provide an introduction to synchrotron techniques available at most synchrotron facilities around the world and steps to become a user of synchrotron research. Included in the presentation will be research highlights of past and current projects related to arsenic in the environment and accumulation in rice accomplished through international collaborations.

3.2 Arsenic transport studies using mutants

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The presence of arsenic (As) in soils and water is a major public concern of environmental impact. In plants, arsenate [As(V)], the most bioavailable form of As, is assimilated through phosphate transporters. Previously, we had identified in *Arabidopsis*, a semidominant mutation at the high affinity phosphate transporter *AtPHT1*. *pht1-1* shows reduced phosphate accumulation and enhanced arsenic content indicating that lowering the uptake rate leads to a more efficient acclimation to arsenate, thus providing increased accumulation capacity. This observation has been confirmed using other Pi transport mutants available in the laboratory. In addition we have shown that As(V) represses more efficiently than Pi the expression of genes involved in Pi uptake while induces others specifically regulated by As(V). Here we will report the transcriptome analysis of the As(V)/Pi interacting responses which allowed the identification of genes potentially involved in these converse signalling pathways that may be relevant in As perception.

3.3 Is the formation of arsenic phytochelatins complexes important for the translocation of arsenic in plants

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Is had been hypothesized for many years that arsenic binds to biothiols in the cytosols due to its thiophilicity. More specifically, in plants arsenic had been proposed to bind to glutathione or phytochelatins, but no molecular identification could have been made due to the lack of analytical methodology. Here we report the development of an analytical method which is capable to identify and quantify those delicate arsenic peptides in plant materials. Using HPLC simultaneous hyphenated to elemental and molecular mass spectrometry (ICP-MS/ES-MS), we were able to determine arsenic phytochelatins complexes in plant extracts for the first time. Additionally the species integrity during the extraction was validated by using XANES and EXAFS. This methodology was then applied to a series of experiments which aimed to study the kinetics of arsenic peptide formation and whether the formation of those peptides are significant for the arsenic translocation from roots into shoots or not. Exposure experiments to the different arsenic species which may appear in soil porewater are conducted with different plants. All plant species show a different pattern of arsenic peptides to be formed with different quantities. However, generally it seems that DMA taken up by the plant does not form a phytochelatin complex, while MA and inorganic arsenic does. Whether this is the reason why DMA is preferentially translocated from roots to shoots when compared to the behaviour of inorganic arsenic will be discussed.

3.4 Breeding plants for desirable traits with respect to arsenic

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Rice accumulates unusually high amounts of arsenic from soils into shoots and grain and can thereby expose humans on high rice diets to health-threatening arsenic intake. Evidence of genetic diversity within rice for arsenate (As^V) tolerance was revealed by measuring root growth in $+As^V$ solution relative to $–As^V$ control. The Bala x Azucena mapping population has revealed a gene model which appears to involve epistatic interactions of three major genes which together explain tolerance. Whole genome transcriptomics has revealed 1200 genes that are differentially regulated in response to As^V. Candidate genes around the tolerance loci have been identified.
Pot experiments in Aberdeen found no relationship between tolerance and shoot or grain arsenic uptake despite the fact that, in the tolerance test Bala (tolerant) has approximately two fold higher shoot but not root arsenic compared to Azucena (sensitive). Field experiments on the mapping population also indicate that Bala accumulates 2 fold more arsenic than Azucena, and quantitative trait loci (QTL)s for shoot and grain arsenic have been revealed. These results, data from literature and planned experiments on a wide range of diverse rice germplasm will be discussed in the context of offering prospects for breeding rice with reduced arsenic impact.

3.5 Engineering plants to alter arsenic metabolism

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Arsenic (As) is an extremely toxic carcinogenic metalloid pollutant that adversely affects the health of more than 500 million people worldwide. Previously, we engineered *Arabidopsis* plants co-expressing the *E. coli arsC* gene (arsenate reductase) in leaves and the *γ-ECS* (γ-glutamylcysteine synthetase) genes, constitutively. These plants showed significantly greater arsenic tolerance and accumulation than control plants (*Dhankher et al., 2002, Nature Biotech. 20:1140-45*; *Dhankher, 2005, New Phytol. 168:503-05*). Arsenate (As^{V}) is a phosphate analog, has been shown to be taken up via the phosphate uptake system and further, most of the As in roots gets reduced to arsenite (As^{III}) endogenously and thus get trapped in roots. To further enhance arsenic movement from roots to the aboveground tissues, we examined the endogenous plant activity that affects the electrochemical state and binding of arsenic in roots. We identified an endogenous arsenate reductase, AtACR2 (*Arath*; CDC25), from *Arabidopsis* that reduces As^{V} to As^{III} in plants. The AtACR2 has a catalytic domain, HCX_5R, required for arsenate reductase activity and a C-terminal cysteine-rich metal-binding domain. Inactivation of AtACR2 by RNAi caused the translocation of 10-16 fold more As from root to shoot tissues when these plants were exposed to As^{V} (*Dhankher et al., 2006, PNAS 103: 5413-18*). Additionally, AtACR2 knockdown plants accumulated 2- to 3-fold less phosphorus in shoots compared with wild type. In order to further characterize AtACR2, we overexpressed the AtACR2 in *Arabidopsis* and the transgenic lines overexpressing AtACR2 were highly resistant to As^{V}, presumably due to binding of resulting As^{III} to cysteine-rich C-terminal domain and also accumulated 2-3 fold less As in shoot tissues. Mutational studies showed that replacement of conserved cysteine residues with serine in the catalytic domain (C72S) and in the C-terminal metal-binding domain (C122, 1227S) abolished the As resistance.

Currently, we are combining the expression of all these genes into a high biomass, fast growing, non-food plant, *Crambe abyssinica*, for phytoremediation of arsenic contaminated soil and water, and food crops such as rice for enhanced resistance and decreased uptake of arsenic. We are also exploring the Rice, Arabidopsis and Crambe genome and isolating genes to understand the molecular mechanism of As tolerance to improve the efficiency of As uptake thereby exploiting the plant for commercial phytoremediation as well reducing the arsenic uptake in plants to block As contamination in food chain.

Poster Abstracts

Listed alphabetically by first author, presenting author is underlined

1. Arsenic disrupts trace element content of rice grain

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Soil, shoot and grain trace element levels were examined in 20 paddy fields in Bangladesh and 20 paddy fields in Ghana. The Bangladesh field sites comprised 10 in an As-contaminated groundwater district (Faridpur) and 10 in a non-affected district (Gazipur), whereas the Ghana field sites comprised 12 in gold mining regions (GMRs) and 8 in non-gold mining regions (NMRs). Analysis of the Bangladesh data showed that As, Cu, Mn and Zn concentrations in the Faridpur paddy soils were significantly higher ($P<0.001$) than in the Gazipur paddy soils. The mean As level in reference soils from Faridpur (8.78 ± 1.15 mg/kg) was also significantly higher than for Gazipur paddy soils (1.1 ± 0.03 mg/kg) but significantly lower ($P<0.001$) than for Faridpur paddy soils (14.5 ± 0.6 mg/kg), thus indicating that the source of As in Faridpur soils is partially geogenic but contamination is compounded by irrigation with As-rich tubewell water. Analysis of the Ghana data also showed higher concentrations of total As in paddy soils irrigated with mining-polluted surface water although the difference in means for GMRs vs. NMRs was not significant. While analysis of the overall Ghana-Bangladesh dataset found that both grain and shoot total As levels show a high positive correlation with soil As ($P<0.001$), a significant negative correlation ($P<0.001$) was observed between soil As and shoot/soil Mn and Zn ratios. Thus, as soil As concentration increases, Mn and Zn uptake by the rice plant decreases. The possible mechanisms by which this disruption occurs are discussed.

2. *Calamagrostis arundinacaea* efficiently reduces arsenic concentration within its rhizosphere soil

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Phytoremediation is considered as the most promising method for the in situ cleanup of arsenic contaminated sites. Here we present the results of a survey conducted to identify and characterize native plant species overgrown the area around the old arsenic/gold mine in Zloty Stok (Poland) with the potential use for phytoremediation of contaminated soil. Plants and soil from their rhizosphere were sampled and analysed for concentrations of As and accompanying Mn, Fe, Mg, Ca, Al, Cu, Zn,Ba, Pb, Ni, Cs, V, Cr, Sr, Rb, Sr, Bi, Mo and U. The soil contained primarily very high As, Pb and Al concentrations (up to 7451 mg/kg, 1058 mg/kg and 31 272 mg/kg respectively). The ability of eight identified species to modify the amount of bioavailable arsenic in their rhizosphere was determined by single extractions of soil samples with the use of water, phosphate buffer, EDTA, and acetic acid. Although As-hyperaccumulators were not found, *Calamagrostis arundinacaea* was identified as a new species which successfully carried out the “natural” phytoextraction of arsenic. This plant was able to increase substantially the arsenic availability in the rhizosphere soil (the As concentration in the water extract was higher by an order of magnitude relative to other studied species), and due to efficient uptake decreased the total As concentration within the root zone by around 40% relative to the reference soil. It appeared that Fe concentration in *Calamagrostis* was also higher. Arsenic in soils is largely associated with Fe oxides/hydroxides, and it is known that grasses releases phytosiderophores to mobilize Fe from the soil. The mechanism behind observed efficient phytoextraction of As could be related to root-induced mobilization processes to increase Fe availability which could lead to co-dissolution of As and its removal as a result of efficient uptake from the soil solution. Phytochelatins production by *Calamagrostis* plants in response to As (as As detoxification system) was also studied.

References: Antosiewicz DM, Escude-Duran C, Wierzbowska E, Sklodowska A. Indigenous plant species with the potential for the phytoremediation of arsenic and metals contaminated soil. *Water, Air, Soil Pollution*, in press.
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3. Tolerance to arsenic in *Pteris vittata* is increased by arbuscular mycorrhizal fungi: cytological and proteomic analyses

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Arbuscular mycorrhizal (AM) symbiosis can affect heavy metals absorption in general and in particular can improve As tolerance and accumulation in *Pteris vittata* plants.
The aim of the present work was to study the influence of the AM fungi *Glomus mosseae* and *Gigaspora margarita* on *P. vittata* plants grown in presence and in absence of 25 ppm of As in hydroponic condition. In particular, the interest was focused on cytological and proteomic changes in the fronds. For this purpose fronds were embedded in EPON resin and analyzed by TEM; for proteomic analysis, leaf proteins were extracted by TCA-acetone method and separated by 2DE. Spots of interest were identified by nano-LC ESI Q-TOF MS/MS. Our results showed cell damages in As treated plants, especially at membrane level (cell membrane proliferation, thylakoid disorganization), partly restored by AM symbiosis. At molecular level As induced the statistical significant change of 179 proteins; in AM plants 165 proteins were modified by the *G. mosseae* symbiosis and 157 proteins were modified by the presence of *Gi. margarita*. Our results show that the two AM fungi differentially modulated protein expression in leaves, both in the presence or not of arsenic.

4. A subgroup of plant aquaporins facilitate the bi-directional diffusion of $As(OH)_3$ and $Sb(OH)_3$ across membranes

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Arsenic is a toxic and highly abundant metalloid that endangers all kind of life forms. Although much is known about transport and handling of arsenite [As(III)] in microbes and mammals, transport systems for As(III) in plants have not been molecularly characterized yet. Biochemical data (Jardine & Meharg, 2003) clearly suggested that plant MIPs function as As(III) channels.
Here we show that a subgroup of Nodulin26-like-Intrinsic-Protein (NIP) homologs, AtNIP5;1 and AtNIP6;1 from *Arabidopsis thaliana,* OsNIP2;1 and OsNIP3;2 from *Oryza sativa,* and LjNIP5;1 and LjNIP6;1 from *Lotus japonicus* are bi-directional As(III) channels. Expression of these NIPs sensitized yeast cells to As(III) and Sb(III), and direct transport assays confirmed their ability to facilitate As(III) transport across cell membranes. On medium containing arsenate [As(V)], expression of the same NIPs improved yeast growth, due to increased As(III) efflux. Our data furthermore provide evidence that NIPs can discriminate between highly similar substrates [As(III) and Sb(III)] and that they may have differential preferences in the direction of transport. Uptake- and toxicity-studies using *Arabidopsis* knock-out mutants are currently under investigation.
This is the first molecular identification of plant As(III) transport systems and we propose that metalloid transport through NIPs is an ancient and indispensable feature.

5. Can selenium contribute to an enhanced arsenic tolerance in plants?

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Arsenic and selenium are two elements which are known to be (phyto-)toxic. Plants are mainly exposed to their oxoanions, arsenite, arsenate, selenite and selenate. The phytotoxicity of the two inorganic arsenic species is based on their interaction with biological processes within plant cells. Selenate, as well as selenite, are metabolised by the same pathway as their sulphur analogues. The resulting non-specific incorporation of selenium into biomolecules, where it replaces sulphur, can be cause of a decreased enzyme activity and is thought to be the main cause for selenium toxicity.

For mammalians an antagonism between these two elements was reported. Applied in the right stoichiometric ratio no toxicity symptoms were observed. No such antagonism between arsenic and selenium was reported for plants so far. But it is conceivable that the replacement of sulphur by selenium in phytochelatins might lead to an enhanced arsenic tolerance in some plant species. The formed As^{III}-PC complexes are less toxic than the free arsenite ions. According to the HSAB principle Se-PCs should form more stable complexes with arsenite than their sulphur analogues do. This increase in stability might contribute to an enhanced arsenic tolerance.

The on-line coupling of HPLC with ES-MS and ICP-MS (oxygen collision cell) provides the necessary analytical tool for the simultaneous speciation analysis of all three elements, arsenic, sulphur and selenium, in plant extracts.

6. RuBisCO expression in arbuscular mycorrhizal and As-stressed fern (*Pteris vittata*): a proteomic study

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We employed a proteomic approach to gain deeper insight into the expression of RuBisCO in arsenic hyperaccumulating *Pteris vittata*, inoculated with the AM fungus *Glomus mosseae*. RuBisCO is a holoenzyme composed of eight large subunits (LSU) and eight small subunits (SSU); it is well known that oxidative stress is responsible for LSU fragmentation, but no information exists on the effect of AM fungi in modulating the RuBisCO expression in the presence or not of metal stress.

Fern pinnae proteins were extracted with TCA/acetone precipitation. All the samples were separated by two-dimensional electrophoresis (2-DE). Spots of interest were excised, digested and identified by nano-LC ESI Q-TOF MS/MS analysis.
A drastic reduction in LSU abundance has been detected when the fern was treated with As, while the co-presence of *G. mosseae* and As brought LSU expression to control values. These results indicate that metalloid and the AM fungus affected the mature expression and assembly of RuBisCO.

7. Effect of the arbuscular mycorrhizal fungus *Glomus intraradices* and arsenic on growth of barley and expression of high-affinity P transporters

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Arsenate (As(V)) is a phosphate (P*i*) analogue and is effectively transported across the plasma membrane of plants via high-affinity P-transporters, apparently competing with P*i*. Studies have shown that application of P*i* and colonization by arbuscular mycorrhizal fungi (AMF) can alleviate the toxic effects of arsenic, but the mechanisms underlying the effects have not been well elucidated.

We used a compartmented pot system to investigate the potential of AMF to ameliorate As toxicity in barley and expression of P transporters upon colonization with *Glomus intraradices* and exposure to As(V). There was no effect of *G. intraradices* on shoot or root P concentrations. Shoot and root P concentrations were significantly higher when As(V) was added to the root-hyphal compartment (RHC) than when no As(V) was added. There was a trend towards lower As concentrations in roots when the plants were colonized by the AMF. When arsenate was present in the RHC we found significantly higher shoot and root S concentrations than when no As(V) was added. These results suggest that the expression of P and S transporters may be affected by the presence of arsenate (to be assessed and presented) and that *G. intraradices* may have the ability to reduce As uptake into barley.

8. Bioavailability of As and P in the rhizosphere of plants inoculated with arbuscular mycorrhizal fungi

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Soil unproductiveness, due to build up of arsenic (As) concentrations in soils from human activities, it's an environmental concern. Elevated soil As concentrations generally decrease the ability of the soil to support an economically viable crop on contaminated sites. Arbuscular mycorrhizal fungi (AMF) have their most significant effect on phosphate uptake, but they have also been shown to alleviate metal toxicity to the host plant. Aim of this work was to describe the roles of AMF on As uptake in lettuce plants growing in a As-polluted soil (total As 250 mg kg^{-1}) collected from Tuscany, Italy. In particularly, we tested the effectiveness of native AMF or inoculation with a commercial inoculum, with or without P fertilizer on the growth and uptake of As by lettuce and their effects on As and P fractions in rhizosphere and bulk soil. Greenhouse pot experiments were established and plants were grown, with or without a commercial inoculum (M+, M-) and two levels of P application (P- at 0 kg P ha^{-1} and P+ at 90 kg P ha^{-1}). Mycorrhizal colonization rate, dry weight and As, P concentrations in plants, as well as As and P fractions, were determined. Commercial inoculum and P application increased plant biomass enormously, by enhancing host plant P nutrition and lowering shoot and root As concentrations compared to plants inoculated with native AMF. In fact, in the rhizosphere of M+P+ plants has been evidenced a P soil depletion respect to plants M-P+. Commercial inoculum protected its host plants from the toxicity of excessive As through P nutrition by absorbing more P from the soil.

9. The suitability of *Eucalyptus cladocalyx* for phytostabilisation of arsenic-rich gold-mine tailings

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The gold extraction process produces physically unstable, contaminated wastes. Phytostabilisation is a proven approach to mitigate environmental contamination from As-rich tailings. Our research focused on the suitability of *Eucalyptus* species for this purpose. To date, knowledge of growth and As accumulation in woody species on tailings is minimal. In our field study we identified *Eucalyptus cladocalyx* as a low As accumulating species that exhibited higher survival and growth than other *Eucalyptus* species. Furthermore, we explored the effects of As concentration and speciation on this species. Interestingly, we found that although increasing As concentration had a strong negative effect on growth, it did not significantly increase As accumulation. Moreover, the effect of As concentration on mortality was minimal, indicating enhanced As-tolerance in this species. We also established that arsenite was more toxic at higher As concentrations and was more readily accumulated at lower As concentrations. Extensive variation in plant response to As highlights the opportunity to select for better performing individuals within the species, thereby enhancing the suitability of *E. cladocalyx* for phytostabilisation of As-rich wastes.

10. Effects of arsenic stress on gene expression and MAP kinase pathways in rice roots

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Plant growth is severely affected by toxic concentrations of arsenic (As^{5+}). However, the molecular mechanisms responsible for the regulation of plant growth by arsenic are not well understood. The aim of this study is to investigate the early signalling pathways activated by arsenic on rice (*Oryza sativa* L.) root. To analyze cellular responses to arsenic, we have taken a large-scale analysis of the rice transcriptome during arsenic stress. We also found that As^{5+}-induced 40- and 42-kDa MBP (myelin basic protein) kinases have the characteristics of a MAP kinase. This study confirmed that the 42 kDa kinase-active band contains the activities of OsMPK6. Using ROS-sensitive dye and Ca^{2+} indicator, we demonstrated that As^{5+} induced ROS production and Ca^{2+} accumulation, respectively. Pre-treatment of rice roots with an antioxidant and a NADPH oxidase inhibitor, ascorbate and diphenylene iodonium (DPI), effectively reduced As^{5+}-induced MAP kinase activation. Moreover, calcium-dependent protein kinase (CDPK) antagonist and phospholipase D (PLD) inhibitor, W7 and n-butanol, attenuated As^{5+}-induced MAP kinase activation. Taken together these data provide an overview of molecular and cellular changes elicited by arsenic exposure.

11. Identification and analysis of arsenite transporter from plant

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Plants take up As(V) via phosphate transporters. However, the molecule(s) which mediates As(III) uptake in plants has not been identified. We expected that loss-of-function mutant of As(III) transporter would be tolerant to As(III), as the As(III) could not enter roots in such mutants. We screened As(III) tolerant mutants using EMS-mutagenized *Arabidopsis thaliana* M2 seeds and obtained 3 independent lines. We also analyzed tolerance of T-DNA mutants of NIPs, which are homologues of aquaglyceroporins. Among the lines tested, those carrying T-DNA in *NIP1;1* showed tolerance to As(III),suggesting possible involvement of NIP1:1 in As(III) uptake. We then sequenced the genomic region of *NIP1;1* of the As(III) tolerant EMS-mutants. Each mutant had an independent mutation in *NIP1;1*, establishing that *NIP1;1* is the causal gene of As(III) tolerance. To examine the As(III) transport activity, we used *Xenopus* oocytes expression system. After exposure to As(III), the oocytes injected with *NIP1;1* cRNA accumulated much As(III) than that of control injected with water. We also performed the promoter-GUS and real-time PCR analysis, and found that *NIP1;1* was highly expressed in roots. From these data, we conclude that NIP1;1 is the major As(III) transporter involved in As(III) uptake into roots.

12. Arsenic in soil and corresponding vegetation of contaminated area in Zarshuran (North West of Iran)

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To assess the potential of the native plant species for arsenic phytoremediation, plant and soil samples were collected from Zareshuran area (West Azerbaijan province, North West of Iran) that have histories of arsenic pollution from mine tailings. Samples were taken in 2006 and 2007 and analyzed for total arsenic by atomic absorption spectrophotometer. Total As in the soil ranged from 114 to 8170 mg kg^{-1}, soluble and extractable As from 0.01 to 9.8 and from 0.04 to 19.8 mg kg^{-1} respectively. The criteria used for selecting plants for As accumulation were there Biological Accumulation Coefficient and Concentration Factor. Among 120 plant species, the highest As contents were found in *Isatis capadocica* (1118 mg kg^{-1}) and *Hesperis persica* (1210 mg kg^{-1}). These species highlight as the most promising to be hyperaccumulate As and used in the remediation of the affected area. In general, Arsenic contents of other plants were low, especially in crops and in the most common wild species.

13. Effects of arsenate and phosphate on their accumulation by *Isatis capadocica*

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Arsenate and phosphate interactions are important for better understanding their uptake and accumulation by plant due to their similarities in chemical behaviors. The present study examined the effects of arsenate and phosphate on plant biomass and uptake of arsenate and phosphate by *Isatis capadocica* a newly-discovered good arsenic accumulator. The plants were grown for 4 weeks in hydroponic systems, which received the combinations of 0, 50, 200 and 1000 μmol L^{-1} arsenate and 20, 80, 320 or 1260 µmol L^{-1} phosphate, respectively. Interactions between arsenate and phosphate influenced their availability in the solution, and thus plant growth and uptake of arsenate and phosphate. At low and medium arsenate levels (50 and 200 μmol L^{-1}), phosphate had slight effects on arsenate uptake by and growth of *Isatis capadocica*. However, phosphate substantially increased plant biomass and arsenate accumulation by alleviating arsenate phytotoxicity at high arsenate levels (1260 μmol L^{-1}). Moderate doses of arsenate increased plant phosphate uptake, but decreased phosphate concentrations at high doses because of its phytotoxicity. Plant arsenate uptake and toxicity depends on both the P/As ratio and phosphate nutrition levels. Based on our result, at the same P/As ratio arsenate is much less toxic at high phosphate levels since more arsenate is taken up by the plants at low phosphate levels. Our findings suggest that phosphate application may be an important strategy for efficient use of *Isatis* to phytoremediate arsenic contaminated soils.

14. Effects of arsenic concentrations and forms on growth and arsenic uptake and accumulation by *Isatis capadocica*

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Isatis capadocica exhibits considerable promise in the phytoremediation of arsenic - contaminated sites due to its ability of accumulating arsenic in the aboveground portions with the accompaniment of good biomass. In this study, *Isatis capadocica* were exposed to 0, 25, 50, 100and 500 µM of organic (dimethylarsinic acid and monomethylarsinic acid) and inorganic (arsenate and arsenite) arsenic compounds for 21-d in hydroponic systems. Isatis capadocica was tolerant of arsenate and survived in hydroponic systems up to 1000 µM l^{-1} arsenate. Addition of 50 µM l^{-1} arsenic was best for Isatis growth and arsenic accumulation resulting in higher biomass. Comparing the availability of arsenic compounds in *Isatis capadocica* increased in order arsenate > arsenite > monomethylarsinic > dimethylarsinic acid. Arsenic toxicity symptoms reflected in suppressed growth of plants were most significant with dimethyl arsenic acid treatments followed by monomethyl arsenic acid, arsenite and arsenate. The results suggest that Isatis may be a good candidate to remediate arsenic contaminated area.

15. Arsenic levels in Libyan soils and grains.

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Surface water is scarce or almost non-existent in arid, southern regions of Libya where ground water is utilised for crop irrigation. Irrigated agriculture in this area started in 1970 when several deep wells were drilled for irrigation purposes to grow wheat and barley. Use of irrigation water has resulted in higher salinity (nitrate, chloride, sodium, calcium, magnesium, ammonia) and some trace elements in the soils, while the impact of high fertilizer applications in this region is unknown, Phosphate fertilizers in particular can have high loadings to toxic elements such as arsenic.

The objective of this study was to establish levels of arsenic concentrations of the grains and soils of the Libyan arable belt. Soil, wheat and barley samples were collected from 3 Libyan agricultural regions: East, West and South. Soil samples were taken from the root zone of each sample, from depths (0–10 cm). After suitable sample preparation and extraction, analysis was performed by inductively coupled plasma mass spectrometry. A one-way analysis of variance showed that the differences in soils and shoots arsenic levels between location means were highly significant ($P<0.001$), The Eastern region recorded the highest mean arsenic level in Libyan soil, with 8.10 mg/kg, but the highest mean shoot arsenic levels were recorded in the southern of Libyan samples at 0.19 mg/kg. Sequential extraction showed that while the southern Libyan soils had lower total arsenic, the plant available fraction was higher than for soils from the other regions. The role of fertilizers and irrigation water in the high plant available fraction in southern Libyan soils is explored.

16. Arsenic tolerance mechanisms in *Holcus lanatus*: is arsenite efflux involved?

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Holcus lanatus L. (Poaceae) growing on As contaminated sites has evolved arsenate resistance mechanisms manifested as suppressed arsenate uptake. Additionally, internal detoxification occurs through arsenate reduction to arsenite, which is then complexed with thiol compounds and sequestered in the vacuoles. In microorganisms, arsenite efflux is also an important mechanism of As detoxification. Recently, it has been shown that plant roots can rapidly efflux arsenite to the external medium. In this study, we tested whether arsenite efflux from root cells is a component of the mechanism of arsenate resistance in *H. lanatus.* Tolerant and non-tolerant ecotypes were grown hydroponically with different arsenate concentrations, and As speciation was determined in nutrient solutions, roots, xylem sap and shoots. At the same arsenate exposure concentration, the non-tolerant ecotype took up more As and effluxed more arsenite to the nutrient solution than the tolerant ecotype. At a similar level of As accumulation in the roots, arsenite efflux was similar in the two ecotypes, indicating that arsenite efflux was proportional to arsenate uptake and was not enhanced in the tolerant ecotype. Most of the As in the roots (86–95%) and majority of the As in xylem sap (55–77%) was present as arsenite.

17. Effect of phosphorus on arsenate tolerance in differentially tolerant rice varieties, Azucena & Bala

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Studies on *Holcus lanatus* showed that it achieves Arsenic(As)-tolerance by down-regulating phosphate-transporters. Here an experiment examining the effects of phosphorus (P) on As-tolerance in rice is presented using an As-tolerant (Bala) and non-tolerant genotype (Azucena) of rice.

Bala and Azucena were grown for 11 days in hydroponics under various treatments: 0 or 13.3μM arsenate, and one of six P concentrations ranging from 0-5mM. Tolerance was calculated as a percentage of root growth under arsenic conditions compared to growth in controls. Shoots and roots were analysed for total arsenic concentrations via Hydride generator and ICP-MS. At P-treatments ≥ 50μM, arsenic tolerance increased and P treatments ≥ 500μM showed lower levels of total root As. At 50μM P an increase in tolerance is observed, however no difference in arsenic content can be seen when compared to lower P treatments. Phosphorus confers tolerance to rice seedlings at P concentrations ≥ 50μM. It appears to convey tolerance partly by reducing arsenic uptake, as roots had reduced arsenic at higher P-concentrations. However, there appears to be another mechanism involved which is independent of this reduced arsenic uptake as increases in tolerances are visible at 50μM P despite no significant reduction in root arsenic concentrations.

18. Arsenic levels in Tanzanian soils and its concentration in maize

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Soils as well as maize shoots and grains were sampled from 40 farms in 8 districts in Tanzania with the objective of assessing the arsenic levels of contamination. Another sample of maize shoots were taken from maize grown in pots with soil amended with vermiculite containing 3.40 mg As/kg to assess its availability. Digestion of the samples was carried out using nitric acid and hydrogen peroxide. Maize shoots and grains were digested in a microwave while soils were digested using a block digest. The arsenic in the digests was determined by ICP-MS. The results showed that more than 95% of the soils and maize samples from Tanzania have very low levels of arsenic. A few elevated arsenic levels were found in samples taken from maize farms which are in the mining areas, research and urban centres. The high levels of arsenic in these farms could be associated with the release of arsenic from weathered rocks, wastes disposal and application of fertilizers and pesticides containing arsenic. Arsenic in vermiculite was found to be unavailable for maize uptake. Vermiculite application reduced shoot arsenic content and thus, makes it safe to use as a growing media or a soil conditioner.

19. Arsenic accumulation in rice grains negative correlation with rates of ROL and root porosity

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Paddy rice suffers from elevated arsenic (As) levels in the soil. Great differences have been commonly observed in As accumulation in aboveground tissues, rates of radial oxygen loss (ROL) from roots, and porosity of roots among rice varieties. We have explored the correlation between As accumulation in grains and straw, rates of ROL, and porosity of roots using 25 rice varieties. Two pot experiments have been conducted: one is that of rice seedlings which are planted in soil added 100 mg As kg^{-1} for analyzing As in grains and straw; the other is that of which are planted in 0.5 strength deoxygenated nutrient solution containing 0.1% agar for measuring rates of ROL and porosity of roots. The results show that there are great differences in As concentrations in grains and straw, rates of ROL, and porosity of roots among the rice varieties. There are significantly negative correlations between As in grains and straw, and ROL/porosity of roots in the rice varieties. ROLs are significant positively correlated with the porosities. Present results indicate that rice varieties with higher porosity of roots trend to have higher rates of ROL, and are more effective in limiting As transferring to grains and straw.

20. Are silver nitrate impregnated silica-gel tubes an efficient trap for biovolatilized arsenic species?

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Arsenic volatilisation in the environment is a scarcely studied phenomenon due to inherent sampling issues (low concentration, stability...) though it is supposed to be an important pathway of release in the atmosphere. Implementing a simple sampling method to detect low concentrations of volatile arsenic species, using flux chamber and silver nitrate impregnated silica gel tubes, will allow us to know in what extent and under which forms arsenic is released from reduced environment, such as rice cultivated paddy fields, and to have a better understanding of the rice rhizosphere influence.

21. Formation of arsenosugars in freshwater green alga and cyanobacteria

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Metabolic processing of arsenic has been studied extensively for algae and fish in marine ecosystems, but there are few studies for freshwater organisms and cyanobacteria. Marine algae are able to accumulate inorganic arsenic in seawater and biotransform it to arsenosugars, which are unique organic arsenic compounds containing ribose in these molecules. We examined the metabolic processing of arsenic in freshwater algae and cyanobacteria to further elucidate the unique properties of these organisms in aquatic environments. Freshwater green alga (*Chlamydomonas reinhardtii* CC125) and cyanobacteria (*Anabaena cylindrica* NIES-19, *Synechocystis sp.* PCC6803) were exposed to arsenate. Intracellular water-soluble arsenic compounds were extracted with water, and then these arsenic compounds were sequentially determined by high-performance liquid chromatography/inductively coupled plasma-mass spectrometry (HPLC/ICP-MS). HPLC/ICP-MS analysis suggested that incorporated arsenate was metabolically converted to dimethylarsinic acid and arsenosugars (glycerol-ribose and phosphate-ribose) in *C. reinhardtii* CC125 and *A. cylindrica* NIES-19, while incorporated arsenate was converted to methylarsonic acid and dimethylarsinic acid in S. PCC6803. Aquatic algae and several species of cyanobacteria may convert incorporated arsenate inherently into arsenosugars when exposed to toxic inorganic arsenic compounds.

22. Relative importance of arsenic in drinking water, raw rice and cooking water as factors contributing to chronic arsenic exposure from groundwaters in West Bengal

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Rice is a major exposure route for arsenic in endemic areas of Bangladesh and West Bengal. To estimate the risk from rice intake we collected raw and cooked rice samples along with cooking and drinking water from 50 households of an arsenic affected block of West Bengal. The contributions to the median risk from drinking water, raw rice and cooking of rice were found to be 48 %, 44 % and 8 % respectively. The total arsenic in raw rice varied from 0.05 to 0.31 mg/kg. Where the arsenic concentration, As_{cw}, in cooking water was less than 10 µg/l, the arsenic content of cooked rice was found to be typically reduced by 5-50% compared to that in raw rice. Conversely, where As_{cw} was greater than 10 µg/l, increases in arsenic content of cooked rice of as much as 200 % were observed. Other researchers (Signes *et al*.,2008), utilising similar cooking methods, observed that the threshold of As_{cw} above which arsenic concentration in rice is increased by cooking is as much as 50 µg/l – much higher than we observed. We therefore speculate that the type of rice may be an important factor controlling the arsenic retained in the cooked rice.

23. Evaluation of arsenic availability for white lupin plants in pyritic contaminated soils

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The estimation of As phytoavailability is one of the key points in the implementation of soil reclamation using plants. A hydroponic culture with different As doses and a soil culture were carried out in parallel, in order to evaluate the actual As dose that plants are receiving when growing on contaminated soils. Arsenic concentration was determined in plant roots and shoots at the end of the experiment, and several extraction methods were used for soil analysis. The low availability of arsenic for lupin plants in these soils was clearly shown, even for 2900 mg total As kg^{-1}soil. Arsenic concentration was compared between soil and hydroponically grown plants. Arsenic concentration in soil-grown plants corresponded to a soluble As concentration (hydroponic experiment) lower than 8 µM As, which could provide an index of the As doses that should be used in hydroponic experiments when evaluating different plant species for soil reclamation. On the other hand, most of the extraction procedures used showed good correlations with As concentration in shoots. The lower R^2 values obtained corresponded to EDTA and $CaCl_2$ as extractants, but $(NH_4)_2SO_4$, low-weight organic acids and pore water (R^2>0.85, p<0.001) can be used to evaluate As bioavailability in this soil. The amounts of As extracted are significantly higher for all the extraction methods than the amounts of As measured in pore water, showing that availability is also related to other As fractions in soils than the dissolved one.

24. Pot experiment showing arsenate dose response in rice

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High arsenic in rice grain poses a threat to health of people on high rice diets. A greenhouse experiment was performed with rice varieties Azucena (arsenate sensitive) and Bala (arsenate tolerant) to assess the impact of soil arsenate on rice growth and tissue arsenic concentration. There were three treatments of arsenate concentrations (0, 10, 50 mg/kg) and plants were harvested at 8 weeks, 15 weeks and at grain harvest.
Initially, plant mass was strongly reduced by arsenate, but this effect became less obvious with time, so that at harvest the plant mass was not significantly lower than the control. There was no genotype by treatment interaction.
Shoot arsenic concentration was significantly elevated by arsenate and was lower at 8 weeks than 15 weeks or at grain harvest. At 15 weeks, Azucena had higher arsenic in the higher arsenate treatment, but lower in the low arsenate treatment. At grain harvest, Bala had higher shoot arsenic than Azucena in both arsenate treatments. Treatments lead to high grain arsenic (0.21 and 0.42 ug g-1 for 10 and 50 ppm treatments respectively) but no genotypic differences were detected.

25. Identification of QTLs for arsenic accumulation and other toxic elements in rice

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Arsenic contamination in rice is a major problem in South and Southeast Asia, with arsenic from soil or irrigation water accumulating in plants and entering the food chain. We present work combining ionomic analysis (ICP-MS and AAS) and quantitative trait loci (QTL) mapping to identify a large number of loci controlling element accumulation in field grown rice from Wuhan, China. The two parents of the mapping population showed a significant difference in arsenic accumulation with Azucena having ~30% more arsenic in its shoots compared to Bala. The mapping population showed transgressive segregation and a broad sense heritability of 61% for arsenic accumulation. We identified 6 QTLs for arsenic accumulation in rice shoots; two on chromosome 1, one on the lower part of chromosome 3, one on chromosome 5 which co-localises with a phosphorus accumulation QTL, and two on chromosome 6 both of which co-localise with other QTLs for toxic elements. The results from this study will be used in conjunction with a number of other field experiments to identify environmentally stable arsenic accumulation QTLs. Once this has been done an attempt will be made to identify the genes underlying these QTLs.

26. PRAMA: Probabilistic Risk Assessment Modelling for groundwater Arsenic mitigation in West Bengal, India

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Arsenic concentrations in groundwaters in West Bengal, other parts of India and many other parts of the world, including Bangladesh and Pakistan, constitute a major health threat to tens of millions of people who utilize these waters – either directly for drinking or indirectly through the irrigation of the food-crops they eat. The PRAMA Project aims to investigate key factors controlling the health risks arising from the use of such waters and to make their findings accessible to key stakeholders. Geostatistical methods have been used to interpolate existing groundwater arsenic concentrations from a database of over 100,000 wells; food frequency questionnaires combined with chemical analysis are being utilized to quantify exposure routes to human receptors; and all these data are being combined with published dose-response relationships to calculate arsenic-attributable health-risks. Key findings to date include: (i) mitigation of drinking water supplies alone is likely to leave a substantial chronic arsenic exposure due to arsenic in rice; (ii) substitution of arsenic-related with pathogen-related health risks needs to be robustly prevented; and (iii) identification of genetic polymorphisms associated with increased susceptibility to arsenic-attributable illnesses provides further evidence that specific groups within the population may be particularly at risk from chronic arsenic exposure.

27. Arsenic metabolism in plants

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Plants take up inorganic and methylated pentavalent arsenic species through their root system. Some of this arsenic is translocated into the shoot. Uptake rate, translocation rate and metabolism are depending on plant species and environmental conditions (soil type, phosphate/sulphate status etc.). Pentavalent arsenic species are then partly reduced within the cells to their trivalent counterparts, which have a strong affinity to sulphydryl-groups of peptides and proteins. There are two groups of these potential ligands to be distinguished in plants, one the non-specific binding to essential sulphydryl-groups of proteins and second the binding to specifically synthesized sulphydryl-containing peptides for “detoxification”. These As-peptide and protein complexes are stable within the cellular environment, but once extracted (during sample preparation) they are easily falling apart. Suitable separation and detection (identification and quantification) methods for this kind of labile metal-complexes were developed. To reduce the problems caused by complex-stability an elemental detector (ICP-MS) and a molecular detector (ES-trapMS) were used simultaneously for quantification of As (ICP-MS) and identification using MS and MS^2 (ES-trapMS).
We present here the identification and quantification of arsenic peptide complexes extracted from plants after exposure to inorganic and methylated arsenic species.

28. Identifying morphological marker for phytoaccumulation of arsenic in *Brassica juncia* L.

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Previous studies suggest that *Brassica juncia* is a good accumulator of arsenic and is an ideal species to use in future breeding programmes related to arsenic phytoaccumulation. This study aims to identify morphological characters that can be used as markers in *B. juncia* breeding programmes that relate to arsenic phytoaccumulation.
A series of greenhouse experiments were conducted to identify distinguishable morphological characters of *B. juncia*, *vars.* RAI and BAR-11, F1 and F2 generations with potential as markers to achieve the project aim. Two varieties, their F1 and RF1 (reciprocal hybrid) were grown hydroponically in 0, 0.5 and 1 mg l^{-1} sodium arsenite.
The preliminary results suggest that *var*. RAI and above RF1 showed significantly higher (p<0.01) uptake of arsenic than BAR-11 and F1. RAI showed deep roots, little root branching, and a smooth stem, while BARI-11 showed shallow roots, profuse root branching, and a hairy stem. This indicates that these characters may be potential markers.
Further trials will be conducted with 0, 15, 30 and 50 mg l^{-1} sodium arsenite with parents and their F1 and F2 hybrids to investigate any association between uptake and identified morphological markers through segregation analysis, to provide evidence of a genetic link, if any.

29. Will reduction of As(V) and rapid transmembrane cycling of As(III) complicate interpretation of arsenic uptake dynamics for plants grown in aerobic soil?

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Plants take up arsenic (As) from nutrient solutions both as arsenite (AsIII) and arsenate (AsV). It is generally accepted that As(III) enters passively through aquaglycerolporins and that As(V) is taken up actively via phosphate (Pi) transporters, competing weakly with Pi. In soils of different pH and redox status, As can exist in a variety of forms, but it has seemed likely that under aerobic conditions As(V) uptake dominates As uptake.

Recently, Xu *et al.* (2007: *New Phytologist* 176: 590–599) have shown that when young tomato and rice plants growing in nutrient solution were supplied with As(V) there was rapid appearance of As(III) in the medium, considered to result from internal reduction of As(V) and active As(III) efflux. Although Xu *et al.* did not directly extrapolate the findings to As uptake from soil, they suggested that net As(III) efflux is part of a detoxification mechanism, and that roots and soil microbes are likely to be engaged in ongoing As(V)-As(III) reduction-oxidation which might result in considerable As(III) cycling between plant and soil.
Here we examine whether such cycling might influence overall As uptake dynamics for plants growing aerobically in soil, and so invalidate past assumptions about As uptake mechanisms.

30. Interaction of arsenic and selenium in plants

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There is evidence that selenium and arsenic interact in some biological systems: arsenicosis may be mitigated by selenium supplementation (and vice-versa), and the formation of the seleno-bis(*S*-glutathionyl) arsinium anion in mammals has been reported. There is no report of the interaction of these two elements in plants, though there is a considerable body of work describing studies involving the individual elements. We have measured the simultaneous uptake of both elements from hydroponic solution by *Helianthus annus* and by *Eichhornia crassipes* and of both elements from soil by *H. annus*. In addition to total elemental concentrations in roots and shoots, we have investigated the formation of metabolites by HPLC with detection by plasma-source mass spectrometry. Preliminary results for *H. annus* indicate that the presence of selenium decreased both the uptake of arsenic and the proportion transferred to the shoots. In the presence of arsenic, both the amount of selenium and the proportion transferred from roots to shoots was also decreased, though the transfer to shoots was much more dramatically decreased. For *E. crassipes*, the presence of selenium significantly increased the total amount of arsenic taken up. Speciation studies indicated no reduction of arsenate to arsenite and only a trace production of selenomethionine.

31. As-speciation in the rhizosphere of *Lolium perenne* grown in a contaminated alluvial soil with and without P fertilization.

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In hydroponics arsenate uptake can be suppressed by increasing P concentration in solution. Soil systems are more complex as several processes with opposite effects on As bioavailability occur simultaneously following P-application: (i) increase in soil solution P-concentration, (ii) increase in soil solution As-concentration, (iii) shift in phosphate-arsenate ratio. These bulk soil processes are superimposed by the formation of concentration gradients which result from the interaction of mass flow, diffusion and root uptake and release of ions. Recently it has been reported for hydroponic experiments that plants alter the speciation of As in the media. Such a process would additionally alter the effect of P-application on As-uptake as arsenite does not interact with phosphate.
In order to elucidate the magnitude of the different processes following the application of different rates of P, a compartment system experiment was conducted with *Lolium perenne* in an As-contaminated alluvial soil.
Increasing rates of P-application resulted in increasing initial P- and As-concentrations in soil solution and increasing phosphate-arsenate ratio. With 100 mg kg^{-1} P-application, highest dry matter production and As-uptake were measured. As-concentrations decreased in root compartment with time and increasing amounts of arsenite were detected in soil solution sampled close to roots. Within the roots arsenite was the dominant As-species.

32. *Atriplex atacamensis*: a xero-halophyte species of interest for phytostabilization on arsenic-affected soils

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Atriplex atacamensis is a xero-halophyte species endemic from Atacama's desert (North Chile). Plants growing on a polluted mining area (Quillagua: 258µg total As g^{-1} soil) contained 8.6 µg g^{-1} of arsenic in leaves, 4.5µg g^{-1} in stem and 35.2 µg g^{-1} in fruits.
Seedlings were maintained during one month to nutrient solution containing 100 µM or 1000µM arsenate (As^{V}), or 20 µM of arsenite (As^{III}). Total arsenic was measured by ICP-OES and speciation was quantified by HPLC-HGAFS. The presence of As excreted in the trichomes covering the leaf surface was analysed by X-ray diffraction and EDAX.
Most of plants remained alive until the end of the treatment. *A. atacamensis* was effective in taking up arsenic (up to 4022 µg g^{-1}) although more than 85% of total arsenic accumulated in roots. In plants exposed to 20 µM As^{III}, less than 20% of arsenic remained in a non-extractible form comparatively to more than 50% in presence of $As(^{V})$. Arsenate constitutes the major form present in the extractible fraction. Only minor traces of As integrated in claudetite were found on the leaf surface.
Since it is resistant to As and stores high amounts As in roots, this plant should be considered for phytostabilisation purposes.

33. Modelling the arsenic transport system in plants: are phytochelatins actually responsible?

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Phytochelatins (PCs) are sulphur–rich peptides, generic formula $[\gamma\text{-Glu-Cys}]_{2\text{-}4}$-Gly, produced by plants to detoxify phytotoxic elements such as cadmium and arsenic. Typically, metal(loid)s are bound and immobilised by PCs within the roots of the plant, but translocation of the metal(loid) to the leaves can also occur. PC-metal(loid) complexes were proposed as the species involved in intercellular translocation. Analysis of the xylem sap of *Brassica juncea* has shown that arsenic is bound to O- and N- bearing ligands, throwing doubt upon the role of PC's as transporters. A feeding trial involving 46 plant species fed either arsenate (As(V), $[AsO_4]^{3-}$), methylarsonic acid (MA, $CH_3AsO(OH)_2$) or dimethylarsinic acid (DMA, $(CH_3)_2AsO(OH)$), has shown that uptake of inorganic arsenate is generally much greater than that of its methylated analogues. In general, whereas arsenate is efficiently immobilised in the roots, significant translocation of methylated As-species to the leaves occurs. This is further complicated in that the plants subdivide into two groups: one with active translocation of methylated-As species to the leaves, the other with enhanced immobilisation in the roots. Using the experimental data retrieved via HPLC-ICP-MS-ESI-MS from the feeding trials, a statistical model shall be built to elucidate the role of PCs in arsenic intercellular translocation.

34. Identification of arsenic tolerance genes of *Arabidopsis thaliana*

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Phytoremediation, using plants to absorb and remove chemical pollutants, has a promising future in our efforts to combat environmental pollutions. While some arsenic-accumulating plant species exist naturally, this technology will become more attractive with plants engineered to have adequate growth and the ability to accumulate large quantities of arsenic. To this goal a better understanding of arsenic-tolerance mechanisms in plants is of critical importance. Our project is aimed at identifying novel plant genes involved in arsenic tolerance in plants. We have been taking a “multicopy suppressor” approach to screen for cDNA clones from the model plant Arabidopsis thaliana which confer elevated resistance to arsenic when transformed into the yeast Saccharomyces cerevisiae. One such cDNA clone has been recovered which gave transformed yeast significantly high levels of arsenic resistance compared to non-transformed yeast. This cDNA contains a full-length open reading frame (ORF) based on sequence annotations in the databases but with no function assigned. Molecular genetic characterisation of the function of the gene encoding this cDNA is underway using in planta approaches.

35. Role of arbuscular mycorrhiza in crop growth in arsenic amended soil

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Agricultural crops are often contaminated with arsenic in Bangladesh. This results from irrigation with arsenic contaminated water and leads to problems in human health. Mycorrhizal fungi can reduce the contamination. To determine the role of arbuscular mycorrhizal fungi in crop growth in arsenic amended soil, three agricultural crops: *Amaranthus gangeticus*, *Raphanus sativus* and *Lycopersicon esculentum* were grown in arsenic amended soils (10ppm, 100ppm and 500ppm arsenic solution) with or without mycorrhizal inoculation. The results indicated that at higher concentrations of arsenic, seed germination was affected. A positive germination response to AM was observed in all the selected plants. Root length and shoot height, leaf number, both fresh and dry weight of roots and shoots were all higher in plants inoculated with AM. Higher nutrient uptake and less arsenic content were recorded in mycorrhiza inoculated plants.

Participants

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Marta Marmiroli	marta.marmiroli@unipr.it	University of Parma		Plant physiology of metals and semimetals
Ernest Marwa	e.marwa@abdn.ac.uk	University of Aberdeen	P18	Use of vermiculite in agriculture. Trace elements, bioavailability, toxicity, dehydroxylation, ICP-MS, DTPA, vermiculite
Steve McGrath	steve.mcgrath@bbsrc.ac.uk	Rothamsted Research	S1.5, S1.6, P16	
Andy Meharg	a.meharg@abdn.ac.uk	University of Aberdeen	S1.8, S3.3, S3.4, P1, P15, P17, P18, P20, P24, P25, P27, P33	
Adrien Mestrot	adrien.mestrot@abdn.ac.uk	University of Aberdeen	P20	Arsenic volatilisation, methylation, rhizosphere, flooded soils, rice
Shinichi Miyashita	shinichi3536@yahoo.co.jp	Tokyo University of Pharmacy and Life Sciences	P21	Arsenosugars, metabolism, alga, cyanobacteria, HPLC/ICP-MS
Debapriya Mondal	Debapriya.Mondal@postgrad.manchester.ac.uk	University of Manchester	P22, P26	Risk assessment from arsenic exposure for the population of West Bengal, India

Participant	Email	Establishment	Abstract No. *	Research Interests
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Amin Uddin Mridha	mridha52@gmail.com	University of Chittagong	P35	Mycorrhizae, Arsenic, Bioremediation, Phytoremediation, Agricultural crop and vegetables
Meher Nigar	m.nigar@abdn.ac.uk	University of Aberdeen	S3.4, P24	Arsenic tolerance and interactions in rice
Gareth Norton	g.norton@abdn.ac.uk	University of Aberdeen	S3.4, P25	Rice Genetics, arsenic tolerance, plant breeding
Erla Olsen	erla@gramar.fo	Gramar Research		Arbuscular mycorrhiza, Atlantic islands, human settlement, manuring practices, climate change
Massimo Pigna	pignamassimo@libero.it	University of Naples	P8	Arsenic, Heavy metals, plant uptake, rhizosphere, kinetics models
David Polya	david.polya@manchester.ac.uk	University of Manchester	P22, P26	Risk assessment analytical chemistry biogeochemistry arsenic hazard
Adam Price	a.price@abdn.ac.uk	University of Aberdeen	S3.4, P17, P24, P25	Rice Genetics, QTLs, candidate genes, abiotic stress
Andrea Raab	che576@abdn.ac.uk	University of Aberdeen	S1.8, S3.3, P5, P27, P33	Arsenic uptake and metabolism of plants, arsenic speciation using LC-ICP-MS/ES-MS
Moupia Rahman	mr2@soton.ac.uk	University of Southampton	P28	Phytoaccumulation, arsenic, *Brassica juncia*, morphological marker, breeding, hydroponic, sodium arsenite
Barry Rosen	brosen@med.wayne.edu	Wayne State Univ. Sch. Med	S1.7	Arsenic, uptake, detoxification, pump, reductase, methylase
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Henk Schat	henk.schat@falw.vu.nl	Vrije Universiteit	S2.1	
Kirk Scheckel	Scheckel.Kirk@epa.gov	US EPA	S3.1	Metals, soils, spectroscopy, sediments, water, bioavailability

Participant	Email	Establishment	Abstract No. *	Research Interests
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Andrew Smith	andrew.smith@adelaide.edu.au	University of Adelaide	S1.3, P7, P29	Arsenic uptake by arbuscular mycorrhizal plants, bioremediation
Koichi Suto	suto@mail.kankyo.tohoku.ac.jp	Tohoku University		Hyperaccumulator, phytoremediation, arsenic metabolism, functional genes, XAS techniques, transport studies
Julian Tyson	tyson@chem.umass.edu	University of Massachusetts	P30	Analytical chemistry, arsenic speciation, water analysis, biogeochemical cycling
M. Kalle Uroic	m.kalle.uroic@abdn.ac.uk	University of Aberdeen		Working on an electrochemical method to measure arsenic in plant xylem sap.
Doris Vetterlein	doris.vetterlein@ufz.de	Helmholtz Centre for Environmental Research - UFZ	P31	Plant arsenic uptake, arsenic speciation, rhizosphere, competitive uptake
Parminder Virk	p.virk@cgiar.org	International Rice Research Institute		Rice Genetics, Rice Breeding, Marker Aided Selection Rice nutrition Quantitative Genetics
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Alan Wood	b.a.wood@abdn.ac.uk	University of Aberdeen	S3.3, P33	Arsenic, phytochelatins, modelling, translocation
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Ren Zhang	rzhang@uow.edu.au	University of Wollongong	P34	Arsenic resistance, phytoremediation, genes, metabolism, bioinformatics
Fangjie Zhao	fangjie.zhao@bbsrc.ac.uk	Rothamsted Research	S1.5, S1.6, P16	
Yong-Guan Zhu	ygzhu@rcees.ac.cn	Chinese Academy of Sciences	S1.1, S1.8	Rhizosphere, metabolism, health risk