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Do heavy metals and metalloids influence the detoxification of organic xenobiotics in plants?

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Abstract
Background, aim and scope Mixed pollution with trace elements and organic industrial compounds is characteristic for many spill areas and dumping sites. The danger for the environment and human health from such sites is large, and sustainable remediation strategies are urgently needed. Phytoremediation seems to be a cheap and environmentally sound option for the removal of unwanted compounds, and the hyperaccumulation of trace elements and toxic metals is seemingly independent from the metabolism of organic xenobiotics. However, stress reactions, ROS formation and depletion of antioxidants will also cause alterations in xenobiotic detoxification. Here, we investigate the capability of plants to detoxify chlorophenols via glutathione conjugation in a mixed pollution situation.
Materials and methods Typha latifolia and *Phragmites australis* plants for the present study were grown under greenhouse conditions in experimental ponds. A *Picea abies* L. suspension culture was grown in a growth chamber. Cadmium sulphate, sodium arsenate and lead chloride in concentrations from 10 to 500 μM were administered to plants. Enzymes of interest for the present study were: glutathione transferase (GST), glutathione reductase, ascorbate peroxidase and peroxidase. Measurements were performed according to published methods. GST spectrophotometric assays included the model substrates CDNB, DCNB, NBC, NBoC and the herbicide Fluorodifen.
Results Heavy metals lead to visible stress symptoms in higher plants. Besides one long-term experiment of 72 days duration, the present study shows time and concentration-dependent plant alterations already after 24 and 72 h Cd incubation. *P. abies* spruce cell cultures react to $CdSO_4$ and Na_2HAsO_4 with an oxidative burst, similar to that observed after pathogen attack or elicitor treatment. Cd application resulted in a reduction in GSH and GSSG contents. When a heavy metal mixture containing Na_2HAsO_4, $CdSO_4$ and $PbCl_2$ was applied to cultures, both GSH and GSSG levels declined. Incubation with 80 μM arsenic alone doubled GSSG values. Based on these results, further experiments were performed in whole plants of cattail and reed, using cadmium in *Phragmites* and cadmium and arsenic in *Typha* as inducers of stress. In *Phragmites australis*, GST activities for CDNB and DCNB were significantly reduced after short-term Cd exposure (24 h). In the same samples, all antioxidant enzymes increased with rising heavy metal concentrations. *Typha latifolia* rhizome incubation with Cd and As leads to an increase in glutathione reductase and total peroxidase activity and to a decrease in ascorbate peroxidase activity. Measurements of the same enzymes in leaves of the same plants show increased GR activities, but no change in peroxidases. GST conjugation for CDNB was depressed in both cattail rhizomes and leaves treated with Cd. After As application increased, DCNB enzyme activities were detected.
Discussion T. latifolia and *P. australis* are powerful species for phytoremediation because they penetrate a large volume of soil with their extensive root and rhizome systems. However, an effective remediation process will depend on active detoxifying enzymes, and also on the availability of

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conjugation partners, e.g. glutathione and its analogues. Species-specific differences seem to exist between the regulations of primary defence enzymes like SOD, catalase, peroxidases, whereas others prefer to induce the glutathione-dependent enzymes. As long as the pollutant mix encountered is simple and dominated by heavy metals, plant defence might be sufficient. When pollution plumes contain heavy metals and organic xenobiotics at the same time, this means that part of the detoxification capacity, at least of glutathione-conjugating reactions, is withdrawn from the heavy metal front to serve other purposes. In fact, glutathione *S*-transferases show strong reactions in stressed plants or in the presence of heavy metals. The spruce cell culture was a perfect model system to study short-term responses on heavy metal impact. Overall, and on the canopy level, this inhibitory effect might result in a lower detoxification capacity for organic pollutants and thus interfere with phytoremediation.

Conclusions We present evidence that pollution with heavy metals will interfere with both the oxidative stress defence in plants, and with their ability to conjugate organic xenobiotics. Despite plant-species-dependent differences, the general reactions seem to include oxidative stress and an induction of antioxidative enzymes. Several processes seem to depend on direct binding of heavy metals to enzyme proteins, but effects on transcription are also observed. Induction of xenobiotic metabolism will be obtained at high heavy metal concentrations, when plant stress is elevated.

Recommendations and perspectives Plants for phytoremediation of complex pollution mixtures have to be selected according to three major issues: uptake/accumulation capacity, antioxidative stress management, and detoxification/binding properties for both the trace elements and the organic xenobiotics. By way of this, it might be possible to speed up the desired remediation process and/or to obtain the desired end products. And, amongst the end products, emphasis should be laid on industrial building materials, biomass for insulation or biogas production, but not for feed and fodder. Each of these attempts would increase the chances for publicly accepted use of phytoremediation and help to cure the environment.

Keywords Detoxification · Heavy metals · Inducible metabolism · Multiple pollution · Organic xenobiotics · Phytoremediation

1 Background, aim and scope

Anthropogenic and especially industrial activities have impacted severely in our environment during the last century. In many cases, environmental pollution by trace elements and especially by heavy metals is accompanied by pollution with organic foreign compounds and vice versa (Pambrun et al. 2008). Surveys alone in Germany resulted in an inventory of more than 80,000 spill sites with potential for pollutant removal (Franzius 1994). Phytoremediation, i.e. the removal of pollutants with the help of plants, but also phytostabilization in such areas affected by multiple pollution is complicated, and only few plant species have been shown to survive under the adverse conditions of mixed contamination. Amongst trace elements, predominantly arsenic, cadmium and lead have been investigated with respect to these effects (Schröder et al. 2002; Memon and Schröder 2009).

Arsenic, a metalloid, frequently included in the heavy metal group, is one of the oldest known inorganic toxins, therapeutics and intentional poisons. Disodium arsenate (Na_2HAsO_4) is a chemical analogue of phosphate and acts as an uncoupler of oxidative phosphorylation and catalase inhibitor. In the environment, arsenate is the predominant form (Peel et al. 1991).

Cadmium (Cd) is a true heavy metal. It evokes a whole array of toxic effects, due to its long biological half-life and storage in plant vacuoles, interacts with photosynthesis and nitrogen metabolism, and causes oxidative damage not only in mammals and plants (Dixit et al. 2001), but also in microbes (Shen et al. 2005).

Lead is one of the most widely used metals in human history and very widespread in the environment (Tully et al. 2000). It is classified as a possible carcinogen, whereby acute toxicity is revealed through its binding to enzyme prosthetic groups and inhibition of heme complex formation.

Contamination of soil or water with these heavy metals is a serious issue with concentration levels exceeding the WHO drinking water guideline values in the lower µg/l regime in many regions of the world.

A plethora of studies exists on heavy metals, metalloids and plants. Trace elements are well known for their ability to interfere with plant metabolism and to induce cellular damage resulting in the formation of reactive oxygen species (ROS), albeit through different reactions. The rise of reactive oxygen species in cell metabolism is a frequently observed phenomenon, and it has been shown that ROS may serve as signalling molecules for a number of defence reactions in plants, including alterations in primary or secondary metabolism.

Tightly connected to the sulphur metabolism in plants is the predominant detoxification pathway of halogenated organic pollutants and herbicides, i.e. glutathione *S*-transferase (GST) dependent conjugation. GST catalyses the conjugation of reduced glutathione to electrophilic centres of such halogenated xenobiotics (Coleman et al. 1997; Schröder 2001). They comprise a very heterogeneous

family of enzymes with diverse tasks in plant metabolism and defence (Dixon et al. 2001). More than 50 GST genes have been identified in model plants like *Arabidopsis*. To date, their functions are not known in too much detail, but it is clear that their expression and activity may be strongly modulated by oxidative stress and ROS.

In order to obtain information on the physiological background of ROS formation under heavy metal stress and its influence on detoxification of organic pollutants, we have investigated reactions of plants used in phytoremediation, e.g. *Typha* and *Phragmites*, but also in a weed, and a plant cell culture that has been used as a biomonitor of stress. The current compilation tries to elucidate detrimental effects of multiple pollution in pulling data together from different studies and exposure of different plant species.[1]

2 Materials and methods

2.1 Plant material

Experiments were performed with three different species: *P. australis*, *Typha latifolia*, and a heterotrophic cell culture of *Picea abies*.

Phragmites australis plants were grown hydroponically in 2-L glass pots under greenhouse conditions in 0.5 L full-strength MS medium. $CdSO_4$ was used to set up final Cd concentrations of 10 and 100 µM in 0.5 L of growth medium. After 24 h of incubation, rhizome and leaf samples were taken; rhizomes were then washed thoroughly with deionised water and frozen immediately in liquid nitrogen. Samples were kept at −80°C before analysis. Each sample consists of three single plants, respectively. For each treatment, three independent replicates were taken.

Typha latifolia plants were grown in a greenhouse at 14 h day, 10 h night and humidity of 50%. After a growing period of 72 days, the plants were treated with three different metals and four different concentrations (10 µM, 50 µM and 250 µM) for 72 h. The control was grown at the same conditions, but not treated with heavy metals. Three plant replicates for each treatment were used.

P. abies L. Karst. heterotrophic suspension-cultured cells have been described elsewhere (Messner and Berndt 1990, Schröder 2001). Media were changed after 3 days and the cultures allowed to grow for four additional days in the presence of heavy metals (Schröder 2001). The cells were then sedimented, the medium discarded, 50% of the cells removed and supplied with new medium, and then used as starting culture for the next experiments. The remaining cells received fresh medium and were further incubated. Upon termination of the incubation period, cells were harvested by removal of media and immediately immersed in liquid nitrogen.

2.2 Incubations

The used metals are: cadmium (as cadmium sulphate), arsenic (as sodium arsenate) and lead (as lead chloride). Plants or freshly transferred cells were incubated with media containing $CdSO_4$, Na_2HAsO_4 or $PbCl_2$ (from 50 to 500 µM). The concentration of particular metals was selected on the basis of previous experiments (Schröder et al. 2002) and levels found in real water samples. To investigate the pathogenesis related oxidative burst in the cell culture, *Rhizosphaera kalkhoffii* was maintained on malt agar (30 g l^{-1} malt extract, 10 g l^{-1} agar (type a, Sigma)). Preparation of the fungal cell wall fraction was performed as described previously (Schröder 2001).

2.3 Protein extraction

The extraction procedure for soluble detoxification enzymes is carried out according to Schröder et al. (2005). Frozen plant material was powdered and extracted (0.1 M Tris/HCl pH 7.8, 5 mM EDTA, 5 mM dithioerythritol DTE, 1% Nonidet P40, 1% insoluble polyvinylpyrrolidone PVP K90), homogenised and centrifuged for 30 min at 20,000 rpm. Proteins in this crude extract were precipitated by addition of ammonium sulphate in two steps of 40% and 80% saturation, respectively. Proteins were centrifuged after each step and the pellet finally resuspended in 2.5 mL of 25 mM Tris/HCl buffer pH 7.8. This step was followed by desalting with Sephadex PD-10 columns (Pharmacia, Germany).

2.4 GSH transferase assay

Spectrophotometer assays for determination of GST activity using the standard substrates 1-chloro-2,4-dinitrobenzene (CDNB, ε_{340nm}=9.6 mM^{-1} cm^{-1}), 1,2-dichloro-4-nitrobenzene (DCNB, ε_{345nm}=8.5 mM^{-1} cm^{-1}), 4-nitrobenzyl chloride (NBC, ε_{310nm}=1.8 mM^{-1} cm^{-1}), *p*-nitrobenzoyl chloride (NBoC, ε_{310nm}=1.9 mM^{-1} cm^{-1}) followed the method of Schröder et al. (2002). Assays with the herbicide fluorodifen (ε_{400nm}=3.1 mM^{-1} cm^{-1}) followed Scalla and Roulet (2002).

2.5 Antioxidative enzymes assay

Glutathione reductase (GR), peroxidase (POX) and ascorbate peroxidase (APOX) were assayed following published

[1] The present paper is based on an oral presentation delivered at the 4th European Bioremediation Conference, held in Chania, Greece, September 3rd to 6th, 2008.

methods. Peroxidase activity was measured according to Drotar et al. 1985 using Guajacol (2-Metoxy-phenol) as a substrate. Measurements were performed at pH 6.0 and 420 nm (ε_{420nm}=26.6 mM^{-1} cm^{-1}). The activity of ascorbate peroxidase and glutathione reductase were determined as described by Vanacker et al. 1998. Ascorbate peroxidase measurement is based on a decrease in extinction at 290 nm through the oxidation of ascorbate (ε_{290nm}=2.8 mM^{-1} cm^{-1}) at pH 7.0. For glutathione reductase, the consumption of NADPH (ε_{340nm}= 6.2 mM^{-1} cm^{-1}) can be followed spectrophotometrically at 340 nm and pH 7.8 according to Schickler and Caspi (1999).

Protein content of the samples was measured by the method of Bradford (1976) with bovine serum albumin as a standard protein.

3 Results

3.1 Visual symptoms

When whole plants were incubated with cadmium sulphate, they showed time and concentration-dependent visual symptoms and alterations in growth patterns, whereas the cell cultures treated with arsenate, cadmium sulphate and lead chloride, due to their short incubation time, remained void of visual symptoms.

The strongest effects of heavy metal exposure were observed in the long-term *Typha* experiment, where incubation for 72 days led to severe changes in biomass production and health (Fig. 1). In order to obtain data for short-term exposure under comparable experimental conditions, a set of *Typha* plants was exposed for 72 h with the same concentrations.

3.2 Oxidative stress

The general response of plants toward heavy metals in concentrations ranging from 10 to 250 µM seems to be an increase in oxidative stress, directly coupled to an induction of enzymes like peroxidase, ascorbate peroxidase and glutathione reductase, i.e. of the Halliwell–Asada cycle, and connected to strong alterations in the glutathione pool.

It had been previously shown that spruce cell cultures react to $CdSO_4$ and Na_2HAsO_4 with a pronounced oxidative burst, similar to that observed after pathogen attack or elicitor treatment (Messner and Schröder 1999; Schröder 2001). H_2O_2 concentrations of up to 45 µM were measured in spruce cells within 8 h after the onset of the incubation. After addition of up to 150 µM $PbCl_2$, the same cells developed only a weak oxidative burst at rates of 1 to 2 µM h^{-1} H_2O_2. In the media of untreated spruce cells, H_2O_2 levels never exceeded 1 µmol h^{-1}. However, and contrary to elicitor treatment, the H_2O_2 extrusion was delayed between 4 and 8 h. This delay seems to indicate that the cellular signal leading to the HM mediated oxidative burst is different from the pathogen answer.

Application of 150 µM $CdSO_4$ resulted in a reduction in GSH and GSSG of 75% and 96%, respectively. When a heavy metal mixture containing 80 µM Na_2HAsO_4, 150 µM $CdSO_4$ and 150 µM $PbCl_2$ was applied to the cultures, both GSH and GSSG levels declined by 60% and 99%, respectively. Incubation with 80 µM arsenic alone doubled

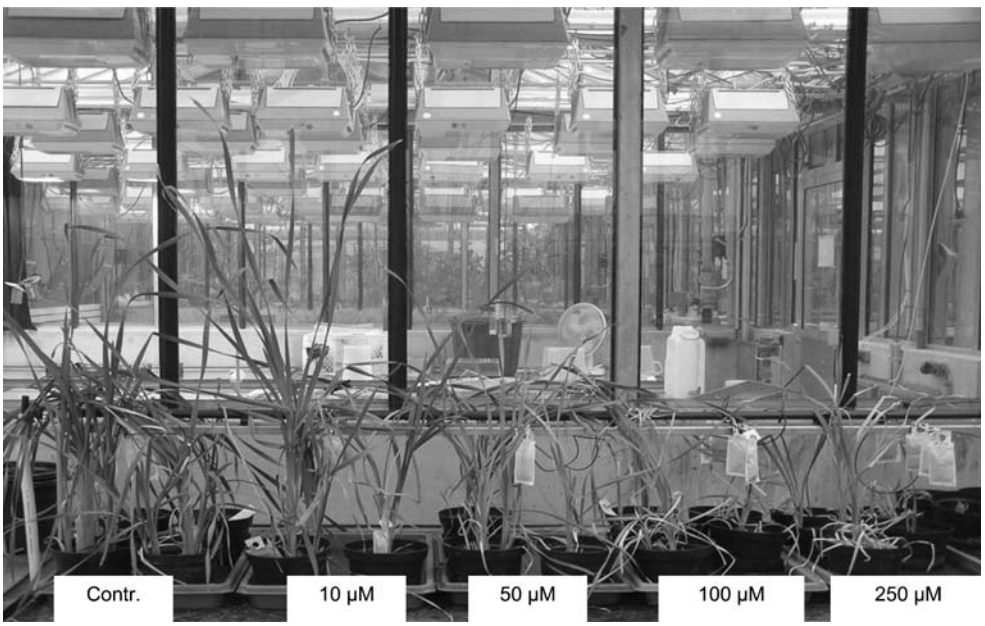

Fig. 1 Visual effects of Cd application in sublethal concentrations to *Typha latifolia* plants grown under greenhouse conditions after 72 days

the GSSG values. Effects of arsenic seem to be interconnected with the rise of reactive oxygen species (ROS) and active oxygen species (AOS) in the cell, leading to SOD or NADH-oxidase catalysed formation of H_2O_2.

Incubating the cells for longer time periods (i.e. 24 h), led to similar effects on thiol contents (data not shown). Incubation with elicitor resulted in the doubling of GSH values within 16 h to 933 nmol g fwt^{-1}, but GSSG concentrations remained unchanged. On the contrary, incubation with 117 mM H_2O_2 decreased GSH levels by 30% and doubled GSSG contents (Schröder 2001). Interestingly, arsenic appears to mimic oxidative stress processes as its effect is comparable to elicitor and H_2O_2 addition to the cell cultures. From these results, it becomes clear that heavy metals influence thiol contents mainly by depleting the pool, e.g. by the larger demand for phytochelatins.

3.3 Induction of enzyme activities

$CdSO_4$ in concentrations up to 500 µM significantly induced GST activities for the four substrates measured ($p<0.05$) in the treated cell culture. Except for EPNP, the inductive effect seemed to be concentration-dependent.

Compared to cadmium, arsenic was a strong inducer. Arsenic concentrations (up to 80 µM) induced GST for all substrates, except EPNP, by a factor of 2 ($p<0.05$), and the effect was strictly concentration-dependent for CDNB, DCNB and pNBC conjugation. It could be clearly shown that EPNP activity of the cells was lost proportionally with increasing metal concentrations.

Cellular responses to $PbCl_2$ contrasted to those of other metals tested, in that, with the exception of EPNP, no significant changes were observed for the conjugation of any of the substrates. EPNP conjugation was depressed by 56% of controls, similar to the treatments with the other heavy metals. In addition to experiments with pure heavy metals, mixtures of the mentioned heavy metals were, irrespective of concentrations applied, always inductive to GST activities of CDNB, DCNB and EPNP. A mixture of 150 µM $CdSO_4$, 150 µM $PbCl_2$ and 80 µM $NaHAsO_4$ led to a doubling of conjugation rates of the two former substrates, but tripled the activity towards the latter (72%, 111%, 231% above controls, $p<0.01$).

Having recorded these effects in a cell culture, we focused on experiments in whole plants with cadmium as the lead heavy metal. We obtained very similar results in *Typha* and in reeds (*Phragmites*). Some reports on Cd-dependent increases in CAT activity, but simultaneously high SOD increases, are available (Vitoria et al. 2001). Current literature on the inducibility of detox enzymes indicates ROS and AOS as potent triggers of GST induction. Interestingly, and different from the general opinion, we found that even mild heavy metal stress might lead to the inhibition of detoxification reactions in reeds (Fig. 2). *Phragmites* glutathione *S*-transferase activity for specific organic substrates (CDNB and DCNB) decreased within 24 h of incubation with cadmium sulphate, whereas the antioxidative enzymes performed at increasingly higher rates. The decline in CDNB activity was around 20%, and 35% in DCNB. Similarly the increases in antioxidative enzymes were around 20% to 30% (Fig. 3). This development depends strongly on the heavy metal to be considered and its concentration, as is reported below.

Ongoing experiments with *Typha* show similar effects (Figs. 4, 5, 6). In rhizomes, antioxidative enzymes like POX and GR are induced with higher HM concentration, but APOX decreases under Cd and As. In the leaves of the same plants, Cd influences only GR activities, whereas APOX and POX remain at more or less the same levels. The massive increase of GR activity points to the consumption of GSH for phytochelatin production. Arsenate, contrary to this, leaves GR activities unchanged, but doubles APOX activities and leads to significant increases of POX. An interesting observation is connected to the fact

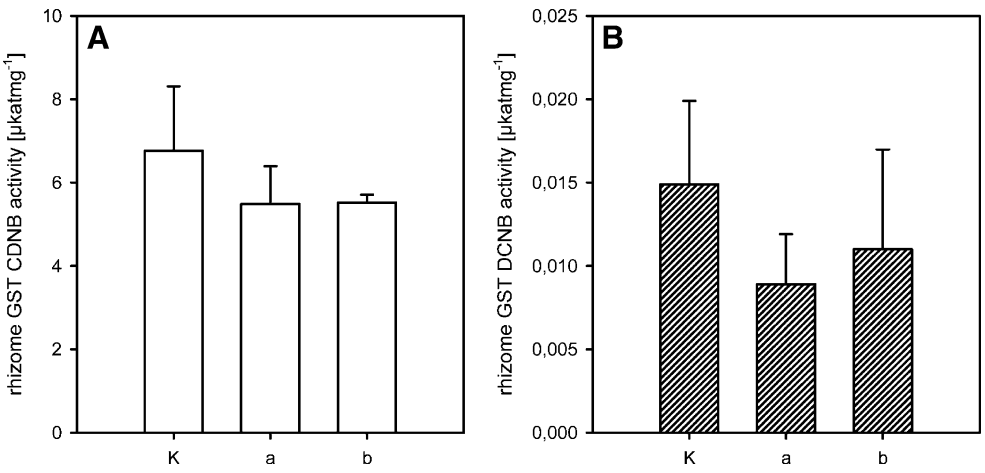

Fig. 2 Activity of glutathione *S*-transferase enzymes in *Phragmites* for chlorobenzene substrates **A**: CDNB (1-chloro-2,4-dinitrobenzene) and **B**: DCNB (1,2-dichloro-4-nitrobenzene) after 24 h in the presence of $CdSO_4$ (*K* control, *a* 10 µM $CdSO_4$, *b* 100 µM $CdSO_4$). Enzyme assays were performed for 5 min, and conjugate formation was recorded at the wavelengths given in M&M. All measurements were done in triplicate, and *error bars* represent standard deviations

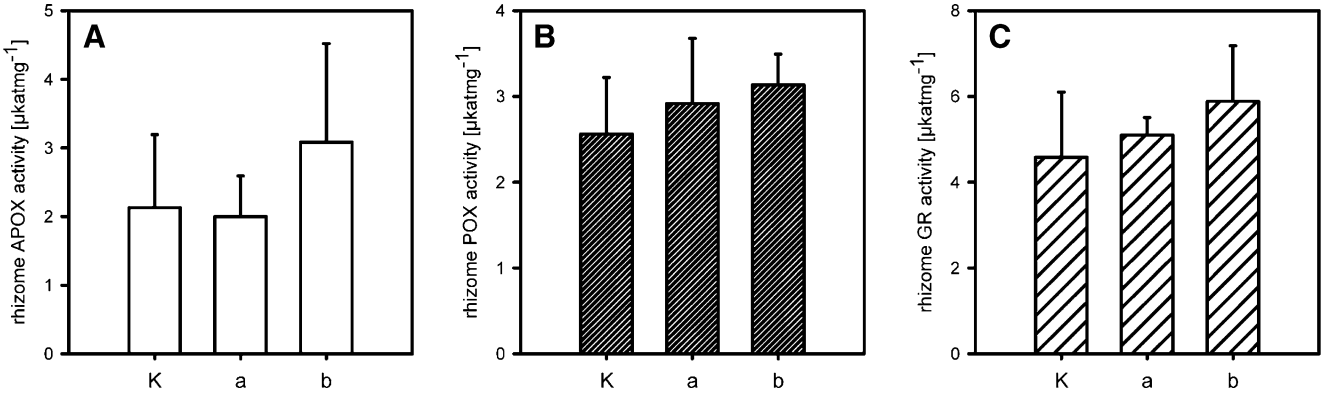

Fig. 3 Activity of antioxidative enzymes, **A**: ascorbate peroxidase (*APOX*), **B**: guajacol peroxidase (*POX*) and **C**: glutathione reductase (*GR*) in *Phragmites* after 24 h in the presence of $CdSO_4$ (*K* control, *a* 10 µM Cd, *b* 100 µM Cd). All measurements were done in triplicate according to the conditions described in M&M, and *error bars* represent standard deviations

that low arsenate concentrations of 10 µM will always lead to an induction of antioxidative enzymes in the leaves, whereas 50 µM and above generally inhibit them. It becomes clear that rhizomes, receiving by far higher concentrations of heavy metals, will react more distinctly to this stress. In leaves of treated plants, additional signalling processes or absence of certain defence genes might modulate the stress response.

The same is principally true for the glutathione *S*-transferases responsible for the detoxification of organic xenobiotics.

GST conjugation is depressed for CDNB in both leaves and rhizomes after Cd treatments, whereas As treatment induces DCNB activity massively. Conjugation of fluorodifen decreases in both treatments, albeit with low significance, except at low concentrations of $CdSO_4$. NBC and NBoC conjugation are both partly induced by cadmium, but generally inhibited strongly by arsenic.

In our experiments with arsenate, conjugation rates under HM influence increased gradually, but did not reach control levels. Even more thrilling is the observation that lead chloride (data not shown) and arsenic (see Fig. 5 C, H) both stimulate the conjugation of the herbicide fluorodifen at lower concentrations. This provokes the question, whether a multiple pollution scenario, including organic pollutants, pesticides and heavy metals at the same time, would increase a given plant's capability for phytoremediation.

In essence, cadmium seems to be inductive to some GST activities in *Typha* at high concentrations, but arsenate exerts inhibitory effects on all GST except the DCNB conjugating form. It is doubtful whether Cd is directly active as GST inducer, rather than depleting GSH and damaging cellular compartments, so that GST induction would occur. Experiments on the direct influence of heavy metals on isolated GST enzymes have shown that decreases in enzyme activity could also be observed when heavy metals directly interfere with catalytic processes (Lyubenova et al. 2007). We used a fraction of partially purified GST from *Calystegia sepium*, common bindweed, because this plant had previously been described as very tolerant against organic pollutants and herbicides, although it produces an extensive rhizome that will allow for relatively high uptake rates of xenobiotics. When *C. sepium* GST was assayed for CDNB and NBOC conjugation in presence of $CdSO_4$, CDNB conjugation was inhibited by 50% at 250 µM, whereas NBOC conjugation remained unaffected. Unexpectedly, the activity of glutathione reductase, the enzyme responsible for the reduction of GSH, was inhibited by 90% already by the lowest HM concentration. Here, it might be speculated that consumption of GSH for heavy metal sequestration and for conjugation of xenobiotics could also lead to additional plant stress.

4 Discussion

Plants growing in serpentine soils, on pollution plumes or in phytoremediation facilities frequently encounter variable concentrations of heavy metals, amongst them cadmium and arsenate species. If these plants do not avoid metal uptake, they are forced to distribute dissolved heavy metals in their tissues and have to cope with the resulting stress. We have investigated several unrelated plant species, amongst them *Typha*, *Picea*, *Phragmites* and *Calystegia* for their response towards cadmium and arsenic, and found some similarities in their reactions. Being typical geophytic plants, the species chosen develop extensive root and rhizome systems that might penetrate a large volume of soil or sediment. Hence, these plants might be of some interest in soil remediation. In any case, the use of cell cultures as model organisms is of importance and will allow to obtain a large number of results under standardised conditions (Langebartels and Harms 1986).

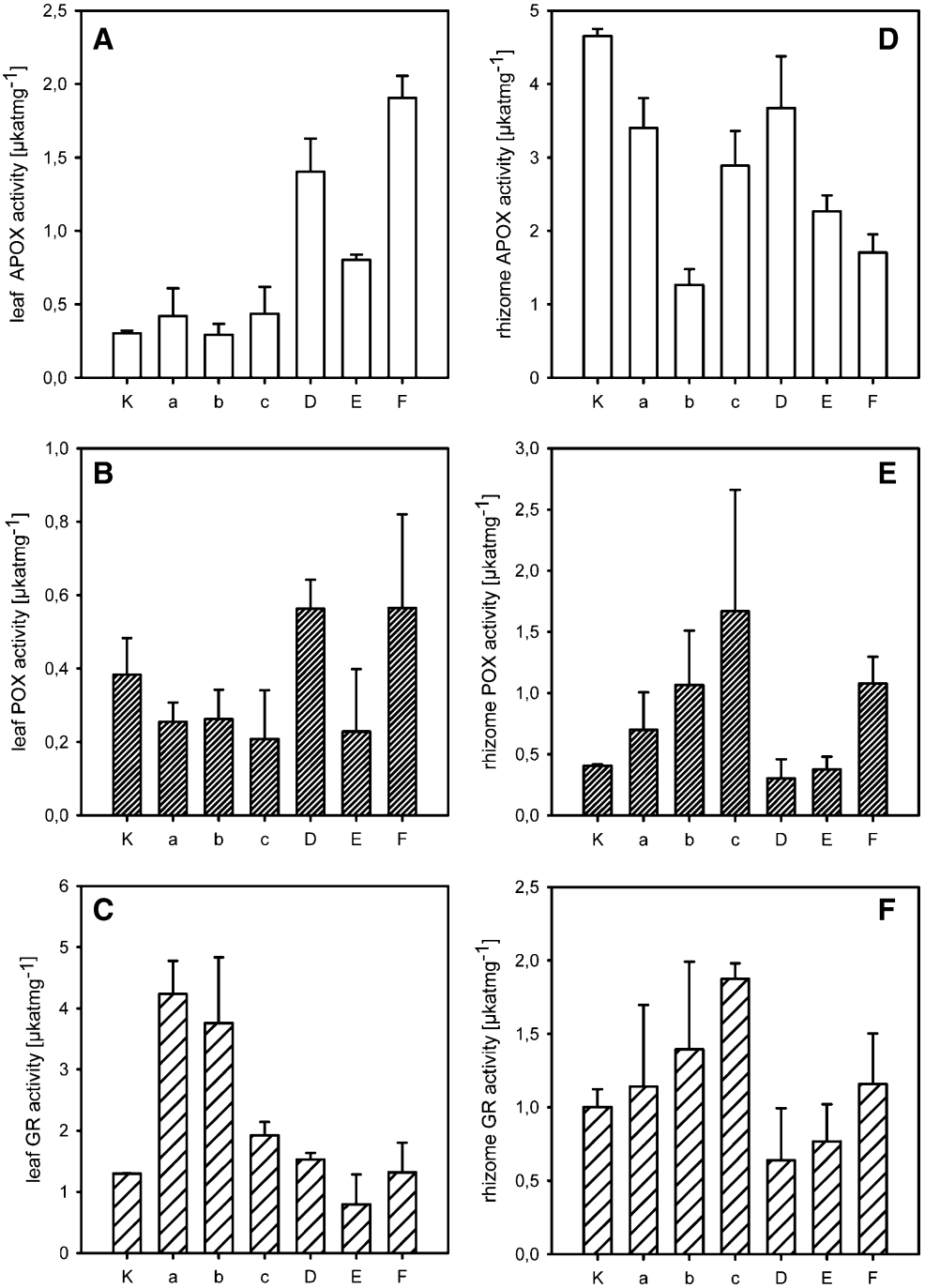

Fig. 4 Influence of cadmium sulphate and sodium arsenate on the antioxidative enzymes ascorbate peroxidase (*APOX*), guajacol peroxidase (*POX*) and glutathione reductase (*GR*) of *Typha latifolia*: *left A–C* (enzyme activities in the rhizome); *right D–F* (enzyme activities in the leaves). All *bars* represent means±standard deviations of a minimum of three independent samples. *K* control, *a* 10 µM Cd, *b* 50 µM Cd, *c* 250 µM Cd, *d* 10 µM As, *e* 50 µM As, *f* 250 µM As

Generally, all investigated species showed increases in most of the antioxidative enzymes of the Halliwell–Asada cycle, obviously after an oxidative burst signal initiating the response few hours after HM uptake. Species-specific differences seem to exist between the regulations of induction: some plants have stronger induction of the primary defence enzymes like SOD, catalase, peroxidases, whereas others prefer to induce glutathione-dependent enzymes that provide the cell with high antioxidant concentrations. Glutathione homeostasis may be decisive in this respect (Noctor et al. 2002). As long as the pollutant mix encountered is simple and dominated by heavy metals only, plant defence might be sufficient. However, most pollution plumes contain both heavy metals and organic xenobiotics at the same time. For a given plant, this means that part of its predisposed detoxification capacity, at least of glutathione-conjugating reactions, is withdrawn from the heavy metal front to serve other purposes. In fact, glutathione *S*-transferases show strong reactions in stressed plants or in the presence of heavy metals. The spruce cell culture was a perfect model system to study short-term responses on heavy

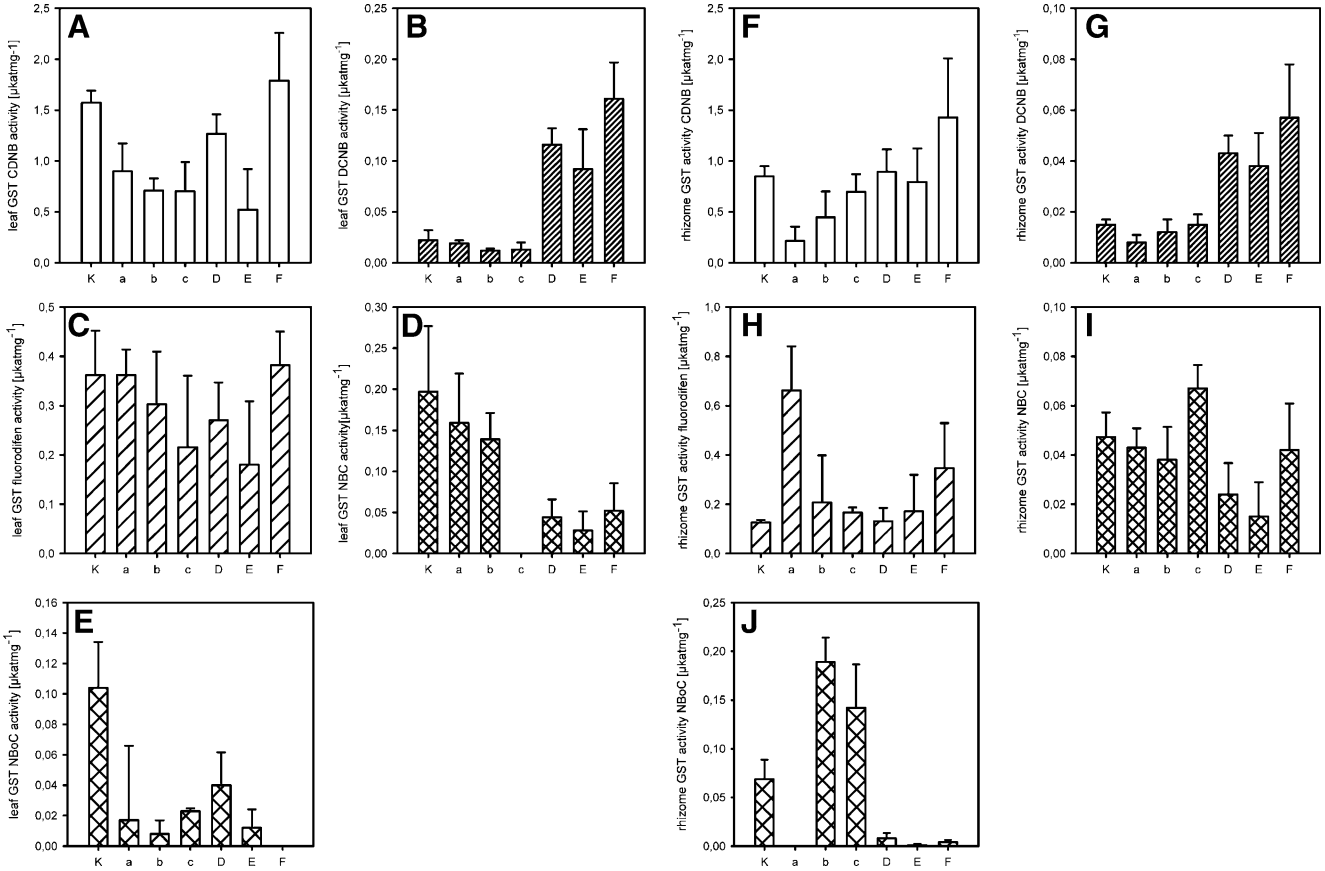

Fig. 5 Influence of heavy metals on GST conjugation of DCNB, CDNB, NBC (nitrobenzylchloride), NBoC (nitrobenz-*O*-yl-chloride) and the herbicide Fluorodifen in *Typha* plants treated with 10, 50 or 250 µM of the respective HM. Graphs *left A–E* (enzyme activities in the rhizome) *right F–J* (enzyme activities in the leaves); Treatments with $CdSO_4$ and $NaHAsO_4$, respectively: (*K* control, *a* 10 µM Cd, *b* 50 µM Cd, *c* 250 µM Cd, *d* 10 µM As, *e* 50 µM As, *f* 250 µM As)

metal impact. It was shown that glutathione pools were depleted, and that several GST isoforms were induced with Cd or Pb, but strongest with As.

Pollution mixtures exerted even more complex reactions (Schröder 2001). An interesting lesson to learn was that the standard model substrate, CDNB, would not yield complete insight in the GST reactions in affected cells. This was also true for the other investigated plant species. Except for the conjugation of CDNB, where no significant changes in conjugation rates are observed, GST activities

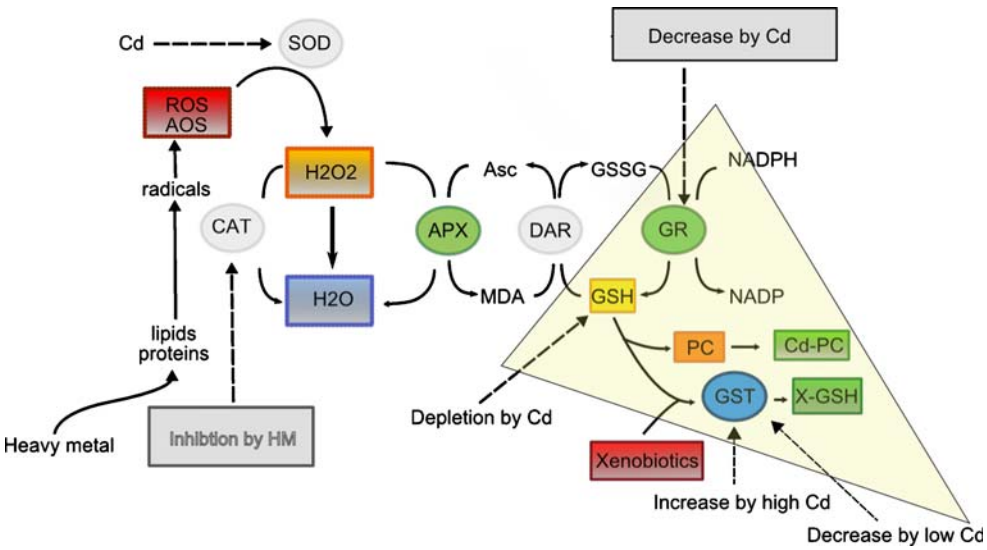

Fig. 6 Sketch of heavy metal influence on plant detoxification, especially on the glutathione-dependent enzymes depicted in the triangle (from Schröder et al. 2002, modified). Abbreviations: *AOS* activated oxygen species, *APX* ascorbate peroxidase, *CAT* catalase, *Cd-PC* cadmium phytochelatin complex, *DAR* dihydroascorbate reductase, *GR* glutathione reductase, *GSH* glutathione (red.), *GSSG* glutathione (ox.), *GST* glutathione *S*-transferase, *HM* heavy metals, *MDA* monodehydroascorbate, *PC* phytochelatins, *ROS* reactive oxygen species, *SOD* superoxide dismutase

in *Typha* control plants are usually lower than conjugation rates after incubation with 10 and 50 µM HM, and a significant increase above control levels was only observed at very high concentrations of 250 µM. In leaves, *Typha* GSTs behave almost identical to *Phragmites* GSTs (see Figs. 2 and 3), being inhibited by increasing HM concentrations, except DCNB conjugation which was induced fourfold by arsenic even at lower concentrations. It is worthwhile to note that DCNB differs only slightly from CDNB.

The inhibition of GST recorded in these experiments raised further questions about the time scale of this reaction, and the reason—did inhibition happen on the transcription level or on active enzymes? So far, inhibition of GST in vitro had only been demonstrated for rat liver (Dierickx 1982). There, at 200 µM/L $HgCl_2$, $CuCl_2$ and $CdCl_2$ inhibited total GST activity by 82%, 50% and 37%, respectively, and seven GST isoforms were affected to different degrees. We were able to show that GST, and even stronger, the GR activities, were inhibited in vivo by cadmium and lead, so that an additive effect with lower transcription rates under long-term stress might lead to severe declines in the activities of glutathione-dependent enzymes.

Hence, growing in a site that is polluted with heavy metals and organic pollutants at the same time requires a potent detoxification system. Any remediation process will depend on active, detoxifying enzymes, and also on the availability of conjugation partners, e.g. glutathione and its analogues. Here, multiple pollution with trace elements like cadmium will be detrimental as it inhibits enzyme activities and withdraws GSH equivalents from the pool. Under real life conditions and multiple pollution scenarios, this might mean that a combination of heavy metals and organic pollutants (a) can only be tackled by the plant, if the correct enzyme activity is induced, or (b) leads to rapid development of stronger stress due to the additional action of the undetoxified organic xenobiotics. Whether these stressed plants would be able to maintain their performance over longer periods has to be proven. It will be of some significance to the whole field of biological pollutant removal to develop sound concepts and expert systems for a plant cover of a given area before explicit measures are taken. By this, it might be possible to speed up the desired biological remediation process and/or to obtain the desired end products. And amongst end products that would be produced from plant material grown at such sites, emphasis should be laid on industrial building materials, biomass for insulation or biogas production, but not for feed and fodder. Each of these attempts would increase the chances for publicly accepted use of phytoremediation and help to cure the environment.

5 Conclusions

T. latifolia and *P. australis* are both powerful species for phytoremediation, because they penetrate a large volume of soil with their extensive root and rhizome systems. However, an effective remediation process will depend on active detoxifying enzymes, and also on the availability of conjugation partners, e.g. glutathione and its analogues. We present evidence that pollution with heavy metals will interfere with both the oxidative stress defence in plants and with their ability to conjugate organic xenobiotics. Experiments indicate that inhibition can depend on direct binding of heavy metals to enzyme proteins, but effects on transcription are also possible. Induction of xenobiotic metabolism will be obtained at high heavy metal concentrations, when plant stress is elevated. Overall, and on the canopy level, this inhibitory effect might result in a lower detoxification capacity for organic pollutants and thus interfere with phytoremediation.

6 Recommendations and perspectives

It has to be proven whether plants that have to face multiple pollution situations are able to maintain their remediation performance over a longer period of time. Developing modular remediation systems carrying plants with different detoxification properties for sequential removal of heavy metals and organic pollutants will be one solution.

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