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Accumulation of four metals in tissues of *Corchorus olitorius* and possible mechanisms of their tolerance

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Abstract

Corchorus olitorius plants treated by 5 µg cm^{-3} of Cd, Pb, Al or Cu in hydroponic culture accumulated in leaves 190, 150, 350 and 325 µg g^{-1}(d.m.) of these metals, respectively, after 6 d of exposure. Exposure of *Corchorus* plants to tested metals resulted in a sharp rise in content of amino acids in leaf tissues, however the magnitude of accumulation was different from one metal to another. Presence of sulphur in the growth medium significantly increased uptake of Cd and Pb and cysteine (cyst) was more effective than K_2SO_4. Similarly, addition of salicylic acid (SA) in the growth medium significantly enhanced the ability of *Corchorus* plants to accumulate all these metals. Growth of *Corchorus* plants was significantly reduced by treatment with any of the four metals except Cu and added cyst, K_2SO_4 or SA alleviated the growth retarding effect of metals.

Additional key words: aluminium, cadmium, copper, cysteine, lead, salicylic acid, potassium sulphate.

Introduction

Heavy metal (HM) pollution of soils and waters, mainly caused by industrial, mining, burning of fossil fuels and sewage disposal operations is a major environmental problem (Cobbett 2002). Heavy metals, unlike organic pollutants, cannot be chemically degraded or biodegraded by microorganisms. Most of heavy metals are toxic at higher concentrations, although some like zinc and copper are required as micronutrients in biological systems. Heavy metals in the environment operate as stress factors in a manner that they cause a change in physiological reactions and reduce vigour or in extreme totally inhibit plant growth.

Some plant species, however, particularly the ones inhabiting areas with chronically high metal concentrations, can absorb and accumulate heavy metals to relatively high concentrations without damage (*e.g.* Gupta and Goldsbrough 1991, Ernst *et al.* 1992, Van Steveninck *et al.* 1992, Fernando and Fernando 1994, Neumann *et al.* 1994, Hall 2002). Despite a large number of reports detailing plant responses to HM toxicity (Rtiegsegger and Brunold 1992, Fernando and Fernando 1994, Kim *et al.* 2002, Kupper *et al.* 2002, Morsch *et al.* 2002, Patsikka *et al.* 2002, Kevrešan *et al.* 2003, Šottníková *et al.* 2003), information on mechanism of HM accumulation, tolerance, and toxicity in higher plants is relatively little (Hall 2002, Schützendübel and Polle 2002, Tari *et al.* 2002). Elucidation of the mechanisms by which plants respond to HM stress is critical if we are to introduce genetic or environmental improvement to HM stress tolerance or to engineer plants suitable for phytoremediation.

Several mechanisms were suggested to contribute to HM tolerance: *1*) production of metal-binding compounds like amino acids (Reilly 1972), citric acid (Thurman and Rankin 1982), malic acid (Brookes *et al.* 1981) and phytochelatins (PCs) (Grill *et al.* 1985, 1987, Rtiegsegger and Brunold 1992, Cobbett 2002, Hall 2002); *2*) metal deposition in vacuoles or excretion by specific glands (Fernando and Fernando 1994); *3*) alterations of membrane structures (De Vos *et al.* 1988), *4*) synthesis of stress metabolites including proteins (Neumann *et al.* 1994); and *5*) deposition in Ca oxalate crystals (Van Balen *et al.* 1980, Franceschi and Schueren 1986, Mazen and El Maghraby 1998). More than one mechanism may be working together in the same species (for review, see Hall 2002).

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Abbreviations: cyst - cysteine; HM - heavy metals; NPTs - non-protein thiols; PCs - phytochelatins; SA - salicylic acid.
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In earlier parts of this study (Mazen 2003, 2004), it was reported that *Corchorus olitorius* accumulate significant amounts of heavy metals after its growth on sewage sludge-amended or on liquid cultures supplemented by Cd, Pb, Al or Cu. Incorporation of accumulated heavy metals into depositions of Ca oxalate as a possible means to detoxify them was examined. Only Al was detected in the formed crystals (Mazen 2004). It was suggested that other mechanisms of tolerance may be working in this plant for other metals and may be for Al too. Based on this result, the work was extended to examine other possible means of metal tolerance in this plant species.

Materials and methods

Fresh seedlings of *Corchorus olitorius* L. were carefully liberated from the soil of a local farm to keep root systems intact. Plants were brought to the laboratory with roots kept continuously immersed in water in a jar until reculturing. Roots were thoroughly washed before transplantation to liquid cultures. Content of total amino acids was determined in leaf tissues of plants which were transplanted and left to grow hydroponically on half strength Hoagland nutrient solutions supplemented with 5 µg cm^{-3} of Cd (as cadmium chloride), Pb (as lead acetate), Al (as aluminum sulphate) or Cu (as copper sulphate). To study effect of exogenous sulfur on ability of *Corchorus* plant to accumulate metals, growth medium contained also 50 µM cysteine (as organic sulphur source) or 1 mM K_2SO_4 (as inorganic sulphur source). Similarly, 200 µM salicylic acid (SA) was added to the growth medium. Plants were allowed to grow at 25 ± 2 °C in growth chamber under irradiance of ~40 µmol m^{-2} s^{-1} for 16-h photoperiod (provided from fluorescent tubes and incandescent lamps). Growth media were aerated continuously using fish aquarium air bubblers. Media were being replaced by fresh ones every other day. Plants were left to grow under treatments for 20 d. At the end of treatment period, plants were washed thoroughly with distilled deionized water and oven dried at 80 °C for 5 d and dry mass was determined.

For heavy metal analysis, dry leaves were ground in a stainless steel mill and sample preparation was carried out according Heckman *et al.* (1987): 1 g powder was dry ashed at 500 ºC for 12 h, dissolved in 4 cm^3 concentrated HNO_3, and evaporated to dryness and the residue was then redisolved in 10 cm^3 3 M HCl, refluxed for 2 h, filtered and diluted to 25 cm^3 with 0.1 M HCl. The determination of heavy metals was carried out with a *Perkin Elmer* 2380 atomic absorption flame spectrophotometer. Total free amino acids were estimated spectrophotometrically following the method described by Lee and Takahashi (1966).

Data were statistically analyzed using *IBM* computer program "*Epistat*" (written by Tracy L. Gustafson, MD, USA). One-way *ANOVA* analysis was followed to test significance of difference.

Results

Corchorus plants treated by 5 µg cm^{-3} of Cd, Pb, Al or Cu accumulated high amounts of these metals (Fig. 1*A*). After 6 d of exposure, the plant leaf tissue contents of Cd, Pb, Al and Cu were more than 190, 150, 350 and 325 µg g^{-1}(d.m), respectively. Accumulation in leaf tissues of the plants started rapidly in the days of treatment, then continued relatively slowly until the day 6 after which it became almost constant.

Exposure of *Corchorus* plants to any of the tested metals resulted in a sharp rise in content of total amino acids in their leaf tissues during early stages of treatment (Fig. 1*B*). Peak values were reached 2 d after initiation of treatment, followed by subsequent gradual decline. Accumulation magnitudes were in the order: Al > Cd > Pb > Cu.

Presence of cysteine or sulphate in the growth medium caused an increase in the uptake of all the four metals (Fig. 2*A*). The effect was significant in cases of Cd and Pb, and insignificant in cases of Al and Cu. Cysteine was more effective on metal accumulation than sulphate. Presence of salicylic acid in the growth medium also significantly enhanced the ability of *Corchorus* plants to accumulate all the toxic metals (Fig. 2*B*) by around 40, 50, 15 and 20 % in cases of Cd, Pb, Al and Cu, respectively.

Treatment with metals significantly reduced growth of *Corchorus* plants except in case of Cu (Fig. 3). At the end of treatment period, this reduction was about 30, 45, 35 and 5 % of reference control in case of Cd, Pb, Al and Cu respectively. Added cyst or K_2SO_4 alleviated the negative effect of metals on growth (Fig. 3). The alleviation effect was significant in case of Cd and Pb but not in case of Al or Cu. Cyst seemed to be more effective than K_2SO_4, only in cases of Cd and Cu. Similarly, presence of SA in the growth medium significantly reduced the metal-alone-retarding effect on growth.

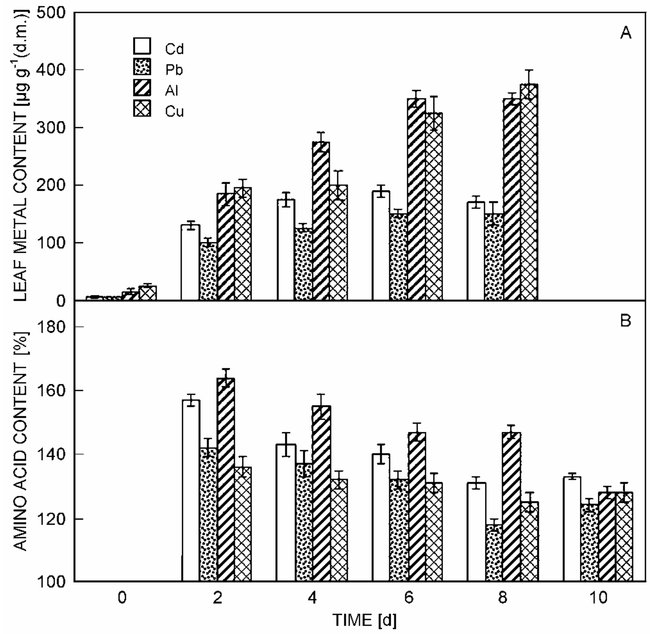

Fig. 1. Contents of Cd, Pb, Al, and Cu (*A*) and of total free amino acids (*B*) in leaf tissues *Corchorus* plants exposed to 5 μg cm^{-3} of these metals. Means of 3 different determinations, *error bars* represent SE. The means in days 2, 4, 6 ad 8 are significantly different ($P < 0.05$) from their counterpart one just before starting treatment (day 0). The amino acid contents are expressed as percentage of those at day 0.

Discussion

Elucidation of the mechanisms how toxic metals act at the cellular level and how plants may defend themselves against these pollutants is receiving increasing attention. Results of this research shows a relationship between ability to accumulate high amount of toxic metals and total amino acids. Thus, one of the possible mechanisms which enable *Corchorus* to tolerate these metals is the synthesis and accumulation of amino acids. Amino acids were reported to play a significant role in metal chelation (Bass and Sharma 1993, Hall 2002). The pattern of amino acid accumulation reported in this research, further suggests that amino acid contribution might be important to metal accommodation in the earlier stages of exposure of plants to metals until other mechanisms start to work. Another possible reason for this pattern could be that *Corchorus* plants exhibit amino acids accumulation only while they can sustain metabolic activities (exposure of small duration).

Prolonged exposure to metals perhaps suppresses the metabolic processes including amino acid formation. Similar to results of this paper, proline accumulation has been reported in seedlings of certain crop plants treated with Cd, Co, Zn and Pb and in *Lemna minor* treated with zinc and Cu where the proline content increased proportionately with an increase in metal concentration (Alia and Saradhi 1991, Bass and Sharma 1993).

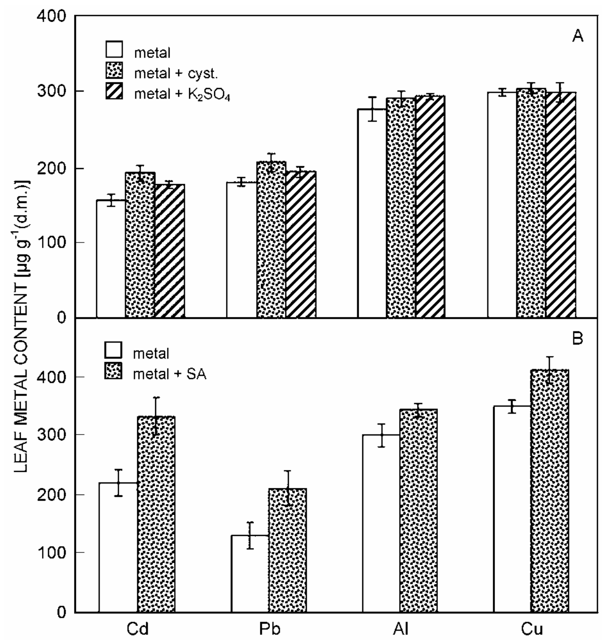

Fig. 2. Effect of exogenously added sulphur (*A*) (organic as cyst and inorganic as K_2SO_4) or 200 μM salicylic acid (*B*) on metal content in *Corchorus* plants, grown at 5 μg cm^{-3} Cd, Pb, Al, or Cu for 20 d. Means of 3 different determinations, *error bars* represent SE.

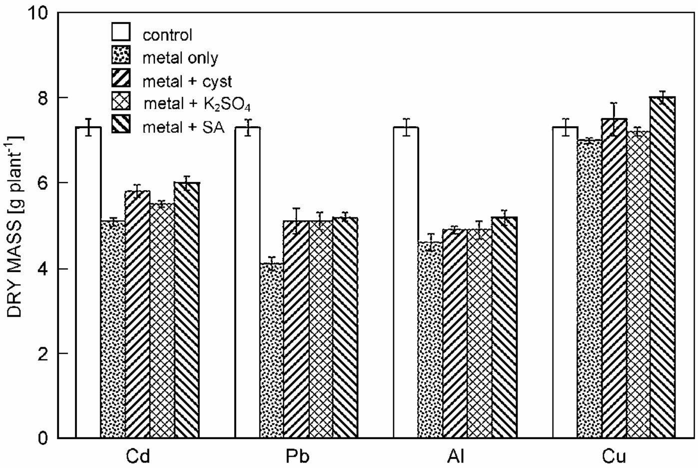

Fig. 3. Growth of *Corchorus* plants as affected by treatment with metal only, metal + cyst, metal + K_2SO_4 or metal + SA. Means of 3 different determinations, *error bars* represent SE.

The ability to tolerate and accumulate toxic metals was also found to be affected by exogenous sulphur and salicylic acid. Through the following discussion, it is suggested that tolerance of *Corchorus olitorius* to toxic metals might be the result of a number of mechanisms acting collectively. The relative contribution of each mechanism seems to be metal dependent.

Sulphur effect indicated in this paper was expected since sulphur is a structural constituent of important amino acids cyst and methionine and several coenzymes and prosthetic groups, such as ferredoxin, which are important for nitrogen assimilation (Petrović and Kastori 1994). Exogenous sulphur (especially cyst) might have also exerted its ameliorative effect on metal toxicity through formation of non-protein thiols (NPTs), which play a pivotal role in heavy metal detoxification in plants. NPTs contain a high percentage of cyst sulfhydryl residues. Glutathione (GSH), synthesized from cysteine, is one of the most important components of NPTs in plant metabolism (Alscher *et al*. 1993, Rennenberg 1995). Exogenously added S compounds were shown to increase total GSH of Cd-treated cells (Mendum *et al.* 1990). GSH may play several roles in heavy metal detoxification, tolerance and sequestration. It protects cells from oxidative stress damage, caused by heavy metals in plants (Bergmann and Rennenberg 1993, Gallego *et al*. 1996, Chaoui *et al*. 1997, Foyer *et al.* 1997, Weckx and Clijsters 1997, Noctor *et al.* Foyer 1998, Hegedûs *et al.* 2001, Tari *et al*. 2002). As an antioxidant, GSH controls the cellular concentrations of cytotoxic H_2O_2 and $O_2^{\bullet-}$ (Weckx and Clijsters 1997, Noctor *et al.* 1998, Schickler and Caspi 1999). GSH is the direct precursor of phytochelatins (PCs) (Rauser 1995, Zenk 1996) PCs are sulfur-rich peptides involved in heavy metal tolerance and sequestration (Steffens 1990, Zenk 1996). They bind metals in the cytosol and sequester them in the vacuole (Rauser 1995, Mehra and Tripathi 2000). Similar to results of this paper, sulphate was reported to increase Zn uptake by barley and cyst did better than SO_4^{2-} when used as substitution for it (Pavanasasivan and Axley 1982). Similarly, low concentrations of added sulphur raised accumulation of selenium in onion plants (Barak and Goldman 1997). Also, Mendum *et al.* (1990) and Popović *et al.* (1996) showed that exogenously added S improved growth suppressed by Cd.

With regard to the effect of SA on tolerance of *Corchorus* plants to toxic metals, results of this research were similar to those reported by Pál *et al*. (2002) who found that when SA and Cd were applied simultaneously, the Cd damage to young maize plants was less pronounced than without SA. Also SA was shown by Szalai *et al.* (2000) to significantly decrease lipid peroxidation and delay senescence and to increase survival in *Arabidopsis* exposed to Cd. These effects of SA might be achieved by SA-induced protein synthesis and increased antioxidants such as superoxide dismutase, peroxidases, glutathione reductase, dehydroascorbate reductase, ascorbic acid and glutathione (Szalai *et al.* 2000). SA was shown to provide protection against certain abiotic stresses, as for example in the case of heat stress in mustard seedlings (Dat *et al.* 1998) or chilling damage in maize (Janda *et al*. 2000).

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