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SHORT COMMUNICATION

UPTAKE OF MERCURY, CADMIUM, URANIUM AND ZINC BY *MIMOSA PUDICA*

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Efficiency of uptake of known pollutant heavy metals Hg, Cd, U and Zn by *Mimosa pudica*, a non-consumable wild plant species, was evaluated. Various concentrations of heavy metals ranging from 1-10, 1-20, 5-20 and 50-200 mM were used and maximum uptake by the roots at 10, 20, 10 and 100 µM recorded for Hg, Cd, U, and Zn, respectively. The translocation of metals from root to stem (48%) and leaves (8%) was highest for Zn, as compared to others. U showed only 35% translocation to stem, where as Cd and Hg translocation to both stem and leaves, was negligible. The results indicated differential uptake for different metals by *Mimosa pudica*. The roots showed maximum uptake capacity for heavy metals, implying the possible utility of *Mimosa pudica* for rhizofiltration.

Key words **: Heavy metal uptake, *Mimosa pudica*, phytoremediation.**

Heavy metal pollution of soils and aqueous streams poses a major environmental threat to human health. Phytoremediation, a plant based technology is an effective method that takes the advantage of the ability of plants to take up elements and compounds from the environment and concentrate them within tissues (Salt *et al*. 1998). There are reports about the plant species like *Chloris barbata* Sw. and *Thlaspi* that are tolerant and also accumulate heavy metals (Patra *et al*. 1994, Escarre *et al*. 2000). Mercury (Hg), Cadmium (Cd), Uranium (U) and Zinc (Zn) are the pollutant heavy metals chosen presently for the uptake studies, as these are the metals which are highly toxic to human and also of great environmental concern (Lakshmanan and Venkateswaralu 1988, Nriagu 1988, Bizili *et al*. 1999, Cobbett 2003).

In order to identify new metal accumulating plants, different plant species need to be investigated for their capacity to take up various heavy metals and their limitations. Hence, *Mimosa pudica* was selected in the present study for uptake of heavy metals because: i) it has short life cycle, ii) it is not consumed by human beings, iii) it has high propagation rate, iv) it is widely distributed and v) it has large shoot biomass. *Mimosa pudica* has been identified out of the 36 plant species screened, to be suitable for phytoremediation of arsenic (Visoottiviseth *et al.* 2002).

Mimosa pudica seeds were collected locally. All the chemicals and reagents used were of analytical grade. The chemicals like $HgCl_2$, $Cd(NO_3)_2$, $U(NO_3)_2$ and $ZnCl_2$ were used as the sources for heavy metals Hg, Cd, U and Zn, respectively. Seven-day-old seedlings of *M. pudica* were transferred to nutrient medium (Steinberg 1953) containing various concentrations of heavy metals. The treatments were given for 8 weeks with concentrations 1, 2, 5 and 10 µM for Hg; 10 weeks with concentrations 1, 2, 5, 10 and 20 µM for Cd; 8 weeks with concentrations 5, 10 and 20 µM for U and 4 weeks with concentrations 50, 100 and 200 µM for Zn. The plants were collected after respective treatments (three

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replications each) and the roots were washed in $Ca(NO_3)_2$ solution to remove the metal adsorbed on the surface. The plants were separated into root, stem and leaves and dried in an oven at 80ºC. The plants treated with U, Cd and Zn were digested in a mixture of perchloric acid-nitric acid (1:5 v/v). The ratio of tissue to acid mixture was kept at 1:10 (w/v). After complete digestion the residue was made acid free by evaporation and was dissolved in distilled water and volume made up to 5 ml. This was used for estimation either directly or after appropriate dilution. The digestion of the plant tissues treated with Hg was carried out in a closed vessel using pressure controlled microwave heating. The equipment used was CEM Microwave sample preparation system 2100, Heavy Duty Vessel Accessory Set. The digestion was done in 5-7 ml of 70% HNO_3 (Suprapure, MERCK). After digestion the sample was diluted to appropriate volume with deionized water. Estimation of Hg was carried out by the cold vapour atomic absorption technique using the instrument GBC 906 AAS equipment with HG3000 accessory (HG 3000 is a Hg hollow cathode lamp á 203.7 nm). The Hg vapour is generated by the reduction of acidified Hg solution with stannous chloride. The sample and reagents get pumped into a mixing manifold using a peristaltic pump that flows through a reduction coil where the Hg vapour is separated using an inert carrier gas. This is passed through a closed Hg cell and light absorbed by Hg analyte atoms is determined using a cold vapour atomic absorption spectrometer. The estimation of uranium was done by the method of Huang *et al.* (1998). The sample volume was made to 0.5 ml, to which 0.1 ml of 4% oxalic acid, 0.1 ml of 0.05% Arsenazo III and 1.8 ml of 4N HCl were added. Absorption was measured at a wavelength of 652 nm. A standard graph was prepared using uranyl nitrate solution in distilled water. The uranium accumulation in plant tissues is expressed as mg/g dry weight of the tissue. Estimation of Cd and Zn were done by Atomic absorption spectroscopy (GBC Model 932 B+, Australia).

The data on uptake of Mercury, Cadmium, Uranium, and Zinc by *M. pudica* is presented in Table 1. The root tissues accumulated Hg about 5.5 mg/g dry weight at 10 µM. The result showed an increase in Hg content for the concentrations upto 10 µM. The stem and leaf showed only negligible amount of Hg when treated upto 10 µM. Almost 100% of Hg that was taken up was found to be retained in the root tissue. Estimation of Cd in various tissues of *M. pudica* showed an increased level of metal in the root as concentration increased. At 20 µM concentration the uptake by root tissue was 8.6 mg/g dry weight, while stem and leaf showed negligible amount of Cd. The highest amount of metal was present in the root tissue followed by stem and the leaf tissue contained the least (Table 1). For U the maximum uptake by the root was 0.574 mg/g dry weight. There was an increase in uptake of U by root from 5 µM to 10 µM, which declined at 20 mM, but was still higher than 5 µM. With increasing concentration more metal got translocated from root to stem. Out of the three concentrations of Zn (50, 100 and 200 µM), the treatment at 100 µM showed the highest Zn accumulation in root tissue (12 mg/g dry weight) whereas, the stem contained 1.2 mg/g dry weight. At 200 µM concentration the absorption showed an increase upto 7.4 mg/g dry weight in the stem, while showing a decrease of uptake upto 6.8 mg/g dry weight in the root. The highest level of Zn in the leaf tissue was 1.2 mg/g dry weight, while in the stem the amount of Zn showed a steady increase upto 200 mM concentration. The distribution of metal in the plant showed the maximum uptake in root (86%) at 50 µM concentration, which declined to 44% at 200 µM. There was a corresponding increase of the metal in the stem tissue at 200 µM (48%). The translocation of Zn from root to leaf was 8% (Table 1). The uptake of Hg and Cd by roots was significant between treatments at concentrations above 2 µM, while in the case of U and Zn the uptake was not significant between treatments, even though the uptake of metal was shown initially (Table 1).

Uranium has been detected in plants including several crop plants even though it does not have any known function. The uptake ranged from 0.22 to 1.77 ppm when grown in the soil with U concentration of 2.3 to 3.27 ppm (Chowdhury and Goswamy 1990, Singh 1997). The distribution of U in different tissues of *M. pudica* was similar to *B. juncea* as shown by Mitra *et al*. (1999), though very high uptake was shown by hairy root cultures of *B. juncea* and *Chenopodium amaranticolor* (Eapen *et al*. 2003). As far as uptake of metal ion is concerned,

Table 1. Uptake of Hg, Cd, U and Zn in *Mimosa pudica*

Metal	Concentration (μM)	Uptake of metal (mg/g dw) Mean ± S. E.		
		Root	Stem	Leaf
Mercury	0 (Control)	0	0	0
	1	0.299 ± 0.017	0	0
	2	0.540 ± 0.050	0	0
	5	2.416 ± 0.756	0.002 ± 0.000	0.003 ± 0.000
	10	5.493 ± 0.398	0.007 ± 0.001	0.007 ± 0.000
	C.D. (0.05)	1.138	0.003	0.001
Cadmium	0 (Control)	0	0	0
	1	0.309 ± 0.019	0.007 ± 0.001	0.004 ± 0.000
	2	0.366 ± 0.023	0.019 ± 0.004	0.071 ± 0.002
	5	2.416 ± 0.047	0.022 ± 0.001	0.013 ± 0.003
	10	6.370 ± 1.168	0.082 ± 0.014	0.041 ± 0.013
	20	8.617 ± 0.117	0. 425 ± 0.026	0.202 ± 0.013
	C.D. (0.05)	1.563	0.377	0.026
Uranium	0 (Control)	0	0	0
	5	0.405 ± 0.068	0.088 ± 0.053	0.019 ± 0.009
	10	0.574 ± 0.059	0.255 ± 0.034	0.047 ± 0.001
	20	0.474 ± 0.095	0.270 ± 0.074	0.038 ± 0.011
	C.D. (0.05)	0.260	0.132	0.022
Zinc	0 (Control)	0.710 ± 0.186	0.413 ± 0.065	0.044 ± 0.001
	50	10.070 ± 0.254	1.254 ± 0.472	0.411 ± 0.079
	100	12.046 ± 1.966	1.208 ± 0.039	0.481 ± 0.051
	200	6.805 ± 1.070	7.408 ± 1.567	1.152 ± 0.332
	C.D. (0.05)	4.897	3.024	0.628

the data gathered to date about plant metal transporters, it is obvious that multiple pathways exist for most metal ions (Clemens 2001). *Brassica* and *Thlaspi caerulescens* were already known to hyper-accumulate for Zn and Cd (Ebbs *et al*. 1997). This species is, however, more effective due to its large biomass. Tolerance to Hg has been shown by *Chloris barbata* Sw. plants and has also shown co-tolerance to Cd and Zn (Patra *et al*. 1994).

A number of hyper-accumulating plants have been identified for various heavy metals for different climatic environments (Baker and Brooks 1989). But it is likely that many more unidentified metal accumulating plants

exist and hence the testing *M. pudica* for metal uptake is relevant. In the dicots, accumulation of Pb was found to be significantly higher than monocots (Huang and Cunningham 1996). In *M. pudica*, the maximum uptake was shown in roots for all the metals studied (Table 1). The translocation of the metals from root to shoot was observed in the case of U and Zn, whereas, accumulation of Cd and Hg was found to be restricted to root. Different plant species possess a range of potential cellular mechanisms that may be involved in the detoxification of heavy metal conferring tolerance to metal stress. Though Zn, Cd and Hg belong to the same group in the periodic table (Group IIA), the uptake and translocation of Zn was found to be different from that of the uptake of Cd and Hg. This may be because Zn is an essential metal for normal plant growth and development, as it is a constituent of many enzymes and proteins. The distribution of metals in different parts of *M. pudica* was found to be similar in the case of U and Zn unlike Cd and Hg in which no transportation of these metals to stem or leaves was observed. This phenomenon can be attributed to the lack of metal specific phytochelation. In the present study, Cd found to be accumulated in the roots while Zn was translocated partly to stem and leaves (Table 1). In the case of *M. pudica* the similarities were shown in the uptake of Cd and Hg, possibly because both belonged to the same group in the periodic table. In contrast, a similar trend in the uptake and transport of U and Zn was observed, even though they belong to different groups. In case of all the four metals studied, the accumulation was highest in the root tissues, indicating the possibilities of the plant species to be used for rhizofiltration of the contaminants.

Identification of a non-crop or non-palatable plant species for phytoremediation of heavy metals is ideal, as this will reduce the chance of its entry into the food chain. *M. pudica* being an unexplored wild plant species, the overall objective of this study was to determine the extent of uptake of different heavy metal species. Our results demonstrate that *M. pudica* responds differently to different heavy metals in their uptake and distribution and this plant species can be a promising candidate for rhizofiltration. Development of hairy root cultures of *M. pudica* for *in vitro* rhizofiltration may be worth exploring, since, the roots efficiently take up the heavy metals used in the present study. Induction of genetic variability for better adaptation of heavy metal stress cannot be ruled out, as *M. pudica* is an economical plant species that requires little agronomic inputs.

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