Journal of Stress Physiology & Biochemistry, Vol. 6 No. 1 2010, pp. 4-12 ISSN 1997-0838

ORIGINAL ARTICLE

Distribution of Bio-accumulated Cd and Cr in two *Vigna* species and the Associated Histological Variations

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Received December 16, 2009

In nutrient culture experiments, bioaccumulation and anatomical effects of cadmium ($CdCl_2$ - 20µM) and chromium ($K_2Cr_2O_7$ - 600 µM) on the structure of root and stem was studied by histochemical and analytical methods in *Vigna radiata* and *Vigna unguiculata*. Each metal exerted specific influences on the anatomy of various tissues in root and stem. Histochemical localisation of cadmium and chromium was observed in the stained sections of root and stem. Atomic Absorption Spectrophotometric study revealed maximum accumulation of cadmium and chromium in the root tissue as compared to shoot with significant variation among the species. Abundant occurrences of densely stained deposits of chromium were seen in the root stelar region of *V. unguiculata* and to a lesser extend in *V. radiata*. Cadmium accumulation in *V. radiata* was comparatively more than that of *V. unguiculata*. The findings also revealed that the accumulation pattern of cadmium and chromium varies between species and hence is species specific.

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Heavy metals are one of the key factors that exert negative influences on man and environment, and their release to environment cause toxicity to plants that are continually exposed to potentially toxic heavy metals. Cadmium is a metal pollutant which enters the environment mainly through anthropogenic and industrial processes and also through fertilizers. (Foy *et al.*, 1978, Sanita di- Toppi and Gabbrielli, 1999, Cakmak *et al.*, 2000). Although cadmium adversely affects plant growth, (Baryla *et al*. 2001, Sandalio *et al*. 2001) root growth is severely affected and results in faster reduction of root biomass compared to the shoot resulting in an increased

shoot/root biomass ratio (Jalil *et al*., 1994, Wojcik and Tukendorf, 1999). According to Stolt *et al*. (2006) the rate of absorption and translocation of cadmium varies from plant to plant and genetic variation exists in the accumulation rate of the cadmium in different parts of the plant. Ederly *et al*. (2004) suggested that in *Phragmitis australis*, cadmium was accumulated mainly in the vacuoles of parenchyma cells of roots.

Chromium toxicity in plants is observed at multiple levels such as reduced yield, inhibited growth of leaves and roots, inhibition of enzymatic activities and mutagenesis (Clijsters and Van Assche, 1985, Bishnoi *et al*., 1993, Shanker *et al*., 2005). Toxic effects of chromium have been reported in water relations of plants (Vazques *et al*., 1987), mineral metabolism, growth and development (Rout *et al*., 1997), seed germination (Peralta *et al*., 2001), enzymatic activities and translocation of sugars (Zeid, 2001). Bioaccumulation of chromium have been reported in cauliflower, beet root and barley (Lahouti and Peterson, 1979), mung bean (Samantary and Das, 1997) and *Albizia amara* (Shanker *et al*., 2005)

Although a number of studies have been documented on the toxic effects of cadmium and chromium, there are very meagre reports on the localisation of these metals in various plant tissues and the concerned anatomical variations, especially in cultivated plants. The present study is an attempt to locate the distribution of cadmium and chromium in the root and stem tissues of *V. radiata* and *V. unguiculata* and see the species variation with regard to the above phenomenon. Further anatomical variations as a result of the accumulation of these heavy metals are also studied.

MATERIALS AND METHODS

Commercially available seeds of *Vigna radiata* (L.) Wilczek and *Vigna unguiculata* (L.) Walp. were used for the study. Healthy seeds were selected and surface sterilized with 0.1 % (w/v) aqueous solution of mercuric chloride for one minute with constant shaking. After decanting the disinfectant, the seeds were then washed thoroughly in double distilled water. Twenty seeds in triplicates were sown in Petri-dishes lined with filter paper and kept for germination.

Modified Hoagland solution (Epstein, 1972) was used for culturing the seedlings. Concentrations of cadmium chloride and potassium dichromate exhibiting 50% growth inhibition were selected for the treatments. ($CdCl_2$- 20 µM, $K_2Cr_2O_7$- 400 µM and $CdCl_2$- 20 µM, $K_2Cr_2O_7$- 600 µM for *V. radiata* and *V. unguiculata* respectively). The germinated seedlings on the petri-dish was further transplanted into polypropylene containers (500 cm^3) containing Hoagland medium along with the respective heavy metals. During transplantation, seedlings were inserted gently through the holes (1 cm^2) made by wire net placed on the container by taking care that only the radicle was immersed in the nutrient solution. The containers were kept at 25 ± 2 °C RH 78%± 2 in the net house condition. Seedlings cultured in nutrient solution in the same type of containers described above without any heavy metal served as the control.

Treated and control seedlings were collected after three days of treatment and sampling was continued at an interval of 3 days up to 12 days. For histochemical studies, uniformly cut pieces of root and stem were fixed in FAA, dehydrated through alcohol- TBA series and embedded in paraffin wax (Johansen, 1940, Berlyn and Miksche, 1976). Using a Leica Rotary Microtome (Model- RM 2125RT) individual blocks were cut at 10 µm thickness. Deparaffinised sections were stained with Delafield's hematoxylin (Berlyn and Miksche, 1976) and photomicrographs were taken by using Nikon camera (Model- ECLIPSE E400)

Cadmium and chromium content in the root and stem tissues were analyzed using Atomic Absorption Spectrophotometer (PERKIN ELMER Model A, Analyst 300). Sample preparation was done according to the method of Allan (1969). The data were analysed statistically and test of significance was done.

RESULTS AND DISCUSSION

Bioaccumulation

In general more accumulation of Cr was observed in *V. unguiculata* in comparison with *V. radiata*, where as higher Cd accumulation was observed in *V. radiata* as compared to *V. unguiculata* (Table 1). Both in *V. radiata* and *V. unguiculata* Cd and Cr accumulation were observed to be the maximum in root tissues compared to shoot tissues. Localization of the cadmium in the roots has been reported in *Phragmites australis* grown in hydroponic medium artificially contaminated with cadmium (Ederli *et al*., 2004). According to Cseh, (2002) considerable amount of cadmium accumulates mostly in the root cell walls and inhibits elongation of root growth. In a study conducted in rice, it has been observed that Cd is highly mobile and gets translocated even to seeds (Reid *et al*., 2003). Even though both species are exposed to the same concentration (20 µM) of $CdCl_2$, the significant differences in the pattern of cadmium accumulation between the species may be due to difference in the tolerance level of these plants towards Cd. According to Yoon *et al*. (2006) metal accumulation varies with plant species and they point out towards the genetic variation as important in this context. The genetic variation may be expressed as differences in morphological and physiological characteristics of genotypes (Hansson *et al*., 2005, Stolt *et al*., 2006). Ishikawa *et al*. (2006) suggested that cadmium accumulation pattern varies with plant species and comparative studies on *Zea mays*, *Brassica juncea*, *Oryza sativa* and *Beta vulgaris* revealed that *Zea mays* displayed the lowest accumulation of cadmium.

Chromium accumulation is comparatively lower in *V. radiata,* therefore it is present in small quantities in the shoot whereas maximum accumulation is shown by *V. unguiculata*. Shanker *et al*. (2005) suggested that generally in plants chromium translocation from root to shoot is very slow. Pulford *et al*. (2001) in a study with temperate trees confirmed that chromium is poorly taken up into the aerial parts and is held predominantly in the roots. In both species of *Vigna* chromium accumulation was very high in the roots compared to the stem (Table 1).

An important reason for enhanced accumulation of chromium in the root may be due the presence of organic acids in the root exudates which form complexes with chromium, there by making them available for the uptake by root. Srivastava *et al*. (1999) suggested that in *Lycopercicum esculentum* carboxylic acid and amino acids present in the root are involved in the enhanced uptake of chromium in the roots.

Anatomical Changes

In general histological variations were more predominant in stem and root of *V. radiata* as compared to *V. unguiculata*, on treatment with Cd, whereas treatment of Cr brought about predominant histological variations in stem and root of both species of *Vigna*. Cd treatment brought about distortion of cortical cell shape in stem and root of *V. radiata* (Fig. 1B&E), whereas tissues of root and stem was unaffected in *V. unguiculata* (Fig. 2B&E). While Cd could disorganize the root epidermis of *V. radiata,* the stem epidermis remained intact (Fig. 1B&E). Cr treatment brought about distortion of epidermis, cortex and stele in stem of *V. radiate* (Fig. 1C), whereas the distortion was limited to the cortex and stele and was to a lesser extend in *V. unguiculata* (Fig. 2C).

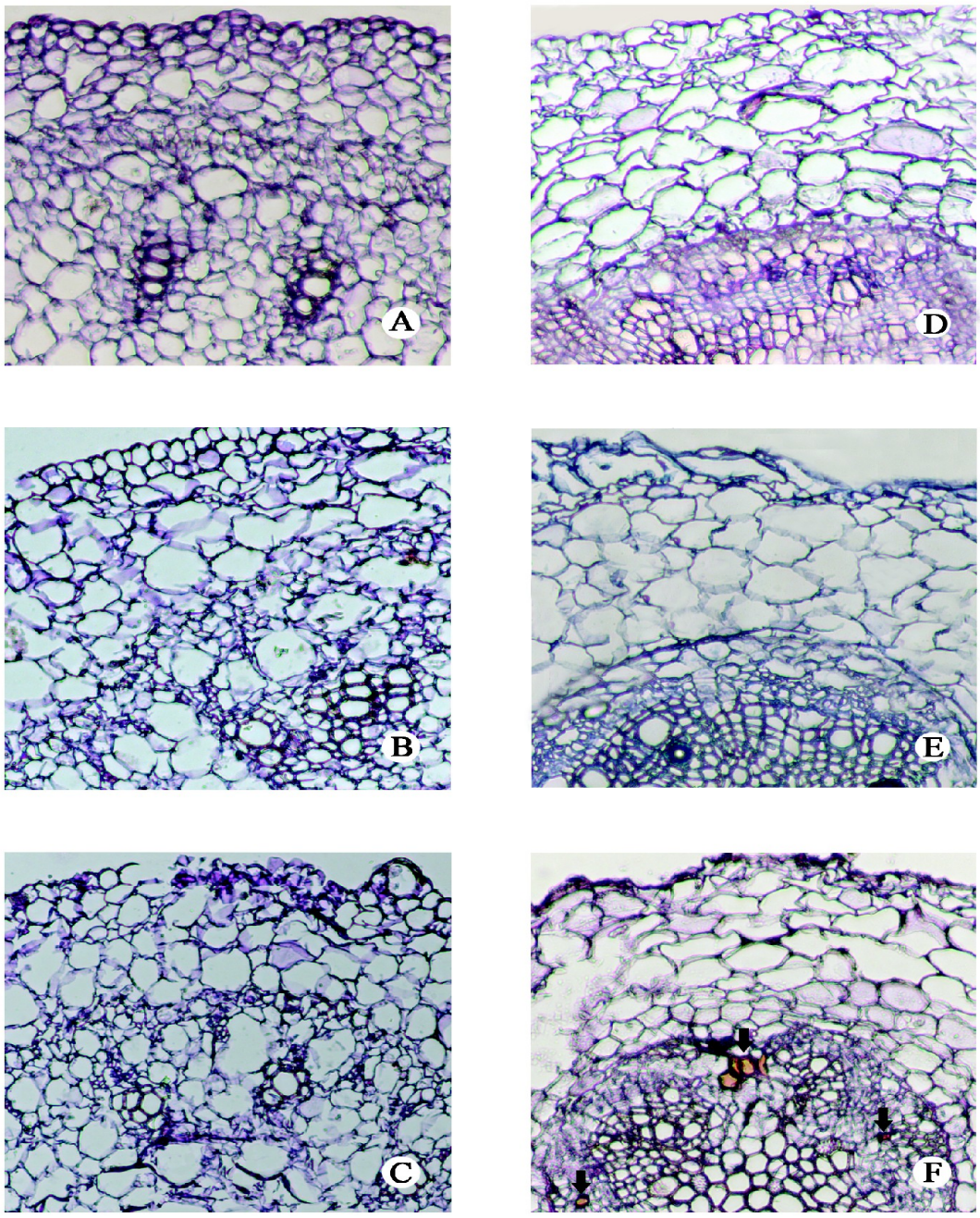

Fig. 1. Histological variations in the stem and root of *Vigna radiata* seedlings treated with Cadmium ($CdCl_2$) and Chromium ($K_2Cr_2O_7$). A, B & C represents *Vigna radiata* stem control, Cd and Cr treated respectively. D, E & F represents *Vigna radiata* root control, Cd and Cr treated respectively. → indicates the densely stained deposits of chromium in the root stelar region

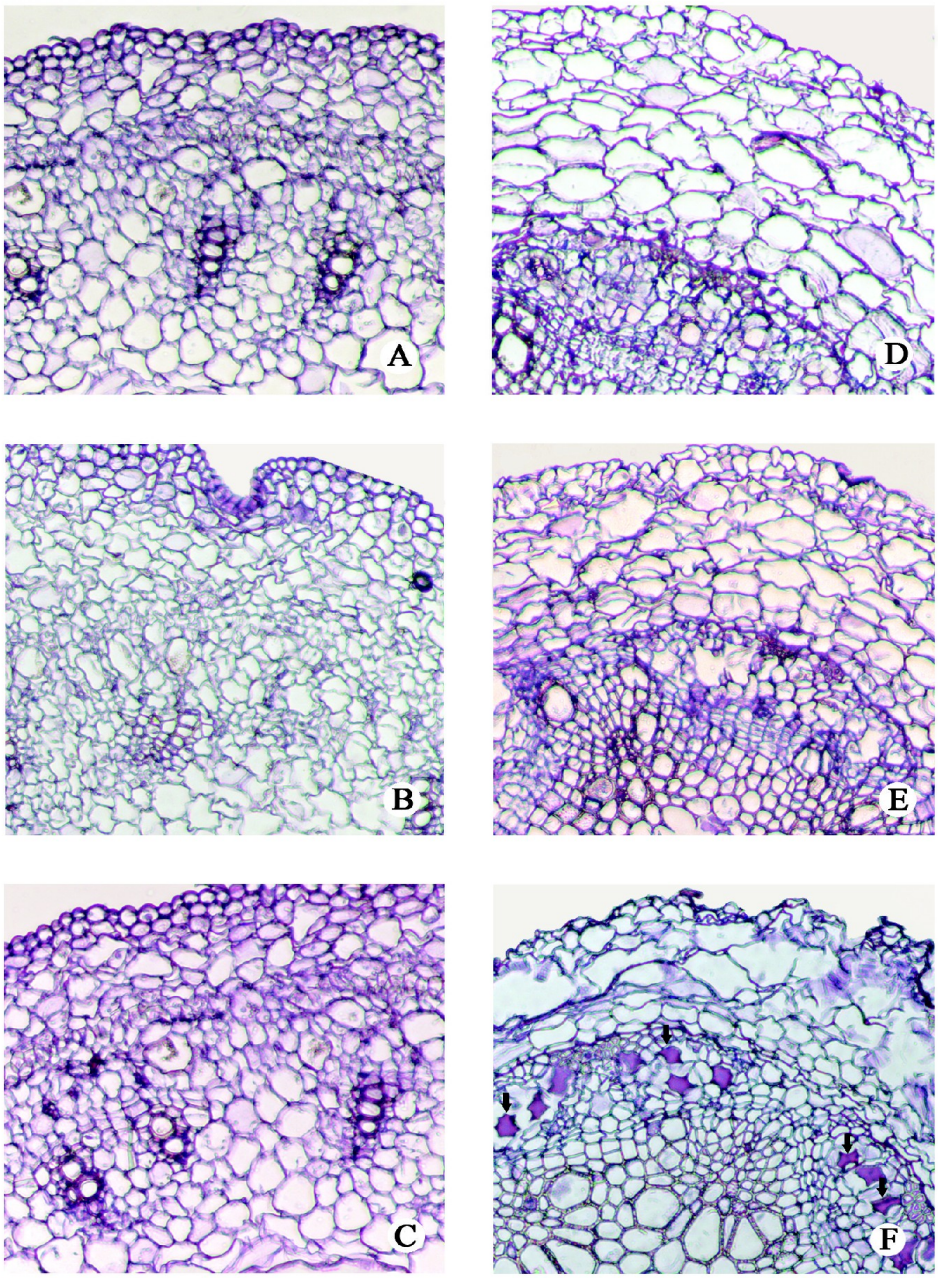

Fig. 2. Histological variations in the stem and root of *Vigna unguiculata* seedlings treated with Cadmium ($CdCl_2$) and Chromium ($K_2Cr_2O_7$). A, B & C represents *Vigna unguiculata* stem control, Cd and Cr treated respectively. D, E & F represents *Vigna unguiculata* root control, Cd and Cr treated respectively. → indicates the densely stained deposits of chromium in the root stelar region

The histological changes in the root treated with Cr resulted in highly distorted piliferous layer and cortex in both species. Even though the stelar portion of root appeared normal in both species, some artefacts in the stele were noticed in *V. radiata* and also densely coloured deposits were observed towards phloem of *V. unguiculata* to a larger extend and to a lesser extend in *V. radiata* (Fig. 1F & Fig. 2F). Thickened cell walls of vessels and pith of *V. unguiculata* was another notable variation (Fig. 2F). The distortion of cells of various tissues may be the result of interference with the cell division or with cell elongation.

Table 1. Distribution of bioaccumlated Cadmium and Chromium in plants treated with $CdCl_2$ and $K_2Cr_2O_7$. The data are the average of recordings from three independent experiments, each with a minimum of three replicates (i.e. n=9). The data represent mean ± standard error.

Treatment		***Vigna radiata***	***Vigna unguiculata***
Cadmium (µg/g dry wt.)	Root	351±23	98±6.4
	Stem	26±1.2	70±4.6
Chromium (µg/g dry wt.)	Root	580±41	2750±98
	Stem	20±1.1	680±35

Earlier it was shown that roots of wheat grown individually with Ni, Cd and Pb, the epidermal cells were disorganized while the cortical cells exhibited distortion of cell shape due to disintegration. These heavy metals were also found to cause breakdown of root vascular tissues (Setia and Bala, 1994). It has also been reported that Cr brings about increased relative proportion of pith and cortical tissue layers (Suseela *et al*., 2002).

This study reveals that significant variation exists between the two *Vigna* species studied, with regard to the distribution of bioaccumlated heavy metals (Cd and Cr) and the associated histological variations. It can be very well presumed that the variation in the distribution may either be due to the differences in the mode of absorption or due to the species specificity as far as the tolerance or sensitivity towards heavy metal toxicity is concerned.

Acknowledgements

We are indebted to The Cashew Export Promotion Council (CEPC), Government of India, for the AAS facilities extended.

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