

ORIGINAL ARTICLE

**Response and Bioaccumulation Potential of *Boerhavia diffusa* L.  
Towards Different Heavy Metals**

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Plants growing in metal enriched- soils take up metals to varying degrees in response to external and internal factors. There is vast literature on analytical data relating to metal up take, illustrating the scale of differences between species and genotypes and between metals in the field and laboratory studies ranging from trace nutrient elements to toxic heavy metals (Foy *et al.*, 1978;

Lepp, 1981; Fitter and Hay, 1983; Borovik, 1990; Friedland, 1990; Cseh, 2002, Pilon-Smits, 2005). Heavy metals like cadmium, chromium, mercury, lead etc. are having no beneficial properties for the plant growth and are highly reactive and consequently toxic to plants.

*Boerhavia diffusa* is a widely used medicinal plant and is an important ingredient of 45 different

Ayurvedic preparations (Sivarajan and Balachandran, 1996). This herb grows wild in all types of soil inclusive of polluted areas, such as drainages and waste lands. It is a diffused perennial herbaceous medicinal plant growing prostrate or ascending upward in habitats like grasslands, agricultural fields, fallow lands, wastelands and residential compounds.

*B. diffusa* (Hogweed) belongs to the family of Nyctaginaceae (Known also under its traditional name as 'Punarnava' in Sanskrit and " Chuvanna thazhuthama" in Malayalam). The plant was named in honour of Herman Boerhaave, a famous Dutch Physician of the 18th Century (Chopra, 1969). The habit, distribution and growth pattern of *B. diffusa* owe maximum chance of pollutant exposure and accumulation of toxic metals in all parts of the plant body and wide medicinal consumption of *B. diffusa* may lead to health hazard. However, the bioaccumulation potential of this plant is not known. Moreover, bioaccumulation of toxic elements by plants leading to severe health hazard is of a concern in the present era because consumption of Ayurvedic medicines is fast increasing.

Cadmium toxicity causes plant growth inhibition, low biomass production, impaired water relations, respiration, photosynthesis and nitrogen metabolism (Sergin and Ivanov, 2001; Perfus-Barbeoch et al., 2002; Linger et al., 2002). Chromium toxicity is observed at multiple levels such as reduced yield, inhibited growth of leaves and roots, metabolism, enzymatic activities and induce mutagenesis (Clijsters and Van Assche, 1985; Bishnoi et al., 1993; Shanker et al., 2005) Mercury shows detrimental effect on plant growth and development (Lenka et al., 1993). The mode of action of this toxic metal includes membrane

distortion (Ouariti et al., 1997). Site competition with metabolites (Perfus-Barbeoch et al., 2002; Moreno et al., 2008). Lead is highly toxic element to plants and it has been reported to inhibit photosynthesis and respiration by affecting electron transport mechanism (Orcutt and Nilsen, 2000). Effect of lead on the physiological aspect such as photosynthesis, translocation, rapid root, growth inhibition, cytological aspects, and bioaccumulation have been investigated in many plants (Gasic et al., 1992; Mohan and Hosetti, 1997; Fodor, 2002).

Effect of heavy metals on medicinal plants and bioaccumulation potential in general and *B. diffusa* in particular has not yet been investigated. The objectives of the present study include standardisation of different concentrations of Cd, Cr, Hg and Pb on *B. diffusa* to impart more or less similar visible morphological symptoms of growth retardation. Analysing growth performances during a period of 20 days in terms of root and shoot length, leaf area, biomass distribution and tolerance index. Treatment of *B. diffusa* plant with four heavy metals is aimed at the assessment of response / tolerance towards these metals and a comparison of growth retardation parameters and bioaccumulation potential.

## MATERIALS AND METHODS

*Boerhavia diffusa* L. cuttings were collected from Calicut University Botanical Garden. Healthy and profusely growing plants were selected for experiments. Healthy cuttings of 10-15 cm length consisting of 3-4 nodes were selected for culture studies. Screening experiments on the effect of treatments of *B. diffusa* cuttings with cadmium chloride ( $\text{CdCl}_2$ ), potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ), mercuric chloride ( $\text{HgCl}_2$ ) and lead acetate ( $\text{CH}_3\text{-}$

COO)<sub>2</sub> Pb 3H<sub>2</sub>O showed that tolerance of *B.diffusa* towards Cadmium, Chromium, Mercury and Lead varied widely. Hence the concentrations in which seedlings survived but exhibited approximately 50% growth retardation were selected for the experiment. Table 1 shows the optimal concentration of each treatment, which brought about 50% growth retardation. Rooted cuttings (3 numbers) were planted in one bottle containing 100 ml of Hoagland solution to which the heavy metal solutions were added to obtain the final concentration as given in Table 1. Minimum 25 bottles were used for each treatment so as to get sufficient tissues for experiments. The hydroponic system was maintained under green house conditions. Plants cultivated in Hoagland solution without any heavy metal salt served as the control.

Samples of treatments and control were collected at comparable interval of four days up to 20 days of growth. At each interval, plants were harvested from each treatment, washed thoroughly in distilled water and blotted to dryness. Morphological parameters such as root/shoot length, leaf area, tolerance index and stomatal index were recorded. For biochemical analyses, root, stem and leaves were sampled. A minimum of 5 plants of each treatment were separately cut into pieces, randomized and sampled in duplicates for each analysis.

Growth of plants were assessed in terms of root length, stem length, and leaf area. Stomatal density on abaxial and adaxial sides of the leaf was counted under a light microscope, by using nail polish impressions of leaf surface. Stomatal index was calculated according to the method of Meidner and Mansfield (1968). Dry weight was determined by using the hot air oven and weighing was repeated until values become constant. Cadmium, chromium,

mercury and lead content of the root, stem and leaf tissues were collected and analyzed according to the method of Allan (1969) using Atomic Absorption Spectrophotometer.

## RESULTS

Growth retardation expressed in terms of reduced root and stem length and leaf area was observed in *Boerhavia diffusa* as a result of cadmium, chromium, mercury and lead treatments. Due to cadmium treatment, root growth was reduced fourth day onwards gradually and the same trend was continued up to 20<sup>th</sup> day. But in plants treated with chromium, root growth retardation was comparatively lower than other metals and more or less similar trend was shown by the plants treated with mercury and lead. Stem growth of *B. diffusa* was adversely affected by cadmium treatment resulting in significant reduction in all stages of growth compared to the control. Chromium treatment resulted in maximum growth reduction of stem compared to other metals. Stem growth retardation due to mercury and lead treatments was significant compared to the control throughout the experimental period. Plants treated with mercury and lead also showed reduced leaf area but low retardation in leaf growth was observed compared to that of cadmium and chromium treatments (Table 2).

Values of tolerance index showed maximum values in the first interval in all treatments and the values showed slight reduction after 8<sup>th</sup> day of growth. Continuous reduction of tolerance index was shown Hg and Pb treatments without any difference between the intervals. Cadmium and chromium showed slight increase in tolerance index values in sample of 8<sup>th</sup> day and significant reduction during further growth in treated plants (Table 3).

Stomatal index of *B. diffusa* plants treated with

all heavy metals showed significant changes. Cadmium treatment resulted in significant increase of stomatal index in the lower epidermis in comparison with that of control, whereas stomatal index of upper epidermis remained almost unchanged. Maximum value of stomatal index of both lower and upper epidermis was shown by the plants treated with mercury compared to the control as well as other treatments. Plants treated with lead showed only slight increase in the stomatal index values of upper and lower epidermis compared to the control (Table 3).

Dry weight distribution of root tissue of *B. diffusa* plants treated with all four heavy metals showed increase compared to the respective controls during all intervals of growth. Stem tissue also exhibited more or less the same trend in the distribution of dry weight content. Only negligible fluctuations were observed in the distribution of dry matter content of leaves of the plants treated

with all metals (Table 5).

There occurred a significant variation in the pattern of bioaccumulation of cadmium, chromium, mercury and lead in *B. diffusa* (Table 5). The accumulation was found to be mainly dependent upon the growth period and related to plant parts like root, stem and leaf. After 2 days all the metals were present only in root tissue where as occurrence of cadmium, chromium and lead was observed in root and stem on 4<sup>th</sup> day. As the growth advanced up to 8<sup>th</sup> day, all metals were present in the root and stem but leaves were devoid of accumulation. Plants collected on 12<sup>th</sup> day exhibited cadmium and mercury only in root and stem but chromium and lead were present in the leaf also. Sixteenth day samples showed all elements except cadmium in root, stem and leaves. Samples of 20<sup>th</sup> day of growth all heavy metals were accumulated in all plant parts.

**Table 1:** Concentrations of Heavy Metal salts used for treatments of *B. diffusa* seedlings.

Heavy metal salts	Concentrations ( $\mu\text{M}$ )
Cadmium chloride	30
Potassium dichromate	400
Mercuric chloride	10
Lead acetate	600

**Table 2:** Effect of Heavy Metals on Root and Stem length (cm) and Leaf area ( $\text{mm}^2$ ) in *Boerhavia diffusa*

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	Root length	3.41 $\pm$ 0.17	4.57 $\pm$ 0.56	6.84 $\pm$ 0.79	8.12 $\pm$ 0.96	11.30 $\pm$ 0.87	13.72 $\pm$ 0.84
	Stem Length	7.53 $\pm$ 0.39	9.62 $\pm$ 0.74	19.5 $\pm$ 0.32	22.6 $\pm$ 0.53	28.30 $\pm$ 0.63	31.62 $\pm$ 0.80
	Leaf area	288.6 $\pm$ 11.6	490.7 $\pm$ 21.6	641.4 $\pm$ 10.0	784.2 $\pm$ 16.3	967.5 $\pm$ 9.50	1158.7 $\pm$ 17.3
Cadmium	Root length	3.41 $\pm$ 0.97	3.96 $\pm$ 0.97	4.28 $\pm$ 0.13	5.89 $\pm$ 0.31	6.13 $\pm$ 0.11	6.89 $\pm$ 0.93
	Stem Length	7.53 $\pm$ 0.39	7.89 $\pm$ 0.72	11.33 $\pm$ 1.43	14.84 $\pm$ 0.92	17.62 $\pm$ 0.81	21.52 $\pm$ 0.76
	Leaf area	288.6 $\pm$ 11.6	323.4 $\pm$ 14.2	386.7 $\pm$ 11.3	423.2 $\pm$ 8.20	567.5 $\pm$ 11.4	593.2 $\pm$ 7.20
Chromium	Root length	3.41 $\pm$ 0.17	4.16 $\pm$ 0.82	5.92 $\pm$ 0.98	7.16 $\pm$ 0.35	9.28 $\pm$ 0.81	10.67 $\pm$ 1.13
	Stem Length	7.53 $\pm$ 0.39	8.39 $\pm$ 0.13	9.67 $\pm$ 0.70	13.23 $\pm$ 0.93	14.77 $\pm$ 0.78	16.39 $\pm$ 0.75
	Leaf area	288.6 $\pm$ 11.6	319.3 $\pm$ 9.30	428.6 $\pm$ 9.3	499.3 $\pm$ 7.82	584.2 $\pm$ 9.31	612.5 $\pm$ 12.4
Mercury	Root length	3.41 $\pm$ 0.17	4.12 $\pm$ 0.35	4.86 $\pm$ 0.56	5.28 $\pm$ 0.69	6.95 $\pm$ 0.52	8.54 $\pm$ 0.25
	Stem Length	7.53 $\pm$ 0.39	8.95 $\pm$ 0.81	17.39 $\pm$ 0.39	19.34 $\pm$ 0.23	23.21 $\pm$ 0.85	24.59 $\pm$ 0.31
	Leaf area	288.6 $\pm$ 11.6	334.7 $\pm$ 11.3	489.8 $\pm$ 14.6	596.2 $\pm$ 10.2	633.4 $\pm$ 12.5	824.2 $\pm$ 15.2
Lead	Root length	3.41 $\pm$ 0.17	4.39 $\pm$ 0.39	5.86 $\pm$ 0.67	6.64 $\pm$ 0.92	9.16 $\pm$ 0.50	10.93 $\pm$ 1.25
	Stem Length	7.53 $\pm$ 0.39	8.34 $\pm$ 1.04	12.39 $\pm$ 0.73	17.38 $\pm$ 0.52	23.28 $\pm$ 0.58	25.34 $\pm$ 0.82
	Leaf area	288.6 $\pm$ 11.6	413.5 $\pm$ 8.95	487.4 $\pm$ 12.2	639.3 $\pm$ 4.43	832.2 $\pm$ 6.30	884.4 $\pm$ 7.43

Values are mean of 5 replicates  $\pm$  standard error

**Table 3:** Effect of Heavy Metals on Tolerance Index percentage pertaining to Root length in *Boerhavia diffusa* during growth.

Treatment	Interval (Days)				
	4	8	12	16	20
Control	100	100	100	100	100
Cadmium	86.65±2.18	72.57±2.51	62.53±2.31	54.20±2.65	50.20±2.19
Chromium	91.02±3.09	86.54±3.42	88.17±3.91	82.12±3.61	77.76±3.41
Mercury	90.15±2.91	71.05±3.14	65.02±2.40	61.50±2.93	62.20±3.10
Lead	96.06±3.61	85.67±3.58	81.77±2.64	81.06±2.98	79.60±3.41

**Table 4:** Effect of Heavy Metals on the Dry weight percentage in *Boerhavia diffusa*

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	Root	8.900±0.13	10.50±0.29	11.74±0.15	12.35±0.11	13.05±0.16	13.56±0.11
	Stem	18.84±0.06	19.57±0.06	19.61±0.02	20.43±0.08	22.21±0.03	22.89±0.01
	Leaf	11.71±0.07	12.37±0.05	13.43±0.11	13.97±0.07	15.38±0.03	16.76±0.10
	Shoot*	30.59	31.94	33.04	34.40	37.59	39.65
Cadmium	Root	8.900±0.13	10.91±0.23	12.26±0.13	13.74±0.06	14.63±0.02	14.98±0.01
	Stem	18.84±0.06	19.32±0.12	19.97±0.21	21.26±0.44	23.39±0.12	24.67±0.41
	Leaf	11.71±0.07	11.91±0.06	12.64±0.03	13.92±0.16	16.72±0.12	17.77±0.04
	Shoot	30.55	31.23	32.61	35.18	40.11	42.44
Chromium	Root	8.900±0.13	11.17±0.21	12.76±0.20	13.93±0.16	15.27±0.11	15.87±0.15
	Stem	18.84±0.06	20.31±0.23	21.93±0.17	23.34±0.13	24.62±0.28	25.33±0.13
	Leaf	11.71±0.07	12.23±0.07	12.94±0.04	14.62±0.03	15.98±0.06	18.31±0.43
	Shoot	30.55	32.54	34.87	37.96	40.60	43.64
Mercury	Root	8.900±0.13	9.320±0.12	9.960±0.11	12.73±0.29	13.67±0.16	15.14±0.23
	Stem	18.84±0.06	19.27±0.17	19.94±0.06	20.76±0.03	21.17±0.11	23.15±0.15
	Leaf	11.71±0.07	12.07±0.11	12.62±0.17	13.33±0.16	15.72±0.13	15.93±0.07
	Shoot	30.55	31.34	32.56	34.09	36.89	39.08
Lead	Root	8.900±0.13	9.730±0.21	10.91±0.11	12.62±0.17	13.55±0.10	14.72±0.24
	Stem	18.84±0.06	18.96±0.06	19.73±0.02	21.68±0.07	23.12±0.21	24.74±0.17
	Leaf	11.71±0.07	11.88±0.03	12.96±0.07	13.88±0.21	15.37±0.01	17.56±0.03
	Shoot	30.55	30.84	32.69	35.56	36.67	42.30

Values are mean of 5 replicates ±standard error, \* Sum of stem and leaf

**Table 5:** Bioaccumulation pattern of Heavy Metals in *Boerhavia diffusa* cultivated in Hoagland solution containing known quantities of Cd, Cr, Hg and Pb.

Interval of sample collection (Days)	Tissues	Bioaccumulation of Heavy metals( $\mu\text{g/g}$ ) Dry weight			
		Cadmium (5.5 $\mu\text{g}$ ) *	Chromium (58.8 $\mu\text{g}$ ) *	Mercury (2.715 $\mu\text{g}$ ) *	Lead (227.6 $\mu\text{g}$ ) *
1	Root	0.454 (8.2)	3.48 (5.9)	0.116 (4.27)	0.230 (0.09)
	Stem	NDR	NDR	NDR	NDR
	Leaf	NDR	NDR	NDR	NDR
4	Root	0.970 (17.6)	3.850 (6.5)	0.310 (11.4)	0.830 (0.35)
	Stem	0.220 (4.0)	0.192 (0.32)	NDR	0.100 (0.04)
	Leaf	NDR	NDR	NDR	NDR
8	Root	1.670 (33.4)	5.000 (8.5)	0.410 (15.1)	6.080 (2.61)
	Stem	0.280 (5.0)	2.790 (4.7)	0.110 (4.1)	0.230 (0.09)
	Leaf	NDR	NDR	NDR	NDR
12	Root	2.179 (39.5)	20.79 (35.3)	0.750 (27.6)	8.870 (3.8)
	Stem	0.937 (17.6)	6.070 (10.3)	0.340 (12.5)	0.380 (0.16)
	Leaf	NDR	0.110 (0.18)	NDR	0.087 (0.03)
16	Root	2.230 (40.5)	23.26 (39.5)	0.970 (35.7)	87.85 (37.7)
	Stem	1.190 (21.6)	6.140 (10.4)	0.410 (15.1)	11.08 (4.7)
	Leaf	NDR	0.142 (0.24)	0.110 (4.1)	0.230 (0.09)
20	Root	2.550 (46.3)	26.02 (44.2)	1.110 (40.0)	107.1 (46.0)
	Stem	1.300 (23.6)	3.410 (5.8)	0.714 (26.2)	26.90 (11.5)
	Leaf	0.104 (18.9)	0.200 (3.4)	0.220 (8.10)	0.377 (0.16)

\*Note: 5.5  $\mu\text{g}$  cadmium (250 ml of 30  $\mu\text{M}$   $\text{CdCl}_2$ ), 58.8  $\mu\text{g}$  chromium (250 ml of 400  $\mu\text{M}$   $\text{K}_2\text{Cr}_2\text{O}_7$ ), 2.715  $\mu\text{g}$  mercury (250 ml of 10  $\mu\text{M}$   $\text{HgCl}_2$ ) and 227.6  $\mu\text{g}$  lead (250 ml of 600  $\mu\text{M}$   $(\text{CH}_3\text{-COO})_2\text{Pb}$   $3\text{H}_2\text{O}$ ). Values in the parenthesis are percentage of accumulation of each metal

## DISCUSSION

Laboratory investigations dealing with heavy metals in plants involve simulation experiments with a wide range of concentration levels and durations. Several simulated experiments on plants with heavy metals have shown that optimal concentration to impart toxicity varies as 25-50  $\mu\text{M}$   $\text{CdCl}_2$  in *Bacopa monnieri* (Ali et al., 1998), 10-100 $\mu\text{M}$   $\text{CdCl}_2$  in *Arabidopsis thaliana*, (Perfus – Barbeoch, et al., 2002), 0.05mM  $\text{CdCl}_2$  in *Triticum aestivum* (Abdel-Latif, 2008), 64  $\mu\text{M}$  chromium in *Bacopa monnieri* (Sinha, 1999), 12-24 mg/l

chromium in *Medicago sativa* (Shanker et al., 2003), 5-10 $\mu\text{M}$   $\text{HgCl}_2$  in *Pisum sativum* (Beauford et al., 1977), 1-2  $\mu\text{M}$   $\text{Hg}(\text{NO})_2$  in *Chromolaena odorata* (Velasco-Alinsug et al., 2005), 1-20  $\mu\text{M}$   $\text{PbCl}_2$  in *Oryza sativa* (Kim et al., 2002) and 100-800 mg/kg lead in *Xanthium strumarium* (Sonmez et al., 2008). By conducting repeated screening experiments under simulated laboratory conditions on *Boerhavia diffusa* cultivated in Hoagland nutrient solution containing different concentration of  $\text{CdCl}_2$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ ,  $\text{HgCl}_2$  and  $(\text{CH}_3\text{COO})_2\text{Pb}$  for different periods, the present author selected the

concentrations of 30  $\mu\text{M}$  cadmium, 400  $\mu\text{M}$  chromium, 10  $\mu\text{M}$  mercury and 600  $\mu\text{M}$  lead to which the experimental plant showed approximately similar visible symptoms of growth retardation retaining their survival in those concentrations. (Table 1). Irrespective of the difference in the concentration of heavy metals, growth retardation is a characteristic feature shown by *B. Diffusa* and magnitude of retardation is not uniform. Growth inhibition has been reported as an important and established visible effect in plants by heavy metals like cadmium in *Cannabis sativa* (Linger, *et al.*, 2005) and *Oryza sativa* (Kim *et al.*, 2002), *Vigna* species (Jamal *et al.*, 2006; Ratheesh-Chandra *et al.*, 2010), lead in *Brassica* species (Hosseini *et al.*, 2007), chromium in *Amaranthus viridis* (Zou *et al.*, 2006), mercury in *Triticum aestivum* (Setia and Bala, 1994) and *Bacopa monnieri*, (Hussain, 2007).

*B. diffusa* exhibits reduced root growth as a result of cadmium, chromium, mercury and lead and more or less similar trend is shown during all intervals of growth (Table 2). In nutrient culture, roots are directly in contact with metal ions and hence the immediate effect is expressed as stunted growth of roots. More inhibition of root growth is affected by cadmium followed by mercury compared to other metals in *B. diffusa*. According to Wilkins (1978) and Wong and Bradshaw (1982), primary toxic effects of heavy metals is root growth inhibition and this parameter is an ideal index to measure the degree of tolerance. Rooted propagules of *B. diffusa* when exposed to the heavy metal shows rapid response and resultant impact as observed in significant changes in root length and tolerance index. Tolerance index calculated on the basis of root length ratio of experimental to that of control (Turner, 1994) shows gradual reduction of

tolerance index during growth in all treatments (Table 3). Although root growth retardation is the symptoms of heavy metals, the metabolic role of heavy metals in root growth impairment phenomenon is not fully known. Linger *et al.* (2005) suggested that cadmium changes the capability of cell division of meristematic cells and the author suggested that this view is highly plausible after scrutinising many reports on adverse effects of heavy metals on cell division and cell elongation. According to Zou *et al.* (2006) inhibited mitotic index in the root apex leads to stunted growth in *Amaranthus viridis*. The sensitivity/ tolerance exhibited by *B. diffusa* towards different concentrations of cadmium, chromium, mercury and lead are depicted in the pattern of root length also (Table 1 & 3).

Significant variations are shown in the stomata index of *B. diffusa* treated with all the metals (Table 3). Although stomatal distribution in relation to heavy metal stress is not well documented, effect of cadmium has been shown to inhibit water stress tolerance in *Phaseolus vulgaris* (Meidner and Mansfield, 1968; Barceleo and Poschenrieder, 1990), reduce cell wall elasticity (Becerril *et al.*, 1989) increase stomatal resistance (Hussain *et al.*, 2006). These views are in consonance with the behaviour of *B. diffusa* in which stomatal index shows only negligible changes in all treatments except mercury. Perturbation of plant- water relationship and osmoregulation of stomatal conductance in *Arabidopsis thaliana* treated with  $\text{CdCl}_2$ , revealed the non – essentiality or requirement of increased stomatal index (Perfus-Barbeoch *et al.*, 2002). Nevertheless, *B. diffusa* under mercury stress, exhibit significantly increased stomatal index in both upper and lower epidermis. This observation is directly related not only to



transpiration but detoxification of mercury also by phytovolatilization process as reported in *Bacopa monnieri* (Hussain, 2007) and in *Brassica juncea* (Moreno et al., 2008).

Irrespective of the significant differences in the concentration of each heavy metal which are applied to the nutrient solution on the basis of visible growth retardation, only slight increase of biomass is shown by all treatments without significant difference between the treatments. Even though the water potential reduction is known to be affected by heavy metal absorption (Costa and Morel, 1994) and resultant stunted growth (Lepp, 1981; Shaw and Rout, 1998; Orcutt and Nilsen, 2000; Fodor, 2002), biomass is expected to be reduced in plants under heavy metal stress. In accordance with several reports, biomass reduction is a typical impact of heavy metal stress in plants which are intolerant to the respective metals (Prasad, 1997; Orcutt and Nilsen, 2000; Cseh, 2002). Similarly biomass reduction has been reported in Sunflower under lead stress (Kastori et al., 1998). Similarly, Zhang et al. (2000) suggested that cadmium induces biomass reduction in Wheat. Notwithstanding, in *Arabidopsis thaliana*, biomass remained unaltered and this observation is interpreted as a detoxification mechanisms of cadmium stress (Perfus-Barbeoch et al., 2002). *B. diffusa* plants exhibits no significant changes in biomass as the response of growth performances to different concentrations of cadmium, chromium, mercury and lead (Table 4) presumably due to the tolerance towards the selected concentrations. According to Baker et al. (1994), Ebbs and Kochian (1997, 1998) biomass production is a significant factor contributing to phytoextraction of metals by plants from polluted soil/water. Pilon-Smits (2005) suggested that phytoextraction is a process defined

as use of plants to clean up pollutants accumulation in harvestable tissues. Due to the treatment with all the four heavy metals *B. diffusa* showed slight increase in biomass though not much significant, indirectly exhibiting mild phytoextraction potential of the plant towards cadmium, chromium, mercury and lead.

Heavy metal accumulation pattern showed a linear increase of cadmium, chromium, mercury and lead in the tissue such as root, stem and leaf only up to a limited period (12 days). Thereafter, accumulation rate is very slow (Table 5). Analysis of metal concentration increments show highest (cumulative) quantity in the samples of 20<sup>th</sup> day. The quantity and accumulation pattern differed among the metals and are found to be dependent on type of metals thereby exhibiting specificity of individual heavy metals in process of absorption, translocation and accumulation (Table 5).

Cadmium accumulation is maximum in the root tissue of *B. diffusa* and content is 46% of the total available cadmium in the growth medium compared to other metals during 20 days of growth while content accumulated in the entire of the total plant is 92%. These findings confirm that *B. diffusa* can accumulate very high cadmium content without much obvious toxic symptoms indicating high tolerance to cadmium toxicity and this view is in accordance with the characteristics of cadmium such as high mobility in the soil-root-system (Sanita-di-Toppi and Gabbrielli, 1999) and accumulation potential depends on available cadmium content (Cobbett and Goldsbrough, 2002; Guo-Sheng et al., 2007). Nevertheless, Sanita-di-Toppi and Gabbrielli (1999) suggested that metal is taken up with the plants more rapidly from the solution than from the soil. In the present study the plants are cultivated in nutrient solution containing

known quantities of the heavy metals and hence the accumulation potential observed need not be comparable or equivalent to comply with the accumulation pattern in the soil system. Cd<sup>2+</sup> ions are fast mobile in plants. Many plants such as *Potamogeton pectinatus* (Rai *et al.*, 2003), *Arabidopsis thaliana* (Perfus-Barbeoch *et al.*, 2002), *Phragmites australis* (Ederli *et al.*, 2004), *Cannabis sativa* (Linger *et al.*, 2005), *Brassica juncea* (Ishikawa *et al.*, 2006; Szollosi *et al.*, 2009) and *Helianthus annuus* (Zou *et al.*, 2008) are reported as hyperaccumulators of cadmium. High mobility of Cd<sup>2+</sup> have been established in rice plant by adding Ca<sub>2</sub>(OH) to induce more mobility (Kim *et al.*, 2003). The authors suggested that Cd<sup>2+</sup> may substitute Ca<sup>2+</sup> resulting in enhanced cadmium accumulation using Ca<sup>2+</sup> channels for the passage of Cd<sup>2+</sup>.

*Boerhavia diffusa* shows considerable accumulation of chromium in the order root>stem>leaf. Progressive accumulation of chromium with more content in roots (10-200 times) than the shoots have been reported in *Lactuca sativa* (Singh, 2001) and *Nelumbo nucifera* (Vajpayee *et al.*, 1999). *Veronica beccabanga* and several hydrophytes showed high chromium removal from the soil. (Zurayk *et al.*, 2001). According to Kabata-Pendias and Pendias (2001), progressive increase of chromium accumulation occur in the roots and shoots of *Helianthus annuus*, *Zea mays* and *Vicia faba*. This observation is comparable to the chromium concentration pattern of *B. diffusa*.

Accumulation of lead in *B. diffusa* is very high in the root, stem and leaf and these values are maximum compared to other metals. This may provide the plant with better tolerance to heavy metal concentration as suggested by Weis and Weis (2004). Studies on the accumulation of lead in *Typha latifolia* showed maximum lead content in

the root while shoot maintained a low level always (Ye *et al.*, 1997). According to Zheljzkov *et al.* (2006) lead accumulation in *Bidens leonorum*, *Melissa* and *Ourianum* was maximum in the root and the accumulation pattern varied from plant to plant and dependant on the availability of the metal in the soil.

The present study reveals that *B. Diffusa* is a potent bioaccumulator of Cd, Cr, Hg and Pb and the accumulation potential and pattern varies from metal to metal and depend on the availability. Since this plant is an important ingredient of many ayurvedic medicine, accumulation of toxic heavy metals causes serious health hazard to the consumers.

## CONCLUSIONS

Non essential heavy metals such as cadmium, chromium, mercury and lead are highly reactive and interfere the normal metabolism and become toxic to plants generating morphological and physiological alterations and modifications. More or less consistent growth performance was exposed by the plants irrespective of the difference of concentration of the heavy metals. Most of the medicinal plants are herbs which are cultivated or naturally growing in soil, contaminated with heavy metals by natural and anthropogenic activities and the plants accumulate considerable quantities of toxic heavy metals. The metals confined in medicinal plants finally reach food chain leading to health hazard in human and animals and get recycled.

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