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ORIGINAL ARTICLE

Accumulation Pattern of Heavy Metals in *Chromolaena odorata* (L.) King & Robins. Grown in Nutrient Solution and Soil

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Accumulation pattern of Al, Cd, Fe, Hg, Cr, Cu, Pb, Ni and Zn in *Chromolaena odorata* plants grown in Hoagland nutrient solution and soil contaminated with known quantities of the above said metals was investigated. Significant variations in the quantity of accumulation as well as distribution among plant parts like root, stem and leaf were shown between the metals. Accumulation of Pb was maximum in the root followed by Fe and Al. Maximum quantity of each metal was accumulated in the root as compared to stem and leaf. Drastic differences in the accumulation pattern of metals between the nutrient solution and soil culture was observed. Comparatively small quantity of metal was accumulated in the plants of soil despite several fold quantity of each metal was given. The results are discussed in terms of BCF, TF, metal specificity as well as detoxification mechanisms.

Key words: bioaccumulation, Chromolaena odorata, heavy metals, metal specificity

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Higher concentrations of essential and non-essential heavy metals in the soil and water lead to toxicity symptoms and inhibit plant growth. A number of physiological and biochemical processes are also affected by higher concentrations of heavy metals. Some plant species naturally develop various extra and intra cellular mechanisms to tolerate heavy metals and utilize a number of metal specific strategies to combat high external metal concentrations. Common strategies adopted by plants include avoidance which limits the uptake of heavy metals and prohibit their entry into the plant tissue (Dalvi and Bhalerao, 2013), immobilization of metal ions by mycorrhizal association (Jentschke and Bold, 2000) and precipitation of metal ions in the rhizosphere due to the presence of root exudates (Marschner, 1997; Pilon-Smits, 2005).

Plants acquire tolerance towards heavy metals by binding them with pectic sites of cell wall carbohydrates and preventing their intake to the cytosol (Ernst *et al*., 1992), or by active efflux of toxic ions (Axelsen and Palmgren, 2000). Another

well documented tolerance mechanism of plants towards heavy metals is synthesis of phytochelatins (PCs) and/or metallothioneins (MTs) which are the derivatives of glutathione and are synthesized by co-ordination with thiol groups of glutathione (Rauser, 1999; Cobbett, 2000; Goldsbrough, 2000). According to Cobbett and Goldsbrough (2002), the PCs form complex with metal ions and are transported to vacuoles by metal/H^+ antiporters or ABC transporters (Rea *et al*., 1998) thus isolating the toxic metals from the metal sensitive enzymes. MTs are known to play a role in the antioxidant protection and plasma membrane repair mechanism (Goldsbrough, 2000).

Increased synthesis of reactive oxygen species (ROS) like singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) is one of the initial responses of heavy metal stress in plants. Higher concentration of ROS damages cells and at the same time synthesis of antioxidants is induced. A number of ROS scavenging enzymes have been reported to decrease oxidative stress due to heavy metals in plants (Vranova *et al*., 2002; Milone *et al*., 2003; Prasad, 2004; Hossain *et al*., 2012). ROS are known to be synthesized as a consequence of excess heavy metal entry to the plant body and cause lipid peroxidation as well as induction of stress enzymes (Foyer *et al*., 1994; Dietz *et al*., 1999; Fodor, 2002; Hall, 2002; Gill and Tuteja, 2010).

Apart from the above mentioned strategies of plants to cope up with heavy metal stress, bioaccumulation of metal ions as a result of absorption, mobilization/compartmentation, translocation to aerial parts and storage in different organs is another adaptation. Plants those absorb and accumulate metals without showing much toxic symptoms are known as accumulators (Yoon *et al*., 2006; Mganga *et al*., 2011). According to those authors, hyperaccumulators are species capable of accumulating metals at levels 100 fold greater than that typically measured in common metal accumulator plants.

Mature plants inclusive of trees, shrubs, vegetables, grasses and weeds can accumulate high concentration of metal into their above ground parts (Baker and Brooks, 1989). Accumulation pattern of heavy metals in plants vary among genotypes, functional behaviour of metals and potential of the plant to limit the entry of metals to the root, root-shoot translocation and availability of ions in the soil. Bioaccumulation potential of native plants have been evaluated under field conditions because metal uptake is influenced by soil factors including pH, organic matter and cation exchange capacity as well as plant factors (Baker and Brooks, 1989; Alloway *et al*., 1990). However, stress tolerance potential of one and the same plant towards different heavy metals is worth investigating.

Chromolaena odorata is an invasive weed and known to accumulate considerable quantity of mercury (Velasco-Alinsug *et al*., 2005) and this plant is considered as phytoremediant since the Hg ions get complexed with sulphur of cysteine producing cinnabar which is quite stable in the environment. Singh *et al*. (2009) reported the potential of *C. odorata* for phytoremediation of ^{137}Cs (radioactive Caesium) from low level nuclear waste. According to them, when plants were incubated in low level nuclear waste, 79% of the activity was removed by plants at the end of 15d. *C. odorata* has been identified to have potential to accumulate heavy metals particularly Pb and Cd from metal pollution caused by solid waste disposal (Agunbiade and Fawale, 2009). However, *C. odorata* is not a hyper

accumulator of heavy metals despite its worldwide distribution and ability to flourish under various soil/climatic conditions and its adaptation to different environment. Even though *C. odorata* has been reported as an accumulator of Cd, Pb and Hg, the response of this species to different heavy metals supplied in nutrient medium and soil was not undertaken so far. The present study envisages the evaluation of bioaccumulation potential of *C. odorata* plant towards essential and non essential heavy metals such as Al, Cd, Fe, Hg, Cr, Cu, Pb, Ni and Zn by cultivating the rooted propagules in Hoagland nutrient medium as well as soil, artificially contaminated with known quantities of these metals.

MATERIALS AND METHODS

Chromolaena odorata twigs of 10-12 cm length, each with four pairs of unfolded leaves were collected from plants wildly growing in Calicut University (C.U.) campus. Cuttings were treated with Indole butyric acid (IBA 50µM) to induce root initiation and rooted cuttings were transferred to 120 ml half strength modified Hoagland solution (Epstein, 1972) taken in glass bottles (8.5 x 5.5 cm). The hydroponic systems were kept in polyhouse at 27±3ºC and RH 78±3. The propagules thus developed were used for treatment with different concentrations of heavy metals under hydroponic conditions and untreated samples served as control. Heavy metals selected for the study includes aluminium, cadmium, iron, mercury, chromium, copper, lead, nickel and zinc. The tolerable limits of each metal salt were determined based on a series of standardization steps. The heavy metal salt concentration which was the tolerance limit as indicated by the growth of *C. odorata* is given in Table 1.

Another study was undertaken by planting rooted propagules in soil in order to elucidate and compare the bioaccumulation potential of *C. odorata*. Poly bags were filled with 2kg each garden soil: sand: dried powdered cow dung in 1:2:1 ratio. The poly bags were placed in polyhouse of Calicut University Botany Department and maintained under conditions of 27±3ºC and RH 78±3 watered regularly. After acclimatization of the propagules, known concentrations of solutions of selected heavy metal salts were added. By trial and error methods, concentrations of metals were standardized which imparted visible growth retardations (Table 1). Seedlings grown in poly bags without any heavy metal addition served as control.

Samples (root, stem and leaf) were collected at selected intervals of 1st day, 15th day and 30th day for the analysis of each heavy metal using atomic absorption spectrophotometer (Perkin Elmer, Analyst 300) in the digest prepared according to Allan (1969).

RESULTS

Plants grown in nutrient medium showed gradual but significant increase of Al accumulation in the root tissue and more or less the same pattern of accumulation were seen in the stem also though the content was low in the stem and leaf as compared to the root (Table 2, Fig. 1a&b).

In the soil even though 100 times more Al was supplied, the accumulation was comparatively very low. After 30 days of supplying $AlCl_3$ only 1.5 µmol of total supply was accumulated in the root. Stem and leaf also contained only very less quantity of Al. When the data of bioaccumulation was expressed in the percentage of total Al supplied, a gradual increasing trend was shown by the root, stem and leaf during growth. Sixty two percentage (62%) of

the total Al was accumulated in the root after 30 days whereas plant cultivated in the soil accumulated only meagre quantity (0.001%) after 30 days.

Cadmium accumulation in the roots of plant cultured in nutrient solution containing $CdCl_2$ was comparatively lower than Al but during further growth significant increase of Cd occurred (Table 2). Very low quantity of Cd was found accumulated in the stem and leaves but after 30 days Cd content was significantly increased in both these plant parts. Plants grown in the soil, exposed to more than 500 times of Cd than that of nutrient culture showed almost twice the amount of Cd in the root on 30th day than under nutrient medium. But on 1st day the cadmium accumulated was nil. Cadmium content of *C. odorata* plant grown in the soil showed very high (several fold) increase in the stem and leaf as compared to the nutrient culture. Cadmium accumulation percentage also showed an increasing trend and maximum (54%) was accumulated in the root after 30 days in the nutrient medium. But Cd accumulation was less than 0.2% in all parts of soil grown plants.

Roots of plants treated with Fe under nutrient culture, exhibited very high content in the root and during further growth the content was increased significantly (Table 2). Stem tissue showed very low Fe content while leaf contained more Fe as compared to stem. Under soil condition, the Fe supplied was more than 100 times higher than the nutrient medium and the root showed very high content of Fe and an increase was noticed during further growth. Fe content of the leaf was less than the root but compared to the stem, leaf contained more Fe. Accumulation percentage of Fe showed an increasing trend during growth both in the culture medium and soil. Maximum (84%) Fe was detected in the roots under nutrient culture whereas in the soil less than 1% of the total supplied Fe was accumulated in the root. Unlike the non essential heavy metals Fe accumulation was observed in the root, stem and leaf of control plants.

Plants cultivated in the nutrient culture exhibited very low Hg content in the roots and accumulation rate of Hg increased during further growth (Table 2). Mercury content of leaf and stem was comparatively lower than that of the root, whereas in the soil in which more than 500 times Hg was added root showed considerable quantity of Hg after 15 days and more than 6 fold increase was occurred after 35 days. Even though the root and stem exhibited more or less the same pattern of Hg accumulation, leaf showed only negligible quantity. Accumulation of Hg was comparatively low in all plant parts, and root showed about 15% under nutrient culture whereas plants cultivated under soil showed 0.5% of the total supplied Hg. In comparison with stem and root, leaves contained only very low Hg content.

Plants treated with Cr under nutrient culture revealed that considerable amount of Cr was accumulated in the root and linear increase was occurred during 30 days of plant growth (Table 2). Stem and leaf showed only low Cr content as compared to the roots. About 300 times Cr was applied to soil in which plants showed no accumulation on 1st day, but after 30 days very high amount of Cr was detected in the roots. Stem exhibited more than 2 fold increase while leaf contained more than 10 fold increase of Cr content in comparison with that of root. Bioaccumulation of Cr was maximum in the roots (73%) of plants cultivated in the nutrient solution after 30 days whereas only 0.5% Cr was accumulated in the root

under soil condition. Considerable quantity of Cr was found to accumulate in the stem (15%) in the nutrient culture whereas trace quantity was accumulated in the stem of soil culture.

Copper content of roots of plants grown in nutrient medium was comparatively very high even after one day and maximum Cu was present in the samples after 30 days (Table 2). Stem showed very low accumulation and least rates of accumulation of Cu was seen in the leaf. In the soil about 280 fold quantity of Cu was applied compared to the nutrient medium and accumulation in the root started after one day and quantities accumulated was comparatively lower than the nutrient culture. Copper content of the stem was almost equal to that of the root after 30 days whereas leaf contained only reduced amount of Cu. Roots of plants cultivated under nutrient solution contained 74% Cu after 30 days whereas in the soil only 0.1% was accumulated. Considerable amount of Cu was detected in the root, stem and leaf of control plants.

Lead accumulation of the roots of plants under nutrient medium was very high and after 30 days maximum Pb was accumulated in the roots (Table 2). After one day stem showed about one half of the amount accumulated in the root and it was reduced after 30 days. Leaf contained only very low lead content. In the soil Pb accumulation was very low in the roots as compared to the nutrient medium even though 500 times higher Pb was added to the soil. Lead content of stem and leaf was very low. Maximum accumulation of Pb was observed in the case of root tissue in comparison with all other metals (93%) whereas in the soil 0.45% only accumulated in the roots. An interesting observation was significant amount of Pb was found to be accumulated in the plant parts of control plants.

Accumulation of Ni in the roots of plants under nutrient culture was maximum after 15 days followed by a significant reduction after 30 days where as Ni content was maximum in the stem (Table 2). Leaf showed only very low amount of Ni. Roots of plant grown in the soil accumulated very feeble amount of Ni in spite of 500 times higher level of Ni salt applied in the soil. Stem and leaf also showed very small quantity of Ni. Maximum accumulation of Ni was occurred in the root tissue. During growth, accumulation rate of Ni was found to be reduced in such a way that minimum accumulation was found after 30 days. Accumulation of Ni was meagre in the soil grown plant parts and trace quantity was observed in the control plants also.

Accumulation of zinc in the nutrient medium enhanced up to 15 days followed by a big drop on 30^{th} day. The same trend was observed in the leaf also even though the accumulated quantity was less in the leaf as compared to that of root. But in the case of stem, accumulation increase was in a linear fashion so that on 30^{th} day there was a four fold increase compared to that of root. In the soil grown plants, 200 times Zn salt was supplied and the metal content of root, stem as well as leaf increased during successive intervals and there was maximum accumulation on 30^{th} day. Root, stem and leaf exhibited 100, 38 and 300 fold increase respectively in comparison with nutrient medium grown plants. Accumulation of Zn in the root tissue showed a reducing trend as growth advanced to 30 days. Percentage distribution of Zn was very low in all plant parts. Presence of Zn was observed in the control plants also.

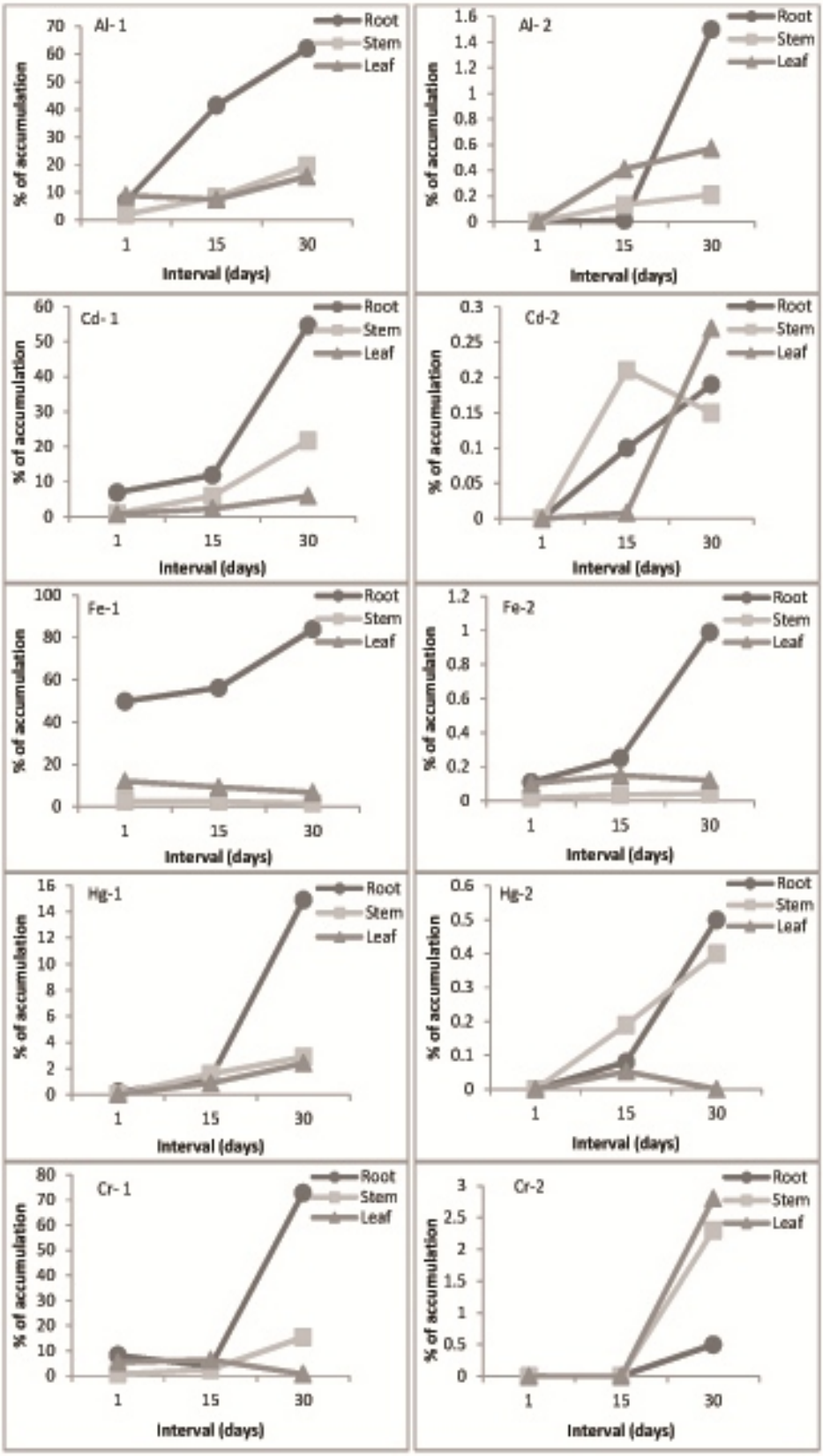

Figure 1a. Percentage accumulation pattern of heavy metals in the root, stem and leaf of Chromolaena odorata. Al_1 to Cr_1= Hoagland, Al_2 to Cr_2=Soil.

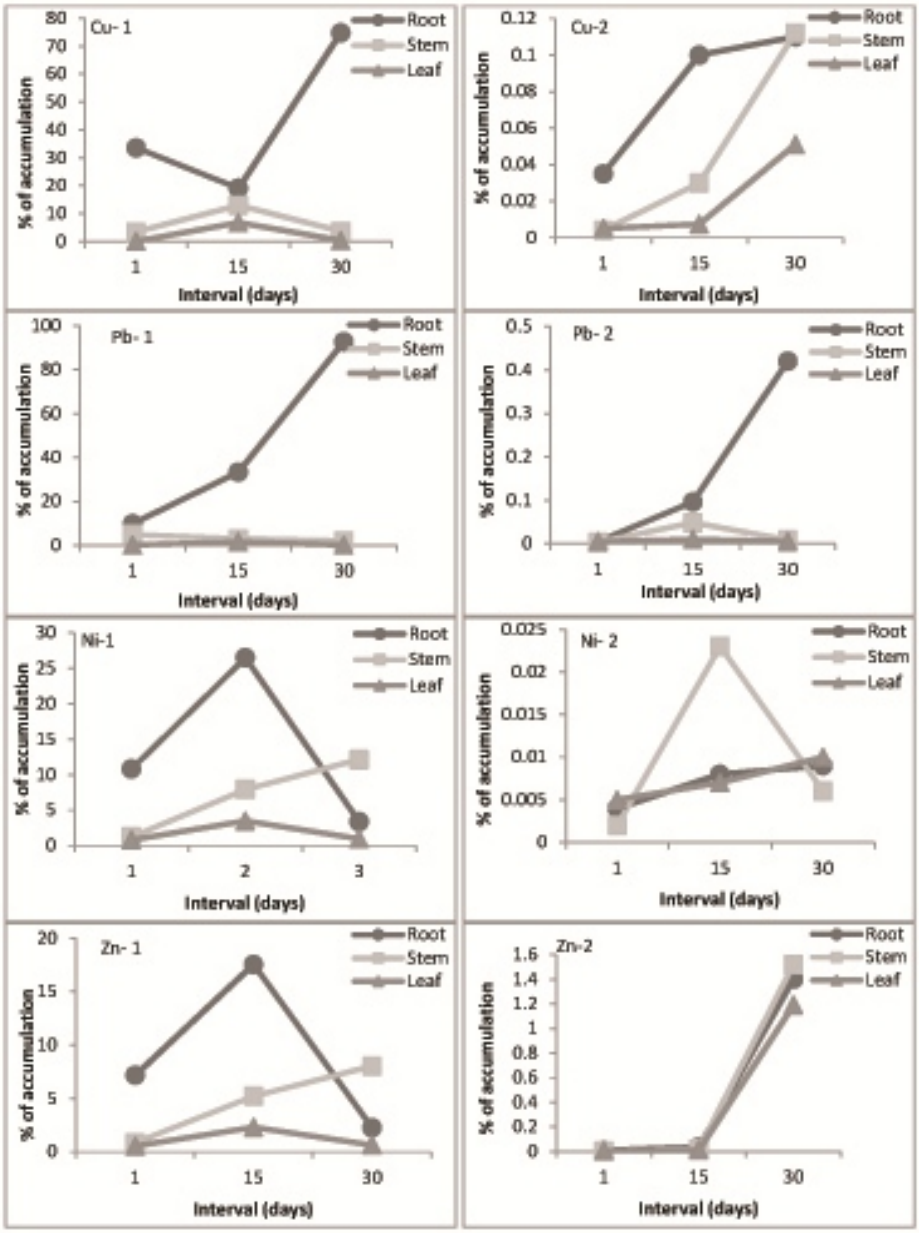

Figure 1b. Percentage accumulation pattern of heavy metals in the root, stem and leaf of *Chromolaena odorata.* Cu_1 to Zn_1= Hoagland, Cu_2 to Zn_2=Soil.

Table 1. Concentrations of heavy metal salts which indicated the tolerance limit of *Chromolaena odorata* propagules grown in Hoagland nutrient medium and soil.

Name of metal	Name and formula of metal salt	Concentration (µM) (Hoagland nutrient)	Concentration (mM) (Soil)
Aluminium (Al)	Aluminium chloride ($AlCl_3$)	1000	100
Cadmium (Cd)	Cadmium chloride ($CdCl_2$)	77	40
Iron (Fe)	Ferric chloride ($FeCl_3$)	900	100
Mercury (Hg)	Mercuric chloride ($Hg\ Cl_2$)	15	8
Chromium (Cr)	Potassium dichromate ($K_2Cr_2O_7$)	140	20
Copper (Cu)	Copper sulphate ($CuSO_45H_2O$)	70	20
Lead (Pb)	Lead acetate $(CH_3COO)_2Pb\ 3H_2O$	1000	20
Nickel (Ni)	Nickel(II) chloride ($NiCl_2\,6H_2O$)	200	20
Zinc (Zn)	Zinc sulphate ($ZnSO_4\,7H_2O$)	250	50

Table 2. Bioaccumulation patterns of heavy metals (µ mol / g dry weight) in *Chromolaena odorata* grown in tolerable limits of heavy metal salts.

Treatments	Plant tissue	Hoagland solution			Soil			
	Interval (days)	1	15	30	1	15	30	control
Aluminium *1000 µmol **100mmol	Root	67.92±3.1 (6.79)	413.5±17.1 (41.35)	619.2±21 (61.92)	NDR	0.6±0.01 (0.0006)	1.5±0.07 (0.001)	NDR
	Stem	20±0.9 (2.0)	83.4±3.5 (8.37)	197.5±8.7 (19.8)	NDR	0.12±0.004 (0.0001)	0.2±0.003 (0.0002)	NDR
	Leaf	89.12±2.5 (8.9)	74.08±2.3 (7.41)	157.2±2.8 (15.76)	NDR	0.4±0.012 (0.0004)	0.56±0.01 (0.0006)	NDR
Cadmium *77 µ mol **40mmol	Root	5.32±0.12 (6.9)	9.12±0.13 (11.86)	41.92±1.5 (54.44)	NDR	40.28±1.7 (0.1)	74.4±2.8 (0.19)	NDR
	Stem	0.64±0.02 (0.82)	4.48±0.16 (5.8)	16.72±0.81 (21.71)	NDR	83.6±2.3 (0.21)	59.8±1.4 (0.15)	NDR
	Leaf	0.56±0.01 (0.72)	1.68±0.05 (2.18)	4.48±0.18 (5.86)	NDR	3.1±0.11 (0.008)	107.6±4.5 (0.27)	NDR
Iron *900 µ mol **100mmol	Root	448.48±10.3 (49.83)	506.32± 6.7 (56.26)	754.69±2.8 (83.85)	112.6±2.7 (0.113)	246±9.3 (0.25)	988± 25.8 (0.99)	5.33±0.04
	Stem	23.84±1.05 (2.64)	22.48± 0.54 (2.49)	15.52±0.2 (1.72)	16.8±0.37 (0.017)	35.2±0.38 (0.035)	41.96± 0.78 (0.042)	0.53±0.01
	Leaf	108.4 ±3.6 (12.03)	83.12± 1.23 (9.23)	60.88±1.7 (6.76)	102.8 ±3.8 (0.103)	153.4±4.5 (0.15)	123.8± 3.7 (0.124)	5.14±0.04
Mercury *15 µ mol **8mmol	Root	0.032± 0.0001 (0.22)	0.184± 0.005 (1.23)	2.14±0.05 (14.27)	NDR	6.4± 0.18 (0.08)	40.2±1.6 (0.5)	NDR
	Stem	0.008± 0.0002 (0.05)	0.232± 0.003 (1.6)	0.42±0.01 (2.9)	NDR	15.6±0.53 (0.19)	31.8±0.9 (0.4)	NDR
	Leaf	0.0032±0.0001 (0.02)	0.128±0.002 (0.85)	0.36±0.009 (2.4)	NDR	4.2±0.08 (0.052)	0.008±0.0001 (0.0001)	NDR
Chromium *140µ mol **20mmol	Root	11.52±0.13 (8.2)	5.12±0.17 (3.66)	102±1.39 (72.84)	NDR	3.4±0.12 (0.008)	201.8±4.7 (0.50)	NDR
	Stem	1.04±0.03 (0.74)	3.52±0.07 (2.5)	21.6±0.31 (15.43)	NDR	2.2±0.07 (0.0054)	927.6±15.8 (2.3)	NDR
	Leaf	7.6±0.08 (5.41)	8.88±0.18 (6.34)	10.4±0.04 (7.4)	NDR	0.2±0.008 (0.0005)	1121.8±34.9 (2.8)	NDR
Copper *70 µ mol **20mol	Root	23.52±0.97 (33.6)	13.36±0.43 (19.08)	52.4±0.85 (74.86)	7±0.87 (0.035)	20.4±0.95 (0.10)	22.8±1.08 (0.11)	2.4±0.09
	Stem	2.4±0.12 (3.42)	9.12±0.25 (13.03)	2.56±0.07 (3.66)	0.82±0.1 (0.0041)	6.4±0.17 (0.032)	22.4±.93 (0.112)	0.296±0.01
	Leaf	NDR	4.72±0.11 (6.74)	0.256±0.01 (0.36)	1.04±0.32 (0.0052)	1.48±0.003 (0.0074)	10.2±0.11 (0.051)	0.352±0.01
Lead * 1000 µ mol **20mmol	Root	98.32±1.87 (9.84)	332.4±10.3 (33.3)	927.9±23.6 (92.79)	0.9±0.03 (0.0045)	19.2±0.32 (0.096)	85±1.8 (0.42)	0.19±0.002
	Stem	48.8±0.98 (4.89)	29.68±.086 (2.97)	20.08±0.67 (2)	0.6±0.02 (0.003)	9.6±0.22 (0.048)	1.74±0.07 (0.0087)	0.144±0.04
	Leaf	1.04±0.02 (0.10)	15.2±0.45 (1.52)	1.2±0.03 (0.12)	0.84±0.03 (0.004)	2±0.09 (0.01)	0.94±0.02 (0.0047)	0.2±0.009
Nickel *200 µ mol **20mmol	Root	21.6±0.98 (10.8)	52.96±2.3 (26.48)	6.8±0.12 (3.4)	0.78±0.02 (0.004)	1.54±0.03 (0.008)	1.82±0.02 (0.0091)	0.24±0.008
	Stem	2.48±0.12 (1.24)	15.76±0.34 (7.9)	24.24±1.02 (12.12)	0.36±0.001 (0.0018)	4.6±0.02 (0.023)	1.14±0.04 (0.0057)	0.08±0.002
	Leaf	1.6±0.05 (0.8)	6.96±0.17 (3.48)	1.92±0.04 (0.96)	1.04±0.01 (0.0052)	1.48±0.01 (0.0074)	2±0.02 (0.01)	0.32±0.005
Zinc *250 µ mol **50mmol	Root	17.92±0.45 (7.17)	43.84±1.2 (17.54)	5.68±0.07 (2.27)	5±0.05 (0.01)	18.4±0.64 (0.037)	700.2±23.9 (1.4)	0.24±0.003
	Stem	2±0.03 (0.8)	13.04±0.35 (5.22)	20.08±0.74 (8.03)	1±0.03 (0.002)	10±0.3 (0.02)	761.6±18.7 (1.52)	0.039±0.001
	Leaf	1.36±0.02 (0.54)	5.76±0.17 (2.3)	1.6±0.04 (0.64)	3±0.05 (0.006)	5.6±0.14 (0.011)	596.2±17.8 (1.19)	0.076±0.002

Values given are mean of 5 replications ± SE, * & **: quantity supplied in Hoagland medium and soil respectively

Table: 3 Effect of Heavy Metals on Bioconcentration (BCF) and Translocation Factor (TF) in *Chromolaena odorata*

Treatments		Hoagland solution			Soil		
		Interval (days)					
		1	15	30	1	15	30
Aluminium	BCF	0.07	0.41	0.62	-	0	0.000015
	TF	**1.61**	0.38	0.57	-	0.87	0.51
Cadmium	BCF	0.069	0.12	0.54	-	0.00026	0.0007
	TF	0.22	0.68	0.50	-	**2.15**	**2.25**
Iron	BCF	0.5	0.56	0.84	0.001	0.0025	0.009
	TF	0.29	0.21	0.1	**1.06**	0.77	0.17
Mercury	BCF	0.002	0.012	0.14	-	0.0008	0.005
	TF	0.35	**2**	0.32	-	**3.09**	0.79
Chromium	BCF	0.082	0.036	0.73	-	0.00017	0.01
	TF	0.75	**2.42**	0.31	-	0.706	**10.15**
Copper	BCF	0.34	0.19	0.75	0.0003	0.001	0.001
	TF	0.10	**1.04**	0.05	0.26	0.39	**1.43**
Lead	BCF	0.098	0.33	0.93	0.00004	0.001	0.004
	TF	0.51	0.135	0.02	**1.6**	0.60	0.03
Nickel	BCF	0.11	0.26	0.034	0.00004	0.00008	0.0009
	TF	0.19	0.43	**3.85**	**1.8**	**3.95**	**1.72**
Zinc	BCF	0.072	0.17	0.023	0.0001	0.0004	0.01
	TF	0.19	0.43	**3.82**	0.8	0.85	**1.94**

BCF= metal concentration ratio of plant roots to medium and TF = metal concentration ratio of plant shoots to roots. Values > **1** are in bold letter

Bioaccumulation factor (BCF) of Al was maximum after 30 days in the nutrient solution whereas in the soil, BCF was negligible (Table 3). Maximum TF value of Al was shown by plants of 1st day in the nutrient culture whereas in soil culture, considerable TF value was shown on 15th day. Maximum TF value was shown by cadmium in the plants cultivated in the soil, whereas the value was comparatively negligible in the case of nutrient culture. BCF of Fe also showed only negligible changes in both the medium, but TF was more in the case of soil culture especially on 1st day. Significantly high TF value was shown by Hg under nutrient culture, but in the soil TF was considerably high. Chromium showed negligible values of BCF in both cultures whereas TF was maximum in the soil (10.15) and in the nutrient culture (2.42). Copper showed low BCF values more being in the nutrient culture as compared to the soil. TF value in many samples of the nutrient culture and soil was maximum in the 30th day samples. BCF value of Pb in the plant samples grown in nutrient solution was more compared to that of the soil grown, whereas TF value was significantly high under soil culture. Nickel showed BCF factor similar to that of Pb, but TF value was high in the plants of nutrient medium on 30th day whereas in the soil TF value was comparatively higher in all the intervals. BCF value of Zn was meagre in plant samples grown in both the media whereas TF value was significantly higher on 30th day under nutrient medium as well as soil.

DISCUSSION

Response and accumulation pattern of *C. odorata* grown in Hoagland solution towards Al, Cd, Fe, Hg, Cr, Cu, Pb, Ni and Zn varies considerably with wide range (15-1000µM) of metals given to impart comparable/similar visible growth retardation (about 50%). Accumulation is a function of uptake capacity and intracellular binding sites and the steps involved are absorption from soil, compartmentation and distribution in aerial parts.

Metal uptake pattern by roots in *C. odorata* shows a linear increase during growth period specifically in the case of all non essential toxic metals-Al, Cd, Hg, Cr and Pb (Table. 2, Fig. 1a&b). Accumulation of essential metals Fe and Cu also showed linear increase in the roots and stem during growth. Accumulation of trace metals Ni and Zn in the root was significantly reduced to about 2.5% after 30 days of growth whereas in the stem linear increase was occurred. These observations revealed that even though Al, Cd, Cr, Hg and Pb are the non essential elements they are absorbed and translocated to the stem and finally reached the leaves. The quantity of heavy metal salts given to the plants grown under nutrient culture varied significantly since these concentrations were selected after standardizing the level to impart about 50% visible growth retardation i.e., plants showed inhibitory effects but survived in those concentrations.

When the concentration rate was expressed as the percentage of metal applied to the medium, Al and Cd showed about 95% and 83% respectively, maximum retaining in the root system. Al and Cd come under redox inactive group of heavy metals (Hossain *et al*., 2012) and cause oxidative stress resulting in interaction with antioxidant defence system at a reduced level compared to redox active metals like Fe, Cu, and Cr which are highly involved in the formation of ROS (Dietz *et al*., 1999). Accumulation Percentage of Fe, Cu and Cr were 85%, 78% and 73% respectively in the root system which indicated comparatively reduced translocation to shoot and resultant toxicity is comparatively reduced coinciding with very low TF values (< 0.3 each). According to Qureshi *et al*. (1995), Wheeler and Power (1995) plants minimize the adverse effect of heavy metals by regulating the distribution and commonly much higher amounts of heavy metals are found in the plant roots. In *C. odorata* root system shows maximum amount of all metals in the roots in general and Al, Fe, Cu and Pb in particular. Notwithstanding, considerable quantities of Fe, Cu, Ni and Zn in the root tissues, and are found to be translocated to stem and leaf (Table 2) because they are essential nutrients for plants.

Absorption and translocation of bioavailable heavy metals to the plant body take place either through the symplast or apoplast depending on the type of the metal. Most of the heavy metal ions enter plant cell by energy dependent process via specific or generic ion carriers or channels (Taiz and Zeiger, 2002). But detection and characterization of these carrier channel protein have been elucidated mainly for essential elements. Perfus-Barbeoch *et al*. (2002) proposed that Cd^{2+} enters the cytosol via calcium channels by conducting patch-clamp studies with *Vicia faba* guard cell protoplasts.

In the soil, in spite of several fold quantity of metal as compared to that of the nutrient culture have been added, concentration in the root, stem and leaves was very low. It seems that absorption and translocation rate is restricted and/or the entry is reduced. One mechanism (avoidance strategy) of lessening the entry is either by precipitation or by complexing of heavy metals in rhizosphere, increasing the pH or excreting anions such as phosphates (Bubb and Lester, 1991). Pinto *et al*. (2008) reported that exudation of malate in sorghum and citrate in maize reduced cadmium uptake. The efficiency of root exudates to bind with heavy metals and thereby preventing the absorption is an important mechanism of stabilizing the heavy metal in the vicinity of the root thus making them unavailable to the plant. In *C. odorata*

more or less a similar mechanism is presumed to occur when plants are cultivated under soil conditions where more than 100, 500,100 times higher Al, Cd and Fe respectively are added compared to the nutrient culture.

Accumulation of Al, Cu, Pb and Ni in the roots of *C. odorata* cultivated in the soil is comparatively lower than the accumulation in the roots of plants grown in nutrient solution in spite of the several fold higher amount of these metals added to the soil. This observation reveals difference in the pattern of heavy metal uptake between culture medium and soil on one hand and between the metals on the other. Plants adopt some exclusion mechanism to avoid/reduce the uptake of heavy metals from the soil by precipitation with root exudates and this precipitation process varies for metal to metal (Delhaize *et al*., 1993; Huang *et al*., 1996).

Another mechanism for the survival of *C. odorata* plants under soil containing very high amount of these toxic metals is found to be related to detoxification. *C. odorata* plant grown in the soil exhibit tolerance to all the metals. The survival of *C. odorata* at comparatively higher concentrations is an indication of this developed tolerance. Such tolerance is acquired either by the plants' ability to exclude them in the roots or by the potential to detoxify within the plant (Rellen-Alvarez *et al*., 2006). On the contrary, plants under nutrient medium are devoid of such detoxification mechanism and the plants are directly exposed to the freely available toxic metal ions in the medium and hence toxicity symptoms are expressed at very low levels of concentrations.

Enhanced accumulation of all metals except Ni and Zn in the roots compared to stem and leaf of nutrient and soil media indirectly indicate that a large fraction of these metals are accumulated in the apoplastic space of the root and this process appears to be an exclusion method as suggested by Tice *et al*. (1992) in wheat under Al stress. Exclusion of metals in general and Al in particular, from the roots has been reported in plants (Kochian, 1995). Osawa and Matsumoto (2001) found that in wheat, malate efflux from root resulted in precipitation of Al and induced inhibition of absorption. Similarly oxalic acid secretion from root tips of buck wheat showed precipitated Al within 30 minutes after exposure to Al (Ma *et al*., 1997). Accumulation of Al is very low and absorption rate is very slow to the roots and resultant toxicity also is comparatively lower. According to Jamal *et al*., (2006), though Al is not an essential metal at low concentration it promotes growth due to slow uptake.

Accumulated Al in the root tissues in *C. odorata* grown in soil shows the least value compared to all other metals (Table 2). So exclusion of Al uptake due to the interference of organic acids of root exudates cannot be ruled out in *C. odorata* plants. Values of BCF and TF of Al accumulation also agrees with the above views. Difference in the distribution of the heavy metals due to comparatively very low or meagre amount of accumulation in the stem and leaf except Al and Cd is suggestive of a tolerance mechanism by excluding the toxic metals from the sensitive metabolic activity in shoot system and in this context *C. odorata* is found to be an accumulator of Al and Cd because 97% and 82% of the total Al and Cd respectively in the nutrient solution is found to be accumulated. In the plant and under soil condition comparatively more Cd is accumulated in the leaf and an increased TF value coincide with the translocation of more Cd to the shoot.

An interesting observation is the accumulation

of exorbitant amount of Fe in the roots (85% of the total) in the plants of nutrient culture and in the soil condition also roots accumulated about 8 times higher Fe in the stem/ leaf. Chen *et al*. (1980) and Liu *et al*. (2008) reported that iron plaque formation due to O_2 and oxidants in the rhizosphere in rice plant roots and the plaque absorb and sequester toxic ions like Cd. So it can be speculated that Fe ions are involved in the plaque formation in the roots of *C. odorata* thereby avoiding more translocation to the shoot system.

Chromolaena odorata plants are comparatively more sensitive to Hg and Cu since toxicity symptoms are shown at 15 µM and 20 µM respectively and these metals showed more translocation and hence TF values are >1 after 15 days of growth though more absorption of the roots followed during further growth. Maximum sensitivity to the stress due to Hg and Cu is found to be due to easier and enhanced translocation to the shoots where metabolism gets impaired. Drastic difference occurs in the accumulation pattern of Hg between the leaves of *C. odorata* given in the nutrient and soil media. Hg^{2+} ions are known to volatilize from the leaves through trichomes in *Bacopa monnieri* (Hussain *et al*., 2010; Hussain and Nabeesa, 2012). In *C. odorata* the mechanism of phytovolatilization do occur in the leaves under soil since <0.0001% in accumulated in the leaves. But in the leaf of plants under nutrient culture, significantly high amount is retained within the leaf plausibly due to the lack of detoxifying mechanism in the nutrient culture.

Nickel is considered as a non essential metal for plant growth. However recent literatures suggest that Ni is an essential element needed for healthy plant growth and get easily transported from roots to shoot and it is a key element for several enzymes and (Khan and Moheman, 2006). *C. odorata* is found to be sensitive to very low content of Ni, and absorption and translocation take place very fastly. At some intervals, more Ni is accumulated in the shoot and TF values are comparatively higher in both growth media. Accumulation of trace metals Ni and Zn also is unique and more or less uniform. Accumulation of these metals are more with shoot showing TF values >1 by plant of nutrient culture and soil.

Hyper accumulation potential of *Thlaspi sp.* toward Cd and Zn (Brown *et al*., 1994), were already reported. The best known Pb hyperaccumulators are *Thlaspi caerulescense, Thlaspi rotundifolim* and *Armeria martima* (Baker and Brooks, 1989). All these observations have unequivocally proved the absorption and translocation of those metals by plants under natural and artificial conditions exhibiting wide specificity among them giving the significant variation in the mode of accumulation/distribution among plant parts. *C. odorata* is not a hyper accumulator of Al, Cd, Fe, Hg, Cu, Cr, Pb, Ni, and Zn. But considerable quantities of all the metals get absorbed and accumulated in this species.

Sensitivity and distribution pattern of essential metals Cu and Zn reveals that *C. odorata* showed tolerance to 250 µM Zn while optimal concentration of Cu was 70 µM. But Cu accumulation was more particularly in the roots compared to Zn accumulation. Zinc ions are known to interact with other mineral nutrients (Boardman and McGuire, 1990) and comparatively low accumulation potential of Zn is presumed to be due to interaction with other metals in the nutrient media in particular.

Bioaccumulation of Pb also is unique among the 9 metals investigated in the present study.

Maximum Pb content is accumulated in the root and leaf. Values < 0.02 of TF are shown by plants of nutrient and soil media indicating low mobility of Pb from root to shoot. Similar observations have been reported in *Phyla nodiflora, Bridens alba and Rubrus fruticosus* growing in metal contaminated soil (Yoon *et al*., 2006) and many wetland plants of mine tailings (Stoltz and Greger, 2002). A unique of observation of Pb in the control plants probably due to the soil contamination of the C.U. campus. Heavy traffic with vehicles using gasoline (probably leaded) for years together might have led to the accumulation of considerable amount of Pb in the soil of C.U. campus.

Translocation factor of Cr to *C. odorata* plants is different from other metals. Maximum TF values are obtained in the plants under nutrient medium after 15 days while in the soil TF value is > 10 revealing very low Cr in the roots. According to Pulford *et al.* (2001), Cr is poorly translocated to aerial parts and held predominating in the roots. Roots are the primary site of metal injury and metal accumulation. Higher uptake of Cr (VI) has been reported in *Nymphaea alba* (Vajpayee *et al*., 2001) and in mung bean (Panda and Choudhury, 2005). According to Barcelo and Poschenrieder (1997), uptake of Cr (VI) in cells takes place via membrane transporters (sulphate carriers).

C. odorata plants are comparatively more tolerant to the non essential elements Al and Pb since the plant exhibit toxicity symptoms at a concentration of 1000 µM each, whereas mode of bioaccumulation do vary among the two metals in such a way that translocation of Al is more, showing comparatively higher TF values and 95% Pb is retained in the root after 30 days of growth in the nutrient medium and comparatively more Pb in the roots of plants cultivated in the soil also.

Accumulation pattern of all the 9 metals investigated using simulating experiments, exposing the plants to individual metals differ significantly. Essential element Cu exhibit very high absorption and translocation. With regard to the mechanisms involved in Cu uptake, the participation of carrier/channel protein of Cu^{2+} (P-type Cu-ATPase) is presumed to occur (Harris, 2000). Similarly nonessential metals particularly divalent Cd and Pb also get accumulated in fairly high amounts in *C. odorata*. In this context, the channels/ carrier proteins of Cu^{2+} may be utilized by Cd^{2+} and Pb^{2+} as already reported by White (1998) and Perfus-Barbeoch *et al.* (2002) according to whom in wheat root Cd^{2+} get their entry via Ca^{2+} channels by mimicking Ca^{2}. More or less similar mechanism is presumed to occur in the accumulation pattern of Fe^{3+} and Al^{3+} ions since the former is an essential element, provided with carrier proteins, and the same path may be followed by the latter also.

BCF values are the ratio of metals accumulated in the root to that of the soil and these values are not relevant to the present study because only known (limited) quantities are given to both the growth media. Nevertheless, a comparison of BCF values between the elements employed in the present study enables to assess the significant variation between the absorption and translocation rates among them.

Chromolaena odorata show significant difference in the potential to accumulate and the order of accumulation in the nutrient solution is Pb > Fe > Cu > Cr > Al > Cd > Hg > Ni > Zn in the root in terms of percentage distribution. In the soil the order is Zn > Fe > Cr > Hg > Pb > Cd > Cu > Ni >Al. The wide range of variation indicate difference in the mode of absorption and translocation of each metal on one hand and the strategy adopted for

detoxification and metal tolerance on the other under different conditions of growth media.

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