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Heavy metal tolerance of weed species and their accumulations by phytoextraction

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Abstract

The ability of the invasive weed species *Parthenium hysterophorus* L. for the accumulation of the heavy metal Cd was studied in a greenhouse experiment. This study aimed at identifying a metal tolerant species from natural vegetation and to assess the phytoextraction potential of the plant. To compare metal concentration in the aboveground biomass to those in roots and in soils,To study their effect on growth and comparison with metal amended soils treated with EDTA. The Cd accumulated by test species was increased significantly after 0.1g / Kg of EDTA was added to the medium. An increase in metal uptake with increase in test concentrations during the early stages and developmental stages was observed which indicates the plant's tolerance to the heavy metal. But a decrease in metal uptake with the maturity of the plant was observed. This indicates that the plant's ability for the metal uptake reduces with time i.e. with maturity. The BCF's of the shoots and roots and TF's being >1 shows the validity of the weeds species for hyperaccumulation of the metal Cd. This is due to the plant's capability to uptake the metal and its tolerance capacity for the heavy metal Cd. Hence, it can be a promising species for phytoextracton of heavy metals and remediation of contaminated soils which is economical and ecofriendly.

Keywords: Phytoextraction, *Parthenium hysterophorus* L., cadmium, BCF, TF.

Introduction

Plants have been frequently used in the search of metal pollution or as accumulators for soil remediation called 'Phytoremediation' which is a cost effective, environmentally friendly, aesthetically pleasing approach most suitable for developing countries (Baker *et al.,* 1994). Phytoremediation, is an emerging technology that employs the use of higher plants for the clean up of contaminated soils, water and atmospheric pollutants. Phytoextraction is gaining great attention as alternative technique for remediation of heavy metal contaminated soils. This is one of the strategies of phytoremediation which employs the uptake and accumulation of metals into plant shoots, which can be harvested and removed from the site. It is necessary to use plants that can tolerate high levels of heavy metals. Plants called hyperaccumulators are preferred for metal decontamination of soils because they take up 100 times the concentration of metals over other plants (Cunningham *et al.,* 1995). They accumulate toxic metals through their roots and transport them to the stems / leaves. These species are used on many sites due to their ability to thrive in highly polluted areas. Once the plants have grown and absorbed the metal could then be recovered and recycled when burned and the ash collected (Comis, 1996). This process is repeated several times to reduce contamination to acceptable levels.

Weed species appear to be a good choice for metal accumulation studies since these hardy, tolerant species can grow in most harsh conditions over large areas and give a good amount of biomass as a secondary product. In most of the contaminated sites hardy, tolerant, weed species exist and phytoremediation through these and other non-edible species can restrict the contaminant from being introduced into the food web. Hence, an attempt was made to identify a metal-tolerant species from natural vegetation in field sites that are contaminated with various heavy metals. Recently, chelating agents have been used as decontaminants in metal-polluted soils facilitating phytoextraction. Early studies showed that application of synthetic metal chelates such as ethylene diamine tetra acetic acid (EDTA) to soils enhances Pb accumulation by plants (Jorgensen, 1993). EDTA was particularly effective in facilitating the phytoextraction of Cd, Cu, Ni, Pb and Zn by plants. EDTA acts by complexing soluble metals present in soil solution. In view of such diverse reports, an attempt was made to examine the enhancement of metal uptake by the test species. The present study was conducted using phytoextraction to explore the accumulation and removal potential of the heavy metal Cd by an invasive weed species *Parthenium hyterophorus* L. belonging to the family Asteraceae wildly grown over large areas in Visakhapatnam city. The test species is one the most popular and dominant indigenous weed species which is well adopted to the polluted environment as affected by the various activities which can accumulate significant amounts of heavy metals. The objectives of the present work were to identify a metal-tolerant weed species from natural vegetation and assess the phytoextraction potential of the plant, to determine the concentration of Cd in soils and plant biomass, to examine the translocation capacity of the plant and also the bioconcentration factor of shoots and roots, to investigate the effect of the metal Cd on the plant growth and the plant's ability to accumulate and tolerate the metal by phytoextraction.

Materials and methods

A green house study was carried out in a n artificially amended sandy soil (Typic Ustochrepts) with different concentrations of 10 (TC1), 50 (TC2) and 100 (TC3) mg/kg Cd $(NO_3)_2$. $4H_2O$.Some selected soil characteristics are as follows: pH (1:2) 8.02 in deionised distilled water; EC 0.116ms; organic carbon 0.41%; calcium carbonate 1.05% and no extractable cadmium was observed. The pot culture experiments were conducted using soil treated with (spiked) cadmium nitrate salt and for comparison an unamended (control). The salt was uniformly mixed with air-dried soil filled in pots (6 kg soil) and left for stabilization for 2 days. One week old seedlings of the weed species *P. hysterophorus* wildly grown in the farmhouse were procured and 5 plants were transplanted in each pot; out of which only 3 were allowed to grow at a uniform distance after survival was confirmed. Another two sets of pots of each treatment were maintained for EDTA application. 0.1g/kg of EDTA in solution form was added at pre-flowering stage (20 d from the day of survival) to examine the EDTA effect on metal uptake. Three replicas of each treatment were considered and care was taken to protect from rain water leaching. Plants were grown under natural conditions of the environment and watering was done at regular intervals with well water. For each concentration 9 plants were harvested at a time interval of pre-flowering (20 d), flowering (40 d) and post flowering (60 d) from 3 replicate pots, without damaging the roots. EDTA treated pots were harvested at an interval of one week (I-scoring) and two weeks (II-scoring) from the day of addition EDTA solution. The soil samples were then dried in the open air, crushed, passed through a 2mm diameter sieve, mixed and analyzed for Cd. Plant samples with maximum recoverable portion of roots were procured and then taken to the laboratory, washed with deionised distilled water, to remove dust and soil mineral particles, separated into root and shoot (including stem, leaves, fruits & inflorescence) parts, dried in an oven at 65-70°C for 72 h (Sampanpanish *et al.,* 2008; Xian and Shokhohiford, 1989). All calculations of Cd and extraction were done on dry basis. The air dried ground, sieved soil samples and the oven-dried ground plant materials were subjected to mixed acid digestion for analysis of the total heavy metal content, the available metal was extracted using acetic acid (2.5% in 1:1 w/v) and the soil pH was measured in 1:2 of soil to water ratio using Systronics digital pH meter (Stewart *et al.,* 1974). Both the total and available metals were determined using Atomic Absorption Spectrophotometer (AAS ZEE nit 700 # 150 Z 70222) (Stewart *et al.,* 1974). The results obtained were statistically analyzed and calculated as a dry mass. Mean values and SE of three replicates of the element content were calculated.

Results and discussion

Cadmium content in root has shown an increase with increase in soil Cd excepting on day-60 where there was an increase from TC1 to TC2 and then decreased from TC2 to TC3. The value in TC1 soils on day-20 was 7.5µg/g, which decreased by day-40 to 5µg/g and has increased rapidly to 15.4µg/g on day-60. The value in TC2 soils on day-20 was 32.82µg/g, which has rapidly declined by day-40 to 25µg/g and thereafter increased to 32.5 µg/g on day 60. The value in TC3 soils on day-20 was 55 µg/g, which has shown a negligible increase by a decimal i.e., to 55.22 µg/g by day-40 and thereafter decreased rapidly to 8.25 µg/g on day-60 (Fig.1a-c). Control was less than all test concentrations on all harvests. In EDTA treatment, the values have shown an increase with increase in soil Cd on both harvests. The value in control was 0.5 µg/g on I harvest which has increased to 2.5µg/g by the II harvest in control soils. In TC1 soils, the value has shown a negligible increase from 7.5 to 7.6 µg/g and in TC2 soils, value has rapidly increased from 37.5 to 50 µg/g. It has increased from 52.5 to 57.5 µg/g in TC3 soils (Fig.1d & e). Control was less than all test concentrations on both harvests. Cd content in shoot has shown an increase with increase in soil Cd on all harvest days including EDTA treatment. The value in TC1 soils on day 20 was 7.5µg/g, which has decreased by day 40 to 5.2 µg/g, which has stabilized showing 5.2 µg/g on day-60 (Fig.1a-c). The value in TC2 soils on day-20 was 17.5µg/g, which has rapidly decreased by day 40 to 15 µg/g and thereafter decreased to7.5 µg/g on day-60. The value in TC3 soils on day 20 was 32.5 µg/g and it has slightly decreased to 15 µg/g by day 40 and stabilized to 31.2 µg/g on day 60. Control was less than all test concentrations on all harvests excepting on day 20 where the values were similar. In EDTA treatment, Cd content of control and test concentrations has shown stabilization in values excepting TC2, which has decreased by II harvest. The control has decreased by II harvest. An increase with increase in soil Cd in test concentrations on both harvests is observed. The value in control was 2.5 µg/g on I harvest which has stabilized by the II harvest in control soils. In TC1 and TC3 soils, the value has stabilized. It has decreased from 35.35 to 32.5 µg/g in TC2 soils (Fig.1d & e).

The mean shoot length of all the test concentrations increased with time. In shoot dry matter, of the total biomass (dry weight) of the above ground parts, around 70% was contributed by leaves. The same trend was observed on all harvest days in all the test concentrations sets. The mean root length of all the test concentrations has also shown an increase with time. The results indicated that the metal content in the root

Fig. 1a-e. Cadmium accumulations in soils shoots and roots of Parthenium hysterophorus on different harvest days including EDTA treatments.

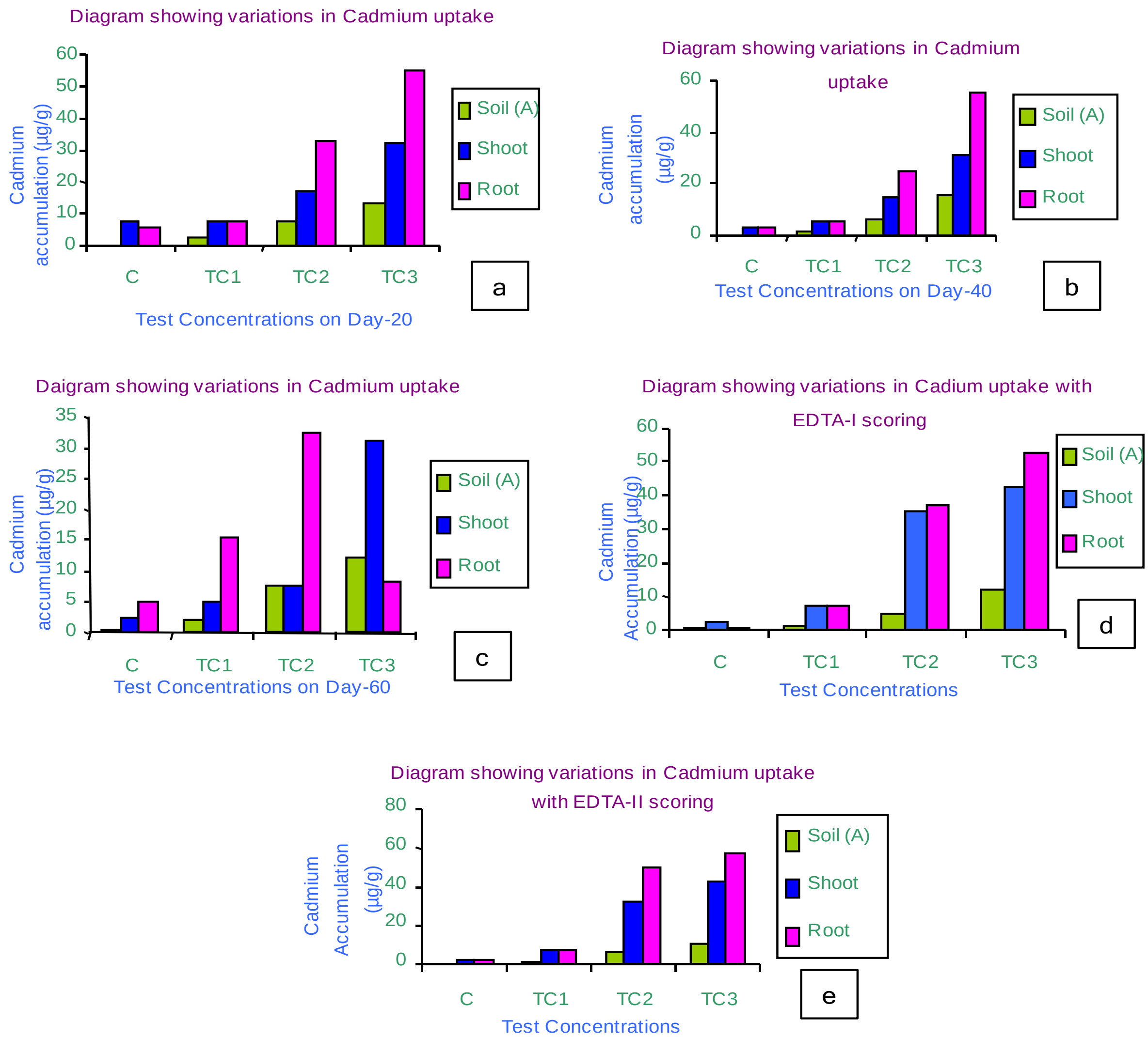

Table 1. The shoot and root bioconcentration factor (BCF) and translocation factor (TF) in different test concentration on different harvest days without EDTA treatment.

Ratio	Harvest days											
	20 Days				40 Days				60 Days			
	C	TC1	TC2	TC3	C	TC1	TC2	TC3	C	TC1	TC2	TC3
BCF (S)	75	3.57	2.24	2.41	16.67	3.45	2.78	2.06	8.33	2.38	1.0	2.6
BCF (R)	53	3.57	4.21	4.1	16.67	3.33	4.63	3.61	16.67	7.36	4.33	0.69
TF	1.41	1.0	0.53	0.6	1.0	1.04	0.6	0.57	0.5	0.32	0.23	3.78

(S)=Shoot, (R)=Root

Table 2. The shoot and root bioconcentration factor (BCF) and translocation factor (TF) in different test concentration on different harvest days with EDTA treatment.

Ratio	Harvest days of EDTA treatment							
	Scoring - I				Scoring - II			
	C	TC1	TC2	TC3	C	TC1	TC2	TC3
BCF (S)	8.33	5	7.86	3.54	8.33	8.33	5.16	4.05
BCF (R)	1.67	5	8.33	4.38	8.33	8.44	7.94	5.48
TF	2.5	1	0.94	0.81	1.0	0.99	0.65	0.74

(S)= Shoot, (R)= Root

dry matter has shown no inhibitory effect with time as expected because its effect should decrease with time. The EDTA application did not produce an important reduction of shoot growth while it increased the concentration of the metal Cd with EDTA caused decrease in root and shoot dry matter.

The available Cd in all the test concentrations showed an increase with increased soil Cd including EDTA treatment. The mean values of test concentrations decreased from day-20 to 40 and then increased by day 60 excepting which increased from day 20 to 40 and thereafter decreased by day 60 (Fig.1a-c). Whereas in EDTA treatment, the values of TC1 and TC3 have decreased and in TC2 they increased by II harvest. The maximum Cd content was observed in TC3 soils on all the harvest days including EDTA treatments. The mean values were 13.5 µg/g on day 20, 15.3 µg/g on day 40, 12 µg/g on day 60, 12 µg/g on I harvest and 10.5 µg/g on II harvest of EDTA treatment. The control was less than all the test concentrations on all harvest days including EDTA treatment (Fig.1d & e).

The total Cd in all the test concentrations has increased rapidly with increased soil Cd including EDTA treatment excepting on day 60 where there was a decrease with increased soil Cd. The mean values of test concentrations have shown decrease from day 20 to 40 and thereafter increased by day 60 excepting TC3 soil which showed stabilization by day 40 and then showed a drastic decrease by day 60 (Fig.1a-c). The maximum Cd content in test concentrations was observed in TC3 soils on all the harvest days including EDTA treatments excepting on day 60 where TC1 shown highest value. The mean values were 42.5 µg/g on day 20 and 40 and 35 µg/g on day 60, 35 µg/g on I harvest and 32.5 µg/g on II harvest of EDTA treatment. The control was less than all the test concentrations on all harvest days including EDTA treatment (Fig.1d & e).

In *P. hysterophorus* with Cd amendment metal concentration was found to be higher in higher treatments and the biomass increased in TC2 soils during developmental stages i.e. day 20 and 40 as also observed in *Plantanus occidentalis* (Roger *et al.,* 1977). The dry matter at higher doses decreased which may be due to increase in certain nutrients as also reported earlier (Roger *et al.,* 1977). The present study has shown variations in accumulation of metals in the plant species. This could be due to differences in the mechanisms of uptake and sequestration of this metal in different plant systems which were also observed in previous studies (Clemens, 2001). However, the plant has the ability to thrive on the test metals contaminated sites. A significant increase in shoot and root concentrations of Cd with increase in test concentrations in the medium shows a positive response of the test species excepting in *L. camara* for its accumulation (Wei *et al.,* 2004). The plant tolerance for Cd was evident in form of increase in plant height and total biomass of the plant. The plants in all the concentrations grew till maturity. An increase in plant height and plant biomass with respect to time was positive excepting in EDTA treatment where shoot and root dry matter have decreased with increased Cd concentration. The increase in biomass was highest during developmental stage, i.e., from day 20 to 40. There was an increase in uptake with increase in test concentrations which is in agreement with earlier studies (Su and Zhu, 2005). The highest uptake is observed in TC3 in both shoot and root with a few exceptions. In EDTA treatment also the highest uptake was observed in TC3 for both shoot and root on both the scorings with very few exceptions.

Early studies showed that application of synthetic metal chelates such as EDTA to soils enhances Pb accumulation by plants (Jorgensen, 1993). The EDTA application did not produce an important reduction of shoot growth while it increased the concentration of the metal Cd with EDTA caused decrease in root and shoot dry matter. A significant increase in shoot and root concentrations of the heavy metal Cd with increase in test concentrations in the medium shows a positive response of the test species for its accumulation (Wei *et al.,* 2004). The concentration of metal accumulated by the test species was increased significantly after 0.1g/kg of EDTA was added to the medium. This is in agreement with the findings in literature (Turgut *et al.,* 2004). While most plants show toxicity symptoms at high Cd accumulations, *P. hysterophorus* accumulated 42.5 ppm in shoot and 57.5 ppm in roots without showing any toxicity as was also observed in *A. sterils* (77ppm) and *I. Tinctoria* (93 ppm) (Felix *et al.,* 1999). Hyperaccumulators have been found that contain more than unity 100 mg/kg Cd shoot biomass (Baker and Brooks, 1989; Baker *et al.,* 1994). The values in plants are several folds higher than the concentration of the metal in the soil and the results are in good agreement with the literature (Joonki *et al.,* 2006). Successful phytoextraction requires plants capable of producing high biomass while accumulating large amounts of contaminants in the biomass from the soil as was also true in the present study (Raskin and Ensley, 2000). Metal ability is depicted as bioconcnetration factor (BCF) which provides an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil substrate (Zayed *et al.,* 1998). It was determined as the ratio of $[Metal]_{shoot}$ / $[Metal]_{soil}$. The TF gives the shoot to root metal concentration and depicts the ability of the plant to translocate the metal species from roots to shoot at different concentrations. It was determined as ratio of $[Metal]_{shoot}$ / $[Metal]_{root}$. It describes that metal in shoot should be >1 i.e TF should be >1 for an accumulator plant (Jorgensen, 1993).The shoot/root of contaminant in all confirmed hyperaccumulators >1 whereas it is invariably <1 for non-accumulators.

The increase in BCF with time was in agreement with literature (Ghosh and Singh, 2005). The translocation property described that the content of heavy metals accumulated in shoot (including stems and leaves) of a plant should be higher than those in its roots, i.e., TF>1. On the basis of our work, endurance property and enrichment factor property should be considered as judging standards of hyperaccumulators too (Zhou *et al.,* 2004, Ma *et al.,* 2004 and Wei *et al.,* 2004). Several workers (Ghosh and Singh, 2005; Tu *et al.,* 2002; Yoon *et al.,* 2006; Shuhe, 2005; Mathe and Anton, 2005) have identified hyperaccumulators of different metals with BCFs greater than one. For e.g.,*Phyla rodiflora* (L.)-6.3 in roots and *Cyperus esculentus* L.-1.1 for Zn, *Solanum nigrum* L.-2.21 in shoots for Cd, 3.63 in shoots(pot studies) and 2.76 in roots (pot studies), *Pteris vittata*- 55 in roots for As (pot), *Ipomoea carnea*- 2 for Cr (pot studies), *Dhatura innoxia*- 1.58 for Cr (pot studies), Willow-3.4 in shoots and 3.12 in roots for Cd, 1.23 in shoots and 1.03 in roots for Zn and Maize-0.39 in shoots and 2.94 in roots for Cd.

The plant species (Das and Maiti, 2007) showing TF greater than one are *Ammania baccifera, Fimbristylis dichotoma, Pycreus flavidus, Typha latifolia and Echinocola cholona* (1.70, 1.45, 2.71, 2.71 & 1.3 for Mn). *Pycreus flavidus* shown a TF of 2.46 for Zn. Heavy metal tolerance species with high BCF and low TF can be used for phytostabilization of a contaminated site together with the vegetative cover (Yoon *et al.,* 2006). The examples of such plants include *G. pennilliana* for Pb, Cu and Zn, *B. alba* for Pb. The results of the present study are in agreement with these results. The accumulator species such as *Brassica* species and other accumulator crop species required special care in field applications. Whereas weeds in the present study required no care to grow, this is a practical problem that will require care in filed applications. The BCF's of shoots and roots and TF's being more than unity (with few exceptions) showing its validity for hyperaccumualtion of the metal can be a promising species for phytoextraction of heavy metals and remediation of metal contaminated soils.

Conclusion

The ability of the plant to accumulate abnormally large quantities of the contaminants from the soil into the above ground biomass, which is known as hyperaccumulator is true in this case. In most of the contaminated sites hardy, tolerant, weed species exist and phytoremediation through these and other non-edible species can restrict the contaminant from being introduced into the food web. Hence, an attempt to identify a metal-tolerant species from natural vegetation in field sites that are contaminated with various heavy metals was successful. The test species *Parthenium hysterophorus* L. not only has strong accumulation to the test metal, but grows rapidly with great biomass as well. Moreover, it has a wide distribution with easy adaptation to different conditions, demonstrating favourable prospect for its application in the phytoremediation of Cd contaminated soils. Hence, it can be considered as the most promising species for phytoextraction of heavy metal contaminated sites and hence phytoremediation.

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