



Screening of selected plants for cadmium and lead phytoremediation

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Abstract

Heavy metal contamination of soil has become a matter of serious concern that requires immediate attention. Phytoremediation is a recent strategy that makes use of plants to remove or stabilize contaminants present in the matrix in which they grow. The present study investigated cadmium and lead phytoremediation potential of the four plants namely *Cosmos sulphureus* Cav., *Crotalaria pallida* Aiton, *Gaillardia aristata* Pursh. and *Gomphrena globosa* L. It was found that with higher B.C.F. values (greater than one) for both cadmium and lead, *G. aristata* and *G. globosa* were good candidates for phytostabilising cadmium and lead. Since all the plants had T.F. values less than one, the potential risk of heavy metals entering the food chain is negligible. The study also tried to find out the effect of 100mg of cadmium and lead per kg of soil on the morphological features like root and shoot length and physiological features like fresh and dry weight of plants. It was found that 100mg of heavy metal (cadmium or lead) per kg of soil did not induce any visible toxicity symptoms like chlorosis, necrosis etc in the plants but caused a decrease in the root and shoot length, fresh and dry weight of plants.

Keywords: heavy metals, bioconcentration factor, translocation factor, phytostabilization, phytoextraction

Introduction

Industrialization and urbanization have led to the release of abundant quantities of heavy metals in the environment during the past couple of years thus polluting the environment (Suman *et al.* 2018; Ashraf *et al.* 2019) [49, 51]. Heavy metals are metallic chemical substances with high atomic numbers, atomic weights and densities. As, Cu, Cd, Pb, Cr, Ni, Hg and Zn are the heavy metals that are commonly found in contaminated areas. Depending on the source, the metals present in different sites may vary (Garbisu and Alkorta 2001; Schmidt 2003; Schwartz *et al.* 2003) [17, 45, 46]. Plants can accumulate heavy metals and thus these can enter the food chain thereby leading to bioaccumulation which causes threat to humans as well as other animals (Sarwar *et al.* 2010; Rehman *et al.* 2017) [44, 41]. Greater concentration of heavy metals in soil affects crop growth by interfering with physiological processes (inhibits photosynthesis, respiration etc), biochemical processes and may even result in destruction of cell organelles thereby leading to death of plants (Garbisu and Alkorta 2001; Schmidt 2003; Schwartz *et al.* 2003) [17, 45, 46]. Heavy metal contamination of soil may also seriously affect the soil microbial community composition which in turn affects the soil characteristics (Giller *et al.* 1998; Kurek and Bollag 2004) [19, 26]. The major problem with heavy metals is their persistence in the environment. This is because their biological destruction is not possible; they only get converted from one oxidation state to another (Garbisu and Alkorta 2001; Gisbert *et al.* 2003) [17, 20]. The presence of cadmium in soil and water has dangerous effects on environment and human health (Salt *et al.* 1995) [42]. Cadmium is more bioavailable compared to lead, copper etc because soil and organic matter has less adsorption capacity for cadmium. Therefore, cadmium is easily absorbed by plants and easily leached into groundwater (McBride 1994, Basta and Sloan 1999, McLaughlin *et al.* 2000, Perronnet *et*

al. 2012) [33, 7, 35, 37]. When plants are grown in cadmium contaminated soil, the water balance of plants is disturbed (Poschenrieder *et al.* 1989) [38]. Cadmium also affects the elasticity of cell wall, stomatal function and photosynthesis in plants (Bazzaz *et al.* 1974; Kirkham 1978; Baszynski *et al.* 1980; Baryla *et al.* 2001) [9, 25, 8, 6]. Once cadmium reaches human body through food chain or other sources, cadmium accumulates there. Kidney damage is a major effect of cadmium toxicity in humans (Salt *et al.*, 1997) [43]. Lead is a carcinogenic heavy metal and once it enters the human body through food chain or other sources, it can cause various ailments. It can cause problems to the nervous system, heart disease, testicular atrophy, anaemia etc. It can affect foetus, babies and young children leading to low IQ. In plants lead toxicity can alter plasma membrane properties (Srivastava and Gupta 1996) [48]. High levels of lead affect the formation of chlorophyll, metabolism of iron, seed germination, stomatal activity, CO₂ uptake, photosynthetic rate etc. (Kacabova and Natr 1986; Poskuta *et al.* 1987) [24, 39]. Due to the various environmental and health risks, strategies to prevent the entry of heavy metals into the environments and organisms and measures to reclaim the contaminated lands have to be undertaken (Gerhardt *et al.* 2017; Hasan *et al.* 2019) [18, 27]. Various approaches like soil washing, electric field application, excavation and landfilling etc have been used for the remediation of heavy metal contaminated soils (Sheoran *et al.* 2011; Wuana and Okieimen 2011; DalCorso *et al.* 2019) [47, 50, 13]. But their limitations like high cost, producing irreversible changes to soil properties, introduction of other pollutants etc make these techniques unattractive (Ali *et al.* 2013; DalCorso *et al.* 2019) [2, 13]. Phytoremediation is an alternative technique that covers all these limitations and is cost effective as well as environment friendly. Phytoremediation which is also known as green remediation, botano remediation or agro remediation is a collection of technologies that uses plants

to eliminate, degrade or stabilize contaminants present in soil, water or sewage (Cunningham *et al.*, 1997; Newman and Reynolds, 2004; Chaney *et al.*, 1997) [12, 36, 10]. Many terrestrial and aquatic plants have been found to have good ability for reclaiming polluted environments (Yoon *et al.*, 2006; Gupta and Sinha, 2007a; Qixing *et al.*, 2011) [22, 40]. There are various strategies for phytoremediation of heavy metal polluted soils which are phytoextraction, phytostabilization, phytovolatilization, phytofiltration and phytodegradation. Phytoextraction is the use of plants to accumulate metals in the harvestable portions of plants and removing contaminants in soil. Phytostabilization is the use of plants to fix contaminants in the root zone or accumulate metals in the root. Phytovolatilization is the use of plants to absorb contaminants and release heavy metals into the atmosphere as fewer toxic forms. Phytofiltration is the use of plants to absorb heavy metals from water. Phytodegradation is the use of plants for breaking down organic pollutants. (Salt *et al.* 1995; Ernst 2005; Marques *et al.* 2009) [42, 15, 3].

Bioconcentration and Translocation Factors

A plant can be called a good phytoextractor if it has high biomass and high bioconcentration efficiency (McGrath and Zhao 2003) [34]. The ability of plants to accumulate metals from polluted soils can be called bioconcentration efficiency. It is estimated by a factor known as bioconcentration factor. Bioconcentration factor is the ratio of metal concentration in the root of the plant to that in the soil/sludge (Yoon *et al.* 2006, Gupta and Sinha 2007b) [14, 51]. The ability of plants to transfer metals from roots to shoots is estimated using translocation factor. It is the ratio of metal concentration in shoot to that in root (Deng *et al.* 2004, Yoon *et al.* 2006) [14, 51]. Plants are chosen for phytoremediation purposes based on their B.C.F. and T.F. values (MacFarlane *et al.* 2007) [30]. If both the B.C.F. and T.F. values of plants are greater than one, the plant can be used in phytoextraction (Yoon *et al.* 2006) [51]. If B.C.F. value is greater than 1 and T.F. is less than one, the plant can be used for phytostabilization (Fitz and Wenzel 2002) [16]. The present work deals with the phytoremediation of the most commonly found heavy metals (lead and cadmium) in contaminated areas. The study was an attempt to investigate the lead and cadmium phytoremediation potential of four common terrestrial plant species.

Materials and Methods

Plant materials

The plant materials used for the present study are listed in Table 1. Seed propagating plants were selected due to the practical feasibility of using such plants for phytoremediation. The seeds of the plants were obtained from Silent Valley Nursery, Thodayad, Calicut and by field exploration.

Table 1: Plants used for the Present Study

Sl No.	Common name	Scientific name	Family
1	Sulphur cosmos	<i>Cosmos sulphureus</i> Cav.	Asteraceae
2	Smooth crotalaria	<i>Crotalaria pallida</i> Aiton	Fabaceae
3	Common blanket flower	<i>Gaillardia aristata</i> Pursh.	Asteraceae
4	Globe amaranth	<i>Gomphrena globosa</i> L.	Amaranthaceae

Experimental design.

Seeds were sown for germination. The seedlings after a month of growth were transferred to growbags filled with 1

kg potting mixture. The potting mixture was prepared by taking soil, cow dung and cocopeat in the ratio 4:1:1. Thirty replicates of seedlings of each plant species were transplanted to the growbags. Seedlings were allowed to grow up for three months in this soil. At the end of three months, ten replicates of each plant species were selected as control plants, ten replicates of each plant species were selected for cadmium as well as for lead treatment.

Heavy metal treatment was provided only once i.e. at the beginning of the fourth month. Since the heavy metals chosen for the present study were cadmium and lead, the solutions of cadmium and lead were prepared. Cadmium solution was prepared by dissolving 4g cadmium nitrate in 2 L distilled water and lead solution was prepared by dissolving 4g lead acetate in 2L distilled water. Each growbag for cadmium treatment was given 50ml of cadmium nitrate solution and each growbag for lead treatment was provided with 50 ml of the lead acetate solution. This means that each growbag containing the plants kept for cadmium treatment received 100mg of cadmium/kg of the soil. Similarly, each growbag containing the plant kept for lead treatment received 100mg of lead per kg of soil. After the one-time treatment of the respective plants with the respective heavy metal solutions, all the plants were watered regularly with 50 ml of water. The chemicals used for the present study were obtained from Hi Media Laboratories Private Limited and Qualigen Chemicals, Mumbai, India.

Preparation of plant samples and heavy metal analysis

After the treatment duration of one month, all the plant samples were harvested carefully. The plants were washed with distilled water to remove dust and mud present in the plants. The root length, shoot length and fresh weight of roots and shoots of all the plants were recorded. Then these plant samples were dried in an oven at 60⁰ C. The dry weights of the samples were recorded when the weight of the samples became constant. The dry weight percentage was calculated using the equation (dry weight/ fresh weight) × 100. The dried samples were ground to powder. The samples for heavy metal analysis were prepared according to Allan's method (1969). 1g ground powder of roots and shoots of each treatment were accurately weighed and digested with a mixture of nitric acid (HNO₃) and perchloric acid (HClO₄) taken in the ratio 4:1. The resulting mixtures were evaporated to dryness and extracted with distilled water. The solutions were heated to boiling and filtered. The volume of the solutions obtained as a result was made up to 50ml. The cadmium and lead concentrations in all the prepared samples were examined by Flame Atomic Absorption Spectrophotometer at CWRDM (Centre for Water Resources Development and Management), Kozhikode, Kerala.

Procedure for heavy metal analysis in soil

Soil samples were collected from control, cadmium treated and lead treated growbags. One gram of each soil sample was digested with repetitive addition of 10ml nitric acid and (HNO₃) and 10ml Hydrogen peroxide (H₂O₂). The solutions were heated to boiling and filtered. 20ml of Hydrochloric acid (HCl) was added to the initial digestate and the sample was refluxed. After filtration, the digestate was diluted to final volume of 100ml. The heavy metal concentration in all

the samples were analysed by Flame Atomic Absorption Spectrophotometer at CWRDM, Kozhikode, Kerala.

Results and Discussion

Morphological features.

An analysis of morphological features like root and shoot length were carried out to find out the effects of Cd and Pb metal stress on the selected plants. The four plants *Cosmos sulphureus*, *Crotalaria pallida*, *Gaillardia aristata* and *Gomphrena globosa* exhibited decreased root and shoot length in the presence of both the heavy metals cadmium and lead when compared to the control plants (Table 2). Aftab *et al.* in 2020^[1] reported that presence of lead reduced the fresh and dry weight and length of root and shoot of *C. sulphureus*. This was in corroborating with our findings related to *C. sulphureus* which exhibited decreased root and

shoot length when exposed to cadmium and lead stress. Even though heavy metal treated *C. pallida* plants showed decreased root and shoot length in comparison to control plants, this decrease cannot be attributed to heavy metal treatment because the plant has accumulated only below detectable levels of heavy metals.

The slight decrease found in root length of the heavy metal treated plants in the present study may be due to higher concentration of heavy metals absorbed in roots (Tables 4, 5).

The aboveground parts of the plants did not exhibit any symptoms of heavy metal toxicity like chlorosis or necrosis. Our results were similar to that of Liu *et al.* (2008)^[28], who reported that *Calendula officinalis* grew normally in soil containing 100mg/kg Cd without suffering phytotoxicity.

Table 2: Effect of Cadmium and Lead on Morphological Parameters of Plants.

SI No	Plant	Treatment	Root length(cm)	Shoot length(cm)
1	<i>Cosmos sulphureus</i>	Control	25.66	112.5
		Cadmium	23	100.66
		Lead	22.66	110
2	<i>Crotalaria pallida</i>	Control	27	115.33
		Cadmium	21	90
		Lead	23	78.521
3	<i>Gaillardia aristata</i>	Control	7.333	60.667
		Cadmium	5.001	58.578
		Lead	5.667	59.673
4	<i>Gomphrena globosa</i>	Control	10.5	61
		Cadmium	8	41.666
		Lead	9	54.33

Physiological features.

The plants *Cosmos sulphureus*, *Crotalaria pallida*, *Gaillardia aristata* and *Gomphrena globosa* were evaluated for the effect of Cd and Pb on the physiological parameters like fresh weight and dry weight.

All the heavy metal treated plants showed a slight lower root and shoot fresh weight (Table 3) when compared to control plants. The roots and shoots of control plants had a higher fresh weight than their respective cadmium and lead treated plants. The root and shoot fresh weight of Pb treated plants was greater than that of Cd treated plants except for *G. globosa* i.e. Pb treated *G. globosa* plants had lower root fresh weight value than Cd treated plants.

The root and shoot dry weight also followed a similar trend. The roots and shoots of control plants had a higher dry weight than their respective cadmium and lead treated

plants. The root and shoot dry weight of Pb treated plants was greater than that of Cd treated plants except for *G. globosa* i.e. Pb treated *G. globosa* plants had lower root-shoot dry weight values than Cd treated plants.

The Cd and Pb treated *C. sulphureus*, *C. pallida*, *G. aristata* and *G. globosa* registered decreased shoot fresh and dry weight in comparison to the control plants (Table 3). Mani *et al.* (2015)^[31] investigated the natural potential of *Chrysanthemum indicum* L., a member of Asteraceae family for the clean-up of lead-contaminated soil under pot experiment. It was found that the maximum applied lead caused reduction in the plant height, root length and dry biomass. In the present study also similar reduction in plant height, root length and dry biomass were observed in the selected plants.

Table 3: Effect of Cadmium and Lead on Physiological parameters of Plants.

SI No	Plant	Treatment	Fresh weight (g)		Dry weight (g)		Dry weight percentage	
			Root	Shoot	Root	Shoot	Root	Shoot
1	<i>Cosmos sulphureus</i>	Control	4.5	28.931	1.867	7.349	41.48	25.4
		Cadmium	3.141	22.402	1.416	5.819	45.08	25.97
		Lead	3.166	23.29	1.823	6.66	57.58	28.59
2	<i>Crotalaria pallida</i>	Control	4.755	32.199	1.578	9.672	33.18	30
		Cadmium	3.931	32.192	1.218	9.091	41.16	28.23
		Lead	4.156	32.190	1.285	9.336	44.86	29.00
3	<i>Gaillardia aristata</i>	Control	1.656	12.456	0.9735	4.9815	58.7	39.99
		Cadmium	1.076	7.723	0.51	3.17	47.3	41.04
		Lead	1.087	11.705	0.6205	4.613	57.08	39.41
4	<i>Gomphrena globosa</i>	Control	1.616	19.401	0.768	4.488	47.52	23.13
		Cadmium	1.322	11.2	0.624	3.606	47.2	32.19
		Lead	1.027	8.936	0.612	1.973	59.59	22.07

Phytoremediation Parameters

The phytoremediation parameters like bioconcentration factor (B.C.F.) and translocation factor (T.F.) were evaluated using the following equations (Yoon *et al.* 2006) [51].

B.C.F. = Metal concentration in root/ Metal concentration in soil

T.F. = Shoot metal concentration / Root metal concentration

If both B.C.F. and T.F. values are greater than one, the plant can be called a hyperaccumulator and it can be used for phytoextraction (Yoon *et al.* 2006) [51].

If B.C.F. value is greater than one and T.F. value is less than one, the plant can stabilize the metal in the root zone or accumulate in the root. Therefore, the plant can be used for the phytostabilization of the metal (Fitz and Wenzel 2002) [16].

If both B.C.F. and T.F. values are less than one, the plant cannot be considered as a good candidate for phytoremediation.

Cadmium

The plants *Cosmos sulphureus*, *Crotalaria pallida*, *Gaillardia aristata* and *Gomphrena globosa* were evaluated for cadmium phytoremediation potential. Atomic Absorption Spectrophotometry was conducted to determine the concentration of cadmium in root, shoot and soil.

All the control samples (root, shoot and soil samples) showed below detectable levels of cadmium which specifies that the samples were not contaminated with cadmium. From the A.A.S. values of cadmium treated samples, except for *C. pallida*, all other plants showed significant cadmium accumulation in roots. *C. pallida* showed below detectable levels of cadmium in roots and shoots even in Cd treated plants. This suggests that *C. pallida* is not a good candidate for Cd phytoremediation.

The roots of *C.sulphureus*, *G.aristata* and *G.globosa* accumulated 186, 143, and 358mg/kg while shoots accumulated 0.5, 68 and 50 mg/kg of cadmium respectively. In the present study, roots accumulated more cadmium than shoots. Our results were in accordance with the reports of Chinmayee *et al.* (2012) [11] who conducted a phytoremediation study on *Amaranthus spinosus* and reported that accumulation of Cu, Pb and Cd was higher in the roots than shoots.

Concentration of cadmium remaining in the soil was 99.73mg/kg, 99.71mg/kg and 99.6mg/kg respectively for these plants. The B.C.F. values were 1.86, 1.43 and 3.59 for *C. sulphureus*, *G. aristata* and *G. globosa* respectively. From the results obtained, it can be concluded that these three plants can be used for phytostabilization of which *G. globosa* with the highest B.C.F. value was the best plant for the phytostabilization of cadmium.

The T.F. values of all the three plants were below 1 (Table 4) so none of the plants were found as cadmium

hyperaccumulator. Among the selected plants *G. aristata* had the highest T.F. value (0.475) so compared to the other plants used for the present study, *G. aristata* has greater potential for phytoextraction of cadmium.

Lead

Lead phytoremediation potential of the four plants *Cosmos sulphureus*, *Crotalaria pallida*, *Gaillardia aristata* and *Gomphrena globosa* were assessed. Atomic Absorption Spectrophotometry was carried out to determine the metal concentration in root, shoot and soil samples.

All the control samples (root, shoot as well as soil samples) had below detectable levels of lead which indicates that neither the plants nor the soil used for the present study were contaminated with lead. The A.A.S. values of the treated samples showed that except for *C.pallida*, all other plants showed quite good accumulation of lead in root. Even lead treated *C. pallida* plants showed below detectable levels of lead in roots and shoots. This suggests that *C. pallida* is not a good candidate for lead phytoremediation. A study by Lindino *et al.* (2012) [27] reported that *Crotalaria spectabilis* (a related species of *C.pallida*) plants presented high rate of survival in soil contaminated by Cd and Pb with reduction in growth and production of dry biomass with below detectable levels of cadmium in the biomass.

In the present study, *C. sulphureus*, *G. aristata* and *G. globosa* plants reduced the concentration of lead in soil to 99.84, 99.3, 99.8mg/kg respectively. Lead accumulation in the roots of *C. sulphureus*, *G. aristata* and *G. globosa* were 62.5, 262, 255 mg/kg respectively. In shoots lead accumulation were 7, 10 and 3 mg/kg respectively (Table 5) i.e. lead concentration was higher in roots than in shoots. This finding was in accordance with the reports of Amouei *et al.* (2012) [4] who investigated the efficiency of remediation of soil contaminated with lead and cadmium by using native plants of Iran which were *Abutilon theophrasti*, *Amaranthus retroflexus* and *Zea mays*. The authors reported that the lead concentration in the roots of plants was more than that in their shoots. In the present study, *G. aristata* accumulated more lead in root as compared to other plants and can be used for phytostabilising lead. The B.C.F. value was also the highest for *G. aristata* which underlines its capacity to phytostabilise lead than the other plants used for the present study. *G. globosa* with a B.C.F. value of 2.57 can also be effective in lead phytostabilization.

Concentration of lead in the shoots of the selected plants was low. In *C. sulphureus* it was 7mg/kg, in *G. aristata* it was 10mg/kg, in *G. globosa* it was 3mg/kg and in *C. pallida* it was below detectable level. None of the plants had T.F. values greater than one which showed that none of the plants used for the present study were hyperaccumulators of lead. But *G. aristata* and *G. globosa* were having a BCF value greater than one, so these plants could be used for the phytostabilization of lead.

Table 4: Cadmium accumulation in control and cadmium treated plants

SI No.	Plant	Control			Cadmium (mg/kg)			B.C.F.	T.F.
		Root	Shoot	Soil	Root	Shoot	Soil		
1	<i>Cosmos sulphureus</i>	BDL	BDL	BDL	186	0.5	99.73	1.86	0.002
2	<i>Crotalaria pallida</i>	BDL	BDL	BDL	BDL	BDL	99.9	-	-
3	<i>Gaillardia aristata</i>	BDL	BDL	BDL	143	68	99.71	1.43	0.475
4	<i>Gomphrena globosa</i>	BDL	BDL	BDL	358	50	99.6	3.59	0.139

BDL: Below Detection Limit

Table 5: Lead accumulation in control and lead treated plants

Sl No.	Plant	Control			Lead (mg/kg)			B.C.F.	T.F.
		Root	Shoot	Soil	Root	Shoot	Soil		
1	<i>Cosmos sulphureus</i>	BDL	BDL	BDL	62.5	7	99.84	0.63	0.112
2	<i>Crotalaria pallida</i>	BDL	BDL	BDL	BDL	BDL	99.9	-	-
3	<i>Gaillardia aristata</i>	BDL	BDL	BDL	262	10	99.3	2.63	0.034
4	<i>Gomphrena globosa</i>	BDL	BDL	BDL	255	3	99.8	2.57	0.012

BDL: Below Detection Limit

Conclusion

Phytoremediation is the use of special type of plants to decontaminate soil or water by inactivating metals in the rhizosphere or translocating them to the aerial parts. This is a novel and eco-friendly technology which can be used for the restoration of polluted sites. Organic as well as inorganic contaminants can be removed from soil, air or water using this technology. But different plants have different capacities to absorb and accumulate metals which may be based on their morphological, anatomical, physiological or genetic characteristics (Lone *et al.* 2008) [29].

The present study was an effort to investigate the cadmium and lead phytoremediation potential of the four plants *Cosmos sulphureus*, *Crotalaria pallida*, *Gaillardia aristata* and *Gomphrena globosa*. From the results obtained, it can be concluded that having B.C.F. values greater than one and T.F. values less than one *G. aristata* and *G. globosa* were effective in phytostabilising cadmium and lead. These plants can be used for holding cadmium and lead in the rhizosphere or in the root itself with less risk of transferring the heavy metals to the food chain.

The study also tried to find out the effect of 100mg of cadmium and lead per kg of soil on the morphological features like root and shoot length and physiological features like fresh and dry weight of plants. It was found that 100mg of heavy metal per kg of soil did not induce any visible toxicity symptoms like chlorosis, necrosis etc in the plants but a reduction in the root length, shoot length, fresh and dry weight were found in all the four plants (*Cosmos sulphureus*, *Crotalaria pallida*, *Gaillardia aristata* and *Gomphrena globosa*). To know more about the effect of heavy metals on the plants, studies should be conducted in the direction of chlorophyll content, electrical conductivity, malonaldehyde content etc.

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