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Phytoextraction of Cadmium and Lead in Three Vegetables Crop Plants

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ABSTRACT

Phytoextraction, is an effective and promising means to cure soil contamination with heavy metals. The present study investigates the ability of three vegetables plants for removal of heavy metals from the contaminated soil and metal mobilization to different plant parts. The three plants selected for the study, *Momordica charantia, Vigna unguiculata* and *Solanum melongena* were grown for 90 days in soil artificially contaminated with cadmium (Cd) and lead (Pb) (50mg metal/kg of soil). The concentrations of the two metals were observed to be higher in roots of *Momordica charantia* and *Vigna unguiculata* than in soil, but root Cd level of *Solanum melongena* was slightly lower than that of soil after 90 days. Translocation potential of the heavy metals indicated higher accumulation of Cd in roots of *Momordica charantia* and *Solanum melongena* than in leaves while the pattern was completely opposite in *Vigna unguiculata*. Lead accumulation was higher in roots than in leaves for all the three plant species studied. The Translocation Factor (TF) of Cd for the three plants was in the range of 1.16 to 2.29 whereas, TF values of Pb remained <1, indicating that only small amount of Pb was translocated from roots to aerial parts.

Keywords: Heavy metals, Phytoextraction, Chlorophyll content, Translocation factor

Environmental pollution caused by heavy metals is a growing concern now-a-days due to its ill effects on plants and animals including human beings (John *et al.* 2008). Various agricultural and industrial activities is on the rise to cope up with the increasing demand of modern civilization, leading to an accumulation of these metals in the water bodies and soil, from which they can easily enter into the food-chain and pose health risks to the human population. Heavy metals are very hazardous pollutants because they are non-biodegradable, extremely toxic even at low concentrations and can change their mobility under different physico-chemical conditions (Mathew, 2001).

Some heavy metals act as essential elements for animals and plants at trace amounts, such as Cobalt (Co), Copper (Cu), Manganese (Mn), Molybdenum (Mo), Vanadium (V), Nickel (Ni) and Zinc (Zn) (He *et al.* 2005; Falusi and Olanipekun 2007). These metals are used for redox processes as components of various enzymes and for regulation of osmotic pressure in cells (Bruins *et al.* 2000). Other heavy metals such as Cadmium (Cd), Lead (Pb), Chromium (Cr), Mercury (Hg), Aluminum (Al), Gold (Au) and Silver (Ag) have no biological function/ beneficial effects (Chang *et al.* 1996). All these heavy metals may be detrimental to living organisms when present in excess amounts in soil and water bodies. Of all toxic heavy metals, cadmium (Cd) ranks the highest in terms of its ability to cause damage to plant growth and pose serious risk to human health via food chain (Shah and Dubey, 1998).

The levels of Cd may rise to toxic levels in the soil due to mining activities, smelting, fuel combustion, as well as the use of phosphate fertilizers, sewage sludge, batteries, pigments, metal coatings, and plastics (ATSDR

2011; Goswami and Das, 2015). Lead is another most abundant toxic metal in the earth crust. Presence of excessive amounts of Cd and Pb in soil affect various plant processes such as growth reduction, especially root growth, chlorosis, disturbances in mineral nutrition and carbohydrate metabolism, photosynthesis, water absorption, and cause wilting of leaves (Moya *et al.* 1993, John *et al.* 2008).

Phytoextraction, an inherent component of phytoremediation, is widely in use to remove heavy metals from soil by growing selective plants and then harvesting those plants for management the contaminated soil (Jadia & Fulekar 2009). Both aquatic and terrestrial plants have been used to reclaim contaminated water bodies and soil (Rahmani and Sternberg 1999, Prasad *et al.* 2001; John *et al.* 2008). The ideal plant used for the purpose of phytoextraction should possess the ability to accumulate metal(s) intended to be extracted, preferably in the aboveground parts, tolerate high metal concentrations in soil, grow at an appreciably fast rate to be considered as an agricultural crop (Goswami & Das, 2015). In the present study, three vegetable crop plants, *Momordica charantia, Vigna unguiculata* and *Solanum melongena* were studied to estimate their bioaccumulation potential for cadmium and lead present in the artificially contaminated soil and to assess the ability of the plants to translocate the metals in shoots and leaves.

Material and Method

Soil sample were collected from field at 20 cm depth, dried, sieved and was then thoroughly mixed. Various soil parameters such as soil texture, pH, EC, Organic Carbon, water soluble sodium, potassium, calcium, exchangeable sodium, potassium, calcium, alkalinity, total nitrogen, water soluble phosphorus, total phosphorus, cation exchange capacity were analyzed. Grain size of the soil was analyzed by Pipette method. Soil pH was determined by potentiometric method using pH meter (Systonic pH-meter 361) in 1:2.5 soil:water suspension. Electrical conductivity was measured in 1:2 soil:water extract using conductivity meter (Thermo Scientic conductivity cell, Orion 013605MD). Organic carbon of the soil was determined by method of Walkely and Black (1934). Available nitrogen was determined using Kjedahl instrument (PlicanKelplus-Distyl) following the method of Subbiah and Asija (1956). Available phosphorus was measured by Bray's method (Bray and Kurtg, 1945). Water soluble anion and cation were determined using Ion Chromatography (Metrohm 797 VA Computrace). For exchangeable sodium, potassium, and calcium, soil samples were extracted by ammonium acetate and the liquid extracts were measured by flame photometry (ELICO CL 361). Cation exchangeable capacity of the soil was determined by following the method of Harada & Inoko (1980).

After analysis of soil parameters plastic pots with holes at the bottom, were filled with approximately 2kg air-dried and sieved soil. The soil was fertilized using 200gm of cow dung and 5gm of urea. The soil was artificially contaminated by lead [Lead nitrate, $Pb(NO_3)_2$] and cadmium [Cadmium nitrate, $Cd(NO_3)_2.4H_2O$] at 50mg of each metal/kg of soil following procedures described by Turan and Esringü (2007). For control pots, similar processes were followed except the addition of metals in the soil. Three edible plant species, *Momordica charantia, Vigna unguiculata* and *Solanum melongena* were cultivated in separate pots (in triplicate) in metal contaminated soil and normal soil (control). Both the control pots and metal contaminated pots were watered 3–4 times per week. Pot experiments were conducted under ambient climatic conditions from May to August, 2013. The maximum and minimum temperature recorded were 29-34^{0}C and the relative humidity ranged from 30-80%. All plant species were harvested after 3 months.

After three months plants were collected from the pots, thoroughly washed with running tap water and rinsed with deionized water to remove any soil particles attached to the plant surfaces. Then plants parts (roots, shoots and leaves) were oven dried at 70°C temperature for 48 hours. The dried samples were weighed and powdered. For the soil samples, upper most soil portion from the pots were discarded. Then the remaining soil were mixed thoroughly and sieved at 2mm. Sieved soil were collected in the zipper bag for metal analysis.

Soil and dry plant samples (1gm) were digested after adding 15 ml of tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$ in 5:1:1 ratio) at 80^{o}C until a transparent solution was obtained (Allen *et al.* 1986). After cooling, the digested sample was filtered using Whatman No. 42 filter paper and the filtrate was finally maintained to 50 ml with distilled water. Metals were analyzed by using Anodic Stripping Voltammetry (Metrohm VA 797 Computraces).

The chlorophyll content was determined according to the method of Arnon (1949). Fresh leaf samples (500mg) were homogenized in 80% cold acetone and centrifuged at 5000rpm for 15 min (Remi Cooler centrifuge C-4), the absorbance was read at 645nm and 663 nm (name of Spec). Values were expressed as mg chlorophyll/gm leaves (fresh weight).

The results were statistically analyzed using one way ANOVA to determine the degree of significance for the studied plants. The analysis was carried out by Microsoft Excel 2013.

RESULTS AND DISCUSSION

The physico-chemical parameters of soil are listed in Table 1. This table shows the results of the different physico-chemical parameters of soil.

Table 1: Physio-chemical parameters of uncontaminated soil

Parameters	Quantity
Soil type	Sandy Loam
pH	6.3
Electron Conductivity	1mS/cm
Organic Carbon	1.09%
Water soluble Cations:	
Sodium	46.9mg/kg
Potassium	60.4mg/kg
Calcium	31.7mg/kg
Exchangeable Cations:	
Sodium	213.2mg/kg
Potassium	17.1mg/kg
Calcium	1537mg/kg
Water Soluble Anions:	
Chloride	350mg/kg
Phosphorus	0.56mg/kg
Sulfate	48mg/kg
Total Nitrogen	209.5mg/kg
Available Phosphorus	2.23mg/kg
Cation Exchange Capacity	7.2meq/100gm
Heavy Metals	
Zinc	80.15mg/kg
Copper	54.85mg/kg
Lead	20.55mg/kg
Cadmium	NA

The three plant species were found to grow well in the heavy metal contaminated soils. There were no visible changes in the external morphology of the plants under the influence of metal stress as compared to control plants; neither any sign of chlorosis was observed in the treated plants (Table 2).

Table 2: Total Chlorophyll content in control and treated experimented plants:

Species Name	Total Chlorophyll mg/g FW	
	Control Plants	**Treated Plants**
Momordica charantia	1.23	1.18
Vigna unguiculata	0.46	0.42
Solanum melongena	0.93	1.01

Earlier observers (Unni *et al.* 1992, Kopittke *et al.* 2007, Siddhu *et al.* 2008, Daniel *et al.*, 2009, Yilmaz *et al.* 2009, Gautam *et al.* 2014) have reported changes like plant growth, germination and peroxidase activity at various doses of Pb/Cd applied separately on the same plants. It may be said that combination of these two metals did not alter the morphology of these plants which is well depicted by the chlorophyll content of the treated and control plants. Experiment conducted by Al-Subu *et al.* (1993), on Marrow vegetables showed that the toxicity of cadmium and lead was mostly antagonistic and sometimes irregular on the parts of root treated or foliar-treated plants.

The value of Cd concentration applied to the soil was higher than the Indian Standard (3-6 mg/kg) but the Pb concentration was much lower than the tolerable limits (250-300mg/kg) as stated by Kabata-Pendias (2001).

Phytoextraction studies showed that the three plant species accumulated higher levels of Cd and Pb in their roots, but their ability for translocation of these heavy metals to the aerial plant parts were considerably different. The levels of Cd were higher in roots of *Momordica charantia* and *Solanum melongena* (36.91 mg/kg and 28.48 mg/kg) followed by the shoot (22.22 mg/kg and 24.97 mg/kg) and leaves (20.63 mg/kg, 11.90 mg/kg). The trend was opposite in case of *Vigna unguiculata* which accumulated higher levels of Cd in leaves (40.43 mg/kg) as compared to root system (29.15 mg/kg). In case of Pb, the highest accumulation was found to occur in root of all harvested plants. *Vigna unguiculata* accumulated highest amounts of Pb in root (78.54mg/kg), followed by *Momordica charantia* (53.72 mg/kg) and *Solanum melongena* (41.99mg/kg). In contrast to Cd, translocation of Pb from root to aerial parts was found to be very poor in *Vigna* and *Solanum* with the exception of *Momordica* which accumulated higher levels of Pb in shoot (34.29). Translocation of Pb from root to the leaves was insignificant in all the three plant species studied (in the range of 2.48 mg/kg – 7.06 mg/kg).

The accumulation of heavy metals in a plant can be quantified by two factors: the Bioconcentration Factor (BCF) and the Translocation Factor (TF) (Selamat *et al.* 2014). The BCF is calculated as the ratio of the element concentration in plant tissues to the concentration of the element in the soil (Zayed *et al.* 1998).

Bioconcentration Factor (BCF) =

$$\frac{\text{Element concentration in plant tissues}}{\text{Element concentration in soil}}$$

TF is calculated as the ratio of metal concentrations in aerial part of the plant to those in roots, indicating the ability of the plant to translocate metals from the roots to the shoots (Roongtanakiat, 2009).

Translocation Factor (TF) =

$$\frac{\text{Element concentration in aerial part of the plant}}{\text{element concentration in root these plant}}$$

Heavy metals concentrations, bioaccumulation factors (BCF) and translocation factors (TF) are listed in Table 4. The BCF values of the Cd and Pb of all the three harvested plants in the metal contaminated soil were >1 except *Solanum melongena* for Pb. The highest BCF value of Cd was observed in *Vigna unguiculata* about 1.92 followed by *Momordica charantia* and *Solanum melongena* ranging about 1.60 and 1.29 respectively. Furthermore, the Cd concentrations in the aerial parts of harvested plants were higher than that of the corresponding roots, i.e. the translocation factors (TF) were >1. The highest TF values of Cd were observed in *Vigna unguiculata*, ranging from 2.18 to 2.38, followed by *Solanum melongena* ranging from 1.17 to 1.38 and *Momordica charantia* ranging from 1.1 to 1.23.

Table 3: Heavy Metals (in ppm) concentration of soil, root, shoot and leaves of three harvested plants

Pt	Cd root	Pb root	Cd shoot	Pb Shoot	Cd leaves	Pb leaves
Momordica charantia	36.91 ± 1.87[a]	53.72 ± 1.55[a]	22.22 ± 1.18[a]	34.29 ± 1.53[a]	20.63 ± 1.05[a]	2.48 ± 0.30[a]
Vigna unguiculata	29.15 ± 0.98[b]	78.54 ± 1.83[b]	26.35 ± 0.83[b]	4.41 ± 1.12[b]	40.43 ± 1.52[b]	7.06 ± 0.61[b]
Solanum melongena	28.48 ± 1.72[ab]	41.99 ± 1.57[c]	24.97 ± 1.53[bc]	10.87 ± 1.47[c]	11.90 ± 1.63[ab]	6.33 ± 0.58[ab]

**The same letter within columns are not significantly different at the 5% probability level by least significant range.*

Table 4: BCF and TF value of harvested plants

	Bioconcentration factors		Translocation factors	
	Cd	Pb	Cd	Pb
Momordica charantia	1.60 ± 0.04	1.29 ± 0.04	1.16 ± 0.03	0.68 ± 0.005
Vigna unguiculata	1.92 ± 0.01	1.29 ± 0.03	2.29 ± 0.04	0.15 ± 0.03
Solanum melongena	1.31 ± 0.03	0.85 ± 0.04	1.29 ± 0.07	0.41 ± 0.05

From the result observed it is clear that BCF and TF values of Pb were less than the values of the Cd. Highest BCF value of Pb was observed in *Momordica charantia* and *Vigna unguiculata* about 1.29 and lowest in *Solanum melongena* about 0.85 (Table 4). Furthermore, the Pb concentrations in the aerial parts of harvested plants were lower than that of their roots, i.e. the translocation factors (TF) were <1. The TF values of the three harvested

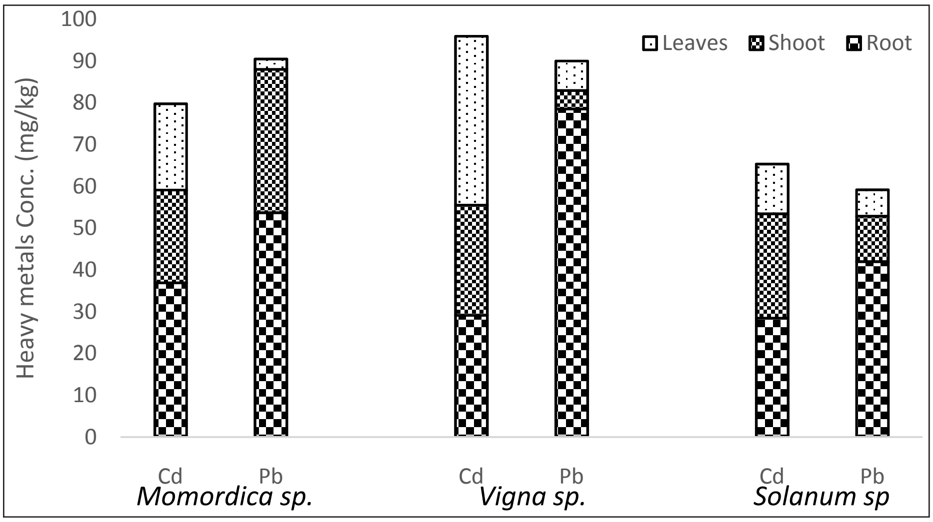

Fig. 1: Cd and Pb concentration in roots, shoots and leaves of three harvested plants

plants *Momordica charantia, Vigna unguiculata* and *Solanum melongena* were 0.67, 0.13 and 0.41 respectively.

The present study showed that the distribution of Cd in the aerial plant parts was found to be significantly higher than roots. The BCF and TF values of Cd were always >1 in all harvested plants. High BCF values indicated that the plants could accumulate Cd from soil. On the other hand, high TF values indicated that plants could take up Cd from the soil and store it in its above ground parts (Selamat *et al.* 2014). Highest BCF and TF values were observed in the *Vigna unguiculata*, which indicated that this plant was capable of accumulating Cd to a greater extent from soil than the other two plants and store the metal in the aerial plant parts. Cadmium was stored in the *Vigna unguiculata* according to following order Leaves>Roots>shoots. Mengel and Kirkby, (1982) stated that Cadmium is readily transported from the soil to the upper parts of plants. Transport of Cd from the soil to plant parts of agricultural crops is significantly greater than other heavy metals except Zinc (Moolenar & Lexmond, 1999).

TF values of Pb was always <1 in all experimental plants, indicating that Pb concentration in the plant root was always greater than the aerial parts. According to Grill *et al.* (1985), this may be due to low mobility of Pb, when it is found in low quantities in the soil, given that the root systems prevents the migration of Pb towards the above ground part of the plant and it only reaches this part if it is found in high concentration.

In the present study also, phytoextraction and bioaccumulation potential of the three plant species *Momordica charantia, Vigna unguiculata* and *Solanum melongena* against Cd and Pb was found to be favorable. Translocation of Cd within the plant system was, however, much higher than that of Pb for all the three plant species studied. Most of the Pb extracted from contaminated soil was localized within the root system while a considerable fraction of extracted Cd was found to be translocated to the aerial parts of the three plant species. Kopittke *et al.* (2007), observed similar result on *Vigna unguiculata*. They observed that, the tissue concentrations of Pb were 10 to 50 times greater in the roots than in the shoots, with the critical Pb concentration being 330 μg/g for the roots and 49 μg/g for the shoots. Another study reported by Daniel *et al.* (2009) that the Cd^{2+} accumulation was greater in seed of Bittergourd than the Pb. Qadir *et al.* (2000) and Chauhan, (2014) observed twofold Cd accumulation in leafy vegetables as compared to others.

CONCLUSION

The results clearly indicate that phytoextraction studies of edible plants are important not only from nutritional point of view, but also from the differential abilities of an individual plant to remove heavy metals from soil and localize them in specific plant parts.

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