PHYTOACCUMULATION OF HEAVY METALS BY *AMARANTHUS SPINOSUS* L. GROWN IN SOIL COLLECTED FROM THREE SELECTED DUMPSITES OF KERALA, INDIA.

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Rapid industrialization, increased urbanization and improved agricultural production methods have led to an increase in heavy metal pollution in the environment. Weedy Amaranthus species commonly found on roadsides and abandoned lands, such as *Amaranthus spinosus* were selected for this study. Seedlings of the experimental plants were grown in soil containing USW (Urban Solid Waste) collected from three selected sites in the state of Kerala, India, namely, Vilappilsala, Chavara and Plachimada. Soil parameters such as soil moisture, soil pH, soil organic carbon and alkalinity were estimated. After measuring the initial heavy metal concentrations at the three selected locations, the test plant seedlings were allowed to grow in soil collected from the respective dumpsites for 60 days, and the percentage reduction in heavy metal content was determined. Post-harvest analysis showed a reduction of more than 50% in heavy metal concentration at each site.

Keywords: *Amaranthus spinosus*, Heavy metals, Post-harvest analysis, Soil, Urban Solid Waste.

Introduction

Soil quality is a crucial parameter for the growth of plants. It act as the source and sink of most of the minerals. Soil polluted with heavy metals due to geologic and anthropogenic activities are serious concern today. Chemical form and speciation are important for the adsorption and transport of heavy metals in soil. Heavy metals are initially adsorbed into the soil followed by slow adsorption reactions and redistributed into different chemical forms with varying bioavailability, mobility and toxicity. The metals remain in the soil for long period of time unlike organic contaminants that may directly affect the vegetation. The source of heavy metals may be natural or anthropogenic. Natural sources by the pedogenesis of parent material and anthropogenic sources by agricultural residues, lead based paints, gasoline, sewage sludge, compost, storm water runoff, petrochemicals and by atmospheric depositions.

The elimination of metals from contaminated soil is rather difficult. The adsorption, desorption, complexation, mobilization and uptake by plants are the main processes noticed in soil [1]. Plants with spreading root system, high biomass, rapid growth and ability to tolerate heavy metals are suitable candidates for phytoextraction and thereby they may be employed for remediating heavy metals from soil. By solubilizing the heavy metals, root exudates make them accessible to plants[2]. The plants may tolerate heavy metals either by exclusion, inclusion or by complexation. Plant based phytoremediation is a cost effective, eco-friendly method to tackle heavy metal pollution in soil.

Material and Methods

Three sites of Kerala with Urban Solid Waste (USW) were selected for the present

investigation Viz., Vilappilsala, chavara and Plachimada (Fig. 1).

Vilappilsala is located in the capital city of Kerala, Thiruvananthapuram district at a latitude of 8^0 52^1 and a longitude of 77^0 04^1. It is a government venture set up in 2000 for dumping and treating the Urban Solid Waste (USW) collected from the city. The seepage of pollutants to nearby aquatic systems occurred during rainy season reducing the water quality. Plastics, batteries and electronic wastes were dumped into the soil. Moreover, the residents of the Panchayat suffered from respiratory and skin disorders leading to agitation by the public so that the government is forced to shut down the processing plant.

Fig. 1: Three Selected Dump Sites of Kerala for Collection of Soil Samples.

Fig .2 *Amaranthus spinosus* L.

Chavara is located in Kollam district of Kerala at a latitude of 9^0 02^1 and longitude of 76^062^1. A public sector company, Kerala minerals and metals Limited (KMML) is located in the area. It is one of the producers of Titanium dioxide in Kerala which generated enormous quantity of sludge. The untreated contaminated sludge and effluents generates soil pollution and the seepage into nearby water bodies resulted in water pollution. The improperly treated sludge contained heavy metals leading to the degradation of the ecosystem.

Plachimada is located in Palakkad district of Kerala at a latitude of 10^0 38^1 and longitude of 76^0 49^1. The factory which is the producer of the soft drink Coca Cola was located in the area. The excessive utilization of ground water resources coupled with the heavy metal pollution above WHO standards lead to the shutting down of the factory.

Analysis of Soil Parameters

Soil with USW from the selected dump sites and garden soil (control) were collected, dried, pulverized and kept in a

desiccator for further analyses. Soil parameters such as Soil moisture, Soil pH, Soil organic carbon and Alkalinity were estimated by standard methods[3].

Evaluation of Heavy Metals in Soil from Dumpsites Before and After Growth of Amaranthus spinosus L.

Amaranthus spinosus (Fig. 2), an invasive weed belonging to Amaranthaceae family luxuriantly distributed in the road sides, waste lands and abandoned area of Kerala commonly known by the name spiny amaranth, prickly amaranth and thorny amaranth was employed for the present study. The plant was identified and herbarium deposited in Kerala University Botanical Herbarium with voucher number KUBH 6032. Polythene lined pots were filled with 3 kg soil, sieved through 2 mm sieve and garden soil was used as control. The initial concentration of heavy metals Viz., Cadmium, Chromium and Lead in the soil collected from three selected sites were estimated. For the preparation of soil samples standard wet digestion method[4] were followed and the concentration of elements were analyzed by Atomic Absorption Spectrophotometer (Perkimelmer, Pinnacle 900H). After the estimation of soil parameters and heavy metals, two-week-old seedlings of *A. spinosus*, three in each polythene lined pots were allowed to grown in the contaminated soil collected from the polluted sites of Kerala.

The experiment was continued till 60 days. After experimental period, the plants were harvested and the soil was again analyzed by using Atomic Absorption Spectrophotometer to find out the final concentration of heavy metals and expressed in mg kg^{-1} [4] followed by the percentage of reduction of heavy metal concentration by the test plant.

Statistical Analysis

All analyses were conducted in triplicate and the results were subjected to statistical analysis by using GraphPad InStat DTCG 3.06 software and the Dunnett's test was performed to find out one-way ANOVA and found out the differences between the treatments at 5% probability level ($p < 0.05$). The results were presented as Mean ± standard error (SE).

Results and Discussion

The physicochemical parameters of soil samples i.e., garden soil and soil collected from selected dumpsites were given in Table 1. The soil parameters like moisture, pH, alkalinity, soil organic carbon influence heavy metal adsorption and bioavailability to plants[5].

Soil pH was found to be acidic in all the sites. pH was relatively low in Plachimada soil, (6.12± 0.31). Soil pH is an indication of acidic or alkaline nature of soil. It possesses a major role in metal dynamics which control the adsorption and precipitation of metals helping in their retention in soil. Reduced pH renders most of the cationic metals in soluble form which make them bioavailable to plants. Heavy metal uptake by plants is mostly dependent on soil pH[6]. High mobility of metals was noticed in low pH[7]. Metals were sparingly soluble in alkaline pH and acidic conditions appear to be favourable for the solubilisation of heavy metals. The pH dependent metal adsorption and dissolution of Fe- Mn oxyhydroxides under reducing conditions was the main mechanism controlling the release of heavy metals from the soil[8]. Metal uptake decreases with increase in pH [9]. The presence of colloids lead to the release of metals at low pH increasing the metal concentration in pore water and roots. It was opined that with increase in metal treatments, pH become acidic and the cation exchange capacity of the soil will become enhanced that make the heavy metals easily available to the plants[10].

Alkalinity was found to be high in Vilappilsala soil (61± 0.28 mEq/L) and was low in Plachimada site (43± 0.46 mEq/L). Plants normally grow well in slightly acidic soil. Soil moisture content was higher in the garden soil as compared to the soil

Table 1: Physico chemical parameters of soil from 3 selected sites.

Soil sample	Moisture content (%)	pH	Soil organic carbon (mg g^{-1})	Alkalinity (mg g^{-1})
Garden soil	4.701±0.01	6.23±0.11	7.76±0.31	46±0.36
Vilappilsala (Site1)	4.411±0.013	6.29±0.19	16.14±0.26	61±0.28
Plachimada (Site 2)	4.052±0.018	6.12±0.31	13.23±0.24	43±0.46
Chavara (Site 3)	4.30±0.13	6.69±0.221	6.08±0.12	56.62±0.192

The results represented as Mean ±SE of three replicates.

Table 2: Initial Concentration of Heavy metals in Soil Samples (µg g^{-1}).

Soil sample	Pb	Cd	Cr
Garden soil	7.173 ±0.1501	0.2533 ±0.0328	2.973 ±0.1091
Vilappilsala (Site1)	11.263 ±0.6986 **	3.443 ±0.18 **	7.030 ±0.2325**
Plachimada (Site 2)	15.320 ±0.6120**	3.510 ±0.176 **	11.870 ±0.7582**
Chavara (Site 3)	19.140 ±0.9557**	5.950 ±0.4389 **	13.127 ±0.9760 **

The results represented as Mean ±SE of three replicates. Significance at $p < 0.05$, according to the Dunnett test.
**Highly significant.

Table 3: Final Concentration of Heavy Metal after Harvest of *Amaranthus spinosus* (µg g^{-1}).

Soil sample	Pb	Cd	Cr
Garden soil	3.760±0.227	0.1033±0.0145	1.138±0.1653
Vilappilsala (Site1)	6.103±0.196**	1.450±0.140 **	3.910±0.1531**
Plachimada (Site 2)	7.723±0.338**	1.583±0.259 **	4.770±0.3439**
Chavara (Site 3)	10.870±0.272 **	3.083±0.269**	7.140±0.1644**

The results represented as Mean ±SE of three replicates. Significance at $p < 0.05$, according to the Dunnett test.
**Highly significant.

Table 4: Percentage of Reduction of Heavy Metal Concentration after Harvest of *Amaranthus spinosus* (%).

Soil sample	Pb	Cd	Cr
Garden soil(C)	48.41	59.21	61.68
Vilappilsala (Site1)	46.18	60.24	46.24
Plachimada (Site 2)	49.58	54.9	59.81
Chavara (Site 3)	43.20	48.18	45.57

collected from various dumpsites. Comparatively higher (4.411± 0.013%) and lower (4.052± 0.018%) moisture content was observed in Vilappilsala and Plachimada soil respectively. Lower soil moisture content may increase the adsorption and the relative adsorption capacity of lead and cadmium increases with decreasing moisture content. Significant positive correlation was reported between heavy metals and soil moisture content[11]. It was reported that the biomass of plants such as *Thlaspi, Alyssium* and *Berkheya* was greater at the higher soil moisture content and they exhibited great metal uptake in such condition[12]. It was also mentioned that the metal such as Zn and Ni was less available at high moisture content and the test plants exhibited greater metal uptake at high moisture conditions.

Soil Organic Carbon (SOC) was very high in Vilappilsala soil (16.14± 0.26 g/kg soil) and low in Chavara soil (6.08± 0.12 g/kg soil). SOC in garden soil was

found to be 7.76 ±0.31 g/kg soil. SOC is the carbon associated with soil organic matter made up of decomposed plant and animal materials as well as microbial organisms. Organic matter content and hydrous ferric oxide found to decrease heavy metal availability since it lead to immobilization of metals[13].

Heavy metals in soil before and after the growth of tolerant species *Amaranthus spinosus*

The initial and final concentration of heavy metals in various soil samples was given in Table 2 and 3. Heavy metal concentration was found to be lower in the garden soil as compared to the soil collected from various dumpsites. Comparatively higher concentration of Pb^{2+} (19.140 ± 0.9557), Cd^{2+} (5.950 ± 0.4389) and Cr^{6+} (13.127 ± 0.9760) was observed in Chavara (site 3) whereas Vilappilsala soil (site1) contained lower concentration of all metals.

Mean concentration of lead in soils normally ranges from 10 to 67 mg Kg^{-1} [14]. Lead is found in rocks as galena at a concentration of 0.1 to 10 mg Kg^{-1}. The concentration of lead in average shale and earth crust was 20 and 12.50 mg Kg^{-1} [15]. During weathering, lead ion can form carbonates and has the ability to be incorporated into clay, iron or manganese oxides as well as organic matter. Organic matter content and pH influences both solubility and mobility of lead. The initial concentration of lead was comparatively low (11.263µg g^{-1}) and high (19.149 µg g^{-1}) in Vilappilsala and Chavara soil respectively. The metal content in soil after the growth of *A. spinosus* (Table 3) was found to be 6.103 µg g^{-1}, 7.723 µg g^{-1} and 10.870 µg g^{-1} in Vilappilsala, Plachimada and Chavara soil respectively. The reduction in lead content (Table 4) was low (<50%) after the growth of *A.spinosus* as compared to the other metals in most of the sites which may be due to the occurrence of lead in significantly less bioavailable form as a soil precipitate[16].

The standard value of cadmium in average shale and earth crust was 0.30 and 0.20 mg Kg^{-1} [15]. The anthropogenic sources of cadmium are industrial effluents, storage batteries, petroleum refineries and fertilizers. The phosphate fertilizers normally contain cadmium content of 10 to 100 µg g^{-1} that increases cadmium level in soil[17]. In the present study, the initial concentration of cadmium was low (3.443 µg g^{-1}) in Vilappilsala and high (5.950 µg g^{-1}) in Chavara soil respectively. The metal content in soil after the growth of *A. spinosus* was found to be 1.450 µg g^{-1}, 1.583 µg g^{-1} and 3.083 µg g^{-1} in Vilappilsala, Plachimada and Chavara soil. The reduction in cadmium content was high (>50%) after the growth of *A. spinosus* as compared to the other metals which may be attributed to the fact that cadmium normally occur in exchangeable and bioavailable form as suggested by Prasad (2004) and also by higher adsorption of Cd^{2+} by *Amaranthus spinosus*[16].

Chromium is present in all phases of the environment like air, water and soil. The naturally occurring chromium ranges from 10 to 50 mg Kg^{-1} depending on the parent material and may even reach up to 125 mg Kg^{-1} [18]. Cr^{3+} and Cr^{6+} are the most stable form of chromium which usually occurs associated with oxygen as chromate and dichromate oxyanions. It has a complex electronic chemistry than other toxic elements which complicate the behaviour of the metal in organisms. The chromium compounds possess multifarious uses in industries and leather and tanning industries are the major contributors of chromium pollution in the environment. The uptake of chromium in plants depends on the speciation of the metal. Cr^{6+} is an efficient competitor with other elements with similar electronic configuration and was found successful at the entry level into the plant[19]. The concentration of chromium was low (7.030±0.2325 µg g^{-1}) and high (13.127±0.9760 µg g^{-1}) in Vilappilsala and Chavara soil respectively. The metal content in soil after the growth of *A.*

spinosus was found to be 3.910 µg g^{-1}, 4.770 µg g^{-1} and 7.140 µg g^{-1} in Vilappilsala, Plachimada and Chavara soil respectively. The reduction in chromium content was moderate after the growth of *A. spinosus* as compared to the other metals.

The metals are found associated with soil solution, inorganic constituents and organic matter, precipitated or embedded in silicate minerals, significant reduction was observed in the metal content in soil collected from three selected sites of Kerala after the growth and harvesting of *A. spinosus*. Among the heavy metals, cadmium showed highest reduction followed by chromium and lead. Among different polluted sites, maximum reduction of Cd^{2+} (60.24%) had occurred in Vilappilsala site where as Pb^{2+} (49.58%) and Cr^{6+} (59.812%) in Plachimada site.

Heavy metals are initially adsorbed into the soil followed by slow adsorption reactions and redistributed into different chemical forms with varying bioavailability, mobility and toxicity. The plants may tolerate heavy metals either by exclusion, inclusion or complexation. In general, the plants exhibit preferential uptake mechanism of some ions over others. The metals move across the biological membrane through transport proteins. The selectivity depends on the property of the transporters to recognize, bind and mediate the transmembrane transport of the metals. These metal transporters or channels are nonspecific and used by different metals[20].

A. spinosus was found to be a moderate accumulator of heavy metals like cadmium, chromium and lead and could reduce the heavy metal content in soil to about 50% in *ex situ* conditions. Further, this property may be used to study remediating potential of the plant in *in situ* conditions by growing the species in the polluted sites. The mechanism become more complex due to the combination effect of more than one heavy metal in soil whose action may be categorized into independent, additive, synergestic or antagonistic effect[21].This may affect the growth and yield of plants depending on the influence of the stressors either independently or in combination. The interrelationship among different heavy metals needs special focus while considering the phytoextraction as an effective remediation technique for heavy metals in soil.

Conclusions

Heavy metal pollution increased enormously due to industrialization and their level significantly exceeded than natural sources. The heavy metal pollution may be effectively tackled with plants possessing the capability to absorb, complex and detoxify the metals in their bioparts. Post harvest analysis of soil after growth of the weedy species *Amaranthus spinosus* showed reduction of 46.32%, 57.66% and 50.54% of Pb^{2+}, Cd^{2+} and Cr^{6+}content of the soil contaminated with Urban Solid Waste (USW) respectively. The study warrants further detailed investigation on to the molecular mechanism behind the efficient uptake of metals by *Amaranthus spinosus* from the soil.

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