



Study of Uptake of Pb and Cd by Three Nutritionally Important Indian Vegetables Grown in Artificially Contaminated Soils of Mumbai, India

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Abstract

The ability of nutritionally important vegetable species as heavy metal accumulators was assessed. The root, stem and leaf content of the two metals lead and cadmium in the three plant varieties Spinach (Spinaciaoleracea), Fenugreek (Trigonellafoenum-graecum) and Red Amaranth (Amaranthuscruentus) was determined using pot experiments. Metal analysis was done using AAS. The metal concentration in different tissues varied according to plant part, type of metal, soil properties, morphological differences in plants, physiological differences in terms of heavy metal content, omission, increased uptake, foliarabsorption, etc. Highest metal concentration was reported in roots of all three plant varieties. Red Amaranth and Spinach showed higher tendency to accumulate lead and cadmium respectively. Among the two metals, Pb was accumulated more than Cd indicating plant affinity towards Pb. Transfer factor index also gave supporting values. None of the transfer factor exceeded 1 showing that none of the three species are hyperaccumulators. But they are certainly accumulating metals in its tissues. The root to shoot translocation factors concluded that metals are getting retained in roots and minimum levels are getting transferred in shoot parts. This indicates that roots which are insignificant for dietary intake of humans contain maximum quantities of heavy metal. Whereas the leaves that are nutritionally important for humans accumulate low levels of metals. Lead concentration in leaves of Red Amaranth and Spinach exceeded Indian standards. While lead concentration in Fenugreek and cadmium concentration in all the three vegetables was detected to be within the prescribed limits. This shows that elevated levels of Cd in soil may not add it significantly in human food chain but the same for Pb may raise its concentration in plants thereby increasing its toxicity.

Keywords: Heavy metals, bioaccumulation, toxicity, phytoremediation, nutrition.

Introduction

Pollution of agricultural soils due to heavy metals is a big concern these days. The heavy metals also called 'trace metals' occur in minute quantities in the earth's crust. However, the ambient environments of developing countries such as China¹ and India^{2,3} have reported higher levels of such metals due to fast and unplanned urban and industrial development. Heavy metal pollution is a problem faced by all countries over the globe. Their exposure to humans from external environments or through bioaccumulation in food chains is a serious concern. Many of these metals are persistent and may accumulate to the levels that affect heterotrophic organisms including humans. Their reactions with natural components of soil or water as well as other anthropogenic pollutants may complicate their removal from environment with the available methods⁴. Hence, the sources are many but for removal of these metals from soil we have to rely largely on physico-chemical methods⁵.

A probable solution to this problem has been the phytoremediation and phyoextraction techniques. Phytoextraction is the ability of plants to remove chemical materials, primarily metals from polluted soil. The metals are

thus absorbed from soil through the roots and deposited in above soil plant parts, e.g. stem or leaf^{4,6}. The most basic use of these techniques may be to provide economical and environmentally friendly mechanisms for removal of heavy metals from polluted environments⁷. Improvements in these techniques along with some other measures such as soil amendments, selection of efficient plant species and augmenting agricultural practices may help to solve heavy metal pollution of soil⁸.

Lead (Pb) and cadmium (Cd) are the metals often considered as primary contaminants of natural environment. The largest worldwide use of these metals has been in batteries, alloys, paints, stabilizers, etc. Human exposure to these metals occurs mainly through food intake or inhalation of soil or dust contaminated with heavy metals. These two metals are highly mobile and are known to cause undesirable effects on metabolic processes of living organisms⁹. They are toxic to both terrestrial as well as aquatic ecosystems. Their deposition in plant tissues may show biochemical as well as physiological changes¹⁰. Extreme values may cause growth inhibition and loss of net production¹¹. Lead and Cadmium both are potential neurotoxins and nephrotoxins¹². Although these metals are considered to be

non-essential for biological processes, they are efficiently absorbed by plants thereby entering into food chain.

The current study has used three nutritionally important vegetables namely Spinach (*Spinaciaoleracea*), Red Amaranth (*Amaranthuscruentus*) and Fenugreek (*Trigonellafoenum-graecum*). These leafy vegetables were selected due to their shorter life cycle, short root length, nutritional importance, wide consumption and suitability to local climate. It is a well-established fact that leafy vegetables exhibit a higher tendency to accumulate toxic metals from soil. Hence the plant shows presence of both essential as well as toxic elements¹³. The objective of current study was to compare the differences in accumulation capacities of the above said vegetable species and to determine their transfer factors and translocation factors for the local environmental conditions.

Material and Methods

The seeds of Spinach (*Spinaciaoleracea*), Red Amaranth (*Amaranthuscruentus*) and Fenugreek (*Trigonellafoenum-graecum*) were obtained from Namdeo Umaji Agritech India Private Limited, Byculla, Mumbai. Good quality seeds were selected. The seeds were washed thoroughly in deionised distilled water. The seeds were germinated later for sprouting.

Clean and new, rectangular plastic pots were selected. (Dimension: length × breadth × height = 50cm × 30cm × 40cm). Plastic pots have an advantage of elimination of possible source of contamination that may arise from soil or cement pots. They are also leakage proof. The size was selected in such a way that the depth is more than maximum root length and area is sufficient for generation of adequate quantities of sample. Soil from a nursery in Tarapur, district Thane, Maharashtra, India was brought in to laboratory. It was screened for dirt, pebbles, stones, leaves, sticks, etc. Fine textured soil was then chosen and filled in pots so that each pot contains around 10Kg of soil.

The soil was then contaminated with nitrate salts of Pb and Cd. (20mg salt per Kg of soil) as solution in distilled water. The two metals were selected because of their least reported synergistic and antagonistic effects. AR grade chemicals from Burgoyne and Co., Mumbai India were used. The soil was homogenized thoroughly for uniform distribution of metals. Half Kg of vermicompost was then added. Use of chemical fertilizers and pesticides was avoided. Such soil was kept idle for 1 week for stabilization. Well germinated seeds were later on planted in each pot and allowed to grow up to harvesting time (3 pots per plant species). The plants were watered daily in morning using distilled water in order to avoid contamination. The pots were kept in ambient sunlight but under shade. They were protected from rain water. The plants took a period of around 60 days to be ready to harvest. A separate pot with untreated soil served as control¹⁴⁻¹⁶.

Sample collection: Plant and soil samples were collected 60 days after sprouting. Plant metal content varies with time and

stage of maturity¹⁷. Hence 60 day period was kept fixed. The samples were collected carefully using plastic hand trowel to dig the soil around the plant and the plants were gently taken out, ensuring that no part of the root was lost. Plant samples were kept in separate self-sealing labelled polythene bags. The plant samples were cleaned initially with tap water and later by deionised distilled water. It helped to remove soil and dirt. The morphological plant part that is root, stem and leaf were then separated by cutting with a steel knife. Soil sample was collected from the same location where plant sample was taken out, up to a depth of 15 cm from soil surface. Samples were kept in self-sealing polythene bags and labelled properly. The plant and soil samples were analysed in the laboratory. Soil was cleaned for dirt, pebbles, plant material, etc. It was dried in oven at 80°C for total moisture removal. It was then passed through 20mm sieve using magnetic sieve shaker to collect fine soil for further processing.

Digestion and determination: 2g. of oven fried vegetation sample was kept for ashing in muffle furnace at 400°C. It was then digested using a mixture of 5ml HNO₃ and 1ml HClO₄. Digestion was performed on hot plate. It was continued till a minimal clear layer of acid was obtained. After cooling, this content was filtered. Final volume of 25ml was made in a volumetric flask with 0.25% HNO₃.

Soil digestion was done in Microwave digestion system (Ethos I Advanced unit). 2g. of oven dried soil was taken in Teflon containers and digested with a mixture of HNO₃: HF in the ratio 8:1. The digested content was centrifuged and filtered. Final volume of 25ml was made using 0.25% HNO₃.

Each sample of soil and vegetation was digested in replicate for consistency of results. The analysis of Pb and Cd was done using dp-ASV. Each sample was analysed twice to obtain typical results and the data reported in mg/Kg.

Results and Discussion

Physicochemical properties of soil: Free metal ion concentration depends on both total metal concentration of soil as well as a number of other factors¹⁸. The soil texture was sandy loam with an average pH recorded to be 7.26 (slightly alkaline). The soil pH was within the permissible levels for appropriate growth and efficient uptake of nutrients materials from soil¹⁹. Organic matter content of soil was determined to be 1.9 g%. The soil CEC was calculated to be 40.2 C mol/Kg. The initial lead concentration of uncontaminated soil was determined to be 0.57 ± 0.03 mg/Kg and the same for cadmium was detected to be 1.13 ± 0.04 mg/Kg. The Pb and Cd concentration of vermicompost and seeds were below detectable level.

Concentration of lead and cadmium in soil and vegetation samples: Metal concentration in soil is a dominant factor in soil to plant transfer of heavy metals²⁰. Heavy metal availability can

also be directly affected by plant itself²¹. The concentration of Pb and Cd in various vegetation tissues suggest that the metal concentration was detected to be maximum in roots followed by leaf and then in stem. It specifies that, stems of these leafy vegetables are merely transporters of metal from root to leaf through its conducting tissues. They themselves store minimal quantities of these heavy metals. Finsteret al²² while working on some fruiting plants and fruit vegetables also recorded highest metal concentration in roots. But subsequently higher concentration was seen in stem and then in leaf which is not observed in current study. This indicates the plant species variances and specificity towards certain metals. Symptoms of chlorosis or necrosis were not seen.

Concentration of lead and cadmium in roots of three plants:

The average concentration of lead in Spinach, Fenugreek and Red Amaranth was detected to be 14.22 ± 2.66 mg/Kg, 10.55 ± 0.11 mg/Kg and 14.26 ± 0.12 mg/Kg respectively. The concentration of the same in control set was determined to be 0.48 ± 0.03 mg/Kg, 0.55 ± 0.03 mg/Kg and 0.68 ± 0.05 mg/Kg respectively. As minimum as 20 times rise in metal concentration was detected in contaminated pot experiment as that of control. This shows that the three plant species actively absorb the metals within its roots if higher concentration of metal is supplied to them through soil. It can be seen from the results that the roots of spinach and red amaranth are similar in their property to absorb lead in its roots. Fenugreek however has shown that, its roots absorbed the least quantities of lead (10.55 ± 0.11 mg/Kg). Thus it can be concluded that among the three plant species, lead is absorbed feebly by Fenugreek, whereas Red Amaranth is a strong absorber of lead. Similar results were obtained by Mellem et al while working on Amaranthusdubius of South Africa¹⁶.

Cadmium concentration of roots was seen highest in Spinach (3.79 ± 0.06 mg/Kg) and the least by Red Amaranth (2.26 ± 0.07 mg/Kg). A similar pattern was observed in control experiment too. It showed a concentration 18 times that of the concentration in control (0.12 ± 0.03 mg/Kg). As per these results, Spinach is best absorber of cadmium while Red Amaranth is least. A common conclusion can be drawn that the three leafy vegetables are showing higher affinity towards lead than cadmium.

Concentration of lead and cadmium in stem of three plants:

The stems of the leafy vegetable plants serve the function of transfer of metals from roots to stem. They are thus an intermediate stage in metal transfer to leaves that are of nutritional important. The average lead concentration in stem was determined highest in Red Amaranth (1.95 ± 0.06 mg/Kg) while the least by Fenugreek (1.64 ± 0.05 mg/Kg). Hence the absorption pattern seen in root uptake in the three plant varieties was observed here too.

Table 1 and table 2 respectively show the concentration of lead and cadmium in soil before (control) and after contamination and the concentration of the metal uptake in root, stem and leaves of the three vegetable species, Spinach, Fenugreek and Red Amaranth.

Average concentration of cadmium in stem was found to be highest in Spinach (1.02 ± 0.09 mg/Kg) followed by Fenugreek (0.93 ± 0.06 mg/Kg) and the least by Red Amaranth (0.9 ± 0.06 mg/Kg). Hence the trend found in Cd concentration in roots was also found in stem of the three plant species. An approximate 8 times rise in Cd concentration was seen in spiked plants than that of control.

Table-1
Concentration of Lead (Pb) in morphological plant parts of Spinach, Fenugreek and Red Amaranth and corresponding soil samples

Sample	Spinach			Fenugreek			Red Amaranth			
	Soil	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Control	0.57 ± 0.03	0.48 ± 0.03	0.23 ± 0.02	0.21 ± 0.02	0.55 ± 0.03	0.13 ± 0.02	0.14 ± 0.03	0.68 ± 0.05	0.15 ± 0.03	0.14 ± 0.02
Set I	19.43 ± 2.36	18.17 ± 2.19	2.51 ± 0.06	2.11 ± 0.05	15.36 ± 0.09	1.91 ± 0.06	1.66 ± 0.05	20.12 ± 0.12	2.65 ± 0.09	2.11 ± 0.13
Set II	20.95 ± 2.19	11.93 ± 3.17	1.17 ± 0.03	3.12 ± 0.06	8.20 ± 0.15	1.36 ± 0.04	2.66 ± 0.03	11.34 ± 0.14	1.58 ± 0.05	3.33 ± 0.03
Set III	20.08 ± 3.15	12.55 ± 2.63	1.61 ± 0.06	3.60 ± 0.05	8.10 ± 0.09	1.64 ± 0.06	2.88 ± 0.06	11.31 ± 0.11	1.62 ± 0.06	3.60 ± 0.05
Avg.	20.15 ± 2.56	14.22 ± 2.66	1.76 ± 0.05	2.94 ± 0.05	10.55 ± 0.11	1.64 ± 0.05	2.40 ± 0.04	14.26 ± 0.12	1.95 ± 0.06	3.01 ± 0.07

Table-2
Concentration of Cadmium (Cd) in morphological plant parts of Spinach, Fenugreek and Red Amaranth and corresponding soil samples

Sample	Soil	Spinach			Fenugreek			Red Amaranth		
		Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Control	1.13 ± 0.05	0.27 ± 0.03	0.13 ± 0.05	0.12 ± 0.03	0.20 ± 0.04	0.11 ± 0.05	0.12 ± 0.05	0.12 ± 0.03	0.11 ± 0.03	0.07 ± 0.02
Set I	23.14 ± 0.16	3.69 ± 0.06	1.21 ± 0.09	1.12 ± 0.09	2.93 ± 0.11	0.95 ± 0.06	0.57 ± 0.06	2.12 ± 0.06	0.89 ± 0.06	0.56 ± 0.07
Set II	25.55 ± 0.15	3.85 ± 0.08	0.79 ± 0.08	1.49 ± 0.06	3.04 ± 0.10	0.95 ± 0.05	0.67 ± 0.05	2.25 ± 0.05	0.93 ± 0.07	0.67 ± 0.08
Set III	24.89 ± 0.13	3.83 ± 0.06	1.05 ± 0.09	1.56 ± 0.08	2.79 ± 0.08	0.89 ± 0.07	0.58 ± 0.07	2.40 ± 0.09	0.87 ± 0.04	0.74 ± 0.07
Avg.	24.53 ± 0.14	3.79 ± 0.06	1.02 ± 0.09	1.39 ± 0.08	2.92 ± 0.09	0.93 ± 0.06	0.61 ± 0.06	2.26 ± 0.07	0.9 ± 0.06	0.66 ± 0.07

Concentration of lead and cadmium in leaf of three plants:

Leaves of all the three plant varieties are nutritionally important to humans than stem and root. Due to their dietary significance, leaves are given utmost attention. In comparison with roots, the leaves of all plant species exhibited lesser concentration. The same tendency was observed in control experiments also. Thus the absorption pattern did not change at elevated metal concentration in soil. The leaves of Red Amaranth showed highest Pb concentration (3.01 ± 0.07mg/Kg) trailed by Spinach (2.94 ± 0.05 mg/Kg) and the least by Fenugreek (2.40 ± 0.04mg/Kg). Thus Red Amaranth is a better absorber of lead but Fenugreek, with the least metal uptake; is suitable for consumption. Concentration of Cd was reported highest in Spinach leaves (1.39 ± 0.08mg/Kg). Cd concentration in Fenugreek and Red Amaranth was seen approximately same. The results can thus be interpreted as among the three plants studied Spinach is the best absorber of Cd but considering dietary importance Fenugreek is better than others.

Hence the concentration of lead was found to be higher in Red Amaranth whereas the concentration of cadmium was detected higher in spinach. Similar results were obtained by Naser et al²³ while working on these two metals. The results are thus in good agreement. The variation in concentration of heavy metal between Spinach, Fenugreek and Red Amaranth species was due to their morphological differences, physiological alterations in terms of heavy metal content, omission, increased uptake, foliar absorption and retention within the matrix²⁴. Among the two metals, concentration of Pb was higher than that of Cd in vegetation samples. Demi rezen, and Aksoy also found a similar pattern while working of leafy vegetables of Turkey²⁵.

Transfer Factor (TF): The transfer factor (TF) is also called as Bioconcentration factor (BCF). This is an index demonstrating the potential of whole plant or its tissues to accumulate metal from soil. High TF value indicates suitability of the plant or its tissue for phytoextraction²⁶. Current study revealed that the TF values varied in the range of 0.02 to 0.71. All values were detected to be less than 1 indicating that none of the plant part is

hyperaccumulating the metals. In Pb, TF value was detected to be highest in roots while the least in stem. Leaves showed intermediate TF values. In case of Cd, roots of all the three plant indicated higher TF. TF of Spinach leaf was more than that of stem. On the contrary, the TF of stem of Fenugreek and Red Amaranth was higher than leaf. Thus it can be said here that, the roots of the three plant species that are nutritionally insignificant to humans are absorbing higher concentration of Pb and Cd. Whereas leaves of these plants that are of highest dietary importance are absorbing relatively lesser concentration of metals. The TF values for Pb were found to be higher than Cd indicating that the three plants show higher affinity towards Pb than Cd. Overall TF values are thus found to be significant.

Translocation Factor (TrF): The translocation factors of all the three plant species (Spinach, Fenugreek and Red Amaranth) for both the metals (Pb and Cd) have been mentioned in the table 4. Translocation Factors closer to 0, exhibit higher concentration of the metal retained in the roots instead of being translocated to shoots of the plant¹⁶. The results show that highest root to shoot Pb translocation was observed in Fenugreek (0.43) and the least by Spinach (0.34). Translocation of Cd was seen highest in Red amaranth (0.69) while the least by Fenugreek (0.53). Hence more than 50% of Cd is actively translocated from root to shoot. But more than 50% of Pb is retained in roots for all the three plant species studied. Thus Pb has higher affinity to bind in the roots while Cd is more mobile²⁷ with its efficient transfer to nutritionally important shoot parts. Plant roots work towards selective absorption of materials from soil and function as an obstruction for translocation of metals to above soil parts²⁸. This may be the reason for root to shoot movement blockage of lead. Cosio C. et al have specified that cadmium appears to be similar to other divalent ions that are necessary for cellular metabolism. Hence Cd uptake probably occurs through those carriers or channels that are actually meant for necessary ions²⁹. Hence despite of roots acting as barriers, due to mediation of carrier molecules Cd translocation in shoots may have increased.

Table-3
Transfer factor (TF) values of plant tissues of three plant species for metals Pb and Cd

Sr. No.	Vegetation type	Plant Tissue	Avg. concentration in vegetation		Transfer Factor (TF)	
			Pb (mg/Kg)	Cd (mg/Kg)	Pb	Cd
1	Spinach	Root	14.22 ± 2.66	3.79 ± 0.06	0.71	0.15
2		Stem	1.76 ± 0.05	1.02 ± 0.09	0.09	0.04
3		Leaf	2.94 ± 0.05	1.39 ± 0.08	0.15	0.06
4	Fenugreek	Root	10.55 ± 0.11	2.92 ± 0.09	0.53	0.12
5		Stem	1.64 ± 0.05	0.93 ± 0.06	0.08	0.04
6		Leaf	2.40 ± 0.04	0.61 ± 0.06	0.12	0.02
7	Red Amaranth	Root	14.26 ± 0.12	2.26 ± 0.07	0.71	0.09
8		Stem	1.95 ± 0.06	0.9 ± 0.06	0.1	0.04
9		Leaf	3.01 ± 0.07	0.66 ± 0.07	0.15	0.03

Note: Conc. in soil: Pb = 20.15 ± 2.56 mg/Kg and Cd = 24.53 ± 0.14mg/Kg, All values reported in dry by dry basis

Table-4
Root to shoot translocation factors (TrF) for three plant species and two metals Pb and Cd

Sr. No.	Vegetation type	Avg. Concentration in aerial plant part		Avg. Concentration in root		Translocation Factor (TrF)	
		Pb (mg/Kg)	Cd (mg/Kg)	Pb (mg/Kg)	Cd (mg/Kg)	Pb	Cd
		(a)	(b)	(c)	(d)	(a/c)	(b/d)
1	Spinach	4.71 ± 0.1	2.41 ± 0.17	14.22 ± 2.66	3.79 ± 0.06	0.34	0.64
2	Fenugreek	4.04 ± 0.09	1.54 ± 0.12	10.55 ± 0.11	2.92 ± 0.09	0.43	0.53
3	Red Amaranth	4.96 ± 0.13	1.55 ± 0.13	14.26 ± 0.12	2.26 ± 0.07	0.38	0.69

Note: All TF values reported in dry / dry basis

Table-5
Coefficient of simple correlation (r) between the concentrations of metals in plants tissues and corresponding soil samples

	Spinach		Fenugreek		Red Amaranth	
	Pb	Cd	Pb	Cd	Pb	Cd
Soil-Root	0.90	0.99	0.80	0.99	0.82	0.99
Soil-Stem	0.77	0.90	0.94	0.99	0.85	0.99
Soil-Leaf	0.92	0.98	0.92	0.99	0.93	0.98

In order to ascertain possible relationship between metal concentration of soils and plant parts analysed, correlation coefficient (r) between these parameters was calculated. The results have been displayed in table 5. The values have been found to be approaching 1. Hence, as per the calculations shown (table 5) across all vegetable samples, a positive relationship between total metal content in soils and vegetables has been obtained²³. The soil – root, soil – stem and soil – leaf relation was seen more promising for Cd with values of r in the range of 0.90 to 0.99. On the contrary, a higher deviation was seen in case of Pb with values of r detected in range of 0.77 to 0.94. For all vegetable types, highest correlation was exhibited by Cd rather than Pb.

Prevention of Food Adulteration Act, 1954 of India and its successive amendments describe the safe intake levels of Pb and Cd from leafy vegetables as 2.5 mg/Kg and 1.5mg/Kg respectively³⁰. The concentration of Pb was marginally lower in Fenugreek while that of Spinach and Red Amaranth was detected beyond the limiting value as compared with control. Pb concentration of control was well within permissible limits.

Hence, at elevated levels of metal concentration in soil, the plant metal concentration may increase beyond the limiting value. Thus it should be taken care that the inflow of metals in agricultural fields needs to be avoided. The concentration of Cd in both experimental as well as control plant leaves was well within prescribed limits. Since affinity by plants for Cd is less than that of Pb, the concentration has been well within the limits. But it is certainly contributing to accumulation of metals in human tissues through food chain transfer.

Conclusion

The lead and cadmium content of the three nutritionally important plant varieties namely Spinach (*Spinaciaoleracea*), Fenugreek (*Trigonellafoenum-graecum*) and Red Amaranth (*Amaranthuscruentus*). The study served its importance due to the dietary importance of the vegetables to humans. The transfer factor (TF) and translocation factor (TrF) were then calculated. It was found that the TF values ranged from 0.02 to 0.71, indicating that none of the three species are hyperaccumulators for the two metals. But the plants are certainly contributing to

the dietary intake of Pb and Cd through food chain transfer. Of the two metals, lead was absorbed by plants in higher concentration than cadmium. The TrF value revealed that the roots of the three plants that are of least dietary importance to humans are retaining higher levels of both the metals. On the contrary, the leaves that are of most dietary importance are absorbing the lower concentration of metal.

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