International Journal of Ecology and Environmental Sciences
www.ecologyjournal.in
Online ISSN: 2664-7133; Print ISSN: 2664-7125; Impact Factor: RJIF 5.28
Received: 26-06-2020; Accepted: 12-07-200; Published: 27-07-2020
Volume 2; Issue 3; 2020; Page No. 83-91

Phytoremediation of metal contaminated soil from Yamuna riverine ecosystem in Delhi region

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Abstract
Soil samples from selected site of Yamuna Riverine Ecosystem in Delhi Region were collected and analysed for various soil quality parameters and heavy metal concentration following standard testing procedures. Half of the Yamuna riverside soil was considered as unpolluted soil and other half was made artificially polluted by spiking of heavy metal salts Pb(CH3COO)2, CuCl2.2H2O, and ZnSO4.7H2O in a concentration of 0.6 gm/kg, 0.7 gm/kg, and 0.8 gm/kg of each of the salts. Experiment was carried out in18 pots sown with coriander and fenugreek seeds separately as well as in combinations. Metal concentration in plants and soil was analysed separately using AAS. Soil pH, NPK was significantly higher ($p<0.05$) for control soil than for the treated one. Organic carbon content had no significant variation ($p>0.05$) with respect to variation in the heavy metal contents in the soils. Soil biological activity, dehydrogenase and respiration were found varying significantly ($p<0.05$) due to the presence of heavy metals. Copper was observed significantly higher ($p<0.05$) in shoots of coriander plants in treated soil. Significantly higher ($p<0.05$) value of Zn was observed in shoots of fenugreek plants grown in treated soil pot and lower in shoots of coriander plants grown in control soil. In case of Pb accumulation, there was no significant variation ($p>0.05$). Accumulation of heavy metals (Cu, Zn, Pb) in roots of coriander and fenugreek plants showed differential behaviour with different treatments of the experimental soil. Significantly higher ($p<0.05$) Cu accumulation in the roots of coriander plants and Pb and Zn in roots of fenugreek plants grown in treated soil was observed. However, there was no significant variation ($p>0.05$) in Pb accumulation in the roots of both the experimental plants.

Keywords: coriander, fenugreek, heavy metals, phytoremediation, soil

Introduction

River Yamuna, the largest tributary of river Ganga in India, has gone polluted to the extent that taking a dip into the river might invite several risks to one's health. Since the last decade the water quality of the Yamuna river, despite huge expenditure and efforts put by the government and other concerned bodies, has deteriorated to a considerable extent. One of the compelling effects of the water pollution is the presence of heavy metals, which at high concentrations are detrimental to health and even toxic, affecting aquatic flora and fauna as well as human life [1, 2].Yamuna water contaminated with toxic and potentially toxic heavy metals also contaminated the riverside soils often used for crop production.

Soil is a natural, non-renewable resource grossly misused as "dumping ground" for by-products which are very toxic and often rich in heavy metals. Accumulation of heavy metals in soil takes place as a result of utilizing sewage water for a long period of time and results in high concentration of these metals in crops grown in this soil. Reclamation of this contaminated soil is the most serious concern and various approaches to reclamation used include biological and physiological ones.

Biological approaches are the natural way of reclamation of contaminated site involving the use of microorganisms to detoxify the presence of heavy metals by breakdown of potential toxic substances into their less toxic form or converting into non-toxic form and use of hyper accumulator plants which actively grow even in metal contaminated soil developing large biomass so as to remove heavy metal from soil by accumulating in aerial parts of the plants and sometimes restricting them in rhizospheric zone in roots, thus helping in reclamation of soil. Physiological approach includes solidification, sedimentation process, soil isolation, stabilization, leaching by acid solution, soil flushing process, vitrification, electro reclamation, and ion exchange methodology. High cost approaches have some side effect as well.

Apart from different traditional approaches, phytoremediation is the best approach as it is the easiest method, reliable, cost effective and eco-friendly approach towards contaminated soil. Phytoremediation is considered as the most promising as it is a plant-based approach with low maintenance and is a convenient technology to degrade contaminants including heavy metals from soil and water and hence helps in reclamation of metal contaminated soil.

Different approaches of phytoremediation include phyto volatilization, phytodegradation, phytoextraction, phytofiltration, phytotransformation involving different modes of action and different mechanisms for extraction of these metals from metal contaminated soil.

In phytovolatilization plants accumulate volatile compounds like mercury and hydrocarbon through roots into aerial part from contaminated soil and transpire some of compounds or metabolites through leaves into atmosphere. Phytodegradation involves the use of microorganisms or plants to degrade the harmful pollutants. Phytoextraction use of plant to hyperaccumulate metals from contaminated soil or water in aerial

part of plant and hence it can be removed and burned or disposed-off away from that site. Phytotransformation is also called phytodegradation involving breakdown of organic contaminants as a result of metabolic process or enzymes produced by a plant. As a result of transformation, heavy metals are converted into less toxic or non-toxic form. Phytoextraction capacity of a plant can be boosted up by use of organic and inorganic agents which enhance the solubility of heavy metals in contaminated soil. With applications of citric acid or sulphur in plants, the concentration of zinc cadmium and uranium was increased significantly in plant biomass [3, 4, 5, 6, 7]. About 45 families and 400 species of plants are identified being hyperaccumulators, that is, the plants which actively grow in metal contaminated soil and have the capacity to accumulate metal concentration present in soil into aerial part of plant at faster rate. Some of the families include Asteraceae, Apiaceae, Brassicaceae, Scorphulacea, Fabaceae, Euphor biaceae, and Laminacea.
With a viewpoint of above background, the present investigation was carried out using Coriander and fenugreek plants for revealing the phytoremediation potential in the removal of metal contaminated soil from Yamuna river bank in Delhi.

2. Materials and Methods

2.1 The Study Area

The area under study was Yamuna river bank (28°32'54.28" N and 77°18'23.53" E) in Delhi at its location of Okhla barrage. Five soil samples were collected randomly from the Yamuna bank at Okhla barrage and its nearby areas in Delhi region in the month of February 2019. All soil samples were mixed together to form a sample pool and bought to laboratory of G.B.P.U.A.T, Pantnagar. The samples were air-dried in open condition and all impurities like stone, pebbles, plant residues were removed. Air-dried soil samples were sieved in 2 mm sieve so as to get fine soil and filled in pots separately (control soil and treated soil). Nine pots were filled with control soil and 9 were filled with treated soil. Three kg of soil was placed in each pot and 18 pots in all were filled so as to proceed with experiment.

2.2 Experimental Design

Seeds of Coriander variety *Pant Haritma* and Fenugreek variety *Pusa Early Bunching* were bought from Vegetable Research Centre of Pantnagar University and sown in control and treated pots. Half of the Yamuna riverside soil was considered as unpolluted soil and other half was made artificially polluted by spiking of heavy metal salts $Pb(CH_3COO)_2$, $CuCl_2.2H_2O$, and $ZnSO_4.7H_2O$ of a concentration of 0.6 gm/kg, 0.7 gm/kg, and 0.8 gm/kg of each of the salts (Figure 1).

Fig 1: Experimental design

2.3 Soil and Plant Analysis

Effect of high concentration of heavy metals on various soil quality parameters like soil biomass nitrogen, phosphorus, potassium, soil organic carbon, soil dehydrogenase activity, soil respiration activity and metal concentration in soil were analyzed following standard testing procedures.
Metal analysis in soil was done twice, first at the time of sowing and second after harvesting of plant so as to know the metal content present in soil and metal which is accumulated by plant. Dried samples of plants and soil, each weighing 0.05 g, were digested with acid, 15 ml HNO_3, H_2SO_4 and HCl (5:1:1), by maintaining constant temperature of 80^0C on a hot plate (Figure 2). The digestion was continued until a transparent solution was obtained. It was allowed for few minutes to cool down and was filtered through Whatsman no. 42 and diluted by adding distilled water, making volume up to 50 ml. Metal concentration in plant as well as soil was analyzed separately using atomic absorption spectroscopy (Figure 3).

Fig 2: Plants grown on control and treated pots and sample preparation on hot plate

Fig 3: Heavy metal (Cu, Pb and Zn) analysis of samples

2.4 Statistical Analyses of Data

All the data were taken in triplicates and analyses was done with the help of Opstats statistical software using Customized Random Design (CRD) by applying one way Anova. All results were expressed in terms of standard error of mean (SEm), critical difference (CD) at level of significance 5%.

3. Results and Discussion

3.1 Physico-chemical analysis of the soil

Biological, chemical and physical characteristics of soil are much dependent upon the presence and concentration of heavy metals. Bioavailability of these potential toxic metals is favoured by various soil factors like soil pH and the organic content present within soil. Solubility of these metals in soil is dependent upon the soil properties like soil pH, cation exchange capacity, organic carbon content present within soil, oxidation state of different minerals and metal concentration within soil [8]. The parameters related to present research for soil are pH, Nitrogen, Phosphorus, Potassium, Carbon, Respiration, Dehydrogenase and accumulation of Zn, Cu and Pb in plants. Heavy metals were more soluble in acidic pH. The concentration of toxic metals is more in acidic soils. The metal availability in soil leads to faster accumulation of these metals into plant. There are many factors responsible for retention and solubility of these metals in soil. Among all the factors, pH has its maximum effect.The soil was found acidic and with artificial application of heavy metals in treated soil reduction of soil pH was noticed. It was observed that pH of the soil from the Yamuna River bank (the control) was slightly acidic. The highest pH was observed in coriander-grown in control soil (6.55) and lowest pH (6.173) was observed in soil that was treated one, having both coriander and fenugreek grown together in the same pot. Using one-way Anova, it was analyzed that pH was significantly higher ($p<0.05$) in control soil and lower in artificially contaminated treated soil (Table 1 and Figure 4).The experiment revealed that there was a decrease in the availability of nutrients in soil treated with heavy metals which might be due to the reason that the presence of high concentration of heavy metal contamination leads to inactivation or reduction in the populations of soil microorganisms that are mainly responsible for soil nutrient transformation. Microorganisms help in enrichment of soil and fixation of soil nutrients by converting their organic form into inorganic forms [9]. Higher value of phosphorus was found for control soil (9.572 kg/ha) in which coriander was grown while lower value was found for treated soil (4.697 kg/ha) in which coriander and fenugreek were grown together in same pots.

Table 1: pH, Nitrogen, Phosphorus, Potassium (kg ha^{-1}) and soil organic Carbon (%) in the control and treated soils used for experiment

Treatment	pH	N	P	K	C (%)
Coriander in control soil	6.55	123.767	9.572	263.984	0.603
Coriander in treated soil	6.20	87.470	5.122	237.776	0.600
Fenugreek in control soil	6.54	123.317	9.333	262.080	0.597
Fenugreek in treated soil	6.183	87.400	5.152	237.478	0.587
Coriander+ Fenugreek control	6.533	126.070	8.801	264.135	0.593
Coriander + Fenugreek treated	6.173	86.333	4.697	236.955	0.586
CD (p=0.05)	0.245	6.027	3.558	5.493	N/A
SEm±	0.80	1.935	0.179	1.763	0.005
CV	2.144	3.169	4.362	4.220	1.315

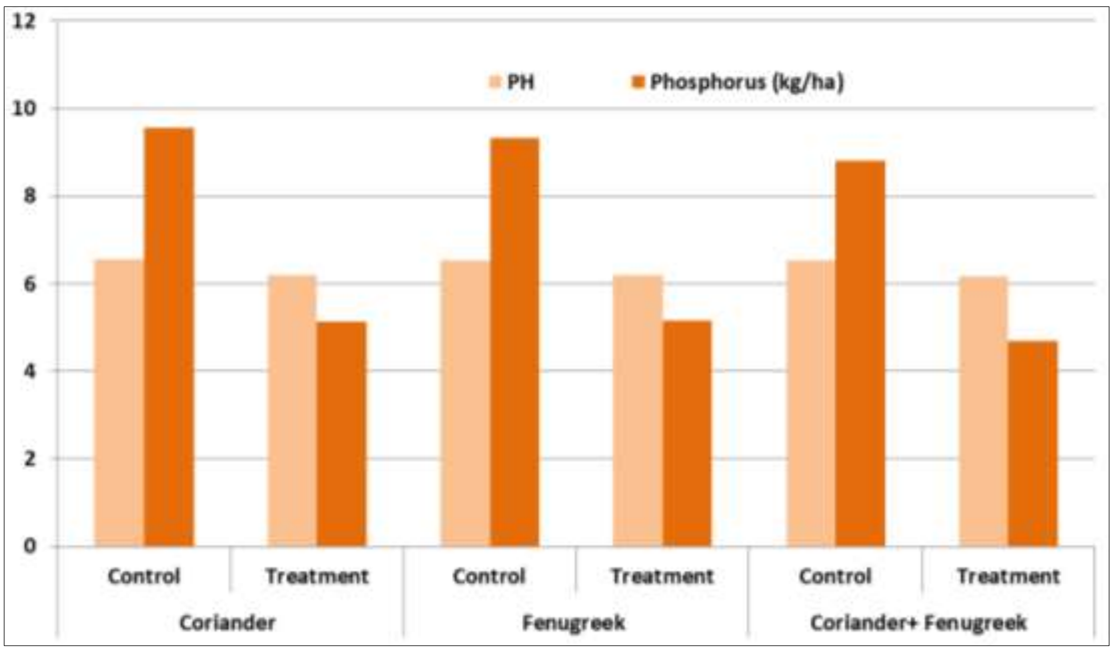

Fig 4: pH and Phosphorus content in experimental plants

It was also observed that high value of nitrogen was 126.070 kg/ha for control soil having coriander and fenugreek grown together and lower value was 86.333 kg/ha for treated soil having coriander and fenugreek together in the same pots whereas Potassium registered higher value (264.135 kg/ha) for control soil in which both plants were grown together in the same pots and lower value (236.955 kg/ha) for treated soil in both plants grown together in same pots (Table 1 and Figure 5). By one way Anova it was analyzed that macronutrient content was significantly ($p<0.05$) higher in control than in the treated soils.

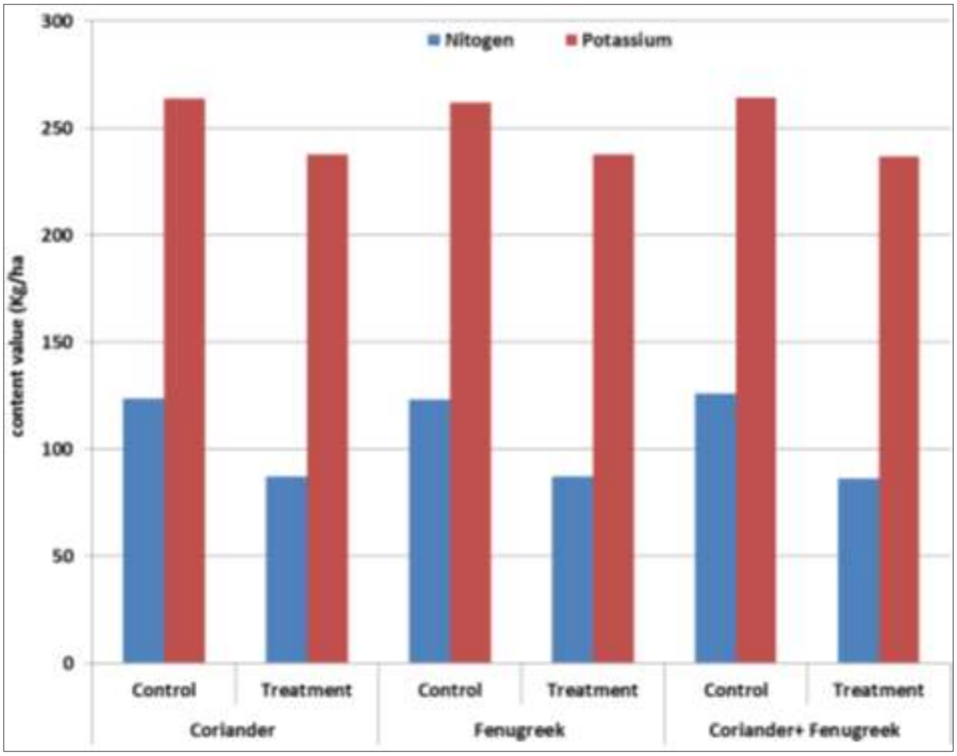

Fig 5: Nitrogen and Potassium content in experimental plants

The three macronutrients most essential for plants' optimum growth and development are nitrogen phosphorus and potassium, commonly known as NPK. A great deal of nitrogen fixation into soil is carried by specific bacteria. Phosphorus is essential for root development of crops, ripening of fruits and development of tolerance within plants towards drought conditions. Phosphorus acts as a limiting factor for all crops generally agricultural crops and even in forage production. Potassium helps in translocation of photosynthetic energy within plants. The energy production during photosynthesis process is stored in plants and potassium regulates this energy in plants for their reproductive processes and for optimum growth. It also provides tolerance to water stress conditions, pest attack and even to frequent temperature fluctuations.

High amount of potassium in soil ensures the plant affinity to grow more smoothly and outgrow pest diseases and provide tolerance to winter freezing. In the absence of these nutrients in soil these are artificially amended in soil as nitrogen, phosphorus and potassium fertilizers to increase soil fertility. The more the soil polluted with heavy metals the more the reduction in carbon and nitrogen contents. Soil organic matter plays a very crucial role in the maintenance of soil fertility and, as a result, in boosting crop yields.Experimental data revealed that the soil organic carbon decreased in artificially treated soil and the lowest amount observed was in pots where hyperaccumulators, both coriander and fenugreek, were grown together. This might be due to the fact that both plants might have generated stress on soil, decreasing microbial activity and ultimately organic matter content. The organic matter content was slightly higher in control soil in compared to that in treated soil. The organic content was higher in coriander grown in control soil and lowest in pots having both coriander and fenugreek plant grown together in the same pot (Table 1, Figure 6). By one-way Anova, however, it was analyzed that there was no significant difference in the values of organic carbon percentage ($p>0.05$).

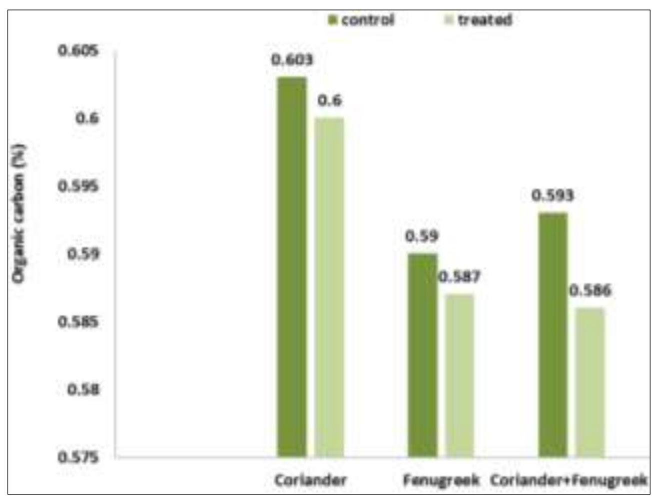

Fig 6: Organic carbon present in experimental pots

Organic carbon is the most essential property of soil as it influences the plant growth and its development by increasing the nutrient availability within soil as a result of mineralization process and serves as major source of the energy for microorganisms present within the soil. Its presence is a result of decomposition of root exudates, residue of plants, soil biota and death and decay of organisms. In a research it was observed that when Pb metal was present in low concentration in soil (500 mg/kg), there was massive increase in biological activity but when its concentration was in high amount, it had negative impact on soil biological activity [10]. It was observed that highest soil respiration value was 23.697 µgCO_2/g/day for control soil in which coriander and fenugreek were grown together in the same pots whereas low total soil respiration value was found in treated pots (10.513 µgCO_2/g/day) having both plants grown together in the same pots whereas soil dehydrogenase activity found higher for control soil (126.070 µg TPF/g soil/day) and lower for treated pots (86.333 µg TPF/g soil/day) in which both plants were grown together (Table 2 and Figure 7). By one way Anova it was analyzed that soil respiration and soil dehydrogenase were significantly ($p<0.05$) higher for control soil than for treated soil. Soil respiration can be used as one of the most important indices for checking the soil biological activity. Soil respiration regulates carbon cycling, soil organic carbon pool and maintenance of carbon flux between an ecosystem and the atmosphere. Basically, it includes all microbial, organic matter and the root respiration all being very useful in the release of CO_2 [11]. Soil dehydrogenase is an index of soil function of number of micro-organisms present within the soil along with their physiological efficiency. Soil respiration can be used as an important indicator of biological activity of soil as it shows the physiological efficiency of microorganisms. Soil dehydrogenase is the most popular test for testing influence of heavy metal concentration on soil microbial activity [12].

Table 2: Soil respiration and soil dehydrogenase activity

Treatment	Soil respiration (µg CO_2/gm/day)	Soil dehydrogenase (µg TPF/g soil /day)
Coriander in control soil	22.833	123.317
Coriander in treated soil	11.257	87.470
Fenugreek in control soil	22.680	123.767
Fenugreek in treated soil	11.130	87.400
Coriander+ Fenugreek control soil	23.697	126.070
Coriander+Fenugreek treated soil	10.513	86.333
CD (p=0.05)	1.881	6.027
SEm±	0.604	1.935
CV	6.085	3.169

Heavy metals in high amount affect soil biota activity and diversity and often get accumulated on organic top soil [13]. In an earlier experiment, it was reported that on comparison of agriculture land and polluted land, dehydrogenase activity of soil was greatly reduced to 10-90% on increasing contamination of heavy metals [14]. Copper concentration (100 mg kg^{-1}) was reported to be sufficient for significant reduction in the soil dehydrogenase activities [15].

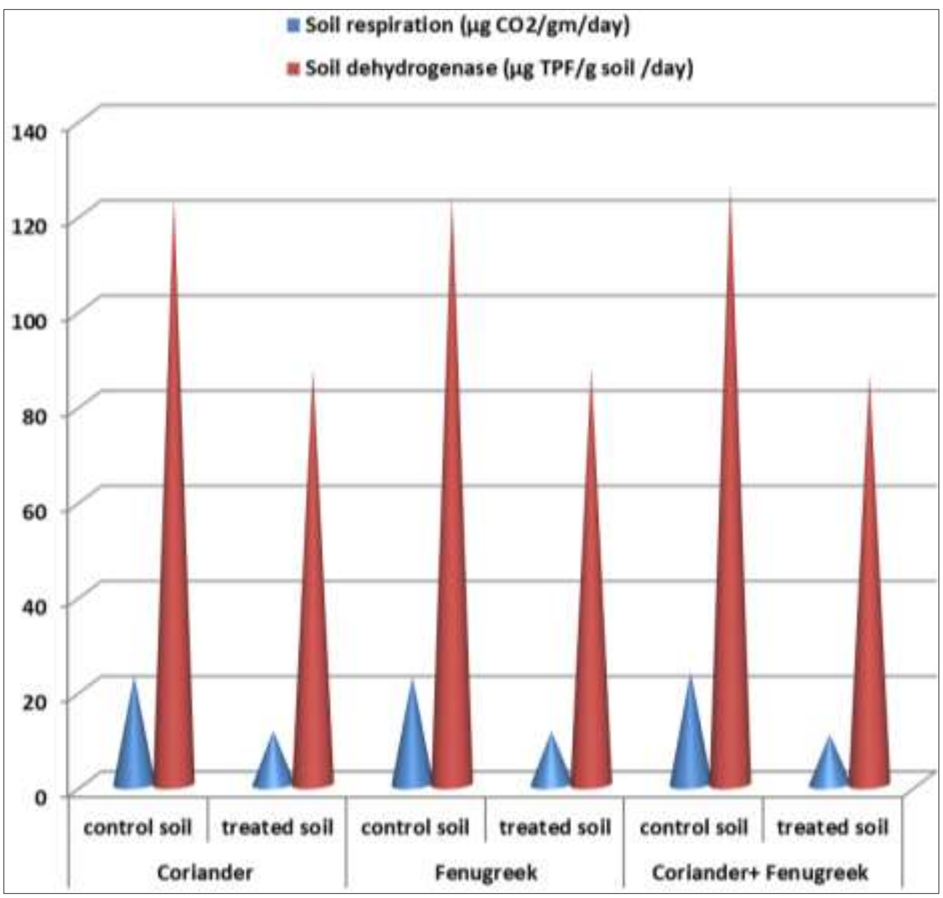

Fig 7: Soil respiration and soil dehydrogenase under controlled and treated conditions

3.2 Heavy Metal Concentration in Control and Treated Soil

Some metals are essential for plants in low concentration even though their presence in high amount leads to toxicity and deterioration of soil quality by disturbing soil fertility, ultimately leading to crop yield reduction. Few hazardous heavy metals include copper (Cu), cadmium (Cd), nickel (Ni), zinc (Zn), lead (Pb), arsenic (As). Their presence in high amount is very toxic to plants [16]. Absorbed from the soil as observed before sowing and harvesting of plants in the pots (Figure 8), these metals get accumulated from the roots to upper parts of the plants. Upon consumption by herbivores they create potential to reach top of the tropic level [17].The heavy metal presence in soil was analyzed by using AAS (Atomic Absorption Spectrophotometer) before sowing and after harvesting of crop.

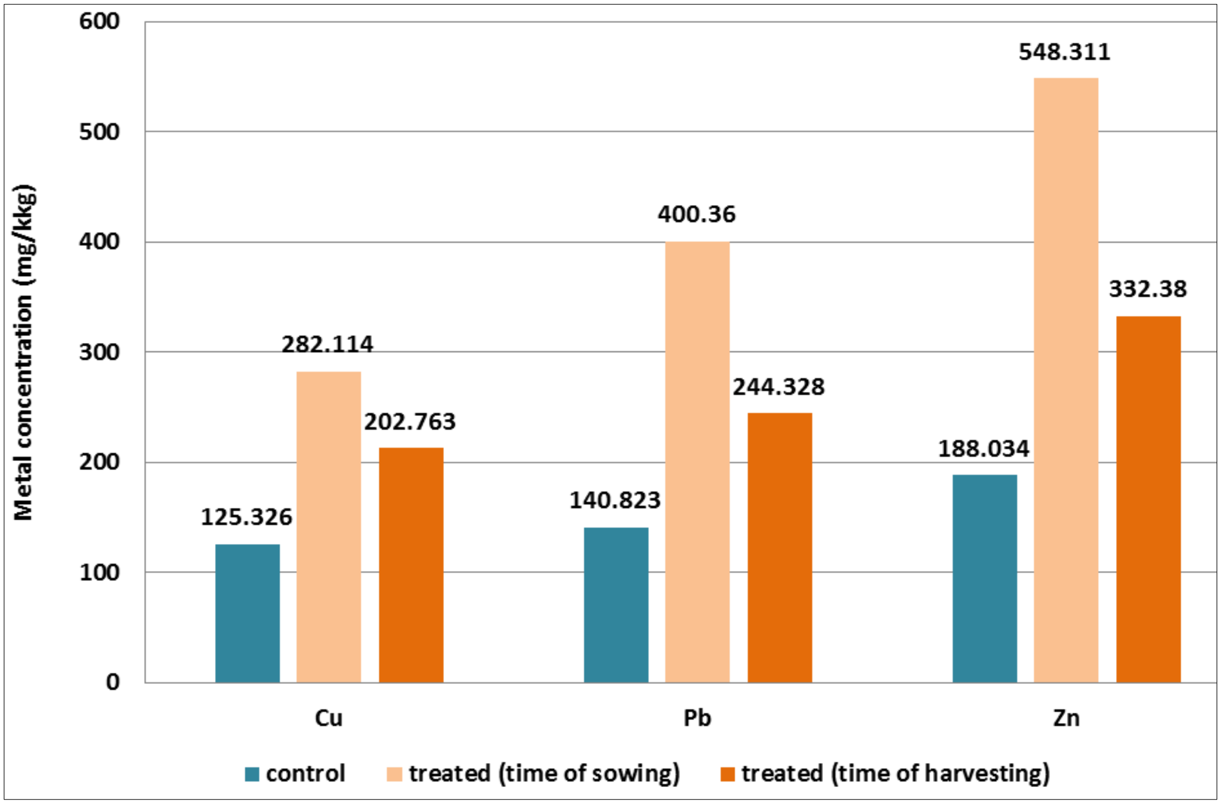

Fig 8: Heavy metal concentration in control, treated soil at the time of sowing and harvesting

3.3 Accumulation of Copper, Lead and Zinc Metals from Soil in Shoots and Roots

Coriander and fenugreek plants are well known metal hyperaccumulators, having special tendency to accumulate heavy metals from soil to different parts of a plant. Shoots and roots of coriander and fenugreek plants were analyzed separately for control and treated soil to understand the accumulation of different metals in different plant parts from control and treated soils. The plants grown together in control soil and treated soil were analyzed separately to show comparison which plant accumulated which metal and the metal concentration accumulated by both the plants. In our experiment related to hyperaccumulation potential of coriander and fenugreek plants in its shoots, it was observed that maximum value of accumulated Cu in the shoots was 25.582 mg/kg in coriander in treated soil and minimum was 18.562 mg/kg in fenugreek plants grown together with coriander plants in the same pots in control soil (Table 3 and Figure 9). Maximum content of Pb (41.352 mg/kg) was in the shoots of coriander plants grown in treated soil and minimum (29.312 mg/kg) in fenugreek plants grown in control soil. Zn accumulation was of highest value (46.931mg/kg)in the shoots of fenugreek plants grown in treated soil and of lowest value (40.038 mg/kg)in the shoots of coriander plant grown in control soil (Figure 9).

Table 3: Cu, Pb and Zn accumulation in coriander and fenugreek shoots (mg/kg)

Treatments	Copper	Lead	Zinc
Coriander-control (shoots)	19.999	34.704	40.038
Coriander-treated(shoots)	25.582	41.352	45.603
Fenugreek-control(shoots)	19.448	29.312	44.846
Fenugreek-treated(shoots)	22.464	35.033	46.931
Coriander+fenugreek-Control (coriander shoots)	19.963	34.528	40.237
Coriander+fenugreek-treated (coriander shoots)	22.221	39.550	44.338
Coriander+fenugreek-control (fenugreek shoots)	18.562	30.427	40.660
Coriander+fenugreek-treated (fenugreek shoots)	20.458	33.768	45.034
CD (p=0.05)	2.548	N/A	4.001
SEm±	3.893	6.942	3.323
CV	6.923	8.492	5.273

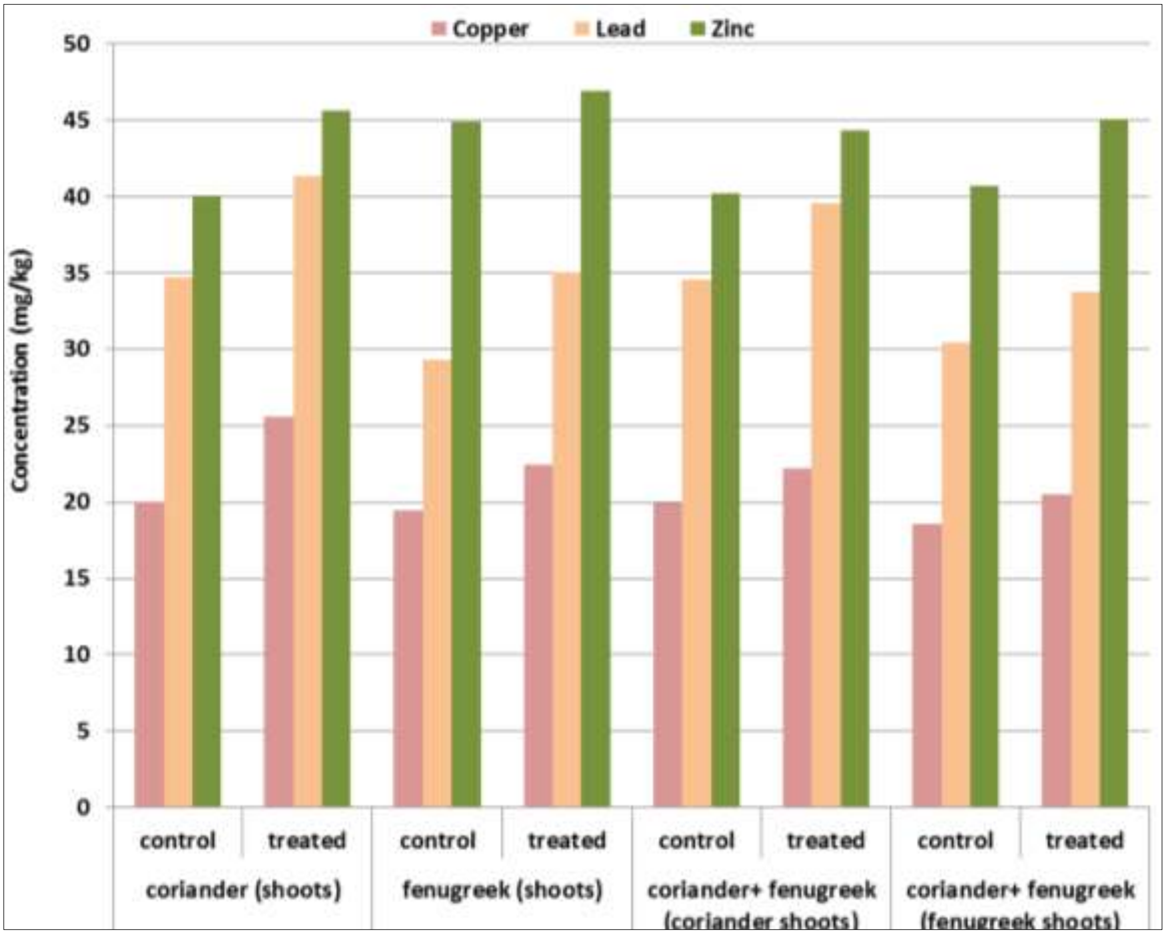

Fig 9: Copper, Lead and Zinc concentration in shoots of control and treated plants

Cu was found significantly higher ($p<0.05$) in shoots of coriander plants grown in treated soil and Zn was significantly higher ($p<0.05$) in shoots of fenugreek plants grown in treated soil. Cu was found significantly lower ($p>0.05$) in fenugreek shoots grown with coriander plants in control soil and Zn was found lowest in coriander plants grown in control soil. No significant variation was observed in accumulation of Pb in all treatments (Table 3). Roots constitute the region from where these heavy metals enter into plants from the soil. Roots translocate these metals to aerial parts of a plant. Coriander plants accumulated more heavy metals in shoots than in roots, whereas fenugreek plant accumulated more in roots than in shoots.

Table 4: Cu, Pb and Zn accumulation in coriander and fenugreek roots (mg/kg)

Treatments	Copper	Lead	Zinc
Coriander-control (roots)	28.104	48.277	45.180
Coriander-treated (roots)	32.729	54.009	48.890
Fenugreek-control (roots)	27.412	55.294	50.288
Fenugreek-treated (roots)	30.570	57.488	54.812
Coriander+fenugreek-control (coriander roots)	21.494	52.213	42.949
Coriander+fenugreek-treated (coriander roots)	23.964	58.507	44.776
Coriander+fenugreek-control (fenugreek roots)	19.497	59.485	47.436
Coriander+fenugreek-treated (fenugreek roots)	24.806	58.922	52.105
CD(P=0.05)	3.553	N/A	5.545
SEm±	1.175	7.241	1.834
CV	7.807	8.587	6.575

Copper accumulation exhibited maximum value (32.729 mg/kg) for coriander plant roots grown in treated soil and minimum value (19.497mg/kg) for fenugreek plant roots grown with coriander in the same pots in control soil (Tables 4 and Figure 10).Accumulation of lead had maximum value (59.485 mg/kg) for the roots of fenugreek plants grown together with coriander plant in the same pots in treated soil and minimum value (52.213 mg/kg) for coriander roots when grown together with fenugreek plants in control soil. Zinc accumulation showed maximum value (54.812 mg/kg)for fenugreek plant roots grown in treated soil and minimum value (42.949 mg/kg) for coriander plant root grown together with fenugreek plant in the same pots in control soil.

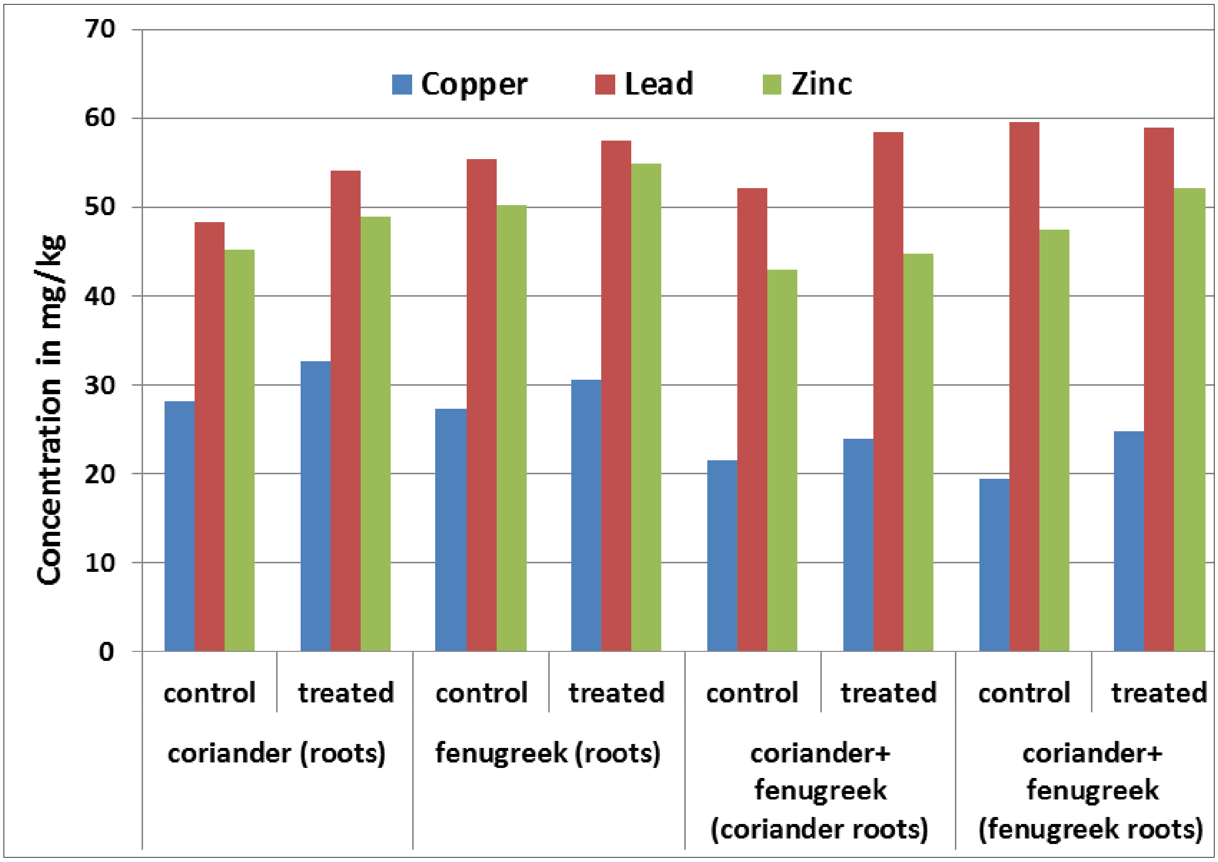

Fig 10: Copper, Lead and Zinc concentrations in roots of control and treated plants

It was clearly revealed that lead and zinc were accumulated in higher concentrations by fenugreek roots and copper was accumulated in higher concentrations by coriander roots. However copper and zinc were found significantly ($p<0.05$) higher in treated soil whereas no significant variation ($p>0.05$) was observed in lead in all treatments of experiments.The data generated in the experiment corroborates with those of [18] in the sense that the nature of heavy metal accumulation by different hyperaccumulator plant species is indifferent. In the process of phytoremediation of heavy metals, the selection of the hyperaccumulators plants would depend on the agro-climatic area they occur in.

4. Conclusion

The accumulation of heavy metals in higher concentration in the riverside soil in Yamuna riverine ecosystem in Delhi Region is as a result of continuous irrigation from metal-contaminated Yamuna river. High metal concentration in soil above permissible limits is harmful to soil, plants and, ultimately, to human health. Phytoremediation is a noble, inexpensive and eco-friendly approach to reduce toxicity of these metals in soil by their accumulation in plant parts. Coriander and fenugreek, the two heavy metal accumulator plant species, can serve as promising crops in reducing heavy metal concentration in the soil. It must be kept in mind that heavy metal contaminated soils must not be used for growing food crops, or the consumers might suffer from health hazards as the foods derived from these crops would carry heavy metals to their bodies. In this experiment the vegetable crops were grown purely for soil remediation purpose. Products to be derived from these experimental crops were not used for consumption purpose. Further, it can also be suggested that the hyperaccumulator plants used for consumption purpose should not be cultivated in the soils infested with heavy metals. There should be stringent legal rules to protect our rivers and other sources of irrigation and water supplies from getting polluted.

5. Acknowledgments

The authors gratefully acknowledge the financial support provided by the University Grants Commission against the project UGC MRP-MAJOR-ENVI-2013-36432.

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