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# In-vitro Assessment of Heavy Metal Removal from Contaminated Agricultural Soil by Native Plant Species

Shazia Akhtar* and Shazia Iram

*Fatima Jinnah Women University, The Mall, Rawalpindi, Pakistan.*

*Corresponding Author Email: shazoo_786@yahoo.com

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**Abstract**

Soil pollution by toxic metals such as cadmium (Cd), lead (Pb), chromium (Cr) and copper (Cu) is a major problem in different agricultural areas of Pakistan especially Gujranwala and Lahore. The objective of this study was to assess the accumulation and uptake of toxic metals from contaminated soil by using local plants such as Soybean (*Glycine max*), Barley (*Hordeum vulgare*), Sunflower (*Helianthus annuus*), Pearl millet (*Pennisetum americanum*), Maize (*Zea mays*), Canola (*Brassica napus*) and Wheat (*Triticum aestivum*). This study was based on the comparison of heavy metal concentrations and their accumulation in different parts of plants. In-vitro experiments were conducted by collecting soil from peri-urban areas of Gujranwala and Lahore being irrigated with untreated wastewater. Different seed varieties of local plants were brought from sale shop at National Agriculture Research Center (NARC) Islamabad. Seeds were germinated and grown in laboratory in ambient environment. Plants were irrigated with different levels of each toxic metal and a control was also run. Plant samples were harvested on 20$^{th}$ day and heavy metal concentrations were checked in roots and shoots of plants. Accumulation of heavy metals in the shoots and roots of local plants was measured using Flame Atomic Absorption Spectrophotometer (FAAS). The evidences provided by this experiment indicated that native crops like maize and canola were effective for phytoremediation of toxic metal polluted soil. In the future further experiments should be performed in order to investigate the phytoremediation potential of these crops for metals.

***Keywords:*** Heavy metals, Native crops, Phytoremediation, Soil.

## Introduction

Heavy metals are natural constituents of earth crust and they are generally found in low concentration. Anthropogenic activities have also increased metals concentration in the environment [1]. Due to rapid growth of population, industrialization, urbanization and increased agricultural practices environmental pollution is also raising. Since 1900 heavy metals pollution has increased sharply [2]. These heavy metals are difficult to destroy biologically however they can be transformed from highly toxic form to less toxic form [3]. Some heavy metals are essential for global ecosystem. These metals exist in the environment with different oxidation states and their oxidation number is related to their toxicity. These heavy metals are ultimately derived from different sources and affecting the whole ecosystem [4]. In Pakistan like other developing countries laws enforcement regarding industrial waste disposal is ineffective and a large number of industries dump their waste into fresh water, which in turn is responsible for soil pollution [5]. Industrial waste contain high concentrations of heavy metals, when added to water bodies and dispersed in irrigation water caused serious type of environmental pollution, causing bad effects on whole biota and ultimately causing a threat to the human being [6]. Parsad and Parsad [7] reported that heavy metals accumulation in nutrient solution and soil results in impaired metabolism and growth retardation in plants. Pakistan peri-urban agricultural soils often received contaminated waste water, polluted or loaded with toxic heavy metals [8].

Some heavy metals are biologically essential even then in excess they become toxic strongly, such type of metals pollution causes inhibition of plants growth and it is highly toxic to plants cells and cause death [9]. McGrath *et al.*, [10] reported that high metals concentration in soil can decrease microbial activities, fertility of soil and also crop yield. Steffens [11] described that near mining operations, landfill or waste disposal sites, on some agricultural sites and natural soil sites plant toxic and lethal metals levels were experienced.

In Pakistan, the use of plant species to decontaminate and remediate polluted soils with heavy metals is very scarce and limited. In Pakistan, study area Gujranwala is also receiving industrial and domestic waste water as an irrigation source. Rhoades [12] has suggested effective species mustard (*Brassica juncea* L.) has potential of concentrating Se in shoots. Some researches has depicted that *Thlaspi caerulescens* can accumulate Cd in shoots [13]. Similarly *Berkheya coddii* can hyper accumulate Ni and have ability to reclamate moderately polluted soil with two crops [14]. Sunflower can also be used for metals polluted soils [15]. Due to high biomass production of sunflower it can be used for phytoextraction process. This study was planned to check the root shoot biomass production and heavy metals uptake by native plants species in metals contaminated soils in laboratory.

## Materials and Methods

### *Soil selection and preparation*

Metals contaminated soil samples of Gujranwala were selected for further experimentation. This soil was selected due to higher level of contamination and bulk sample was taken for the experiments. The soil was air-dried on open air, grinded and sieved through a 2-mm sieve for further experimentation.

### *Seed germination*

Seeds of native plants (vegetable and crops) of Gujranwala were surface sterilized in 1.5% sodium hypochlorite for 5 minutes then washed thoroughly with distilled water (Gupta, 2003). Then seeds were placed on filter paper in Petri dishes containing 20 mL distilled water for 48 hours. Every plant seeds were sown in triplicate under laboratory conditions. Seeds were considered germinated when their radical was at least 2 mm out.

### *Plant growth*

After germination plants were shifted in plastic cups having 75 grams soil and allowed to grow in-vitro under condition of constant amounts of water dissolved urea and sulphate of potash (SOP). On 20$^{th}$ day plant samples were analyzed for heavy metal concentrations because they received maximum heavy metals in the soluble form and had large biomass production. Both shoot and root portions of plant samples were processed for total heavy metal analysis [16]. All the collected plant samples were washed thoroughly under running tap water and distilled water so that no soil particles remained, air dried and then oven dried at 70$^{o}$C for 10 hours. After that dried roots and shoots were grinded separately with pestle and mortar. The grinded plant samples (0.25g) were added in 10 mL of double acid $HNO_3$:$HClO_4$ (2:1 ratio) in conical flask (100 mL) and placed on hot plate in fume hood at 250$^{o}$C. After 3 hours plant samples were digested and all samples were filtered and diluted up to 15 mL [16]. Filtered samples were analyzed for heavy metals Cu, Pb, Cd, and Cr. For quantification, stock solutions were prepared using salts of corresponding metal ions; $CuSO_4$, $Pb(NO_3)_2$, $Cd(NO_3)_2$ and $Cr(NO_3)_3$ of analytical grade supplied by Merck and Sigma Aldrich. Concentration (0.5–4 µg/mL) vs absorbance calibration graph were constructed by running various concentrations of each metal ion on Flame Atomic Absorption Spectrophotometer using air-acetylene as flame (PerkinElmer Analyser 800 model). Regression (R) values were in the range of 0.997-0.999.

## Results and Discussion

Present study consisted of laboratory experiments in which the ability of local plants to uptake and accumulate toxic metals from the contaminated soils of Gujranwala and Lahore was checked. Seeds of Wheat (*Triticum aestivum*), Soybean (Glycine max), Barley (*Hordeum vulgare*), Sunflower (*Helianthus annuus*), Pearl millet or Pearl millet (*Pennisetum americanum*), Maize (*Zea mays*) and Mustard (*Brassica campesstris)* were grown in-vitro.

### *Growth of native plant roots and shoots*

Shoot length of native plants is presented in (Table 1). Maximum shoot length was shown by barely crop at 150 mg $kg^{-1}$. On the other hand minimum shoot length was shown by mustard crop. The order of shoot length of remaining crops was wheat, maize, pearl millet, sunflower and soyabean. Maximum shoot length of wheat (12cm) was noticed at 50 mg $kg^{-1}$ of Cu concentration. In case of barely shoot length was almost similar at 0, 50 and 150 mg $kg^{-1}$concentration of Cu, but length reduced at 250 mg $kg^{-1}$. It was shown that Cu application enhanced barely growth at lowest concentration and become toxic at higher concentration (250 mg $kg^{-1}$). By growing maize crop in laboratory, it was observed that control pot showed more shoot growth than Cu applied pots (50, 150 and 250 mg $kg^{-1}$). Minimum growth was noticed at 250 mg $kg^{-1}$ which was 1.1 cm. Maximum shoot length of pearl millet (20.60 cm) was noticed at 150 m.

Maximum root length was shown by barely crop then mazie, pearl millet, wheat, sunflower, soyabean and mustard. Wheat root length was observed maximum (7.6 cm) at 50 mg $kg^{-1}$ concentration of Cu and then the length was reduced when concentration was increased. Maximum barely root length was observed at control. Maximum maize growth was observed at mg $kg^{-1}$. Similar was the case of sunflower and pearl millet. However mustard and soybean growth was enhanced at 50 mg $kg^{-1}$ (Table 2).

In the present study mustard root and shoot lengths and their fresh and dry weights were decreased with the treatment of higher Cu, Cd, Pb and Cr. This finding is supported from the results of Turan and Esringü [17] that adverse effects of Cu on roots are related to severe reduction in the elongation growth of the longest root as well as root plasma membrane permeability of the seedlings.

***Table 1.*** **Effect of different concentrations of metals on native plants shoots length.**

| Native Crops | Metals mg $kg^{-1}$ | Shoot length (cm) | | | |
|---|---|---|---|---|---|
| | | ***Cu*** | ***Cd*** | ***Cr*** | ***Pb*** |
| **Wheat** | **Control** | 10.8 | 21.8 | 7.2 | 16.0 |
| | **50** | 12.0 | 21.2 | 19.2 | 15.4 |
| | **150** | 9.3 | 18.2 | 10.2 | 20.7 |
| | **250** | 7.7 | 0.0 | 2.2 | 1.3 |
| **Barely** | **Control** | 22.2 | 19.4 | 21.7 | 20.0 |
| | **50** | 22.2 | 17.0 | 17.3 | 20.7 |
| | **150** | 22.1 | 22.8 | 20.7 | 20.2 |
| | **250** | 18.6 | 16.3 | 21.0 | 19.9 |
| **Maize** | **Control** | 10.0 | 0.0 | 16.6 | 25.3 |
| | **50** | 6.6 | 27.2 | 14.2 | 14.0 |
| | **150** | 3.6 | 24.8 | 4.1 | 26.0 |
| | **250** | 1.1 | 19.4 | 1.0 | 9.1 |
| **Sunflower** | **Control** | 5.4 | 4.7 | 9.0 | 14.5 |
| | **50** | 2.6 | 4.6 | 6.5 | 13.1 |
| | **150** | 2.9 | 3.9 | 1.4 | 7.0 |
| | **250** | 1.5 | 0.3 | 1.3 | 13.4 |
| **Pearl millet** | **Control** | 15.6 | 15.6 | 2.9 | 5.9 |
| | **50** | 16.2 | 14.4 | 7.4 | 7.2 |
| | **150** | 20.1 | 7.7 | 6.4 | 5.4 |
| | **250** | 10.2 | 5.7 | 10.6 | 6.9 |
| **Mustard** | **Control** | 2.3 | 0.7 | 2.9 | 3.8 |
| | **50** | 3.5 | 4.1 | 3.0 | 2.2 |
| | **150** | 3.4 | 1.1 | 2.6 | 1.7 |
| | **250** | 6.7 | 1.4 | 2.0 | 1.4 |
| **Soyabean** | **Control** | 5.0 | 1.7 | 9.8 | 3.3 |
| | **50** | 3.6 | 3.5 | 8.9 | 1.3 |
| | **150** | 3.5 | 2.0 | 8.1 | 1.7 |
| | **250** | 3.4 | 0.0 | 0.4 | 1.0 |

***Table 2.*** **Effect of different concentrations of metals on native plants roots length.**

| Native Crops | Metals mg $kg^{-1}$ | Root length (cm) | | | |
|---|---|---|---|---|---|
| | | ***Cu*** | ***Cd*** | ***Cr*** | ***Pb*** |
| **Wheat** | **Control** | 7.3 | 7.8 | 2.4 | 14.9 |
| | **50** | 7.6 | 7.3 | 7.2 | 12.3 |
| | **150** | 5.4 | 10.8 | 4.7 | 8.2 |
| | **250** | 6.6 | 0.0 | 0.4 | 0.0 |
| **Soyabean** | **Control** | 3.8 | 1.0 | 7.0 | 3.1 |
| | **50** | 2.8 | 3.9 | 5.3 | 0.9 |
| | **150** | 2.1 | 1.3 | 6.5 | 2.3 |
| | **250** | 2.8 | 0.0 | 1.1 | 1.2 |
| **Barely** | **Control** | 18.4 | 5.2 | 12.4 | 9.7 |
| | **50** | 16.1 | 6.0 | 9.0 | 13.3 |
| | **150** | 17.1 | 5.3 | 10.1 | 10.7 |
| | **250** | 16.8 | 3.5 | 10.9 | 10.2 |
| **Sunflower** | **Control** | 13.3 | 3.8 | 10.3 | 9.7 |
| | **50** | 4.3 | 4.7 | 9.1 | 9.3 |
| | **150** | 9.0 | 3.6 | 0.5 | 8.2 |
| | **250** | 4.0 | 0.0 | 2.2 | 9.6 |
| **Pearl millet** | **Control** | 17.0 | 9.3 | 5.0 | 7.7 |
| | **50** | 11.1 | 8.1 | 1.5 | 6.0 |
| | **150** | 17.2 | 8.7 | 1.8 | 6.0 |
| | **250** | 12.6 | 6.7 | 3.4 | 7.7 |
| **Maize** | **Control** | 10.8 | | 8.3 | 20.6 |
| | **50** | 11.5 | 8.6 | 8.0 | 8.5 |
| | **150** | 7.4 | 14.0 | 2.5 | 16.0 |
| | **250** | 2.9 | 12.7 | 1.0 | 6.9 |
| **Mustard** | **Control** | 0.6 | 0.3 | 4.6 | 4.5 |
| | **50** | 3.8 | 4.7 | 1.3 | 2.3 |
| | **150** | 3.7 | 0.6 | 1.4 | 0.7 |
| | **250** | 4.0 | 0.2 | 0.6 | 0.1 |

***Effect of heavy metals on growth of native plant***

Effect of Cu, Cd, Cr and Pb concentrations on the biomass of wheat, barely, maize, sunflower, pearl millet, mustard and soybean, in terms of fresh and dry weight were investigated (shoot fresh biomass of all seven crops tested in-vitro till 20th day of their growth was included in the study). It was revealed from the results that maximum shoot biomass of wheat (0.122 g) was at 50 mg $kg^{-1}$of Cu concentration. Cd contaminated soil of Gujranwala showed maximum wheat shoot biomass at control level (0.329 g) after this level with increasing Cd doses wheat shoot biomass reduced sequentially. Maximum wheat shoot biomass was observed at 50 mg $kg^{-1}$of Cr level and Pb polluted soil showed maximum shoot biomass at 150 mg $kg^{-1}$ of Pb level and biomass decreased at 250 mg $kg^{-1}$. Dry biomass of wheat shoot showed behaviour corrosponding to fresh shoots weight.

The seed germination of wheat in copper was extremely slow as tiny radicals appeared on 6th day this shows that Cu exerted the adverse effects on seed germination similar results were found in the other studies [18]. In case of sunflower shoots the uptake and accumulation process with changing concentration of Pb was high which indicated greater potential of sunflower to store Pb in roots.

Barely crop was grown in contaminated soils of Gujranwala and it was shown that barely fresh shoots showed maximum biomass (0.119, 0.245 and 0.108 g) at 150 mg $kg^{-1}$of Cu, Cr and Pb respectively. This was optimum level for three metals to obtain maximum barely shoot biomass. However in Cr contaminated soil maximum fresh shoot biomass was noticed at control level and with increasing concentration biomass decreased. So, the overall analysis results revealed maximum biomass at control and it was decreased when concentrations of metals were increased. Barley growth was significant in almost all concentrations of heavy metals. In Cu, Cr and Pb the growth of barley shoots and roots was higher than control plants as well as crops. It was noticed that inhibitory effect of Cu and Pb on seed germination shoots and roots growth was very low on barley as compared to other crops.

Maize fresh shoot biomass was obtained by growing it in metals contaminated soils of Gujranwala. Maximum maize shoot biomass was obtained (1.79, 0.56 and 3.15 g) at 50 mg $kg^{-1}$of Cu, Cd and Pb concentrations, respectivly. These results showed that maize shoot biomass could be produced maximum at 50 mg $kg^{-1}$of Cu, Cd and Pb. Cr showed more toxicity for the maize shoots. Maximum biomass was showed at control (1.11 g) and at 50, 150 and 250 mg $kg^{-1}$of Cr. Minimum maize shoot biomass was obtained 1.1g at 250 mg $kg^{-1}$. Dry shoot biomass also showed similar trend. Sunflower was another native crop grown in contaminated soils of Gujranwala and it was shown that sunflower fresh shoots showed maximum biomass (0.625, 0.303, 0.632 and 0.699 g) at 0, 50 mg $kg^{-1}$of Cu, Cd, Cr and Pb, respectively. Cu and Cr were more toxic for sunflower as with increasing metals concentrations shoot biomass was decreased. In case of Pb, biomass was slightly reduced at 50 and 150 mg $kg^{-1}$ of Pb concentartion but again increased (0.623 g) at 250 mg $kg^{-1}$. Dry shoots were also showed biomass production in same pattern.

Pearl millet fresh shoot biomass was obtained by growing it in metals contaminated soils of Gujranwala. It was observed from the results that maximum shoot biomass of pearl millet (0.102 g) found at 150 mg $kg^{-1}$ of Cu concentration. Cd contaminated soil of Gujranwala indicated maximum pearl millet shoot biomass (0.071 g) at concentration of 50 mg $kg^{-1}$. After this level, with increase in Cd doses pearl millet shoot biomass was reduced. Maximum pearl millet shoot biomass was observed at 250 mg $kg^{-1}$of Cr level while Pb polluted soil showed maximum shoot biomass at 0 and 150 mg $kg^{-1}$level and biomass decreased at 250 mg $kg^{-1}$. Dry biomass of pearl millet shoot showed behaviour corrosponding to fresh shoot weights.

Mustard was another native crop grown in contaminated soils of Gujranwala, mustard fresh shoots showed maximum biomass (0.623, 2.067, 1.8 and 3.356 g) in control pots of Cu, Cd, Cr and Pb applied soils respectively. Mustard was more effective for Pb uptake. Overall 250 mg $kg^{-1}$ dose of all four metals was more toxic to mustard crop. Fresh shoot biomass of soyabean crop in Cu and

Pb contaminated soils was found maximum at control levels. Cd and Cr metals showed maximum shoot biomass at 50 mg $kg^{-1}$ of metals. Dry shoot biomass also depicted similar trend.

Fresh and dry weights of wheat, barley, maize, sunflower, pearl millet, mustard and soyabean grown in Cu, Cd, Cr and Pb contaminated soils was recorded. These crops were harvested after $20^{th}$ day of sowing.

Wheat fresh and dry weights were recorded. It was noticed that maximum root fresh biomass in the Cu contaminated soil was found 0.061 g at 250 mg $kg^{-1}$ of Cu concentration. In case of Cd application control pots showed more growth of wheat root biomass as compared to other concentrations of Cd. This presented that Cd was more toxic for wheat root growth. Cr level of 50 mg $kg^{-1}$ initially enhanced root growth after this concentration wheat root growth decreased at 150 and 250 mg $kg^{-1}$. Similarly, in case of Pb doses maximum wheat growth (0.086 g) was observed at 150 mg $kg^{-1}$ of Pb level and at 250 mg $kg^{-1}$ concentration wheat root biomass decreased. Similar behavior was observed in case of wheat dry root biomass.

Barley fresh and dry root biomass was measured after in-vitro experiment. It was observed that with increasing Cu concentration barley fresh roots biomass was increased and maximum biomass was found at 250 mg $kg^{-1}$ of Cu concentration. Similar behaviour was depicted by the Cd and maximum root biomass was observed at 250 mg $kg^{-1}$ of Cd concentration (0.19 g). In case of Cr application, barley root biomass was slightly decreased from 0 to 50 mg $kg^{-1}$ of Cr level (from 0.16 to 0.1 g) and then started to increase to 0.13 and 0.25 grams at 150 and 250 mg $kg^{-1}$ of Cr respectivly. In contrast to these results Pb showed different behaviour and maximum root biomass was recorded at control (0.17 g) and after that it was decreased by increasing concentration of Pb.

Maize was another native crop tested in the laboratory. It was obeserved from the result that maximum maize root biomass was observed at 50 mg $kg^{-1}$ of Cu (0.79 g). Similarly for Cd maximum maize root biomass was noticed 0.88 g at 50 mg $kg^{-1}$ of Cd level. In case of Cr control pot showed greater root biomass production ( 0.079 g) and with increasing Cr level maize root biomass was reduced consequently. Similar trend was shown by the Pb application and maximum root biomass was noticed at control (0.079 g).

Sunflower fresh roots produced maximum biomass at concentration of 50 mg $kg^{-1}$ of Cu (0.16 g). Cd contaminated soils showed similar behaviour and produced maximum root biomass at 50 mg $kg^{-1}$ of Cd level but it proved more toxic to sunflower crop and 250 mg $kg^{-1}$ of Cd concentration inhibited the sunflower root growth completely. In case of Cr control pot showed greater root biomass (0.13 g) and with increasing level of Cr sunflower roots growth decreased. Pb contaminated soil showed maximum root biomass (0.159 grams) at mg $kg^{-1}$.

Pearl millet fresh and dry root biomass was measured after in-vitro experiment. It was observed that with increase of Cu concentration pearl millet fresh roots biomass was increased first and reached to maximum at 50 mg $kg^{-1}$ of Cu concentration.It was then decreased till 250 mg $kg^{-1}$ of Cu. In Cd contaminated soil maximum root biomass was observed at control levelof Cd concentration (0.25 g). In case of Cr application, pearl millet root biomass increased from 0 to 250 mg $kg^{-1}$ of Cr level ( 0.14 g). Analogous behaviour was revealed by Pb and showed maximum root biomass 0.19 g after that it decreased with increasing Pb levels.

Mustard root growth enhanced by the addition of Cu and maximum root biomass was obtained at 250 mg $kg^{-1}$ of Cu concentration. Cd contaminated soil showed greater root biomass production at 50 mg $kg^{-1}$. In case of Cr mustard root growth exhibited by the Cr doses and maximum biomass was observed at 150 mg $kg^{-1}$ of Cr concentraion ( 0.8 g). Pb was proved toxic and growth of mustard roots decreased with increasing Pb levels and control pot showed greater biomass (016 g). Soyabean roots showed greater biomass at control level of Cu ( 0.79 g) then decreased at 50 mg $kg^{-1}$ of Cu after that a little increase was obsrved but that value was lower than control. Cd contaminated soil showed maximum root biomass

0.11 grams at 50 mg $kg^{-1}$ of Cd. Similar trend was noticed in the case of Cr and 0.25 grams root biomass was produced at control level. In contrast to this Pb application enhanced the soyabean growth and maximum biomass was noticed 0.68 grams at 250 mg $kg^{-1}$ of Pb concentration.

The uptake and accumulation potential of heavy metals was observed in native plants tissues. The accumulation of heavy metals is dependent on crop type, plant organ i.e. shoots or root and type of heavy metal present in the soil. Plant species differ widely in their ability to uptake and accumulate heavy metals according to [19]. In the present study root and shoot lengths and fresh and dry weights of maize were decreased with the treatment of higher Cu, Cd, Pb and Cr concentrations respectively. This was in accordance with the finding of Gupta and Abdullah (2011) that root, shoot, seed germination and dry biomasses were decreased when treated with Cd and Cu doses of 50, 100, 150 and 200 mg $L^{-1}$.

Heavy metals analysis in roots and shoots tissue of wheat indicated that most accumulated metal was Pb (146.88 µg $g^{-1}$) as compared to other metals. The order of accumulation was Pb>Cr>Cu>Cd. It was also noted that root parts were more efficient in uptake of metals. Maximum Cr was noticed 115.08 µg $g^{-1}$ in wheat roots; maximum Cd and Cu concentration were 86.7 and 107.28 µg $g^{-1}$ in root parts of wheat. In case of Cd, Cu and Pb metal uptake by wheat crop increased with increasing their concentrations in soil from 0 to 50, 150 and 250 mg $kg^{-1}$ of soil. Whereas Cr uptake was maximum at 50 mg $kg^{-1}$ of soil concentration and decreased at 250 mg $kg^{-1}$ of soil (Fig. 1).

Barley roots were more efficient in Cu, Cd, Cr and Pb uptake as compared to shoots. Results showed that Pb was highest accumulated metal by barley crop than Cu, Cr and Cd. Fig. 2 depicted that Cu, Cd and Pb removal by barley crop from soil increased with increasing initial metals concentration in soil but Cr was decreased after 150 mg $kg^{-1}$ to 250 mg $kg^{-1}$ of soil. Barley shoot removed maximum (9.48 µg $g^{-1}$) Cu at control while maximum concentrations of Cd (54.0 µg $g^{-1}$), Cr (11.16 µg $g^{-1}$) and Pb (115.86 µg $g^{-1}$) was removed at 150 mg $kg^{-1}$and 250 mg $kg^{-1}$ (Cr, Pb) of soil.

The order of metals removal from soil by maize plants was Cd>Cr>Pb>Cu. Among all the levels applied 150 mg $kg^{-1}$ dose of Cd was optimal level for Cd removal from soil. Similarly, Pb and Cr were removed efficiently at 250 mg $kg^{-1}$of soil while Cu was removed at control and 150 mg $kg^{-1}$ of soil. By these values it was found out that maize crop was best accumulator for Cd metal then for Cr, Pb and Cu. Maize shoots were more efficient in uptake of Cu (22.53 µg $g^{-1}$), Cd (141.93 µg $g^{-1}$) and Cr (97.02 µg$g^{-1}$) while Pb (69.3 µg$g^{-1}$) was highly removed by maize roots (Fig. 3).

Copper is also essential to plant growth, but may cause toxic effects when shoots or leaves accumulate Cu levels exceeding 2 mg $kg^{-1}$. Cu play an important role in regulating normal metabolism of plants. At its high concentration Cu is strongly toxic metal to plants cells and may cause plants growth inhibitor and sometimes cause death of the plant.

Figure 4 depicted that sunflower shoots were good accumulator of Cd and Pb whereas higher concentrations of Cu and Cr were accumulated by sunflower roots. Maximum Pb (206.49 µg $g^{-1}$) uptake was noticed in shoots. Maximum Pb (118.68 and 119.76 µg $g^{-1}$) uptake was observed by roots. Sunflower seeds were unable to germinate at 250 mg $kg^{-1}$ of Cd concentration in soil and uptake of Cd at this level was also shown zero. Variable behavior was observed with increasing initial metals concentrations (Fig. 4).

Heavy metals analysis in roots and shoots tissue of pearl millet indicated that most accumulated metal was Cd (104.4 µg $g^{-1}$) in roots at 250 mg $kg^{-1}$ as compared to other metals. The order of accumulation was Cd>Pb>Cu>Cu>Cr. It was also noted that root parts were more efficient in uptake of metals. Maximum Cr was 11.16 µg $g^{-1}$ in pearl millet roots; maximum Pb and Cu concentration were 115.86 and 66.76 µg $g^{-1}$ respectively in root parts of pearl millet. In case of Cd, Cu and Cr metals uptake by pearl millet crop increased with increasing their concentrations in soil at 0, 50, 150 and 250 mg kg$^{-1}$ of soil. Whereas maximum Pb removal was observed at 150 mg kg$^{-1}$ of soil concentration and decreased at 250 mg kg$^{-1}$ of soil (Fig. 5).

Results obtained from mustard crop at different levels of applied metals showed that Cu, Cr and Pb were concentrated more in roots of mustard, while Cd was found high in shoot part of mustard crop. Maximum Cu concentration was noticed 24.72 µg g$^{-1}$ in roots of mustard. Highest accumulated levels of Cd, Cr and Pb were 39.22, 38.46, and 39.3 µg g$^{-1}$ respectively. Cu uptake increased with increasing initial applied concentration, however Cd removal was maximum at 150 mg kg$^{-1}$ (Cd) and Cr and Pb were translocated efficiently at 50 mg kg$^{-1}$ (Fig. 6).

Figure 7 presented that Cu and Pb were accumulated more in root part where as Cd and Cr were concentrated more in shoot of soybean. Maximum Cu (16.62 µg g$^{-1}$) and Cr (34.83 µg g$^{-1}$) concentrations were recorded at applied concentration of 250 mg kg$^{-1}$ and Cd was noticed 34.83 µg g$^{-1}$ at 50 mg kg$^{-1}$ of soil concentration. Overall Pb (82.75 µg g$^{-1}$) was observed as highest concentrated metal in soybean crop among all four tested metals. Cu, Cr and Pb uptake was increased with increasing initial metal concentration but Cd uptake increased firstly at 0 to 50 mg kg$^{-1}$ of soil then decreased from 150 to 250 mg kg$^{-1}$ of soil concentrations (Fig. 7). The accumulation and distribution of heavy metals in plants depends on the environmental factors, such as plant species, element species, chemical and bioavailability, redox, pH, cation exchange capacity, dissolved oxygen, temperature and secretion of roots [19]. In the present study maize accumulated greater cadmium and lead in its roots as compared to chromium and copper. It has been reported that cadmium is a highly mobile metal, easily absorbed by the plants through root surface and moves to wood tissue and transfers to upper parts of plants [20].

Among the selected local plants maize and mustard were effective plants for phytoremediation purpose. Maize was best for phytoremediation of Cd and Pb polluted soils and canola for phytoremediation of Cd. All the selected plants were more sensitive to cadmium as compared to other metals. Increase in the concentrations of copper, cadmium, lead and chromium brought up changes in most of the growth parameters of plants. Among all metals cadmium affected more the plant seed germination, seedling growth, shoot and root elongation of selected local plants.

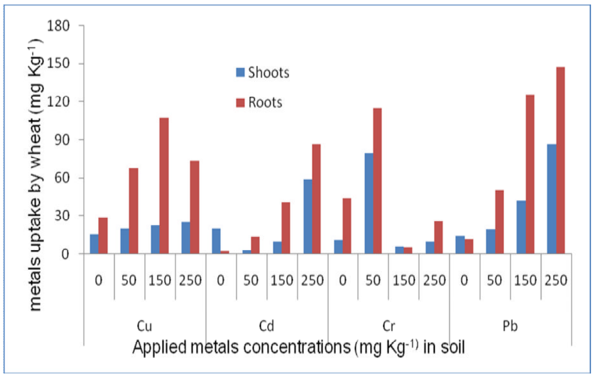


***Figure 1.*** **Heavy metals accumulation in shoot and root of wheat**

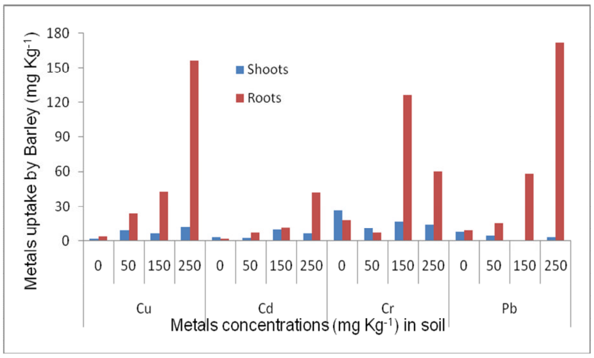


***Figure 2.*** **Heavy metals accumulation in shoot and root of Barley**

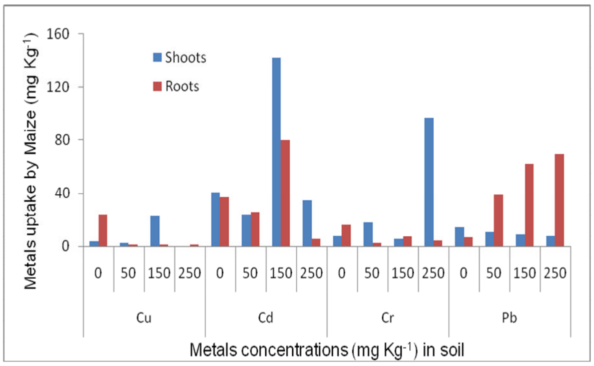


***Figure 3.*** **Heavy metals accumulation in shoot and root of Maize**

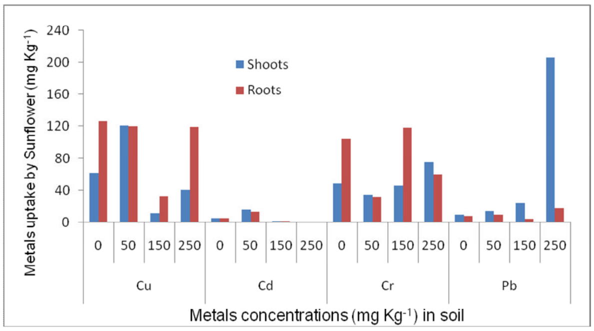


***Figure 4.*** **Heavy metals accumulation in shoot and root of Sunflower**

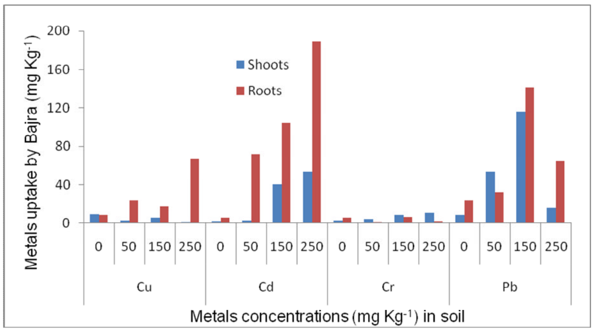


***Figure 5.*** **Heavy metals accumulation in shoot and root of Pearl millet**

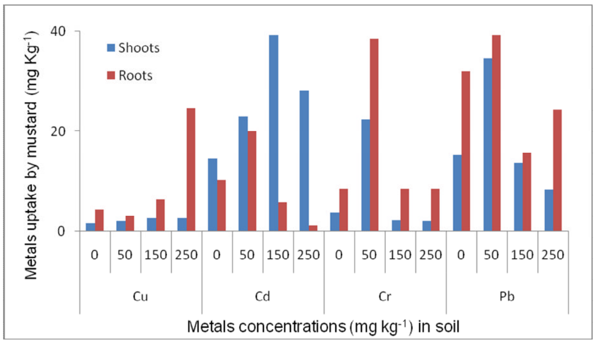


***Figure 6.*** **Heavy metals accumulation in shoot and root of mustard**

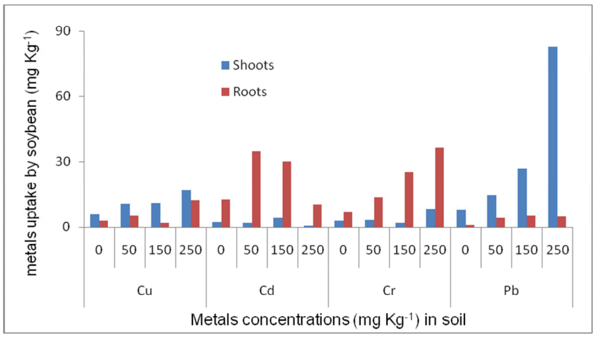


***Figure 7.*** **Heavy metals accumulation in shoot and root of Soybean**

## Conclusion

In overall study conducted, it is concluded that maize and mustard were more shoot biomass producing crops. Maximum root biomass was produced by barely and pearl millet crops. Maximum shoot and root length was also produced by barley crop. Sunflower and barley were noticed more effective accumulators of Cu. Maize, wheat and barley were proved effective accumulators for Cd and Cr accumulation from heavy metals contaminated soils. Phytoremediation can be a good option because different native crops have the ability to accumulate metals e.g maize, mustard, wheat, barley and sunflower etc. Efficient native plants should be grown in the contaminated soil near the industrial areas so that they efficiently phytoremediate the contaminated soils. Crops used in the laboratory experiments (maize, wheat, mustard, canola and sunflower etc.) could be used for multi-tasking like for the management of heavy metals polluted soils (phytoremediation) as well as for biomass production which can be ultimately used for biogas and biofuel production.

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