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# TOXICITY AND BIOACCUMULATION OF HEAVY METALS IN SPINACH SEEDLINGS GROWN ON FRESHLY CONTAMINATED SOIL

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**Abstract**

This study was conducted to investigate the toxicities of 3 different doses of cadmium (Cd), lead (Pb), and zinc (Zn) in both single and mix forms on the growth parameters (shoot and root fresh and dry weights, root and shoot lengths, shoot diameter, number of leaves, cell size and mitotic index) of spinach seedlings. Soil from historically uncontaminated site was taken and after air drying and sieving (2 mm mesh sieve) was spiked with selected metals. Plastic pots filled with spiked soil and seedlings of spinach were prepared in greenhouse environment. Growth parameters were calculated according to standards methods and heavy metals (HMs) were extracted from plant tissues in digestion block with the help of acid mixture (15 ml) of $HNO_3$, $HClO_4$, and $H_2SO_4$ (5:1:10), and concentrations of HMs were measured using atomic absorption spectrometer (Analyst 700 of PerkElmer). Increasing concentrations of Cd, Pb and Zn in both single and mixture forms significantly ($p<0.05$) reduced growth parameters of *S. oleracea* seedlings. The reduction in growth parameters of *S. oleracea* seedling showed the dose response for Cd, Pb and Zn in both single and mixture forms. The uptake patterns of Cd, Pb and Zn in Cd/Pb, Cd/Zn and Pb/Zn showed antagonistic impacts on each other and uptake pattern was reflected in growth of the seedlings. Toxicities caused by selected HMs were highest for Cd followed by Pb and Zn. Highest toxicity was observed in plant seedlings grown on Cd/Pb treated soil.

## Introduction

Many heavy metals (HMs) including Cd and Pb are nonessential and toxic for plant growth (Zhiqiang *et al*., 2009; Lia *et al*., 2012), but plant takes them up rapidly when present in growing medium like soil (Fusconi *et al*., 2006). Cd is phytotoxic in nature and not only inhibits the growth parameters of plants but also causes death of plant (Toppi *et al*., 1999). Different plant processes such as respiration and photosynthesis are badly affected by Cd and also decreased water and nutrient uptake by plants (Kuo & Kao, 2004). Cd reduces root growth because of the reduction of the rate of new cell production (Liu *et al*., 2004), inhibits the activities of antioxidative enzymes of plants (Correa *et al*., 2006) and induces oxidative stress in cells (Sandalio *et al*., 2001). Similarly, Pb causes retardation of plant growth and inhibition of seed germination (Iqbal & Shazia, 2004). Pb also has significant negative impacts on seedling biomass, root and shoot lengths (Joseph *et al*., 2002). Like Cd, Pb also adversely affects the process of photosynthesis, respiration and metabolism of plants (Paolacci *et al*., 1997).

Zinc is one of the essential elements for many physiological processes in plants, but its higher concentration, makes it toxic (Baccio *et al*., 2005). Zn significantly changes mitotic activity (Rout & Das, 2003), disturbs membrane integrity and permeability (Stoyanova & Doncheva, 2002) and finally kills the plant cell (Change *et al*., 2005). Zn causes necrosis of shoot (Mahmood *et al*., 2005) and inhibits root development (Lingua *et al*., 2008). Furthermore, Cd, Pb and Zn also reduce the plant uptake rate of essential elements like Mn, Fe, K, Mg and Ca (Wu *et al*., 2007), which further accelerating toxicity in plants in term of reduction in plant weight and height.

Seedling and seed germination stage of plant life are sensitive to environmental factors such as HMs pollution (Abedin & Meharg, 2002). A mixture of HMs are present in the soil, the presence of one pollutant can increase or decrease the adverse impacts of other pollutant. In the past, the effects of the single metal on plants were investigated by many researchers (Cao *et al*., 2009; He *et al*., 2008; Yang *et al*., 2009). However, the study of combine toxicity of HMs on plants seedlings needs more attentions by researchers through the world. The joint affects of HMs were investigated by few researchers (Farooqi *et al*., 2009; Yizong *et al*., 2009; An *et al*., 2004). The present study was conducted to investigate the toxicological effects of Cd, Pb and Zn alone and in mixture forms (Cd/Pb, Cd/Zn and Pb/Zn) on biomass (shoot and root fresh and dry weights), lengths (shoot and root lengths), cell size, shoot diameter, number of leaves and mitotic index (MI) of *S. oleracea* seedlings. Furthermore, accumulation of HMs by *S. oleracea* seedlings was also investigated.

## Materials and Methods

**Soil preparation:** Table 1 summarizes the basic properties of soil used for this control environment experiment. Soil was collected from historically uncontaminated site of Peshawar, Pakistan. After transportation to laboratory, the soil was air dried and passed through a 5mm sieve. The sub-soil was spiked (mg/kg) with Pb (Pb1= 300, Pb2= 400 and Pb3= 500, Cd

(Cd1= 0.5, Cd2= 1 and Cd3 1.5), Zn (Zn1=250, Zn2= 500, Zn3= 700), Cd/Pb (Cd1/Pb1= 0.5/300, Cd2/Pb2=1/400, Cd3/Pb3= 1.5/500), Cd/Zn (Cd1/Zn1= 0.5/250, Cd2/Zn2=1/500, Cd3/Zn3=1.5/700) and Pb/Zn (Pb1/Zn1= 300/250, Pb2/Zn2= 400/500, Pb3/Zn3= 500/700). The spiked soil was thoroughly mixed with remaining soil, air dried and passed through a 5 mm sieve again. The plastic pots were filled with 500 g of HMs spiked soil and 4 replicates were prepared for each treatment.

**Table1. Basic properties of soil used for this study.**

| Parameter | Values |
|---|---|
| pH | 7.7 |
| Organic matter (%) | 1.6 |
| EC (dS/m) | 6.71 |
| **Soil particle size (%)** | |
| < 20 µm | 7.6 |
| 20-62 µm | 66 |
| 62-250 µm | 25.7 |
| 250 µm – 1 mm | 0.577 |

**Seedling preparation:** The seeds of *S. oleracea* were disinfected with 30% (w/w) $H_2O_2$ solution for 10 min, followed by thorough washing with deionized water. The disinfected seeds were germinated in petri dishes inside the folds of wet filter papers at 28±1°C. After four days, uniform seedlings (4) were cultivated in each plastic pot. This experiment was performed in a greenhouse with day temperature of 25±4°C and night temperature of 19±3°C. The plants were under sun light of 12 h and relative humidity of 65±2%. The seedlings were irrigated with deionized water and pots were re-randomized on regular basis so that each plant can get the same amount of light and temperature. After 2 weeks of cultivation, the seedlings were harvested and washed with deionized water to remove the adhered soil particles.

**Growth parameters:** Fresh weights, lengths and diameter of root and shoot were recorded with the help of electronic balance, ruler and screw gage, respectively. The plants were dried in oven at $80^0$C for 3 days and dry weights were also recorded.

**Cell size:** The leaf cell sizes were recorded using microscope with a micro-scale on its eye piece.

**Mitotic index:** Fifteen root tips of germinated seedlings of each treatment were fixed in a mixture of ethanol-acetic acid (3:1) for 24 h, hydrolyzed in 1M HCl for 10 min and stained in 2% aceto-orcine. The root tips were cut off in a drop of 45% acetic acid, macerated and squashed (Sharma & Sharma, 1980). The prepared slides were carefully observed under light microscope and MI were calculated with the following formula.

$$\mathrm{MI}\,(\%) = \frac{\text{Number of dividing cells}}{\text{Total number of cellls}} \times 100$$

**Heavy metals analysis:** Dried plants were powdered and 0.5 g weighted into acid washed and dried digestion tube. Acid mixture (15 ml) of $HNO_3$, $HClO_4$, and $H_2SO_4$ (5:1:10) was added and the samples were left overnight. Next day, the digestion tubes were placed on a digestion block at a temperature of 80°C for 1 h and then raised to 120-130°C till clear solution obtained. After completion of digestion, the transparent solutions were filtered into acid washed volumetric flasks and make the volume 50 ml with double deionized water. The concentrations of HMs in the digested solutions were measured using atomic absorption spectrometer (Analyst 700 PerkElmer) in the laboratory of National Center of Excellence in Geology, University of Peshawar, Pakistan.

**Statistical analysis:** The data were statistically analyzed using statistical package (SPSS 17). The data were analyzed by one way of variance (ANOVA) to confirm the variability and validity of results. Duncan's multiple range test (DMRT) was performed to determine significance between the treatments with a significance level of $p<0.05$. Linear regression analysis was also performed to establish the relationships between the HM concentrations in the plants tissues and the HM concentrations in the soil.

## Results

**Heavy metal effects on plant biomass:** Figures 1, 2, 3 and 4 present the toxicological effects of Cd, Pb and Zn both individually and mixture forms on plant biomass (fresh and dry weights). HM concentrations had a significant ($p<0.05$) adverse impacts on the biomass of *S. oleracea* seedlings (Table 2). At the highest application rate of Cd, the shoot and root fresh and dry weights were decreased by 49.2, 50, 38 and 41%, respectively (Table 3) as compared to control. Similarly, at the highest dose of Pb shoot and root fresh and dry weight decreased by 44, 45, 30.8 and 35% (Table 3) as compared to control, respectively. The addition of high dose of Zn decreased shoot and root fresh and dry weights by 40, 47.5, 29.7 and 32%, respectively (Table 3) as compared to control.

Under the influence of highest dose of Cd/Pb, the reduction in shoot and root fresh and dry weights was 60, 68, 61 and 63%, respectively as compared to control. Similarly, at the highest dose of Cd/Zn, shoot and root fresh and dry weights decreased by 57, 62.5, 35 and 64.3%, respectively (Table 3) as compared to control. The addition of highest dose of Pb/Zn decreased shoot and root fresh and dry weights by 32, 35, 33 and 57.1%, respectively (Table 3) as compared to control.

Soil amended with the mixture of Cd and Pb showed highest toxicity on biomass of seedlings but was less than the sum of the toxicity caused by both Cd and Pb individually. However, the toxicity on seedling biomass under the influence of combine Cd/Zn was less than the toxicity due to Zn and more than the Cd toxicity alone. The combine toxicity of Zn/Pb was less than toxicity of Zn and Pb alone on *S. oleracea* biomass. The dry weights of both shoot and root were more affected than their fresh weights of *S. oleracea* seedlings. The root fresh and dry weights were more affected than the shoot fresh and dry weights.

**Table 2. ANOVA analysis of growth parameters of *S. oleracea.***

| Parameters | | Sum of squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|---|
| Leaves | Between groups | 103.112 | 18 | 5.728 | 7.307 | .000 |
| | Within groups | 44.688 | 57 | 0.784 | | |
| | Total | 147.799 | 75 | | | |
| Shoot length | Between groups | 28.843 | 18 | 1.602 | 48.103 | .000 |
| | Within groups | 1.899 | 57 | 0.033 | | |
| | Total | 30.742 | 75 | | | |
| Shoot fresh weight | Between groups | 47.566 | 18 | 2.643 | 191.880 | .000 |
| | Within groups | .785 | 57 | 0.014 | | |
| | Total | 48.351 | 75 | | | |
| Shoot dry | Between groups | 10282.455 | 18 | 571.248 | 6897.875 | .000 |
| | Within groups | 4.720 | 57 | 0.083 | | |
| | Total | 10287.176 | 75 | | | |
| Shoot diameter | Between groups | 14.357 | 18 | 0.798 | 77.544 | .000 |
| | Within groups | .586 | 57 | 0.010 | | |
| | Total | 14.943 | 75 | | | |
| Root length | Between groups | 148.518 | 18 | 8.251 | 171.959 | .000 |
| | Within groups | 2.735 | 57 | 0.048 | | |
| | Total | 151.253 | 75 | | | |
| Root fresh weight | Between groups | .827 | 18 | 0.046 | 14.388 | .000 |
| | Within groups | .182 | 57 | 0.003 | | |
| | Total | 1.010 | 75 | | | |
| Root dry weight | Between groups | 4.358 | 18 | 0.242 | 5.140 | .000 |
| | Within groups | 2.685 | 57 | 0.047 | | |
| | Total | 7.043 | 75 | | | |
| Cell size | Between groups | 63.277 | 18 | 3.515 | 21.396 | .000 |
| | Within groups | 9.365 | 57 | 0.164 | | |
| | Total | 72.642 | 75 | | | |
| Mitotic index | Between groups | 13784.621 | 18 | 765.812 | 373.192 | .000 |
| | Within groups | 116.968 | 57 | 2.052 | | |
| | Total | 13901.589 | 75 | | | |

**Table 3. Comparison of reduction of growth parameters (%) of different HM treatments and control of *S. oleracea* seedlings.**

| Treatments | Seedlings biomass | | | | Length | | | | No of leave | MI |
|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot fresh weigh | Root fresh weight | Shoot dry weight | Root dry weight | Shoot length | Root length | Shoot diameter | Cell size | | |
| Cd 1 | 26.9 | 29 | 21.5 | 25.0 | 25.9 | 34.2 | 3.00 | 20.0 | 25.0 | 7.30 |
| Cd2 | 48.8 | 49 | 35.0 | 36.0 | 26.7 | 37.8 | 6.30 | 30.0 | 31.2 | 18.9 |
| Cd3 | 49.2 | 50 | 38.0 | 41.0 | 29.3 | 56.9 | 7.80 | 45.0 | 50.0 | 22.7 |
| Pb 1 | 26.0 | 28 | 16.9 | 19.0 | 19.0 | 13.8 | 2.80 | 20.0 | 21.8 | 6.40 |
| Pb 2 | 39.0 | 32 | 29.0 | 31.0 | 24.1 | 23.1 | 5.80 | 26.0 | 29.9 | 15.0 |
| Pb 3 | 44.0 | 45 | 30.8 | 35.0 | 25.0 | 46.2 | 7.10 | 42.5 | 41.0 | 20.3 |
| Zn 1 | 25.0 | 27.5 | 12.0 | 15.0 | 14.9 | 11.3 | 2.30 | 18.0 | 19.0 | 8.10 |
| Zn 2 | 37.0 | 37.5 | 27.0 | 30.9 | 19.2 | 16.5 | 4.50 | 21.0 | 25.0 | 13.0 |
| Zn 3 | 40.0 | 47.5 | 29.7 | 32.0 | 22.0 | 31.8 | 5.10 | 35.0 | 37.5 | 18.9 |
| Cd1/Pb 1 | 50.0 | 55.0 | 38.0 | 40.0 | 37.9 | 41.8 | 3.90 | 22.0 | 43.7 | 8.00 |
| Cd2/Pb 2 | 55.0 | 59.0 | 51.0 | 55.7 | 48.3 | 57.3 | 9.20 | 33.0 | 50.0 | 24.7 |
| Cd3/Pb 3 | 60.0 | 68.0 | 61.0 | 63.0 | 55.2 | 66.9 | 10.2 | 65.5 | 62.5 | 27.0 |
| Cd1/Zn 1 | 48.0 | 50.0 | 34.0 | 35.7 | 27.5 | 10.0 | 7.10 | 46.0 | 37.5 | 7.80 |
| Cd2/Zn 2 | 50.2 | 52.5 | 34.9 | 50 | 30.0 | 12.0 | 8.10 | 52.0 | 43.7 | 18.5 |
| Cd3/Zn 3 | 57.0 | 62.5 | 35.0 | 64.3 | 36.9 | 40.0 | 9.10 | 58.0 | 50.0 | 24.1 |
| Zn1/Pb 1 | 26.6 | 30.0 | 23.0 | 25.7 | 20.0 | 36.0 | 4.30 | 32.5 | 25.0 | 9.80 |
| Zn2/Pb 2 | 28.1 | 34.0 | 30.7 | 32.9 | 22.0 | 40.0 | 7.10 | 45.0 | 31.2 | 13.3 |
| Zn3/Pb 3 | 32.0 | 35.0 | 33.0 | 57.1 | 23.8 | 58.0 | 8.10 | 55.0 | 37.5 | 16.6 |

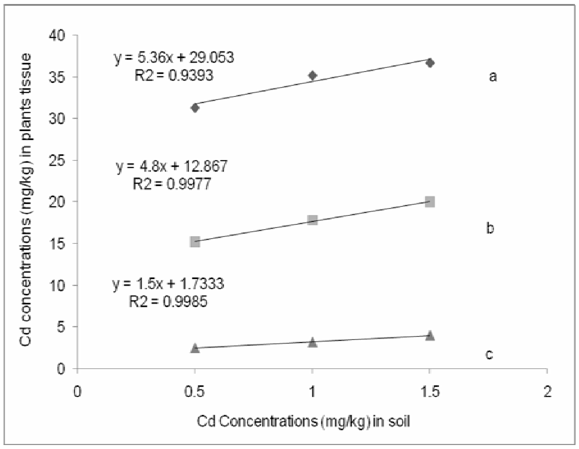


Fig. 1. Linear regressions model for Cd concentrations in soil and seedlings tissues on dry weight basis. Relationships between Cd concentration in soil and seedlings a) single Cd treatments; b) Cd/Pb treatments and c) Cd/Zn treatments.

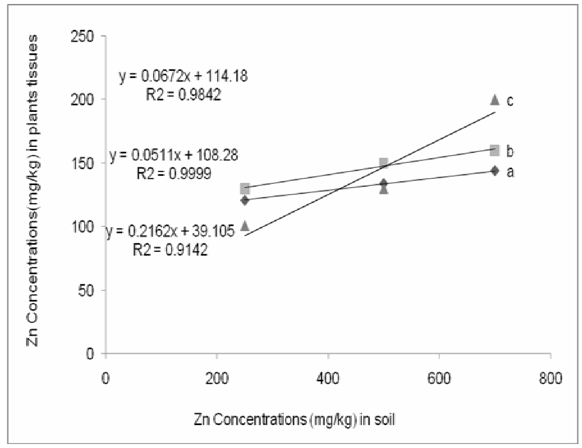


Fig. 3. Linear regressions model for Zn concentrations in soil and seedlings tissues on dry weight basis. Relationships between Zn concentration in soil and seedlings a) single Zn treatments; b) Zn/Pb treatments and c) Zn/Cd treatments.

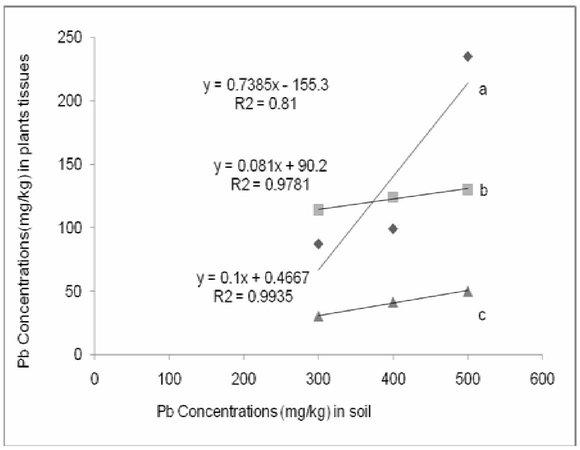


Fig. 2. Linear regressions model for Pb concentrations in soil and seedlings tissues on dry weight basis. Relationships between Pb concentration in soil and seedlings a) single Pb treatments; b) Pb/Cd treatments and c) Pb/Zn treatments.

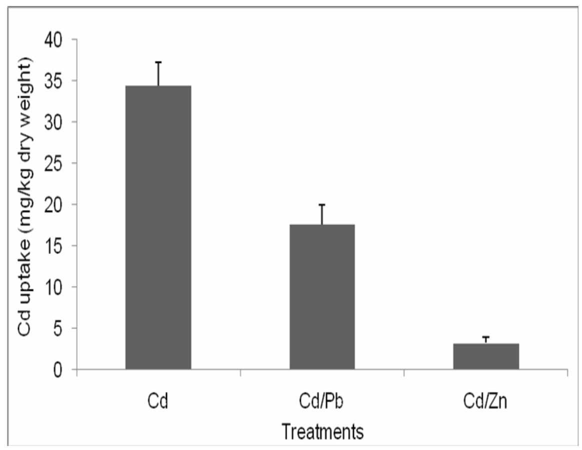


Fig. 4. Comparison of Cd uptake by plant seedlings grown on different amended soils. The error bars indicate the standard deviation.

**Heavy metals effects on shoot and root lengths:** Different concentrations of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn had a significant ($p<0.05$) toxicological impacts on shoot and root lengths of *S. oleracea* seedlings (Table 2). Like seedling biomass, the increasing concentrations of HMs in the soil resulted a decrease in the shoot and root lengths (Table 3). At the highest dose of Cd, the shoot and root lengths were decreased by 29.3 and 56.9%, respectively as compared to control. Similarly, at the highest dose of Pb, the shoot and root lengths were decreased by 25 and 46.2%, respectively (Table 3) as compared to control. At the highest dose of Zn the shoot and root lengths were decreased by 22 and 31.6%, respectively (Table 3) as compared to control.

Under the influence of highest dose of Cd/Pb, the reduction in the shoot and root lengths was 55.2 and 66.9%, respectively as compared to control. The combine toxicity of Cd/Zn in the shoot and root lengths were 36.9 and 40%, respectively, while the reduction in shoot and root lengths were 23.8 and 58%, respectively under the combine toxicity of Pb and Zn. In general, the root lengths were more affected by HMs amendments as compared to shoot lengths.

**Heavy metals effects on shoot diameter:** Table 2 summarizes the effects of HMs on shoot diameter of the selected plant seedlings. Different concentrations of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn had a significant ($p<0.05$) toxicological impacts on shoot diameter of *S. oleracea* seedlings. The increasing concentrations of HMs in the soil resulted in a decrease in the shoot diameter of *S. oleracea* seedlings (Table 3). Similarly, the reduction in shoot diameter was 7.8, 7.1, 5.1, 18.2, 9.1 and 8.1% under the influence of highest doses of Cd, Pb, Zn, Cd/Pb Cd/Zn and Pb/Zn, respectively (Table 3) as compared to control.

**Heavy metals effects on cell size, leaves and MI:** The increasing concentrations of HMs in the soil resulted in a decrease in the cell size, number of leaves and root MI of *S. oleracea* seedlings (Table 3). The reduction in cell size, number of leaves and root mitotic index of *S. oleracea* seedlings was significant ($p<0.05$). At the highest dose of Cd, the cell size, number of leaves and MI showed a reduction of 45, 50 and 22.7%, respectively (Table 3) as compared to control. Similarly, at the highest dose of Pb, the cell size, number of leaves and MI showed a reduction of 42.5, 41 and 20.3%, respectively (Table 3) as compared to control. At the highest dose of Zn, the cell size, number of leaves and MI showed a reduction of 35, 37.5 and 18.9%, respectively (Table 3) as compared to control. The reduction in the cell size, number of leaves and MI were 65.5, 62.5, and 27%, respectively as compared to control under the influence highest dose of Cd/Pb. In Cd/Pb treatments cell size, number of leaves and MI were further decreased but the decrease was less than the sum of reduction due to both Cd and Pb alone. The reduction in the cell size, number of leaves and MI were 58, 50, and 24.1%, respectively as compared to control under the influence highest dose of Cd/Zn. The reduction in the cell size, number of leaves and MI were 55, 37.5, and 16.6%, respectively as compared to control under the influence highest dose of Cd/Zn.

**Plant uptake of HMs:** Figure 1 shows the relationships between the Cd concentrations in soil and seedling tissues. In plant seedlings, the concentration of Cd increased with increasing concentrations of Cd in soil amended with single and mixture of Cd. Seedlings were accumulated Cd at rate of 3440, 1760 and 323% from the soil amended with single Cd, Cd/Pb and Cd/Zn, respectively (Fig. 4). Similarly, the concentrations of Pb in seedlings showed an increase with increasing concentrations of Pb in soil. The regression analysis showed positive relationships between the concentrations of Pb in soil and seedlings in all treatments (Pb, Pb/Cd and Pb/Zn) as shown in Fig. 2. Plant seedlings accumulated Pb at rate of 35.03, 30.65 and 10.12% from the soil spiked with Pb, Pb/Cd and Pb/Zn, respectively (Fig. 5). The regression analysis showed strong positive relationship between Zn contents in soil and seedlings (Fig. 3). Zn was accumulated in seedlings at arte of 27.54, 26.9 and 24.8% from the soil amended with Zn, Zn/Cd and Zn/Pb, respectively (Fig. 6).

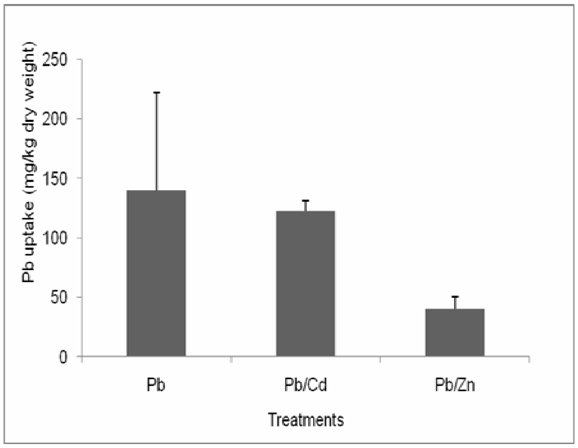


Fig. 5. Comparison of Pb uptake by plant seedlings grown on different amended soils. The error bars indicate the standard deviation.

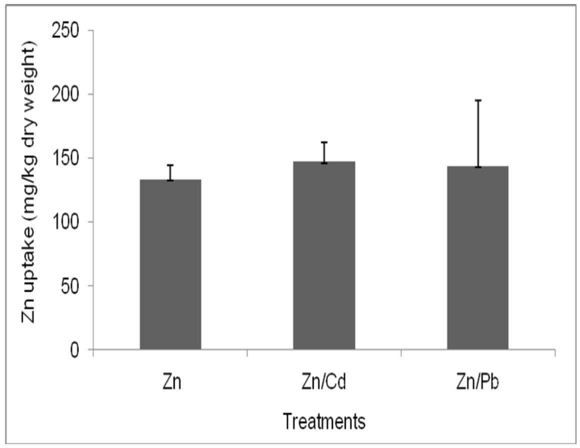


Fig.6. Comparison of Zn uptake by plant seedlings grown on different amended soils. The error bars indicate the standard deviation.

## Discussion

Results of the present and previous studies (Lingua *et al*., 2008; Farooqi *et al*., 2009; Khan *et al*., 2008; Devi *et al*., 2007; An *et al*., 2004; Sun *et al*., 2008; Nedjimi *et al*., 2009; Ahmad *et al*., 2012) show that plants grown on HM contaminated soil contain high concentrations of HMs in their tissues. HMs has adverse affects on plant growth, which further lead to decrease plant yield and inhibition of enzymatic activities (Zeng *et al*., 2008; Shafeeq *et al*., 2012).

Cadmium and Pb are highly phytotoxic metals when present at high concentrations in soil ecosystem and/or irrigating water. In the present study, increasing concentrations of Cd and Pb has significantly ($p<0.05$) reduced shoot and root fresh and dry weights. These findings are agreed with the results of previous studies. An *et al.*, (2004) observed a drop in shoot and root growth in *S. oleracea* with increasing concentrations of Cd and Pb in its growing medium. Increasing concentrations of Cd in growing medium decreases shoot and root biomass as observed by Shukla *et al.*, (2003). Similarly, Devi *et al.*, (2007) also observed reduction of root and shoot dry weights in rice with increasing concentrations of Cd. Farooqi *et al.*, (2009) found a decrease in seedling dry weight of *A. lebbeck* with increasing concentrations of Pb. Zayed & Amin, (2002) observed a decrease in dry weights of *T. Alexendrium* with increasing uptake of Pb However, the results of the present study are inconsistent with the results of few studies previously conducted on plant seedlings. Vogel-Mikus *et al.*, (2006) have not observed any toxicity in *Z. mays* and *H. annuus* with increasing concentrations of Cd. Similarly, Lin *et al.*, (2007) observed that Cd concentrations from 0.3 to 33 mg/kg enforced the growth of *T. aestivum* seedlings.

These different responses of HMs on plant seedlings depend on characteristics of soil and physiology of plants.

Increasing concentrations of Cd and Pb in soil produced significant ($p<0.05$) toxicity on *S. oleracea* seedlings in terms of shoot and root lengths. The present results of root and shoot lengths are consistent with the findings of Shukla *et al.*, (2006) who found a reduction in root and shoot lengths. Increasing concentrations of Pb adversely affect seedlings length in *A. marina* (MacFarlane& Burchett, 2002). Similarly, Liu *et al.*, (2004) also reported a reduction in formation of new cells under the influence of Cd and Pb which leads to reduction in shoot and root lengths.

In this study, other growth parameters like number of leaves, cell size, shoot diameter and MI were also significantly ($p<0.05$) affected with increasing concentrations of Cd and Pb in soil ecosystem. The toxicity in term of shoot diameter was not severe as compared to other parameters. In case of cell size and MI, Cd and Pb are toxic for *S. oleracea* seedlings. Jun-yu *et al.*, (2008) also observed reduction of MI in *O. sativa* with increasing concentrations of Cd. Cd causes cellular damage like swelling of mitochondria, cracking of epidermis, rough endoplasmic membrane (Jin *et al*., 2008), which may be the reason of reduction in cell size.

Zn is an essential element for plants, but its excess can significantly damage plant growth (Wang *et al.*, 2009). This study showed that increasing concentrations of Zn were responsible for increase toxicity in seedlings. Shoot and root fresh and dry weights were reduced with increasing Zn concentrations. Zn reduced plant biomass because of creation of deficiency of macronutrients such as phosphorous (Marschner, 1995). Mahmood *et al.*, (2005) found a reduction in growth of corn with increasing concentrations of Zn. In the present study, Zn has also significantly reduced MI in *S. oleracea* seedlings which are agreed with those findings reported in the literature (Rout & Das, 2003).

Metals in the soil ecosystem are always present in mixture form (Spurgeon *et al*., 1994), therefore, bioaccumulation of one metal in the mixture inhibits or enforces the accumulation of other metal (Peralta-Videa *et al*, 2002). The uptake pattern of Cd and Pb in Cd/Pb showed that both Cd and Pb affect the uptake of each other in antagonistic way. The growth parameters of *S. oleracea* seedlings showed the dose response for Cd and Pb. The uptake pattern of both Cd and Pb were reflected in the growth parameters of seedlings. The toxicities of Cd and Pb in form of Cd/Pb in terms of almost all growth parameters were more than the individual toxicities of Cd and Pb but less than their additive toxicities. Kalavrouziotis *et al.*, (2009) reported the same results in Broccoli at low Cd concentrations, while An *et al.*, (2004) produced the same result in studying *C. sativus.* Zhiqiang *et al.*, (2009) found additional effects on biomass, root and shoot lengths of *B. rapa* with increasing concentrations of Cd along with Pb. On the other hand, Luan *et al.*, (2008) found no response in shoot growth with the combine addition of Cd/Pb in *G. max*.

Both Zn and Cd compete with each other because of their similar chemical and physical properties. Results of this study showed antagonistic behavior between Cd and Zn. These results are consistent with the results reported by Weihong *et al.*, (2009) who observed a reduction in the uptake of Cd in the presence of Zn. Similarly, Sharma *et al.*, (1999) also observed reduction in the uptake of Zn in the presence of Cd. The uptake pattern of Cd and Zn was reflected in the growth parameters of *S. oleracea* seedlings. Auda & Ali, (2010) found a reduction in the inhibitory effects of Cd on *D. carota* growth and dry matter with addition of Zn. Similarly, Sharma *et al.*, (2007) observed synergistic interaction of Zn with Cd at its low concentrations, while antagonistic at high Zn concentrations.

Characteristically, Pb and Zn also interact with each other if present in mixture form. In case of *S. oleracea* seedlings both of Pb and Zn antagonistically affected the uptake rates of each other, which consistent with the results reported by MacFarlane & Burchett, (2002), who observed that the accumulation of Zn enhanced the accumulation of Pb in leaves and vice versa. The uptake pattern of Zn and Pb in Zn/Pb amended soil showed that both Zn and Pb affect the uptake of each other in antagonistic way. The uptake pattern is reflected in the growth parameters of *S. oleracea* seedlings. These results are consistent with the results observed by Boedeker *et al.*, 1993.

## Conclusion

On the basis of findings it is concluded that increasing concentrations of Cd, Pb and Zn in both single and mixture forms produced significant ($p<0.05$) toxicity on *S. oleracea* seedlings in terms of shoot and root lengths, shoot and root biomass, number of leaves, MI, cell size and shoot diameter. Sever toxicity on seedlings was shown by Cd treatment as compared to toxicities produced by Pb and Zn. The uptake pattern of Cd and Pb in Cd/Pb showed that both Cd and Pb affect the uptake of each other in antagonistic way. The toxicities due to Cd and Pb in form of Cd/Pb in terms of almost all growth parameters were more than the toxicities due to individual Cd and Pb but less than their additive toxicities. Cd and Zn also affect the uptake rate of each other in antagonistic way. Similarly, Pb and Zn also affect the uptake of each other in antagonistic way in case of *S. oleracea.*

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