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Metals toxicity and its bioaccumulation in purslane seedlings grown in controlled environment

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ABSTRACT

We aimed to find the toxicological impacts of Cd, Pb and Zn in single dozes and in combinations on Purslane (*Portulaca oleracea*) seedling. The Pursolane seedlings grown in pots in a green house were treated with different soil treatments spiked (mg/kg) with Pb (300, 400 and 500), Cd (0.5, 1 and 1.5), and Zn (250, 500, 700) alone and then in specified combinations/concentrations *i.e.*, Cd/Pb (0.5/300, 1/400, 1.5/500), Cd/Zn (0.5/250, 1/500, 1.5/700) and Pb/Zn (300/250, 400/500, 500/700). The results indicated that increasing concentrations of the studied HMs in seedlings tissues significantly ($p < 0.05$) reduced the seedlings growth. Cd was more toxic to *P. oleracea* seedling, compared to Pb and Zn. Roots of *P. oleracea* seedlings were more sensitive to the studied HMs in comparison with shoot. The uptake patterns showed antagonistic impacts on each other and were reflected in response to growth parameters. The combine toxicities of Cd, Pb and Zn (Cd/Pb, Cd/Zn and Pb/Zn) were more than the toxicity due to single dose of each element but less than their additive sums.

Keywords: Heavy Metals; Seedlings Toxicity; Portulaca Oleracea; Heavy Metals Toxicity

1. INTRODUCTION

The vegetables irrigated with wastewater contain HMs, which are not only dangerous to plant life but also to animals and human beings [1,2]. For instance HMs accumulated in soil disturbs the soil ecosystem and plants development by reducing plant yield, affecting leaf & root growth and inhibiting enzymatic activities [3-5].

With wastewater irrigation, normally HMs accumulates in the soil to high levels, which are toxic and can lead ecological consequences [6]. HMs enter to human body in different moods but the food chain contamination is one of the most important pathway, which contributes 90% compared to other routes like inhalation and dermal contact [7].

Many HMs like Cd and Pb are nonessential and toxic for plant growth [8], but plant takes them up rapidly when present in growing medium [9]. Cd is phytotoxic in nature, and it not only inhibits the growth of plants but also causes cell senescence [10]. Moreover Cd badly affects respiration and photosynthesis and decreases water and nutrient uptake by plants [11]. Cd reduces root growth by decreasing cell division [12], inhibits the activities of anti-oxidative enzymes of plants [13] and induces oxidative stress in cells [14].

Similarly, Pb causes retardation of plant growth and inhibition of seed germination [15], also it has significant negative impact on seedling bio mass, root length and shoot length [16]. Same as Cd, Pb also adversely affects on photosynthesis, respiration and metabolism of plants [17]. Zn, which is one of the essential elements for many physiological processes, in higher concentrations affects mitotic activity [18], disturbs membrane integrity and permeability [19]. In high concentration Zn causes necrosis of shoot and inhibits root development [20,21]. Cd, Pb and Zn jointly reduce the uptake of other essential elements like Mn, Fe, K, Mg and Ca in plants [22]. This reduction causes low productivity and toxicity in plants in term of reduction in plant biomass.

According to Abedin & Mehrage [23] both seedling and seed germination stage are important for the study of impacts of HMs. The study of the effects of individual

HMs in control environment provide us useful information about HMs, but these information can not reflect the real exposure of plants to HMs in natural environment as the natural environment contains a mixture of different HMs. Thus effects of individual HMs on plants are different from the joint effects of mixture of HMs [24]. The combine effects of mixture of HMs depend on the components of mixture; either they have antagonistic or synergistic relationship [25]. So to evaluate the impacts of HMs on plants it is necessary to study them in combination doses as well. In the past the effects of the single HMs on plants were investigated by many researchers [26,27] however there are few studies on joint effects of HMs on plants [28-30].

This paper investigates the toxicological effects of individual Cd, Pb and Zn and their combination (Cd/Pb, Cd/Zn and Pb/Zn) on seedling biomass (shoot and root fresh and dry weights), seedling lengths (shoot and root lengths), cell size, shoot diameter, number of leaves and mitotic index (MI) of *P. Oleracae* seedlings.

2. METHODOLOGY

2.1. Soil Spiking with Heavy Metal

Sub-soil from an uncontaminated site of Peshawar, Pakistan was collected with basic properties mentioned in **Table 1**. Soil was dried in open air and passed through a 5 mm sieve. The dried and sieved soil was spiked (mg/kg) with Pb (300, 400 and 500, Cd (0.5, 1 and 1.5), Zn (250, 500, 700), Cd/Pb (0.5/300, 1/400, 1.5/500), Cd/Zn (0.5/ 250, 1/500, 1.5/700) and Pb/Zn (300/250, 400/500, 500/ 700). The spiked soil was then dried, thoroughly mixed and again sieved with a 5 mm sieve.

2.2. Pot Filling

Plastic pots were filled with 500 gm spiked soil and four replicates were prepared as required for scientific analysis.

Table 1. 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

2.3. Plant Material

P. Oleracae seeds were kept in 30% (w/w) H_2O_2 solution for 10 min for disinfection and then washed with deionized water. Seeds were germinated in Petri dishes for four days at 28˚C ± 1˚C temperature. Four uniform seedlings were cultivated in each plastic pot. Pots were kept in a greenhouse with day temperature of 25˚C ± 4˚C and night temperature of 19˚C ± 3˚C. Plants were under sun light of 12 h and relative humidity of 65% ± 2%. Deionized water was used to irrigate the seedlings. The pots were re-randomized for getting equal amount of sunlight and heat. The plants were harvested 2 weeks after cultivation.

2.4. Recording Growth Parameters

Fresh weight, length and diameter of the root and shoot were recorded and after drying in oven at 80˚C for 3 days, dry weights were also recorded.

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2.6. Mitotic Index

Germinated seedlings were fixed in a mixture of ethanol-acetic acid (3:1) for 24 h, hydrolyzed in 1 M 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 following Sharma & Sharma [31] methodology. The prepared slides were carefully observed under light microscope and MI was calculated with the following formula.

MI (%) = (Number of dividing cells/Total number of cells) × 100.

2.7. Heavy Metals Extraction and Analysis

The Grinded seedlings were weighted and about 0.5 g was put in acid washed and dried digestion tube. The powdered seedling with a mixture of (15 ml) of HNO_3, $HClO_4$, and H_2SO_4 (5:1:10) were left overnight. On following day the digestion tubes were placed on a digestion block at a temperature of 80˚C for 1 h and then raised to 120˚C - 130˚C till clear solution obtained. The digested, transparent solutions were filtered into acid washed volumetric flasks and make the volume 50 ml with double deionized water, with the help of atomic absorption spectrometer (Analyst 700 PerkElmer).

2.8. Quality Control

For verification the accuracy and precision of digestion procedure a reagent blank and standard reference soil and vegetable sample were included in our samples.

2.9. Statistical Analysis

The data were taking as the means of four replicates and statistically analyzed using SPSS. Analysis of variance (ANOVA) and Duncan Multiple Range test (DMRT) was used for confirmation variability and validity and statistical significance between the treatments respectively. Linear regression analysis was performed to establish the relationships between the HM concentrations in the plants tissues and the HM concentrations in the soil.

3. RESULTS

3.1. Heavy Metals Impacts on Plant Biomass

P. Oleracae seedling was significantly ($p < 0.05$) affected with increasing concentrations of Cd, Pb and Zn when applied alone or in cobination. Maximum high dose of Cd remarkably decreased the shoot and root fresh weight (42.3% and 38.1%) as well as its dry weights (34.9% and 39.2%). Pb high doses has the similar impacts while the addition of Zn high dose, decreased shoot and root fresh weight with a bit mild ratio (37.5%, 29.4%) compared to other toxicants in the study. The combine application of Cd/Pb in growing medium reduced shoot and root fresh and dry weights (up to 49.17%) from control.

Shoot and root fresh and dry weights were also significantly reduced ranging from 36.6% to 43.0%, respectively in comparison with the control due to highest dose of Cd/Zn. Similarly at the highest dose of Pb/Zn shoot and root fresh and dry weights were reduced by 47.8%, 40.3%, 34.2% and 39.2%, (**Table 2**) respectively. It is notable that the dry weights of both shoot and root were more affected than their fresh weights of studied seedlings.

3.2. Heavy Metals Impacts on Plant Height

Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn had a significant ($p < 0.05$) toxicological impacts on shoot and root lengths of *P. oleracea* seedlings. The highest dose of Cd and Pb and Zn reduced shoot lengths by 24.4%, 22.9% and 21.4%, respectively (**Table 2**). It means that Pb had the most adverse impact on plant height. Similarly shoot

Table 2. Percent reduction in growth parameters due to HM treatments control group.

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		
Cd 1	13.1	25.6	18.2	20.0	22.6	25.0	18.4	34.1	7.10
Cd 2	33.1	30.6	24.2	25.8	23.5	29.2	19.6	40.9	11.7
Cd 3	42.3	38.1	34.9	39.2	24.4	29.2	24.9	45.5	13.5
Pb 1	10.0	23.1	14.0	18.0	17.9	21.3	16.0	25.9	4.20
Pb 2	15.0	25.6	16.3	23.5	19.8	25.8	17.4	27.5	6.60
Pb 3	38.1	33.1	32.6	38.3	22.9	27.2	19.5	29.5	9.60
Zn 1	9.00	20.3	12.1	16.7	10.2	19.6	13.6	22.3	4.40
Zn 2	14.0	23.5	14.5	20.7	19.0	24.6	15.1	23.4	5.70
Zn 3	37.5	29.4	24.7	37.3	21.4	25.0	17.5	28.6	9.30
Cd1/Pb 1	15.0	30.0	20.6	25.3	23.1	27.3	20.8	36.9	11.9
Cd2/Pb 2	35.0	33.3	27.3	27.8	27.6	37.9	23.3	45.5	12.5
Cd3/Pb 3	45.2	40.0	40.4	49.17	30.2	39.1	27.4	47.3	15.1
Cd1/Zn 1	15.4	26.9	35.5	40.5	23.5	26.1	19.3	36.4	9.50
Cd2/Zn 2	34.6	33.0	26.3	27.6	27.4	32.1	23.1	43.6	13.9
Cd3/Zn 3	43.6	40.0	36.6	40.0	27.3	32.4	26.6	47.9	15.1
Zn1/Pb 1	11.2	24.6	16.7	20.0	21.8	25.9	20.8	28.6	6.60
Zn2/Pb 2	17.2	27.0	18.9	27.3	31.2	27.3	20.0	28.2	7.40
Zn3/Pb 3	47.8	40.3	34.2	39.2	29.0	30.4	23.4	31.8	10.5

and root lengths were reduced by 30.2% and 39.1% due to the highest dose of Cd/Pb.

Similarly at the highest dose of Cd/Zn root lengths were reduced by 32.4%. The addition of highest dose of Pb/Zn reduced root lengths by 30.4% (**Table 2**).

3.3. Heavy Metals Impacts on Plant Secondary Growth

Apart from vertical growth, heavy metals also adversely affected the longitudinal growth as the diameter of *P. oleracea* plants parts showed in response to different concentrations of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn. Shoot diameters were reduced by 24.9%, 19.5%, 17.5%, 27.4%, 26.6% and 23.4% due to the highest doses of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn, respectively. In all three treatments of combine toxicity (Cd/Pb, Cd/Zn, Pb/Zn) the shoot diameters were more affected as compare to their single dose.

3.4. Heavy Metals Impacts on Plant Number of Leaves and Mitotic Index

The increasing concentrations of HMs resulted in a significant decrease in the cell size, number of leaves and root mitotic index of *P. Oleracae* seedlings as our result showed (**Table 2**). At the highest dose of Cd, Pb and Zn the number of leaves and MI were drastically reduced. The reduction in number of leaves and MI were 47.3% and 15.1% due to the highest dose of Cd/Pb. Similarly at highest dose of Pb/Zn, number of leaves and MI were reduced by 31.8% and 10.5%, respectively as compared to control.

3.5. Heavy Metal Uptake by Plants

The relationships between the Cd concentrations in soil and in seedling tissue are given in **Figure 1**. All three relationships, *i.e.* between the single Cd concentration in soil and seedlings (**Figure 1(a)**), between Cd/Pb in soil and in seedlings (**Figure 1(b)**) and between Cd/Zn in soil and in seedlings (**Figure 1(c)**), show that with increasing concentrations of Cd in soil whether in single form or in mixture, accumulate in large amount in seedling tissues. Seedlings accumulated Cd, 42.3, 16.1 and 10.1 times of its concentration in the soil spiked with single Cd, Cd/Pb and Cd/Zn treatments, respectively (**Figure 2**). Similarly, Pb concentrations in seedlings showed increasing trend. The regression analysis showed positive relationships between the concentrations of Pb in soil and in seedlings in all the three treatments *i.e.* single Pb, Pb/Cd and Pb/Zn. (**Figures 3(a)-(c)**). **Figure 4** presents that seedlings accumulated 35%, 31% and 10.1% of Pb from single Pb, Pb/Cd and Pb/Zn treatments, respectively. The regression analysis showed strong positive relationship between the

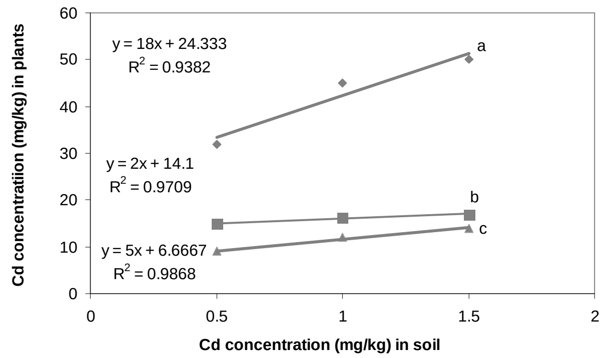

Figure 1. Cd concentrations in soil and seedlings tissues on dry weight basis; (a) Cd concentration in soil and seedlings in single Cd treatments; (b) Cd concentration in soil and seedlings in Cd/Pb treatments; (c) Cd concentration in soil and seedlings in Cd/Zn treatments.

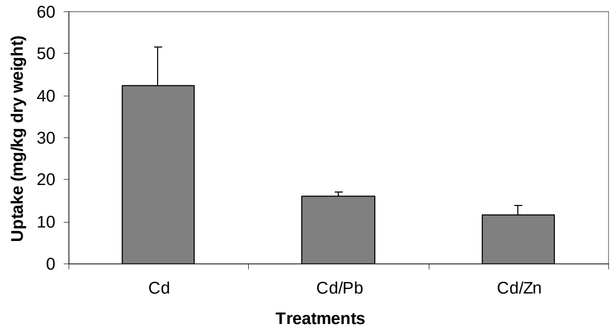

Figure 2. Comparison of Cd uptake from different treatments.

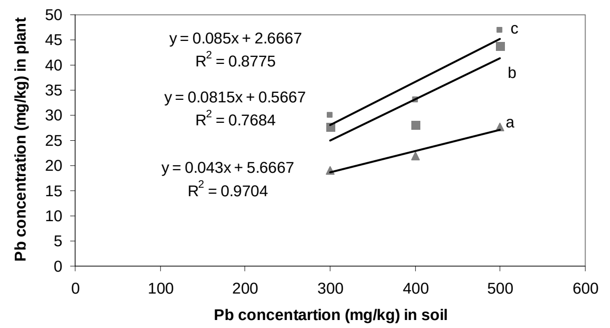

Figure 3. Pb concentrations in soil and seedlings tissues on dry weight basis; (a) Pb concentration in soil and seedlings in Pb/Zn treatments; (b) Pb concentration in soil and seedlings in Cd/Zn treatments; (c) Pb concentration in soil and seedlings in Single Pb treatments.

Zn concentration in soil and seedlings in single Zn, Zn/Cd and Zn/Pb treatments (**Figures 5(a)-(c)**). *P. Oleracae* seedlings accumulated 27.5%, 26.9% and 26.8%, of Zn concentration in the soil in single Zn, Zn/Cd and Zn/Pb treatments, respectively (**Figure 6**).

4. DISCUSSION

Plants have the ability to accumulate HMs in their

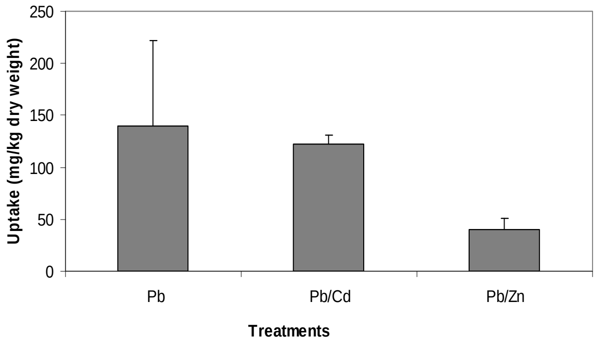

Figure 4. Comparison of Pb uptake from different treatments.

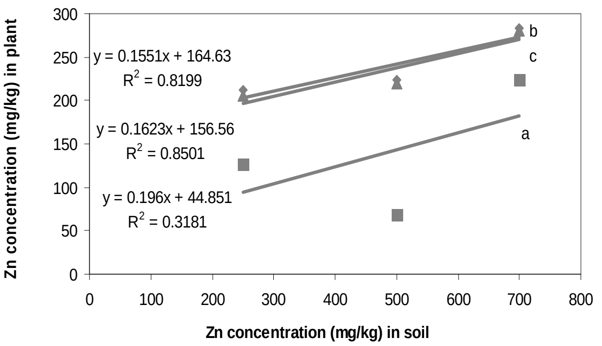

Figure 5. Zn concentrations in soil and seedlings tissues on dry weight basis; (a) Zn concentration in soil and seedlings in Zn/Cd treatments; (b) Zn concentration in soil and seedlings in Zn/Pb treatments; (c) Zn concentration in soil and seedlings in single Zn treatments.

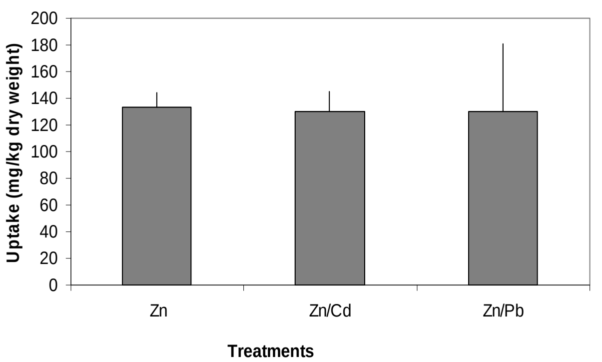

Figure 6. Comparison of Zn uptake from different treatments; Note: The error bars indicate the standard deviation.

body tissues when grow on HMs contaminated soil [2,4]. HMs are plants toxicant and affect plants badly. Zeng *et al*. [5] reported that HMs can reduce plant yield, affect leaf and root growth and inhibit enzymatic activities.

Cd inhibited plant growth and the toxicity increased with increasing concentration in soil. In the present study, increasing concentrations of Cd significantly reduced shoot and root fresh and dry weights. The results of present study are in line with the results of previous studies, for example Shute & Macfie [32], Auda *et al*. [33], Xiaoli & Shu-zhen [34] observed a reduction in biomass and lengths of soybean, carrot and wheat due to high concentration of Cd. On the other hand Fei-bo *et al.* [35] have not observed any reduction in plant growth however. Similarly other growth parameters like number of leaves, shoot diameter and MI were adversely affected with increasing concentrations of Cd which were also reported by Jun-yu *et al.* [36] in rice plant with increasing concentrations of Cd. The increasing concentration of Cd is responsible for change the rate of photosynthesis. This reduces the synthesis of carbohydrates, protein and finally decreases the growth of plants [33].

Increasing concentrations of Pb significantly ($p < 0.05$) reduced biomass, length, number of leaves, diameter and mitotic index of *P. Oleracae* seedlings. The results of the present study are consistent with the results of previous studies. For example in a study on different concentration of Pb in growing medium resulted a retarded growth of shoot and root in *C. sativus* [30]. Farooqi *et al*. [28] found a decrease in seedlings dry weight of A. *lebbeck* with the increasing concentrations of Pb. The toxicity of Pb in *P. Oleracae* seedling in the form of reduction in shoots and root fresh and dry weights was less than the Cd. It may be due to high uptake of Cd compared to Pb.

Zn is an essential element for plants, but its excess can significantly damage plant growth [37]. The results of present study also revealed that increasing Zn concentrations are responsible for retarded growth in seedlings. Shoot and root fresh and dry weights were reduced with increasing Zn concentrations. Zn reduced plant biomass due to deficiency of macronutrients uptake as reported by Marschner [38], Mahmood *et al*. [20] and Rout & Das [18].

The toxicities of HMs depend on their mobility from soil to root and then root to shoot [30]. The presence of Pb in growing medium reduced the uptake of Cd by plants and vice versa. The uptake pattern of both Cd and Pb was reflected in the growth parameters of *P. Oleracae* the results are agreed with the studies of [8,39]. The adverse impacts of Cd/Pb were more severe than the negative impacts on growth due to Cd and Pb in single form but less than the additive of the toxicity due to Cd and Pb. Zn and Cd are usually found together in ores because of similar physical and chemical properties. Our results are in line with the findings Koleli *et al.* [40] and Sharma *et al*. [41] who reported that due to the presence of Cd in growing medium, reduce the uptake of Zn and vice versa. The uptake pattern of Cd and Zn is reflected in the growth parameters of *P. Oleracae* seedlings. The toxicities due to Cd and Zn in all its three treatments were in order of Cd/Zn > Cd > Zn, but toxicity due to Cd/Zn was less than the additive sum of toxicities due to Cd and Pb.

Weihong *et al*. [42] for *V. zizanioides* and Xu *et al*. [43] for ryegrass produced the same results.

5. CONCLUSION

Result concluded that increasing Cd, Pb and Zn concentrations (single and mixture forms) produced significant ($p < 0.05$) toxicity on *P. oleracea* seedlings. Result revealed that shoot and root lengths, shoot and root biomass, shoot diameter, number of leaves, MI and cell size of *P. oleracea* were significantly affected. The co-existence of Zn and Pb like other HMs also result the change in the uptake of them. Zn and Pb interact each other when present together. The uptake pattern of Zn and Pb in Zn/Pb showed that both Zn and Pb affected the uptake of each other in antagonistic way. The uptake pattern was reflected in growth parameters. In case of *P. Oleracae* seedlings the toxicities due to Zn and Pb in all its three treatments were in order of Zn/Pb > Zn > Pb.

6. ACKNOWLEDGEMENTS

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