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Cadmium–Zinc Interaction in Relation to Lettuce Growth

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ABSTRACT
A field experiment was conducted on alluvial soil (Entisols) of Sheila Dhar Institute Experiment Farm, Prayagraj to find out the possible effect of Zinc on yield and phytoaccumulation of Cadmium in Lettuce. Cd was applied as $CdCl_2$ @ 0, 20 and 40 mg kg^{-1} and Zn was applied as $ZnSO_4$ @ 0, 40 and 80 mg kg^{-1}. It was observed that Zn application up to 80 mg kg^{-1}increased the dry biomass yield of lettuce by 20.37% over the control and decreased Cadmium concentration particularly in shoot and root of lettuce. However, the applications of higher dose of Cadmium without Zn reduced the yield of lettuce and increased the Cd concentration in shoot and root of lettuce by 3.92 and 4.73mg kg^{-1}respectvely. The reduced uptake of Cadmium was observed in Zinc treated plots. The authors conclude that the application of $ZnSO_4$@ 80 mg kg^{-1} reduced the Cd uptake in shoot and root of lettuce grown in Cd-contaminated soils.
Keywords: *Cadmium, Zinc, Lettuce, phytoaccumulation*

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INTRODUCTION

Toxic heavy metals are accumulated in agricultural soils due to human activities such as industrialization, mining, and agricultural practices[1,2,3,4]. Among these metals, cadmium (Cd) needs special attention due to its high mobility mainly in the soil-plant system as well as Cd toxicity to humans even at very low concentrations[5,6]**.** Cadmium enters into the food chain mainly through food crops grown in Cd-contaminated soil[7,8,9,10]. Higher Cd concentrations in plants caused toxicity at physiological, morphological, and molecular levels[11,12,13].

The rapid development of urbanization and the expansion of industrial sectors in and around urban cities are characteristically caused by the accumulation and contamination of urban soil heavy metals, which are greatly affected by the urban soil environment. Typically, soil heavy metals are extremely introduced into the urban environment through urban waste, waste disposal, industrial effluents, vehicle emissions, construction waste, and huge usage of agrochemicals [14, 15, 16, 17]. As a results, larger than five million sites are severely contaminated by various soil heavy metals around the world[18,16]**.**

Vegetables are an important source of human diets, and their contamination can cause serious health problems [19,20,21,22,23,24]. Leafy vegetables like lettuce are considered as potential hyperaccumulators of heavy metals [25]. One of the properties of green leafy vegetables is the accumulation of heavy metals in their tissues without exhibiting any toxicity symptoms [26]**.**

The risk of Cd uptake by crops, followed by the transfer in the food chain, is an issue of high concern is now a day. Lettuce is a worldwide important crop, one of the most consumed leafy vegetables in the human diet [27] and a high Cd-accumulating species [28]. The bioavailability of heavy metals in a plant varies for different plant organs, and the absorption and bioaccumulation rate is highest for roots as compared to other parts [29].The mean heavy metal uptake by plants increases as the contents of these metals increase in the soil environment [30].

Several studies have been conducted on the effect of Cd and Zn interaction on lettuce but the studies regarding the impact of Zinc on the Cd- contaminated soil are hardly observed. In recent years, the concentrations of heavy metals are continuously increasing considerably in this area due to untreated sewage irrigation. However, Zn deficiency has also been reported in the study area. Lettuce is a leafy vegetable that can uptake a high concentration of heavy metals than other crops. Therefore, the present study was undertaken to assess the effect of Cadmium, Zinc interaction on dry biomass yield of lettuce and uptake of Cadmium in the shoot and root of lettuce.

MATERIAL AND METHODS

Plant material and experimental layout

The Sheila Dhar Institute Experimental Site covers an area of 1 hectare, is located at Prayagraj in northern India at25° 57, N latitude, 81°50, E longitude and at 120 ± 1.4 m altitude. A sandy clay loam soil, derived from Indo-Gangetic alluvial soils, situated on the confluence of rivers Ganga and Yamuna alluvial deposit,was sampled for the study. The texture was sand (>0.2 mm) 55.54 %, silt(0.002–0.2 mm) 20.22 % and clay (<0.002 mm) 24.24 %.The physical properties were : pH-(7.8 ± 0.2), EC(dS/m) at °C -(0.28 ± 0.03), (%)- 0.038 ± 0.01, Total Cd (mgkg^{-1}) 0.17 ± 0.02 DTPA-extractable Cd (mg kg^{-1}) 1.56 ± 0.57organic carbon % -(0.56 ± 0.15), CEC [C mol (p+)/kg]- 19.6 ± 0.6, Total nitrogen (%)-0.07 ± 0.02.

(Note- ± values indicate standard error having three replications, EC- electrical conductivity, CEC-cation exchange capacity, DTPA diethyl)

After a systematic survey factorial experiment was conducted to study the effect of Cadmium-Zinc Interaction in relation to lettuce growth. The experiment was replicated thrice with nine treatments and conducted in a completely factorial Randomized Block Design (factorial RBD). After 24 hrs of the treatment seeds were sown. Soil moisture was maintained by irrigating the crops at an interval of 5-6 days. Lettuce was grown successively in the 27 plots (each of 1m^2 in the area). The treatments of Cd × Zn consisted of 0, 20, 40 mg kg^{-1} Cd along with0, 40,80 mg kg^{-1} Zn. The source of Cd and Zn were $CdCl_2$ and $ZnSO_4$.

Soil sampling

The larger fields were divided into suitable and uniform parts, and each of these uniform parts was considered a separate sampling unit. In each sampling unit, soil samples were drawn from several spots in a zigzag pattern, leaving about a 2-m area along the field margins. Silt and clay were separated by the pipette method and fine sand by decantation [31]

Soil chemical analysis

Soil pH was measured in a suspension of 1:2.5 soil water ratio by Elico digital pH meter (ModelLI127, Elico Ltd, Hyderabad, India). Double Distilled Water was used for preparing of all kinds of solutions. Organic carbon of soil was determined by rapid chromic acid digestion method of Walkley and Black. Cation exchange capacity (CEC) was determined by neutral 1N ammonium acetate. Total nitrogen was determined by micro-Kjeldahl method (Glass Agencies, Ambala, India) containing digestion mixture of sulphuric acid, selenium dioxide and salicylic acid. Available sulphur extracted with$CaCl_2$ solution (0.15%) was determined by turbidimetric method. Total phosphorus was determined by hot plate digestion using HNO_3 (16M, 71%) and extracted by standard ammonium molybdate solution [31].

Extraction for Cadmium (Cd) content in the soil

For total Cd content, one gram of soil was mixed in 5 ml ofHNO_3 (16 M, 71 %) and 5 ml of $HClO_4$ (11 M, 71 %). The composite was heated up to dryness. The hot distilled water was added. The contents were filtrated, and the volume was made up to 50 ml. The clean filtrate was used for the estimation of heavy metals (Cd) by atomic absorption spectrophotometer (AAS) (Analyst 600, PerkinElmer Inc., MA,USA). For available Cd, 5 gram of soil was mixed with 20 ml DTPA solution {Di-ethyl-triamine-penta acetic acid (DTPA) solution [1.97 g (0.05 M) DTPA powder, 13.3 ml (0.1 M) Tri-ethanol amine and 1.47 g(0.01 M) CaCl2 were dissolved in distilled water [32] and were made up to 1 l after adjusting the pH to 7.3] was added} and the contents were shaken for2 h and then filtered through Whatman filter paper No. 42. The clean filtrate was used for the estimation of Cd by the aforesaid spectrophotometer.

Processing plant samples

Plants were harvested after 60 days having higher phytochemicals at their maturity stage as suggested by Mani *et al*. (2012). Plant samples were carefully rinsed with tap water followed by 0.2 % detergent solution, 0.1 N HCl, deionizer water and double-distilled water. Samples were then soaked with tissue paper, air-dried for 2–3 days in adjust and contaminant-free environment, placed in clean paper envelopes, dried in a hot-air oven at a temperature of 45 C, and ground to a fine powder. Plant biomass dry weights were recorded. Shoot and root were separated and analyzed.

Determination of Cadmium in plant extract

One gram of ground plant material was digested with15 ml of a tri-acid mixture containing conc. HNO_3 (16 M,71 %), H_2SO_4 (18 M, 96 %) and $HClO_4$ (11 M, 71 %) in5:1:2). The composite was heated on a hot plate at low heat (60 C) for 30 min, and the volume was reduced to about5 ml until a transparent solution was obtained. After cooling, 20 ml distilled water was added and the content was filtered through Whatman filter paper No. 42[33]Total Cd was determined by the AAS.

Data analysis

The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. Graph pad Prism (version 9, Graph Pad Software, USA) software was used for drawing Figures.

RESULTS AND DISCUSSION

Effect of Cd × Zn interaction on the yield of lettuce:

Data (fig.1) indicate a significant influence of Cd, Zn& and Cd ×Zn interaction on the dry biomass yield of lettuce. The application of the maximum dose of Zn 80 mg kg^{-1}(T_3) increased the highest yield of plant 20.37% over the control. Application of Zn boosts the yield of lettuce and was found to play an ameliorative role in Cd-contaminated soil. Treatment of Cd 20 mg kg^{-1} and 40 mg kg^{-1} in without Zn treated plot significantly decreased the dry matter 15.99% and 21.89% content over the control respectively as the dose of Cd increased from 0-40 mg kg^{-1}. Combined treatment of Cd 20 mg kg^{-1} and Zn 40 mg kg^{-1} (T_5) and Cd 40 mg kg^{-1} and Zn 40 mg kg^{-1}(T_7) decreased the yield 3.40% and8.68 % respectively. Integrated application of Cd 20 mg kg^{-1} and Zn80 mg kg^{-1}(T_6) and Cd 40 mg kg^{-1} and Zn 80 mg kg^{-1}(T_9) increased the yield of plant 7.92 %and 3.77% over the control respectively.

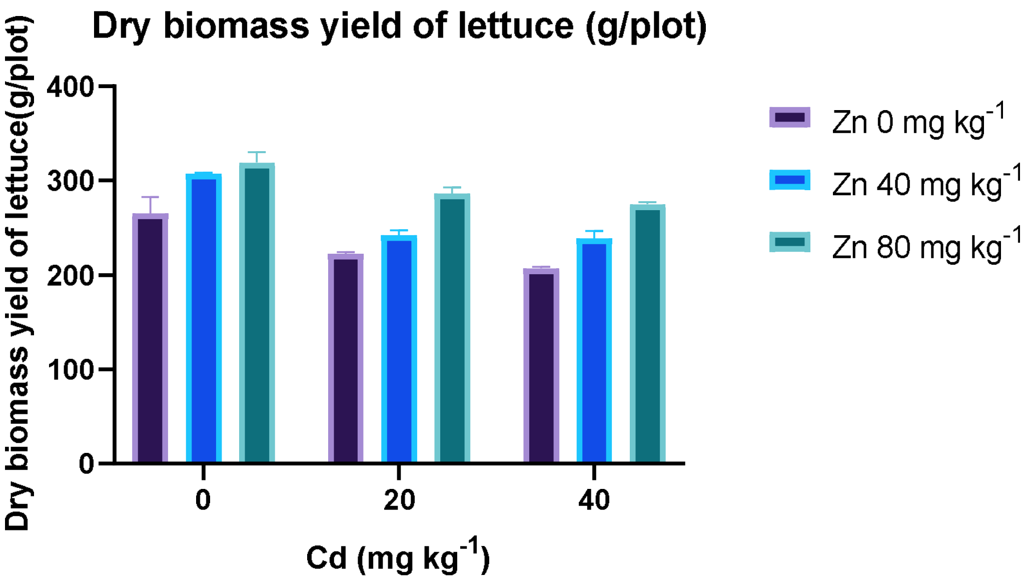
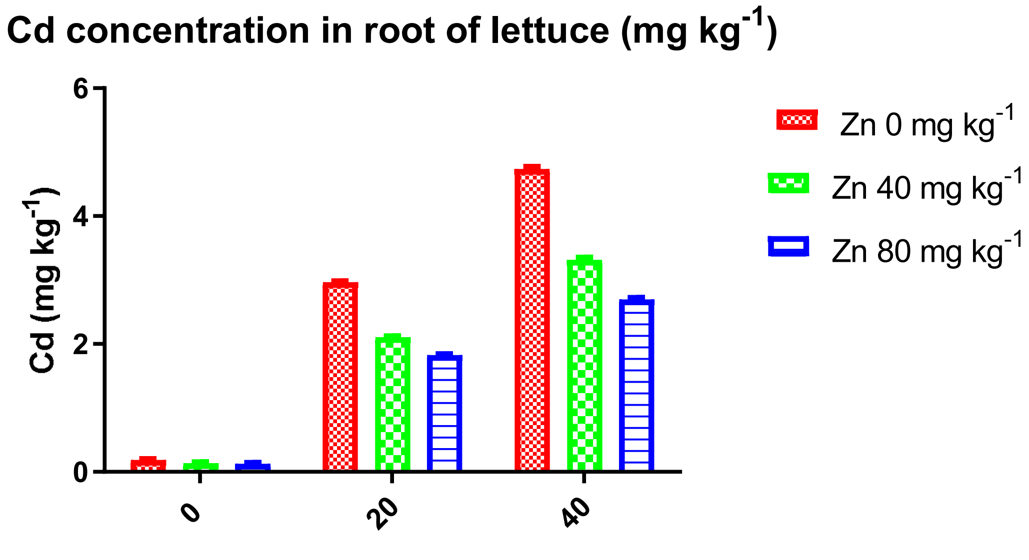


Fig.1: Effect of Cd × Zn interaction and ZSB on dry biomass yield of lettuce
[Note-Treatment combinations used under the pot experiment at Sheila Dhar, T_1-Control, T_2-Cd0 mg kg^{-1}+Zn40mg kg^{-1}, T_3-Cd 0 mg kg^{-1}+ Zn 80,T_4-Cd20 mg kg^{-1} ,T_5-Cd20 mg kg^{-1}+ Zn 40 mg kg-1,T_6-Cd20 mg kg-1+Zn 80 mg kg-1, T_7-Cd 40 mg kg^{-1}, T_8-Cd 40 mg kg^{-1} +40 mg kg^{-1},T_9-Cd 40 mg kg^{-1} +80 mg kg^{-1}.]

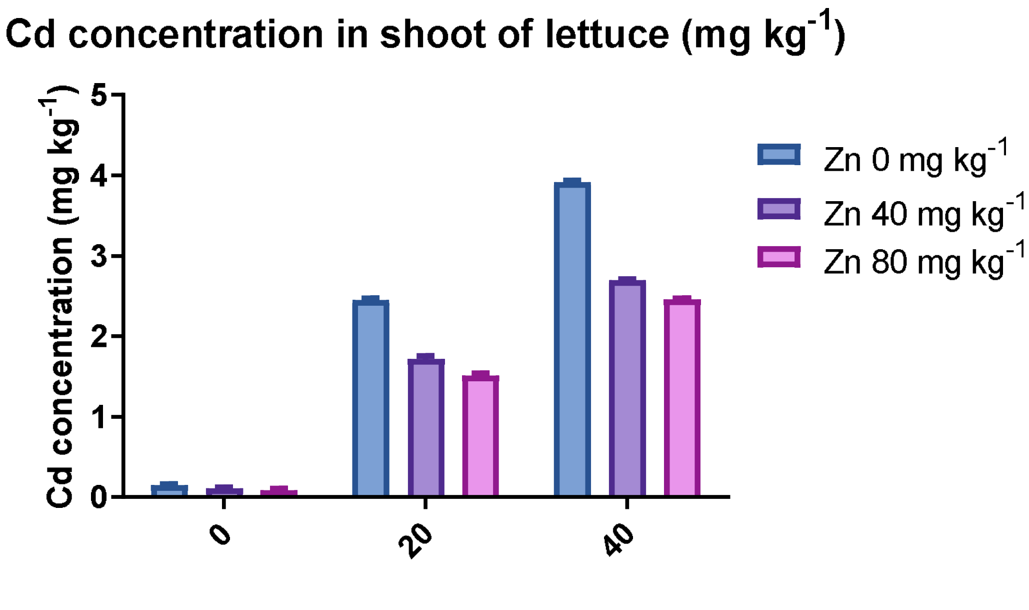
Heavy metals have significantly negative effects on plant growth [34] other toxic effects may include root browning, alteration of mineral concentrations, and changes in photosynthesis [34].Plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips and finally death[35,36,37,38].

Effect of Cd × Zn interaction on Cd concentration in root and shoot of lettuce

The data graphically presented in figures (2a) and (2b) indicated that the effect of different treatments on the uptake of Cd in shoot contaminated and root of lettuce at experimental sites was observed highly significant. Cadmium concentration varies from 0.09 mg kg^{-1} to 3.92 mg kg^{-1} and 0.12 mg kg^{-1} to 4.73 mg kg^{-1} in the shoot and root respectively. Individual application of Zinc 80 mg kg^{-1}(T_3) decreased the minimum concentration in the shoot and root of the plant by 0.09 mg kg^{-1} and0.12 mg kg^{-1}. Treatment of maximum dose of Cd 40 mg kg^{-1} (T_7) without Zn was observed the maximum concentration of 3.92 and 4.73 mg kg^{-1} in the shoot and root of lettuce. Combined application of Cd 20 mg kg^{-1} and Zn 80 mg kg^{-1}(T_6) reduced Cd uptake 1.52 and 1.82 mg kg^{-1} in shoot and root of the plant. Integrated application of Cd 40 mg kg^{-1} and Zinc 80 mg kg^{-1}(T_9) mitigates Cd uptake 2.46 and2.69 mg kg^{-1} in the shoot and root of lettuce, respectively. Zinc decreased Cd translocation to shoots of wheat[39] and lettuce [40].The plant accumulates a large portion of heavy metal in root followed by stem and leaf. The dry biomass of both roots and shoots was significantly reduced in Cd-treated plants compared to the control plants[41].



(Cd applied in mg kg^{-1})
Fig. 2a: Effect of Cd × Zn interaction on concentration in the root of lettuce



(Cd applied in mg kg^{-1})
Fig.2b: Effect of Cd × Zn interaction on Cd concentration in the shoot of lettuce

CONCLUSION

Application of maximum dos of Zn increased the highest potential to dry biomass of the plant and decreases the concentration Cd in lettuce due to reduce the solubility of Cd in soil. Thus a more detailed study is required to grow lettuce crops in Cd- soil and evaluated their growth and distribution of Cd contamination in the different edible parts of the plant. Cd is not beneficial in plant growth and reduces the plant growth and yield of the plant because it tends to accumulate in the shoot of the vegetables grown in Cd polluted soils. Application of Zn reduces Cd accumulation in plant and a high concentration of Zn (80 mg kg^{-1}) should be applied in highly Cd-polluted soil. Application of Zn increases the growth and yield of plants and minimizes Cd-accumulation in plants.

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