

## Evaluation of the effect of vermicompost and EDTA application on sunflower's uptake of heavy metals, growth and biometric parameters under the soil having Cd & Pb

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### ABSTRACT

A study was conducted to evaluate the interaction between Cd × vermicompost, Cd × EDTA and Pb × vermicompost, Pb × EDTA for Cd and Pb clean-up efficiency of sunflower (*Helianthus annuus* L.) plant on the Cd and Pb contaminated Indo-Gangetic alluvial soil of Sheila Dhar Institute (SDI) experimental farm at Prayagraj, Uttar Pradesh, India. The results showed that application of vermicompost maximum increased height (23.42% for Cd and 18.83% for Pb) and dry biomass yield (32.57% for Cd and 32.90 for Pb) whereas EDTA decreased the height (10.43% for Cd and 8.56% for Pb) and dry biomass yield (9.51% for Cd and 13.68% for Pb) of sunflower plant Cd and Pb content at 30 mg kg<sup>-1</sup> contamination level with respect to the control. The application of vermicompost and EDTA maximum increased the concentrations of Cd and Pb in the shoot (24.51, 64.33, and 47.36, 71.98%) and in the root (21.39, 63.30 and 44.91, 70.61%, respectively) were recorded Cd and Pb content at 30 mg kg<sup>-1</sup> contamination level with respective control. In conclusion, the application of vermicompost and EDTA enhanced the clean-up efficiency of sunflower on the Cd and Pb contaminated Indo-Gangetic alluvial soil. Thereafter findings need for research in contaminated soils to sustain the ecosystem for sustainable development.

**Keywords:** Cadmium, Contaminated soil, Efficiency, Lead, Sunflower

### INTRODUCTION

Contamination with heavy metals associated with agricultural activities and expanding rapid industrialization has become a serious environmental problem on a global level (Chaoua *et al.* 2019, Sandeep *et al.* 2019). When high industrial development is combined with demographic population growth, substantial environmental involves. Humans have long added significant amounts of pollutants to the soil, water, and atmosphere biotopes as a result of industrial operations such mineral mining, gas emission, pesticide use, and creation of municipal waste (Shah *et al.* 2020). Several environmental and ecological issues are heavy metal pollution of agriculture, including the introduction of dangerous heavy metals into the food chain, a decrease in crop growth and production, deterioration of soil function, and a change in the microbial community (Sharma and Archana, 2016). Cadmium (Cd) and lead (Pb) are most harmful heavy metals for the soil pollution (Guo *et al.* 2010). High Cd and Pb levels can hinder plant growth by interfering with metabolic functions, nutritional balance, and enzymatic activities. Furthermore, heavy metals

are transported up the food chain via crops, endangering the health of people and animals (Singh *et al.* 2015, Khan *et al.* 2016).

Vermicompost (VC) is an organic fertilizer that is rich in nutrients and microorganisms and when organic matter is broken down by the interaction of earthworms and microorganisms as a result of a non-thermophilic biodegradation (Beykkhormizi *et al.* 2016). Using hyperaccumulator plants and associated rhizospheric bacteria, phytoremediation is a green method for stabilizing, transferring, or degrading contaminants in soil, water, and the environment (Liu *et al.* 2020). As an alternative to the more expensive conventional purification processes, plants are being employed more and more for the restoration of polluted soil and water (Nguyen *et al.* 2017). Sunflower (*Helianthus annuus* L.) is an annual plant in the member of Asteraceae family. It is one of the target species with the greatest potential as a phytoextractor due to its important agronomic characteristics, such as tolerance to high and low temperatures and adaptation to a variety of soils. It is one of the common environmental plants that is used in a variety of situations because of its ability to grow quickly with high biomass and it can hyper

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accumulate heavy metal-materials (Kamil *et al.* 2019). Vermicompost and Zn-EDTA have been studied for their potential to improve sunflower plant clean-up efficiency. The research presented here is new in that it evaluates the effectiveness of Cd and Pb clean-up individually to eliminate competition between these contaminants for accumulation by sunflower plants. The differing results that the identical treatments had on the Cd and Pb concentrations in the sunflower plants were the other novel aspect. Therefore, the goal of this study was - (i) to assess the physico-chemical properties of Indo-Gangetic alluvial soils (ii) to assess the effect of vermicompost and EDTA on uptake of Cd and Pb by *Helianthus annuus* L. plant (iii) to assess the effect of treatments on the biometric parameters of plants, such as plant height and total biomass of sunflower plant and to assess the effect of treatment to remediate Cd and Pb from contaminated Indo- Gangetic alluvial soils.

## MATERIALS AND METHODS

The study was performed in Sheila Dhar Institute (SDI) experimental farm Mumfordganj situated between latitudes 25°20'N and longitudes 81°52'E with comparable slopes of 101m at Sheila Dhar Institute of Soil Science, University of Allahabad, Prayagraj, India. The average rainfall is 850-934mm and sub-humid climate with temperature approximate 20-25°C in the winter and 40-46°C in the summer. The average relative humidity is 66% and its vary from around 42% during summer and 87% during monsoon period. Soil samples were collected at a depth of 0-20 cm at the research experimental farm of SDI at Mumfordganj, Sheila Dhar Institute of Soil Science, University of Allahabad, Prayagraj, India. The samples were air dried, sieved with <2 mm sieve and the concentrated di-acid mixture of concentrated HNO<sub>3</sub> and HClO<sub>4</sub> (1:4 v/v) was used to digest the total Cd and Pb in the soil (Kumar and Mani 2010). The soil in the chosen experimental plots is predominantly tropical sub humid Indo-Gangetic alluvium (Entisols), with a little amount of filler soil. Sunflower plant (*Helianthus annuus* L.) was raised as a test crop and harvested when it reached maturity level. The experimental study was consisted of 16 treatments in the alluvium soil including a control plot. The goal of the current study was to investigate the relationship between Cd and Pb in a

comprehensive manner using EDTA and vermicompost to combat Cd and Pb accumulation in sunflower plants on the alluvium soil of SDI experimental farm.

The replicates were performed by factorial randomized complete block design (Factorial RBD). The experimental design was as follows: (1) without EDTA and vermicompost (control), (2) vermicompost (Four levels- 0, 400, 800, 1200 g m<sup>-2</sup>), (3) Zn-EDTA (four levels- 0, 20, 40, 60 ppm) (4) CdCl<sub>2</sub> and Pb (NO<sub>3</sub>)<sub>2</sub> (Four levels- 0, 10, 20, 30 mg kg<sup>-1</sup>) respectively. The height of plant was measured 65 days after sowing and the plants were harvested. Root and shoot of plants were separated and external particles were removed and brought to the laboratory. The plants were rinsed with deionized water and then oven-dried at 125°C to constant weight prior to chemical analysis. The plant samples were ground to a fine powder and one gram of each sample (root and shoot) separately was digested in a tri-acid mixture of concentrated HNO<sub>3</sub> (16M, 71%), HClO<sub>4</sub> (18M, 96%) and H<sub>2</sub>SO<sub>4</sub> (11M, 71%) (5:2:1 by volume, respectively) (Kumar and Mani 2010). After digestion the concentrations of available Cd for determined by atomic absorption spectrophotometer (AAS) (A Analyst 400, PerkinElmer Inc., MA, USA) at National Botanical Research Institute, Lucknow, Uttar Pradesh, India by using their respective Lumin<sup>TM</sup> Lamps with a limit of detection of 0.053 (Cd) and 0.025 (Pb) parts per million.

## STATISTICAL ANALYSIS

Statistical analyses were performed by one way analysis of variance (ANOVA) and Turkey's test was used to differentiate means of three replicates for physical, chemical, Cd, Pb, plant height and dry biomass. All statistical analyses were analyzed significance difference at the 5% (P <0.05) level of significance using IBM SPSS statistics (Version 28.0.1.0, IBM Corp., USA). The graphs were plotted using GraphPad Prism 9.3.1.471 (MSI Version 2.0, USA).

## RESULTS AND DISCUSSION

### ***Physico-chemical characteristics of the vermicompost (VC) and soil used in the study***

The main characteristics of soil and cattle vermicompost are used shown in Table 1. The

vermicompost was derived from cattle manure which had been prepared by earthworm species *Eisenia fetida* (Family-Lumbricidae) for 60 days. It was not treated with chemical and no material was added to the during vermicomposting for enriching it. Sand, silt and clay percentage of soil samples were varied from 61.50±0.60, 21.40±0.50 and 17.10±0.28% respectively. pH of VC and soil samples were ranged from 6.7±0.20 and 7.9±0.07 respectively. The value of electrical conductivity, organic carbon and cation exchange capacity of VC and soil samples were found ranging from 1.08±0.06 dSm<sup>-1</sup>, 12.80±0.60%, 92.62±0.69 cmol (p<sup>+</sup>) kg<sup>-1</sup> and 0.35±0.03 dSm<sup>-1</sup>, 0.64±0.03%, 25.80±0.18 cmol (p<sup>+</sup>) kg<sup>-1</sup> respectively. Further, addition of soil organic carbon could be due to accumulation of residual lignin in vermicompost directly flow soil

structural and metabolic carbon pool enhanced the microbial activities, organic carbon and mineralization process (Chang *et al.* 2007). Nitrogen and phosphorus content of VC and soil samples were found in varied from 1.36±0.06, 0.52±0.04 and 0.11± 0.02, 0.13±0.03% respectively. The sulphur content of VC was found in ranged of 0.54±0.08%. Cadmium (Cd) and lead (Pb) concentrations in VC and soil samples were varied from 0.03±0.01, 0.06±0.02 and 0.48±0.06, 0.72±0.05 mg kg<sup>-1</sup> respectively. The presence of heavy metals (Cd and Pb) beyond their prescribed limit and binding tendency towards sulphur and carboxylic acid (R-COOH) and minor groups of protein disturb many enzymatic activities of biota in the environments (Slavin *et al.* 2017).

Table 1: Mean (±SD) concentrations of physicochemical characteristics of the studies contaminated soil properties and cattle vermicompost at Sheila Dhar Institute (SDI) Experimental Farm, Prayagraj, India

Parameters	Unit	Soil	Vermicompost
Sand	%	61.5±0.60	-
Silt	%	21.4±0.50	-
Clay	%	17.1±0.28	-
pH	-	7.9±0.07	6.7±0.20
EC at 25°C	dSm <sup>-1</sup>	0.35±0.03	1.08±0.06
Organic carbon	%	0.64±0.03	12.8±0.60
CEC	cmol(p <sup>+</sup> ) kg <sup>-1</sup>	25.8±0.18	92.62±0.69
Total nitrogen	%	0.11±0.02	1.36±0.06
Total phosphate	%	0.13±0.03	0.52±0.04
Sulphure (SO <sub>4</sub> )	%	-	0.54±0.08
Cd	mg kg <sup>-1</sup>	0.48±0.06	0.03±0.01
Pb	mg kg <sup>-1</sup>	0.72±0.05	0.06±0.02

### **Effect of vermicompost and EDTA on growth and biometric properties of the sunflower plant**

The results indicated that for the contaminated soil with Cd and Pb the application of vermicompost significantly (p <0.05) increased and EDTA significantly (p <0.05) decreased plant dry biomass yield compared to control (Fig. 1 & 2). These are numerous reasons for improvement of plant biometric properties under the effect of vermicompost and these include changes physical, chemical and biological properties of soil for plant growth. At 30 mg kg<sup>-1</sup> Cd and Pb contaminated level in the Indo-Gangetic alluvial soil, the treatments of vermicompost led to an increasing the plant height by 8.52, 16.72, 23.42 and 9.67, 16.26,

18.83% and dry biomass yield by 14.70, 26.17, 32.57 and 13.78, 25.53, 32.90% of Cd and Pb respectively, when compared to the control (Fig. 1 & 2). Vermicompost impact on plant biometric parameters has improved for a combination of factors, including modifications to the physical, chemical and biological properties of soils for plant growth. The use of vermicompost improved plant growth, increased root length and growth-promoting rhizobacteria in the rhizosphere of chickpea (*Cicer arietinum*), when compared to the control, the plants fresh and dry weights and shoot length gradually increased (Sahni *et al.* 2008).

The application of EDTA dramatically reduced the plant height and dry biomass yield of both parts of the plant whereas vermicompost increased plant height and dry biomass yield

with respect to control. On the other hand, the biometric properties of the plants were reduced by the application EDTA at each contamination level of Cd and Pb in the Indo-Gangetic alluvial soil but vermicompost increased these properties (Fig. 1 & 2). At the 30 mg kg<sup>-1</sup> Cd and Pb contaminated level in the Indo-Gangetic alluvial soil, the application of EDTA significantly ( $p < 0.05$ ) decreasing the plant height by 4.16, 8.41, 10.43 and 3.33, 5.39, 8.56% and plant dry biomass yield by 3.09, 6.41, 9.51 and 7.36, 9.85, 13.85% of Cd and Pb respectively, when

compared to their respective control (Fig. 1 & 2). Despite the fact that high EDTA concentrations are hazardous to plants and ultimately lower plant development, dry biomass production, and metal concentrations in the shoot, they reported that the EDTA level led to a larger total accumulation of heavy metals. Reduced seed germination, chlorosis, necrosis abscission, shoot desiccation and transpiration caused low plant biomass in plants exposed to high levels of both free heavy metals (Cd, Pb) and free chelates (Nascimento *et al.* 2006).

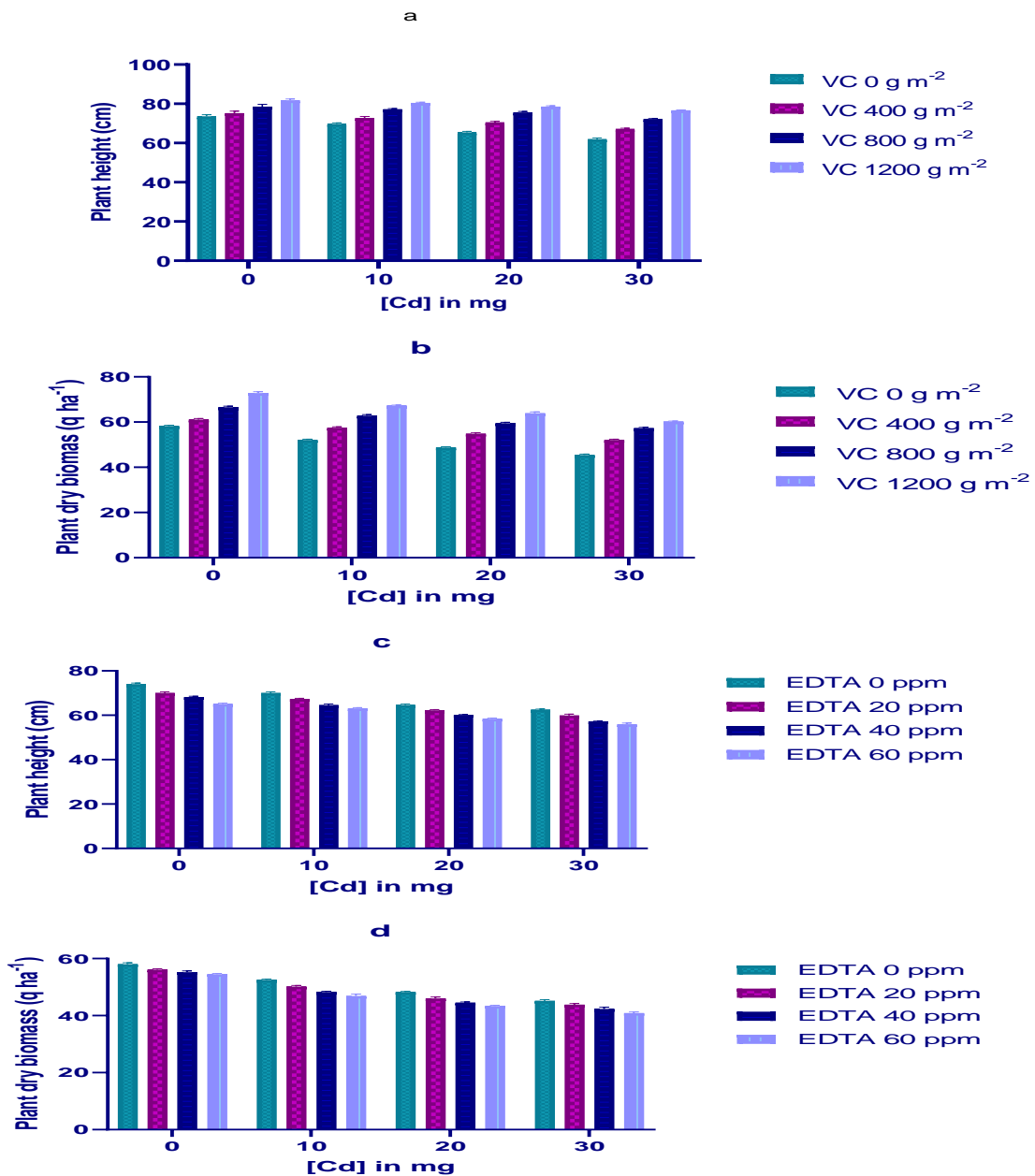


Fig. 1 Showing the effect of VC and EDTA application on the plant height (a, c) and dry biomass yield (b, d) in sunflower plant grown in Cd contaminated Indo-Gangetic alluvial soils. All the values three replicates ( $n=3$ , mean $\pm$ SD). All the represent data are significant difference at  $p < 0.05$ .

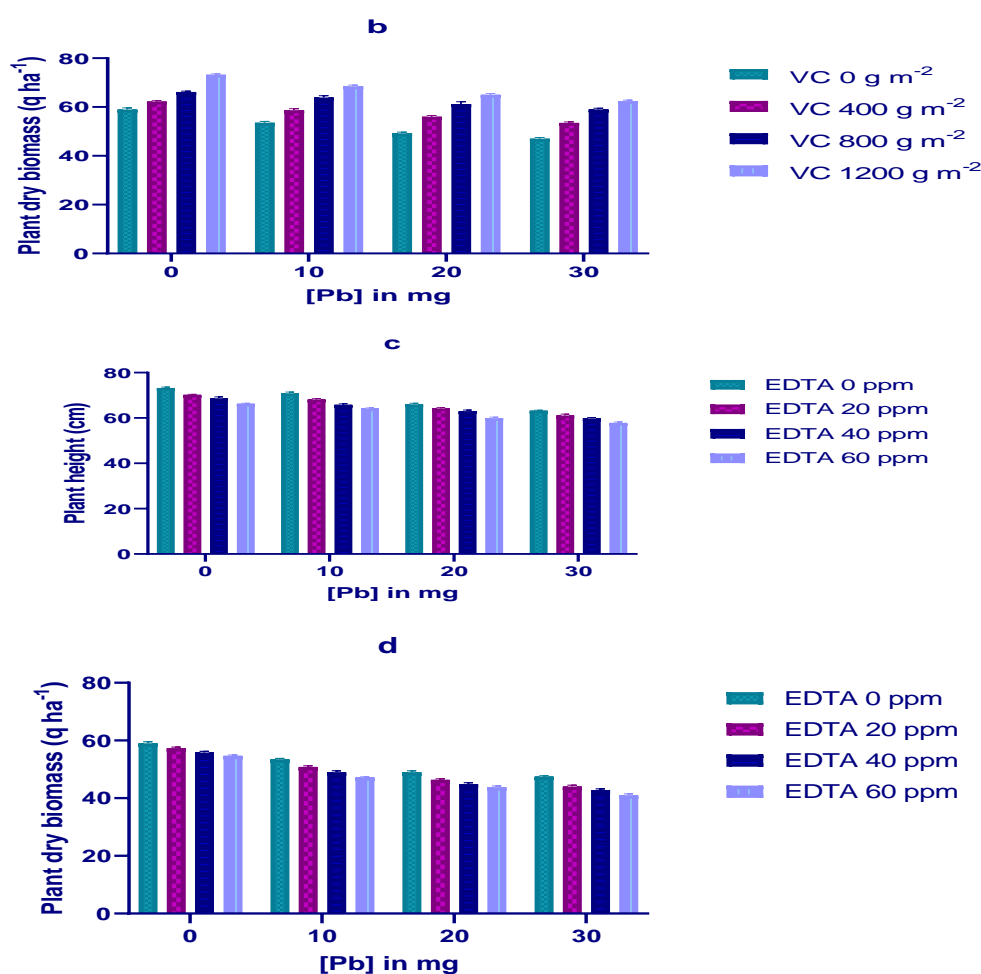


Fig. 2: Showing the effect of VC and EDTA application on the plant height (a, c) and dry biomass yield (b, d) in sunflower plant grown in Pb contaminated Indo-Gangetic alluvial soils. All the values three replicates ( $n=3$ , mean $\pm$ SD). All the represent data are significant difference at  $p < 0.05$

### **Effect of vermicompost and EDTA on concentrations of Cd and Pb in tissues of sunflower plant**

The results of this study show that application of vermicompost and EDTA significantly ( $p < 0.05$ ) increased the Cd and Pb concentrations in the shoot and root of sunflower plant when compared to control for each contamination level in the Indo-Gangetic alluvial soil (Fig. 3 & 4). Furthermore, on increasing the contamination level of Cd and Pb in Indo-Gangetic alluvial soil, the concentrations of these heavy metals in both parts shoot and root of sunflower plant increased. At a level of  $30 \text{ mg kg}^{-1}$  Cd and Pb contamination in Indo-Gangetic alluvial soil, the application of vermicompost increased the Cd and Pb concentrations in the shoot by 8.25, 21.35, 24.51 and 5.26, 36.84, 47.36% and in the root by 8.95, 14.41, 21.39 and

14.17, 35.32, 44.91% respectively, with respect to the corresponding control (Fig. 3 & 4). The adsorption Pb by vermicompost application was markedly higher than Cd (Zhu *et al.* 2017). The application of sheep manure extract increased the bioavailability of Cd in the soil and also enhanced the heavy metal accumulation in the shoot and root of sunflower plant (Rizwan, Ali, Rizvi *et al.* 2016).

The solubilization of contaminated soil Cd and Pb for root accumulation and translocation in the above ground of the aerial tissues under the effect of EDTA is one of the reasons for the increasing in Cd and Pb concentrations in the shoot parts of sunflower plant. In the plots contaminated with  $30 \text{ mg kg}^{-1}$  Cd and Pb, the application of EDTA increased the Cd and Pb concentrations in the shoot by 36.87, 49.63, 64.33 and 42.14, 58.08, 71.98% and in the root by 35.55, 55.96, 63.30 and 40.81, 50.61, 70.61%

respectively with to the control (Fig. 3 & 4). In this experiment described here, it was found that higher concentrations of Cd and Pb in the sunflower root than in the shoot after all treatments (Fig. 3 & 4). EDTA has a great potential to accumulate and increase the concentrations of Cd and Pb in the shoot and root of sunflower plant. This could be due to

altering physico-chemical properties of soil, plants rhizospheric processes and its mechanisms (Shittu *et al.* 2015). In addition, the chelating agents (EDTA) of these compounds help in the translocation of heavy metals (Cd and Pb) from roots to the aerial parts of plant (Rostami and Azhdarpoor 2019).

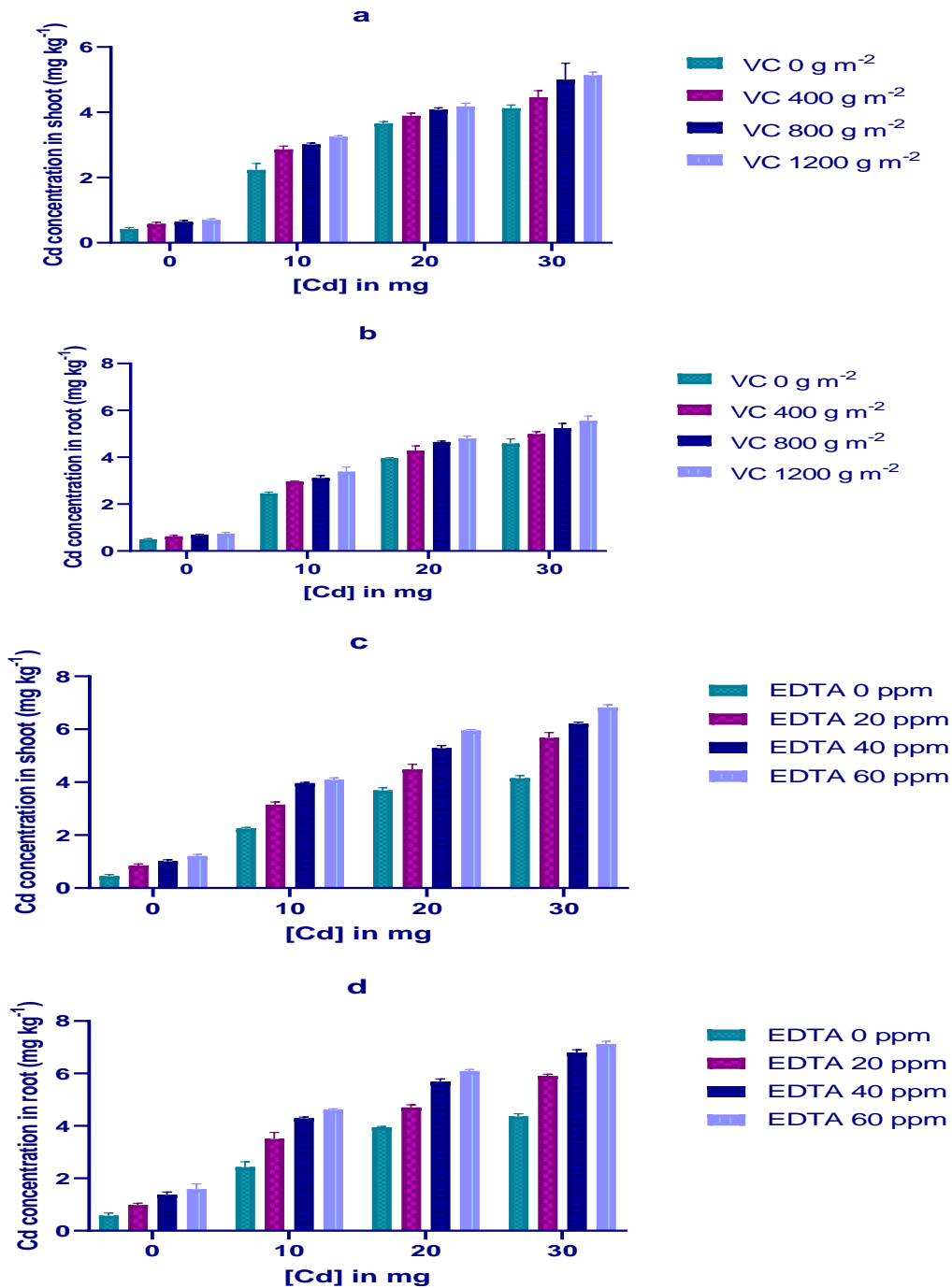


Fig. 3: Showing the effect of VC and EDTA application on the accumulation of Cd in the shoot (a, c) and root (b, d) of sunflower plant grown in Cd contaminated Indo-Gangetic alluvial soils. All the values three replicates ( $n=3$ , mean $\pm$ SD). All the represent data are significant difference at  $p < 0.05$ .

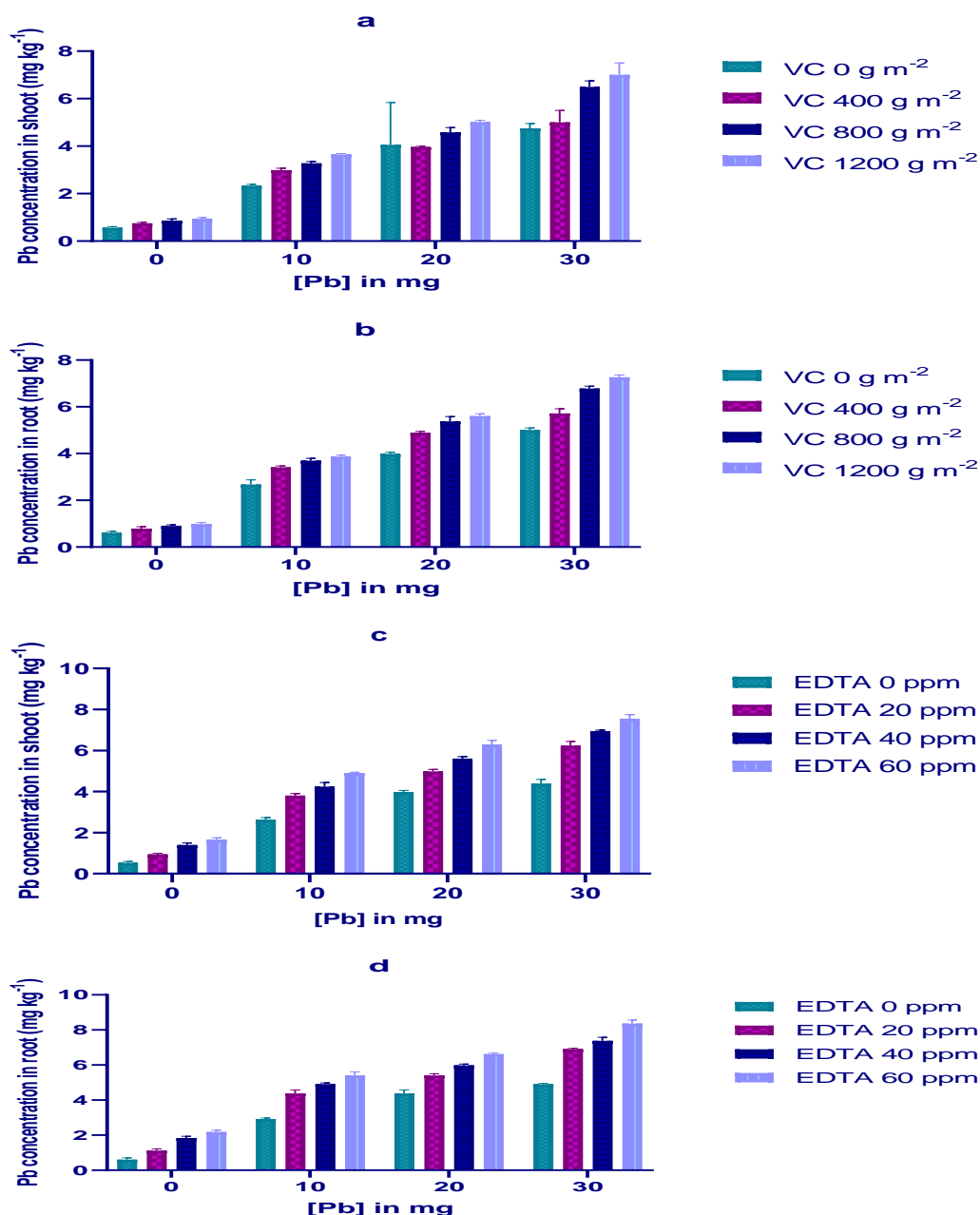


Fig. 4: Showing the effect of VC and EDTA application on the accumulation of Pb in the shoot (a, c) and root (b, d) of sunflower plant grown in Pb contaminated Indo-Gangetic alluvial soils. All the values three replicates ( $n=3$ ,  $\text{mean}\pm\text{SD}$ ). All the represent data are significant difference at  $p < 0.05$

## CONCLUSION

This study revealed that the application of vermicompost stimulated the growth, biometric properties and biomass of sunflower plant whereas EDTA decreased these properties. The concentrations of Cd and Pb in the shoot and root parts of sunflower plants significantly ( $<0.05$ ) increased with the application vermicompost and EDTA compared with control. EDTA proved to be advantageous in increasing the maximum concentrations of Cd

and Pb compared to vermicompost in both parts of the sunflower plant and it also gave higher bioaccumulation index but it had negative effects on plant height and dry biomass yield. According to the results, it is recommended that vermicompost and EDTA may be applied in the heavy metals (Cd and Pb) contaminated Indo-Gangetic alluvial soil in order to enhanced clean-up efficiency of sunflower plant to sustain the ecosystem and minimize the venomous influence on environment.

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