



THE EFFECT OF Cd × Ca INTERACTION AND ORGANIC MATTER ON SUNFLOWER

Dinesh Mani, Bechan Singh* and Vipin Sahu

Sheila Dhar Institute of Soil Science, Department of Chemistry, University of Allahabad,
Prayagraj - 211002 (U.P.), India.

Abstract

An investigation was conducted to study the interaction between Cd and Ca, organic matter for Cd phytoremediation in sunflower (*Helianthus annuus* L.) on alluvial soil (ENTISOLS) of Sheila Dhar Institute of Soil Science experimental farm, Prayagraj. Cd was applied as CdCl₂ @ 0, 10, 20 mg kg⁻¹, Ca was applied as CaCO₃ @ 0%, 0.5% and 1%, Organic matter as compost @ 0, 1, 2 kg/m². It was observed that Ca application 1% and compost 2 kg/m² increased the biomass yield and seed yield of sunflower by 12.99% and 8.52% over the control and decrease Cd concentration in shoot and root of sunflower. However application of Cd without Ca and compost reduced yield and increase the Cd concentration in shoot and root of sunflower by 2.95 mg kg⁻¹ and 3.2 mg kg⁻¹ respectively. The reduced uptake of Cd was observed in Ca and compost treated plots. The author concludes to apply high dose of Ca and compost to enhance yield of sunflower and phytoremediation of Cd-contaminated soils through soil-plant rhizospheric process.

Key words: Cadmium, Calcium, Phytoremediation, Interaction, Organic matter

Introduction

The problem of heavy metal contamination in the environment is widespread. Taken up by plants, heavy metals may enter the food chain, and therefore, humans can also be exposed to them (Intawongse and Dean 2006). Due to their properties such as toxicity, persistence, and non-biodegradation, contamination with metals has become a serious and widespread environmental threat, particularly in urban areas (Yang-Guang *et al.*, 2016). The soil may be contaminated by the accumulation of toxic metals through emissions from the industrial activities, land application of fertilizers, pesticides, spillage of petrochemicals, wastewater irrigation, and atmospheric deposition (Khan *et al.*, 2008; Zhang *et al.*, 2010). Increasing levels of soil contamination with heavy metals may be transformed and transported to plant and from plants pass into animals and humans (Atayese *et al.*, 2010).

Heavy metal pollution has become an issue of serious international concern. One of the heavy metal cadmium (Cd) is known to be a widespread environmental contaminant and a potent toxin that may adversely affect

human health (Chellaiah 2017). Cadmium (Cd) is a nonessential toxic heavy metal. In recent decades, anthropogenic activities, such as mining, smelting, and application of sludge and fertilizer, have resulted in Cd contamination of agricultural lands worldwide (Duan *et al.*, 2018). The main source of Cd contamination in the soil and environment is emissions, batteries and commercial fertilizers (Liu *et al.*, 2015). Plants grown in Cd-contaminated soils can accumulate heavy metals at high concentration causing serious risk to human health when consumed. The accumulation of Cd in plants may cause several physiological, biochemical and structural changes (Khan *et al.*, 2007) such as alters mineral nutrients uptake (Hossain *et al.*, 2010), disturbs the Calvin cycle enzymes, photosynthesis and carbohydrate metabolism (Hossain *et al.*, 2010) changes the antioxidant metabolism (Khan *et al.*, 2009) and lowers the crop productivity (di Toppi and Gabbrielli 1999). It inhibits various biochemical and physiological activities causing visible symptoms of toxicity leading to a reduction in biomass growth of plants.

The addition of organic matter amendments, such as compost, fertilizers and wastes, is a common practice

**Author for correspondence* : E-mail : bechansinghch@gmail.com

for immobilization of heavy metals and soil amelioration of contaminated soils (Clemente *et al.*, 2005). The effect of organic matter amendments on heavy metal bioavailability depends on the nature of the organic matter, their microbial degradability, salt content, and effects on soil pH and redox potential, as well as on the particular soil type and metals concerned (Walker *et al.*, 2003, 2004). Essential nutrients are required for normal growth of plants (Hussain *et al.*, 2019). Calcium (Ca) is an essential plant macronutrient that involved in various plant physiological processes, such as plant growth and development, cell division, cytoplasmic streaming, photosynthesis, and intracellular signalling transduction. Organic amendments like compost may effectively reduce the bio-availability of heavy metals in soils due to its high content of organic matter. Calcium is very effective in decreasing the metal bio-availability due to the introduction of additional binding sites for heavy metals and due to pH effects.

Plants have differed in their ability to accumulate heavy metals for this concern, the selection of plant species for phytoextraction of heavy metals depends mainly on the ability of tolerant capacity and the biomass of the selected plant. Sunflower (*Helianthus annuus* L.) is a Native Americans annual plant belonging to the family of Asteraceae with a large flowering head (inflorescence) and it grows in a wide range of soil types. *H. annuus* was selected in this study based on its high biomass, fast growth rate and its ability to remove heavy metals from contaminated soils (Forte and Mutiti, 2017).

Therefore, the present study was undertaken to assess the effect of Cd × Ca interaction and organic matter on dry biomass yield, seed yield, the concentration of Cd in root and shoot of sunflower.

Materials and Methods

Plant material and experimental layout:

The Sheila Dhar Institute Experimental Site covers an area of 1 hectare, is located at Allahabad (Prayagraj) in northern India at 25°57' N latitude, 81°50' E longitude and at 120 ± 1.4m 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) 55.53%, silt (0.002-0.2 mm) 20.22% and clay (<0.002mm) 24.25%. The physical properties were: pH-(7.8 ± 0.2), EC(dS/m) at °C -(0.28 ± 0.03), organic carbon % -(0.56 ± 0.15), CEC [C mol (p+)/kg]- 19.6 ± 0.6, Total nitrogen (%) -0.07 ± 0.02, phosphorus (%) -0.038 ± 0.01, Total Cd (mg kg⁻¹) 0.17 ± 0.02.

(Note- ± values indicate standard error having three replications, EC electrical conductivity, CEC cation

exchange capacity).

Experimental layout:

After a systematic survey factorial experiment was conducted to study the effect of Cadmium- Calcium Interaction and Organic matter on sunflower. The experiment was replicated thrice with ninth treatments and conducted in a completely factorial randomized block design (factorial RBD). After 24 hr of the treatment seeds were sown. Soil moisture was maintained by irrigating the crops at an interval of 5-6 days. Sunflower was grown successively in the 27 plots (each of 1m² in the area). The source of Cd and Ca were CdCl₂ and CaCO₃. Cd as CdCl₂ @ 0, 10 and 20 mg kg⁻¹, Ca as CaCO₃ @ 0%, 0.5% and 1% and Compost @ 0, 1 and 2 kg/m².

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 zig zag 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 (Chopra and Kanwar 1999).

Extraction for Cadmium content in the soil:

For total Cd content, one gram of soil was mixed in 5 ml of HNO₃ (16 M, 71%) and 5 ml of HClO₄ (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) (AAnalyst 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 (Lindsay and Norvell 1978) and were made up to 1 l after adjusting the pH to 7.3] was added} and the contents were shaken for 2 h and then filtered through Whatman filter paper No. 42. The clean filtrate was used for the estimation of Cr by the aforesaid spectrophotometer.

Soil pH:

Soil pH was measured with 1: 2.5 soil-water ratio using Elico digital pH meter (Model LI 127, Elico Ltd., Hyderabad, India) at the Laboratory of Sheila Dhar Institute of Soil Science, University of Allahabad, Prayagraj - 211002, Uttar Pradesh, India. Double distilled water was used for the preparation of all solutions.

Organic Carbon:

One gram soil was digested with 10 ml of 1 N potassium dichromate ($K_2Cr_2O_7$) solution and 20 ml of concentrated sulphuric acid (18 M, 96%). The solution was shaken well for 2 min and kept for half an hour and then diluted with 200 ml of distilled water. Then, 10 ml of ortho-phosphoric acid (15 M, 85%) and 1 ml of diphenylamine indicator were added in solution. The solution became deep violet, and further, it was titrated against N/2 ferrous ammonium sulfate solution, till the violet color changed to purple and finally to green (Chopra and Kanwar 1999).

Cation exchange capacity (CEC):

CEC was determined by using a neutral 1 N ammonium acetate solution. A known weight of soil (5 g) was shaken with 25 ml of the acetate solution for 5 min and filtered through Whatman filter paper No. 42 (Chopra and Kanwar 1999).

Total Nitrogen:

One gram soil was digested with 10 ml of digestion mixture containing sulphuric acid and selenium dioxide. Salicylic acid was also added to include the nitrates and nitrites. Digestion was carried out until the soil colour changed to white. The N in the digest was estimated by using the micro-Kjeldahl method, Glass Agencies, Ambala, India (Chopra and Kanwar 1999).

Total Phosphorus:

Two-gram soil was taken with 4 ml $HClO_4$ (11 M, 71%) in a 50-ml beaker covered with a watch glass and put on a hot plate, and digestion was carried out till the soil colour changes to white. Ten ml HNO_3 (16 M, 71%) was added to the filtrate solution. Ammonia was added to saturate the solution. Then 30 ml standard ammonium molybdate solution was added in the solution to extract the total phosphorus content from soil (Kumar and Mani 2010).

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 with 15 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%) in 5 : 1 : 2. The composite was heated on a hot plate at low heat (60°C) for 30 min, and the volume was reduced to about 5 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 (Kumar and Mani 2010). Total Cd was determined by the AAS.

Data analysis:

The experimental results were expressed as mean \pm standard error of mean (SEM) of three replicates. Graph pad Prism (version 8, Graph Pad Software, USA) software was used for drawing Figures.

Results and Discussion

Effect of Cd \times Ca interaction and organic matter (O.M.) on dry biomass yield and seed yield of sunflower

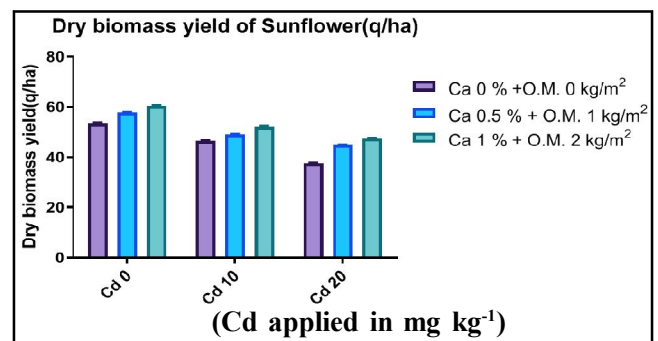


Fig. 1: Effect of Cd \times Ca interaction and organic matter (O.M.) on dry biomass yield of sunflower.

In the control and amendments (calcium and organic matter) treated plots plant seems healthy whereas plant grown in maximum dose of cadmium

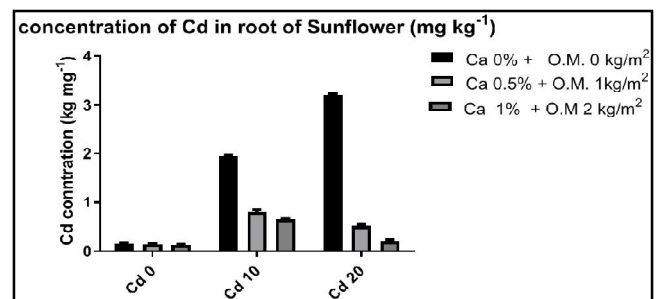


Fig. 3: Effect of Cd \times Ca and interaction and organic matter (O.M.) on Cd concentration in root of sunflower.

Fig. 1, 2 indicate that cadmium was effective for decreasing dry biomass yield and seed yield of plant whereas reduced in all plant grown in Cd-contaminated plots compared to control and amendments treated plots. Application of Ca 1% and organic matter 2 kg/m²

increased the dry biomass yield and seed yield of sunflower by 12.99% and 8.52% respectively over the control. The application of 20 mg kg⁻¹ Cd maximum reduced dry biomass yield and seed yield of sunflower by 30.04% and 34.07% compared to control. Many studies had reported an inhibitory effect of various heavy metals, including Cd and Pb, on plant growth (Sharma and Dubey 2005; Mishra *et al.*, 2006). According to previous reports, the results of this study showed that the growth of sunflower plants is reduced by Cadmium.

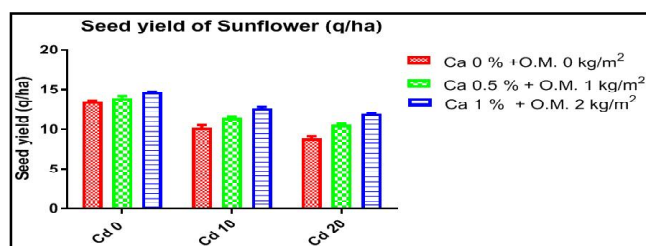


Fig. 2: Effect of Cd × Ca interaction and organic matter (O.M.) on seed yield of sunflower.

Effect of Cd × Ca and interaction and organic matter on Cd concentration in root and shoot of sunflower

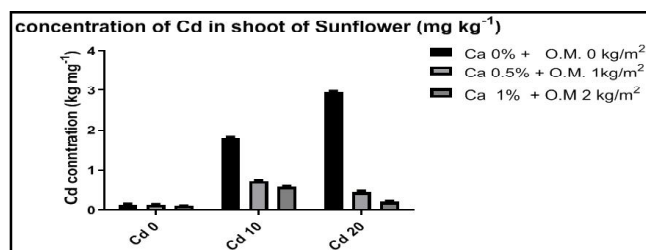


Fig. 4: Effect of Cd × Ca and interaction and organic matter on Cd concentration in shoot of sunflower.

The data graphically presented in Fig. 3 and 4 indicated that the effect of different treatments on the uptake of Cd in shoot and root of sunflower at experimental sites was observed highly significant. Cadmium concentration varies from 0.11 mg kg⁻¹ to 2.95 mg kg⁻¹ and 0.16 mg kg⁻¹ to 3.2 mg kg⁻¹ in shoot and root respectively. Treatment of Calcium 1% mg kg⁻¹ with organic matter decreased the minimum concentration in the shoot and root of the sunflower plant by 0.20 mg kg⁻¹ and 0.21 mg kg⁻¹. Treatment of maximum dose of Cd 20 mg kg⁻¹ without amendments was observed the maximum concentration of 2.95 and 3.2 mg kg⁻¹ in the shoot and root of sunflower. Mani *et al.*, (2007) investigated the interaction between Cd and Ca, Zn and organic matter for Cd-phytoremediation in sunflower and suggested the phytoremediation of Cd-contaminated soil through soil-plant-rhizospheric processes. The data revealed that application of Ca and organic matter reduce uptake of Cd below 0.22 mg kg⁻¹ and 0.21 mg kg⁻¹ in the root and shoot of plants.

Conclusion

Therefore, Ca 1% and organic matter 2 kg/m² application may be recommended to enhance dry biomass yield and seed yield of sunflower. The response of Ca and organic matter was observed ameliorative in Cr-contaminated plots. Organic amendments like compost may effectively reduce the bio-availability of heavy metals in soils due to its high content of organic matter. Calcium is very effective in decreasing the metal bio-availability due to the introduction of additional binding sites for heavy metals and due to pH effects.

The present study suggests that sunflower with high biomass has efficiently removed cadmium demonstrate a suitable potential plant for the cleaning of Cd metal-polluted environment. The use of soil amendments like manures could be one of the approaches to immobilize metal in the soil for reducing its toxicity to plants.

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