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# Phytoaccumulation, Interaction, Toxicity and Remediation of Cadmium from *Helianthus annuus* L. (sunflower)

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**Absract** An investigation was conducted to study the interaction between Cd and Ca, Zn and organic matter for Cd-phytoremediation in sunflower on the alluvium soil of the Sheila Dhar Institute (SDI) experimental farm, Allahabad (India). Application of 40 ppm Zn produced 11.18% extra dry matter (DM) content and 5.8% extra seed yield over the control. We recommended 1.0% Ca, 40 ppm Zn and 20 tons/ha of compost to enhance dry matter yield and diminish the Cd accumulation in 15 ppm Cd- ethylenediaminetetraacetic (EDTA)-treated plots up to 1/12 folds in sunflower (<0.21 ppm), which indicated phytoremediation of Cd-contaminated soil through soil-plant-rhizospheric processes.

**Keywords** Cadmium · Phytoremediation · Sunflower · Toxicity

The need for environmental management in both industry and agriculture has been widely accepted. We have therefore chosen to consider appropriate practicable suggestions for combating Cd accumulation in the ecosystem for sustainable agriculture. The increasing load on the environment is increasing the density of metals in the soil, which may become fatal not only for plants but also for animals and human (Eric, 2003). Cadmium is considered one of the most important metal contaminants since the Itai-itai disease took place in Japan (Cai and Braids, 2002). Therefore, it is wise to take steps to curb the accumulation of heavy metals in soil, while scientific analysis of soil must estimate their accumulation/mobility, which in turns affects vegetation. Metal-hyperaccumulating Thlaspi species have attracted significant research effort over the last decade (Roser et al., 2006). $CaCO_3$ and soil pH are also two crucial factors in terms of availability of these heavy metals in sewage-irrigated soils (Laetitia et al., 2002). Ca is among the least researched nutrients in India. Ca content was found to be relatively low in sewage-water-irrigated soil samples compared to tube-well-irrigated soils (Tiwari et al., 2003). To combat Cd accumulation, Zn at higher concentrations has been found to interfere with the transport of Cd into phloem cells from companion cells (Cakmak and Marschner, 1988). The present research work was therefore undertaken to assess the effect of Ca, Zn and organic matter (OM) on the uptake of Cd by sunflower.

## Materials and Methods

After a systematic survey, the experiment was laid out in factorial design in order to study the interaction and management practices at the SDI experimental farm, Allahabad, India. Soils in the selected plots are composed mostly of recent and old alluvium (Entisols) with some filler soil. The physicochemical properties of the experimental soil have been given in Table 1a. Sunflower (*Helianthus annuus* L.) was grown as a test crop and harvested at maturity. The experimental work consisted of 16 treatments to soil by weight, including a control. The present investigation was conducted with a view to study the interaction between Cd and Ca, Zn and organic matter to combat Cd accumulation in sunflower on the alluvium soil of the SDI

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**Table 1(a)** Mean (±SD*) concentrations of physicochemical properties of soils at Sheila Dhar Institute (SDI) Experimental Farm, Allahabad region, India

| Parameters | Values | Parameters | Values (ppm) |
|---|---|---|---|
| Texture % (Sand, silt and clay, sandy clay loam) | 56 ± 5.5, 20 ± 2.6 and 24 ± 5.2, respectively | DTPA-extr. Cd | 0.38 ± 0.23 |
| pH | 8.0 ± 0.3 | DTPA-extr. Cr | 0.34 ± 0.25 |
| EC (d $Sm^{-1}$) at 25°C | 0.28 ± 0.07 | DTPA-extr. Cu | 0.36 ± 0.21 |
| Organic carbon (%) | 0.56 ± 0.14 | DTPA-extr. Pb | 0.64 ± 0.16 |
| CEC [C mol ($p^+$) $kg^{-1}$] | 19.8 ± 8.45 | DTPA-extr. Zn | 12.80 ± 5.7 |
| Total nitrogen (%) | 0.08 ± 0.03 | DTPA-extr. Fe | 13.50 ± 6.8 |
| Total phosphate (%) | 0.09 ± 0.04 | DTPA-extr. Mn | 10.60 ± 5.9 |

(**b**) Detection levels: evaluation data obtained for calculating the LOD and LOQ values for the extraction/analysis using the 3(RMSE)/slope method

| Analyte | Sensivity for AAS (μg $mL^{-1}$) | ELOQ (mg $kg^{-1}$) | LLMV (mg $kg^{-1}$) | Av. recovery (R) ± standard deviation (%) | Calc. MDL (mg $kg^{-1}$) | Calc. MQL (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|
| Cd | 0.03 | 0.03 | 0.06 | 94 ± 4.3 | 0.015 | 0.045 |
| Cr | 0.2 | 0.05 | 0.10 | 96 ± 5.3 | 0.03 | 0.09 |
| Cu | 0.1 | 0.01 | 0.02 | 93 ± 3.8 | 0.005 | 0.015 |
| Pb | 0.2 | 0.02 | 0.05 | 101 ± 6.5 | 0.01 | 0.03 |
| Zn | 0.03 | 0.05 | 0.10 | 104 ± 7.8 | 0.05 | 0.15 |
| Fe | 0.1 | 0.01 | 0.02 | 107 ± 7.1 | 0.04 | 0.12 |
| Mn | 0.06 | 0.02 | 0.04 | 95 ± 4.5 | 0.05 | 0.15 |

*Standard differences were calculated among three regions (Mumfordganj, Naini and Jhusi) from Allahabad, India

LOD: level of detection, LOQ: level of quantification, RMSE: root mean square error, ELOQ: estimated level of quantification, LLMV: lowest level of method validation, MDL: method detection level, and MQL: method quantification level. The $t_{99(n\text{-}1)}$ $s$ELOQ method was used to calculate the MDL and MQL

experimental farm, Allahabad, India. Four levels of Ca (0, 0.2, 0.5 and 1.0%), Zn (0, 40, 60 and 80 ppm), OM (0, 0.5, 1.5 and 2.0 $kg/m^2$) and Cd (0, 5, 10 and 15 ppm) were applied as $CaCO_3$, Zn-ethylenediaminetetraacetic acid (Zn-EDTA), compost and $CdCl_2$+EDTA (1:1 molar ratio), respectively. 2.5 mmol EDTA $kg^{-1}$ soil is the application rate used in phytoremediation studies (Collins et al. 2001). All experimental plots contained 100 ml of 1 M excess EDTA (above the total concentration of trace metals) to buffer micronutrient free-ion activities. Concentrated solutions of EDTA in a 1:1 molar ratio with Cd were prepared separately and allowed to equilibrate overnight before addition to the soil solution (Schaider et al. 2006). Both root and shoot portions of the plant samples and soils were brought to the laboratory safely for chemical analysis. Plant samples were dried at 105°C for five hours. Silt and clay were separated by the pipette method and fine sand by decantation. Ca was determined by the EDTA method, organic carbon by the chromic acid digestion method and cation exchange capacity (CEC) by using neutral 1 N ammonium acetate solution (Chopra and Kanwar, 1996). Diethyltriaminepentaacetic acid (DTPA) solution [1.97g (0.05M) DTPA powder, 13.3ml (0.1 M) Tri-ethanol amine and 1.47g (0.01 M) $CaCl_2$ were dissolved in distilled water made up to 1 liter after adjusting the pH to 7.3] was prepared (Lindsay and Norvell, 1978) to extract the available heavy metals in the soil samples. 5g of soil was shaken with 20 mL of the reagent for two hours. The clean filtrate was used for the estimation of Cd, Cr, Zn, Cu, Fe and Mn by atomic absorption spectroscopy (AAS). Heavy metals in plants were determined by tri-acid mixture (conc. $HNO_3$, conc. $H_2SO_4$ and $HClO_4$ in a 5:1:2 ratio) with inductively coupled plasma (Model-LABTEM) spectrometry (ICP-AES) (Li et al., 1995) at the central environmental pollution control laboratory of Indian Farmers Fertilizer Cooperative Ltd. (IFFCO-Phulpur), Allahabad. For the nitrogen analysis, a known weight of soil (1 g) was taken in a 150 mL conical flask and treated with 10 mL of digestion mixture containing sulfuric acid and selenium dioxide. Salicylic acid was also added to include the nitrates and nitrites. Digestion was carried out until the soil color changed to white. The nitrogen in the digest was estimated by steam distillation using a micro-Kjeldahl apparatus. For total P, a 2 g soil sample was taken with 4 mL $HClO_4$ (70%) in a 50 mL beaker covered with a watch glass and placed on a hot plate and digestion was carried until the soil color changed to white (Arora, 2002). The detection limit [method detection level (MDL)] was calculated using the formula: $MDL = t_{99\ (n-1)} \times S_{ELOQ}$. Where, $t$ is Student's $t$ value for a 99% confidence level [$t$ observed 3.143 for seven replicates ($df$ = 6)], and $S_{ELOQ}$ is the standard deviation of the replicate analyses from the estimated level of quantification. Recovery expressed as a percentage was calculated as (Table 1b): $R = [(CF - CU)/CA] \times 100$ %. Where, $CF$ is the concentration of the analyte in the unfortified sample and $CA$ is the concentration of the analyte added in the fortified sample (Eurachem, 1998). Data were analyzed by factorial analysis of variation (ANOVA) using various treatments as independent factors with the help of the sum of square (SS) and degree of freedom (DF). The standard error (SE) is given by $SE = \sqrt{\frac{2V_E}{n}}$, where, $V_E$ is the variance due to the error, $n$ is the number of replications, and the critical difference (CD) is given by CD = $SE_{diff.} \times t_{5\%}$ ($t_{5\%}$= 2.042 at $DF_{error}$= 30 was observed). The regression equation for estimating the value of $y$ for various characteristics $x$ was determined by $Y_c = \bar{y} + b_{yx}(x - \bar{x})$, where, $\bar{x}$ is the mean of $x$, $\bar{y}$ is the mean of $y$ and $b_{yx}$ is the regression of $y$ on to $x$. The slope, intercepts, goodness of fit ($r^2 = b_{yx} \times b_{xy}$) and standard deviation ($S_{yx}$) were determined in accordance with Motulsky and Christopoulos, 2003.

## Results and Discussion

The effect of Cd toxicity on sunflower was apparent from visual symptoms after 40 days of sowing. These symptoms were exhibited in the form of light white–yellow spots on the interveinal areas of the lower leaves. The management practices related to the interaction between Cd and Ca, Cd and Zn; and Cd and OM for combating Cd phytoaccumulation for sustainable agriculture were investigated and are summarized below

### The Effect of Cd × Ca interaction on dry biomass and yield of sunflower

The data (Fig. 1a and 1b and Table 2) indicated a significant influence of Cd, Ca and Cd × Ca interaction on the dry matter (DM) and seed yield of plants. Either the control plots or the Ca-treated plots registered the highest DM yield (57 q/ha) and seed yield (12.93 q/ha). Application of Ca in the Cd-EDTA-treated plots boosted the DM yield of sunflower and was found to play an ameliorative role in Cd-contaminated soil. The $r^2$ values between [Cd] and yield decreased from 0.79 to 0.16 in dry biomass and from 0.87 to 0.18 in seed yield as [Ca] increased from 0 to 1.0%, while successive doses of Ca treatment decreased the significance level ($F$ values) of the influence of Cd on the growth and yield parameters of sunflower plants (Table 2).

Application of Cd-EDTA in nil-Ca-treated plots significantly decreased the DM content by 28.07% and decreased the seed yield by 32.53% over the control as the dose of Cd

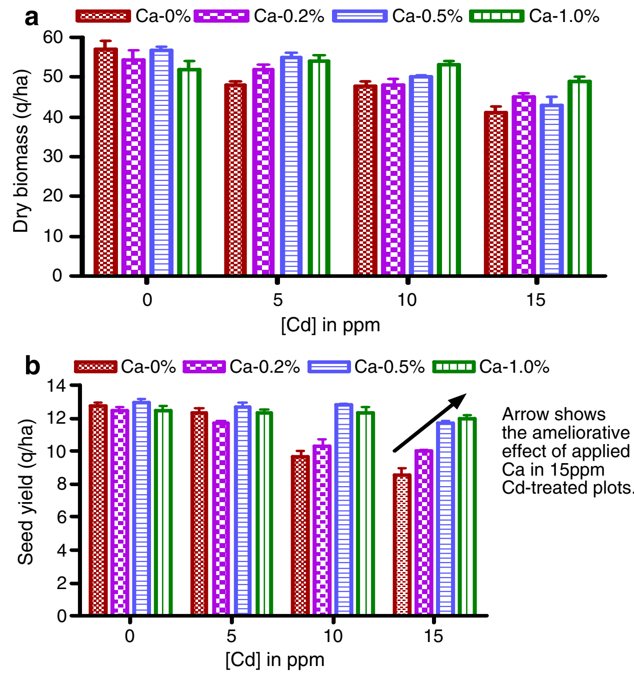


**Fig. 1** (a) Effect of Cd × Ca interaction on dry biomass of sunflower (b) Effect of Cd × Ca interaction on seed yield of sunflower

increased from 0 to 15 ppm. The hazardous effect of Cd-EDTA was observed to be minimized in Ca-treated plots, which might be due to the ameliorative role of Ca in the physiology of plants (Sakal et al., 1992; Cameron et al., 1997).

### The Effect of the Cd × Zn interaction on dry biomass and seed yield of sunflower

The data (Fig. 2a and 2b and Table 3) indicated the highly significant effects of Cd, Zn and the Cd × Zn interaction on the DM content and seed yield of sunflower, which decreased linearly as the doses of Cd increased up to 15 ppm. However, application of Zn up to 40 ppm either singly or in combination increased the DM content of all the plots, resulting in 11.18% and 5.8% extra DM content and seed yield over the control, respectively. However, higher levels (beyond 60 ppm) had a negative effect on DM content and the seed yield of the plants. The pronounced effect on DM content was observed only in plots treated with Cd-EDTA, recorded to be 26.67% lower than the control. The adverse effect of Cd- EDTA on the DM of plants was observed to be higher than that of Zn-EDTA.

Zn has been observed to have an encouraging effect on Cd-contaminated plots (Gupta and Potalia, 1990). The decrease in yield may be due to a reduced photosynthetic rate and internal water deficit in shoot system due to poor root development, leading to a drop in chlorophyll content of the affected leaves. Since Zn and Cd are chemically

**Table 2** Linear regression values of Cd × Ca interaction on dry biomass (seed yield) of sunflower

| Values | 0% Ca | 0.2% Ca | 0.5% Ca | 1% Ca |
|---|---|---|---|---|
| Slope | –0.967 ± 0.155 | –0.6400 ± 0.1266 | –0.9200 ± 0.1268 | –0.2000 ± 0.1451 |
| | (–0.302 ± 0.037) | (–0.177 ± 0.0224) | (–0.072 ± 0.0234) | (–0.0301 ± 0.0201) |
| *Y* intercept (at $x = 0$) | 55.67 ± 1.45 | 54.63 ± 1.184 | 58.07 ± 1.186 | 53.50 ± 1.36 |
| | (13.09 ± 0.3455) | (12.45 ± 0.2095) | (13.07 ± 0.2194) | (12.52 ± 0.1880) |
| x intercept (at $y = 0$) | 57.59 | 85.36 | 63.12 | 267.5 |
| | (43.39) | (70.45) | (180.5) | (415.4) |
| $r^2$ | 0.7947 | 0.7189 | 0.8404 | 0.1596 |
| | (0.8696) | (0.8616) | (0.4879) | (0.1835) |
| $S_{yx}$ | 3.008 | 2.451 | 2.455 | 2.811 |
| | (0.7152) | (0.4337) | (0.4542) | (0.3892) |
| *F* | 38.72*** | 25.57*** | 52.67*** | $1.899^{NS}$ |
| | (66.71)*** | (62.28)*** | (9.529)** | $(2.248)^{NS}$ |

***Significant deviation from zero ($p < 0.0005$); **significant ($p < 0.005$), NS = not significant. Data in brackets are linear regression values between Ca and the seed yield of sunflower

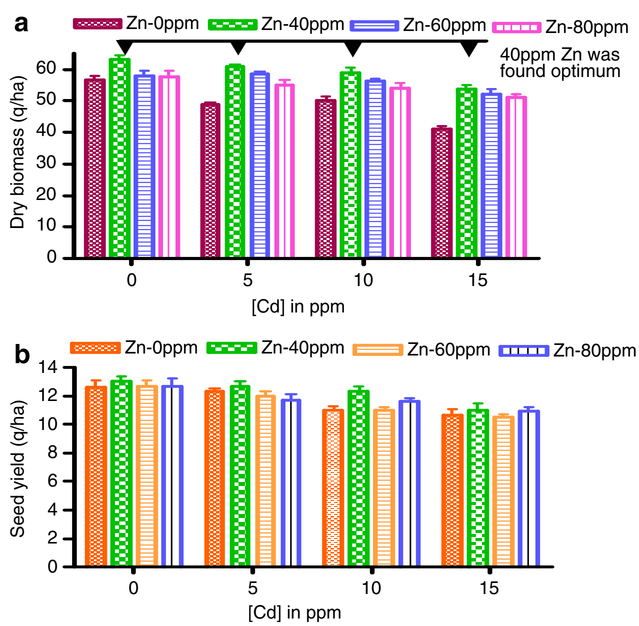


**Fig. 2** (a) Effect Cd × Zn interaction on dry biomass of sunflower (b) Effect of Cd × Zn interaction on seed yield of sunflower

similar, they can compete for similar binding sites within the apoplasmic cell wall. Therefore, the application of 40 ppm of Zn may be recommended to increase the DM content and seed yield of sunflower (Cakmak and Marschner, 1988). The $r^2$ values between [Cd] and yield decreased from 0.77 to 0.53 in dry biomass and from 0.69 to 0.54 in seed yield as [Zn] increased from 0 to 80 ppm. Successive doses of Zn treatment decreased the significance level (*F* values) of the influence of Cd on the growth and yield parameters of sunflower plants (Table 3).

## Effect of the Cd × OM interaction on dry biomass and seed yield of sunflower

The data (Table 4) reveal that the application of various levels of OM significantly increased the DM content and seed yield of sunflower. The interaction effect was observed to be non-significant. Application of OM at a level of 20 t/ha was found to be most effective at boosting the DM content and seed yield of the plants. Application of OM at 5, 15 and 20 ton/ha remarkably and significantly increased the DM and yield of plants (Table 4). The maximum yield was found in the plot where OM was added at a rate of 20 t/ha. Even 15 tons of OM per hectare gave higher yields compared to the control plots. Addition of OM individually increased the seed yield of sunflower by 7.89% compared to the control (Eriksson, 1989; Sakal et al., 1992). Application of OM was found to play an ameliorative role in soils treated with Cd-EDTA in terms of increased DM content and seed yield of the crops.

## The Effect of the Cd × Ca interaction on uptake of Cd by sunflower

The data (Fig. 3a and 3b) indicated a highly significant effect of Cd, Ca and the interaction of Cd × Ca on the uptake of Cd by both the roots and shoots of the plants. There is an indication that the relative Cd uptake is often greater in the control than in the plots treated with Cd-EDTA. The application of 15 ppm Cd registered the highest phytoaccumulation of Cd (2.50 ppm and 2.37 ppm in roots and shoots, respectively). The data revealed that application of Ca competitively reduced the uptake of Cd below 0.21 ppm (in the roots) and 0.20 ppm (in the shoots) of plants in almost all the plots. A significant negative correlation was observed, as reported by Garg and Totawat (2005) near a stream polluted with effluent from a zinc smelter in Udaipur, Rajasthan. The $r^2$ values between the amount of [Cd] added and the uptake of Cd decreased from 0.99 to 0.41 in roots and 0.97 to 0.77 in shoots as [Ca] increased from 0 to 1.0%; successive doses of Ca treatment linearly decreased the significance level

**Table 3** Linear regression values for the effect of the Cd × Zn interaction on dry biomass (seed yield) of sunflower

| Values | Zn 0 ppm | Zn 40 ppm | Zn 60 ppm | Zn 80 ppm |
|---|---|---|---|---|
| Slope | –0.9133 ± 0.156 | –0.5933 ± 0.1210 | –0.5933 ± 0.1210 | –0.4200 ± 0.1252 |
| | (–0.144 ± 0.03) | (–0.125 ± 0.035) | (–0.149 ± 0.024) | (–0.107 ± 0.031) |
| *y* intercept (at *x* = 0) | 55.93 ± 1.46 | 63.53 ± 1.132 | 59.30 ± 1.104 | 57.57 ± 1.171 |
| | (12.75 ± 0.28) | (13.19 ± 0.33) | (12.67 ± 0.23) | (12.55 ± 0.29) |
| *x* intercept (at *y* = 0) | 61.24 | 107.01 | 145.08 | 137.1 |
| | (88.52) | (105.2) | (84.84) | (116.9) |
| $R^2$ | 0.7725 | 0.7063 | 0.5428 | 0.5296 |
| | (0.6939) | (0.5626) | (0.7912) | (0.5381) |
| $S_{yx}$ | 3.035 | 2.343 | 2.285 | 2.424 |
| | (0.5857) | (0.6767) | (0.4698) | (0.6090) |
| *F* | 33.96*** | 24.05** | 11.87* | 11.26* |
| | (22.67)** | (12.86)** | (37.89)*** | (11.65)* |

***Significant deviation from zero ($p < 0.0005$); ** = significant ($p < 0.005$); *significant ($p < 0.05$). Data in brackets are values of the linear regression between cadmium and the seed yield of sunflower

**Table 4** The effect of the Cd × OM interaction on dry biomass and seed yield of sunflower (q/ha)

| Compost (kg/m$^2$) | [Cd] (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 5 | | 10 | | 15 | |
| | Dry biomass | Seed yield | Dry biomass | Seed yield | Dry biomass | Seed yield | Dry biomass | Seed yield |
| 0 | 57.00 | 12.67 | 49.00 | 12.33 | 50.33 | 10.93 | 41.00 | 10.33 |
| 0.5 | 60.00 | 12.67 | 58.33 | 11.67 | 57.33 | 11.67 | 55.33 | 11.33 |
| 1.5 | 63.67 | 13.83 | 62.67 | 14.67 | 59.00 | 14.00 | 59.00 | 13.33 |
| 2.0 | 70.67 | 13.67 | 70.00 | 14.67 | 68.33 | 14.00 | 69.00 | 13.67 |
| SE | | Cd | | | OM | | Cd × OM | |
| | | Dry biomass | | Seed yield | Dry biomass | Seed yield | Dry biomass | Seed yield |
| | | 1.72 | | 0.30* | 1.72** | 0.30** | 3.44 | 0.60 |
| CD (0.05) | | NS | | 0.61 | 3.51 | 0.61 | NS | NS |

SE: standard error; CD: critical difference ($p = 0.05$); NS: not significant; *significant at the 5% level; **significant at both levels (5% and 1%)

(*F* values) of the influence on Cd uptake by the plants (Table 5).

These findings indicate the interference of Ca in Cd toxicity to sunflower plants. This might be due to the chemisorption of Cd in the soil due to the lower ionization potential and electronegativity of the soil, which facilitates the formation of strong complexes with the metal cation according to their ability to form covalent bond in the order: Pb > Cr > Cd > Zn (Xian and Shokohifard, 1989; Rattan et al., 2002).

## The Effect of the Cd × Zn interaction on the uptake of Cd by sunflower

The data (Fig. 4a and 4b) indicated that the effect of Cd, Zn and the Cd × Zn interaction were highly significant. The accumulation of Cd in the roots and shoot of the plants significantly increased and indicated greater relative Cd uptake in plots treated with Cd-EDTA over the controls. However, application of Zn either singly or in combination reduced the Cd uptake in the plants. Application of Zn at levels up to 80 ppm resulted in reduced accumulation (<0.21 ppm and <0.12 ppm) of Cd in the roots and shoots of the plants. Application of the recommended dose of Zn in crops would be beneficial for combating Cd toxification. Maintenance of the ratio Zn:Cd at a high level of 100:1 in Cd-containing wastes would be helpful in the exclusion of Cd from the food chain (Gupta and Potalia, 1990; Lingg et al., 1996). It appears that application of Zn up to a lower level (40 ppm) slightly increased Cd uptake by shoots in some plots. However the application of Zn either singly or in combination reduced the Cd uptake in plants. The $r^2$ values between the amount of [Cd] added and the uptake of Cd decreased from 0.98 to 0.52 (in roots) and from 0.97 to 0.013 (in shoots) as [Zn] increased from 0 to 80 ppm; successive doses of Zn treatment decreased the significance level (*F* values) of the influence of Cd on the Cd uptake by plants (Table 6), which indicates that Zn in the soils and plants plays an important role in Cd accumulation in crops (Ramachandran and D' Souza, 1998).

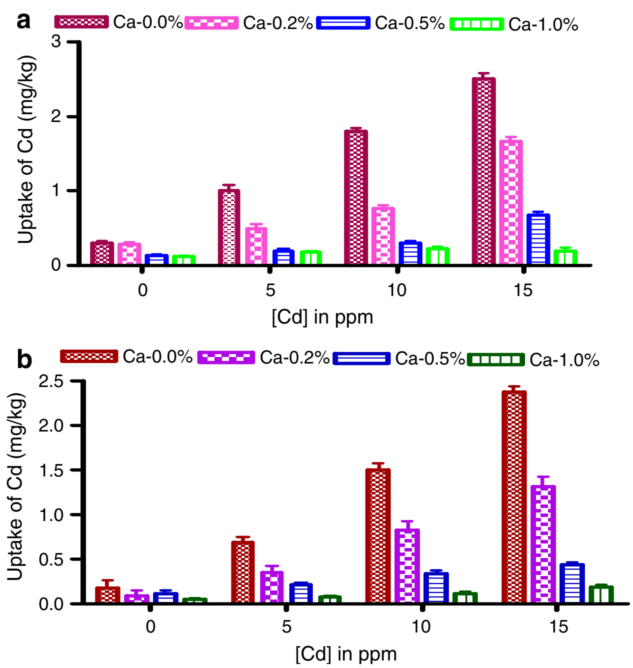


**Fig. 3** a Effect of Cd × Ca interaction on the uptake of Cd by roots of sunflower (ppm) b Effect Cd × Ca interaction on the uptake of Cd by shoots of sunflower (ppm)

The effect of the Cd × OM interaction on the uptake of Cd by sunflower

The application of 15 ppm Cd registered the highest accumulation of Cd (1.97 and 2.23 ppm in root and shoot, respectively) in the plants. The study reveals that Cd, OM and the Cd × OM interaction influence Cd uptake in plants significantly even at a 1% level of significance. Cd-EDTA applied individually enhanced the Cd uptake by 5.3 and 8.26 times over the control in roots and shoots, respectively, as the doses of Cd increased from 0 to 15 ppm (Table 7). Application of OM at the lower level of 5 t/ha increased the Cd phytoavailability in sunflower (Green-Ruiz, and Páez-Osuna, 2003) and thereby indicated a significant positive correlation (Patel et al., 2004). Since metal-EDTA complexes can dominate soil solution metal speciation, the results of our study imply that it is likely that chelated metals will comprise the majority of metals taken up by plants during chelate-enhanced phytoextraction (Schaider et al. 2006).

These results indicate that the presence of acetic and citric acids in the leaves of *H. annuus* might be related to its Cd accumulation (Sun et al. 2006). On the other hand the addition of OM at a rate of 20t/ha in plots treated with Cd at a dosage of 15ppm,diminished Cd accumulation by 1/9.85 and 1/8.26 times in the roots and shoots of the plants, respectively (Narwal et al., 1992; Moreno et al., 1998; Almas and Singh, 2001). Application of OM was found to play an ameliorative role in Cd-contaminated soil. It may be suggested that application of 20 t/ha OM retarded the Cd accumulation below or equal to 0.30 ppm in the plants.

## Reaction Mechanisms

Reactions between metal ions and water to form hydrolysis products (i.e., $MeOH^+$) can be expressed in the general form: $xM^{z+} + yH_2O = [M_x\,(OH)^{(xy-z)+}] + yH^+$

As the pH increases, aqueous metal cations hydrolyze, resulting in metal-hydroxyl complexes. $Ca^{2+}$ ions or the pH

**Table 5** Lin Reg values of Cd × Ca interaction on the uptake of Cd by roots and shoots of sunflower

| Values | 0% Ca | 0.2% Ca | 0.5% Ca | 1.0% Ca |
|---|---|---|---|---|
| Slope | –0.1475 ± 0.005 | –0.0881 ± 0.011 | –0.0347 ± 0.005 | –0.0063 ± 0.002 |
| | (0.1475 ± 0.008) | (0.0837 ± 0.008) | (0.022 ± 0.003) | (0.009 ± 0.002) |
| *Y* intercept (at $x = 0$) | 0.2977 ± 0.046 | 0.1487 ± 0.103 | 0.067 ± 0.048 | 0.135 ± 0.022 |
| | (0.079 ± 0.075) | (0.019 ± 0.073) | (0.115 ± 0.024) | (0.039 ± 0.015) |
| *X* intercept (at $y = 0$) | –2.018 | –1.688 | –1.929 | –21.49 |
| | (–0.5345) | (–0.2229) | (–5.309) | (–4.275) |
| $R^2$ | 0.9889 | 0.8650 | 0.8180 | 0.4141 |
| | (0.9711) | (0.9188) | (0.8763) | (0.7786) |
| $S_{yx}$ | 0.096 | 0.213 | 0.100 | 0.046 |
| | (0.1560) | (0.1525) | (0.0497) | (0.030) |
| *F* | 887.1*** | 64.05*** | 44.96*** | 7.06* |
| | (336.5)*** | (113.1)*** | (70.81)*** | (35.16)*** |

***Significant deviation from zero ($p < 0.0005$); *significant ($p < 0.05$). Data in brackets are linear regression values between applied Cd and uptake of Cd by shoots of sunflower plants

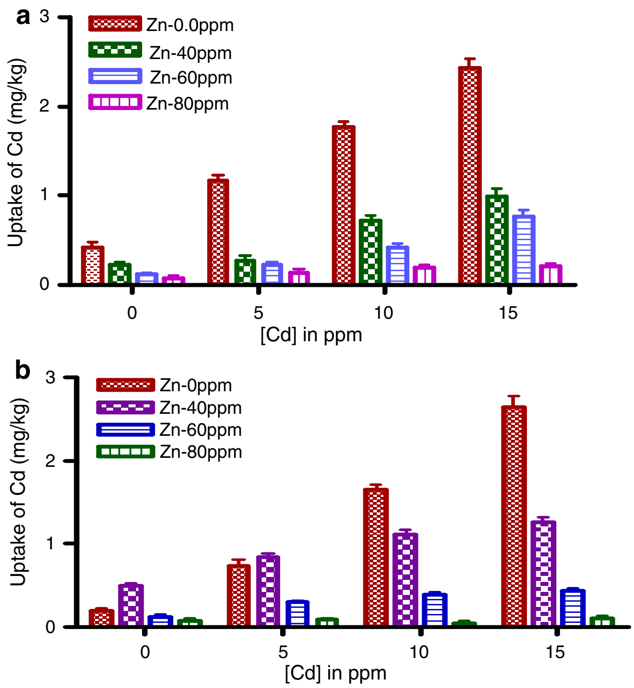


**Fig. 4** a Effect of Cd × Zn interaction on uptake of Cd in roots of sunflower b Effect of Cd × Zn interaction on the uptake of Cd by shoots of sunflower (ppm)

of the soil may have reduced the availability of Zn by the substitution reaction: $Ca^{2+}$ + Zn-EXCH ⇔ $Zn^{2+}$ + Ca-EXCH (Where, EXCH = exchangeable). Other reactions include other mineral precipitation reactions, such as the precipitation of a Ca-$ZnCO_3$, or other adsorption reactions, such as adsorption onto Fe or Mn oxides or clay (Gomes et al., 2001). The supply of adequate quantities of Ca prevents any shift in soil pH. Furthermore, this study also indicates that Cd adsorption was strongly pH dependent.

$$CdCl_2 + CaCO_3 \rightarrow CdCO_3 + CaCl_2 \text{ or}$$
$$Cd^{2+} + CaCO_3 \Longleftrightarrow CdCO_3 + Ca^{2+}$$

$$CdCl_2 + 2NH_4OH \rightarrow Cd(OH)_2 + 2NH_4$$

The solubility of $CdCO_3$ and Cd $(OH)_2$ is very low in the neutral to alkaline range of the experimental soil. This may be ascribed to the substitution of ions like $H^+$, $Cd^{2+}$ and $Fe^{2+}$ due to the ionic strength of $Ca^{2+}$ (Tyler and Olsson, 2001). The following type of ionization reactions for adsorption may be possible under these conditions:

$$10\,Ca^{2+} + 6\,PO_4^{-3} + 2\,OH^- \leftrightarrow [Ca_{10}\,(PO_4)_6\,(OH)_2],$$

$$10\,Zn^{2+} + 6\,PO_4^{-3} + 2\,OH^- \leftrightarrow [Zn_{10}\,(PO_4)_6\,(OH)_2],$$

$$10\,Cd^{2+} + 6\,PO_4^{-3} + 2\,Cl^- \leftrightarrow [Cd_{10}\,(PO_4)_6\,(Cl)_2],$$

$$10\,Cd^{2+} + 6\,PO_4^{-3} + 2\,OH^- \leftrightarrow [Cd_{10}\,(PO_4)_6\,(OH)_2],$$

and

$$Zn_{10}\,(PO_4)_6\,(OH)_2 + 10\,Cd^{2+} + 6\,PO_4^{-3} \leftrightarrow [Cd_{10}\,(PO_4)_6\,(OH)_2] + 10\,Zn^{2+}$$

In alkaline soil reactions, Zn dissolves in caustic alkalis with the formation of zincates and the evolution of hydrogen gas at a very low redox potential (<–200mV) according to the reaction [Zn] + 2NaOH → [$Na_2$ $ZnO_2$] + $H_2$

Thus, the rate-controlling factor of heavy metals may be the dissociation of apatites in soil solution (Kathryn et al., 2002). Application of Zn up to 60 ppm either singly or in combination increased Zn uptake in the plants. Zn mobility in neutral to alkaline soils may also be expressed by

**Table 6** Linear regression values for the effect of the Cd × Zn interaction on the uptake of Cd in sunflower

| Values | Zn 0 ppm | Zn 40 ppm | Zn 60 ppm | Zn 80 ppm |
|---|---|---|---|---|
| Slope | 0.1320 ± 0.0063 | 0.0554 ± 0.0071 | 0.043 ± 0.005 | 0.0087 ± 0.026 |
| | (0.1651 ± 0.0095) | (0.0515 ± 0.0046) | (0.02 ± 0.0027) | (0.0008 ± 0.0022) |
| $Y$ intercept (at $x$ = 0) | 0.1353 ± 0.066 | 0.4600 ± 0.059 | 0.0620 ± 0.047 | 0.0903 ± 0.025 |
| | (0.0687 ± 0.089) | (0.5427 ± 0.043) | (1640 ± 0.025) | (0.077 ± 0.02) |
| $X$ intercept (at $y$ = 0) | –3.485 | –2.443 | –1.451 | –10.34 |
| | (–0.4160) | (–10.53) | (–8.013) | (–96.67) |
| $r^2$ | 0.9776 | 0.8587 | 0.8763 | 0.5249 |
| | (0.9681) | (0.9251) | (0.8564) | (0.01328) |
| $S_{yx}$ | 0.1224 | 0.1376 | 0.0983 | 0.0508 |
| | (0.1835) | (0.08978) | (0.05133) | (0.04222) |
| $F$ | 436.2*** | 60.78*** | 70.87*** | 11.05* |
| | (303.3)*** | (123.5)*** | (59.63)*** | $(0.1346)^{NS}$ |

***Significant deviation from zero ($p$ < 0.0005); *significant ($p$ < 0.05); NS=not significant. Data in brackets are values of the linear regression between applied Cd and the uptake of Cd by the shoots of sunflower plants

**Table 7** Effect of the Cd × OM interaction on the uptake of Cd by sunflower (ppm)

| Compost (kg/m$^2$) | [Cd] (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 5 | | 10 | | 15 | |
| | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot |
| 0 | 0.37 | 0.27 | 1.13 | 1.13 | 1.83 | 1.83 | 1.97 | 2.23 |
| 0.5 | 0.43 | 0.47 | 0.73 | 0.73 | 0.80 | 0.80 | 0.93 | 1.00 |
| 1.5 | 0.20 | 0.37 | 0.20 | 0.27 | 0.33 | 0.33 | 0.57 | 0.63 |
| 2.0 | 0.07 | 0.20 | 0.13 | 0.30 | 0.17 | 0.23 | 0.20 | 0.27 |
| SE | | Cd | | | OM | | Cd × OM | |
| | | Root | | Shoot | Root | Shoot | Root | Shoot |
| | | 0.05** | | 0.06** | 0.05** | 0.06** | 0.10** | 0.12** |
| CD (0.01) | | 0.146 | | 0.175 | 0.146 | 0.175 | 0.292 | 0.350 |

SE: standard error; CD: critical difference ($p$ = 0.01); **significant at 5% and 1% levels

substitution reactions. $Zn(OH)^+$ is a dominant solution species of Zn that may absorb into soil and replace one $H^+$, according to the reaction $Zn(OH)^+ + LH \Leftrightarrow [Zn(OH)L] + H^+$, where L is an organic ligand in the soil capable of complexing with Zn. The chemistry behind the adsorption of Zn with OM is given by the reaction (Tlustos et al., 2001):


A decrease in the concentration of Zn can also be due to alkaline (pH = 8.1 ± 0.1) reaction and adsorption by the clay content of the experimental soil (Saha et al., 2002). Cd salts also form $[Cd(NH)_3]^{2+}$ in the presence of excess ammonia. Zn and Cd form complex salts with alkali cyanides in which the metal is present in the form of complex anions such as $[Zn(CN)_4]^{2-}$ and $[Cd(CN)_2]^{2-}$, respectively. The application of 15 ppm Cd registered the lowest dry matter (DM) yield (41 q/ha), the lowest seed yield (8.58 q/ha) and the highest accumulation of Cd (2.5 ppm and 2.65 ppm in the roots and shoots of sunflower, respectively). Cd-EDTA applied individually reduced the DM yield by 26–28%, decreased the seed yield by 32.5% and increased the uptake of Cd by almost 8–10 times over the control as levels of Cd were increased to 15 ppm. This mechanism indicates that the application of the recommended doses of Ca and Zn in crops would be beneficial for combating Cd toxification and maintaining Ca and Zn content in plants (Miller et al., 1995).

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