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Acceleration of Cadmium Phytoextraction by Sunflower (*Helianthus annuus* L.) In Collaboration of Ethylenediamine Tetraacetic Acid (EDTA)

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Abstract: Industrialization and excessive use of fertilizers incorporates heavy metals in soils of agricultural lands that are responsible for soil contamination and toxicity of food chain. The aim of this work was to check the effect of cadmium on sunflower growth, its accumulation in plant parts along with translocation potential of ethylenediamine tetraacetic acid (EDTA). Sunflower seeds were subject to 450 mg Cd /kg sand and six levels of EDTA (0.25, 0.5, 0.75, 1, 1.25 and 1.5 g EDTA /kg sand) with an untreated control. The seedlings were harvested ten days after emergence. Application of EDTA enhanced the accumulation of Cd more in roots than shoots as compared to control. Low level of 0.25 g/kg of EDTA translocated 54.63 and 9.65% Cd in sunflower roots and shoots, while 1 g/kg of EDTA extract more Cd up to 192 and 168%, respectively as compared to its control. High translocation of Cd by 1g/kg of EDTA reduced sunflower height, fresh and dry weights of both shoot and root. Roots are the major metal sinks suggesting defense mechanism of plant to avoid Cd translocation in seeds. The use of sunflower along with 1 g/kg EDTA is an active and potential accumulator of Cd for phytoextraction of metals from contaminated soils. Sunflower in collaboration of selected EDTA level should be tested in fields to verify their suitability for phytoremediation of agricultural lands.

Key words: Translocation factor · Sunflower · Phytoextraction · EDTA · Phytotechnologies

INTRODUCTION

Cadmium is a non-essential water soluble heavy metal that contaminates food chain by accumulating in plant parts as it is easily mobile in soil and bioavailable [1] eventually transiting and accumulating in top consumers of food chain i.e. human [2]. Anthropogenic activities such as mining, industry and excessive use of impure low cost fertilizers and municipal wastes polluted soil and water reservoirs [3, 4]. The Cd backlog in environment causes many diseases like, cancer, bone mineralization, lungs damage, damages in tubular and glomerular parts of kidney, increased osteoporosis and nephritis [5, 6, 7]. Conventional remediation approaches of metal pollution is not economically suitable solution therefore phytoremediation is alternatively found to be very attractive strategy, being low cost, environment friendly and sustainable [8].

Phytoextraction is a friendly in-situ decontamination process based on plants ability to remove sufficient amount of heavy metals from contaminated soil and water and translocate to harvestable parts [9]. Another strategy is the use of chelators for the extraction of metals by the acidification and solubilization of soil [10, 11]. EDTA has proved the most efficient chelating agent for enhancing metals uptake and transport in above ground parts of the plants [12, 13] and has positive affinity for Cd [14]. Sunflower, a fast growing high biomass crop [15, 16] is capable of accumulating significant amount of Cd and many other metals as well [17].

A lot of reports are available on Cd accumulation by sunflower [18, 19], while this metal accumulation capacity of sunflower can be enhanced with the help of chelating agents such as EDTA, EDDS and DTPA etc. [17, 20]. Thus in context of toxic effects of Cd on plants and human being, the objective of the study was to explore the potential and optimum concentration of EDTA for arbitrating the Cd extraction by sunflower for rehabilitation of Cd affected soils or sites.

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MATERIALS AND METHODS

This experiment was carried out on sunflower (*Helianthus annuus* L.) hybrid SF-187 obtained from National Agriculture Research Center (NARC) Pakistan were sown in sand filled plastic pots under continuous white fluorescent light (*PAR* 300 µmol m^{-2} s^{-1}) in growth room maintained at 28±2°C. Plants were subject to 450 mg Cd /kg sand along with six different levels of EDTA corresponding to 0.25, 0.5, 0.75, 1, 1.25 and 1.5 g EDTA /kg sand with untreated sand as a control. The sand was thoroughly washed thrice and air dried. The pots, after filling, were sealed at bottom to prevent loss of water. The experiment was set up in a completely randomized design and each treatment was replicated thrice. The seedlings were harvested ten days after completed emergence of seedlings. Roots and shoots were separated and treated with ice cold solution of $CaCl_2$ having strength 5 mM to eradicate extracellular Cd [21] and then washed with deionized water. Both shoot and root lengths were measured and weighed after blotting with filter paper. All oven dried samples (70°C for 48 h) of both roots and shoots, were digested by using concentrated HNO_3; after that 50 mL of distilled water was added to the sample volume. The resulting solutions were analyzed for Cd concentration by using Atomic Absorption Spectrophotometer (Aanalyst-330, Perkin Elmer and Germany). The heavy metal translocation capability from root to shoot of plant is known as translocation factor was calculated as Cd concentration in shoots dry matter divided by Cd concentration in the roots. Statistical analysis was performed by Statistix 8.1. All variables were tested with two way ANOVA followed by Duncan's Test.

RESULTS

The effects of Cd were adverse on different morphological attributes of sunflower shoot and root. Length, fresh and dry biomass of shoot and root experienced notable significant reduction under Cd contamination. The percent reduction in case of shoot and root length under Cd stress was 31.15 and 38.96%, respectively (Fig. 1a, d). Cadmium at 450 mg/kg caused 38.54 and 44.25% reduction in shoot fresh and dry weight of hybrid SF-187 respectively (Fig. 1b, c). Similarly the reduction in root fresh and dry weight was 30.50 and 44.8% respectively (Fig. 1e, f).

Application of EDTA alone up to 0.5 g/kg caused gradual improvement in all morphological parameters of hybrid SF-187 as compared to control. The maximum shoot length (26.2 cm), shoot fresh (4.88 g) and dry biomass (3.58 g) was recorded at 0.5 g/kg, however further increase in EDTA dose resulted in gradual reduction in growth parameters. Root growth was also affected at higher EDTA levels i.e. from 0.75 to 1.50 g/kg, while lower levels exerted positive effects, so maximum root length (23.3 cm), root fresh weight (3.29 g) and root dry weight (2.69 g) was recoded at 0.5 g/kg EDTA application followed by 0.25 g/kg EDTA (Fig. 2).

Table 1: Correlation between growth attributes and Cd concentration in hypogenous, epigenous parts of sunflower treated with 450 mg Cd/kg sand and EDTA

Growth Attributes	Cd S (µg/g)	Cd R (µg/g)
Shoot length (cm)	-0.64 ns	-0.83 *
Root length (cm)	-0.66 ns	-0.85 *
Shoot fresh weight (g)	-0.63 ns	-0.85 *
Shoot dry weight (g)	-0.6 ns	-0.8 *
Root fresh weight (g)	-0.63 ns	-0.83 *
Root dry weight (g)	-0.6 ns	-0.81 *
Translocation factor	0.15 ns	-0.28 ns

* Significant; ns non-significant

Low concentration of Cd was recorded in plants with no application of EDTA and Cd i.e. in shoot (0.097 µg/g) and root (1.32 µg/g), however addition of Cd and EDTA alone in growth medium elevated Cd contents in root and shoot (Fig. 2). Addition of EDTA in Cd contaminated growth medium i.e. 0.25, 0.5, 0.75 and 1 g/kg further promoted the Cd uptake in both organs (Fig. 2) but root had more concentration of Cd than shoot in each treatment. The maximum concentration with proper plant growth was recorded when EDTA was applied @ of 1 g/kg along with Cd that increased 168% more Cd contents in shoot and 192% in root, however further increase in EDTA level significantly lowered the Cd contents of both studied organs (Fig. 2). Parallels drawn between Cd concentrations of root are significant ($P<0.01$) and negatively correlated with growth attributes of both epigenous and hypogenous portions of sunflower while shoot Cd was non-significant ($P>0.05$) (Table 1).

Translocation factor (TF) was used as a tool to assess the potential of sunflower to remediate the Cd contaminated soil. EDTA application enhanced translocation potential from 0.073 (control) up to 0.451, 0.515, 0.537, 0.611, 0.634, 0.618, 0.597 at 0.25, 0.5, 0.75, 0.1, 1.25 1.0 and 1.5 g/kg. Results showed that addition of EDTA in growth medium gradually enhanced the TF value and the maximum value of TF i.e. 0.63 was recorded at 1 g EDTA/kg with better plant as compared to high EDTA levels (Fig. 3).

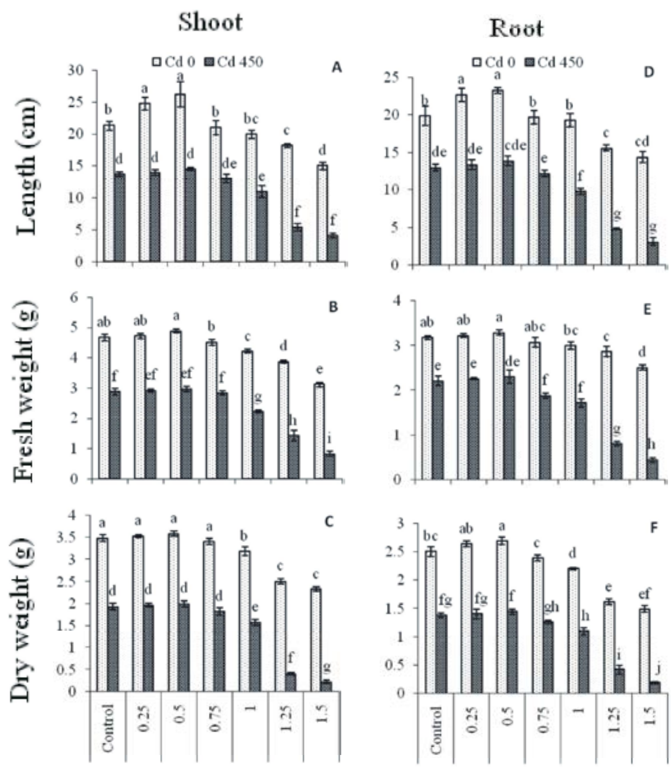

Fig. 1: Effect of 450 mg/kg Cd along with different levels of EDTA on sunflower growth attributes at germination stage. Values and means (n=42)±SE, bars followed by letters showed significant differences (P<0.01) according to Duccan's Test

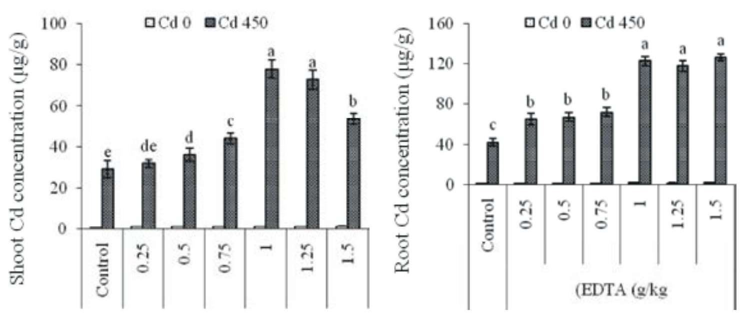

Fig. 2: Changes in Cd concentration in sunflower plant portions under 450 mg/kg Cd along with different levels of EDTA at germination stage. Values and means (n=42)±SE, bars followed by letters showed significant differences (P<0.01) according to Duccan's Test

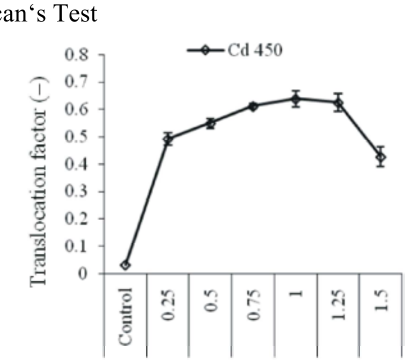

Fig. 3: Cd translocation factor in relation to EDTA different levels at germination stage. Values and means (n=42)±SE

DISCUSSION

Metal toxicity becomes a major constraint due to expansion of industrial cities and its mergence with agricultural lands. *Brassica juncea, Zea Mays and Helianthus annuus* remove toxic metals by accumulating them in their epigenous (old leaves) and hypogenous (roots) parts [19, 22]. Cd accumulation in the roots resulted in reduced growth and disruptions of mineral nutrients status [23, 24]. Reduced mitotic activity and hanged up apex length are the direct reason of growth reduction [25]. Cd exerted deleterious effects on root length and root fresh biomass, reduced shoot and root fresh and dry weights [26, 27] shoot length [28] and shoot fresh as well as dry biomass [29]. Biomass reduction by Cd toxicity is the direct outcome of chlorophyll synthesis inhibition and limited photosynthetic process [30] as enzymes of carbon reduction cycle are sensitive to Cd toxicity [31]. The more reduction in sunflower root growth was the result of accumulation of higher concentration of Cd in root than shoot which as a consequence inhibits the mitotic cell division in meristematic cell zone by stalling the metaphase [32], also the roots are the first and direct victim of metal toxicity [33]. Retention of more Cd in root than shoot and its less translocation to upper parts may be credited to the plant tolerance of Cd. The cause of affected seedling growth under metal treatments may be the result of decline in the ratio of meristematic cells and suppressed activity of some enzymes present in endosperm and cotyledons. By the action of amylase and protease starch and proteins are converted into sugars and amino acids, respectively, than these primary biomolecules i.e. sugars and amino acids are transported to the actively growing shoot and root tips. So, under metal treatment the activity of these hydrolytic enzymes is disturbed as metals attack the active groups of enzymes as a result sufficient food supply do not reach to the root and shoot apices, thereby seedling growth is reduced [34]. The present study showed that lower EDTA levels i.e. 0.25 and 0.5 g/kg exerted positive effects on all morphological parameters i.e. shoot and root fresh and dry biomass and length, while higher levels from 0.75 to 1. g/kg caused gradual reduction in growth parameters. EDTA at its lower concentration of 0.269 μmol/L inserted positive effects on growth attributes of *Microcystis aerugonisa* and *Scenedesmus quadricola* [35], while higher dose of 30 mg L^{-1} of EDTA caused notable reduction in plant fresh weight, plant height and chlorophyll contents [36]. The better plant growth under EDTA application can be advocated by the argument that chelating agent i.e. EDTA promotes the macro and micro nutrients uptake and improves the plant nutrient status and ultimately exerts positive effects on plant growth and development [37, 38].

Addition of EDTA in Cd contaminated growth medium i.e. 0.25, 0.5, 0.75 and 1 g/kg further promoted the Cd uptake in both organs but root had more concentration of Cd than shoot in every treatment as *Calamagrostis epigejos* root accumulated more Cd than shoot [39]. Similarly the roots of some other plants like wheat [40] and maize [41] accumulated higher contents of Cd than shoot. Retention of more Cd in root than shoot is Cd detoxification strategy of plants. Cd mainly retained in roots when applied at low concentration however, at higher concentration its translocation to the shoot or epigenous parts was significant due to overloading of Cd in roots [42]. Phloem mobility of Cd is limiting factor in its translocation from root to shoot [43]. Application of 1 g EDTA/kg of sand is the best suited dose for maximum uptake of Cd with proper plant growth, while further increase in EDTA levels reduced the Cd uptake potential of sunflower. As high EDTA levels are toxic for plant growth and resulted in reduced uptake of Cd by decreasing the plant biomass [44, 45]. 1 g/kg EDTA is the best optimal doze to accumulate highest concentration of Cd i.e. 210.3 mg/kg in *R. globosa* [46]. Metal sequestration in roots cells and their transport efficiency to xylem play a key role in determining their translocation to aerial parts [44]. Cd uptake under EDTA application is that the chelator has increased the soluble fraction of Cd to plants resulted in elevated Cd uptake [47, 48]. Translocation Factor (TF) shows the usefulness of plants to translocate appreciable amount of metal to the upper harvestable parts [37]. In the present study the TF value was less than unity i.e <1 which proved sunflower as a good phytostabilizer for Cd [19].

CONCLUSION

Our results indicated that EDTA is a good choice of chelator for rehabilitation of Cd contaminated soil or area to enhance the phytoextraction potential of plants. Applying EDTA at 1 g/kg soil found to be very effective treatment for accelerating Cd phytoextration along with sunflower. High concentration of Cd in roots and low translocation factor values from shoot to root suggested as a defense mechanism to avoid toxicity in apical active tissues. It is recommended to investigate sunflower plants along with 1 g/kg EDTA chelator in open fields of metal contaminated soils, by using standard agronomic practices in order to improve phytoremediation technologies and restore heavy metal affected soils.

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