

Original Research

Uptake and Leaching of Cu, Cd, and Cr after EDTA Application in Sand Columns Using Sorghum and Pearl Millet

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Abstract

In a greenhouse experiment using sand columns, sorghum and pearl millet were grown and spiked with metal solutions of Cu, Cd, and Cr in two concentrations. The chelating agent EDTA was applied to one-month-old plants and metal mobilization was observed through uptake by the plants or leaching through the columns during a period of one month. Growth was much better in pearl millet than sorghum under metal stress. Metal uptake was significantly higher in sorghum and was in the order root>shoot>leaves in both the plants. Metals were differentially mobilized in the order of Cu>Cr>Cd as shown by plant uptake and leaching through the columns. The root to shoot translocation of Cd was significantly improved after EDTA application, and to some extent for Cu but not for Cr. The leaching of metals was the maximum in the second week of application and was almost negligible by the fourth week conforming to the amount of EDTA detected in the leachate. Leaching of Cr differed in the maximum amount at the time of chelant application, while Cu and Cd showed maximum leaching after 7 days. As compared to sorghum, pearl millet was better at controlling leaching because of its extensive root system.

Keywords: chelants-assisted phytoremediation, chelant persistence, EDTA, leaching hazard

Introduction

Metal contamination is a matter of serious concern throughout industrial areas of the world. Most industrially polluted regions are laden with heavy metals and among them Cu, Cd, and Cr are the most important especially in tannery- and textile-polluted areas. The

process of phytoextraction can be enhanced with biological or chemical assistants. Among the chelating agents, EDTA has been the most effective in hastening the process of phytoextraction [1, 2].

A few plant species with high biomass show the capacity to translocate high amounts of heavy metals from roots to shoots [3]. Zhivotovsky et al. [4] have reported that complexation of metals with EDTA reduces their bioaccumulation in roots and thus enhances translocation of metals to aerial parts of plants. Nevertheless, Barrutia et al. [5] argued that

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EDTA-enhanced metal uptake is affected by plant type, age, and mode of EDTA application. EDTA is thought to be phytotoxic and the plausible explanation for this is the elevated metal uptake due to its application [6]. EDTA and metal-EDTA complexes are non-biodegradable persisting even for months in the field [7, 8]. Neugschwandtner et al. [9] have reported that EDTA can persist in soil for as long as six months. It is important to determine how long it takes to stop leaching of heavy metals after EDTA application.

Most of the pioneers in chelant-assisted phytoremediation have advocated a non-selective nature of EDTA as a metal chelant [10-12]. However, some others are of the view that certain elements are selectively chelated by EDTA [13-17]. The purpose of this research was to figure out the extent of metal mobilization and translocation by EDTA in sand columns. The metal selection was mainly based upon their high prevalence in the tannery and textile effluents that have polluted a major area of soil in the Punjab province of Pakistan, including Cu, Cd, and Cr.

There are some specific plants that show hyperaccumulation of specific metals, and fast-growing plants often show this capability. Plants in the grass family are vigorous and show quick growth, due to which they can prove to be suitable for growth and phytoextraction in industrially polluted habitats. Padmapiya et al. [18] have stated that sorghum is an ideal plant for remediation of heavy-metal pollution. Similarly, pearl millet has shown bioaccumulation of a number of heavy metals present in tannery solid waste [19]. This research was aimed at studying the impact of EDTA retention after its application using sorghum and pearl millet.

Material and Methods

Set-up of the Experiment

Coarse-grained sand was purchased for the experiment from a construction material outlet. It was washed, dried in an oven, and then sterilized by autoclaving. Then it was filled in plastic columns of 25 cm size, having a diameter of 8 cm, specially designed for the experiment. The columns had one cm diameter removable stoppers at the base, where they were lined with nylon gauze of 0.25 mm before being filled with sand.

Certified seeds of sorghum (*Sorghum bicolor* var. PSC 1010) and pearl millet (*Pennisetum glaucum* var. Hp-50) were obtained from the Seed Certification Centre in Lahore, Pakistan. Seeds were germinated in sterilized petri-plates on moist filter papers, and one-day-old seedlings were transferred into sand-filled plastic columns. The plants were grown in a wire house with a glass roof under natural light and dark conditions. The temperature range was 25-35°C, Relative humidity was 30-40% and the light intensity range during the

experiment was 8000-12000 lux. The nutrients for growth were applied in the form of Hoagland nutrient solution applied twice a week, and the columns were maintained at pot capacity with distilled water daily. After the establishment of plants during 15 days, 1 mM and 2 mM metal solutions were applied to the plants (30 ml each). Applied metal solutions were prepared from 1 M stock solution from analytical-grade Cu, Cd, and Cr (Merck). Five replicates of each metal treatment were maintained.

The experiment was set in a "Randomized Complete Block Design" [20], and comprised of five replicates for each treatment. Three sets of EDTA treatments for each metal treatment were prepared from disodium salt dehydrate of EDTA ($C_{10}H_{14}N_2O_8 \cdot 2H_2O$), without EDTA, 1 mM EDTA, and 2.5 mM EDTA. The sand columns were daily supplemented with water and after every two days with Hoagland's Solution. EDTA treatment was given to 35-day-old plants in a single application and the leachate was collected right after application and then at weekly intervals. Plants were harvested after four weeks of EDTA application at the age of 56 days. At the time of leachate collection, 150 ml water was added to each pot and about 50-60 ml leachate was collected depending upon soil moisture and humidity.

Post Harvest Analysis

Plants were harvested after about 56 days and various morphological parameters (i.e., number of leaves, number of roots, shoot length, root length, fresh weight, and dry weight) were noted at the time of harvest. The roots, shoots, and leaves of plants were separated and air dried and later in an oven at 60°C until a constant weight was obtained. The roots, shoots, and leaves were separately acid-digested with the nitric and perchloric acid digestion method described by Greenberg et al. [21]. For the estimation of metals (Cu, Cd, and Cr), samples were analyzed on an atomic absorption spectrophotometer (Model GBC SAVAANT AA, Australia). The AAS was calibrated for each element using standard solutions of known concentration before sample injection. Instructions for equipment setting, calibration, and assay for specific elements (using Cu as a standard) after every ten samples for quality control as recommended in the operational manual (by the manufacturer) were strictly followed. The leachate was also digested in the same way and metal estimations were carried out.

Bio-concentration Factor, Translocation Factor, and Tolerance Index

The bio-concentration factor (BCF) was calculated as the amount of metals was different in soil and is defined as the ratio of metal concentration in plant roots or aerial tissues to that in the soil. The translocation factor (TF) indicated the ability of plants to translocate

metal from the roots to the shoots. BCF, TF, and tolerance index (TI) were calculated as follows [22]:

- $BCF = C_{root}/C_{soil}$ (where C_{root} is the concentration of metal in root and C_{soil} is the concentration of total metal in soil).
- $TF = C_{aerial}/C_{root}$ (where C_{aerial} is the concentration of metal in aerial parts).
- $TI (\%) = \frac{\text{Plant root or shoot biomass in soil with metal}}{\text{Plant root or shoot biomass in soil without metal (control)}}$

Determining EDTA in the Leachate

Determining EDTA was done at the time of application and at weekly intervals after application. The method of Belal et al. [23] was followed. Suitable quantities of EDTA over a concentration range of 0.1-1.0 and 2.0-5.0 mg L⁻¹ were taken in separate 25 ml conical flasks. Then 1.5 ml of 100 mg L⁻¹ magnesium atomic absorption standard solution and 5 ml of ammonia buffer (pH 10) were added to the flask.

The solution was diluted to 25 ml with deionized distilled water. The whole content of the flask was then run on a cation exchanger (Amberlite IR-120) column 10 cm in length and 1 cm² in area. The Mg-EDTA sequestrate was obtained by centrifugation of samples at 16,000 rpm in a centrifuge (Model, SBC0060-230V, USA). The Mg-EDTA sequestrate was aspirated into the atomic absorption spectrophotometer and the absorbance was measured at 285.5 nm [23]. A standard curve was computed from the values obtained. The leachates obtained at weekly intervals were subjected to the same procedure as given above and the unknown concentration of EDTA was obtained from the curve.

Statistical Analysis

Statistical analysis of the data was carried out using the software SPSS 11.5 applying two-way ANOVA (Duncan's multiple range test) for different metal and EDTA treatments.

Table 1. Growth parameters of 56 days old plants of sorghum and pearl millet grown under different treatments of Cu and EDTA.

Growth Parameters	Conc. of Cu	Sorghum			Pearl millet		
		0mM EDTA	1mM EDTA	2.5mM EDTA	0mM EDTA	1mM EDTA	2.5mM EDTA
No. of leaves	0mM	13.00±0.6 b	11.33±0.6 bc	10.33±0.6 bc	17.00±2.0 a	12.00±3.0 b	14.00±2.0 b
	1mM	8.00±1.0 c	5.00±0.0 e	3.00±1.0 f	11.00±2.0 bc	9.00±1.0 c	8.00±1.0 c
	2mM	5.67±0.6 de	4.33±0.6 e	2.33±0.6 f	9.00±1.0 c	7.33±0.6 d	6.00±1.0 de
No. of roots	0mM	9.00±0.6 b	8.67±0.6 b	6.67±0.6 c	14.00±1.0 a	13.33±0.6 a	12.00±2.0 ab
	1mM	7.67±0.6 c	6.33±0.6 c	5.33±0.6 d	12.00±2.0 ab	11.00±1.0 ab	8.67±1.5 b
	2mM	7.00±1.0 c	5.33±0.6 d	4.00±1.0 d	11.33±3.05 ab	9.33±4.04 b	7.00±4.35 c
Shoot length (cm)	0mM	23.80±1.0 de	23.20±0.7 de	20.67±1.5 e	38.00±2.00 a	37.00±1.0 a	34.33±1.2 b
	1mM	21.27±1.4 e	17.63±0.4 f	15.33±0.35 fg	34.17±1.0 b	28.00±1.0 c	24.00±1.0 d
	2mM	17.93±1.8 f	13.93±0.9 g	12.43±1.0 g	25.33±0.6 d	21.00±1.0 e	15.00±1.0
Root length (cm)	0mM	5.90±0.7 a	5.57±0.6 bc	4.53±0.6 c	12.20±0.8 a	10.00±1.0 ab	9.33±0.6 ab
	1mM	5.73±0.9 bc	4.40±0.3 c	2.87±0.2 d	8.10±0.8 b	6.50±0.5 bc	5.50±1.0 bc
	2mM	5.30±0.8 bc	3.70±0.2 c	2.23±0.1 d	7.20±0.3 b	5.30±0.1 bc	3.77±0.3 c
Seedling length (cm)	0mM	29.70±0.6 d	28.77±0.6 d	25.20±1.6 e	50.20±1.6 a	47.00±1.7 a	43.67±0.6 b
	1mM	5.73±0.9 g	4.40±0.3 gh	2.87±0.2 h	42.27±0.3 b	34.50±1.3 c	29.50±1.4 d
	2mM	5.30±0.8 g	3.70±0.2 gh	2.23±0.1 h	33.73±2.1 c	26.30±1.0 e	18.77±0.9 f
Fresh weight (g)	0mM	7.20±0.1 a	6.87±0.05 ab	6.20±0.1 ab	8.20±0.1 a	7.63±0.06 a	6.40±0.1 ab
	1mM	6.33±0.06 ab	5.10±0.1 b	4.60±0.1 b	6.93±0.15 ab	5.30±0.1 b	4.63±0.06 b
	2mM	3.33±0.15 c	2.20±0.2 cd	1.60±0.1 d	3.43±0.15 c	2.60±0.1 cd	1.83±0.06 d
Dry weight (g)	0mM	2.70±0.1 a	2.37±0.06 a	2.03±0.06 ab	3.60±0.1 a	2.83±0.06 a	2.10±0.1 ab
	1mM	2.13±0.06 ab	1.70±0.1 b	1.50±0.1 b	2.33±0.06 a	1.87±0.06 b	1.43±0.06 b
	2mM	0.90±0.1 bc	0.50±0.1 c	0.50±0.1 c	1.03±0.15 bc	0.80±0.1 bc	0.52±0.4 c

Values with the same lowercase letters within each parameter are not statistically significant at $P = 0.05\%$, according to Duncan's multiple range test.

Results and Discussion

Effect of Heavy Metal and EDTA Treatment on Plant Growth

Chemically enhanced phytoextraction had been suggested as an efficient strategy for heavy metal removal from soil by the help of plants. EDTA is one of the most widely used chemical compounds for improving bioavailability of different contaminants in different soils [24]. Lingua et al. [25] reported that chelant amendment to highly Cu- and Zn-polluted soils enhanced the heavy metal uptake by the plants, and also favored the phytostabilisation or phytoextraction of these metals.

In this study, the growth experiment continued for 56 days and growth parameters of sorghum and pearl millet were noted at the time of harvest. Morphological parameters showed an inverse relationship with increasing concentration of metals. However, no toxicity symptoms were observed on the plants. A high amount

of heavy metals caused visual toxicity symptoms on sunflower leaves, such as necrosis and chlorosis, and the toxic effects increased with increasing accumulation of heavy metals in the plants [26]. The growth of pearl millet was much better than sorghum under the metal application of Cu and Cd, but no difference in both was observed under Cr application. The plants exhibited poor growth with Cd and Cr, but relatively better growth was observed with Cu (Tables 1-3).

Within each treatment, EDTA caused a decrease in all growth parameters. The most significant impact of EDTA application could be observed on root length and shoot length, which decreased with increases in doses of EDTA (Tables 1-3). The decrease in plant length following EDTA amendment can be attributed to EDTA toxicity and the formation of metal-chelant complexes. The heavy metal mobilization can badly affect cell wall elasticity and viscosity, reduce cell division and transpiration, and damage the cell membranes, which are generally maintained by Zn^{2+} and Ca^{2+} [27]. Various negative effects, including growth reduction, necrosis

Table 2. Growth parameters of 56-day-old plants of sorghum and pearl millet grown under different treatments of Cd and EDTA.

Growth Parameters	Conc. of Cd	Sorghum			Pearl millet		
		0mM EDTA	1mM EDTA	2.5mM EDTA	0mM EDTA	1mM EDTA	2.5mM EDTA
No. of leaves	0mM	15.00±0.6 a	13.00±1.0 ab	11.33±0.6 b	13.00±2.0 ab	11.00±1.0 b	10.00±1.0 b
	1mM	10.33±0.6 b	8.00±1.0 bc	6.00±1.0 c	11.00±1.0	7.67±1.2 bc	5.33±0.6 c
	2mM	8.67±0.6 bc	5.33±0.6 c	3.33±0.6 d	9.67±1.2 b	5.00±1.0 c	3.33±0.6 d
No. of roots	0mM	10.00±0.6 bc	10.00±1.0 bc	9.67±0.6 bc	16.00±1.0 a	12.00±1.0 b	10.00±2.0 bc
	1mM	8.67±0.6 c	6.00±1.0 cd	4.33±0.6 d	8.67±1.5 c	6.00±1.0 cd	4.00±1.0 d
	2mM	7.33±0.6 c	4.33±0.6 d	3.33±0.6 d	8.33±0.6 c	9.33±1.15 bc	3.33±0.6 d
Shoot length (cm)	0mM	23.70±1.2 b	21.77±1.4 bc	21.03±1.0 bc	27.8±0.8 a	24.03±1.0 b	20.83±1.3 bc
	1mM	19.33±0.6 c	14.67±0.6 d	12.33±1.5 de	23.17±1.7 b	18.83±1.3 c	12.50±1.0 de
	2mM	16.43±1.1 cd	12.10±1.1 de	11.40±0.9 e	20.00±2.0 c	12.63±1.0 de	10.00±0.6 e
Root length (cm)	0mM	7.10±0.2 ab	6.40±0.4 b	5.50±0.4 b	12.50±0.5 a	10.67±0.4 a	8.67±0.6 ab
	1mM	5.47±0.5 b	4.50±0.4 bc	3.43±0.3 b	7.00±1.3 ab	5.53±0.7 b	4.57±0.5 bc
	2mM	4.73±0.3 bc	3.37±0.3 c	2.73±0.2 c	5.93±1.0 b	3.63±0.6 c	2.83±0.4 c
Seedling length (cm)	0mM	30.80±1.4 c	28.17±1.3 c	26.53±0.8 cd	40.30±0.8 a	34.70±1.4 b	29.50±1.9 c
	1mM	24.80±0.5 d	19.17±0.9 e	15.77±1.3 f	30.17±0.8 c	24.37±1.8 d	17.07±1.5 ef
	2mM	21.17±0.9 e	15.47±0.9 f	14.13±0.9 f	25.93±1.9 cd	16.27±1.0 f	12.83±0.8 g
Fresh weight (g)	0mM	9.90±0.1 a	8.50±0.1 ab	6.87±0.06 b	10.30±0.1 a	8.80±0.1 a	7.20±0.1 b
	1mM	6.60±0.1 b	5.60±0.2 b	5.53±0.06 b	6.80±0.1 b	6.00±0.1 b	5.23±0.1 bc
	2mM	5.00±0.1 bc	4.23±0.06 bc	2.87±0.06 c	5.50±0.1 b	4.57±0.15 bc	3.13±0.15 c
Dry weight (g)	0mM	4.90±0.1 a	3.70±0.1 b	2.47±0.06 b	5.10±0.1 a	3.90±0.1 b	3.10±0.1 b
	1mM	2.30±0.1 bc	1.83±0.2 c	1.83±0.06 c	2.40±0.1 bc	2.00±0.1 c	1.77±0.06 c
	2mM	1.57±0.06 c	1.03±0.06 cd	0.57±0.06 d	2.00±0.1 c	1.47±0.06 cd	0.70±0.1 d

Values with the same lowercase letters within each parameter are not statistically significant at $P = 0.05\%$, according to Duncan's multiple range test.

Table 3. Growth parameters of 56-day-old plants of sorghum and pearl millet grown under different treatments of Cr and EDTA.

Growth Parameters	Conc. of Cd	Sorghum			Pearl millet		
		0mM EDTA	1mM EDTA	2.5mM EDTA	0mM EDTA	1mM EDTA	2.5mM EDTA
No. of leaves	0mM	20.00±0.6 a	18.33±0.6 ab	16.00±1.0 b	22.00±1.5 a	18.33±0.6 ab	16.00±1.0 b
	1mM	14.33±0.6 b	10.33±0.6 c	8.33±0.6 cd	13.00±1.0 bc	9.00±1.0 c	6.33±0.6 d
	2mM	12.00±1.0 bc	7.67±0.6 cd	4.67±0.6 e	10.67±0.6 c	7.67±0.6 cd	4.67±0.6 e
No. of roots	0mM	16.00±0.6 ab	14.33±0.6 b	13.00±1.0 bc	18.00±1.0 a	15.67±0.6 ab	14.00±1.0 b
	1mM	12.33±0.6 bc	9.00±1.0 c	6.00±1.0 d	11.00±1.0 c	7.67±1.5 d	6.67±1.5 d
	2mM	9.67±1.5 c	6.00±1.0 d	4.67±1.1 e	8.67±0.6 cd	6.67±1.5 d	4.67±1.2 e
Shoot length (cm)	0mM	28.6±1.0 b	25.10±0.8 c	22.00±1.0 d	31.20±1.2 a	25.50±0.5 c	23.67±0.6 d
	1mM	22.93±0.8 d	19.10±0.8 de	15.03±0.3 e	24.53±0.8 d	19.23±0.8 de	14.40±0.5 ef
	2mM	20.77±0.7 de	14.03±0.6 ef	12.07±0.8 f	22.33±1.0 d	15.17±1.2 e	12.10±1.0 f
Root length (cm)	0mM	14.10±0.8 ab	12.80±0.7 b	11.87±1.0 b	16.00±1.0 a	14.13±0.8 ab	13.67±0.6 ab
	1mM	12.00±0.7 b	8.67±0.6 c	5.80±0.2 d	13.40±0.7 ab	9.00±1.0 c	6.37±1.0 d
	2mM	10.00±0.4 bc	5.73±0.7 d	4.13±0.7 e	10.23±1.4 bc	5.03±1.7 de	4.13±0.7 e
Seedling length (cm)	0mM	42.80±1.5 b	37.90±1.0 c	33.87±1.5 d	47.20±1.3 a	39.63±1.2 bc	37.33±0.6 c
	1mM	34.93±1.4 d	27.77±1.4 e	20.83±0.2 f	37.93±0.4 c	28.23±1.8 e	20.77±1.1 f
	2mM	30.77±0.3 de	19.77±0.3 f	16.2±0.6 g	32.57±1.8 de	20.20±2.5 f	16.23±0.7 g
Fresh weight (g)	0mM	12.10±0.2 a	11.00±0.2 a	10.07±0.1 ab	12.60±0.1 a	11.43±0.06 a	10.50±0.1 ab
	1mM	9.33±0.06 b	7.87±0.1 b	7.20±0.1 bc	9.67±0.06 b	8.20±0.1 b	8.50±0.1 b
	2mM	7.10±0.1 bc	4.80±0.06 cd	3.10±0.2 d	6.77±0.15 c	5.30±0.2 c	4.23±0.06 cd
Dry weight (g)	0mM	6.10±0.2 a	5.40±0.1 a	4.77±0.01 ab	6.30±0.1 a	5.93±0.06 a	5.10±0.1 a
	1mM	4.03±0.01 ab	2.97±0.01 b	2.50±0.1 b	4.57±0.06 ab	3.40±0.1 b	3.70±0.1 ab
	2mM	2.30±0.1 b	1.70±0.1 c	0.70±0.1 d	2.40±0.1 b	1.70±0.1 c	1.23±0.06 c

Values with the same lowercase letters within each parameter are not statistically significant at $P = 0.05\%$, according to Duncan's multiple range test.

etc., after EDTA treatment, were possibly because of a large amount of heavy metal mobilization in soil solutions.

Pearl millet showed better growth as compared to sorghum in Cu-contaminated columns. Increasing concentrations of Cu and EDTA showed a decrease in all growth parameters in both plants. Kolbas et al. [28] also observed that increasing Cu concentration in the growth medium linearly decreased the root length of sunflowers. The overall growth parameters resembled in both plants under Cu stress (Table 1).

A similar pattern of growth was observed in both the plants under Cd stress (Table 2). Dry biomass of both plants was significantly affected with the amount of metal. Increasing the Cd concentration in the hydroponic condition and soil culture decreased the root length, leaf area, and fresh and dry weights of shoots and roots of sunflowers as compared to controls [29]. Similarly, root biomass, stem diameter, and total leaf area in sunflowers was observed to be strongly affected with Cd treatments [30]. The dose of EDTA did not

affect growth significantly, and the biomass of both plants was similar.

A similar pattern of growth was observed under Cr stress as for Cu and Cd. The biomass of both plants was nearly similar in the presence of Cr, and the dose of metal as well as EDTA strongly checked the growth in both plants (Table 3). These findings are in accordance with Ding et al. [31], who observed less root growth, seedling growth, and biomass in *Hibiscus cannabinus* grown under Cr stress.

Effect of EDTA on Metal Uptake in Plants

The effect of chelant was examined by determining metal uptake in various plant parts, i.e., roots, shoots, and leaves. The concentration of Cu was observed to be the highest in roots in both plants. However, metal uptake was much better in sorghum (up to 2,400 mg kg⁻¹) as compared to pearl millet (up to 1,719 mg kg⁻¹) (Fig. 1). Cu uptake was in the order of root > shoot > leaf. The uptake showed a significant

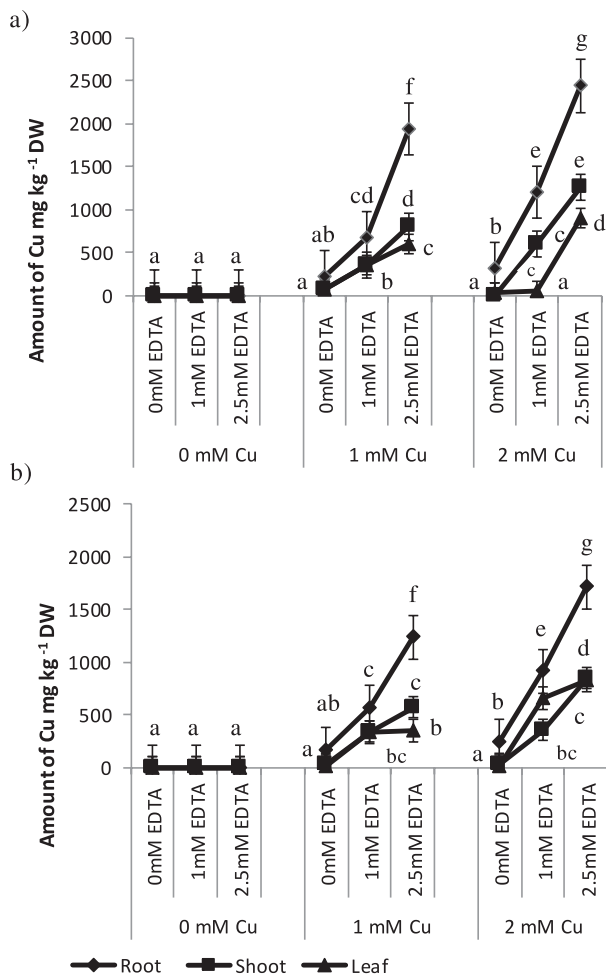


Fig. 1. Copper content of different plant parts of a) sorghum and b) pearl millet grown under different treatments of EDTA. The points with similar lowercase letters indicate non-significant values according to DMRT at $P = 0.05\%$

enhancement in both plants with increasing EDTA concentration, from 320 mg kg⁻¹ to 1,200 mg kg⁻¹ to 2,440 in sorghum, and from 247 mg kg⁻¹ to 920 mg kg⁻¹ to 1,719 mg kg⁻¹ in millet under 0 mM to 1 mM to 2.5 mM EDTA doses, respectively. However, the increase in uptake was less significant in shoots and leaves due to EDTA.

Cadmium uptake efficiency was significantly higher in sorghum (583 mg kg⁻¹) compared to pearl millet (253 mg kg⁻¹). In both plants, metal uptake was maximum in roots, followed by shoots and then leaves (Fig. 2). Lonardo et al. [32] also reported that metal uptake in roots was found to always be greater than in the shoots, which suggests a metal exclusion approach of reproductive tissues and stem by arresting heavy metals in the roots to avoid harm. Similar findings on metal partitioning in plant parts in hydroponics were also reported by Zacchini et al. [33]. EDTA showed a significant influence on metal uptake in roots and shoots, but not in the leaves. Sinhal et al. [34] observed that, as compared to the control, the EDTA amendment profoundly enhanced the uptake of Zn, Cd, Pb, and

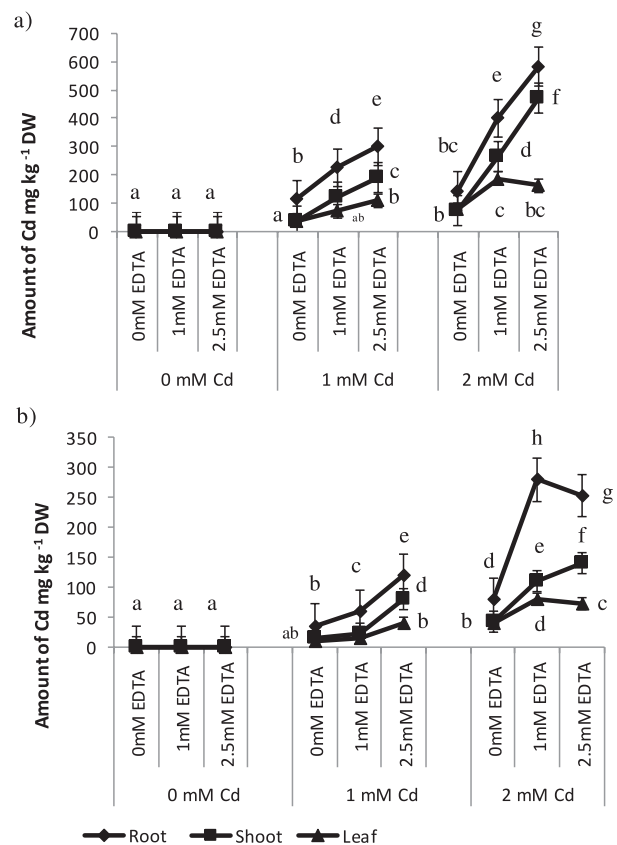


Fig. 2. Cadmium content of different plant parts of a) sorghum and b) pearl millet grown under different treatments of EDTA. The points with similar lowercase letters indicate non-significant values according to DMRT at $P = 0.05\%$

Cu in roots, stems, and leaves of *Tagetes* sp. Similar findings were also reported by Ali et al. [27].

EDTA application significantly enhanced metal uptake in sorghum but not in pearl millet. There was a much higher uptake of Cr (797 mg kg⁻¹) in sorghum compared to pearl millet (430 mg kg⁻¹). However, root to shoot partitioning was much greater in sorghum. EDTA application did not affect Cr uptake by shoots and leaves of sorghum, but significantly affected metal uptake in shoots of millet (Fig. 3). There was hyperaccumulation of Cu and Cr in the roots and Cd in all plant parts in sorghum without EDTA. However, hyperaccumulation in the aerial parts was attained for Cu after EDTA application. Similarly, in pearl millet, hyperaccumulation of Cu, Cd, and Cr occurred only in the roots but after EDTA application, Cu and Cd hyperaccumulation occurred in the aerial parts and of Cr in the shoots as given by Ding et al. [31].

To evaluate the ability of sorghum and pearl millet to extract and accumulate heavy metals, the bio-concentration factor (BCF) was determined. The BCF is a general index used for estimating a plant's capability to extract heavy metals from the substrate and for comparing the phytoextraction potential of plants [35]. The bioconcentration factors of both plants under different metal and EDTA treatments are given in

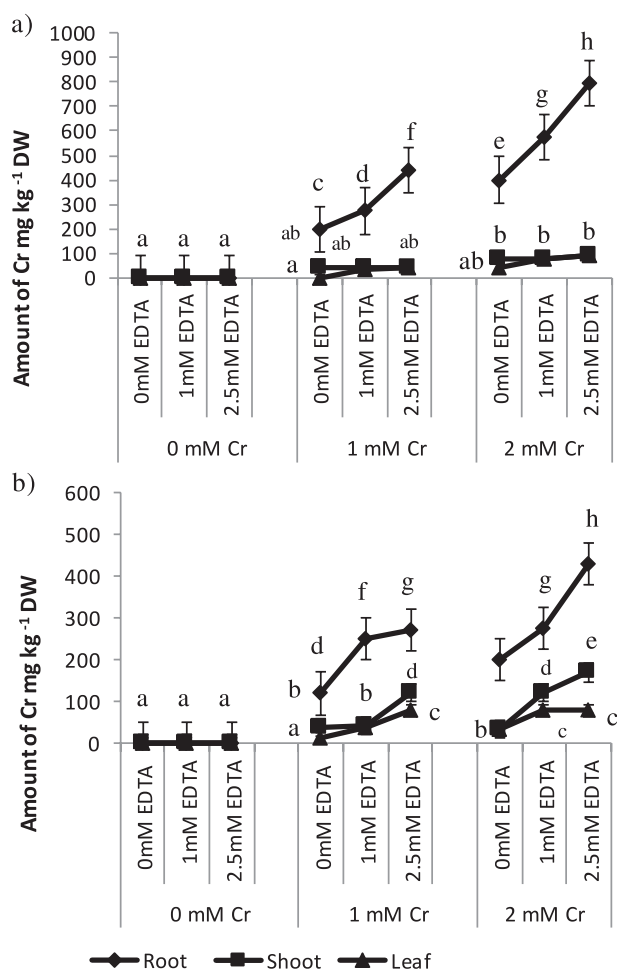


Fig. 3. Chromium content of different plant parts of a) sorghum and b) pearl millet grown under different treatments of EDTA. The points with similar lowercase letters indicate non-significant values according to DMRT at $P = 0.05\%$

Table 4. Higher BCF values were observed in sorghum for all metals as compared to pearl millet. Accumulation in both the plants was in the order $\text{Cu} > \text{Cd} > \text{Cr}$. Higher bioaccumulation was observed under less metal concentration and it increased with increasing concentration of EDTA (Table 4).

Metal translocation is presented as the ratio between the amount of metal in the shoots and that in the roots [36]. High values of translocation factor (TF) are indicative of a plant's ability to uptake metal from the substrate and store it in the aboveground parts [37]. In the present study, it marks the ability of EDTA to influence the transfer of Cu, Cd, and Cr from root to shoot. The translocation factors were improved after EDTA treatment in both the plants for Cu and Cd, but not in Cr (Table 5). This is in line with Chen and Cutright [38], who reported effective root to shoot translocation of Cd and Ni but not of Cr. Meers et al. [39] have also shown that translocation of Cr was not affected by EDTA application in sunflower. Increasing EDTA concentration did not have a significant impact on improving the translocation. Therefore, a 1 mM dose of EDTA was enough to mobilize the metal. Similarly, a reverse trend was noticed between translocation factor and chelating agent at high doses of EDTA by Ebrahimi [40]. A significant decrease in metal TF was found upon the addition of 1.5 mmol kg^{-1} of EDTA.

EDTA mobilized the metals in the order of $\text{Cu} > \text{Cr} > \text{Cd}$ despite the use of inert sand columns and equal doses of metal solutions and EDTA. Greater translocation as recorded with EDTA treatment in artificially spiked Cu soil could be due to decreased metal binding to the root tissues. Comparatively stable Cu complexes, because of having high affinity with EDTA, are easily translocated to harvestable plant parts as compared to free metal ions. This complexation would decrease the free metal ions binding to (negatively charged) carboxyl groups located in cell walls of xylem [41]. Restricted Cd movement over plant tissues is possible because of the interactions with exchange sites present in vacuolar compartments and cell walls [36].

This research indicated a differential chelation of EDTA with Cu, Cd, and Cr. Turgut et al. [42] observed that EDTA caused a selectivity of metals from $\text{Cr} > \text{Cd} > \text{Ni}$ to $\text{Cd} > \text{Cr} > \text{Ni}$ with the change in dose from 0.1 g/kg to 0.3 g/kg in sunflower. According to them, selectivity is dependent upon changes in plant cultivars, chelator type, and dose and the soil type. January et al. [43] have also observed a differential effect of EDTA on

Table 4. Bioconcentration Factors (BCF) of different metals in roots of plants under different doses of EDTA.

Heavy Metal	Conc. of Metal	BCFs under different conc. of EDTA in:					
		Sorghum			Pearl millet		
		0mM EDTA	1mM EDTA	2.5mM EDTA	0mM EDTA	1mM EDTA	2.5mM EDTA
Cu	1mM Cu	1.50	7.90	9.80	1.36	6.40	9.10
	2mM Cu	0.30	4.10	9.38	0.79	3.40	7.47
Cd	1mM Cd	0.26	0.80	2.25	0.14	0.38	1.71
	2mM Cd	0.17	1.00	2.50	0.16	0.65	0.90
Cr	1mM Cr	0.16	0.20	0.55	0.12	0.36	0.46
	2mM Cr	0.14	0.20	0.39	0.14	0.34	0.86

Table 5. Translocation Factor (TF) of different metals in plants under different doses of EDTA.

Heavy Metal	Conc. of Metal	TFs under different conc. of EDTA in:					
		Sorghum			Pearl millet		
		0mM EDTA	1mM EDTA	2.5mM EDTA	0mM EDTA	1mM EDTA	2.5mM EDTA
Cu	1mM Cu	0.32	0.54	0.41	0.20	0.60	0.46
	2mM Cu	0.00	0.50	0.51	0.10	0.38	0.49
Cd	1mM Cd	0.35	0.54	0.64	0.40	0.38	0.60
	2mM Cd	0.50	0.66	0.80	0.54	0.39	0.55
Cr	1mM Cr	0.20	0.15	0.09	0.33	0.17	0.44
	2mM Cr	0.20	0.14	0.11	0.18	0.44	0.39

a group of three metals, so that the order of selection changed from Cd = Cr > Ni (without EDTA) to Cr > Cd > Ni with EDTA. However, the order of selection of metals did not change in the current study without and with EDTA.

The tolerance indices of metals were higher for lower doses of metals. Overall, the tolerance indices decreased with increasing concentrations of EDTA (Table 6), indicating that higher metal concentration lowers the TI plants because of higher uptake due to the action of EDTA.

Effect of EDTA on Metal Leaching in Plants

While using desorption techniques and chelating agents for decontaminating metal-polluted soils, there are a lot of issues to be addressed. These soil amendments can possibly induce mobilization of non-targeted metals/metalloids that could be phytotoxic (like Mn and Al). Furthermore, the enhanced solubilization of targeted metals/metalloids can lead to enhanced leaching to groundwater, particularly when there is no plant growth. Much lysimetric and column research has presented increased concentrations of heavy metals/metalloids in the leachates after the addition of different synthetic chelates [44]. In this study, the three metals showed leaching over time that

significantly increased with the application of EDTA. Leaching was observed at weekly intervals after EDTA application. Cu and Cd showed similar behavior, and more leaching was observed in the second week as compared to the time of EDTA application in both plants. But the behavior of Cr was different in both the plants, and significantly greater leaching was observed right at the time of EDTA application, which decreased over time. In fact, the decrease was directly proportional to time. When Jean-Soro et al. [45] observed leaching of Ni and Cr with EDTA and citric acid, more Cr was leached with citric acid than with EDTA.

Regarding the leaching behavior of Cu, it was significant at the time of EDTA application and slightly increased during the first week (up to 1,800 mg L⁻¹ in sorghum and 3,100 mg L⁻¹ in millet), but significantly decreased in the second week and was almost negligible in the fourth week (Fig. 4). A reverse correlation was observed between the amount of Cu absorbed and leached. A greater amount of Cu was absorbed and less leached by sorghum as compared to pearl millet in which less Cu was absorbed and a higher amount was leached. In addition to enhanced Cu mobilization and plant uptake, Cu leaching was also noticed by Komarek et al. [46] after EDDS addition of 3 and 6 mmol/kg in *Populus nigra*.

Table 6. Tolerance Indices (TI) of different heavy metals in plants under different doses of EDTA.

Heavy Metal	Conc. of Metal	TIs under different conc. of EDTA in:					
		Sorghum			Pearl millet		
		0mM EDTA	1mM EDTA	2.5mM EDTA	0mM EDTA	1mM EDTA	2.5mM EDTA
Cu	1mM Cu	0.78	0.71	0.73	0.64	0.66	0.68
	2mM Cu	0.33	0.21	0.24	0.28	0.28	0.24
Cd	1mM Cd	0.46	0.49	0.74	0.47	0.51	0.57
	2mM Cd	0.32	0.27	0.23	0.39	0.37	0.22
Cr	1mM Cr	0.66	0.55	0.52	0.72	0.57	0.72
	2mM Cr	0.37	0.31	0.14	0.38	0.28	0.24

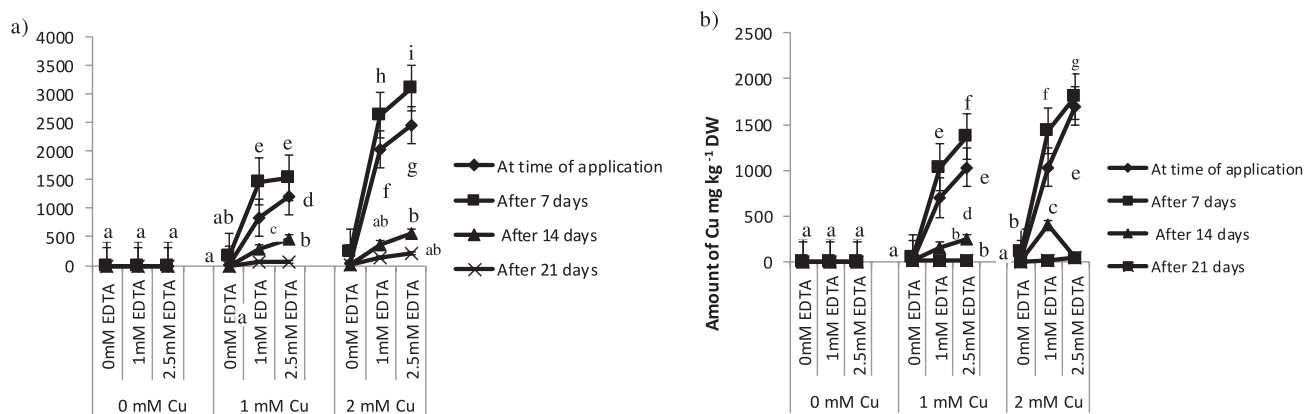


Fig. 4. Copper content of leachate at four different intervals from the date of application of EDTA in columns with a) sorghum b) pearl millet. The points with similar lowercase letters indicate non-significate values according to DMRT at $P = 0.05\%$

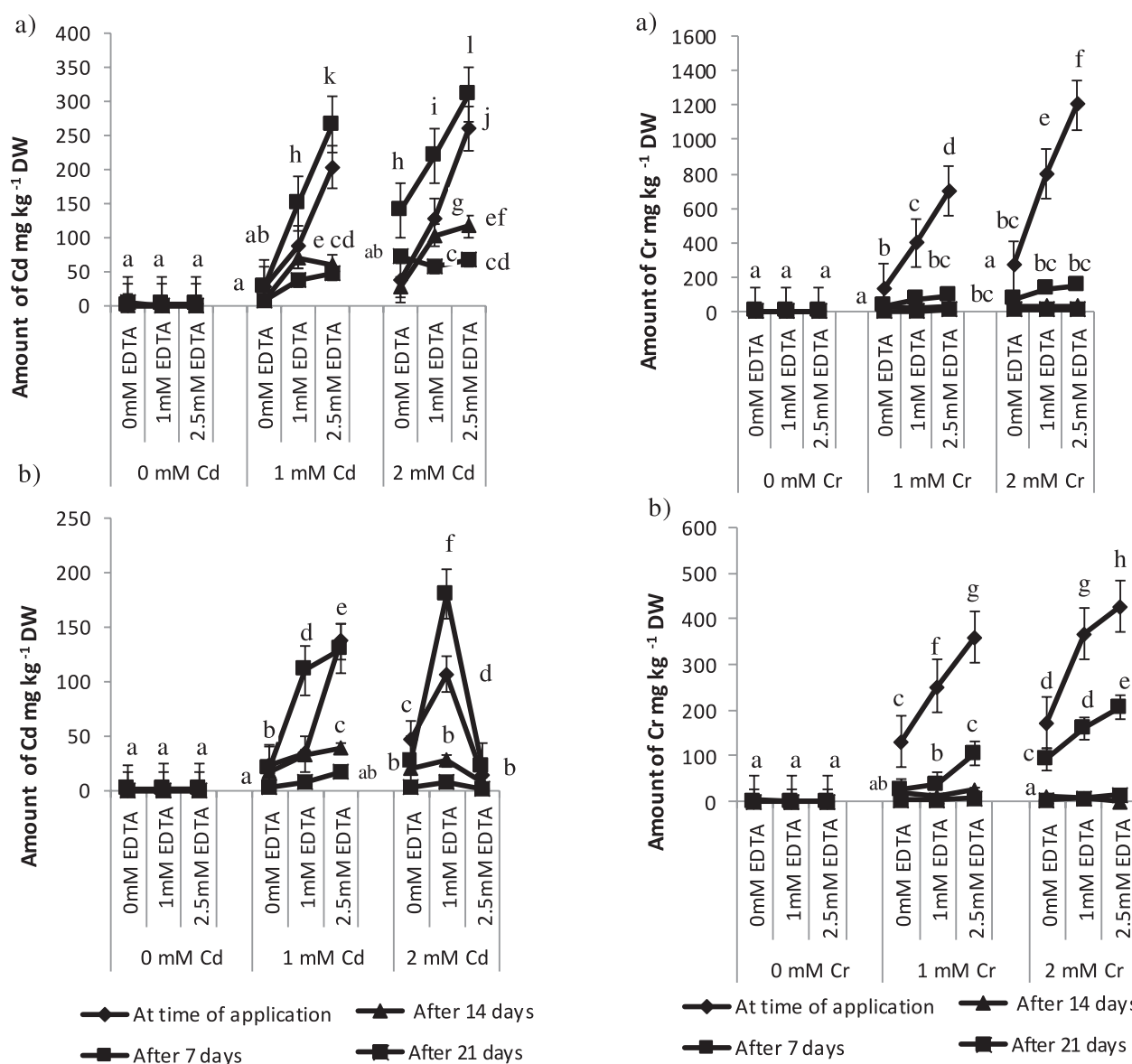


Fig. 5. Cadmium content of leachate at four different intervals from the date of application of EDTA in columns with a) sorghum b) pearl millet. The points with similar lowercase letters indicate non-significate values according to DMRT at $P = 0.05\%$

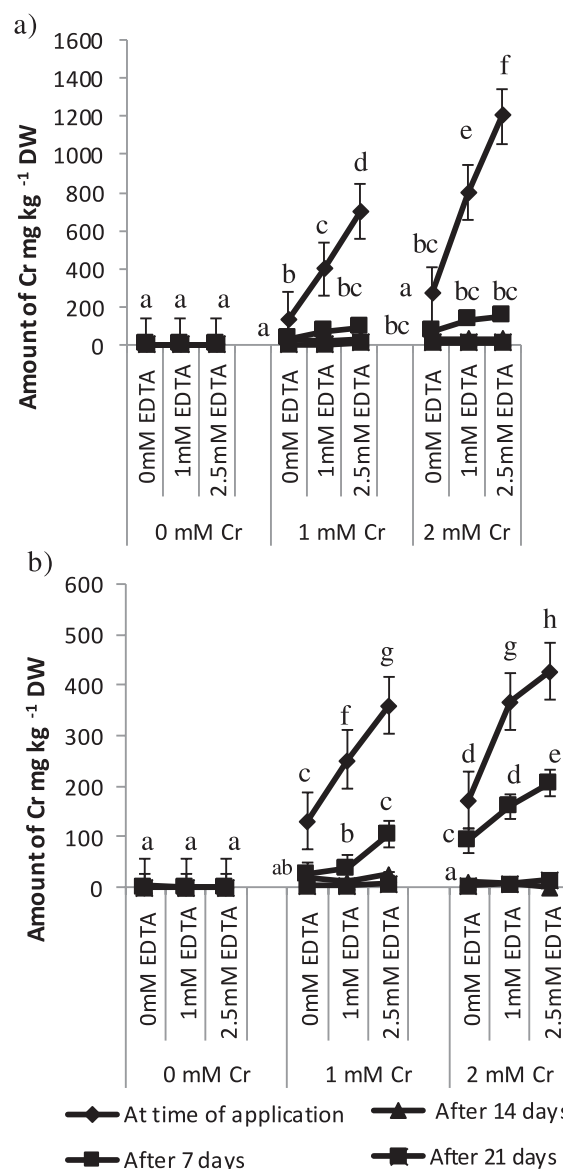


Fig. 6. Chromium content of leachate at four different intervals from the date of application of EDTA in columns with a) sorghum b) pearl millet. The points with similar lowercase letters indicate non-significate values according to DMRT at $P = 0.05\%$

Table 7. Amount of EDTA (mM kg⁻¹sand) in the leachate in sand columns having sorghum and pearl millet at weekly intervals under different metal and EDTA treatments.

Plant	Metal Conc.	1 mM EDTA				2.5 mM EDTA			
		1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
Sorghum	1mM Cu	0.30 ab	0.40 a	0.08 b	BDL	0.40 a	0.40 a	0.10 c	0.01 c
	2mM Cu	0.40 a	0.20 ab	0.20 ab	0.01 c	0.50 a	0.50 a	0.04 bc	0.05 bc
	1mM Cd	0.08 b	0.08 b	0.07 b	0.03 c	0.10 b	0.10 b	0.06 bc	0.04 bc
	2mM Cd	0.08 b	0.10 b	0.08 b	0.05 bc	0.10 b	0.20 ab	0.08 b	0.01 c
	1mM Cr	0.20 ab	0.06 bc	0.02 c	BDL	0.30 ab	0.08 b	0.02 c	BDL
	2mM Cr	0.30 ab	0.08 b	0.02 c	0.01 c	0.40 a	0.08 b	0.02 c	0.01 c
Pearl millet	1mM Cu	0.30 a	0.40 a	0.10 b	0.06 bc	0.40 a	0.50 a	0.20	0.06 bc
	2mM Cu	0.60 bc	0.60 a	0.20 ab	0.08 b	0.50 a	0.50 a	0.30 ab	0.10 b
	1mM Cd	0.04 b	0.08 b	0.03 c	BDL	0.08 b	0.08 b	0.03 c	0.01 c
	2mM Cd	0.08 b	0.09 b	0.02 c	BDL	0.01 c	0.02 c	BDL	BDL
	1mM Cr	0.10 ab	0.04 bc	0.01 c	BDL	0.20 ab	0.08 b	0.02 c	BDL
	2mM Cr	0.20	0.09 b	BDL	BDL	0.20 ab	0.10 b	0.02 c	0.01 c

Values with the same lowercase letters within each parameter are not statistically significant at $P = 0.05\%$, according to Duncan's multiple range test.

BDL = Below detection limit

The amount of Cd in the leachate was strongly influenced with EDTA application in both plants, especially in sorghum. The amount of Cd reached up to 310 mg L⁻¹ in 20.5 mM EDTA and 180 mg kg⁻¹ in millet with 1mM EDTA (Fig. 5). The maximum amount of Cd was leached after 7 days, followed by the time of application, and decreased significantly after 14 and 21 days. In sorghum, metal leaching was directly proportional to the dose of EDTA, while in pearl millet 1 mM EDTA seemed to be the most effective dose (Fig. 5).

There was a much higher Cr uptake in sorghum as compared to pearl millet. However, root-to-shoot partitioning was much greater in sorghum. EDTA application did not improve Cr uptake by the shoots and leaves of sorghum while it significantly affected shoot uptake in millet (Fig. 6). The amount of Cr in the leachate was greater in sorghum (799 mg L⁻¹) and the maximum amount of Cr was leached at the time of EDTA application decreasing after 7 days and becoming negligible after 14 days of application. In millet, there was a high amount of Cr in the leachate (367 mg kg⁻¹), even after 7 days, but became negligible after 14 days of application.

Among the three metals, Cu showed leaching in the highest amount, followed by Cr and finally Cd. The metals absorbed by the plants were also in the same order Cu>Cr>Cd. This shows the selective behavior of EDTA in mobilizing the metals because inert sand columns were used in these experiments to minimize adsorption to soil particles. Moreover, the metal and

EDTA doses were also constant. Another important observation was that higher doses of EDTA did not show as much impact on Cu and Cd as on Cr. The amount of Cr in the leachate showed a direct correlation with the amount of EDTA.

Pearl millet proved to be a better plant at controlling metal leaching – especially that of toxic Cd and Cr as compared to sorghum because of its more expansive root system having greater length and number of roots. This conforms the findings of Xu et al. [47], indicating that roots of sorghum are inefficient in the uptake of EDTA-chelated lead.

Estimating EDTA in the Leachates

EDTA is thought to persist in soil for several weeks or months in the field because EDTA and metal-EDTA complexes are non-biodegradable [7, 8]. The amount of EDTA in the leachate decreased over time (Table 7), and the decrease was significant in the fourth week of application. In the fourth week, it was not detectable in some cases. When the amount of metal in the leachate was compared with the amount of EDTA in the leachate, a very high correlation was observed in all metals and both doses of EDTA (Table 8). This shows that a single application of metals is enough to mobilize the metal, and split applications can increase the leaching hazard for a longer period. Similar observations have been made by Jean-Soro et al. [45], that as compared to a single chelant injection, alternative injection of water and chelant leads to greater leaching of metals.

Table 8. Pearson's correlation between the amount of metal in the leachate and the amount of EDTA detected in the leachate.

Plant	Metal	1 mM EDTA	2.5 mM EDTA
Sorghum	1mM Cu	0.996606	0.976695
	2mM Cu	0.99156	0.999076
	1mM Cd	0.762675	0.945828
	2mM Cd	0.933772	0.904533
	1mM Cr	0.990057	0.98909
	2mM Cr	0.995834	0.997976
Pearl millet	1mM Cu	0.984411	0.999004
	2mM Cu	0.973902	0.979549
	1mM Cd	0.982594	0.997365
	2mM Cd	0.953879	0.932928
	1mM Cr	0.967416	0.992094
	2mM Cr	0.999558	0.999654

This study also shows that leaching continues so long as EDTA remains in the soil. However, after a month its amount is almost negligible in the soil. Thus, leaching is a short-term hazard that also depends on the dose of EDTA. Therefore leaching can be managed by using a low dose of 5 mmol per kg of soil [48] and as low as 1 mmol per kg soil, but only when using high biomass metal-bioaccumulating plants [49, 50]. This study indicates that even a small dose can mobilize the metal enough for enhancement in metal uptake. The increased mobility of heavy metals is probably due to the residual chelant present in soil [45]. If a small dose of EDTA is used, most part of it will be used in forming chelates with the metal cations and thus less metal will leach down. However, so long as the EDTA remains in the soil, it will disturb soil structure [51], cause toxicity to the plants, and subsequently reduce plant biomass and shoot metal concentration by causing metal leaching. Plant roots might be badly affected due to disruption of cell membranes by the chelating agent having concentrations greater than 10 mmol chelant kg⁻¹. Furthermore, groundwater contamination is also caused by higher doses of chelant. Therefore, lower doses are always recommended in any case.

Conclusions

Chelating agents are quite helpful in improving efficiency of phytoextraction. The addition of EDTA results in increasing metal bioavailability, consequently enhancing metal uptake in plants. Among the three metals spiked on sand columns, with sorghum and pearl millet, EDTA selectively mobilized them in the order Cu>Cr>Cd. This was manifested both by the

plant uptake and metal leaching through the column. Sorghum performed better in metal uptake while pearl millet was better at controlling leaching.

However, enhanced metal solubilization in the soil contributes to groundwater contamination by leaching down of heavy metals. In order to deal with this increasing menace, EDTA should be used in low concentrations as it is found that less EDTA concentration is quite effective in metal mobilization and it also lessens the risk of groundwater contamination. A dose of one mM EDTA in a single application is appropriate for sufficient metal mobilization. Besides, plants with thick and extensive root systems can be helpful in enhancing phytoextraction and in slowing down metal leaching in the soil.

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Conflict of Interest

The authors declare no conflict of interest.

References

- SAIFULLAH, MEERS E., QADIR M., DE CARITAT P., TACK FMG., DU LAING G., ZIA M.H. EDTA-assisted Pb phytoextraction. *Chemosphere*. **74**, 1279, **2009**.
- MIAO Y., XI-YUAN X., XU-FENG M., ZHAO-HUI W., FENG-YONG G. Effect of amendments on growth and metal uptake of giant reed (*Arundo donax* L.) grown on soil contaminated by arsenic, cadmium and lead. *Trans. Nonferrous Met. Soc.* **22**, 1462, **2012**.
- ARSHAD M., SILVESTRE J., PINELLI E., KALLERHOFF J., KAEMMERER M., TARIGO A., SHAHID M., GUIRESSE M., PRADERE P., DUMAT C. A field study of lead phytoextraction by various scented *Pelargonium* cultivars. *Chemosphere*. **71**, 2187, **2008**.
- ZHIVOTOVSKY O.P., KUZOVKINA YA., SCHULTHESS C.P., MORRIS T., PETTINELLI D. Lead uptake and translocation by willows in pot and field experiments. *Int. J. Phytoremed.* **13**, 731, **2011**.
- BARRUTIA O., GARBISU C., HERNÁNDEZ-ALLICA J., GARCÍA-PLAZAOLA J.I., BECERRIL J.M. Differences in EDTA-assisted metal phytoextraction between metalicolous and non-metalicolous accessions of *Rumex acetosa* L. *Environ. Pollut.* **158**, 1710, **2010**.
- HADI F., BANO A., FULLER M.P. The improved phytoextraction of lead (Pb) and the growth of maize (*Zea mays* L.): The role of plant growth regulators (GA₃ and IAA) and EDTA alone and in combinations. *Chemosphere*. **80**, 457, **2010**.
- GOEL S., GAUTAM A. Effect of chelating agents on mobilization of metal from waste catalyst. *Hydrometallurgy*, **101**, 120, **2010**.
- ZHAO Z., XI M., JIANG G., LIU X., BAI Z., HUANG Y. Effects of IDSA, EDDS and EDTA on heavy metals

- accumulation in hydroponically grown maize (*Zea mays* L.). J. Hazard. Mater. **181**, 455, **2010**.
9. NEUGSCHWANDTNER R.W., TLUSTO'S P., KOM'AREK M., SZ'AKOV'A J., JAKOUBKOV'A L. Chemically enhanced phytoextraction of risk elements from a contaminated agricultural soil using *Zea mays* and *Triticum aestivum*: Performance and metal mobilization over a three year period. Int. J. Phytorem. **14**, 754, **2012**.
 10. BARONA A., ARANGUIZ I., ELIAS A. Metal associations in soils before and after EDTA extractive decontamination: implications for the effectiveness of further clean-up procedures. Environ. Pollut. **113**, 79, **2001**.
 11. GRCMAN H., VODNIK D., VELIKONJA-BOLTA S., LESTAN D. Ethylenediaminedisuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. J. Environ. Qual. **32**, 500, **2003**.
 12. EVANGELOU M.W.H., EBEL M., SCHAEFFER A. Chelate assisted phytoextraction of heavy metals from soil: Effect, mechanism, toxicity, and fate of chelating agents, Chemosphere. **68**, 989, **2007**.
 13. BLAYLOCK M.J., SALT D.E., DUSHENKOV S., ZAKHAROVA O., GUSSMAN C., KAPULNIK Y., ENSLEY B.D., RASKIN I. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ. Sci. Technol. **31**, 860, **1997**.
 14. SHEN Z.G., LI X.D., WANG C.C., CHEN H.M., CHUA H. Lead phytoextraction from contaminated soil with high biomass plant species. J. Env. Qual. **31**, 1893, **2002**.
 15. MUHAMMAD D., CHEN F., ZHAO J., ZHANG G., WU F. Comparison of EDTA- and citric acid-enhanced phytoextraction of heavy metals in artificially metal contaminated soil by *Typha angustifolia*. Int. J. Phytorem. **11**, 558, **2009**.
 16. BAREEN F., TAHIRA A. Efficiency of seven different cultivated plant species for phytoextraction of toxic metals from tannery effluent contaminated soil using EDTA. Soil Sed. Contam. **19**, 160, **2010**.
 17. GHEJU M., PODE R., MANEA F. Comparative heavy metal chemical extraction from anaerobically digested biosolids. Hydrometallurgy. **108**, 115, **2011**.
 18. PADMAPIYA S., MURUGAN N., RAGAVENDRAN C., THANGABALU R., NATARANJAN D. Phytoremediation potential of some agricultural plants on heavy metal contaminated mine waste soils, Salem district, Tamilnadu. Int. J. Phytorem. **18**, 288, **2016**.
 19. BAREEN F., SHAFIQ M., JAMIL S. Role of plant growth regulators and a saprobic fungus in enhancement of metal phytoextraction potential and stress alleviation in pearl millet. J. Hazard. Mater. 237-238, 186-193, **2012**.
 20. STEEL R.G., TORRIE J.H. Principles and Procedures of Statistics, A Biometrical Approach, 2nd ed., McGraw Hill International Book Company, UK, **1998**.
 21. GREENBERG A.E., CLESSERI L.S., EATON A.D. Standard Methods for the Examination of Water and Waste Water, 20th ed., American Public Health Association, USA, **1998**.
 22. SHI G., CAI Q. Cadmium Tolerance and accumulation in eight potential energy crops. Biotechnol. Adv. **27**, 555, **2009**.
 23. BELAL F., ALY F.A., WALASH M.I., KENAWY M.I., OSMAN A.M. Determination of ethylenediaminetetraacetic acid (EDTA) in pharmaceutical dosage forms using flame atomic absorption spectroscopy. IL Farmaco. **53**, 365, **1998**.
 24. MEERS E., QADIR M., DE CARITAT P., TACK F.M.G., DU LAING G., ZIA M.H. EDTA-assisted Pb phytoextraction. Chemosphere. **74**, 1279, **2009**.
 25. LINGUA G., TODESCHINI V., GRIMALDI M., BALDANTONI D., PROTO A., CICALTELLI A., CASTIGLIONE S. Polyaspartate, a biodegradable chelant that improves the phytoremediation potential of poplar in a highly metal-contaminated agricultural soil. J. Environ. Manage. **132**, 9, **2014**.
 26. JUNIOR C.A., MAZZAFERA P., ARRUDA M.A.Z. A comparative ionomic approach focusing on cadmium effects in sunflowers (*Helianthus annuus* L.). Environ. Exp. Bot. **107**, 180, **2014**.
 27. ALI S.Y., CHAUDHURY S. EDTA-enhanced phytoextraction by *tagetes* sp. and effect on bioconcentration and translocation of heavy metals. Environ. Proc. **3**, 735, **2016**.
 28. KOLBAS A., MARCHAND L., HERZIG R., NEHNEVAJOVA E., MENCH M. Phenotypic seedling responses of a metal-tolerant mutant line of sunflower growing on a Cu-contaminated soil series: potential uses for biomonitoring of Cu exposure and phytoremediation. Plant Soil, **376**, 377, **2014**.
 29. PRITSA T.S., FOTIADIS E.A., LOLAS P.C. Corn tolerance to atrazine and cadmium and sunflower to cadmium in soil and hydroponic culture. Commun. Soil Sci. Plant Anal. **39**, 1168, **2008**.
 30. CORNU J.Y., BAKOTO R., BONNARD O., BUSSIÈRE S., CORIOU C., SIRGUEY C., STERCKEMAN T., THUNOT S., VISSE M.I., NGUYEN C. Cadmium uptake and partitioning during the vegetative growth of sunflower exposed to low Cd concentrations in hydroponics. Plant Soil. doi:10.1007/s11104-016-2839-8, **2016**.
 31. DING H., WANG G., LOU L., LV J. Physiological responses and tolerance of kenaf, (*Hibiscus cannabinus* L.) exposed to chromium. Ecotoxicol. Environ. Saf. **133**, 500, **2016**.
 32. DI LONARDO S., CAPUANA M., ARNETOLI M., GABBRIELLI R., GONNELLI C. Exploring the metal phytoremediation potential of three *Populus alba* L. clones using an in vitro screening. Environ. Sci. Pollut. Res. **18**, 82, **2011**.
 33. ZACCHINI M., PIETRINI F., SCARASCIA MUGNOZZA G., IORI V., PIETROSANTI L., MASSACCI A. Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics. Water Air Soil Pollut. **197**, 234, **2009**.
 34. SINHAL V.K., SRIVASTAVA A., SINGH V.P. EDTA and citric acid mediated phytoextraction of Zn, Cu, Pb and Cd through marigold (*Tagetes erecta*). J. Environ. Biol. **31**, 255, **2010**.
 35. ZAHER H., GHANAYAT T., GHABRICHE R., CHMINGUI W., LAKHDAR A., LUTTS S., ABDELLY C. EDTA-enhanced phytoremediation of lead-contaminated soil by the halophyte *Sesuvium portulacastrum*. Environ. Sci. Pollut. Res. Int. **21**, 7607, **2014**.
 36. GUNAWARDANA B., SINGHAL N., JOHNSON A. Amendments and their combined application for enhanced copper, cadmium and lead uptake by *Lolium perenne*. Plant Soil. **329**, 283, **2010**.
 37. NORLEELASELAMAT S., ABDULLAH R.S.S., IDRIS M. Phytoremediation of lead (Pb) and arsenic (As) by *Melastoma malabathricum* L. from contaminated soil in separate exposure. Int. J. Phytorem. **16**, 694, **2014**.

38. CHEN H., CUTRIGHT T. EDTA and HEDTA effects on Cd, Cr and Ni uptake by *Helianthus annuus*. *Chemosphere*. **45**, 21, **2001**.
39. MEERS E., RUTTENS A., HOPGOOD M., LESAGE E., TACK F.M.G. Potential of *Brassica rapa*, *Cannabis sativa*, *Helianthus annuus* and *Zea mays* for phytoextraction of heavy metals from calcareous dredged sediment derived soils. *Chemosphere*. **61**, 561, **2005**.
40. EBRAHIMI M. Effect of EDTA and DTPA on phytoremediation of Pb-Zn contaminated soils by *Eucalyptus camaldulensis* Dehnh and effect on treatment time. *Desert*. **19**, 65, **2014**.
41. CHIGBO C., BATTY L. Chelate-assisted phytoremediation of Cu-pyrene-contaminated soil using *Z. mays*. *Water Air Soil Pollut.* **226**, 1, **2015**.
42. TURGUT C., PEPE K.M., CUTRIGHT T.J. The effect of EDTA and citric acid on phytoremediation of Cd, Cr, and Ni from soil using *Helianthus annuus*. *Environ. Pollut.* **131**, 147, **2004**.
43. JANUARY M.C., CUTRIGHT T.J., KEULEN H.V., WEI R. Hydroponic phytoremediation of Cd, Cr, Ni, As, and Fe: Can *Helianthus annuus* hyperaccumulate multiple heavy metals? *Chemosphere*. **70**, 531, **2008**.
44. WANG A., LUO C., YANG R., CHEN Y., SHEN Z., LI, X. Metal leaching along soil profiles after he EDDS application - A field study. *Environ. Pollut.* **164**, 204, **2012**.
45. JEAN-SORO L., BORDAS F., BOLLINGER J.C. Column leaching of chromium and nickel from a contaminated soil using EDTA and citric acid. *Environ. Pollut.* **164**, 175, **2012**.
46. KOMÁREK M., VANĚK A., MRNKA L., SUDOVÁ R., SZÁKOVÁ J., TEJNECKÝ V., CHRASTNÝ V. Potential and drawbacks of EDDS-enhanced phytoextraction of copper from contaminated soils. *Environ. Pollut.* **158**, 2428, **2010**.
47. XU Y., YAMAJI N., SHEN R., MA J.F. Sorghum roots are inefficient in uptake of EDTA-chelated lead. *Ann. Bot.* **99**, 869, **2007**.
48. ZHAO S., LIAN F., DUO L. EDTA-assisted phytoextraction of heavy metals by turfgrass from municipal solid waste compost using permeable barriers and associated potential leaching risk. *Biores. Technol.* **102**, 621, **2011**.
49. HERNANDEZ-ALLICA J., GARBISU C., BARRUTIA O., BECERRIL J.M. EDTA-induced heavy metal accumulation and phytotoxicity in cardoon plants. *Environ. Exp. Bot.* **60**, 26, **2007**.
50. BAREEN F. Chelate assisted phytoextraction in the oilseed *Brassicaceae*. In: N.A. Anjum I. Ahmad, M.E. Pereira, A.C. Duarte, S. Umar, N.A. Khan, (Eds.). *The Plant Family Brassicaceae: Contribution towards Phytoremediation*. Environmental Pollution Book Series, Vol. No. **21**, Springer: Dordrecht, the Netherlands. 289, **2012**.
51. TSANG D.C., ZHANG W., LO I.M. Copper extraction effectiveness and soil dissolution issues of EDTA-flushing of artificially contaminated soils. *Chemosphere*. **68**, 234, **2007**.

