

EDTA-enhanced phytoextraction of Cd and Pb in spiked soil with Marigold and associated potential leaching risk

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Abstract— EDTA is a common chelating agent used in phytoextraction for enhancing the trace elements uptake from soil to plants. But application of EDTA can cause ground water contamination, which is potentially a risk for human health. In this study EDTA-enhanced phytoextraction of Cd and Pb by Marigold. was evaluated. Application of EDTA could significantly increase Cd and Pb accumulation in the various part of plants, but EDTA addition also increased the Cd and Pb concentration in leached water about 8 to 12 time and 50 to 75 times respectively compared to the control. So, insitu application of EDTA can be potentially risk to ground water contamination with trace elements.

Keywords— EDTA, leached water, Marigold, phytoextraction.

I. INTRODUCTION

Trace elements (TE) are of major concern in recent time. TEs accumulate in the soil and water due to human activities, such as mining and smelting, industrialization, use of TEs containing waste water, fertilizer, pesticides, fossil fuel burning etc. Some TEs like Zn, Cu, Co, Mn etc. are essential for human health; these elements are used for redox processes as components of various enzymes and for regulation of osmotic pressure in cells [1]. But all TEs are toxic at higher level.

Phytoremediation is one most cost effective engineering based technologies for remediation of trace elements that use plant and their root-bound microbial communities to remove or absorb trace elements from soil [2]. Phytoextraction is one of the process of phytoremediation defined as the use of the plants that can accumulate, transport and concentrate trace elements from the soil to the roots and aerial plants parts[3]. Natural hyperaccumulating plants are capable to accumulate high amount of TEs, but all hyperaccumulating plants cannot used for phytoextraction because of their low biomass and limited growth [4], [5].

Chelating agent such as ethylenediaminetetraacetic acid (EDTA), diethylenetrinitriolpentaacetic acid (DTPA), nitrilotriacetic acid (NTA), ethylenediaminedisuccinate (EDDS) have been used for long time in nutrient solutions to increase the solubility and plant availability of trace elements [6].

EDTA has been the most effective chelating agent tested because of its less biodegradable nature, but it might cause groundwater contamination with TEs [7, 8, 9]. According to Thayalakumaran et al., [6]in situ application of such chelates can increase the risk of water pollution by promoting uncontrolled metal solubilisation and leaching. The objective of this study was to evaluate the effect of various concentration of EDTA application to TEs containing spiked soil on plant uptake and leaching of Cd and Pb.

II. MATERIAL AND METHOD

2.1 Pot –Culture experiment

Soil samples were collected from agricultural field at 20 cm depth, dried and sieved. Soil parameters such as soil texture, pH, EC, Organic Carbon, water soluble sodium, potassium, calcium, exchangeable sodium, potassium, calcium, alkalinity, total nitrogen, water soluble phosphorus, total phosphorus, and cation exchange capacity were analyzed which shown in Table 1. After analysis of soil parameter, 1 kg of soil/pot was taken in plastic pots which have a hole. The pots with soil were separately contaminated artificially with aqueous solution of Lead nitrate and Cadmium nitrate [$\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$] to get the concentration 100mg Pb/kg of soil and 100mg Cd/kg of soil respectively. Pots were watered 4 times per week with 200ml distilled water. After soil preparation one marigold (*Tagetes* sp.) plant was transplanted into the each pot. After 30 days EDTA di sodium salt was added to the soil as aqueous solution at dose of 2g Na_2EDTA /kg of soil (E2) and 4g Na_2EDTA /kg of soil (E4). Each treatment had 3 pots against control where no EDTA was added (E0). Leached water from pots was collected in a beaker during the experimental period. This water was filtered with Whatman 42 filter paper and acidified with HNO_3 for analysis of TEs.

TABLE 1
VARIOUS PHYSIOCHEMICAL PARAMETERS OF EXPERIMENTAL SOIL

Soil Parameters	Values and type
Soil Type	Sandy loam
Organic carbon	0.97%
Electron conductivity	1.02mS/cm
Na	40mg/kg
K	56.6mg/kg
Ca	30.2mg/kg
Chloride	296mg/kg
Sulphate	45mg/kg
Phosphorus	2.56mg/kg
Total Nitrogen	192mg/kg
Cation Exchange Capacity	10.2meq/100g
Zn	80.15
Cd	Below detectable
Pb	20.55 mg/kg
Cu	54.85 mg/kg

2.2 Collection and Sample Preparation

After three months, plants were collected from the pots, thoroughly washed with running tap water and 0.1M HCl and rinsed with deionized water to remove any soil particles attached to the plant surfaces. Then the plant samples were dried at 70°C for 48 hours [10]. The dried plants samples were weighed and ground for analysis of metal concentration. Sequential extraction of soil samples after harvesting was done by method proposed by Tessier et al., [11] (Table 2).

TABLE 2
FIVE STEP SEQUENTIAL EXTRACTION PROCEDURE FOR CHEMICAL SPECIATION, PROPOSED BY TESSIER ET AL. (1979)

Fraction	Extraction chemical for 1gm of soil	Temperature and Mixing
Exchangeable (F1)	8ml 1M MgCl ₂ at pH 7	1 hr. at 25-30°C
Bound to Carbonate (F2)	8ml 1M NaOAc, pH 5	5 hrs. at 25-30°C
Bound to Fe-Mn oxides (F3)	20ml 0.04M NH ₂ OH.HCl in 25% (v/v) HOAc	6 hrs. at 96±3°C
Bound to organic matter (F4)	3ml 0.02M HNO ₃ + 5ml 30% H ₂ O ₂	2 hrs. at 85±2°C
	3ml 30% H ₂ O ₂	2 hrs. at 85±2°C
	5ml 3.2M NH ₄ OAc in 20% (v/v) HNO ₃	30 min. at 25-30°C
Residual (F5)	4ml HNO ₃ , 1ml HClO ₄ and 10ml HF	8 hrs. at 100°C
	1ml HClO ₄ and 5ml HF	6 hrs. at 190°C

2.3 Heavy metals analysis of soil and plants

Soil and plant samples (1 g) were digested separately with 15 ml of tri-acid mixture (HNO₃, H₂SO₄, and HClO₄ in 5:1:1 ratio) at 80°C until a transparent solution was obtained [12]. After cooling, the digested sample was filtered using Whatman No. 42 filter paper and the filtrate was finally made up to 50 ml with distilled water. Trace elements of digested plant samples and leached water were analyzed by using Anodic Stripping Voltammetry (Metrohm VA 797 Computraces).

III. STATISTICAL ANALYSIS

All treatments were replicated three times in the experiments. The means and standard error (SE) were calculated by the Microsoft Office Excel 2013. One-way ANOVA and Duncan test was carried out with SPSS20.

IV. RESULT AND DISCUSSION

4.1 Speciation of soil Cd and Pb after harvesting of Marigold

From previous study, it was found that the high portion of Cd was associated with exchangeable fraction of soil particles [13, 14]. Application of chelating agent like EDTA could significantly enhance exchangeable fraction of Trace elements [14]. The results of sequential extraction test performed after the harvesting of plant to evaluate heavy metals distribution of soil are shown in Fig. 1. Application of EDTA salt, total heavy metal content for all metals in soil significantly decrease compared to no chelator applied soil.

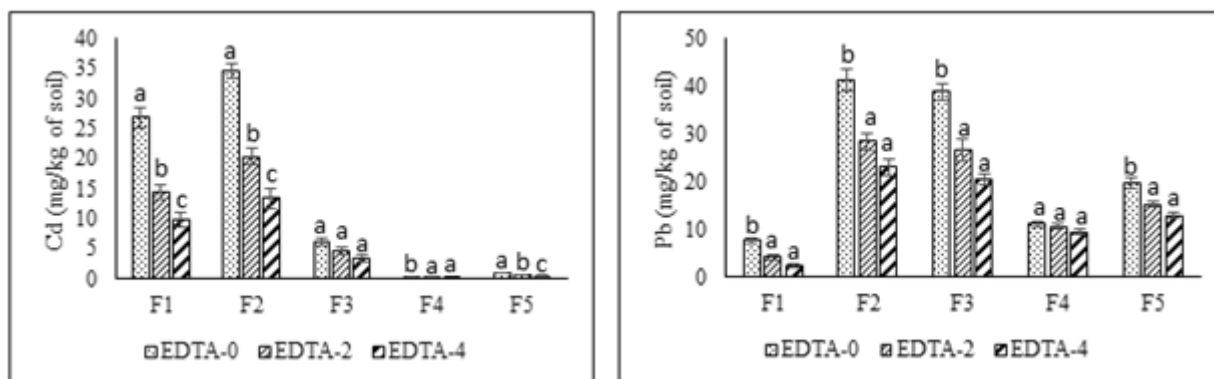


FIG. 1: EFFECTS OF APPLICATION OF EDTA ON SPECIATION OF TES IN SOIL AFTER HARVESTING. SAME ALPHABET INDICATED NO STATISTICALLY SIGNIFICANT DIFFERENCE ACCORDING TO DUNCAN TEST AT $P < 0.05$.

4.1.1 Cadmium

Previous study reported that the highest amount Cd content in the spiked soil was present in non-residual fraction [15]. For this reason mobility of Cd is higher than the other metals. The result in Figure 1 showed that large amount of Cd was present in carbonate bound fraction (F2) which is about half of the total metal in the soil when no chelator was applied (Fig. 1). But EDTA application significantly decreased the Cd concentration about 1.7 and 2.6 fold under the treatment of EDTA-2 (2g Na_2EDTA /kg of soil) and EDTA-4 (4g Na_2EDTA /kg of soil) respectively compared to EDTA-0 (no chelator applied soil). The second most important fraction was exchangeable fraction (F1), in which about one fourth Cd was present at EDTA-0. However, application of EDTA significantly reduce the Cd content to about 1.86 and 2.74 fold under the treatment of EDTA-2 and EDTA-4 respectively compared to EDTA-0. The exchangeable fraction has special importance due to the high mobility of toxic trace metals into the environment and biomass; thereby it has the potential to contaminate the ecosystem [16]. Very low amount of Cd is present in Fe-Mn (F3) bound fraction, organic matter bound fraction (F4) and residual fraction (F5) at EDTA-0 soil. Cd content also reduce in these fraction to about 1.27 and 1.72 fold in Fe-Mn oxide (F3) fraction, 2 and 2.56 fold in oxide bound fraction (F4) and 1.5 and 1.98 fold in residual fraction under the treatment of EDTA-2 and EDTA-4 respectively, compared to EDTA-0.

4.1.2 Lead

Unlike Cd, very small amounts of Pb were present in exchangeable fraction (F1) which were about 6.4%, 3.57% and 1.95% in EDTA-0, EDTA-2 and EDTA-4 respectively (Fig. 1). The highest concentration of Pb in all samples was in the Fe/Mn oxides bound fraction (F3). This trend is already reported by some authors in the mine contaminated soil [17], street sediment [18] and metal polluted river sediment [19]. Fe-Mn oxides are excellent scavengers of Pb [16]. According to Salomons, [20] this metal is predominantly adsorbed by the hydrous oxides of Fe/Mn and changes to redox conditions which may cause the release of Pb into the environment. Fig. 2 showed that after addition of EDTA Pb content decreased in all fraction, about 1.83 and 3.27 fold in F1, 1.45 and 1.78 fold in F2, 1.45 and 1.9 fold in F3, 1.06 and 1.17 fold in F4 and 1.29 and 1.51 fold in F5 under the treatment of EDTA-2 and EDTA-4 respectively.

4.2 Cd and Pb accumulation by Marigold

The addition of EDTA resulted in a significant increase in Cd and Pb accumulation. Cd accumulation increased by 2.41 and 3.4 fold in shoots and 11.7 and 15.7 fold for leaves at the concentration 2g EDTA/kg and 4g EDTA/kg of soil respectively compared to E0 (no chelator applied soil) (Fig. 2). According to Prusty et al. [21] Pb is less bioavailable and less

mobile than the other metals, for this reason highest amount of Pb concentrated in root and very little amount of Pb was found in aerial part of plants. But application of EDTA significantly enhanced the Pb accumulation by 2.28 and 3.74 fold for shoot and 3.46 and 6.01 fold for leaves at the concentration of 2g EDTA/kg and 4g EDTA/kg of soil respectively compared to E0 (no chelator applied soil).

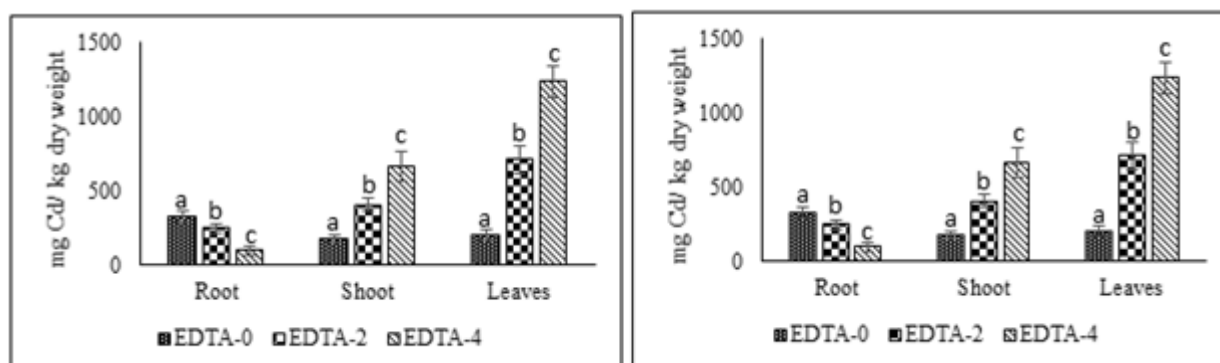


FIG. 2: EFFECTS OF APPLICATION OF EDTA ON THE UPTAKE OF Cd AND Pb IN MARIGOLD. SAME ALPHABET INDICATED NO STATISTICALLY SIGNIFICANT DIFFERENCE ACCORDING TO DUNCAN TEST AT $P < 0.05$.

4.3 Cd and Pb concentration in leached water

Table 3 showed that the Cd and Pb concentration of leached water. After addition of EDTA Cd concentration increased by 0.7 mg/L in EDTA-0 to 5.73 mg/L and 8.28 mg/L under the treatment of EDTA-2 and EDTA-4 respectively. Similarly, Pb concentration also increased from 0.05 mg/L to 2.5 mg/L and 3.77 mg/L after addition of EDTA-2 and EDTA-4 respectively. Because of high mobility of Cd, the Cd concentration in leached water was so much higher than the Pb, which is less mobile.

TABLE 3
Cd and Pb CONCENTRATION OF LEACHED WATER AFTER APPLICATION OF VARIOUS EDTA CONCENTRATION

Trace Elements	Cd (mg/L)	Pb (mg/L)
EDTA-0	0.7±0.18 ^a	0.05±0.01 ^a
EDTA-2	5.73±1.23 ^b	2.5±0.64 ^b
EDTA-4	8.28±1.44 ^c	3.77±0.86 ^c

V. CONCLUSION

It may be concluded from the present study, that application of EDTA could increase the mobility of the trace elements in the soil. It also revealed that addition of EDTA could significantly increase the TEs accumulation in the various parts of the marigold compared to the no chelator applied soil. However, TEs accumulation by plant, after application of EDTA was insignificant compared to the amount of TE present into the soil. Most of trace elements were leached with water to the surrounding soil which is a risk of ground water pollution with trace elements.

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