original article

Influence of electrical conductivity on the phytoremediation of contaminated soils to Cd²⁺ and Zn²⁺

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ABSTRACT

Aims: This research was conducted to study the effects of the electrical conductivity (EC) of irrigation water and compost on the Cadmium (Cd) and Zinc (Zn) uptake by sunflower, *Helianthus annuus*. The transfer of Cd and Zn from soils close to the Zn mine, to the sunflower tissues, and the interactions between the two concerned metals, were also investigated.

Materials and Methods: For this purpose, 10% weight/weight from municipal composts was applied to raw soils that were randomly collected from the mine region. Series analyses were also implemented by irrigation water, with EC values in the range of 0, 2, 4, and 6 dS/m.

Results: The maximum uptake rate of Cd, with EC levels of 6 dS/m, in plant samples was $4.82 \mu g/g$ for the roots, $6.14 \mu g/g$ for the stems, and $5.4 \mu g/g$ for the leaves; and the maximum uptake of Zn, in plants irrigated with tap water, was 241 $\mu g/g$ by the roots, $624 \mu g/g$ by the stems, and 229 $\mu g/g$ by the leaves, respectively.

Conclusions: Results showed that high EC levels of irrigation water increased Cd accumulation and decreased Zn accumulation in the shoots. The presence of high EC levels in irrigation water negatively affected biomass production by plants. Chlorine ion (Cl-) had a positive influence on Cd accumulation in the harvestable parts of the plant.

Key words: Cadmium, phytoremediation, salinity, sunflower, zinc

INTRODUCTION

The environment may be contaminated with heavy metals that are related to anthropogenic activities such as mining, electroplating, the metal smelting operations, energy and fuel manufacture, severe cultivation, sludge dumping, power transmission,^[1,2] wastewater sludge or municipal composts, fertilizers, emissions from public waste incinerators, and other human activities.^[3,4]

There are several methods for the removal heavy metals

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from contaminated soils, such as, soil washing, soil structure, and fertility that involve significant engineering costs, and they have an adverse effect on biological activity.^[5,6] Phytoremediation has received increasing recognition, as an environment friendly and low-cost technology.^[7,8] On the other hand, an *in situ* approach of phytoremediation is attractive, as it offers site restoration and protection of the biological activity, physical structure, and chemical properties of the soil's fractional sanitization.^[5,9,10] This technology, which is an emerging technology,^[11] has a strong potential as a natural, solar energy-driven remediation approach for the treatment of polluted sites,^[12] which use green plants and their related rhizospheric microorganisms to remove, degrade, or contain contaminants located in the soil, sediments, groundwater, surface water, and even the atmosphere, to remove metals from the soil or environment.^[11,13,14]

There are numerous different alternatives for soil phytoremediation^[13,15,16] such as, phytostabilization

and phytoextraction. Phytostabilization consists of the immobilization of metals in the soil or roots, and phytoextraction includes the uptake of contaminants from the soil, which results in the translocation from the roots to the part of the plant above the ground.^[13,15]

The characteristics of the plants that apply for phytoremediation include: Their ability to accumulate the metals intended to be extracted, preferably in the parts above the ground; high tolerance to the metals; fast growth and high biomass yield; cultivation as a crop; and easily harvestable.^[17,18]

Ozkutlu *et al.*, showed that irrigation of crops with saline waters enhanced the mobilization of Cd from the soil and translocation of Cd from the shoots to the grains.^[19] Hattori *et al.*, expressed that use of Cl⁻ led to a remarkable enhancement in Cd uptake, in sunflower and kenaf plants. It could be a promising method for the phytoremediation of Cd, in combination with the soil pH adjustment, depending on the tolerance of the plant species to low pH.^[20]

The purpose of this study was to determine the effect of salinity on the phytoremediation of Cd and Zn from the contaminant soil.

MATERIALS AND METHODS

Experimental design

This study was conducted between 15 June and 17 August, 2010, in a greenhouse at Isfahan (32°39'N, 51°43'E) University of Medical Sciences in Iran. The sampled soil was from the Bama's mine, the third biggest lead and zinc mine of Iran, located in the 20 km interval, at southwest of Isfahan, with a height of 1750 m above sea level, and an average annual rainfall of 140 mm. The municipal solid waste (MSW) compost came from the Isfahan compost plant, after a thorough composting process. The physicochemical characteristics of the compost, sludge, and soil samples that were air-dried and sieved through a 2 mm sieve are shown in Table 1. The experimental site consisted of 12 plots that had an inner diameter of 30 cm and a height of 30 cm, with two variables, amendment type, four EC levels, and three replications. Sunflower Helianthus annuus could accumulate metals and had about the same biomass as the Indian mustard.^[13] Each pot was filled with 10% W/W municipal compost and dry soils. Tap water was used for irrigation

Characterization of soil and amendments

All chemicals used were purchased from the Merck Company and had analytical purities. The sand, silt, and clay content in the soil were analyzed by the hydrometer method.^[21] The organic matter (OM) content in the samples was measured using the Walkley and Black method.^[22] The cation exchange capacity (CEC) was determined by NH₄⁺ saturation at pH 7.^[23] Total nitrogen (TN) was determined by the Bremner and Mulvaney method,^[24] and total phosphorus (TP) was determined by the Olsen method.^[25] Samples were extracted with neutral 1-N ammonium acetate^[26] and the potassium concentrations in the extracts were determined by a flame photometer. The soil pH values were determined in a fraction of 1:2 soil-to-water suspensions, with a pH meter model 262, and electrical conductivity (EC) was determined in a fraction of 1:2 soil-to-water suspensions with an EC meter model 644.^[27] Heavy metals were determined by the tri-acid digestion method, following the American Public Health Association (APHA) by atomic absorption spectrometry (AAS; Perkin-Elmer 3030 Atomic Absorption Spectrophotometer).^[28] For pH and EC measurements, the 1:5 (W/V) municipal compost-to-water to water suspensions were prepared. Next, the pH was measured before filtration, as also the EC on the filtrate.

Plant harvesting

Sampling was started 60 days after sowing (DAS) the plants. Plant harvesting, which paid attention to the roots, took care to extract the plants completely from the soil. Subsequently, all the plant fractions (roots, leaves, and shoots) were carefully washed with deionized water. The fundamental plant growth parameters, such as plant height and fresh and dry biomass of the plant fractions were measured. The dry biomass of the plant fractions were measured after 48 hours at 60°C in an AirForce oven.

Heavy metal content in soil and plant fractions

For determining the heavy metal concentrations, the soil or two amendment samples from each pot were collected, air-dried, screened by means of a 2 mm sieve, and finally oven dried (60°C for 48 hours). Subsequently, these prepared samples were acid-digested and analyzed for Cd and Zn by atomic absorption spectrometry. For plant fractions, the samples were homogenized in particle size by a grinder and their heavy metal content was determined by the dry ashing method.^[29]

Data analysis

The data are presented in the text as the average of at least three replicates per treatment. The mean values±standard

| Table 1: Basic physicochemical characteristics of soil,sludge, and compost | | | | | |
|--|-------|-----------|--|--|--|
| Parameter | Trea | Treatment | | | |
| | Soil | Compost | | | |
| рН | 7.0 | 7.0 | | | |
| Sand (%) | 55 | - | | | |
| Silt (%) | 34.17 | - | | | |
| Clay (%) | 10.83 | - | | | |
| Organic C (%) | 0.22 | 20.88 | | | |
| Organic matter (%) | 0.37 | 39 | | | |
| CEC (C.mol. kg ⁻¹) | 71.8 | 46 | | | |
| EC (dS.m ⁻¹) | 1.867 | 10.16 | | | |
| P %)) | 0.138 | 0.22 | | | |
| K (%) | 0.023 | 1.06 | | | |
| Total-N (%) | 0.084 | 1.61 | | | |
| Total-Cd (mg.kg ⁻¹) | 4.95 | 5.22 | | | |
| Total-Zn (mg.kg ⁻¹) | 490 | 542.8 | | | |

deviation is reported. The data have been subjected to a one-way analysis of variance (ANOVA), and have been used to examine the effects of the EC levels.

RESULTS

Soil properties and total contents

The basic physicochemical characteristics of the compost, as amendment and soil, and Zn and Cd fractionation in the studied matrixes, are summarized in Table 1.

Metal accumulation in plants

Cadmium uptake by the plants

The results in Table 2 show the Cd uptake by the plants. ANOVA showed that there was a difference between the EC levels and Cd uptake in the roots and leaves ($P_{value} < 0.05$), but not in the stems ($P_{value} > 0.05$). The maximum uptake rate of Cd with an EC level of 6 dS/m in the plants sample was 4.82 µg/g for the roots, 6.14 µg/g for the stems, and 5.4 µg/g for the leaves, respectively.

Zinc uptake by the plants. Data regarding the Zn uptake by plants is shown in Table 3. The maximum uptake of Zn in plants irrigated with tap water (EC level of 0 dS/m) was, 241 µg/g by the roots, 624 µg/g by the stems, and 229 µg/g by the leaves, respectively. ANOVA showed the difference between the EC levels and the Zn uptake in leaves ($P_{value} < 0.05$), but it not in the roots and stems ($P_{value} > 0.05$).

Growth parameters

Dry weight of the root, stem, and leaves of the plant, and the plant height of the sunflower, grown under non-saline water and saline water irrigation, are shown in Figures 1 to 4. The results show that the highest dry weight of the root, stem, and leaves occurs in the sunflower grown under non-saline water irrigation. A significant effect between the EC levels in the dry weight and height of the plant is shown by ANOVA ($P_{Value} < 0.05$).



Figure 1: Variation in the dry weight of the roots of sunflowers in three replicates under different NaCl levels (0, 2, 4, and 6 dS m⁻¹) after 60 DAS. The data are the mean \pm SD (n=3); P_{value} <0.05

Heavy metals in the soil

Cadmium mobility in the soil

Cadmium mobility in the soil with NaCl irrigation and without NaCl irrigation, after 60 DAS, is shown in Figure 5. ANOVA has not shown a significant difference between the EC levels and type of treatments in the mobility of cadmium in the soil ($P_{value} > 0.05$).

Zinc mobility in the soil

Results of the zinc mobility in the soil are shown in Figure 6. ANOVA did not reveal a significant effect of the mobility of

Table 2: Total Cd (μ g/g dry weight) uptake by root, stem, and leaves of sunflower (*Helianthus annuus*) in a plant sample that was irrigated with tap water and 2,4, 6 dS/m NaCl solution 60 days after planting; at harvest

| | No EC | With EC, dS/m | | |
|--------------|------------------|-------------------|-----------------|----------------|
| | 0 | 2 | 4 | 6 |
| Root* | 2.87 ± 0.4 | 3.43 ± 0.1 | 4.7 ± 0.8 | 4.8 ± 1.1 |
| Stem * * | 4.6 ± 0.05 | 4.7 ± 1.1 | 5.8 ± 0.0 | 6.14 ± 0.0 |
| Leaves* | 4.11 ± 0.0 | 4.3 ± 0.6 | 5.23 ± 0.1 | $5.40~\pm~0.3$ |
| Data are the | maana of three r | nligatos + standa | rd doviation *P | |

Data are the means of three replicates \pm standard deviation. ${}^*P_{\rm value}{<}0.05;$ ${}^*P_{\rm value}{>}0.05$

Table 3: Total Zn (μ g/g dry weight) uptake by roots, stems, and leaves of sunflower (*Helianthus annuus* L.) in a plant sample that was irrigated with tap water and 2,4, 6 dS/m NaCl solution, 60 days after planting; at harvest

| | No NaCl | W | With NaCl, dS/m | | |
|----------|---------|-------|-----------------|-----------|--|
| | 0 | 2 | 4 | 6 | |
| Root* | 241 ± | 232 ± | 219 ± | 147 ± | |
| | 10.3 | 52.0 | 63.9 | 39.7 | |
| Stem* | 624 ± | 521 ± | $501 \pm$ | 407 ± | |
| | 52.8 | 178.7 | 133.4 | 118 | |
| Leaves** | 229 ± | 154 ± | 137 ± | $106 \pm$ | |
| | 79.0 | 67.0 | 20 | 51.4 | |

Data are the means of three replicates \pm standard deviation. $*{\it P}_{\rm value}{\rm >}0.05;$ $**{\it P}_{\rm value}{\rm <}0.05$



Figure 2: Variation in the dry weight of the stems ofsunflowers in three replicates under different NaCl levels $(0, 2, 4, and 6 dS m^{-1})$ after 60 DAS. The data are themean±SD (n=3); $P_{value} < 0.05$

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Figure 3: Variation in the dry weight of the leaves ofsunflowers in three replicates under different NaCl levels(0, 2, 4, and 6 dS m⁻¹) after 60 DAS. The data are themean±SD (n=3); $P_{\rm value} < 0.05$



Figure 5: Variation levels in the Cd mobility in soil, in three replicates, under different NaCl levels (0, 2, 4, and 6 dS m⁻¹) after 60 DAS. The data are the mean±SD (n=3); P_{value} >0.05

zinc in the different EC levels ($P_{value} > 0.05$).

DISCUSSION

Soil properties and total content

As shown in Table 1 the texture of the soil was Sandy Loam., and due to the vicinity of the local Zn-mine, high contents of Zn and Cd were found in the raw soil. On account of Low soil pH is a main reason help the uptake of heavy metals.^[30]

Metal accumulation in plants

Cadmium uptake by the plants

The results in Table 2 reveal that Cd uptake rises as the salinity level increases. The obtained results in the current research are in good agreement with the previous ones reported by Ozkutlu,^[19] who reported that with rising NaCl, the Cd concentration increased in flag leaves. The study by Khoshgoftar^[31] showed



Figure 4: Variation in the heights of sunflowers in three replicates under different NaCl levels (0, 2, 4, and 6 dS m⁻¹) after 60 DAS. The data are the mean±SD (n=3); $P_{value} < 0.05$



Figure 6: Variation levels in the Zn mobility in soil, in three replicates, under different NaCl levels (0, 2, 4, and 6 dS m⁻¹) after 60 DAS. The data are the mean \pm SD (n=3); $P_{value} > 0.05$

that with increasing salinity, the Cd concentration in the grain shoots increased proportionally with the level of NaCl. With an enhanced NaCl level in irrigation water, the Cl- concentration in the soil and the Cd uptake by plants increased. The Cl- ion formed complex ions with Cd to form CdCl+, CdCl,-, and CaCl₄. The application of Cl⁻ might dissolve the adsorbed soil Cd to form complex ions. It was assumed that the Cd dissolved by Cl- was absorbed by sunflowers and moved to the leaves through the xylem, in the form of complex ions, and accumulated there.^[20] The results of this experiment revealed that the absorption of cadmium in plants irrigated with saline water was more than in plants irrigated with non-saline water. CdCl²⁻ⁿ complexes could dominate the Cd solution chemistry and take it up directly across the plasma membrane, thus increasing the phytoavailability of Cd to the plant and enhancing the root uptake of Cd.^[32] The main factor of Cd uptake enhancement was the formation of the Cd-Cl complex.^[33]

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Zinc uptake by the plants

CONCLUSIONS

Data regarding Zn uptake exhibited that as the salinity increased, the concentration of Zn in the plant decreased [Table 3]. The results from this investigation showed that Zn uptake by the plant decreased with increasing Cd uptake. Therefore, our findings were very much in line with the results obtained by researchers like Khoshgoftar,^[31] Ozkutlu,^[19] and Bunluesin,^[34] who expressed that increasing the absorption of cadmium, caused a decline in the absorption of zinc, in plants. There was a strong competition between these two metals for the same membrane-transporters.^[35] A study showed that the effects of Zn on plant Cd uptake relied on the concentration of Cd and Zn, plant variety, and type of plant tissue.^[34] The analysis that was done by Nan et al. showed the Cd-Zn interaction mechanism, and they concluded that the effects of the two metals were synergistic to each other in the field conditions.^[36]

Growth parameters

The data, in general, showed that the dry weight of plants and the plant height of sunflower grown under saline water irrigation significantly decreased, as compared with those grown under non-saline water irrigation [Figures 1 to 4]. The results in the present study were in agreement with those reported by Mohamedin et al.,^[37] who stated that salinity stress caused a significant reduction in the growth parameters studied and also the macro- and micronutrient concentrations in plants. Figures 1-4 reveal a considerable decrease in the dry weight of roots, stems, leaves, and heights of sunflower, under different NaCl levels. This was because of the effects of salinity on the reduction of water absorption and metabolic activities of the plants. The presence of high concentrations of the metals in the plant tissues and high salt concentrations negatively affected the plant health and growth reduction.^[38]

Heavy metals in the soil

Cadmium mobility in the soil

Although ANOVA did not show any significant effect on the EC of cadmium mobility in the soil, the result pertaining to the Cd concentration in the soil, after 60 DAS, showed that with NaCl irrigation, the mobility of Cd in the soil was more than in the soil without NaCl irrigation [Figure 5], and by increasing the NaCl levels in the irrigation water, the mobility of heavy metals in the soil increased. Soil mobility of cadmium depended on different characteristics of the soil, such as, pH, organic matter, cation exchange capacity, clay content, chloride level, and cadmium and zinc concentrations.^[39] Saline water enhanced the mobility of heavy metals.^[40]

Zinc mobility in the soil

With increasing EC levels, the metal mobility in the soil increased However, ANOVA did not show any significant effect of the EC levels on Zinc mobility in the soil [Figure 6].

By application of NaCl in the shoots, the Cd concentration in this zone of the plants was increased. On account of the high mobility of Cd with NaCl, the results presented in this article showed that, remobilization of the Cd deposited in the stems or other plant tissues were promoted in the presence of Cl, by the formation of mobile CdCl_n²⁻ⁿ complexes. On the other hand, our data indicated that irrigation of sunflower with saline water containing high NaCl concentrations could not only mobilize Cd from the soil, but also increase the translocation of Cd from the roots to the shoots. The effect of NaCl on the other hand was significant, and proportionally decreased Zn accumulation in the shoots.

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