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Application of sheep manure and potassium fertilizer to contaminated soil and its effect on zinc, cadmium and lead accumulation by alfalfa plants



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ABSTRACT

In Jebel Ressas mining area (Southern of Tunisia), the dispersion of particles that contain Pb, Zn and Cd results in the contamination of the surrounding agricultural soils. These soils have high concentrations of Pb (970 mg kg^{-1}), Zn (9641 mg kg^{-1}) and Cd (53 mg kg^{-1}). This glasshouse study examined the effect of application of fertilizers, i.e., organic fertilizer as local sheep manure and inorganic fertilizer as potassium chloride (KCl), on the growth, uptake and translocation of Cd, Pb, and Zn of alfalfa (*Medicago sativa* L.) grown on a contaminated soil. Obtained results showed that alfalfa could tolerate high Cd, Pb, and Zn concentrations in soil and had very good growth performance. Regarding to biomass generation it was observed, in every case, that plant growth is not affected in the treated soil compared with blanks sown in an untreated control soil; improvement ranged from 80% for the KCl to 97% for sheep manure. Application of sheep manure increased electrical conductivity and reduced DTPA-extractable metal concentrations in the soils. But KCl fertilizer favored their accumulation in plants. So, KCl could be a useful amendment for phytoextraction of metals by accumulator species, while sheep manure can be very useful for phytostabilisation.

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1. Introduction

Mining activity contributes significantly to metal contamination due to the discharge and the natural spread of mine wastes by wind, water and rainfall into adjacent agriculture soils, food crops and water ecosystems [1]. These heavy metals accumulate in soils and vegetation, which is a health risk to humans and animals, plant growth and soil microbial activity [2,3].

Numerous studies have pointed to phytoremediation as an alternative, environmental friendly method for remediation of polluted soils [2,4,5]. These technologies are based on the use of plants, with agronomical practices for reclamation of soils. The combination of tolerant plants adapted to edaphoclimatic conditions, together with soil amendments to improve soil properties has been used for reclamation of polluted soils. Among the different alternatives for soil phytoremediation, two stand out: phytostabilization and

phytoextraction. Phytostabilization denotes the use of plants to stabilize pollutants in the soil, whereas phytoextraction is a technique which lies in using plants to remove pollutants. These pollutants are taken up and accumulated in plant tissues. Then, the above-ground plant parts are harvested and, in this way, the pollutants are removed [6]. Efficiency of phytoextraction is, however, limited by the low mobility and bioavailability of some heavy metals (especially Pb) in polluted soils [7]. Thus, a large number of heavy metal phytoextraction studies have focused on the use of fast growing crops (e.g., alfalfa, *Helianthus annuus* L., *Zea mays*, tomatoes) with high biomass yields combined with the enhancement of heavy metal mobility and bioavailability through addition of different kinds of amendments [8,9]. Miller et al. [10] reported that alfalfa had ability of accumulating Cd in soils receiving high dosage of sewage sludge (equivalent to $4.6 \text{ kg Cd hm}^{-2}$).

Since contaminated soils with heavy metals are often characterized by a low content of organic matter, low levels of nutrients (nitrogen, potassium and phosphorus) and other physical anomalies [11], the addition of various amendments such as organic materials or inorganic fertilizer is required for plant growth. The utilization of amendments is an effective approach to increase pH, stabilize metals, form water stable aggregates, augment microbial

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life, and supply organic matter and nutrients that are critical to establish new vegetation. The addition of amendments such as fly ash, pig manure, sewage sludge, is effective in lowering the metal toxicity of soil and provides a slow release of nutrient sources such as N, P, K to support plant growth [12,13]. The incorporation of organic amendments can stabilize metals in soil, reducing metal availability and mobility but increase their amounts in plant shoots overall because of increasing biomass [14]. The addition of fertilizers can enhance microbial growth such as rhizosphere plant growth-promoting bacteria [15]. These will enhance the availability of heavy metals to plants. In addition, fertilizers are an essential ingredient for successful restoration of mine wastes [16].

Based on the above considerations, the aim of the present work was to evaluate the effects of the use of KCl and sheep manure, on the growth and heavy metal accumulation of alfalfa (*Medicago sativa* L.) in a Cd, Pb and Zn mine soil.

2. Materials and methods

2.1. Soil sampling and analysis

Soil was collected from agricultural land adjacent to the abandoned Jebel Ressay mine located 30 km Southern of Tunisia. The study site was contaminated by lead, cadmium and zinc. The sample of soil was taken from the upper 20 cm after removing the first layer of surface soil. Soon after collection, the soil sample was carefully transferred to clean and dry self-sealing polyethylene bags for transport to laboratory. After being air-dried, soil sample was sieved through 2 mm sieve in order to remove large particles and obtain a homogeneous soil size.

Soil texture was determined following Bouyoucos methodology [17]. Soil pH and electrical conductivity (EC) were determined using 1:2.5 soil/H₂O [18]. Cation exchange capacity (CEC) was determined using the ammonium-saturation and distillation method [19]. The total Kjeldahl nitrogen was determined by the Kjeldahl method. The available phosphate and potassium were determined by the colorimetric method [20] and the FAA spectrophotometer [21], respectively. The organic matter content was determined by wet combustion with sodium dichromate and sulfuric acid without heat application [22]. Total metal concentrations were measured by acid digestion with HNO₃, HCl and HF in a microwave digester. All the heavy metal analyses were performed using an inductively coupled plasma atomic emission spectroscopy (ICP-AES). The available Pb, Zn and Cd in the soil after 4-wk equilibration were determined by the DTPA method according to Lindsay and Norvell [23]. All the analyses were carried out in triplicate.

2.2. Pot experiment

The pot experiment was performed in a greenhouse in plastic pots. Seeds of alfalfa (*M. sativa* L.) were first cultivated in peat moss for about 1–2 wk until their seedlings emerged. Then, they were transferred to pots filled with test soil. There were three treatments: control C (Cd, Pb and Zn contaminated soil without application of amendments), T1 (C + KCl solution, applied at the rate of 1 g kg⁻¹ before seeding), T2 (C + local sheep manure 10% w/w). For KCl, 1 g kg⁻¹ was added according to the average application amount of K₂O in the soil for crop system (0.34 g kg⁻¹ K₂O). All pots were watered and kept at the field capacity moisture throughout the growing season. After growing for four months, the whole plant in each pot was taken out of the soil, and the root was cleaned and cut. Subsequently, both the root and the aerial parts were drying in a 50 °C oven for 3 d, weighed on the balance, and were then ground into powders for further chemical analyses. Each treatment was replicated three times.

2.3. Remediation efficiency calculations

The Bioconcentration Factor (BCF) of each metal in plants was calculated by dividing the metal concentration (mg kg⁻¹) of the harvestable plant material (foliage) by the total metal concentration of the soil (mg kg⁻¹) [24]. Further, the Translocation Factor (TF) was calculated by dividing the total metal content in the foliage by the total metal content in roots [24]. Both factors were calculated on a dry weight basis.

2.4. Statistical analyses

Data were statistically analyzed by SPSS v.13 package using One-Way analysis of variance. Data presented are mean values of replicates ± standard deviation (n = 2 or 3 depending on the experiments).

3. Results and discussion

3.1. General characteristics of the mine soil and the sheep manure

Basic physico-chemical characteristics of the mine soil and the sheep manure are summarized in Table 1. It is seen that the mine soil contained 25% clay, 34% silt, and 41% sand. Their texture thus belongs to sandy clay loam. The mine soil was near neutral pH (7.6). High contents of Pb, Cd and Zn were found in the mine soil due to the contamination caused by the local Pb–Zn smelter. Concentrations of three metals were significantly higher than limit values in soils which are 2 mg kg⁻¹ soil for Cd, 300 mg kg⁻¹ soil for Zn and 100 mg kg⁻¹ soil for Pb [25]. The sheep manure contained much higher levels of total N, P and K (63, 74 and 14 times, respectively, higher than those in the mine soil).

3.2. Effect of treatments on aboveground biomass production

Table 2 shows the final dried biomass of alfalfa after harvesting. In general, no evident symptoms of metal toxicity were observed during the experiments. The total dry mass of alfalfa was affected by soil amendments, decreasing in the order: T2 (C + local sheep manure 10% w/w) > T1 (C + KCl solution, applied at the rate of 1 g kg⁻¹ before seeding) > C (Cd, Pb and Zn contaminated soil without application of amendments) (Table 2). These results showed that the application of KCl or sheep manure had beneficial effects on the plant growth in contaminated soil. Analysis of sheep manure in this study showed 42 times more organic matter, 63, 74 and 14 times more N, P and K than in the contaminated soil, respectively. These could enhance growth in alfalfa. Sandalio et al.

Table 1

Some physicochemical properties of mine soil and sheep manure used in the pot experiment (mean n = 3; ±sd).

Parameter	C	Sheep manure
Clay (< 0.002 mm), %	25 ± 0.8	
Silt (0.002–0.05 mm), %	34 ± 1.1	
Sand (0.05–2 mm), %	41 ± 1.3	
Organic matter, %	0.86 ± 0.1	36.2 ± 0.7
pH (H ₂ O)	7.6	7.9
CEC, meq 100 g ⁻¹	10.6 ± 0.23	14.6 ± 0.61
Total Kjeldahl N, g kg ⁻¹	0.31 ± 0.01	19.6 ± 0.19
Total P, g kg ⁻¹	0.36 ± 0.02	26.7 ± 0.14
Total K, g kg ⁻¹	0.55 ± 0.02	7.7 ± 0.43
Total Pb, mg kg ⁻¹	970 ± 16	–
Total Cd, mg kg ⁻¹	53 ± 4.6	–
Total Zn, mg kg ⁻¹	9641 ± 58	0.7 ± 0.03

C: control.

(–) not detected.

Table 2

Average biomass of plants and total heavy metals concentrations in roots and aerial parts of plants under various soil conditions.

Treatment	Biomass (g) ^a	Zn (mg kg ⁻¹)			Cd			Pb		
		Roots	Aerial parts	Total	Roots	Aerial parts	Total	Roots	Aerial parts	Total
C	8.1 ± 0.5	213.9 ± 8.2	285.3 ± 7.9	499.2 ± 8.0	3.3 ± 0.2	8.3 ± 0.7	11.6 ± 0.4	14.1 ± 0.3	45.3 ± 0.7	59.3 ± 0.5
T1	14.6 ± 1.8	281.6 ± 9.4	388.8 ± 8.8	600.4 ± 9.1	3.4 ± 0.4	9.7 ± 0.5	13.1 ± 0.4	28.1 ± 0.6	172.2 ± 2.4	200.4 ± 1.5
T2	16 ± 1.8	184 ± 6.5	229.6 ± 7.1	413.6 ± 6.8	1.2 ± 0.2	3.7 ± 0.3	4.8 ± 0.3	6.9 ± 0.2	19.7 ± 0.4	26.6 ± 0.3

^a This is the averaged dry mass of a whole plant, including its roots and aerial parts, after growing for 120 d. C: control, T1: C + KCl solution, T2: C + local sheep manure.

[26] reported that K element enhanced sunflower resistance to heavy metals in the Cd and Zn-contaminated soil by reducing the damage to membranes and proteins in plant. Tanhan et al. [27] also found the similar results for *Chromolaena odorata* seeds. Rotkittikhun et al. [28] reported the improved growth of *Vetiveria zizanioides* grown in lead contaminated soil with pig manure.

3.3. Heavy metal concentrations in plant tissues

The total concentrations of Pb, Zn, and Cd accumulated in the entire plant tissues of plants growing in the controlled soils (C) and in two test soils (T1 and T2) after four months are shown in Table 2. In general, plants in KCl amended soils can accumulate higher concentrations of metals than plants in the control soils. Zhao et al. [29] reported that addition of K₂SO₄ and KCl significantly increased Cd concentrations in spring wheat. Plants grown in sheep manure-treated soil showed significantly lower concentrations of Pb, Zn and Cd compared to the control (Table 2).

Whether accumulated metals are translocated to the aerial parts or remained in the roots of the plants is also a subject of importance [30]. The Pb, Zn and Cd concentrations in alfalfa plants growing in the KCl or sheep manure amended soils are presented in Table 2. From observing data in Table 2, it is evident that the addition of soil amendment to polluted soil significantly affects the accumulation of heavy metals in plants tissues. The concentrations of Pb, Zn, and Cd were higher in the aerial parts as compared to the roots (Table 2). This showed that Pb, Zn, and Cd elements were transferred easily from root to the aboveground part of alfalfa. Compared with the control, KCl fertilizer markedly increased Pb, Zn and Cd concentrations in the different tissues of alfalfa. The incorporation of sheep manure was able to induce a reduction on metal accumulation in aerial parts and roots of tested plants (Table 2). There were reductions of 55% in Cd, 57% in Pb and 20% in Zn concentrations in the aerial parts of alfalfa grown in sheep manure-treated soil compared to those grown in control soil (Table 2). The concentrations of heavy metals in the roots of alfalfa were reduced by 62% in Cd, 51% in Pb and 14% in Zn in sheep manure-treated soil as compared to the plants grown in the control soil. Singh et al. [30] have reported that the concentrations of heavy metals in palak were reduced by 12% for Cd, 17% for Cu, 9% for Ni, 14% in Pb, 9% in Zn, 4% in Mn and 14% in Cr in farmyard manure treated soil as compared to the plants grown in the control soil.

The application of sheep manure and KCl to the contaminated soil resulted in an increase in the organic matter and the EC value of

soil (Table 3). Sheep manure raised also the soil pH. These modifications of soil properties affected the concentrations of DTPA extractable metals. Sheep manure reduced DTPA-extractable Pb, Cd and Zn concentrations in soil (up to 34% reduction of three metals in comparison with non amended soils). This may be due to chelation, complexation, and adsorption between metals in soils and the organic matter present in sheep manure and also the dilution effect when they are mixed with soils [13,31]. Pérez-Esteban et al. [32] also reported that the application of sheep-horse manure reduced Cu and Zn availability by metal re-distribution from labile to less available soil fractions especially to the organic fraction in the case of Cu and to the Fe–Mn oxides fraction in the case of Zn.

Soil treated with KCl showed an increase in the concentrations of DTPA extractable metals (up to 33% increase of Pb, 70% increase of Cd and 67% increase in Zn). Zhao et al. [29] also showed that, anions Cl⁻ and SO₄²⁻ increase Cd uptake by spring wheat. Grant et al. [33] considered that the effect of K fertilizers may be due more to the accompanying anions of the salt. Chloride is well known for forming relatively stable complexes with Cd as CdCl⁺ and CdCl₂⁰, which are available to plants, almost equivalent to Cd²⁺ [34,35].

3.4. Bioconcentration factor and translocation ratio

The BCF and TF values of metals are shown in Fig. 1. The BCF is a common index used to estimate plants ability to pump heavy metals from the substrate and to compare species for phytoextraction potentials. Under our experimental conditions, BCF values are lower than 1 for the two treatments and for the three metals (Fig. 1a), thus indicating the limited ability of alfalfa plant to extract Pb, Zn and Cd from this soil. The BCF is reported to decrease with increasing metal concentration in soil [36], and low values are considered normal when plants are grown on polluted soils. Compared with the control, sheep manure decreased the BCF of Pb, Cd and Zn. This was consistent with the result of Singh et al. [30] and Hao et al. [37] that the lower BCF was correlated with the lower availability of soil heavy metals due to manure application. Contrary to that, following the KCl addition the Pb, Zn and Cd BCFs were increased 4.25, 1.34 and 1.2 folds, respectively compared to the control treatment. Zhao et al. [36] also reported that potassium fertilizers application resulted in the increase of Cd extraction capacity of spring wheat due to increase of plant uptake.

Metal translocation indicates the ability of amendments to affect metal transfer from roots to shoots and is expressed as the ratio of metal in the shoots to that in the roots. In the present study, values of TF were more than 1 for all the heavy metals, suggesting that heavy metals are readily transported from the root to shoot (Fig. 1b). Both sheep manure and K fertilizer increased Pb, Cd and Zn translocations from root to aboveground part of alfalfa compared to the control.

4. Conclusions

Based on the findings of this study, the application of sheep manure and KCl to the heavy metals mine soil could increase

Table 3

pH, EC, total and DTPA extractable Pb, Zn and Cd (mean ± sd, n = 3) in the different treatments before plant growth experiment.

Treatment	pH	EC (dS m ⁻¹)	Pb DTPA (mg kg ⁻¹)	Cd DTPA (mg kg ⁻¹)	Zn DTPA (mg kg ⁻¹)
C	7.6	0.19 ± 0.01	29.1 ± 1.8	3.4 ± 0.0	290 ± 7
T1	7.7	0.27 ± 0.02	38.8 ± 2.1	5.8 ± 0.0	483 ± 9
T2	7.8	0.46 ± 0.03	19.7 ± 1.3	2.2 ± 0.0	193 ± 6

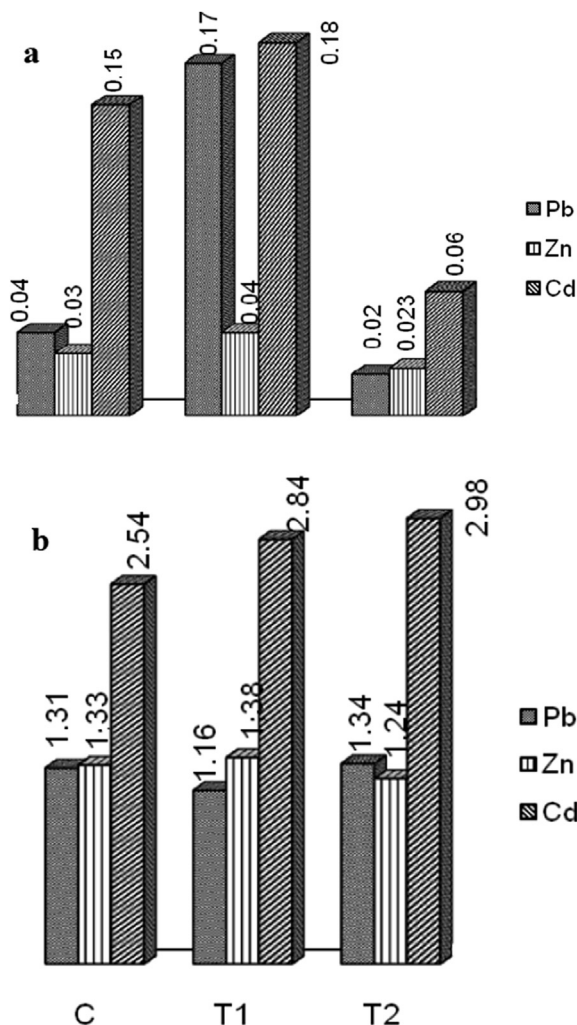


Fig. 1. (a) Bioconcentration factor (BCF) and (b) Translocation factor (TF) values of heavy metals in alfalfa under different treatments.

nutrients for plant growth. Data obtained also show that the applications of sheep manure and inorganic fertilizer affects the concentration of heavy metals found in plants. In particular, sheep manure decreased Pb, Cd and Zn concentrations in plant tissues. On the contrary, KCl markedly increased alfalfa heavy metals concentration. Additionally, alfalfa plants, which grown on mine soils could not be feasible for the phyto-extraction of Cd, Pb and Zn because BCF values were lower than 1. Sheep manure was effective to be used in the immobilization of metals, in this type of mine contaminated soil. Under these conditions the sheep manure amendment could reduce metal toxicity and metal uptake by plants with a low risk of leaching into groundwater. Efficiency of sheep manure on the immobilization of heavy metals should be tested and evaluated in polluted soils under field conditions in the future.

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