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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.

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 afalfa had ability of accumulating Cd in soils receiving high dosage of sewage sludge (equivalent to 4.6 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 Ressas 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/H_2O [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 HNO_3^-, 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 K_2O in the soil for crop system (0.34 g kg^{-1} K_2O). 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_2O)	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.