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Bioaccumulation of Heavy Metals from Soil into Alfalfa.

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ABSTRACT

In the present study, Cd, Pb and Zn uptake alfalfa grown in contaminated soils with heavy metals amended with zeolite was studied. For this purpose, a greenhouse experiment was conducted with soils from three different regions of Thessaly (industrial area, rural area amended with sewage sludge and non-polluted area). In all pots zeolite was added at two different levels (0 and 1% zeolite / kg soil), planted with alfalfa seeds. The plants were grown for 90 days from germination. During this time, became three cuttings of alfalfa, at 30 days each. The morphological characteristics of plants were identified. The total concentration of Cd, Pd and Zn in the above ground plants part and the available concentration of metals in soil were determined using atomic absorption spectrophotometry. Furthermore, the bioaccumulation factor for the accumulation of heavy metals in the tissues of plants, was determined. The concentration of heavy metals in the tissues of the first and third cutting plants, followed the order: Zn> Cd> Pb, while in the case of the second cutting plants the ranking of Cd and Pb was reversed. The higher concentration of heavy metals was found in the plants, which grown in soil from industrial area. Plants which were developed in soils with zeolite, had the lowest concentration of metals studied. Also, the available concentration of Zn in soil was reduced with zeolite. The bioaccumulation factor of alfalfa first, second and third cutting, followed the order: Zn> Cd> Pb.

Keywords: cadmium, lead, zinc, alfalfa, zeolite, bioaccumulation factor

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INTRODUCTION

Soil pollution with heavy metals has become a global problem, threatening besides the environment, human health [1, 2]. Abnormally high concentrations of heavy metals observed in the soil, mainly due to human activities related to the industrial development [3] and application of sewage sludge to agricultural land [4].

Various remediation methods of soil pollution with heavy metals may be applied [5], but most of them have proven to be costly [6]. The latter can be eliminated by phytoremediation and / or using natural and inexpensive adsorbent materials such as zeolite.

The phytoremediation has been found at least 40% less expensive than other in situ and ex situ remedial approaches [7]. It is a method easy to apply and environmentally friendly as it used plants to extract and transfer metals to their harvestable parts (phytoextraction) with the aim to reduce the concentration of metals in contaminated soils to regulatory levels [8]. There are plant species tolerant in metals and others (hyperaccumulators) are characterized by their ability to accumulate high quantities of metals in their tissues (hyperaccumulators) [9]. Hyperaccumulators are plants that achieve that achieve a plant-to-soil metal-concentration ratio (bioaccumulation factor) greater than one. This ability of the plant may vary from metal to metal and plant to plant [10].

Various techniques have been used in combination with phytoremediation, in order to increase the effectiveness of soil remediation [11, 12]. Zeolite use can reduce significantly the availability of Cd, Pb and Zn in soil, through the adsorption process [13, 14], thereby limiting the concentration of metals extracted from soil through plants in a phytoremediation program.

The aim of this study was to investigate the suitability of alfalfa plants in combination with zeolite incorporation in soil to reduce cadmium (Cd), lead (Pb) and zinc (Zn), in an economic and environmentally friendly remediation method.

MATERIALS AND METHODS

Selection of Soils

The studied soils were obtained from an industrial area and a rural area amended with sewage sludge, of Thessaly, central Greece. A non-polluted area in Larisa, central Greece, was chosen to obtaining soil, which was used as a control for comparison purposes. Soils were sampled from the 0- to 30-cm layer, air dried and passed through a 2-mm sieve. All soil samples were analyzed for their physicochemical properties and their content of Cd, Pb, Zn (Table 1).

Greenhouse Pot Experiment and Crop Growth

A greenhouse pot experiment was conducted in five replicates, as follows: PVC pots were filled with 2 kg soil form industrial area (S1), soil from rural area in which sewage sludge applied (S2) or soil from non-polluted area (S3). In all pots zeolite was added at two different levels (0 and 1% zeolite / kg soil), and plant seeds of alfalfa. The experimental design was complete randomized blocks with six treatments (Table 2). The pots were irrigated with deionized water and the moisture content was kept at 60% of field capacity. The plants were grown for 90 days from germination. During this time, became two consecutive cuttings of alfalfa, at 30 days each. After 90 days, the whole plants and soils were removed from the pots, and the aerial parts were separated from the roots and prepared for analyses. Soil samples were air dried, ground handily and selected for analyses.

Table 1: Properties of the studied soils.

Soil Properties	Study areas		
	S1	S2	S3
Sand (%)	49	33	30
Silt (%)	37	42	44
Clay (%)	17	25	26
pH, 1:1 water	8.4	7.9	7.7
TCCE (%)	2.2	1.32	0.9
OM (%)	1.54	1.27	1.1
CEC (cmol(+)kg^{-1})	16.5	35.6	26.6
Total N (Kjeldahl) (g/100g)	0.1	0.09	0.13
Olsen P (mg kg^{-1})	8.7	38.2	8.7
Exch. K (cmol(+)kg^{-1})	0.56	0.55	1.8
Total heavy metals (mg kg−1)			
Cd	1.3	1.6	ND*
Pb	17.6	18.8	14.00
Zn	99.8	63.7	54.00

Notes: pH (1:1 H_2O) in soil; OM, organic matter; CEC, cation exchange capacity (cmol(+) kg^{-1}); TCCE, total CaCO3 equivalent.
*ND: Not Detected

Table 2: Treatments of the pot experiment.

Code	Treatment
S1. A.	2 kg soil from Industrial area plus Alfalfa
S1. Z. A.	2 kg soil from Industrial area plus Zeolite (1% zeolite / kg soil) plus Alfalfa
S2. A.	2 kg soil from Area in which sewage sludge applied plus Alfalfa
S2. Z. A.	2 kg soil from Area in which sewage sludge applied plus Zeolite (1% zeolite / kg soil) plus Alfalfa
S3. A.	2 kg soil from non-polluted area plus Alfalfa
S3. Z. A.	2 kg soil from non-polluted area plus Zeolite (1% zeolite / kg soil) plus Alfalfa

Preparation and Analyses of Plant Samples

After harvesting the aerial parts of plants separation from the roots. All the alfalfa plants (1st, 2nd and 3rd cutting) were washed successively with distilled water and 0.001 M hydrochloric acid (HCl). Afterward, the morphological characteristics of plants such as height and dry weight were identified and the total Cd, Pd and Zn were determined, by using atomic absorption. Specifically, the above ground plants part was dried in an oven at 70 ◦C for 48 h and the dry weight of plants was determined. After determining the dry weight, the plants were ground in a stainless steel mill. One g of the aboveground biomass of each sample was dry ashed in a muffle at 450 ◦C for 5 h and diluted with 50 mL of 20% hydrochloric acid solution. The concentrations of the studied trace elements (Cd, Pb, Zn) in the solutions were determined by atomic absorption spectrometry (Varian, SpectrAA-400 Plus, Varian Inc., Australia) [15]. In addition, the bioaccumulation factor (BF) for the accumulation of heavy metals in the tissue plants, was determined, as follows:

$$BF = \frac{concentration\ of\ metal\ in\ plant}{concentration\ of\ metal\ in\ soil}$$ [10].

Preparation and Analyses of Soil

After harvesting the plants, soil samples were air dried ground to pass through a 2-mm sieve and analyzed for trace elements availability. A soil subsample was separated from each sample and the available trace elements were extracted by using diethylenetriaminepenta acetic acid (DTPA) [16]. The statistical analysis was carried out by applying the method of analysis of variance (ANOVA, F-test), for a significance level of 95%. Significantly different treatments are labeled with different lowercase letters while nonsignificant differences share the same lowercase letter.

RESULTS AND DISCUSSION

Morphological characteristics of plants

The height of first cutting alfalfa plants ranged from 12 to 17.28 cm (Table 3). The greatest height had the plants which grown in soil from non-polluted area (S3) and the lowest height had the plants which grown in soil with zeolite from industrial area. Significant increase was recorded the height of alfalfa in the second and third cutting. Also, significant was the positive effect of zeolite at the height of plants which grown in contaminated with heavy metals soils from S1 and S2 study area, regardless cutting. The dry weight of first cutting alfalfa was ca 0.5 g, regardless of treatment and almost doubled in the second and third cutting (Table 3). Also, the dry weight of the first cutting plants from S1, S2 and S3 study area, respectively, increased by applying the zeolite in soil.

The comparison of results on the morphological characteristics of alfalfa (Table 3) shows that the growth of plants (height of first, second and third alfalfa cutting and shoot dry biomass of third alfalfa cutting) was affected negatively by the presence of Cd, Pb and Zn in soil. The reduction of plant growth, caused by heavy metals, is a consequence of the photosynthetic mechanism inhibition and of cell division, as well as of the destruction of certain enzymes related to growth [17]. Similar studies reached at the same result [18, 19]. However, the toxic effect of metals in plants growth was limited by addition of zeolite in soil. Similar study in the past, reached at the same result [20]. Specifically, the presence of zeolite in cadmium polluted soil reduced the negative effect of metal on soybean development. This is due to conversion part of available concentration of cadmium to unavailable for uptake by plants, after amending the soil with zeolite [21].

Table 3: Height and dry weight of the first, second and third cutting of alfalfa plants.

Treatment	Height (cm)			Dry weight (g)		
	1st cutting	2nd cutting	3rd cutting	1st cutting	2nd cutting	3rd cutting
S1. A.	12.00a*	16.60a	17.73a	0.47a	0.78a	0.80a
S1. Z. A.	14.35b	20.58bc	20.85ab	0.51a	0.89a	0.94ab
S2. A.	13.05ab	18.03ab	18.40a	0.46a	0.80a	0.86a
S2. Z. A.	14.00b	21.75cd	22.28bc	0.55a	0.95a	0.91ab
S3. A.	16.38c	23.63cd	24.88c	0.48a	0.93a	0.99ab
S3. Z. A.	17.28c	24.35d	25.23c	0.54a	1.20a	1.28b

*Different letters at each column denote significant different means (F-test, $P < 0.05$); data presented are mean values.

Metal contents in the above ground plants part

Very high was the concentration of Zn in the above-ground part of the first cutting of alfalfa plants, exceeding the limit of 100 ppm, which according to reference [25], should not exceed the plants intended for consumption as animal food (Table 4). Also, higher than the 1 ppm limit for Cd in cattle sheep and goats, was found to be the concentration of Cd in the above-ground part of the first cutting of alfalfa plants, which were grown in the soil from S1 and S2 study area [23]. Significantly lower was the concentration of Cd, in the second and third cutting of plants. Also, the concentration of Zn in the above-ground part of alfalfa, which was grown in soil from S1 study area, decreased by 72% from the first to the second cutting and 49% from the second to the third cutting. On the contrary, a different picture was presented by the Pb, the concentration of which in plants increased between the 1st and 2nd cutting, but eliminated in the third cutting. The lower concentration of metals in the second and third cutting plants, may be due to the effect of dilution of metals in their tissues, as the produced biomass was almost double in the second and third cutting, compared to the first cutting (Table 4). A similar result had the previous research [24], according to which, the concentration of Cd and Zn was lower in the alfalfa of the third cutting, compared to the first cutting plants, due to the difference in weight.

Table 4: Concentration of cadmium, lead and zinc in the above-ground part of the 1st, 2nd and 3rd cutting alfalfa.

Treatment	Concentration of heavy metals in alfalfa (mg / kg dry weight of plant)								
	1st cutting			2nd cutting			3rd cutting		
	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn
S1. A.	1.25c*	0.07a	238.16d	0.32b	3.16b	65.54d	0.44c	ND**	33.41f
S1. Z. A.	0.94b	0.02a	145.19c	0.27b	2.21ab	47.99c	0.25b	ND**	28.01e
S2. A.	1.29c	0.05a	124.51bc	0.30b	2.45ab	43.05c	0.30b	ND**	26.13d
S2. Z. A.	1.00b	0.02a	102.09abc	0.33b	2.30ab	16.88a	0.25b	ND**	22.27c
S3. A.	0.02a	ND **	97.00ab	0.02a	0.82ab	25.02b	0.09a	ND**	20.30b
S3. Z. A.	0.01a	ND **	71.32a	0.04a	0.31a	19.27ab	0.08a	ND**	14.76a

*Different letters at each column denote significant different means (F-test, $P < 0.05$); data presented are mean values.
** Not Detected

Generally, the concentration of heavy metals in the tissues of the first and third cutting plants, followed the order: Zn> Cd> Pb, while in the case of the second cutting plants the ranking of Cd and Pb was reversed. The plants which were grown in soil from S1 study area, presented the highest concentration, regardless the cuttings. Lower was the concentration of Cd, Pb and Zn in plants grown in soil from S2 study area. Application of zeolite in the soil, caused 39%, 18% and 27% reduction in concentration of Zn in the first cutting plants, which were grown in soil from S1, S2 and S3 study area, respectively. Also, the application of zeolite reduced the concentration of Zn in the second and third alfalfa cutting. Similar were the results of earlier studies [25, 14], according to which, the zeolite adsorbing heavy metals, reduces their availability for uptake by plants, while, at the same time, reduces the risk of contamination of the food chain with heavy metals.

The Bioaccumulation Factor for Cd, Pb and Zn of first, second and third alfalfa cutting, is presented below (Table 5).

A plant is considered hyperaccumulators of metals when characterized of BF greater than one (BF>1) [12]. On the contrary, plants which are characterized by 0.1 <BF <1, are among the "moderate" accumulators and those with 0.01 <BF <0.1 belong to the "low" accumulators [26]. Based on the above categorization and experimental results (Table 5) show that, alfalfa acts as a moderate accumulator of Cd. The BF for Zn in first cutting alfalfa was found to be greater than one as depicted in Table 5. This means that these plants are hyperaccumulators of Zn, although this capacity was moderated in the second and third cutting. Similar were the results of other researchers, who found that alfalfa accumulates a significant amount of Zn from soil [27, 7]. However, according to the results of another study [28], to be successful and economically viable the bioaccumulation, required the use of plants with bioaccumulation factor at least ten (10), as well as, a capability of producing biomass at least 20 tn / ha or different plants with BF at least 20 and biomass production at least 10 tn / ha. The BF for alfalfa, is visibly away from the above prices. Finally, the ability of alfalfa to accumulate Pb, is characterized low, making it unsuitable for efficient use in a phytoremediation program of contaminated with Pb soil.

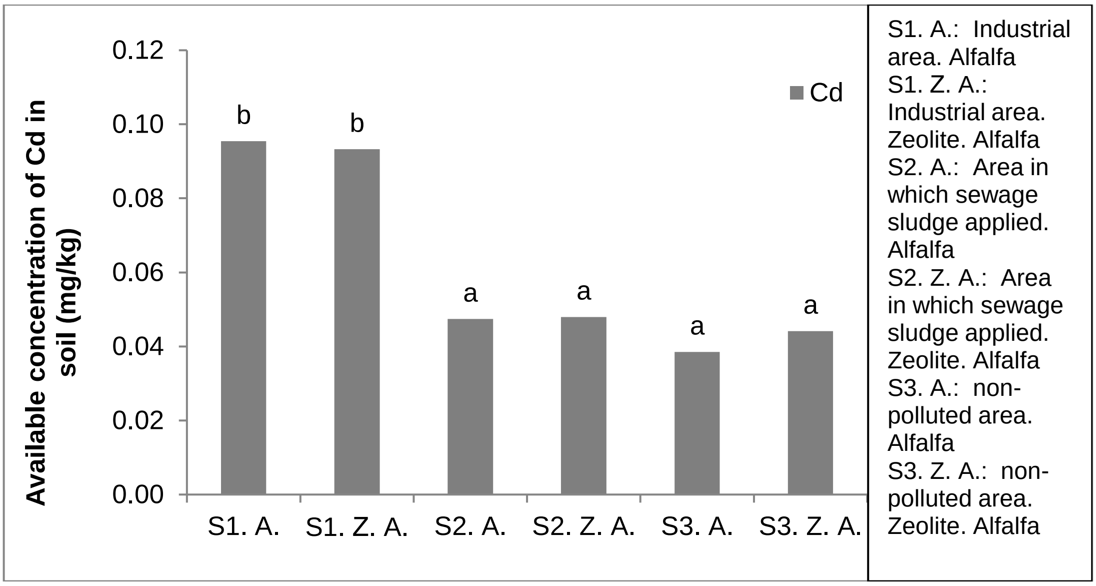
Table 5: Bioaccumulation Factor for Cd, Pb and Zn of 1st, 2nd and 3rd cutting alfalfa.

Treatment	Bioaccumulation Factor								
	1st cutting alfalfa			2nd cutting alfalfa			3rd cutting alfalfa		
	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn
S1. A.	0.96b*	0.00	2.39b	0.24a	0.18a	0.66c	0.34b	0.00	0.33b
S1. Z. A.	0.72ab	0.00	1.45a	0.20a	0.13a	0.48b	0.19a	0.00	0.28a
S2. A.	0.81ab	0.00	1.95ab	0.19a	0.13a	0.68c	0.19a	0.00	0.41c
S2. Z. A.	0.62a	0.00	1.60ab	0.21a	0.12a	0.26a	0.16a	0.00	0.35b

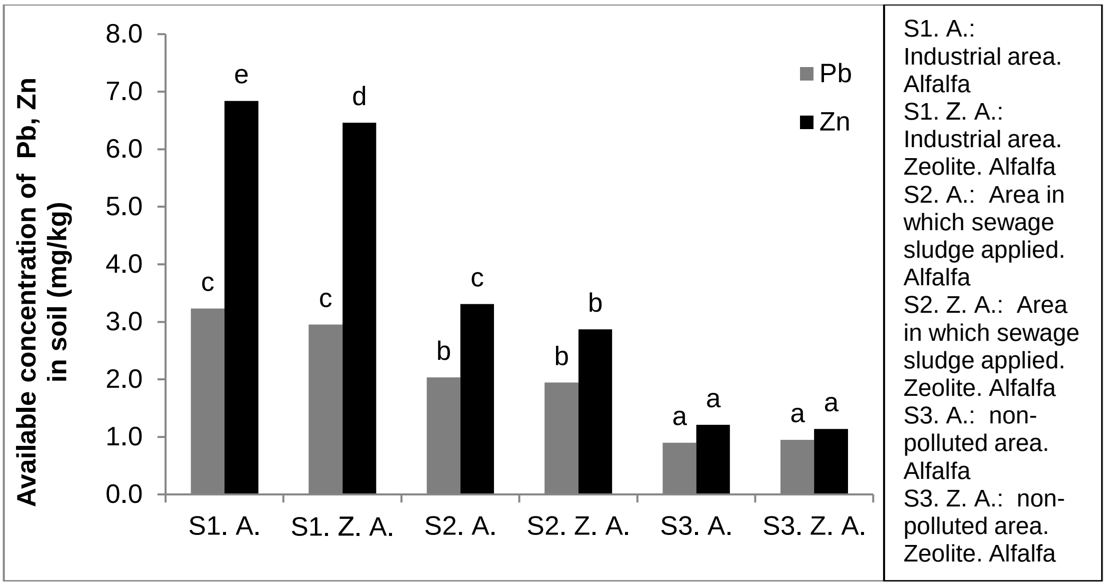
*Different letters at each column denote significant different means (F-test, $P < 0.05$); data presented are mean values.

Available concentration of metals in soil

The available concentration of study metals in the soil after harvesting the plants, followed the order: Zn> Pb> Cd, regardless of treatment (Figure 1). The availability of Cd, Pb and Zn was higher in the soil from S1 area (0.10, 3.23 and 6.84 mg Cd, Pb, Zn / kg soil, respectively) compared with the other two study areas (0.05, 2.03, 3.31 mg Cd, Pb, Zn / kg soil from S2 area and 0.04, 0.90, 1.21 mg Cd, Pb, Zn / kg soil from S3 area, respectively). The greater availability of metals in soil from S1 study area is associated with a greater concentration of metals in the tissues of plants which grown in this soil (compare Table 4 and Figure 1). The application of zeolite in soil did not reduce the availability of Cd and Pb, but significantly limited the availability of Zn in soil of the 1st and 2nd study area. The result agrees with previous study [29].



(a)



(b)

Figure 1: Available concentration of a) cadmium, b) lead and zinc, in the soil of pots, after harvesting the plants.

CONCLUSIONS

The aim of this study was to examine if alfalfa may be used for remediation the soil pollution with heavy metals. Data showed that on soils contaminated with small quantities of metals may be used alfalfa as phytoaccumulator crop for the phytoremediation of soils contaminated with Cd and Zn. Zeolite could be combined with a program of phytoremediation soil pollution resulting in reducing the decontamination time, as found that decreases the availability Zn in soil. Meanwhile, the zeolite decreasing the availability of Zn in soil, decreases the uptake of metal by plants, and contributes to the safe for human health production of agricultural products. Finally, zeolite application on the soil, positively affects to the development of alfalfa plants.

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