Bulgarian Chemical Communications, Volume 47, Special Issue D (pp. 167 - 172) 2015

Phytoremediation of cadmium, lead and zinc by *Medicago sativa L.* (alfalfa): A study of different period

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Received June 26, 2015; Revised September 10, 2015

Medicago sativa L. (alfalfa) has been reported to be extremely resistant to contaminants as well as a bioaccumulator. However, heavy metals accumulated by alfalfa in different period was less analyzed and reported. In the present experiment, the alfalfas grew for 10 days, 40 days and 80 days in different soils which were polluted by Cd, Pd, and Zn. It was found that: alfalfa could be applied for phytoremediation of soil polluted moderately by Cd, Pb, and Zn. Alfalfa could transfer heavy metals from roots to shoots in seedling period. Alfalfas had the higher biomass in maturation period. However, biomass of alfalfa was not significant difference in the same period. In different period, alfalfa showed the similar ability of accumulating Cd, Pb and Zn in roots and shoots. Compared with the shoots, the contents of Cd, Pb and Zn in the roots were higher. BCF (bioconcentration factor) values of alfalfa in Cd, Pb and Zn were higher in maturation period. Alfalfa had a better ability of accumulating Cd. TF (translocation factor) values of alfalfa were low and tended to decrease with the increasing contents of Cd, Pb and Zn in the soils.

Keywords: Heavy metals, Biomass, Bioconcentration factor, Translocation factor, *Medicago sativa L*

INTRODUCTION

Anthropogenic activities have concentrated some heavy metals in certain areas up to dangerous levels for living organisms [1]. Activities such as mining and agriculture have polluted extensive areas throughout the world [2, 3, 4, 5]. As well known, Cadmium (Cd) and lead (Pb) have long been considered as a major contamination problem, not only for working conditions but also for the environment [6, 7]. Cd and Pb were environmental pollutants mainly of anthropogenic origin [8, 9]. It was well known that zinc (Zn) in low concentrations was essential for plant growth [10, 11], nevertheless, beyond certain a threshold concentration, it became toxic for most plant species [12, 13].

Recently, there have been some studies on the ability of biomaterials or biomass to extract heavy metals from the environment, and phytoremediation has emerged as one of the alternative technologies for removing pollutants from the environment. Interest in using plants for environmental remediation was increasing due to their natural capacity to accumulate heavy metals and degrade organic compounds [14]. Phytoremediation was defined as the use of plants to remove pollutants from the environment or to render them harmless [15]. Alfalfa was also found to show highly resistant to contaminants as well as a bioaccumulator [16]. Previously, Angle and Chaney [17] reported that alfalfa has the ability to accumulate concentrations of heavy metals well above the tolerance levels of other plants. Alfalfa may be a good source of plant tissues because it has been found to tolerate heavy metals and grow well in contaminated soils [18]. Further, alfalfa was shown to be used as a potential source of biomaterials for the removal and recovery of heavy metals [19]. In this work, the accumulation of Cd, Pb, and Zn by alfalfa in different period (seedling period, vegetation period, and maturation period) was investigated.

EXPERIMENTAL

Soil sources and preparation

The soil samples were collected from the Bohai University in Jinzhou city of Liaoning Province, China. The climate style belongs to the warm temperate zone, the annual average parameters were as follows: temperature 9.2 °C, rainfall 436.7 mm. The major heavy metals in the soil were Cd, Pd, and Zn. The physical and chemical properties of the soil were shown in Table 1.

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The salts used in this study included $Cd(NO_3)_2$, $Pb(NO_3)_2$, and $Zn(NO_3)_2$. The collected soils (5 kg) were added into each pot. The salts were separately diluted with deionized water, and all solutions were adjusted to pH 7.5-7.7 with HNO_3 and NaOH before being added into pots. The urea was added into soils of the pot to keep the N at the same level. Treatments were prepared at the concentrations of (1) control, Cd 7.843 mg/kg + Pb 55.120 mg/kg + Zn 221.078 mg/kg (following as S0); (2) treatment 1, Cd 8.143 mg/kg + Pb 105.120 mg/kg + Zn 271.078 mg/kg (following as S1); (3) treatment 2, Cd 8.843 mg/kg + Pb 155.120 mg/kg + Zn 346.078 mg/kg (following as S2); (4) treatment 3, Cd 17.843 mg/kg + Pb 355.120 mg/kg + Zn 471.078 mg/kg (following as S3); (5) treatment 4, Cd 27.843 mg/kg +Pb 555.120 mg/kg + Zn 771.078 mg/kg (following as S4).

Table 1. The physicochemical properties and patterns of soils.

Parameter	Soil
pH	7.63
organic matter	5.26 %
CEC	8.64 cmol/kg
total N	0.12 %
total P	0.06 %
total K	1.13 %
Cd	7.84±2.38 mg/kg [b]
Pb	55.12±5.07 mg/kg [a]
Zn	221.08±10.55 mg/kg [a]

[a]Significant at $p < 0.01$.
[b]Significant at $p < 0.05$. n=10.

Plant culture

Seeds of alfalfa were obtained from Tai Yuan Seed Company. Seeds were surface sterilized by immersion in 2% (v/v) H_2O_2 and shaken at 144 rpm on an orbital shaker in sterile deionized water for 6 h [20]. They were then sown onto stainless plates with aseptic gauze in incubator, and the temperature and moisture were kept at 25 °C and 60%, respectively. Seedlings grew at a length of 2 cm and then 60 seedlings were transplanted to each pot in a green house. After 2 days, pots were migrated and grew under natural conditions.

Analysis

In the experiment, 10 alfalfas were harvested by clipping the shoots and pulling out the roots randomly after they have grown for 10 days (seedling period), 40 days (vegetation period), and 80 days (maturation period), respectively. After the roots and shoots were washed with deionized water and dried in an oven at 70 °C for 72 h, the dry weights were recorded by electronic balance. The roots and shoots of alfalfa were digested at 150 °C for 200 min with 10 ml mixture of $HNO_3/HClO_4$ (4:1) [21]. The digestion was accomplished using the simple setting- furnace digestion and big cuvettes. Subsequently, the volume of digested sample was adjusted to 20 ml with double deionized water and analyzed using ICP-OES.

Two bio-concentration factors, BCF (bio-concentration factor, defined as concentration ratio of plant shoot to soil) and TF (translocation factor, defined as concentration ratio of shoot to root) computed from the treatments concentrations would be used to discuss the results of this study. All the data obtained from this study were analyzed by SPSS 13.0. The Duncan multiple range test was used to determine the statistical significant ($P < 0.05$). Controls and treatments were in triplicates for analysis.

RESULTS AND DISSCUSION

Biomass of alfalfa

After 10 days, 40 days and 80 days, 10 alfalfas were dried and recorded by electronic balance (table 2). The biomass of alfalfa, grown in soil polluted by different concentration of heavy metal, was not significantly different in the same period ($P < 0.05$). Appropriate heavy metals could promote alfalfa to accumulate the biomass, but high contents of heavy metals were harmful to alfalfa. It meant the alfalfa growing has been affected under the contents of the Cd, Pb, and Zn in soil.

Bioaccumulation of Cd, Pb, and Zn by alfalfa

After 10 days, 40 days and 80 days, alfalfa could uptake Cd, Pb, and Zn from the soil. Figure 1-3 show the total mass of heavy metals in the alfalfa's shoots and roots in three periods.

Table 2. Biomass of alfalfa.

Time	S0	S1	S2	S3	S4
10 days	12.98±1.12[b]	14.57±1.39[b]	13.30±0.82[a]	13.26±1.01[a]	11.12±0.78[a]
40 days	39.64±2.99[a]	46.70±2.63[a]	41.29±1.93[a]	43.71±2.67[a]	32.06±1.48[a]
80 days	56.32±3.23[a]	68.15±3.71[a]	62.37±2.54[a]	65.22±2.48[a]	40.80±3.85[b]

Unit was mg/plant. [a]Significant at $p < 0.01$. [b]Significant at $p < 0.05$. n=10.

The contents of Cd, Pb, and Zn in alfalfa were the highest after alfalfas have grown for 80 days. Alfalfa accumulated more Cd and Zn with the increasing contents of Cd and Zn in the soils, but the contents of Cd, Pb, and Zn in alfalfa were not significant different in seedling period. Compared with the contents of Cd, Pb, and Zn in alfalfas which have grown for 80 days, 65.54%-94.58% of Zn, 63.98%-86.90% of Cd, and 60.38%-93.51% of Pb have been accumulated by them which have grown for 40 days. The contents of Cd, Pb, and Zn were higher in the roots than in the shoots, which may be it is related to both heavy metals uptake from the soils and xylem translocation from roots to shoots. The contents of Cd, Pb and Zn in alfalfa under treatment 3 level were highest in each period. It meant that Cd, Pb and Zn could be absorbed by alfalfa in soil polluted moderately. Alfalfa showed the similar ability of accumulating Cd, Pb and Zn in roots and shoots in the whole period. The accumulation of Cd, Pb, and Zn in both shoots and roots of alfalfas was increased with the increasing contents of Cd, Pb, and Zn in the soils, but the accumulation was decreasing after the contents of Cd, Pb, and Zn (especially for Pb) were achieved treatment 3 level in three periods, respectively. In general, Cd has greater toxic effects on plants than Pb and Zn. The damage to plants made by Cd could affect the growth of plants and tolerance to heavy metals much more than Pb and Zn. Thus, the content of Zn in the shoots was significantly higher than Cd and Pb, which was in accordance with the previous results found for alfalfa [22]. The content of Cd in roots of alfalfa was higher than Pb, and this result was agreed with that found for alfalfa [23]. The content of Pb in the alfalfa was low, and it was not agreed with the finding of alfalfa adsorbing metal order: Pb > Zn > Cd [24], this may be due to the pH of soil and Pb bound by soil or alfalfa, the metal binding affinities for ligand in the soils, the antagonism of ions, chemical competition for multi-metal and physicochemical properties of Pb in the soils. Bioaccumulation depends not only on the characteristics of the organism itself, but also on the characteristics of the substance and the environment factors. With increasing contents of heavy metals in the soils, alfalfa showed performance to avoid and resist.

BCF and TF of alfalfa in Cd, Pb and Zn

BCF values were studied in each sample (Figure 4-6). BCF values of alfalfa in Cd, Pb and Zn were higher while alfalfas were grown, and BCF values of roots were higher than those of shoot. Also, concentrations of heavy metals in soils could influence the abilities of phytoextraction, higher concentration maybe increase the contents of heavy metals in tissue compartments of plants [25].

The BCF values of shoots were nearly equivalent compared with roots in seedling period, since the xylem has not got formed during seedling period. After alfalfas have grown for 40 days, BCF values for Cd, Pb and Zn were higher. After alfalfas have grown for 80 days, BCF values for Cd were from 5.72 to 71.85 in shoots and from 13.75 to 571.97 in roots; BCF values for Pb were from 0.25 to 9.61 in shoots and from 0.34 to 98.25 in roots; BCF values for Zn were from 1.91 to 20.64 in shoots and from 2.75 to 43.65 in roots; the average BCF values of alfalfa for Cd, Pb, and Zn were 30.66, 3.63, and 7.47 in shoots and 250.21, 39.32 and 16.31 in roots, respectively. Values of BCF can be an index for the plant's ability to accumulate heavy metals. BCF values are different in response to concentrations, kinds of heavy metals, the accumulation ability, physiological factors of plants and environmental conditions. In this experiment, it was apparent that alfalfa had better ability of bioaccumulate Cd than Pb and Zn, the result was agreed with that found for alfalfa [20]. Alfalfa could uptake much Zn and Pb, but BCF values were not the highest, which may be due to the physiological and morphological characteristics of alfalfa, concentrations of heavy metal and conditions of environment.

The average values of TF were studied in each sample (table 3). The higher values of TF indicated that plants could move and distribute more heavy metals. The TF values of alfalfa were low, it showed that there were less Cd, Pb and Zn moved into shoots. The TF values of alfalfa tended to decrease with the increasing contents of Cd, Pb and Zn in the soils. The TF values in Zn were higher than Cd and Pb, maybe it is related to the characteristics of the alfalfa and characteristics of Cd, Pb and Zn in the soils. Since movement of heavy metal from roots to shoots was likely to occur via the xylem and to be driven by transpiration from the leaves, alfalfa could move much heavy metal from roots to shoots in seedling period. The study of heavy metals translocation and accumulation occurred predominantly via the phloem (Popelka et al., 1996). Cellular sequestration of Cd or Pb could have a large effect on the levels of free Cd or Pb in plants, thus, it can potentially influence the movement of Cd or Pb throughout the plant [20].

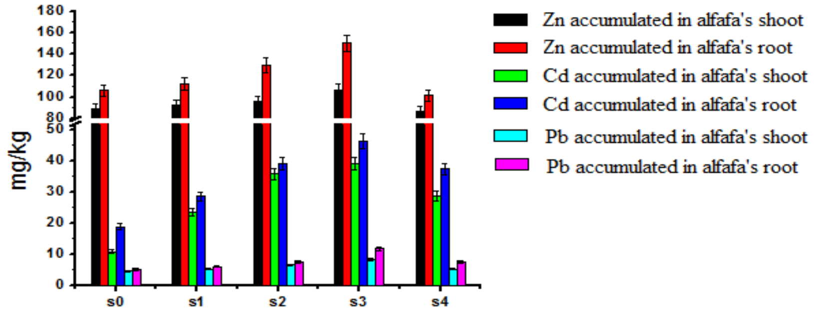

Fig. 1. Contents of Cd, Pb, and Zn accumulated in alfalfas after alfalfas have grown for 10 days. Significant at $p < 0.05$.

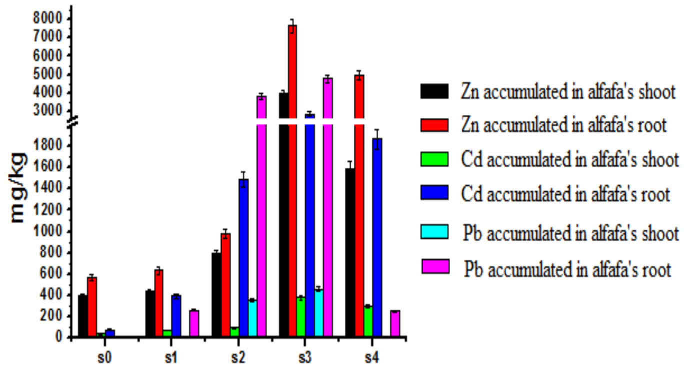

Fig. 2. Contents of Cd, Pb and Zn accumulated in alfalfas after alfalfas have grown for 40 days, Significant at $p < 0.05$.

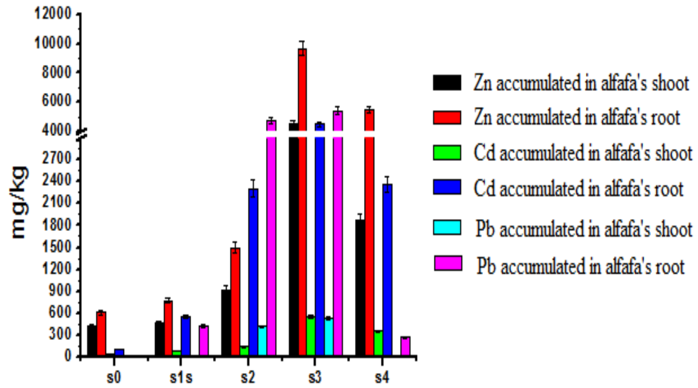

Fig. 3. Contents of Cd, Pb and Zn accumulated in alfalfas after alfalfas have grown for 80 days, Significant at $p < 0.05$.

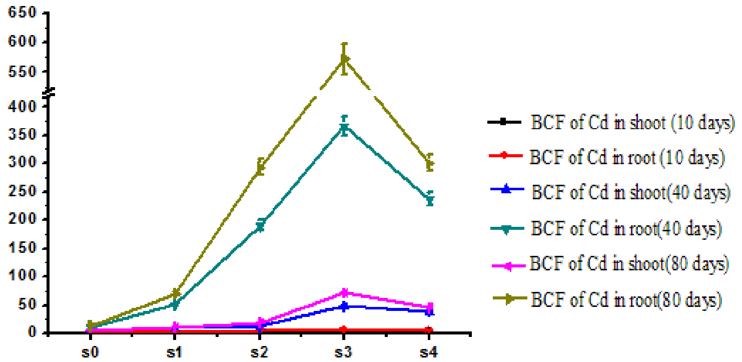

Fig. 4. Values of BCF of alfalfa for Cd. Significant at $p < 0.05$.

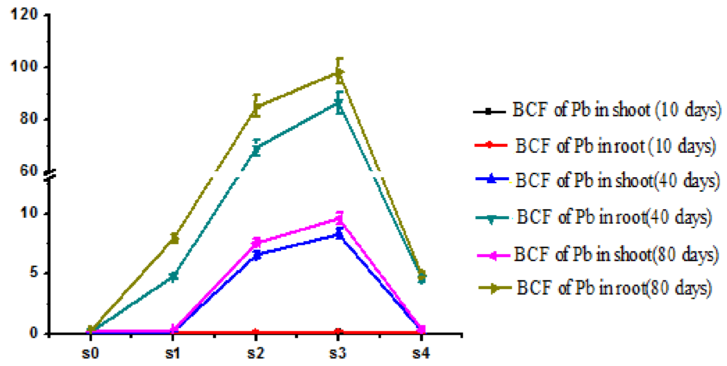

Fig. 5. Values of BCF of alfalfa for Pb. Significant at $p < 0.05$.

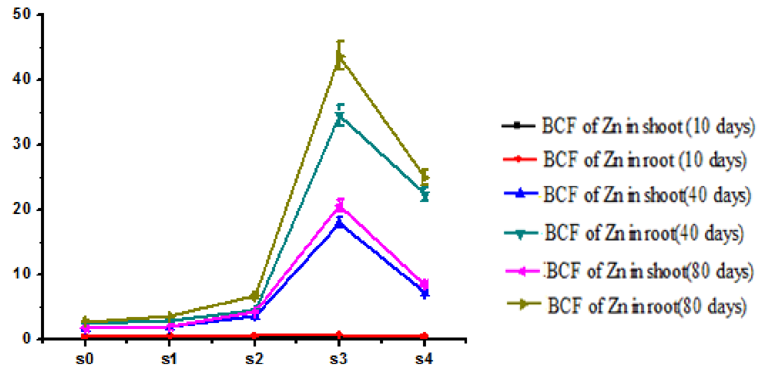

Fig. 6. Values of BCF of alfalfa for Zn, Significant at $p < 0.05$.

Table 3. The average TF values of alfalfa in Cd, Pb, and Zn.

days	Cd			Pb			Zn		
	10	40	80	10	40	80	10	40	80
S0	0.57	0.49	0.42	0.91	0.58	0.75	0.84	0.70	0.69
S1	0.82	0.18	0.15	0.87	0.06	0.05	0.83	0.69	0.61
S2	0.91	0.07	0.06	0.88	0.09	0.09	0.74	0.81	0.62
S3	0.85	0.13	0.13	0.71	0.10	0.10	0.71	0.52	0.47
S4	0.77	0.16	0.15	0.69	0.06	0.06	0.86	0.32	0.34

CONCLUSIONS

In this work, the accumulation of Cd, Pb, and Zn by alfalfa in different period was investigated. The results indicated that alfalfa had the higher biomass while alfalfa was grown. However, biomass of alfalfa, grown in soil polluted by different concentration of heavy metals, was not significantly different in the same vegetation period; the contents of Cd, Pb, and Zn in alfalfas were the highest after they have grown for 80 days. Alfalfa accumulated further Cd, Pb, and Zn with increasing contents of Cd, Pb, and Zn in the soils, but the contents of heavy metal in alfalfa were not significantly different in seedling period. Alfalfa had better ability of bioaccumulate Cd than Pb and Zn; the TF values of alfalfa were low and tended to decrease with the increasing contents of Cd, Pb, and Zn in the soils. The TF values in Zn were higher than Cd and Pb. Alfalfa could be applied for phytoremediation of soil moderately polluted by Cd, Pb, and Zn.

Acknowledgements: *The authors acknowledge the National Natural Science Foundation of People's Republic of China (51379078, 51579101, 51409103 and 51379079), Science-tech Innovation Talents in University of Henan Province (15HASTIT044) and National Key Technology Support Program (2012BAC19B03).*

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