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Original Research

Accumulation and Tolerance of Pb in Some Bioenergy Crops

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Abstract

Contamination of agricultural soil is a worldwide problem, with heavy metals being a major part of the concern. Bioenergy crop production is also a profitable phytoremediation strategy using biofuel crops for both utilization and remediation of contaminated soil. To investigate lead (Pb) accumulation and tolerance of three different energy crop cultivars, three-week-old healthy seedlings were grown in Hoagland solution supplemented with five different concentrations of Pb 0, 25, 50, 100, and 150 mg/kg. At the end of 30 days, Pb content and translocation, tolerance index, bioconcentration factor, and growth parameters of the plants were evaluated in the study. Results showed that increasing Pb concentrations did not affected the growth and development of Sunburst (*Panicum virgatum* L.) and Dinçer (*Carthamus tinctorius* L.) cultivars. The highest Pb contents were also found in roots and shoots of Sunburst and Tarsan-1018 (*Helianthus annuus* L.) cultivars. Dinçer cultivar has a high ability to transfer Pb from root to shoot when compared to others. These results suggest that these cultivars may be good candidates for remediation of Pb-contaminated areas for use in biofuel production.

Keywords: **b**ioenergy crops, heavy metal, lead, phytoremediation

Introduction

Among alternative renewable energy sources, biomass seems to have taken the lead as it competes most favorably in all countries of the world and has a dominant role [1-7]. Besides erosion, desertification, and depletion problems, bringing up of these species as biomass energy sources may also produce integrated solutions for energy demand. The energy plants, as a clean and environment friendly source, have the potential to supply global energy needs.

Soils contaminated with metal and metalloid elements pose a major environmental and human health risk [8]. Increasing the production of energy plants will serve both global energy need and the depuration of soil contamination. For this purpose, plant-based technologies for cleaning contaminated soils can be used, and this technology is called phytoremediation, which covers a wide range of pollutants such as inorganic chemicals, including heavy metals [9], metalloids [10], including persistent organic pollutants [11], and radioactive elements [12-13].

Plants have a remarkable position for depuration of soil and water due to their different physiological, genetic, and biochemical characteristics. Researchers are also exploring the use phytoremediation biomass as a renewable energy source [14]. The main characteristics of plants used for phytoremediation are [15]:

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- Ability to accumulate heavy metals in aboveground parts.
- Tolerance to high metal concentrations in soils.
- Fast growth and high accumulating biomass.
- Easy to grow as an agricultural crop and easily harvestable.

Switchgrass is a warm-season perennial C4 grass native to North American tallgrass prairies and a member of the subfamily Panicoideae of the Poaceae family [16]. Switchgrass has a large root system and adapts to a wide range of ecosystems as well as marginal lands [17-20]. It has been reported that switchgrass has the ability to accumulate some heavy metals [21]. Annual sunflower and safflower have fast growth rates and large production areas as oil crops; they also have been used in bioenergy production and have the potential to be accumulator crops [22-23].

Among heavy metals, lead (Pb) is one of the most hazardous pollutants of the environment, and Pb pollution in air, water, and agricultural soil is an ecological concern due to its impact on human health and the environment [24]. Highly contaminated soil does not promote plant development. On the other hand, low- and medium-level toxic metal-contaminated areas can be remediated by growing metal storage plants. The purpose of this study was to evaluate the ability of bioaccumulation of Pb by three cultivars of Sunburst (switchgrass), Dinçer (safflower), and Tarsan-1018 (sunflower) in hydroponic culture, and to determine the ability of growth of the cultivars belonging to three energy plants in different Pb concentrations (Tolerance Index, TI). However, depending upon capacity of accumulation (Bioconcentration Factor, BCF, and Translocation Factor – TF), it is aimed to assess how much these cultivars can be used for remediation of Pb-contaminated soil.

Material and Methods

Selection and Preparation of Plant Materials

We used three energy crops in the experiments: Sunburst switchgrass cultivar, Dinçer safflower cultivar, and Tarsan-1018 sunflower cultivar. Seeds of Sunburst were obtained from the Blade Seed Company (USA). Seeds of Dinçer and Tarsan-1018 cultivars, with high adaptability to the conditions in many parts of Turkey, were obtained from the Agricultural Research Institutes in Turkey. After surface sterilization with 0.1% (w/v) sodium hypochlorite solution for 15 min, seeds were initially sown in vials filled with a sterile perlite moistened with distilled water and kept for three weeks in a greenhouse under natural light. The uniform seedlings were selected for hydroponic experiments in the greenhouse located at the Department of Field Crops, Faculty of Agriculture, Yuzuncu Yil University in Van, Turkey.

Hydroponic Experiment

After three weeks of growth, the uniform seedlings were transplanted into 1.5 L polypropylene pots filled with 1.2 L of 25% strength modified Hoagland solution [25]. The pH of the nutrient solution was maintained at 5.5 ±0.1 by the addition of dilute sulfuric acid or 0.1 mol/L NaOH. A full-strength Hoagland solution contains 1 mM KH_2PO_4, 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 92 µM H_3BO_3, 1.5 µM $ZnSO_4$, 0.6 µM $CuSO_4$, 0.2 µM MoO_3, 18 µM $MnSO_4$, and 3.6 µM $FeSO_4$ [26]. Three seedlings were placed in one pot containing the nutrient solution for a one-week acclimation. Then the seedlings were exposed to the Pb-treated solutions for 30 days. $Pb(NO_3)_2$ was used to provide Pb pollution. Tested Pb concentrations (0, 25, 50, 100, and 150 mg/kg) were added to the hydroponic culture. The plant containers were randomly distributed in a greenhouse at 25/20ºC during the day/night with a relative humidity of 70%, and the photoperiod was set at an approximate 16/8 h day/night cycle. All of the treatments were conducted in triplicate, and the nutrient solution in the containers was continuously aerated with air pumps. When the volume of the treatment solution in the pots decreased by 10%, Hoagland solution was added to the initial volume.

Sampling and Measurement

Thirty days after transplanting, three seedlings in each plastic container were harvested and washed thoroughly with running tap water and rinsed with distilled water to remove any perlite particles attached to the plant surfaces. The roots and shoots were then separated and its lengths were measured. The samples were oven-dried at 70ºC until a constant weight was reached. The dried samples were weighed and ground into a powder. Then the plant samples were wet-digested in HNO_3:$HClO_4$ (6:2 v/v) by an advanced microwave digestion system from Ethos Easy. The Pb content in roots and shoots was analyzed by ICP-OES (iCAP 6000 SERIES, ICP Spectrometer).

The tolerance index (TI) was expressed on the basis of plant growth parameters and calculated as the following [27]:

$$\mathrm{TI} = (\text{growth parameters}_{\mathrm{Pb}}) / (\text{growth parameters}_{\mathrm{control}}) \times 100$$

The translocation factor (TF) of Pb from root to shoot and bioconcentration factor (BCF) were calculated as follows [28-29]:

$$\mathrm{TF} = (\mathrm{Pb}_{\mathrm{shoot}}) / (\mathrm{Pb}_{\mathrm{root}})$$
$$\mathrm{BCF} = (\mathrm{Pb}_{\text{shoot or root}}) / (\mathrm{Pb}_{\text{nutrient solution}})$$

Statistical Analysis

All data were presented as means ± standard errors (SEs). Analysis of variance was performed using SPSS

Table 1. Variables of related plant growth of Tarsan-1018, Sunburst, and Dinçer cultivars exposed to different Pb concentrations (mean ±S.E., n = 9).

	Treatments (mg/kg)	Shoot length (cm)	Shoot biomass (g/plant)	Root biomass (g/plant)	Total biomass	Root/Shoot
Tarsan-1018	0	55.0±1.0a	2.66±0.03a	0.44±0.04a	3.10±0.02a	0.16±0.02c
	25	37.7±4.5c	0.61±0.07c	0.14±0.04c	0.75±0.12c	0.23±0.04ab
	50	37.3±3.3c	0.07±0.03c	0.14±0.02c	0.84±0.08c	0.19±0.02bc
	100	45.1±4.3b	1.18±0.16b	0.30±0.06b	1.49±0.22b	0.25±0.02a
	150	37.2±5.5c	0.62±0.08c	0.16±0.02c	0.78±0.09c	0.26±0.02a
Sunburst	0	35.4±1.8ab	0.06±0.01	0.02±0.01b	0.08±0.02b	0.31±0.03b
	25	34.8±7.1ab	0.04±0.02	0.05±0.03b	0.09±0.02b	1.52±0.60ab
	50	34.9±3.7ab	0.13±0.10	0.02±0.01b	0.14±0.11b	0.19±0.09b
	100	28.9±7.6b	0.08±0.01	0.16±0.05a	0.25±0.05a	1.99±0.72a
	150	39.4±1.0a	0.08±0.01	0.02±0.00b	0.10±0.01b	0.24±0.02b
Dinçer	0	35.6±1.4a	1.33±0.34a	0.35±0.08a	1.68±0.40a	0.26±0.04a
	25	30.1±5.8ab	0.84±0.36b	0.14±0.09c	0.99±0.44bc	0.17±0.05b
	50	29.4±1.1ab	1.14±0.16ab	0.31±0.09ab	1.46±0.25ab	0.27±0.04a
	100	30.8±4.0ab	1.00±0.14ab	0.21±0.06bc	1.20±0.21ab	0.20±0.03ab
	150	26.1±0.5b	0.39±0.07c	0.10±0.00c	0.49±0.07c	0.26±0.05a

Means in the same column for three crop cultivars by the same letter are not significantly different at $p<0.05$ based on Duncan test.

Version 22.0 software (SPSS Inc., USA). Duncan's multiple range tests were used to compare the significant differences of means. The level of statistical significance was set at $p<0.05$.

Results and Discussion

Shoot length, root and shoot biomass, total biomass, and root/shoot ratio of Tarsan-1018, Dinçer, and Sunburst cultivars exposed to different Pb concentrations are shown in Table 1. A significant decrease in shoot length of Tarsan-1018 was detected, while no remarkable decrease in shoot length of Sunburst and Dinçer cultivars was observed with increasing Pb concentration. When compared to the control group, root and shoot biomass of Tarsan-1018 remarkably decreased, and all concentrations except Pb 100 mg/kg were among the same group. There was not any significant effect of applied concentrations on shoot biomass of Sunburst. When root biomass of Sunburst was investigated, the highest growth was obtained in the 100 mg/kg Pb application and other applications were found in the same group. For Dinçer, control and 50-100 mg/kg Pb concentrations released the highest growth level, while the 150 mg/kg Pb concentration yielded the lowest growth level. Also, control and 50 mg/kg Pb concentrations were among the same group for root development in Dinçer. In a study on wheat with different contaminants and concentrations, it is stated that seedlings exposed to the same contaminant by hormesis and paradoxal effect may have non-monotonic responses [30]. In a conducted study related to above data, the ryegrass plants showed that the growth of plants was affected by Pb stress. As soil Pb level increased, plant height decreased; however, shoot fresh weight and root fresh weight initially increased before decreasing, with the maximum value at 300 mg Pb/kg [31].

When total biomass is taken into consideration, with increasing Pb concentrations a remarkable decrease in growth of Tarsan-1018 was observed, while Sunburst and Dinçer cultivars were not affected so much. Amer et al. [32] investigated plant growth with increasing Pb concentrations. At a concentration of 5 mg/L it seemed that shoot weight (SW) is higher than control with a stimulating effect on the growth of *A. halimus, P. oleracea,* and *M. lupulina*. In treatment Pb10 no significant effect on SW was observed for *A. halimus*, while a stimulating effect was shown on *M. lupulina* and a toxic one on *P. oleracea*. Treatment Pb50 prevented *A. halimus* and *P. oleracea* from growing, but did not exert any detrimental effect on SW of *M. lupulina*, being that this parameter is not statistically different from control [32].

Effects of Pb toxicity on root/shoot rate were specifically observed for each of the cultivars. When compared to control, the root/shoot ratio of Tarsan-1018 cultivar increased with increasing Pb concentrations; for Sunburst cultivars an increase in root/shoot ratio was detected in 25-100 mg/kg concentrations and a decrease was detected in other concentrations with increasing Pb concentrations (Table 1). No effect of different

Table 2. Tolerance index (TI) of Tarsan-1018, Dinçer, and Sunburst cultivars exposed to different Pb concentrations (mean ±S.E., n = 9).

	Treatments (mg/kg)	TI Shoot length	TI Shoot biomass	TI Root biomass	TI Total biomass
Tarsan-1018	25	68.5±8.1	22.9±2b	32.6±9b	24.3±3b
	50	67.9±5.9	26.3±2b	31.1±3b	27.0±2b
	100	81.9±7.7	44.6±5a	68.2±12a	48.0±6a
	150	67.6±10.1	23.3±2b	37.1±3b	25.3±2b
Sunburst	25	98.2±19	72.9±3	257.4±83b	116.0±19b
	50	98.6±10	211.3±17	94.4±38b	183.9±45ab
	100	81.5±11	141.2±5	916.6±91a	322.5±69a
	150	111.4±2	137.3±11	109.3±16b	130.7±12b
Dinçer	25	84.6±16	63.4±16 a	41.3±6b	58.8±26ab
	50	82.5±3	85.5±12a	90.2±12a	86.4±14a
	100	86.7±11	75.1±10a	58.2±9ab	71.5±12a
	150	73.2±1	28.9±4b	28.7±0.3b	28.8±3b

Means in the same column for three crop cultivars by the same letter are not significantly different at $p<0.05$ based on Duncan test.

concentrations on root/shoot rate of Dinçer was observed. In a study on *Brassica chinensis*, an increase of root and shoot growth level was achieved for Pb concentrations up to 500 mg/kg. It is known that lead has a stimulatory effect on high metal-accumulating plants [33].

For Tarsan-1018 the tolerance index (TI) value was increased at 100 mg/kg Pb concentration and no difference was observed at other concentrations. High TI levels were observed for Sunburst at all Pb concentrations. For Dinçer cultivar, when compared to control, high TI values were detected for all concentrations except 150 mg/kg concentration (Table 2). We observed growth and development criteria and that Sunburst and Dinçer cultivars were not affected by increasing Pb concentrations (Table 1). Pb concentrations used in the study did not affected growth levels for Sunburst and Dinçer. It is thought that Pb has a remarkable stimulatory effect on these two cultivars. Although TI varies with increasing Pb concentration, all three varieties of *S. integra* can be defined as highly tolerant (TI>60) to Pb [34]. For Yizhibi

Table 3. Pb content in shoots and roots, bioconcentration factor (BCF), and translocation factor (TF) of Tarsan-1018, Dinçer, and Sunburst cultivars grown in Pb-treated (mean ±S.E., n = 9).

	Treatments (mg/kg)	Pb content (mg/kg)		BCF (%)		TF (%)
		Shoot	Root	Shoot	Root	
Tarsan-1018	25	9.4±0.4d	492±46d	37.7±1.5a	1,971±186d	1.93±0.24a
	50	18.3±0.3c	1,328±44c	36.5±0.6a	2,657±89c	1.38±0.02b
	100	31.8±0.9b	3,328±214.3b	31.8±0.9b	3,328±214b	0.96±0.09c
	150	45.1±0.7a	5,798±114.5a	30.1±0.4b	3,865±76a	0.78±0.02c
Sunburst	25	16.3±0.7d	419±101d	65.3±2.7a	1,679±107d	4.03±0.92a
	50	32.8±1.5c	1,757±60c	65.5±3.0a	3,514±121c	1.87±0.02b
	100	41.9±1.0b	8,639±208b	41.9±1.0c	8,639±208b	0.49±0.02c
	150	72.1±4.6a	16,252±56a	48.0±3.0b	10,834±370a	0.44±0.03c
Dinçer	25	4.8±0.2d	87.5±6c	19.1±0.8d	349±27d	5.48±0.33a
	50	8.0±0.9c	286±26c	16.0±1.9c	573±52c	2.83±0.61b
	100	23.8±0.9b	1,039±73b	23.8±0.9a	1,039±73b	2.30±0.17b
	150	33.5±2.3a	2,614±321a	22.3±1.5a	1,743±114a	1.30±0.20c

Means in the same column for three crop cultivars by the same letter are not significantly different at $p<0.05$ based on Duncan test.

and Weishanhu, the TIs decreased significantly at 196 μM Pb, whereas at 123 and 178 μM Pb they were only slightly reduced compared to the treatment with 47 μM Pb. For Dahongtou, there were no significant differences in TI among the treatments. Weishanhu had the highest TI at all Pb concentrations except at 47 μM, when the highest TI was measured for Yizhibi [34]. There are differences of tolerance against Pb among species and even varieties of plants that they show against Pb. The results of our work are also consistent with this situation.

As Pb level increased in the environment, Pb levels both in the root and in the shoot increased in all cultivars. Higher levels of Pb were detected in roots than in shoots in all cultivars (Table 3). A study on rice plants showed that lead level absorbed by roots was 1.7-3.3 times higher than it was in shoots [35]. In another study, *A. halimus, P. oleracea,* and *M. lupulina* accumulated much more Pb in their roots than in shoots, with *A. halimus* by far being the most efficient among the tested plants in root Pb uptake [32].

For all energy plant cultivars used in our study, lead levels in roots and shoots increased depending on increasing concentrations of Pb. The highest lead levels in roots and shoots were detected for Sunburst, while lower Pb concentrations were detected for Tarsan-1018 and Dinçer cultivars when compared to Sunburst.

Bioconcentration factor (BCF) index of all cultivars are given in Table 3. High BCF levels were observed at roots of all three bioenergy plants. With increasing Pb concentrations, the increase in BCF value was higher for Sunburst. Sunburst was followed by Tarsan-1018 and Dinçer cultivars, sequentially. BCF value was found as %10834 for Sunburst at 150 mg/kg Pb concentration. Remarkably, lower BCF values were detected in shoots when compared to roots for all three energetic plants. Higher shoot BCF levels were detected again in Sunburst when compared to other cultivars. BCF value is an important indicator of metal accumulation capacity [36-37]. It is known that plants with BCF values over 1,000 have good metal accumulation. Although in our study root BCF values of Tarsan-1018 and Sunburst exceeded 1,000 for all Pb concentrations, for Dinçer, at Pb 25-50 mg/kg concentrations, BCF values under 1,000 were detected in the roots.

The translocation factor (TF) of cultivars used in our study is presented in Table 3. For all cultivars, translocation of Pb from roots to shoots was detected as quite low. For Tarsan-1018 and Sunburst, TF values for Pb 100-150 mg/kg concentrations of less than 1 were observed. TF value of Dinçer was more than 1 at all concentrations. In Sunburst and Tarsan-1018 cultivars, Pb retention at roots was higher but translocation of Pb to shoots was limited. Dinçer cultivar showed a better performance of Pb translocation when compared to the other two cultivars. The activation of affinity transport system at low metal concentrations, saturation of metal uptake in xylem, and transport rate of metals highly influence a plant's TF and EC values [38-40].

Lastly, while using energy crops for phytoremediation of contaminated land, it should be kept in mind that plant species should be perennial in nature. As Pandey et al. [41] suggested, we have considered phytoremediation with energy crops to be a safe and sustainable approach.

Conclusions

Tarsan-1018, Sunburst, and Dinçer cultivars, which have the potential to be bioenergy plants, showed differences in terms of tolerance to Pb toxicity. Although Tarsan-1018 showed repression in growth with increasing Pb concentrations, it was able to absorb high levels of Pb. Dinçer cultivar did not showed growth retardation with increasing concentrations of Pb but was able to absorb less Pb. Additionally, Dinçer was more able to transfer Pb from root to shoot when compared to Tarsan-1018 and Sunburst. When compared to other cultivars, Sunburst reflected a higher Pb accumulation without growth retardation. Root-to-shoot transfer was low for Sunburst cultivars. All the three bioenergy plant cultivars studied may be good accumulator candidates for phytoremediation and fuel production on Pb-contaminated soil by their different lead tolerances.

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