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## BIOACCUMULATION OF LEAD IN SOYBEANS GROWN IN NATURALLY POLLUTED SOILS

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### Abstract

The ability of plant species to take up lead from polluted soils may vary due to soil properties and the level of pollution. This study aimed to investigate the capability of soybeans to take up and translocate Pb in different plant parts, such as roots, stems, shoots, pods, and seeds. For that purpose, five soybean varieties with different vegetation periods were grown in naturally polluted soils. Soil and plant samples were digested and analyzed by ICP-MS. Calculated biotransfer factors indicate a low ability of soybeans to distribute Pb in the above-ground parts of plants. It was noticed that the content of Pb in the soil has the greatest influence on its accumulation compared to the soil acidity. The ability of the soybean to distribute Pb in the seed was the lowest. Anyway, despite this, the results suggest that soybeans grown in highly polluted soil pose a potential risk for human and animal health due to their ability to store lead in seeds above the permitted concentrations.

**Keywords:** soybean, lead, bioaccumulation, soil pollution, soil acidity, health risk.

## **1. INTRODUCTION**

Soybean (*Glycine max*) is a leguminous plant native to East Asia that belongs to the family *Fabaceae*. It is rich in proteins and contains no starch, which makes it valuable for diabetics. Soybeans are known for their ability to fix nitrogen through a symbiotic relationship with soil bacteria, which makes them valuable in crop rotations. They're widely cultivated for their high protein content (making them essential in animal feed and plant-based diets) and oil, which is extracted for use in cooking, biodiesel, and industrial products. Additionally, soybeans are used in many forms, such as tofu, soy milk, and tempeh, and are a key ingredient in various food products around the world. Its high nutritional value and low price make it a valuable source in the food industry. Additionally, literature data indicate that soybeans are capable of bioaccumulating some heavy metals from the soil like lead (Pb), cadmium (Cd), and arsenic (As), suggesting soil remediation activity (Zhao et al., 2011). Although many studies showed that lead readily accumulates in the roots than in the fruiting parts of crops like beans (Pourrut 2011), some researchers have found the highest concentrations of Pb in the soybean seed (Aranisola 2013). This study aimed to investigate soybeans' ability to take up and translocate Pb in the plant parts, like roots, stems, leaves, pods, and seed, when grown in high or extremely polluted soils with lead.

## **2. MATERIALS AND METHODS**

### **2.1. Investigated varieties**

Five different soybean cultivars were included in this investigation. Cultivars Avigea and Pela, with a short vegetation period, originate from Bulgaria and Macedonia, respectively. Cultivar Balkan with a medium vegetation period, originating from Serbia. Cultivars Ilindenka and Pavlikeni with long vegetation period originate from Macedonia and Bulgaria, respectively (Table 1).

### **2.2. Field settings**

The experiment was performed in a pot with dimensions that allow the undisturbed development of the plant in order to express its genetic potential. Soil samples were collected from the soil top layer (25 cm depth) near the mining area of „Zletovo“ with Pb-Zn mineralization, located in the Kratovo-Zletovo volcanic region of the Vardar zone (40°76' N; 021°10' E; 375 m a.s.l.) and investigated for the presence of element content. Pots were placed in a greenhouse situated in the agronomic area Amzibegovo near the town of St. Nikole. The experiment was conducted in a greenhouse in a controlled environment in order to exclude the influence of other agro-ecological conditions, like the accumulation of heavy metals through the leaf surface and uncontrolled deposition of heavy metals by the rain. Each cultivar was planted in four repetitions in two

different soils. The pots were randomly placed in the greenhouse and irrigated with the same amount of distilled water.

### 2.3. Sample preparation

The total and bioavailable content of Pb in the soil were determined before sowing of soybean varieties according to ISO 14869-1 using open wet digestion with a mixture of acids. The content of Pb in plant parts was determined using ICP-MS (model 7500cx, Agilent Technologies, Santa Clara, CA, USA). Quality assurance was assumed using certified reference material JSAC 0401 for soil and NMIJ CRM 7511-A for plants.

### 2.4. Soil metal contamination assessment

The level of soil pollution was evaluated using the geoaccumulation index (Igeo) according to Müller (1969):

$$I_{geo} = \log_2 [C_n / (1.5 \times B_n)] \dots\dots\dots \text{Equation 1}$$

Where  $C_n$  represents the measured content of the examined metal  $n$  in the soil sample,  $B_n$  is the natural background content of the metal  $n$  in the Vardar zone of the Republic of Macedonia (Stafilov and Shine, 2016) and the factor 1.5 is used because of the possible variations in background values due to lithological variability. The degree of metal contamination of the samples was classified as: Grade 0 ( $I_{geo} \leq 0$ ), uncontaminated; Grade 1 ( $0 \leq I_{geo} \leq 1$ ), uncontaminated to moderately contaminated; Grade 2 ( $1 \leq I_{geo} \leq 2$ ), moderately contaminated; Grade 3 ( $2 \leq I_{geo} \leq 3$ ), moderately to heavily contaminated; Grade 4 ( $3 \leq I_{geo} \leq 4$ ), heavily contaminated; Grade 5 ( $4 \leq I_{geo} \leq 5$ ), heavily to extremely contaminated; and Grade 6 ( $5 \leq I_{geo} \leq 6$ ), extremely contaminated.

### 2.5. Assessment of bioaccumulation in plant tissues

The degree of the bioaccumulation of Pb in soybean varieties was expressed as a bioaccumulation factor (BAF), defined as the ratio between total content of the element in the plant (sum of the content in roots, shoots, leaves, pods, and seeds) and the content of the element in corresponding soils (Equation 2). In steady state, BAF is equal to zero (Arnot and Gobas 2006).

$$BAF = C_{plant} / C_{soil} \dots\dots\dots \text{Equation 2}$$

The ability of the plants to accumulate Pb from the soil (substrate) was investigated by the Biotransfer Factor (BTF). It is defined as the ratio between the content of the element in the aerial plant parts and that of the roots (Equation 3).  $BTF > 1$  represents that transfer of the element was effectively made from the root to the

aerial part of the plant, which is a characteristic for high accumulation crops (Baker and Brooks 1989; Zhang et al. 2002).

$$\text{BTF} = \text{Cplant part} / \text{Croot} \dots \dots \dots \text{Equation 3}$$

## 2.6. Statistical analysis

Statistically significant difference between soybean varieties was investigated using ANOVA single-factor analysis followed by the Post-Hoc T-test for equal variances. The Bonferroni Adjustment was performed to control the Type I errors when multiple comparisons are made.

## 3. RESULTS

### 3.1. Soil quality

The investigated soil was characterized by low organic matter content with almost neutral acidity pH = 6.9. The content of the Pb was 437 ppm for total and 83.5 ppm for available Pb content. The Igeo value reveals that the soil is heavily to extremely contaminated with Pb (Table 1).

**Tabela 1.** Soil quality from the Zletovo mining area

	<b>Pb (total) ppm</b>	<b>Pb (available) ppm</b>	<b>pH</b>	<b>OM %</b>	<b>K2O mg/110g</b>	<b>P2O5 mg/100g</b>	<b>Loam %</b>	<b>CEC cm/mol</b>	<b>EC mS/cm</b>
<b>soil</b>	437	83.5	6.9	1.47	13.7	12.0	10.2	9.0	0.41

### 3.2. Pb content in the soybean plants

#### 3.2.1. Total Pb content in the plants

ANOVA single-factor analysis showed a statistically significant difference between some of the investigated varieties (Table 2). The new critical value after the Bonferroni Adjustment was 0.01. To identify the differences, the Post-Hoc T-test for equal variances was performed, and the obtained result showed that all investigated varieties differ between themselves, regarding their ability to take up Pb from the soil, except the varieties of Balkan and Pela. The BAF didn't indicate for possible bioremediation activity. The greatest ability to uptake Pb from the soil was observed at the variety of Ilyndenka followed by Avigea, Balkan, Pela, and Pavlikeni.

**Table 2.** Two-tailed p-values obtained from the Post-Hoc T-test for equal variances, performed to identify differences between the investigated varieties to uptake Pb from soil ( $\alpha_{crit} = 0.01$ ).

	Avige a	Avige a	Avigea vs	Avigea vs	Pela vs	Pela vs	Pela vs	Balkan vs	Balkan vs	Ilyndenk a
	vs Pela	vs Balkan	Ilyndenk a	Pavliken i	Balka n	Ilyndenk a	Pavliken i	Ilyndenk a	Pavliken i	vs Pavlikeni
p	0.002	0.007	0.01	$2.6e^{-4}$	0.1	$1.5e^{-4}$	0.002	$3.4e^{-4}$	0.001	$3.3e^{-5}$

### 3.2.2. Pb content in the roots

Ilyndenka has the greatest content of Pb in the roots, followed by Balkan, Avigea, Pela, and Pavlikeni. The greatest ability to store Pb in roots was observed in Balkan (90.39%), which means that this variety has the lowest ability to translocate Pb in the above plant parts. Ilyndenka stored around 70% of accumulated Pb in roots, while Avigea, Pavlikeni, and Pela stored 66.72%, 56.14% and 55.21%, respectively. The ability of Avigea to store Pb in roots significantly differs from Pela and Pavlikeni. Pela also differs from all other varieties while Balkan differs from Ilyndenka and Pavlikeni, only. A statistically significant difference was observed between Ilyndenka and Pavlikeni, also (Table 3).

**Table 3.** Two-tailed p-values obtained from the Post-Hoc T-test for equal variances, performed to identify differences between the investigated varieties to store Pb in roots ( $\alpha_{crit} = 0.01$ ).

	Avige a	Avige a	Avigea vs	Avigea vs	Pela vs	Pela vs	Pela vs	Balkan vs	Balkan vs	Ilyndenk a
	vs Pela	vs Balkan	Ilyndenk a	Pavliken i	Balka n	Ilyndenk a	Pavliken i	Ilyndenk a	Pavliken i	vs Pavlikeni
p	$6.2 e^{-4}$	0.26	0.02	$4.6 e^{-5}$	$5.7 e^{-4}$	0.001	0.007	0.01	$2.1 e^{-5}$	$5.3 e^{-4}$

### 3.2.3. Pb content in the stem

Regarding the ability of the investigated varieties to translocate Pb in the stem, Pela was identified as the most potent, followed by Pavlikeni, Avigea, Ilyndenka, and Balkan. The statistical analysis showed that most of the varieties didn't significantly differ, except Balkan with Avigea, Pala, and Ilyndenka (Table 4).

**Table 4.** Two-tailed p-values obtained from the Post-Hoc T-test for equal variances, performed to identify differences between the investigated varieties to store Pb in stem ( $\alpha_{crit} = 0.01$ ).

	Avige a	Avige a	Avigea vs	Avigea vs	Pela vs	Pela vs	Pela vs	Balkan vs	Balkan vs	Ilyndenk a
	vs	vs	Ilyndenk	Pavliken	Balka	Ilyndenk	Pavliken	Ilyndenk	Pavliken	vs
	Pela	Balkan	a	i	n	a	i	a	i	Pavlikeni
p	0.56	$5.3 \times 10^{-4}$	0.17	0.03	0.003	0.55	0.03	$6.0 \times 10^{-4}$	0.04	0.01

#### 3.2.4. Pb content in the leaves

The greatest ability to translocate Pb from the roots to the stem was observed in Pavlikeni, followed by Avigea, Pela, Ilyndenka, and Balkan. Avigea differed significantly from Pela, Balkan, and Pavlikeni, while Pela and Pavlikeni differed from Balkan and Ilyndenka (Table 5).

**Table 5.** Two-tailed p-values obtained from the Post-Hoc T-test for equal variances, performed to identify differences between the investigated varieties to store Pb in leaves ( $\alpha_{crit} = 0.01$ ).

	Avige a	Avige a	Avigea vs	Avigea vs	Pela vs	Pela vs	Pela vs	Balkan vs	Balkan vs	Ilyndenk a
	vs	vs	Ilyndenk	Pavliken	Balka	Ilyndenk	Pavliken	Ilyndenk	Pavliken	vs
	Pela	Balkan	a	i	n	a	i	a	i	Pavlikeni
p	0.002	$9.7 \times 10^{-5}$	0.17	0.03	$2.6 \times 10^{-4}$	0.001	0.04	$7.4 \times 10^{-6}$	$7.1 \times 10^{-4}$	$1.8 \times 10^{-4}$

#### 3.2.5. Pb content in the pods

The greatest ability to translocate Pb in pods was observed in Pela followed by Balkan, Ilyndenka, Avigea and Pavlikeni. Statistical analysis reveals significant differences between Avigea with Pela and Pavlikeni, and Pela with Balkan, Ilyndenaka, and Pavlikeni (Table 6).

**Table 6.** Two-tailed p-values obtained from the Post-Hoc T-test for equal variances, performed to identify differences between the investigated varieties to store Pb in roots

	Avige a	Avige a	Avigea vs	Avigea vs	Pela vs	Pela vs	Pela vs	Balkan vs	Balkan vs	Ilyndenk a
	vs	vs	Ilyndenk	Pavliken	Balka	Ilyndenk	Pavliken	Ilyndenk	Pavliken	vs
	Pela	Balkan	a	i	n	a	i	a	i	Pavlikeni
p	$7 \times 10^{-4}$	0.84	0.22	0.01	0.001	0.004	$1 \times 10^{-4}$	0.31	0.02	0.01

#### 3.2.6. Pb content in the seeds

The greatest ability to translocate Pb in the seed was observed in Pavlikeni and Ilyndenka, followed by Avigea, Balkan, and Pela. Ilyndenka significantly differs from Pela, Balkan, and Pavlikeni (Table 7).

**Table 7.** Two-tailed p-values obtained from the Post-Hoc T-test for equal variances, performed to identify differences between the investigated varieties to store Pb in leaves ( $\alpha_{crit} = 0.01$ ).

	Avige a	Avige a	Avigea vs Ilynden a	Avigea vs Pavliken i	Pela vs Balka n	Pela vs Ilynden a	Pela vs Pavliken i	Balkan vs Ilynden a	Balkan vs Pavliken i	Ilynden a vs Pavlikeni
p	0.08	0.11	0.02	0.24	0.38	0.002	0.53	0.002	0.82	0.005

#### 4. CONCLUSION, DISCUSSION, AND RECOMMENDATIONS

The obtained results from this study showed that the investigated soybean varieties can tolerate high amounts of Pb in the soil and develop without any difficulties in highly polluted soils with Pb. Furthermore, great differences were observed between the investigated varieties regarding their capabilities to take up Pb from the soil, store it in the roots, and translocate it in the above-ground plant parts. No similarities were observed between varieties from the same maturity group. Overall, all varieties have the greatest ability to store Pb in roots and the lowest ability to translocate it in the seed. Despite that, the amount of Pb in the seeds in all varieties exceeds the maximum permitted levels of 0.2 ppm for soybean seed intended for human consumption, set by the European Food Safety Authority (EFSA) under the EU Regulation No. 1881/2006, no matter of whether their capability was high or low. This outcome suggests that although soybeans tolerate high concentrations of Pb in the soil, extreme caution should be exercised as concentrations can exceed permissible values. Further research is needed to establish the safe threshold for Pb concentrations in soils with varying pH levels, as heavy metals tend to be more bioavailable in acidic soils.

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