THE POTENTIAL OF CORN (*ZEA MAYS*) FOR PHYTOREMEDIATION OF SOIL CONTAMINATED WITH HEAVY METALS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
Heavy metal pollution is a significant environmental problem and has a negative impact on human health and agriculture. Phytoremediation has recently emerged as an efficient heavy metal remediation technology. To examine the phytoremediation potential, an experiment was conducted, where the influence of high and low concentrations of heavy metals, lead, cadmium and zinc (Pb, Cd and Zn) on the phenological characteristics of corn (*Zea mays*), as well as their accumulation in the underground and aboveground part was monitored. The experiment was carried out in outdoor conditions in pots in which corn was planted and the soil was contaminated with heavy metals in concentrations below and above the maximum allowable concentration prescribed by the Ordinance. The experiment lasted 45 days. By processing the results, statistically significant differences in plant development were determined.The highest concentration of Zn was recorded in the aboveground part of the plant, the stem in the amount of 24.443 mg/kg, and the lowest concentration in the leaf 0.216 mg/kg. The highest concentrations of Cd and Pb were recorded in the root, for lead 26.610 mg/kg and in the stem for cadmium 30.490 mg/kg, which is a statistically significantly higher established value compared to other parts of the plant.

KEYWORDS: phytoremediation, Corn (*Zea mays*), heavy metals

INTRODUCTION

Phytoremediation has recently emerged as an efficient heavy metal remediation technology. If phytoremediation of urban areas is taken into account, pruning-tolerant species are preferred. Plant species useful for phytoremediation must meet some requirements: rapid growth, high-yield biomass production, pollution tolerance.

If phytoremediation of urban areas is taken into account, pruning-tolerant species are preferred. The species recommended for phytodegradation, which is usually found in the root, should have a large and dense root system. Root systems with a sufficiently large surface area are also preferred for microbial communities that take place together with the plant phytostimulation process [1].

The grass family (*Poaceae*) is one of the most important for the phytoremediation of heavy metals and organic pollutants such as PAH (polyaromatic hydrocarbons) and petroleum hydrocarbons. The advantage of the plant from this family is that after cutting and drying, the plant material is not brittle. They have a rather shallow root system, but they are very dense and penetrate well into the soil, so these plants are very effective in phytoextraction of the upper part of the soil level.

The goal of remediation of contaminated areas due to human activity is to restore such ecosystems to their original state. Due to the application to a large number of pollutants, as well as the possibility of implementation on various surfaces, and due to lower environmental impact and lower costs, phytoremediation is considered a green technology. The toxicity of heavy metals in plants varies depending on plant species, metal specificity, metal content, chemical form, soil composition and pH. Some metals, including Cu, Mg, Co, Zn, and Cr, are essential for plants in trace amounts, but only when metals are present in bioavailable forms, and in increased amounts can become toxic to plants [2]. Others, such as Mg, Co, and Zn, have unknown biological functions and can cause disturbances even at relatively lower concentrations [3].

Phytoremediation consists of four different technologies used by plants and each has a different mechanism for remediation of soils, sediments and waters contaminated with heavy metals. These include:

1. Phytoextraction - the use of plants, with large biomass and the ability to accumulate metals and appropriate soil additives to transport and concentrate metals from the soil to the aboveground parts of plants, which will then be removed through conventional agronomic measures.
2. Phytostabilization - the use of plants to reduce the bioavailability of pollutants in the environment; in this case the plants stabilize the contaminated soil rather than clean it.
3. Rhizofiltration - the use of the root system of plants for the absorption and adsorption of pollutants, mainly metals, from water.
4. Phytovolatilization - the use of plants for the extraction of certain, volatile, metals from the soil, and then their release through the leaves into the atmosphere.

Zinc (Zn) is an essential trace element necessary for all living things while cadmium (Cd) is toxic and has no physiological or biological role in the body. They have very similar chemical properties and their uptake into the root and acropetal movement can be controlled by the same mechanisms [4].

Mojiri [5], was chosen cadmium, zinc as pollutants because they are widespread in the environment, especially as soil pollutants. The experiments were performed under natural environmental conditions where the reaction to *Zea mays* stress conditions, caused by the presence of heavy metals, was monitored. Today, the environment is filled with large amounts of toxins, including heavy metals of various shapes. Heavy metal pollution is a significant environmental problem and harms human health and agriculture. Heavy metals from the soil accumulated in plants enter the food chain and their excessive amount can be toxic to humans and animals.

Recently, a lot of work has been done on finding plants that can absorb, distribute and accumulate heavy metals in the aboveground part of plants. During several years of growing these plants, the concentration of heavy metals in the soil is reduced to the allowed value, thus cleaning the soil, and will create conditions for normal agricultural production. Therefore, in order to recultivate such and similar soils, in the last twenty years, the method of biological reclamation - phytoremediation, ie cultivation of annual and perennial herbaceous plants to reduce concentrations of heavy metals (extraction into the aboveground part of plants), increasing numbers and activities soil microflora as well as their detoxification (conversion of metals into forms that are not harmful to plants).

Plants growing on soils damaged by heavy metals may not accept metals, ie be tolerant to high concentrations of metals in the soil; to absorb metals, and to accumulate them in the root system and mycorrhizal root flora; to adopt metals and accumulate them in the aboveground part [6].

Currently, phytoremediation is an efficient and affordable technological solution used to extract or remove inactive metals, such as soil and water pollutants. This technology is environmentally friendly and potentially cost-effective [7].

Zinc (Zn) and cadmium (Cd) are elements with very similar chemical properties. They have similar ionic structures, a similar degree of electronegativity and a relative density above 5 g cm-3, based on which they are included in the group of "heavy metals". Both metals are present in very low amounts (mg/kg, and Cd and less) in almost all soils and most living organisms.They can be classified in the group of trace elements. Despite the chemical similarity, the biological and physiological importance of these elements is very different. Zn belongs to the group of metals necessary for more plants and mammals while Cd is unnecessary and toxic. To date, no physiological role of Cd in plant tissue has been established. The presence and accumulation of Cd, even in very low concentrations, significantly affect a number of physiological and biochemical processes in plant tissue, and its toxicity is ultimately manifested through slowed and impaired growth and development and reduced yields [8]. Wangstrand et al. [9], state that in humans the primary source of Cd is food. It is estimated that about 80% of Cd in the human diet comes from grains, vegetables and potatoes.

Unlike Cd, Zn is essential for a number of enzymes in nitrogen metabolism, energy transfer, and protein synthesis. Zn is biochemically and physiologically involved in several processes in the plant, ranging from water uptake and transport, through membrane stability and ion transport to the biosynthesis of auxins, RNA, DNA, protein, and carbohydrate and lipid metabolism. However, at too high concentrations like other heavy metals and Zn can be toxic, inhibiting cell growth and photosynthesis.

Zn is present in almost all cells of the human body, it is necessary for the immune system and the smooth functioning of cellular functions, and its deficiency causes hair loss disorders, skin health problems, memory loss, weakening of intellectual abilities, weakness and decreased immunity, fetal development problems. , male infertility and congenital diseases. It is estimated that almost 60% of the world's population has health problems due to micronutrient malnutrition, among which the most critical iron, zinc and vitamin A deficiency [4].

Corn (Zea mays)

Is a genus from the family *Poaceae*, generally known as the grass family. "*Zea*" is derived from the ancient Greek name for food grass. The genus *Zea* has four species, the most economically significant of which being *Zea mays L*. Maize has been shown to accumulate significant amounts of heavy metals when induced by the addition of metal chelates. The crop is tolerant to heavy metals, has a high ability to accumulate metals in foliar parts with moderate bioaccumulation factor. Because of such characteristics, maize may continuously phytoextract metals from polluted soil by transporting them from roots to shoots [10].

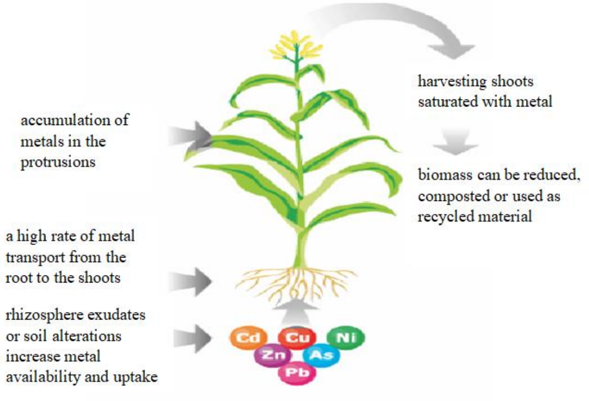

Figure 1. Schematic representation of phytoextraction of metals from soil, adapted, [11]

The aim of this study was to determine:

1) The ability of maize, (*Zea mays*) genus from the family *Poaceae*, as a phytoremediator of soil contaminated with lead (Pb), zinc (Zn) and cadmium (Cd).

2) Possibility of accumulation of heavy metals (Pb and Cd) in its underground or aboveground parts Zn.

MATERIALS AND METHODS

Plastic containers had been used to set up the experiment, volume 5 liters, filled with soil, from the experimental plots of the Biotechnical Faculty of the University of Bihać, then left in external conditions, of the Biotechnical Faculty. The soil in the containers has been contaminated with high and low concentrations of heavy metals (Pb, Cd, and Zn), and the maximum permitted amounts of pollutants prescribed by the Ordinance on determining the permitted amounts of harmful and dangerous substances in the soil and methods of their examinations were used as criteria (Official Gazette of the Federation of B&H, 72/09). For experimental research, a plant culture was selected, corn (*Zea mays*), which shows a very rapid adaptation to conditions in the new and foreign environment, but also a great ability to reproduce. It is adaptable to almost all possible habitats conditions. Corn seeds were left to germinate in a petri dish on a moist substrate, and four days later were planted in plastic containers. Solutions of the corresponding salts were prepared; solution of lead nitrate $Pb (NO)_3$, 0.1 mol/L, cadmium sulfate $CdSO_4$ 0.05 mol/L zinc sulfate $ZnSO_4$ 0.1 mol/L, and then diluted and adjusted to the mass of the earth to define the exact mass concentrations of ions present, which are eliminated by a remediation process. Low concentration (LC) of lead ions (Pb^{2+}) was 1000 mg, high concentration (HC) of lead ions (Pb2+) was 2000 mg, low concentration (LC) of cadmium ions (Cd^{2+}) 20 mg, high concentration, cadmium ions (Cd^{2+}) 100 mg per kilogram of soil, low concentration (LC) of zinc ions (Zn^{2+}) was 1000 mg, high concentration (HC) of zinc ions (Zn^{2+}) was 5000 mg, per kilogram of soil. LC, low metal concentrations, were 5 to 10 times higher than the maximum allowable concentrations, according to regulations [12]. HC, high metal concentrations, were 20 to 25 times higher than the maximum allowable concentrations, according to regulations [12]. Containers with soil without the addition of heavy metals were treated as a control. The prepared metal solutions were applied directly to plastic containers, each of which contains 5 kg of soil with known chemical properties. (Table 1). The plants were grown for 45 days from mid-July to the end of August under natural light temperature conditions. All treatments in three replications (HC, LC, Control) were tested after 45 days.

PLANT GROWTH MONITORING, MEASUREMENTS AND ANALYSIS OF HEAVY METAL CONCENTRATIONS

During the experiment, the growth and development of *Zea mays* were monitored. After the experiment (45 days) plants were less than 30 cm tall. The concentration of heavy metals in the aboveground organs (stem and leaves) and underground organs (roots) was determined following the instructions [13]. The concentration of heavy metals in the aboveground organs (stem and leaves) and underground organs (root) was evaluated after air-drying the collected and treated plant components. Samples of accurately weighed mass (about 1 g), previously homogenized dry plant material, were transferred to a porcelain pot and heated in an electric oven for 8 hours, afterward the temperature was kept constant for the next 12 hours. The samples are then calcined to determine the mineral content in a muffle furnace at a temperature of 600 ° C until white or pale green ash is obtained. The cooled sample was dissolved in 4 ml of 10% HNO_3, filtered through filter paper (blue stripe), and made up to 50 ml with deionized water. In the sample prepared in this way, the values on the Atomic Absorption Spectrophotometer, Perkin Elmer AAS 800, were read by flame technique. The analyzes were performed in the Laboratory of the Biotechnical Faculty of the University of Bihać.

RESULTS AND DISCUSSION

Before conducting the experiment, a chemical analysis of the soil was performed (from the experimental plots of the Biotechnical Faculty), the results are shown in Table 1.

Table 1. Chemical analysis of the soil used in the research

Parameters	Unit of Measurement	Results
Depth	cm	0-30
Hygroscopic moisture (Hy)	%	4.70
Organic matter	%	21.42
Mineral matter	%	78.58
Humus	%	0.54
Active acidity		7.07
pH KCl-u		6.95
NH_3-N	mg/ kg	3.68
NO_3^--N	mg/ kg	7.05
NO_3^-	mg/ kg	30.38
P	mg/ kg	1.62
PO_4^{3-}	mg/ kg	4.88
P_2O_5	mg/ kg	3.79
SO_4^{2-}	mg/ kg	27.13
K_2O	mg/ kg	9.22
K	mg/ kg	7.59
Ca^{2+}	mg/ kg	553.48
Mg^{2+}	mg/ kg	81.39

The obtained results of the analyzed plant material with standard deviation and the results of statistical data processing (One-Way ANOVA and Tukey test) depending on the applied concentration and part of the plant are presented in Tables 2, 3, and 4.

The plants looked healthy during the control treatment in the first stages of growth and development. By monitoring the parameters of plant growth and development, no significant changes were observed on the young leaves of the plant, while the plants were grown in soil contaminated with low concentrations of metals, which developed normally and achieved the expected development characteristic of the mentioned species.

Based on a one-way analysis of variance (One-Way ANOVA and Tukey test), a significant effect of metal concentration (High Concentration-HC and Low Concentration-LC) on phytoremediation potential depending on the part of the plant (root, stem and leaf) was determined, ($p \leq 0{,}05$).

Table 2. Values of tested heavy metal, lead, Pb in plant material (mg/kg)

Pb	Root	Stem	Leaf
Control	15,123±0,38^{a}	3,982±0,99^{b}	4,082±0,98^{c}
Low concentration, LC	21,66±0,19^{b}	16,19±0,03^{a}	13,59±0,73^{a}
High concentration, HC	26,610±0,29^{b}	16,47±0,03^{a}	16,12±0,59^{b}
ANOVA	$p \leq 0{,}05$	$p \leq 0{,}05$	$p \leq 0{,}05$

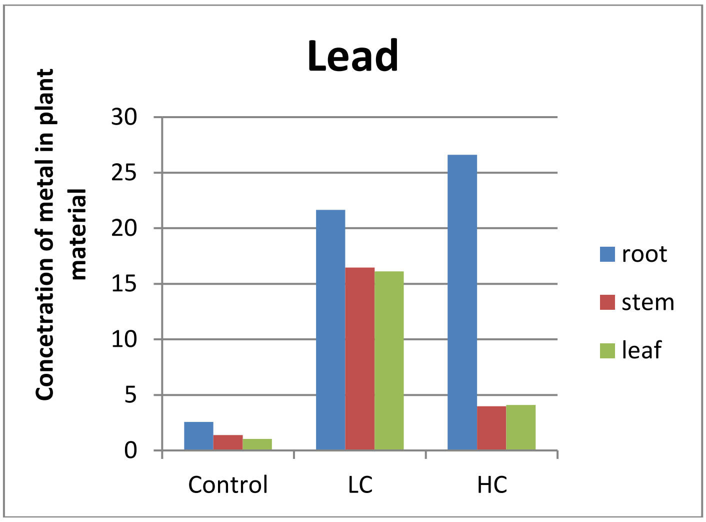

Figure 2. Concentration of Lead, Pb in plant material

Table 3. Values of tested heavy metal, cadmium, Cd in plant material (mg/kg)

Cd	Root	Stem	Leaf
Control	2,231±0,64^{a}	12,52±1^{a}	5,904±0,97^{a}
Low concentration, LC	19,18±0,07^{b}	13,94±0,71^{a}	12,40±0,95^{b}
High concentration, HC	20,398±0,54^{b}	30,490±0,09^{b}	13,09±0,87^{b}
ANOVA	p≤0,05	p≤0,05	p≤0,05

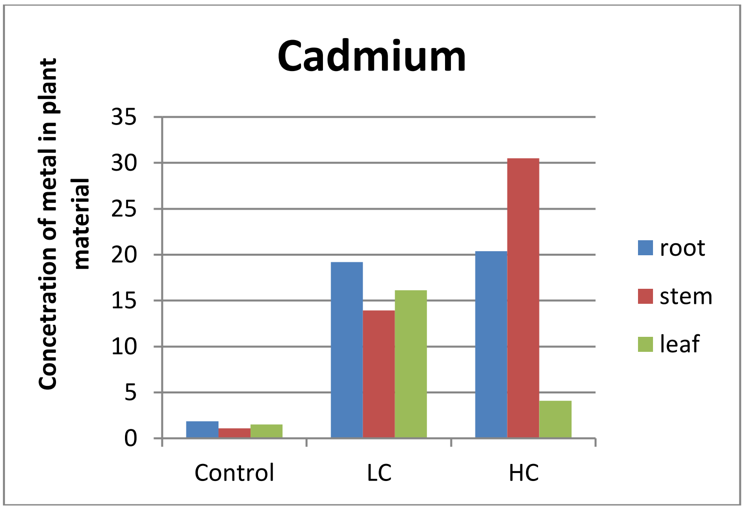

Figure 3. Concentration of cadmium, Cd in plant material

Table 4. Values of tested heavy metal, zinc, Zn in plant material (mg/kg)

Zn	Root	Stem	Leaf
Control	4,37±0,382^{a}	9,06±1,01^{a}	0,216±0,97^{a}
Low concentration, LC	9,421±0,662^{b}	9,50±0,910^{a}	5,46±0,95^{b}
High concentration, HC	11,426±0,762^{b}	24,443±0,080^{b}	6,15±0,92^{b}
ANOVA	p≤0,05	p≤0,05	p≤0,05

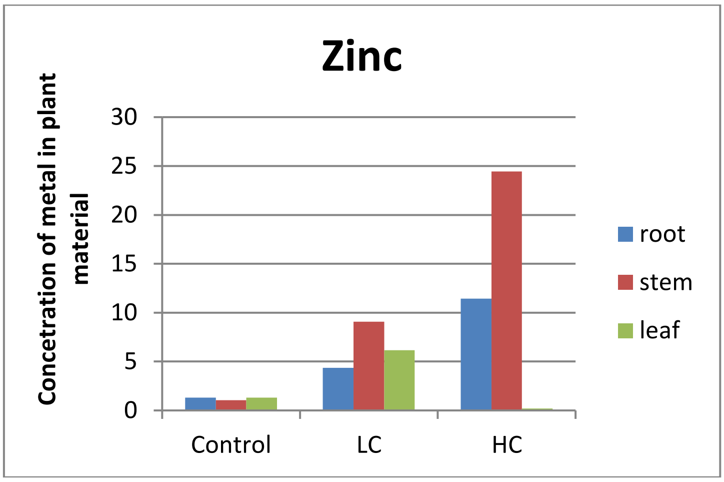

Figure 4. Concentration of zinc, Zn in plant material

Plants with phytoremediation ability are characterized by the accumulation of lead in the root system, and according to [14], this in a way represents a form of protection of the aboveground part. Natural concentrations of lead in plants range from 5 to 10 mg/kg,[15]. Lead concentrations in the tested plants during treatment with low and high concentrations are significantly higher. Lead belongs to the metals that accumulate in the underground parts of plants. Generally, the lead content in plant organs tends to decrease in the following order: roots> leaves> stems> flowers [16]. Factors that most affect the accessibility of Cd and Zn plants are concentration of Cd and Zn in the soil and the form in which they are, soil pH, content of organic matter, clay and calcium carbonate, microbiological activity in the rhizosphere, soil moisture, soil temperature, concentration of other elements, especially phosphorus and climate. The root helps the plant to absorb cations by secreting low molecular weight compounds, which function as metal chelators in the soil. Depending on the plant species, these are most often organic acids, which dissolve inaccessible forms of nutrients in the soil and phytosiderophores, which affect the enhanced work of the microflora and the faster transition of metals into the accessible form. An increase in the concentration of Cd in the soil can reduce the availability of certain metals from the soil as well as reduce the number of populations of soil microorganisms. Such action of Cd can reduce the uptake of Zn into the root, which is not an example of direct competition of Cd and Zn, but the reduced uptake of Zn is a consequence of the creation of unfavorable conditions for the uptake of Zn. It is well known that iron (Fe) in plants of the *Poaceae* family is absorbed into the root by phytosiderophores [17] found that Zn, except in the form of Zn^{2+} is adopted.

Several studies indicate that ZIP proteins play a significant role in the uptake of Zn in cereals [18], [19], [20] play a significant role in the uptake of Zn in cereals. hence Cd^{2+}, but that each transporter is specifically controlled by one particular metal [21]. Since Cd is a non-essential toxic element, it was considered that this is the reason why plants do not have a specific system for its adoption. It has been hypothesized and confirmed by numerous studies [21], [22] that Cd, like other toxic cations, is adopted together with essential elements, and because of its chemical similarity to Zn, it is assumed that they are adopted into the plant by the same mechanisms [23],[24]. In addition to common uptake mechanisms with other cations, the existence of Cd-specific transporters has been established [4]. Lead mainly accumulated in the underground parts of corn while the aboveground parts contained smaller amounts of this metal.

Among the studied metals, the concentration of Cd was found in the largest quantities, in the aboveground part of the stem.

Furthermore, the results of the research showed that corn shows tolerance to heavy metals. Even high concentrations of heavy metals (Cd and Pb) in the soil did not completely eliminate the ability to grow. Some metals such as Pb did not have a negative effect on maize growth and maize grew at a similar rate as control plants. Only at higher doses of Cd (100 mg/kg) was growth-retarded observed, indicating symptoms of "metal stress" in plants involving morphological and structural changes in aboveground shoots, such as leaf deformation and chlorosis, leaf necrosis, red and purple leaf color, brown leaf edges, reduced leaf number and size, reduced biomass, inhibited growth and eventual death [25].

Conducted research [26], have shown that *Helianthus annuus L.* has the ability to accumulate Pb, Cd and Zn in its tissue (shoots and root) depending on the type of metal and the applied concentration. The highest concentrations of Pb were recorded in the root of the plant (24.03 mg / kg), while higher concentrations of Cd (16.13 mg / kg) and Zn (14.696 mg / kg) were recorded in the aboveground part of the plant (leaf). The accumulation of heavy metals and their distribution depends on the type of plant, plant organs, phenological phase, degree of contamination and combination of metals in the soil. Some research, [27] showed that the increased concentration of heavy metals in the soil stimulated the growth of the Japanese knotweed, *Reynoutria japonica*. This fact proves that *R. japonica* accumulates heavy metals efficiently when, it is grown in soils contaminated with heavy metals. This was especially observed in the case of Cd. In the aboveground parts of the plant from the samples with the addition of high concentration (HC), Cd 100 mg kg-1, 537 times more Cd was found compared to the control.

According to [15], 3-8 mg/kg Cd in the soil is a critical concentration for plant growth. Analysis of metal concentrations in plant tissues showed a high potential of corn for the uptake of heavy metals and their intensive accumulation in aboveground parts (in the case of Cd, Zn) and underground parts (in the case of Pb).

Translocation factor is defined as the ratio of the metal concentration in the aboveground part of the plant with respect to the metal concentration in the root and is used to determine the efficiency of the plant in translocating heavy metal from root to aboveground part. The translocation factor indicates an enhanced ability of plants to bioaccumulate heavy metals compared to the control sample.

Table 5. Translocation factor (TF)

Element	Low Concentration	High Concentration
Pb	0,76	0,14
Cd	0,72	1,49
Zn	2,07	2,14

$$TF = \frac{metal\ (\ shoot)}{metal\ (\ root)}$$

Translocation factor is defined as the ratio of the mass fraction of metal in the aboveground part of the plant with respect to the share of metal in the root and is used to determine the efficiency of the plant in translocating heavy metal from root to aboveground part. The translocation factor indicates an enhanced ability of plants to bioaccumulate heavy metals compared to the control sample. The obtained values for TF are the most important test that can be used to assess the phytoremediation potential of the plant [28].

Plants that have a translocation factor less than 1, can be used as photo stabilizers (metal concentration in the underground part of the plant), and as phytoextractors if they have a translocation factor greater than 1, transport and concentrate metals from soil to aboveground parts of plants [29].

The results of this study show that in maize from the control sample for all three heavy metals TF is greater than 1, and based on this indicator, corn can be classified as an accumulator of these heavy metals (Pb, Cd and Zn).

CONCLUSIONS

The experimental study was conducted to identify the ability of maize (*Zea mays*) to remove heavy metals (Pb, Cd and Zn) from contaminated soil. The main mechanism of remediation of heavy metals using the corn (*Zea mays*) is based on the extraction of contaminants from the soil (phytoextraction, followed by translocation and accumulation of contaminants in the aboveground part of the plant).

Analysis of the collected and processed data proved that corn (*Zea mays*) has the ability to accumulate Pb, Cd and Zn in its tissue (shoots and root system) depending on the type of metal and the applied strength of concentration. The highest concentrations of Pb were recorded in the root system of the plant (26.61 mg/kg), while higher concentrations of Cd (30.49 mg/kg) and Zn (24.43 mg/kg) were recorded in the aboveground part of the plant (stem). The accumulation of heavy metals and their distribution depends on the type of plant, plant organs, phenological stage, degree of contamination and combination of metals in the soil.

Based on the obtained results, it can be concluded that corn (*Zea mays*) is a tolerant plant species in soils contaminated with heavy metals (Pb, Cd and Zn) and can be successfully used in the phytoremediation of soil contaminated with these heavy metals.

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