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Original scientific paper

PHYTOREMEDIATION OF SOIL CONTAMINATED WITH HEAVY METALS USING THE SUNFLOWER (*HELIANTHUS ANNUUS L.*)

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ABSTRACT: Phytoremediation has proven to be a suitable method for removing heavy metals from the soil with the help of plants. To examine the phytoremediation potential, the experimental study monitored the influence of high and low concentrations of heavy metals (Pb, Cd, and Zn) on *Helianthus annuus L.*, as well as their accumulation in seeds, roots, stems and leaves. The experiment was carried out during one growing season in outdoor conditions, in pots with a volume of 5L in which *Helianthus annuus L.* was planted, and the soil was contaminated with these heavy metals in concentrations below and above the maximum allowable concentration prescribed by the Rulebook on Determining Permissible Quantities of Harmful and Dangerous Substances in Soil and Methods of Their Testing. After growth and development of the plant, the experimental research examined the accumulation potential of the plant, the growth of the plant itself and its ability to survive depending on different concentrations of heavy metals. By processing the obtained results, statistically significant differences of heavy metals were determined in individual parts of the plants, depending on the applied concentration. The highest concentration of Zn was recorded in the leaves of the plant in the amount of 18.21 mg/kg, and the lowest concentration in the stem, 3.92 mg/kg. The measured values of heavy metals Cd and Pb differ from the above because the lowest concentrations of these heavy metals were recorded in the seed, and were 12.02 mg/kg for Pb and 9.20 mg/kg for Cd, which is a statistically significantly lower determined value relative to other parts of the plant.

Keywords: phytoremediation, *Helianthus annuus L.*, heavy metals.

INTRODUCTION

Heavy metals are a major group of inorganic contaminants. Significantly large areas of land are contaminated with heavy metals due to the use of sludge or municipal compost, pesticides, fertilizers, emissions from municipal waste incinerators, car exhaust, metallurgical industry, residues from mining and military activities, and smelting industry (Abdelhafez et al., 2012.). Unlike organic pollutants, heavy metals are not biodegradable and pose a problem to living organisms and the environment due to their carcinogenic effects (Wu et al., 2018.). The presence of heavy metals in the soil in high concentrations negatively affects plant growth and agricultural productivity (Roy et al., 2005.). Plants differ in their tolerance to heavy metals; however, most growing plants are not hyperaccumulators for heavy metals (Peixoto et al., 2001; Hall, 2002.). Hyperaccumulation is defined as the uptake and sequestration of exceptional concentrations of an element in the aboveground parts of a plant under field conditions (Pollard, 2000.). Hyperaccumulation depends on the plant species, soil physicochemical properties (pH, cation exchange capacity, organic matter content, electrical conductivity (E.C), and different types of heavy metals. In hyperaccumulators, there is a rapid and active translocation of the heavy metals to the shoot via the xylem, which could probably be up-regulated by transpiration (Chaudhary et al., 2016.). Some plants are known for their ability to accumulate abnormally high concentrations of metals, such as Zn, Ni, Mn, or Pb, in their aboveground parts to more

than 1% of their dry weight. These plants are called hyperaccumulators. About 450 plant species have been identified as hyperaccumulators for different heavy metals (Balafrej et al., 2020.).

Cd, Cr, Cu, Hg, Pb and Zn are the most common heavy metals that occur as contaminants (Jing et al., 2007.). Phytoremediation is a method that should be considered for remediation of contaminated sites due to its cost-effectiveness, aesthetic benefits, and long-term applicability (Marques et al., 2008.). Phytoremediation is a method of remediation, recovery, cleaning of the soil (water or air) using plants. Phytoremediation technology includes phytoextraction, phytostabilization, and phytovolatilization to remove metal ions from soil and water (Alaboudia et al., 2018.). Phytoextraction is an effective way to remove heavy metals from contaminated soil into plant tissue (Abbas and Abdelhafez, 2013.); after which it can be easily and safely processed or recycled (Lasat, 2002.). Plants differ in their ability to accumulate heavy metals (Kacalkova et al., 2015.). The choice of plant species for phytoextraction of heavy metals depends mainly on the tolerant capacity and biomass of the selected plant (Rezania et al., 2016.). Some plant species can accumulate high concentrations of heavy metals in their tissue; however, they produce little biomass and grow slowly, making them impossible to use for phytoremediation. Plants used for phytoextraction of metals from contaminated soil must have certain characteristics, including (i) tolerance to high concentrations of metals; (ii) short growing season and efficient accumulation of metals in biomass; (iii) metal concentration in the aboveground part of the plant; and (iv) easy to pick (Nehnevajova et al., 2007.). Plant species that generate large amounts of biomass, such as *Helianthus annuus L.*, have the potential to remove large amounts of heavy metals that accumulate in the aboveground part of the plant (Safari Sinegani and Khalilikhah, 2008.).

HELIANTHUS ANNUUS L.

Helianthus annuus L. is an annual plant native to America that belongs to the *Asteraceae* family with a large flower head (inflorescence) and grows in a wide range of different soil types. The flower stalk can grow to a height of 3 m, with a flower head reaching up to 30 cm in diameter (Alaboudia et al., 2018.). *Helianthus annuus L.*, a fast-growing crop, is tolerant of heavy metals. It is used for rhizofiltration because it shows a large intake of heavy metals through the roots, but also shows low efficiency in their translocation from root to shoot (Zou et al., 2008.). The ability of *Helianthus annuus L.* to absorb heavy metals was tested in the field, container, and hydroponic conditions with different concentrations of pollution. *Helianthus annuus L.* has been found to accumulate large amounts of metals (Zn, Pb, Cu) (Angelova, 2016.). According to most authors, heavy metals mainly accumulate in *Helianthus annuus L.* root (Lin et al., 2003; Marchiol et al., 2007.). Other authors have reported that some metals move from the root to the aboveground part of the plant (Adesodun et al., 2010; Herrero et al., 2003.). According to Rivelli et al. (2012) during the cultivation of *Helianthus annuus L.* on soil contaminated with cadmium (Cd) and on soil with combined pollution (Cd, Zn, and Cu), a significant impact on the physiology and growth of the plant was observed only in the variant where the soil was contaminated with more metals, with significant accumulation of metals in the tissue, especially in the roots and in the old leaves. Nehnevajova et al. (2005) evaluated the potential use of the *Helianthus annuus L.* for phytoremediation and reported that *Helianthus annuus L.* can be used for phytoextraction of soils contaminated with heavy metals.

This study aimed to examine the phytoremediation potential of the *Helianthus annuus L.* in soil contaminated with high and low concentrations of heavy metals (Pb, Cd, and Zn), as well as accumulation of the same in the seed, root, stem, and leaves of the plant.

MATERIAL AND METHOD

The experiment was conducted during one growing season in pots with a volume of 5L in outdoor conditions, on the property of the Biotechnical Faculty of the University of Bihać and consisted of control (soil without heavy metals) and soil contaminated with high and low concentrations of heavy metals (Pb, Cd, and Zn). *Helianthus annuus L.* was used as a test plant. It was chosen because is known to be a fast-growing deep-rooted industrial oil crop that can remove heavy metals such as zinc or copper from a polluted environment (Nehnevajova et al., 2005.). Before conducting the experiment, a chemical analysis of the soil was performed, the results of which are shown in Table 1. For the purposes of the research, three pots were used, in three replications. 4 *Helianthus annuus L.* seeds were sown in each pot. Pots with soil and *Helianthus annuus L.*, without the addition of heavy metals, were treated as a control. The soil in other pots was treated with a certain concentration of heavy metals (Pb, Cd, and Zn).

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

| Parameter | Measuring unit | Analysis results |
|-------------------------------|----------------|------------------|
| Depth | cm | 0-30 |
| Hygroscopic moisture (Hy) | % | 4.70 |
| Organic part | % | 21.42 |
| Mineral part | % | 78.58 |
| Humus | % | 0.54 |
| Active acidity | | 7.07 |
| pH in KCl | | 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 ₂ O ₅ | mg/kg | 3.79 |
| SO ₄ ²⁻ | mg/kg | 27.13 |
| K ₂ O | mg/kg | 9.22 |
| K | mg/kg | 7.59 |
| Ca ²⁺ | mg/kg | 553.48 |
| Mg ²⁺ | mg/kg | 81.39 |

As a criterion for determining the concentration of heavy metals added during the research, the maximum permitted amounts (MPA) of pollutants prescribed by the Rulebook on Determining Permissible Quantities of Harmful and Dangerous Substances in Soil and Methods of Their Testing were taken (Official Gazette of the Federation of BiH, 72/09). Accordingly, solutions of the corresponding salts (solution of lead nitrate Pb(NO₃)₂ 1 mol/L; cadmium sulfate CdSO₄ 0.1 mol/L and zinc sulfate ZnSO₄ 1 mol/L) were prepared and then diluted and adjusted the mass of the soil in order to define the exact mass concentration of the ions present, which are removed by the remediation procedure. The low concentration (LC) of lead

ions (Pb^{2+}) was 1000 mg, cadmium (Cd^{2+}) 20 mg, and zinc (Zn^{2+}) 1000 mg per kilogram of soil. The high concentration (HC) of lead ions (Pb^{2+}) was 2000 mg, cadmium (Cd^{2+}) 100 mg, and zinc (Zn^{2+}) 2000 mg per kilogram of soil. Prepared solutions of heavy metals were applied directly to the pots. This experiment lasted 30 days. After 30 days, the preparation of plant material for determining the concentration of heavy metals in the seeds, roots, stem, and leaves of the plant was started. Preparation, storage, and analysis of soil samples were done according to the requirements of ISO standards. The roots were dug up and separated from the soil by washing. Shoots are divided into stem, leaf, and head of the *Helianthus annuus L.* The samples were packed in plastic bags and transferred to the laboratory of the Biotechnical Faculty. In the laboratory, they were washed with water, cut into pieces, and then dried for 78 hours at 60°C. To determine the concentration of heavy metals, the samples had to be prepared properly. Atomic air/acetylene flame absorption spectroscopy using ready-made standards for all three heavy metals (F-AAS, Flame AAS) (Perkin Elmer, AA800) was used to determine the concentration of Pb, Cd, and Zn.

RESULTS AND DISCUSSION

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.

Table 2. Recorded Pb values in plant material

| Pb (mg/kg) | root | stem | leaf | seed |
|---|-------------|-------------|--------------|-------------|
| Control | 7.332±0.32b | 3.029±0.99a | 11.914±0.98c | 12.02±0.99c |
| Low concentration | 17.11±0.51b | 16.87±0.83b | 21.32±0.93a | 16.41±0.98b |
| High concentration | 24.35±0.08a | 22.05±0.23b | 22.03±0.59b | 18.97±0.97c |
| ANOVA $p \leq 0.05$ $p \leq 0.05$ $p \leq 0.05$ $p \leq 0.05$ | | | | |

Plant culture (*Helianthus annuus L.*) was chosen for experimental research due to its high biomass, rapid growth rate, and plant ability to remove heavy metals from contaminated soil (Forte and Ronca, 2017). 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, significant changes were observed on young leaves, leaf chlorosis, plants remained low and poorly developed, which is not the case with plants grown in soil contaminated with low metal concentrations, which developed normally and achieved the expected development characteristic of the mentioned type. Symptoms of plant toxicity with Pb, Cd, and Zn in higher concentrations were reflected in the formation of dark green leaves, necrosis of tissue of older leaves at the end of vegetation, but also the formation of a significantly shorter root system compared to control treatment, extremely dark in color. Based on the results of the analysis of the variance of the conducted research, a significant influence of the treatment (high and low concentration of the solution) on the phytoremediation ability of the plant and the uptake of heavy metals from the soil into various plant organs was determined. According to Mitić et al., (2013), plants are more difficult to absorb lead in inorganic form and move to aboveground organs, except on acidic soils, while it is much easier to absorb organic lead compounds and transported to aboveground parts of plants. Plants with phytoremediation ability are characterized by the accumulation of lead in the root system, and according to Mitić et al., (2013), 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 (Radojević and Bashkin, 1999). The concentrations of lead in the tested plants during the treatment with low and high concentrations are significantly higher. Research by Angelova et al., (2016) and the determination of *Helianthus annuus L.* potential, as well as its phytoremediation ability, depending on soil type and fertilization applications, showed that the highest lead concentration was recorded in leaves (59%) and significantly lower in seeds (1%). The conducted studies are in accordance with the research of Angelova et al., (2016) because at low concentrations of lead in the soil the highest concentration of this heavy metal was recorded in leaves (21.32 mg/kg), while at treatment with high concentrations of lead the highest value in the root system (24.03 mg/kg).

Table 3. Recorded values of the tested heavy metal Cd in plant material

| Cd (mg/kg) | root | stem | leaf | seed |
|---------------------------------|--------------|-------------|-------------|-------------|
| Control | 2.626±0.64a | 9.44±1c | 9.20±0.97c | 4.638±0.96b |
| Low concentration | 10.32±0.07 b | 9.10±0.71 a | 11.92±0.95c | 9.16±0.83a |
| High concentration | 11.82±0.54c | 11.97±1.19c | 16.13±0.47a | 12.91±0.98b |
| ANOVA p≤0.05 p≤0.05p≤0.05p≤0.05 | | | | |

Studies conducted by Alaboudia et al., (2018) prove that generally, *Helianthus annuus L.* accumulates a higher amount of Pb in the root compared to the shoot. However, *Helianthus annuus L.* also accumulates large amounts of Cd in its shoots compared to Pb, but an increase in soil Cd concentration above 20 mg/kg leads to a decrease in BCF (bioconcentration factor) below 1, which affects the potential phytoremediation capacity of plant species (Zu et al., 2005.). The obtained results showed that *Helianthus annuus L.* can accumulate large amounts of Cd in its tissues in relation to Pb and therefore this plant can be effectively used for the remediation of soils contaminated with cadmium. The obtained results of the conducted experimental research, where the highest concentrations of Cd in the treatment of HC and LC were measured in the aboveground part of the plant (leaf), and the amounts for LC 18.21 mg/kg and HC 14.96 mg/kg, agree with previously conducted studies. Lee et al., (2013) and Forte and Mutiti (2017), who found that the plant *Helianthus annuus L.* is significantly more favorable for the accumulation of Cd in its tissues compared to other heavy metals.

Table 4. Recorded values of the tested heavy metal Zn in plant material

| Zn (mg/kg) | root | stem | leaf | seed |
|---------------------------------|--------------------------|-------------------------|--------------------------|-------------------------|
| Control | 4.64±0.09 ^a | 4.638±0.99 ^a | 8.54±0.66 ^c | 7.25±0.99 ^b |
| Low concentration | 7.05±1.64 ^a | 9.10±1.55 ^c | 8.21±0.69 ^b | 7.74±0.99 ^b |
| High concentration | 10.337±0.34 ^a | 11.97±0.25 ^b | 14.696±1.32 ^c | 12.64±0.79 ^b |
| ANOVA p≤0,05 p≤0,05p≤0,05p≤0,05 | | | | |

Zinc belongs to the group of elements whose mobility through the plant is mediocre when both the ascending and descending flow is in question. In the case when the concentration of Zn in the soil is extremely low, the consequence is a low intensity of transfer from older to younger parts of the plant. In cases when its concentration in the soil is high, it mainly settles in the roots of plants. If its concentration

decreases to 10 to 20 mg/kg, a latent (hidden) deficiency occurs, and if it decreases below 10 mg/kg, an acute deficiency occurs with symptoms of deficiency and plant death (Živanović, 2010.). Symptoms of Zn toxicity in plants are manifested in reduced growth, tiny leaves with reddish-brown spots, and reduced roots (Alloway, 2008). The results show that the lower concentration of Zn below 10 mg/kg remained mostly in the stem, which resulted in the formation of small leaves covered with spots, while at high concentrations of Zn the highest value was recorded in the leaves (14.69 mg/kg) and slightly lower in seeds (12.56 mg/kg).

Table 5. Translocation factor (TF)

| Element | Control | Low concentration | High concentration |
|---------|---------|-------------------|--------------------|
| Pb | 1.62 | 1.24 | 0.90 |
| Cd | 3.50 | 1.15 | 1.36 |
| Zn | 1.84 | 1.16 | 1.42 |

$$TF = \text{metal (shoot)} / \text{metal (root)}$$

The translocation factor is defined as the ratio of the mass fraction of metal in the aboveground part of the plant concerning 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 (Angelova et al., 2016.). The results of this study show that in *Helianthus annuus L.* from the control sample for all three heavy metals TF is greater than 1, and based on this indicator, *Helianthus annuus L.* can be classified as an accumulator of these heavy metals (Pb, Cd, and Zn). Angelova et al. (2016) in their study, which examined the phytoremediation potential of *Helianthus annuus L.* for heavy metals (Pb, Cd, and Zn), also obtained values of $TF > 1$, and their results showing the highest value of TF in the control sample for Cd, and the smallest for Pb.

CONCLUSIONS

The experimental study was conducted to identify the ability of *Helianthus annuus L.* to remove heavy metals (Pb, Cd, and Zn) from contaminated soil. The main mechanism of remediation of heavy metals using the plant *Helianthus annuus L.* 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 showed that *Helianthus annuus L.* 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 (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 stage, degree of contamination, and combination of metals in the soil.

Based on the obtained results, it can be concluded that *Helianthus annuus L.* is a tolerant plant species in soils that are low to moderately contaminated with heavy metals (Pb, Cd, and Zn) and can be successfully used in phytoremediation of soil contaminated with these heavy metals.

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