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EXAMINATION OF THE POSSIBILITY OF ENHANCING THE PHYTOREMEDIATION POTENTIAL OF PLANTS THROUGH COMPLEXATION OF ALKALINE SOIL

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ABSTRACT

This study presents the results of applied phytoextraction as a soil phytoremediation method, based on the analysis of selected heavy metal content in soil and plant material. The selected locations where phytoextraction was applied as a phytoremediation method (Gradišće, Podbrežje, and Tetovo) are situated in the city of Zenica, in proximity to the ArcelorMittal d.o.o. Zenica plant, identified as a potential major source of soil contamination in the surrounding area. The plant species used as potential phytoremediators were maize, Swiss chard, and alfalfa. The study analyzed the heavy metal content (Zn, Ni, Pb, Cd, Cr, and Cu) in soil samples after plant material extraction, as well as in the root samples of the plant material. Additionally, the study presents the pH values of the soil before sowing (initial state) and after plant extraction. The primary objective of this research was to determine whether soil complexation with an aqueous EDTA solution contributed to the increased uptake of selected heavy metals from soil into the selected plants under real environmental conditions. To assess this, one portion of the land plots was treated with a 0.1 M EDTA solution (from sowing until the late growth stage), while the other portion was left untreated. The results presented in this study indicate that soil complexation with the EDTA solution did not significantly enhance the phytoremediation potential of plants in the majority of analyzed samples. One of the key reasons for the reduced mobility of heavy metals from soil into plant material may be the alkaline nature of the soil at all three study locations, with a pH > 8. The mobility of heavy metals is significantly higher in acidic soils compared to alkaline soils.

Keywords: Phytoextraction, complexation, EDTA solution, heavy metals, plants, soil

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1. INTRODUCTION

Soil contamination by heavy metals is a worldwide problem for human health and safe food production. Except for uncommon geogenic origins, heavy metal contaminants are inadvertently introduced to soils through anthropogenic activities such as mining, smelting, warfare and military training, electronic industries, fossil fuel consumption, waste disposal, agrochemical use, and irrigation [1]. A promising approach to addressing heavy metal pollution through the use of plants is possible due to the phenomenon of metal hyperaccumulation in certain plant species, which, in addition to their tolerance,

possess the enhanced ability to accumulate heavy metals from the soil into plant tissues [2]. Phytoremediation is an emerging technology that employs the use of higher plants for the cleanup of contaminated environments [3]. The concept of phytoremediation was first introduced in 1983 and still, this technique is at the testing stage [4]. Unlike plants that are merely tolerant to heavy metals, hyperaccumulators actively absorb large amounts of one or more different heavy metals, translocate them to aboveground plant organs, and accumulate them at concentrations 100 to 1,000 times higher than non-hyperaccumulating plants. Unlike roots, metal-enriched shoots can be harvested and safely disposed of, with the potential for metal extraction. Phytoextraction is a phytoremediation method by which contaminants (heavy metals) are absorbed from the soil through the root system and then translocated to other plant tissues [5]. Soil decontamination via phytoextraction can be accelerated by adding complexing chemical compounds (chelators), which enhance metal solubility and mobility in the soil, leading to greater metal uptake by plants [6]. The time required for remediation depends on the concentration of heavy metals in the contaminated soil, the growing season, and the selected plant species, ranging from 1 to 20 years. This method is particularly suitable for decontaminating large areas of contaminated soil at shallow depths with low to moderate levels of contaminants. In soil, heavy metals are present in chemically bound forms (oxides, sulfides, sulfates, and carbonates) that are practically insoluble in water. However, since water is a universal solvent due to the polarity of its molecules, chemical compounds binding heavy metals eventually become soluble in water. The objective of this study is to determine whether soil complexation with an aqueous EDTA solution enhances the uptake of selected heavy metals into plants under real environmental conditions. Complexing agents increase metal solubility and, consequently, the mobility of heavy metals in the soil, allowing for greater accumulation in plants, as plants can absorb nutrients and other substances only in aqueous solutions. EDTA solution is a weak acid, and its solutions exhibit mildly acidic properties. Previous studies conducted under controlled (laboratory) conditions have demonstrated that soil complexation with an EDTA solution can significantly enhance the phytoremediation potential of certain plants, particularly increasing lead accumulation in maize [6]. However, it is important to note that in these controlled studies, in addition to EDTA soil complexation, the soil pH was deliberately lowered using an $Al_2(SO_4)_3$ solution, which was not the case in the present research [6].

2. EXPERIMENTAL PART

Experimental soil plots of 300 m² each were selected at three locations in the Zenica area (Gradišće, Podbrežje, and Tetovo). Each experimental plot was divided into two sections, where maize, Swiss chard, and alfalfa were sown from seed. In one section of each plot, the soil was complexed with a 0.1 M EDTA solution (from the sowing period to the late growth stage), while in the other section, no soil complexation was performed. At the end of the growth period, plant material was manually sampled (aboveground plant parts along with the root), packed in plastic bags on-site, and transported to the laboratory. Soil sampling was conducted by Article 35 of the Regulation on the Determination of Permissible Quantities of Harmful and Hazardous Substances in Soil and Methods for Their Testing (*Official Gazette of the Federation of Bosnia and Herzegovina*, No. 72/09), while soil sample preparation followed Article 36 of the same regulation. A composite soil sample (~2 kg) was collected from 10 to 20 individual samples, taken using a chromed soil probe and plastic hand tools. Soil samples for laboratory analysis were taken from a depth of 0–20 cm. Determination of the total and available content of heavy metals (Zn, Ni, Pb, Cd, Cr, Cu) was performed using an AAS method (using an atomic absorption spectrometer,

PERKIN ELMER 3110). Chemical analysis of heavy metals was done according to standard ISO 11 466. The above analyses were carried out at the "Kemal Kapetanović" Institute at the University of Zenica.

3. RESULTS AND DISCUSSION

The objective of this study is to determine whether soil complexation with an aqueous EDTA solution contributes to an increased uptake of selected heavy metals from the soil into selected plant species (enhancing the phytoremediation potential of plants) under real environmental conditions. Tables 1 and 2 present data on heavy metal concentrations in soil samples (Table 1) and plant material (Table 2). Table 1 provides data for analyzing the effect of soil complexation on the uptake of selected heavy metals by plants indirectly, by comparing heavy metal content in non-complexed and EDTA-complexed soil across different plots. Table 2 provides data for analyzing the effect of soil complexation on the uptake of selected heavy metals by plants directly, by comparing heavy metal content in plants grown in non-complexed and EDTA-complexed soil across different plots.

Table 1. Heavy metal concentrations in soil samples after plant material removal ($mgkg^{-1}$)

Heavy metals	ZPK	ZPKE	ZTK	ZTKE	ZGK	ZGKE	ZPL	ZPLE	ZTL	ZTLE	ZGL	ZGLE	ZGB	ZGBE
Zn	70	80	180	250	**140**	**120**	90	100	170	250	**120**	**110**	**100**	**80**
Ni	**220**	**200**	100	140	70	90	**210**	**190**	**140**	**120**	50	70	60	70
Pb	50	70	100	180	**220**	**80**	70	80	100	150	**80**	**70**	**70**	**60**
Cd	1	1	**2**	**1**	<1	2	1	1	1	2	1	2	1	1
Cr	260	260	70	70	**70**	**60**	**270**	**240**	70	90	70	70	**70**	**40**
Cu	30	20	50	50	40	40	30	30	50	50	**50**	**40**	40	40

Z-Soil, P-Podbrežje, T-Tetovo, G-Gradišće, K-Maize, B-Swiss Chard, L-Alfalfa, E-Complexed with EDTA

Table 2. Heavy metal concentrations in plant root samples ($mgkg^{-1}$)

Heavy metals	KP	KPE	KT	KTE	KG	KGE	LP	LPE	LT	LTE	LG	LGE	BG	BGE
Zn	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	14	<1	<1
Ni	53	41	**<1**	**16**	13	<1	29	14	**11**	**14**	14	13	39	17
Pb	53	20	52	16	**13**	**14**	15	15	<1	<1	<1	<1	<1	<1
Cd	<1	<1	2	<1	<1	<1	**<1**	**3**	**1**	**3**	1	1	2	1
Cr	**26**	**41**	**<1**	**16**	13	<1	44	44	21	<1	<1	<1	<1	<1
Cu	26	20	**25**	**33**	39	29	<1	<1	<1	<1	<1	<1	<1	<1

P-Podbrežje, T-Tetovo, G-Gradišće, K-Maize, B-Swiss Chard, L-Alfalfa, E-Complexed with EDTA

Analyzing the effect of soil complexation on the uptake of selected heavy metals by plants, indirectly through heavy metal content in the soil, the bolded results in Table 1 show that complexation with the EDTA solution had the greatest effect on soil samples from the Gradišće location, when analyzing all three plant species and all heavy metals. At the Gradišće location, it was observed that the concentration of heavy metals in the soil complexed with the EDTA solution decreased in nine out of the eighteen comparisons, when comparing samples from non-complexed soil with those from EDTA-complexed soil. In four of the eighteen comparisons, there were no changes in concentration, while in five comparisons, a counter-effect was recorded, meaning a reduction in the uptake of selected heavy metals by the plants. At the other locations (Podbrežje and Tetovo), soil complexation did not show the desired effect. One possible reason for the more significant impact of soil complexation on the uptake of selected heavy metals at the Gradišće location could be the soil pH (Tables 3 and 4). The soil from Gradišće shows slightly lower pH

values compared to the soils from Tetovo and Podbrežje. The solubility of heavy metal compounds increases with lower pH, which in turn increases their mobility in the soil in this case [7]. Analyzing the effect of soil complexation on the uptake of selected heavy metals by plants, directly through
heavy metal content in the plants, the bolded results from Table 2 show that complexation with the EDTA solution did not affect increasing the uptake of selected heavy metals by the plants (phytoremediation potential) in the majority of the analyzed samples. These results were unexpected, given that literature data suggests that soil complexation with an EDTA solution can significantly increase the accumulation of certain heavy metals in specific plant species, particularly the increased accumulation of lead in maize [6]. One of the key reasons for the reduced mobility of heavy metals from the soil into plant material is the alkaline soil present at all three locations (Table 3).

Table 3. pH values of soil samples before planting (initial condition)

pH values	ZP	ZT	ZG
pH in H_2O	8.27	8.32	8.10
pH in KCl	7.35	7.66	7.34

P-Podbrežje, T-Tetovo, G-Gradišće

As mentioned, the pH value has a significant impact on the mobility of heavy metals in the soil, and consequently, on the availability of metals to plants. Reducing the pH by one unit increases the solubility, and thus the mobility, of heavy metals in the soil, such as zinc, cadmium, and nickel, by two times [7, 8]. Changing the pH value alters the solubility of these metals, whether they precipitate (usually with an increase in pH) or dissolve from poorly soluble oxides or hydroxides, forming inorganic and organic complexes in the soil. The mobility of lead increases when the pH value drops below 6.5 [9]. Complexing the soil with the EDTA solution did not significantly decrease the soil pH at all locations (Table 4) when compared to the pH values of the soil in its initial condition (Table 3).

Table 4. pH values of soil samples after removal of plant material

Sample	pH in H_2O	pH in KCl
ZPK	8.0	7.6
ZPLE	8.0	7.6
ZPKE	8.1	7.5
ZPL	8.1	7.5
ZTL	8.1	7.8
ZTB	8.0	7.6
ZTLE	8.0	7.7
ZTK	8.0	7.8
ZTKE	8.1	7.6
ZGB	8.0	7.6
ZGBE	8.0	7.7
ZGL	8.0	7.5
ZGLE	7.8	7.5
ZGK	8.1	7.7
ZGKE	8.1	7.7

Z-Soil, P-Podbrežje, T-Tetovo, G-Gradišće, K-Maize, B-Swiss Chard, L-Alfalfa, E- Complexed with EDTA

By conducting experiments in controlled laboratory conditions, the soil in pots was further acidified with an $Al_2(SO_4)_3$ solution [6]. On large land areas, under uncontrolled conditions, it is almost impossible to acidify the soil to the desired pH value. We believe that much better effects in terms of heavy metal mobility in the soil, and thus metal availability to plants, would be achieved if soil parcels with acidic soil were selected.

4. CONCLUSIONS

The goal of the research conducted in this study was to determine whether soil complexation with an EDTA solution contributed to increasing the uptake of selected heavy metals from soil into selected plants, all under real environmental conditions. Analyzing the effect of soil complexation on the uptake of selected heavy metals in the plants, directly through the heavy metal content in the plants, the results show that soil

complexation with the EDTA solution did not have an effect on increasing the uptake of selected heavy metals by the plants (phytoremediation potential) in the majority of analyzed samples. One of the key reasons for the reduced mobility of heavy metals from the soil into plant material is the alkaline soil at all three study locations, with a pH > 8. The mobility of heavy metals is much higher in acidic soils than in alkaline ones. Soil complexation with the EDTA solution did not significantly affect the reduction of soil pH. Possible evidence for this claim is provided by the bolded results in Table 1, which show that soil complexation with the EDTA solution had the greatest effect on soil samples from the Gradišće location, which, in turn, showed a slightly lower pH value compared to the soil pH at the other places.

The general conclusions related to the conducted research are as follows:

- The effect of significantly increasing the mobility of certain heavy metals in the soil, and consequently the availability of metals in certain plants, through soil complexation with an EDTA solution is more likely to occur under controlled laboratory conditions, where it is possible to significantly lower the soil pH, as demonstrated in some studies.
- The effect of increasing the mobility of certain heavy metals in the soil, and thus the availability of metals in certain plants, through soil complexation with an EDTA solution, is likely possible even under uncontrolled conditions, but only in acidic soils. This requires further investigation, and existing literature supports this possibility.

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Conflicts of Interest

The authors declare no conflict of interest.

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