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HEAVY METALS ACCUMULATION AND DISTRIBUTION PATTERN IN MAIZE PLANTS

SUMMARY

This paper investigates the pattern of heavy metal accumulation and distribution in maize plants grown in a field near Sarajevo International Airport. Soil and maize plant samples from the studied soil were analyzed for selected heavy metals, i.e., chromium (Cr), cadmium (Cd) lead (Pb), zinc (Zn), copper (Cu) nickel (Ni) and manganese (Mn). The pattern of their accumulation and distribution were assessed using bioaccumulation factor (soil-root transfer) and translocation factor (root-leaves transfer). In this study, all the heavy metals studied (except Zn and Mn) exhibited higher contents in the analyzed root tissues than in the above-ground parts of maize (stem, leaves and kernels), suggesting that maize plants have versatile mechanisms to restrict transport of toxic heavy metals Cr, Cd, Pb, Cu and Ni from root to other parts of maize plants. On the other hand, Zn and Mn were more efficiently transferred from the maize roots to leaves. These results were expected, since Zn and Mn play an important role in various metabolic processes in plants. The study results also indicate that maize plants have a strong ability to absorb Cd ions from soil and accumulate them in roots, suggesting that maize is an efficient plant in phytostabilizing Cd.

Keywords: bioaccumulation, root-leaves transfer, toxic heavy metals, translocation

INTRODUCTION

Maize (*Zea mays* L.) is a member of Poaceae family, originally from Central America. It was introduced into Europe at the end of the 15th century, from where it spread through the Old World. It is now one of the most important and widely-grown cereal crops worldwide. Maize is not only an important food and feed crop, but also an important raw material for the manufacture of many industrial products, including maize starch, oil, syrup and biofuels. Global

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demand for maize products is therefore expected to rise worldwide in the future (Revilla *et al.,* 2022). To achieve it's ever rising demand for maize products, farmers apply different agricultural practices including among others the use of enormous amounts of chemical pesticides and fertilizers that can pollute soils, groundwater and the atmosphere (Tudi *et al.,* 2021). Natural sources can also increase soil heavy metal pollution including soil parent materials, volcanic eruptions, and rock weathering. In any case, heavy metals in excess amounts in soils lead to reduction in food quality and yields and can cause serious effects to human health through the food chain (Alengebawy *et al.,* 2021). Therefore, food security, particularly in the context of important crops such as maize, has become a global concern.

Many studies have reported that the effects of heavy metals on plant growth and development greatly depend on soil physicochemical properties and both the form and concentration of heavy metals in the soil (AL-Huqail *et al.*, 2022; Xu *et al*., 2022). However, there are relatively few studies investigating the physiological mechanisms of plant responses to heavy metal toxicity. In this light, plants have developed different strategies to overcome elevated levels of heavy metals in soils during evolution (Sladkovska *et al.*, 2022). The best-first strategy is to minimize heavy metal uptake from the soil through root exudates, such as oxalic acid or citric acid, which have a high affinity to form stable heavy metal complexes in the rhizosphere. In addition, some root exudates have the capacity to change the pH of rhizosphere, leading to precipitation of heavy metals, thereby limiting their availability to plant roots (Yan *et al.*, 2020). At next stage, if these strategies fail and heavy metals enter the root, plants may activate different tolerance mechanisms for heavy metal detoxification. This includes among others the low translocation of heavy metals from roots to the aerial parts, the embedding of heavy metals in the plant cell walls as well as chelation and compartmentalization of heavy metals in different intracellular compartments such as vacuoles where they are stored without toxicity (Emamverdian *et al.*, 2015). Selection of strategy to cope with heavy metal toxicity primarily depends on plant genetic background and on environmental factors that affect plant growth (Viehweger, 2014).

The current study was designed to study the effect of soil characteristics and its heavy metals content on heavy metal accumulation and detoxification mechanisms in maize plants. A better understanding of these mechanisms can help farmers and scientists to predict and explain the effects of heavy metals on maize production. Understanding these mechanisms also creates a predisposition to make the right decision about the possibilities of maize production on soils contaminated by heavy metals.

MATERIAL AND METHODS

Study area

This study was carried out in 2022 at the agricultural experimental station of the Faculty of Agriculture and Food Science located near Sarajevo

International Airport (Bosnia and Herzegovina). The site is located at 43°49′34.42″ N and 18°19′18.48″ E, at an altitude of 505 m above sea level (Figure 1).

Figure 1. Location map of the study area

The study area has a continental humid climate typified by cold and not so humid winters and warm and humid summers, with a mean annual temperature of 9.6 °C and a mean annual precipitation of 899 mm. This climate is classified as Dfb (warm-summer humid continental climate) according to the Köppen-Geiger climate classification (Kottek *et al*., 2006).

The experimental area is divided into three soil plots. Each plot was 3 m wide by 10 m long consisting of 4 rows. After preparing the soil for sowing, the domestic maize hybrid: BL 43 was sown (5 May 2022), maintaining 75cm×20cm plant spacing. The experimental field was fertilized with 180, 100 and 150 kg/ha of N (nitrogen), P (phosphorus) and K (potassium), respectively. The sources of N, P and K were urea, superphosphate and potassium chloride, respectively. N was applied in three equal splits, i.e. one day before sowing, 30 days and 60 days after sowing. The entire amounts of P and K were applied one day before sowing. Weeding was done manually as and when necessary during maize cultivation. Control measure for pests and diseases was not applied in this study.

Plant material

BL 43 is a dent type, mid-ripening and high-yielding maize hybrid. It is tolerant to drought and resistant to major maize diseases and leaf pests and aphids. It has very strong and elastic stalks, resistant to lodging and breakage. The ear is cylindrical, with 16-18 rows of kernels, while the grain is yellow and large, tightly attached to the cobs and has excellent quality.

Soil sampling and analysis

For the current study, soil samples from field site (the surface soil layer at a depth of 0-30 cm) were collected one month before the maize sowing (2 April 2022) using stainless steel spoon auger and then analyzed using standard soil testing methods. pH was measured in 1 mol/L potassium chloride solution using the LLG-7 pH Meter (solution-soil ratio 2.5:1). Organic matter was determined by oxidation method with potassium dichromate (ISO 14235, 1998), total N content by Kjeldahl method (Bremner, 1960), and the available P and K content by Egnér-Riehm method (Egnér *et al.,* 1960). The contents of heavy metals in soil samples were determined by atomic absorption spectrophotometry, after digestion of the samples with aqua regia solution (mixture of nitric acid and hydrochloric acid in a ratio of 1:3).

The digestion of soil samples for heavy metals analysis was done as follows: 3 g of air-dried soil sample was carefully weighed and transferred into a 250 mL flat bottom flask, after which 28 mL of aqua regia solution was added. The flask was covered with a watch glass and left for 16 h at room temperature in a fume hood. Then, the flask was connected to the reflux system and refluxed on a hot-plate for 2 h. After cooling to room temperature, the mixture was filtered through Whatman No.42 filter paper into a 100 mL volumetric flask and diluted to the mark with deionized water (ISO 11466, 1995).

The contents of investigated heavy metals in digested soil samples were determined using an atomic absorption spectrophotometer (Shimadzu AA-7000, Japan) according to the instructions specified in the ISO 11047 method (ISO 11047, 1998). The quantification of investigated heavy metals was carried out using an external calibration curve. The external calibration curves were prepared by diluting the stock solutions of 1000 mg/L of each element supplied from Merck (Darmstadt, Germany). All calibration curves were characterized by a high correlation coefficient ($r>0.995$).

Plant sampling and analysis

Three maize plants (including root, stem, leaves and kernels) from each experimental soil plot were carefully collected at the stage of full maturity. Leaves, stem, root, and fruits/kernels of each plant were separated, dried at room temperature, grinded and then stored in paper bags until analyses. The contents of heavy metals in plant samples were also determined by atomic absorption spectrophotometry, after wet digestion of the plant sample with mixture of nitric-perchloric acid in a ratio of 2.5:1.

The digestion of plant sample for heavy metals analysis was done as follows: 1 g of air-dried plant sample was carefully weighed and transferred into a 100 mL flat bottom flask, after which 10 mL of nitric acid and 4 mL of perchloric acid was added. The flask was covered with a watch glass and left for 2 h at room temperature in a fume hood. Then, the flask was gently heated on a hot plate for 30 min. After cooling to room temperature, the mixture was filtered through Whatman No.42 filter paper into a 50 mL volumetric flask and diluted to the mark with deionized water (Lisjak *et al*., 2009).

Bioaccumulation and translocation factor calculation

Bioaccumulation factor (BAF) is defined as the ratio of heavy metal content in plant roots to the soil. It was calculated by dividing the heavy metal content in the root sample by the heavy metal content in the soil sample. Translocation factor (TF) explains an ability of a plant to translocate the heavy metal from roots to above-ground parts of plant. In this study, it was calculated by dividing the heavy metal content in the leaf sample by the heavy metal content in the root sample of maize (Ahmed and Slima, 2018).

$$BAF = \frac{C\ roots\ (heavy\ metal\ content\ in\ the\ root)}{C\ soil\ (heavy\ metal\ content\ in\ the\ soil)} \quad (1)$$

$$TF = \frac{C\ leaves\ (heavy\ metal\ content\ in\ leaves)}{C\ roots\ (heavy\ metal\ content\ in\ roots)} \quad (2)$$

According to BAF values, the plants can be classified into four categories, namely no phytoaccumulation (BAF<0.01), low phytoaccumulation (0.01-0.1), moderate phytoaccumulation (0.1-1) and high phytoaccumulation (BAF>1) (Sekabira *et al*. 2011). In general, the plant species with BAF values >1 and TF values <1 have the potential to be used in phytostabilization, whereas plant species with both BAF >1 and TF >1 have the potential to be used for phytoextraction (Mohotti *et al.*, 2016).

Statistical analysis

All analyses were carried out in triplicate and the results were expressed as mean value ± standard deviation. Analysis of variance and significance of difference among means were tested by one-way ANOVA and least significant difference (LSD) using the Microsoft Excel 2010 package program (Office 2010, Redmond, WA, USA). Statistical significance was considered with a 95% confidence interval (P<0.05).

RESULTS

Heavy metal contents and basic chemical properties of studied soil

Heavy metal contents in studied soil plots varied from 24.12 to 24.20 mg/kg for Cr, 0.29 to 0.30 mg/kg for Cd, 39.13 to 39.50 mg/kg for Pb, 37.11 to 38.22 mg/kg for Zn, 20.11 to 20.63 mg/kg for Cu, 34.11 to 35.01 mg/kg for Ni, and 622.66 to 697.31 mg/kg for Mn. The total contents of Cr, Cd, Pb, Zn, Cu and Ni did not exceed the proposed heavy metal limits for agricultural soils in Bosnia and Herzegovina (80, 1, 80, 150, 65 and 40 mg/kg, respectively). Limit value of Mn in soils is not established by legislative (OG FBiH, 2009) since it is not considered as directly contaminating element. However, the content of Mn in examined soils was lower than toxic level of Mn in soils (850 mg/kg) reported by Pais and Jones (1997).

Results obtained from soil analysis also showed that the examined soil plots have a slightly acid reaction (from 6.0 to 6.2), moderate level of organic carbon (from 3.0 to 3.2), and low content of available P (from 6.0 to 7.1) and K (from 9.1 to 12.2). The studied soil has a clay loam texture with a good balance between air-porosity and water-holding capacity, and is therefore considered ideal for maize production. According to World Reference Base for Soil Resources (IUSS, 2015), the studied soil can be classified as Alluvium. Alluvium soils develop on alluvial deposits near the river and are mostly characterized by a weak surface horizon and by parent material derived from river.

Heavy metal contents in maize plants

Heavy metals contents (Cr, Cd, Pb, Zn, Cu, Ni and Mn) in different parts of maize plants are presented in Table 1.

Table 1. Heavy metal contents in the maize plant samples

The parts of maize plant	Heavy metals (mg/kg dry mass)						
	Cr	Cd	Pb	Zn	Cu	Ni	Mn
root	6.34 ± 0.99[a]	1.23 ± 0.21[a]	0.09 ± 0.09[a]	37.1 ± 19.4[a]	28.9 ± 7.1[a]	14.7 ± 7.9[a]	43.2 ± 12.7[b]
stem	1.10 ± 0.21[b]	0.22 ± 0.13[b]	0.02 ± 0.05[b]	20.9 ± 11.6[b]	7.2 ± 2.6[bc]	1.16 ± 1.2[b]	9.8 ± 10.3[c]
leaves	1.17 ± 0.19[b]	0.27 ± 0.11[b]	0.02 ± 0.04[b]	39.2 ± 17.7[a]	12.5 ± 8.1[b]	1.44 ± 0.7[b]	77.9 ± 20.8[a]
kernels	0.62 ± 0.11[b]	0.10 ± 0.04[c]	0.01 ± 0.01[b]	20.1 ± 6.1[b]	3.1 ± 2.1[c]	1.13 ± 0.8[b]	6.1 ± 1.7[c]
$Lsd_{0.05}$[1]	0.59	0.13	0.05	14.41	5.66	3.67	13.43

[1]*Averages denoted by the same letter in the same column indicate no significant difference (P<0.05)*

The results showed that the contents of all examined heavy metals in the kernels i.e. edible parts of maize were below the maximum permissible value of heavy metals in food crops set by FAO/WHO (2001). Accordingly, the maximum permissible contents of Cr, Cd, Pb, Zn, Cu and Ni in the edible parts for human consumption are 2.3 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 100 mg/kg, 40 mg/kg and 4 mg/kg dry mass, respectively. The maximum permissible value for Mn in edible plant parts is not reported by FAO/WHO since Mn has relatively low toxicity for human health. The obtained results also showed that the content of Cr, Cd, Pb, Cu and Ni was significantly higher in the roots than in other parts of the maize plants.

Translocation of heavy metals from soil to maize roots and from roots to leaves of maize

The values of bioaccumulation factor (soil-root transfer) and translocation factor (root-leaves transfer) for the investigated heavy metals in the maize plants are presented in Table 2.

Table 2. Bioaccumulation and translocation factor values for the investigated heavy metals

Soil plot		Cr	Cd	Pb	Zn	Cu	Ni	Mn
1	BAF[1]	0.25	4.43	0.003	0.77	1.44	0.93	0.08
	TF[2]	0.20	0.20	0.27	0.98	0.50	0.10	1.69
2	BAF	0.29	3.83	0.002	0.88	1.59	0.65	0.06
	TF	0.14	0.23	0.14	1.28	0.48	0.11	1.72
3	BAF	0.25	4.13	0.001	1.21	1.22	0.59	0.06
	TF	0.22	0.12	0.17	1.01	0.32	0.09	2.01

[1]*Bioaccumulation factor (soil-root transfer) value*
[2]*Translocation factor (root-leaves transfer) value*

The bioaccumulation values (BAF) for the investigated heavy metals from soil to maize roots decreased in the order: Cd>Cu>Zn>Ni>Cr>Mn>Pb. The BAF value for Cd was much higher than 1, suggesting that the maize plants are good candidates for phytostabilization of Cd-polluted soil. Present study also showed that the translocation value (TF) was highest for Mn, followed by Zn and Cu, indicating that maize plants have a natural ability to translocate these elements from roots to above-ground parts.

DISCUSSION

Soils polluted by heavy metals can have unfavorable effects on plant growth and productivity. Moreover, heavy metals at toxic levels in soils can pose a significant health risk to food crops and consequently to humans via the food chain. To overcome elevated levels of heavy metals in soils, plants have evolved numerous detoxification mechanisms (Dalvi and Bhalerao, 2013). However, different plant species activate different detoxification mechanisms at physiological, biochemical and molecular levels, which mainly depend on plant genotype, soil properties and heavy metal concentration and availability in soils (Gill *et al.*, 2022). An understanding of these interrelated detoxication mechanisms in plants is important to predict the possibilities of plant growth on soils polluted with heavy metals.

In this study, all the heavy metals studied (except Zn and Mn) exhibited higher contents in the analyzed root tissues than in the above-ground parts of a maize plant. This finding strongly suggested that maize plants have versatile mechanisms to restrict transport of toxic heavy metals Cr, Cd, Pb, Cu and Ni from root to other parts of maize plants. Some of these mechanisms could be as follows: (1) heavy metal ion binding to the cell wall, (2) chelation of heavy metals in the cytosol with strong ligands, (3) heavy metal sequestration in the specific root cells and (4) efflux of heavy metal from the cytosol of a root cell, either into the apoplast or into the vacuole. The metal translocation restriction phenomenon for maize plants was most pronounced for Ni, followed by Cr, Cd and Pb. This result was expected, since above-mentioned heavy metals have no known physiological function in plants. Moreover, they are toxic for plants even at very low concentrations (Jaishankar *et al.,* 2014).

In this study, Zn and Mn were more efficiently transferred from the maize roots to leaves as compared with Cr, Cd, Pb and Ni. Similar results were found by Shafiq *et al.* (2020) and Abedi *et al*. (2022). These results were also expected since both Zn and Mn play an important role in various metabolic processes tightly associated with photosynthesis and therefore the plant tends to translocate them to the leaves (Hänsch and Mendel, 2009). Among others, Mn participates in the structure of photosynthetic proteins (Alejandro *et al.,* 2020), while Zn acts as a cofactor of numerous enzymes involved in photosynthesis and sugar formation (Umair Hassan *et al.,* 2020). In the light of the above mentioned, it is obvious that heavy metal distribution within plant differs considerably, primarily depending on the heavy metal toxicity and their role in plant metabolism. Numerous studies have also confirmed this observation (Angulo-Bejarano *et al.,* 2021; Riyazuddin *et al.,* 2021).

In the present study, the BAF value for Cd was the highest one. This value was much higher than 1 (ranged from 3.83 to 4.33), suggesting that maize is a promising species for phytostabilization of Cd-contaminated soil. The obtained results are in an agreement with those of Li *et al.* (2012) and Rizwan *et al.* (2017). Huang *et al.* (2020) reported that Cd uptake by root cells occurs through plasma membrane transporters involved in the uptake of essential elements such as Ca, Mg, Fe and Zn. It is therefore not surprising that many plants, including maize, can easily absorb Cd from the soil (Sun *et al.,* 2022). However, the maize plants have the strong ability to prevent or slow down the Cd translocation from roots to above-ground parts, including the grain. This finding indicates that maize can grow healthy on soils contaminated with certain level of Cd. This hypothesis has, in fact, been confirmed by many other scientists (Seifikalhor *et al*., 2020; Sterckeman and Thomine, 2020).

This study indicated that the BAF value for Zn and Cu in maize plants was also at a higher level. Taking into account that both Zn and Cu are essential for plant metabolism, these results were as expected.

The BAF value for Pb was the lowest (ranged from 0.001 to 0.003), suggesting that maize plant roots can uptake only small amounts of Pb from the studied soil. This data is highly desirable, since the Pb is one of the most toxic metals for living organisms, even when absorbed in small amounts (Collin *et al.,* 2022). However, this finding is not consistent with the findings of several studies where the values of Pb soil-root transfer for maize and other plants were much higher (Aladesanmi *et al*., 2019; Chiwetalu *et al*., 2022). It is obvious that the Pb uptake by roots differs significantly among different plant species and even within a species (Bassegio *et al*., 2020). It depends primarily on soil chemical properties, including among others, pH, organic carbon content, redox potential and cation exchange capacity (Luo *et al*., 2014). Soil microbes could also affect Pb mobility and availability to plant roots mainly through alteration of soil pH (Gladkov *et al*., 2023). The pH value of studied soil was found to be slightly acidic, which is not favorable for release and mobility of Pb in soil. This is probably one of the most important reason for low Pb mobility in the analyzed soil and consequently for low Pb transfer from soil to maize roots. Namely, Pb is more soluble in acidic soils than in slightly acidic, neutral and alkaline soils, where Pb precipitates as hydroxides and carbonates, rendering it less accessible to plants (Li *et al.*, 2021).

From the health point of view, an interesting finding is that the maize grown in the studied soil accumulate lower levels of toxic heavy metals (Cr, Cd, Pb and Ni) in kernels than in the other parts. Moreover, the contents of above-mentioned heavy metals in kernels i.e. the most edible part of the plant were much lower than the permissible limit set by FAO/WHO (2001). Similar findings were reported by Zhou *et al.* (2022), indicating that maize relieve health risks of heavy metal-polluted soils. Future studies will be needed to verify this hypothesis.

CONCLUSIONS

Overall, the results of the present study indicate that maize plants have an ability to reduce Pb uptake from soil to roots as well as to restrict transport of toxic heavy metals Cr, Cd, Pb, Cu and Ni from root to above-ground parts under experimental conditions. The study also showed that maize plants have a great potential to absorb Cd ions and accumulate them in roots, suggesting that maize is an efficient plant in phytostabilizing Cd. In this study, the accumulation pattern of all tested heavy metals in maize organs was arranged as: roots>leaves>stem>kernels. Also, the levels of all heavy metals, in maize grains, were lower than the permissible limits set by FAO/WHO, indicating that their translocation to the edible part was comparatively low or absent. In general, the study highlights that the consumption of maize grown on studied soil, from the point of view of heavy metals, should not be dangerous to human health, suggesting that maize relieve health risks of heavy metal-polluted soils. However, further investigations are needed to confirm this hypothesis.

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