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ESSENTIAL HEAVY METALS ACCUMULATION AND DISTRIBUTION PATTERN IN CUCUMBER PLANTS

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Abstract: The main purpose of this study was to assess the uptake levels and distribution of essential heavy metals Cu, Zn, Mn, and Fe within the cucumber plants (*Cucumis sativus* L. 'Opalit F1'). Essential heavy metal levels in soil and plant samples were determined using atomic absorption spectrophotometry. The concentration of total and available forms of all examined heavy metals in the studied soil was lower than the permissible value prescribed by legislation or scientific literature. The Mn and Zn accumulation were higher in the root and leaves than in the stem and fruits, while the Cu and Fe accumulation was significantly higher in the root than in the all above-ground parts of a cucumber. Since the heavy metals concentration in cucumber was below the recommended limits set by World Health Organization, the consumption of cucumber grown on the studied soil, from the point of view of contamination of the soil with tested heavy metals, should not be dangerous to human health.

Keywords: root, leaves, stem, fruit, distribution.

INTRODUCTION

Heavy metals represent a heterogeneous group of elements widely varied in their biological functions. Several heavy metals (e.g., cadmium, chromium, lead, mercury) are harmful to plants even in small quantities, while some other heavy metals such as copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe) are essential for plant growth and development (Nagajyoti et al., 2010). Cu is a constituent of several enzyme systems involved in oxidative stress responses, it participates in photosynthetic electron transport, mitochondrial respiration, and assists in plant metabolism of lignin, carbohydrates and proteins (Yruela, 2005). Zn plays an important role in plant metabolism by acting as a co-factor for several enzymes involved in the proper functioning of the antioxidant defense system. Zn is also important in activating many enzymes involved in protein synthesis, stabilization of cellular membranes, auxin synthesis and pollen formation (Hacisalihoglu et al., 2003; Marreiro et al., 2017). Mn plays a vital role in photosynthesis by participating in the structure of photosynthetic proteins and enzymes. Also, Mn is essential for antioxidant defense system in plants as an enzyme antioxidant-co-factor (Millaleo et al., 2010). Fe plays an important role in metabolic processes such as DNA synthesis, respiration, and photosynthesis. In addition, Fe acts as a major constituent of many enzymes involved in plant defense systems against free radicals (Tripathi et al., 2018). However, the presence of Cu, Zn, Mn and Fe in excess in soils may cause damage to the plant by increasing the production of reactive oxygen species or decreasing the quality of harvested plant products. The absorption of these elements by plant and distribution within the plant depends not only on their concentration in the soil, but also on the type of soil, its chemical and physical properties, and the plant genetic background.

During the last decade, cucumber has become one of the most popular vegetable crops among consumers in Bosnia and Herzegovina, resulting in a gradual increase in presence of this vegetable in greenhouse vegetable production. Unfortunately, there is not much research in Bosnia and Herzegovina related to dynamics of essential heavy metals Cu, Zn, Mn and Fe in the soil-plant system in intensive cucumber production. Considering that the high presence of Cu, Zn, Mn and Fe in soils can cause negative effects on vegetable safety, and thus on human health, it is very important to study the dynamics of these elements in the soil-plant system in areas where vegetables produce intensively. The Srebrenik municipality in the north-eastern Bosnia is one of the areas where the vegetable production has been rapidly increasing, and hence this area was selected as the object of this study.

The main purpose of this study was to assess the uptake levels and distribution of essential heavy metals Cu, Zn, Mn, and Fe within the cucumber plants (*Cucumis sativus* L. 'Opalit F1').

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MATERIALS AND METHODS

Study area

The experiment was carried out from March to July 2018 in a double-span polyethylene-covered greenhouse with natural ventilation in Gornji Moranjci (44° 40′48″ N, 18° 25′54″ E), Srebrenik municipality, Bosnia and Herzegovina. Greenhouse has a width of 10 m, length of 22 m, eaves height of 2.2 m and ridge height of 3.6 m. The greenhouse was equipped with two side roll-up vents due to humidity regulation. Green knitted shade cloth was used to prevent excessive light intensity and reduce greenhouse temperature during warm day.

The climate in studied site is classified as Cfb by Köppen and Geiger climate classification (Rubel and Kottek, 2010). The average annual temperature is 11.1°C, and precipitation here averages 856 mm, with significant rainfall even in the dry months.

The soil of the experiment site was described from a profile within the experiment area and classified as Dystric Cambisol, according to FAO Soil Classification (FAO, 1998). Acid reaction, base saturation less than 50% and a medium nutrient storage capacity is a typical characteristic of this type of soil. The experimental soil was loam in texture with granular and crumb structures in the upper horizons and moderate water- holding capacity in root zone. The depth of the arable soils profile was 50 - 60 cm. The experiment area in greenhouse was divided into four equal plots. The size of each plot was 10 m x 4 m.

The seeds of *Cucumis sativus* L. 'Opalit F1' were sown on February 23, 2018 and seedlings were transplanted 20 days later. All agro-technical measures needed for optimum cucumber growth (fertilization, pest control measures, irrigation) were performed identically on all experimental plots until the time of technological maturity of fruits.

Soil sampling and analysis

The soil sample from each plot was collected a few weeks before planting cucumbers in March 2018 from 0-30 cm depth, using clean steel shovel. The collected soil samples were mixed thoroughly to form the greenhouse soil average, and thereafter was air-dried, sieved (< 2 mm) and stored in paper bags until analysis.

The following parameters of soil chemical properties were subject of analysis: soil reaction (soil pH), content of organic matter (OM), content of available forms of phosphorus (available P) and potassium (available K), and content of total and available forms of Cu, Zn, Mn, and Fe. Soil pH was determined by pH meter in 1:2.5 (v/v) suspensions of soil in water (pH H2O) and in a 1 M KCl solution (pH KCl) according to ISO 10390 method (ISO, 2005). OM was determined by chromic acid digestion method (ISO, 1998), available forms of phosphorus and potassium by ammonium lactate method (Egner et al., 1960), and the total and available forms of Cu, Zn, Mn and Fe by atomic absorption spectrophotometry (AA-7000 Shimadzu, Japan) according to the instructions specified in the ISO 11047 method (ISO, 1998).

Previous extraction of total forms of Cu, Zn, Mn and Fe from soil sample was made according to ISO method (ISO, 1995) as follows: 3 grams of air-dried soil (fraction smaller than 1 mm) was weighed and transferred to 250 ml of round bottom flask and thereafter 28 ml of freshly prepared aqua regia (mixture of nitric and hydrochloric acid in 1:3 ratio) was added. The flask was covered with a watch glass, allowed to stand at 16 h (overnight) at room temperature. After that, the flask with solution was refluxed on hotplate for 2 h, allowed to cool, filtered through quantitative filter paper into 100 ml flask, and then diluted to the mark with deionized water.

Previous extraction of available forms of Cu, Zn, Mn and Fe from soil sample was made with EDTA solution according to a procedure described by Trierweiler and Lindsay (1969). 10 g of air-dried soils was weighed and transferred to 100 ml plastic bottle, and thereafter 20 ml EDTA solution (0.01 M ethylenediaminetetraacetic acid (EDTA) and 1 M $(NH_4)_2CO_3$, adjusted to pH 8.6) was added. The mixture in bottle was shaken for 30 min at 180 rpm in an orbital shaker, filtered through quantitative filter paper into 25 ml flask and diluted to the mark with deionized water.

After determining the concentrations of total and available forms of tested heavy metals in soil samples availability ratio was calculated. Availability ratio (AR) represent the percentage available fraction of total metal concentration in soil. The following formula was applied for the calculation of the availability ratio (Massas et al., 2010):

$$A = {}^{Ca}/_{Ct}\ 10^2$$

Ca - the available metal concentration
Ct - the total metal concentration

Plant sampling and analysis

At the time of technological maturity of fruits, from each examined plot five cucumber plants (whole plant with root) were carefully collected. Leaves, root and fruits of each plant were separated, dried, grinded and then stored in small paper bags until measurements.

Atomic Absorption Spectroscopy was also used to determine the concentration of Cu, Zn, Mn, and Fe in plant samples. Extraction of heavy metals from the plant sample was performed using HNO_3-H_2SO_4 solution as follows: 1 g of air-dried plant sample was weighed and placed in 100 ml flat bottom flask, and then 10 ml HNO_3 and 4 ml H_2SO_4 were added. The flask was covered with a watch glass, allowed to stand a few hours at room temperature and then gently heated on a hot plate for thirty minutes. The mixture allowed to cool, then filtered through quantitative filter paper into 50 ml flask and thereafter diluted with deionized water to the mark (Lisjak et al., 2009).

Statistical analysis

All measurements with plant material were done in triplicates and the results were presented as mean ± standard deviation. The results were processed statistically using one-way ANOVA and differences between means were tested using the least significance difference (LSD) test at $P < 0.05$. Means that differed at $P<0.05$ were considered as significantly different.

RESULTS

Chemical properties of the greenhouse soil

Analyzed soil was acidic to slight acidic with moderate level of organic matter, while the determined values for available phosphorus and potassium content indicates high supply of examined soil with these macro-elements (Table 1).

Table 1.

Results of soil chemical analysis

Parameter	Unit	Measured value
pH H_2O	pH unit	6.4
pH KCl	pH unit	5.3
OM	%	2.81
P_2O_5	mg/100 g	16.84
K_2O	mg /100 g	80.9

In accordance with these results the following fertilizer recommendations were given and carried out identical to all plots: 200 kg/ha superphosphate fertilizer as part of the autumn fertilization and 100 kg/ha urea and 200 kg/ha NPK 8:12:26 as part of fertilization during the growing season (amounts of fertilizers were recalculated based on examined soil plot area).

Concentration of essential heavy metals in the studied soil

Determined values of total and available forms of Cu, Zn, Mn and Fe in the examined greenhouse soil are listed in Table 2.

Table 2.

Cu, Zn, Mn and Fe concentrations in soil (mg/kg dry weight)

Soil	Concentration							
	Total Cu	Available Cu	Total Zn	Available Zn	Total Mn	Available Mn	Total Fe	Available Fe
Determined value	15.76	1.37	36.95	0.94	246.54	3.21	16849.9	16.2
Permissible value	80	5	100	10	850	10	-	20
Availability ratio	8.69		2.54		1.3		0.1	

The concentration of all examined heavy metals was lower than the permissible value in agricultural soils established by the legislation in Bosnia and Herzegovina (Official Gazette of FBIH, 2009). The limit value of Fe in agricultural soils is not in the legislative rules or the main topic of discussion among scientists, because Fe is not a direct contaminant of the soil, but its concentration in the soil has considerably exceeded the average value of Fe concentration in soils (0.6%) reported by Kabata-Pendias et al. (1999).

The available concentrations of all examined elements were below the threshold value reported by Kaur et al. (2006). Accordingly, the threshold value for Cu, Zn, Mn and Fe available concentrations in soils is 5, 10, 10 and 20 mg/kg, respectively.

The availability ratio (AR) for heavy metals in soils is mainly dependent on the composition of the parent rock materials and the degree of weathering as well as the chemical and physical properties of soil. The highest AR was established for Cu, followed by Zn, Mn and Fe.

Essential heavy metals concentration in cucumber

The mean concentrations of Cu, Zn, Mn and Fe in the cucumber fruit decreases in the following order: Fe > Zn > Mn > Cu (Table 3).

Table 3.

Cu, Zn, Mn and Fe concentrations in cucumber plant (mg/kg dry weight)

Plant parts	Concentration			
	Cu	Zn	Mn	Fe

Root	10.68 ± 2.62[a]	41.35 ± 10.35[a]	53.91 ± 9.42[a]	533.21 ± 33.23[a]
Stem	4.93 ± 1.24[b]	28.01 ± 6.73[c]	10.92 ± 2.47[c]	30.12 ± 5.62[d]
Leaves	5.04 ± 0.55[b]	37.72 ± 3.99[ab]	38.82 ± 6.89[b]	55.94 ± 8.79[b]
Fruit	6.12 ± 2.37[b]	31.89 ± 1.35[bc]	7.42 ± 1.36[c]	55.55 ± 5.68[bc]
$LSD_{0.05}$	1.85	6.63	5.88	18.87

Values with the same letter in each column are not significantly different ($p<0.05$)

In addition, their concentration in cucumber fruits were all below the recommended limits set by FAO/WHO. Accordingly, the maximum recommended value for Fe, Zn and Cu in food crops is 425 mg/kg, 99.4 mg/kg, and Cu 73.3 mg/kg, respectively.

The results of this study revealed that all examined heavy metals accumulated in higher amounts in the roots of cucumber plants, especially Fe. Moreover, the Fe concentration in root was several times higher in comparison with Fe concentration in other parts of a cucumber plant.

DISCUSSION

Monitoring of Cu in the studied soil and cucumber

Many studies have shown that the total Cu concentration in soil at >100 mg/kg may cause phytotoxicity to plants and thus decrease their productivity (Yang et al., 2002; Gharbi et al., 2005; Zhou et al., 2005). For the assessment of Cu toxicity to plant, the data of available Cu forms in soil is also important. The values of 5 mg/kg and above for Cu available forms in soils have been considered as excess. In these circumstances, it is possible that plants absorb too much Cu, resulting in disturbances in essential cellular processes (Chen et al., 2015).

In the present study, the determined values of total and available forms of Cu were not even close to above-mentioned threshold value, indicating that the observed Cu concentrations in tested soil were low and therefore no toxic for cucumber growth and development. These results suggest that the parent material i.e. lithological structure of the examined soil and wider area of Srebrenik municipality is not rich in Cu-bearing minerals. The results of many scientists who investigated the petrographic and geotechnical properties of soils from Srebrenik municipality confirm this observation (Cipurković et al., 2011; Babajić et al., 2017). Although the Cu total and available forms were relatively low, the availability ratio for Cu in the studied soil was high compared to other examined elements indicating that some chemical and physical properties of soil favour the release of strongly bound Cu from colloidal soil material to soil solution. It is well known that the Cu availability is higher in acidic soils because H^+ ions contributes to displacing the cations from adsorption soil complex, reducing the cation exchange capacity, and thus increasing the concentrations of Cu^{2+} and other cations in the soil-water system (Bradl, 2004). In addition, neutral to alkaline soil have high presence of carbonates that favour Cu precipitation and adsorption, thereby reducing its mobility. So, the high availability ratio for Cu in the studied soil could be primarily explained by soil reaction.

Besides pH value, organic matter is also known to be strongly associated with Cu mobility in soils. Matijević et al. (2014) reported that organically complexed Cu^{2+} is bound tighter than any other divalent transition metal and that these Cu forms are not available to plants. Cu complexation with organic matter is therefore considered as the most significant mechanism of Cu^{2+} retention in soil, resulting in limiting Cu bioavailability. Accordingly, it is assumed that the relatively low content of organic matter in the studied soil (below optimum) also contributes to higher Cu mobility and thus higher value of Cu availability in the present study.

As shown in Table 3, the cucumber accumulates Cu more in the roots than in the above-ground parts of a plant, and this finding is consistent with findings of Alaoui-Sossé et al. (2004) who noted that plant activate different mechanisms to prevent or slow down the transport of Cu and other potential toxic elements from root to other part of plants. Some of these mechanisms are binding of metals by strong ligands and their compartmentation in specific root cells (Leitenmaier et al., 2013).

Monitoring of Zn in the studied soil and cucumber

In soils, the total Zn concentration was reported to be in the range from 10 to 300 mg/kg (Kiekens, 1995). The total Zn concentration in the studied soils was 36.95 mg/kg, and this value was lower compared to the permissible Zn value for agricultural soils established by the legislation in Bosnia and Herzegovina, but also compared to the worldwide average value for Zn in soils. Ure et al. (1982) calculated an average Zn concentration of 60 mg/kg from 7402 worldwide soils, while Kabata-Pendias et al. (1992) reported a worldwide mean Zn concentration of 64 mg/kg (range 17-125 mg/kg). The concentration of available Zn in the studied soil was also lower than threshold value of 10 mg/kg that has been considered as excess (Kaur et al., 2006). Based on above-mentioned threshold values, the studied soil was regarded as adequate for cucumber growth and development.

The results of this study showed that the availability ratio for Zn in the studied soil had value of 2.4 which means that 2.4% of total Zn in soils occurred in forms that available to plants. This finding is in line with findings of Dvorak et al. (2003) who reported that available Zn forms in soils ranged from 0.4 to 4.9% in relation to the total Zn.

Clark et al. (2000) reported that soil pH influences Zn availability more than any other factor. Whereas low pH (<5) shifts the equilibrium towards free and thus available Zn ions, the higher pH (>6.5) favor the formation of stable carbonate or hydroxyl complexes with Zn, resulting in decreased Zn availability in soils.

Many scientists have found that soils high in clay or organic matter had higher adsorptive capacities for Zn, resulting in the formation of stable organically complexes with Zn, thereby limiting Zn mobility and thus its availability (Adiloglu et al., 2006; Babazadeh et al., 2011; Huang et al., 2014).

In the present study, the accumulation of Zn was higher in root than in other parts of cucumber, but this increase was not statistically significant in relation to Zn concentration in leaves. This result lead to the conclusion that cucumber tends to translocate Zn from roots to the leaves, because Zn is necessary for many physiological processes, including chlorophyll production, and growth hormone production (Hussein et al., 2018).

Monitoring of Mn in the studied soil and cucumber

The Mn total concentration in the studied soil was relatively low compared to the toxic value of Mn in soils (850 mg/kg) reported by Pais et al. (1997), as well as compared to the worldwide average value of 440 mg/kg reported by Emsley (2001). The concentration of available forms of Mn in the studied soil was also low as compared to the threshold value of 10 mg/kg that has been considered toxic for plant (Kaur et al., 2006).

The availability ratio for Mn in the studied soil was 1.3, which means that 1.3% of total Mn in soils is available for plant. This value is a relatively high, considering that only 0.1 - 1% of total Mn in soil is available to plants according to Vukadinović and Vukadinović (2011). Certainly, the high availability ratio for Mn in the studied soil is the result of the influence of soil chemical properties on the release of Mn from colloidal soil material to soil solution. Many scientists reported that the Mn availability increases as soil pH, organic matter and soil aeration decreases (Scăeţeanu et al., 2013; Rengel, 2015).

Considering that fact, it is assumed that the acid reaction of the studied soil (pH in KCl 5.3) contributed to the transformation of insoluble Mn^{3+} and Mn^{4+} ions to soluble Mn^{2+} and thus available forms for plants. The results of this study also indicate that the mobility of Mn and heavy metals in general is much higher in acidic soils, and results of many studies confirm this hypothesis (Kukier et al., 2004; Adamczyk-Szabela et al., 2015).

The results of the present study also showed that cucumber mostly accumulates Mn in the leaves and root, and much less in the stem and fruits, indicating that Mn distribution within the plant is very similar to Zn, but not to Cu. It is evident that the nutrient distribution and accumulation in cucumber differs considerably, depending primarily on the nutrient toxicity as well as nutrient needs for the maintenance of essential physiological processes in plant.

Monitoring of Fe in the studied soil and cucumber

The Fe total concentration in the studied soil was much higher than the average value of Fe concentration in soils of 0.6% reported by Kabata-Pendias et al. (1999). This result indicates that the parent material of the studied soil is characterized by Fe-bearing minerals. This hypothesis has, in fact, been confirmed by many scientists (Cipurković et al., 2011; Babajić et al., 2017). Although the total Fe concentration was much higher than the worldwide average value, the concentration of available Fe in the studied soil was below the threshold value of 20 mg/kg that has been considered as excess (Kaur et al., 2006).

The availability ratio for Fe in the studied soil was the lowest, indicating that the total concentrations and availability of the studied heavy metals do not follow a similar spatial distribution. For example, the availability ratio for Cu and Zn in the studied soil was more than eighty and twenty times higher as compared to availability ratio for Fe, respectively. Colombo et al. (2014) reported that the magnitude of Fe available forms is very low and is governed by very low solubility of Fe oxides. They also noted that some general rules governing the behavior of Fe in soils are the soil redox potential and pH. Neutral pH and aerobic conditions promote Fe oxidation and precipitation of Fe oxides, whereas reducing and acid conditions promote the Fe mobilization from minerals. Furthermore, Shenker et al. (2005) reported that the interaction of Fe minerals with plant, microbes, and organic matter can improve the formation of soluble Fe forms and increase the availability of Fe for plant.

The results of this study also showed that the Fe concentration was even ten times higher in the root than in other parts of cucumber, while the difference in accumulation among root and other parts of cucumber was much lower for Mn, Zn and Cu. This observation indicates that Fe in high amount is potential harmful for essential physiological processes in leaves and fruit and that cucumber tends to prevent or reduce its transport from roots to the to the above-ground parts of plants.

CONCLUSIONS

Generally, the studied greenhouse soil was not polluted with potentially toxic heavy metals Cu, Zn and Mn but it contained relatively high Fe concentration as compared to the average worldwide value.

The cucumber mostly accumulates Mn and Zn in the root and leaves, and less in the stem and fruits, while the Cu and Fe accumulation was several times higher in the root than in the above-ground parts of a cucumber.

Considering the content of examined heavy metals in cucumber was below the recommended limits set by Food and Agriculture Organization/World Health 0rganization (FAO/WHO), it can be concluded that the consumption of cucumber grown on studied soil, from the point of view of heavy metals, should not be dangerous to human health.

AUTHORS CONTRIBUTION

Murtić S. and Zahirović C. conceived the idea, did the experimental work, analyzed the data and wrote the manuscript (the contribution to work was 70%). Jurković J., Karić L. and Koleška I. facilitated the research and critically reviewed the manuscript (the contribution to work was 30%).

FUDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study, in the writing of the manuscript and in the decision to publish the results.

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