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Zinc and Copper Dynamics in the Soil - Plant System in Intensive Strawberry Production

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SM and HC designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors IK and RO managed the analyses of the study. Authors FB and JA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The term heavy metal, when related to its impact on the life of the plant, almost always implies negative connotations. However, some heavy metals like copper (Cu) and zinc (Zn) are essential to maintain the metabolism of plant, and without them the plant would not be able to successfully complete its life cycle. The aim of this study was to examine the dynamics of Zn and Cu in the soil - plant system in intensive strawberry plantation on pseudogley soil in Northwestern Bosnia, area of

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Gradacac. The content of Zn and Cu in the examined soil, leaves and fruits of strawberries was determined by atomic absorption spectrophotometry. Zn and Cu contents (means +/- SEM) were 82.06 +/- 14.07 and 8.45 +/- 2.35 in soil, 100.34 +/- 4.61 and 0.41 +/- 0.11 in leaves, 91.72 +/- 6.32 and 0.32 +/- 0.18 in fruits expressed as mg/kg dry matter (DW), respectively. Uptake, translocation and accumulation of Zn in the leaves and fruits of strawberries was at a satisfactory level in accordance with the plant's needs for this element, which was not the case when the dynamics of Cu was studied. Some of the main reasons for that were: a low Cu content in the examined soil, low mobility of Cu in the plant, and antagonistic relationship between Zn and Cu in soil.

Keywords: Fruit; leaf; nutrient; soil fertility; health.

1. INTRODUCTION

Lately, there is evidence of growing number of studies that have been conducted in order to determine the content of heavy metals in soils, and finding answers to how and what influences their accumulation in plants. A special attention was given to the dynamics of the toxic heavy metal like cadmium (Cd), chromium (Cr) and lead (Pb) in the system soil - plant because their presence in consuming parts of the plant can cause serious health problems [1,2,3,4].

Unlike Pb, Cd and Cr, the dynamics of Zn and Cu in soil - plant system has not been the object of research as much, particularly because their presence in consuming parts of the plant does not necessarily mean toxic effect on the human health. Moreover, their presence in human body are necessary for the maintenance of certain physiological processes. Zn is important for cell replications and for nucleic acid and amino-acid metabolism, while Cu is necessary for the growth and formation of bone, formation of myelin sheaths in the nervous systems, and it helps in the transfer of iron from tissues to the plasma. Also, Cu is an essential component of several antioxidant enzymes like cytochrome oxidase, catalase, peroxidase, and ascorbic acid oxidase which plays an important role in antioxidant defense system in human body [5].

Apart from its importance for human, Zn and Cu are very important for the functioning of the plant. In fact, both elements are constituent of many enzymes which are required in the process of photosynthesis, mitochondrial respiration, oxidative stress response, and some other physiological processes of plant. Also, Zn is essential for plant growth because it plays an important role in the synthesis of plant hormones auxins [6], while the Cu assists in plant metabolism of carbohydrates and proteins [7]. Although necessary, the higher concentration of Cu and Zn can have very negative impact on plant. The toxicity of Zn for the plant occurs when the content of Zn in plant exceeds toxic limit 150 mg/kg DW, and such concentrations of Zn are very rarely reported in the cultivation of crops. In these cases, the plants manifest lower growth and reduction of the root system, smaller leaves with reddish-dark spots [8]. Cu toxicity in the plants is manifested when the content of Cu in plant exceeds toxic value 15 mg/kg DW, and the excess Cu is visually manifested with chlorosis of either the whole leaf or between the veins of the new leaves. As the deficiency symptoms of Cu progress, the leaves may wilt [9,10].

Uptake of Zn and Cu by plant and their accumulation in certain plant parts depends on many factors: primarily on the content and form of Zn and Cu in the soil [11], but also on the type of soil, its chemical properties, and genetic characteristics of the plant itself [12]. Also, soil texture (sand, silt and clay content) is important factor for Zn and Cu availability [13].

Given the above mentioned, the objectives of this research were: to determine the content of Zn and Cu in the examined soils, to determine their accumulation in the leaves and fruits of strawberries, and based on analysis of all the results, to give an opinion on the dynamics of Zn and Cu in the system soil - plant under conditions of intensive strawberry cultivation on the examined site.

2. MATERIALS AND METHODS

2.1 Field Experiment

The experiment was set up in 2015 in intensive plantation of strawberry (*Fragaria x ananassa* Duch. cv. 'Maya') on pseudogley soil. This type of soil is characterized by respective wet and dry phases of soil resulting in the occasional excessive wetting, which is dictated by its profile, ie. typical sequence of horizons in pseudogley soils. Their natural profile may be designated principally as Oi-A-Eg-Btg-Cg [14], and it is mainly correlate with Stagnosols [15]. Upper horizons are of loam-clay composition, while the lower horizons are of very colloidal and clay composition, which results in their lower porosity [16]. The poorly permeable soil horizons are mainly the result of pedogenic processes of acidification, eluviation and illuviation of fine clay particles. Hence, these soils are acid or very acid and the amount of exchangeable calcium ions is often low in these soils [17].

Experimental field was divided into three plots and each plot was subdivided into three replicates (5 m x 4 m).

The results of basic pedo-mechanical properties for average samples of examined soil horizons are shown in Table 1.

2.2 Soil Chemical Analysis

Composite soil samples (1 - 2 kg) were collected before cultivation from five sites from each replicate at a depth of 0 - 25 cm using a soil sampler probe. The following parameters of soil fertility were subject of chemical analysis: soil acidity (pH), organic matter (OM), content of available forms of phosphorus (available P), content of available forms of potassium (available K), and content of Zn and Cu in the soil. pH was determined by pH meter [18], OM by colorimetric method [19], the content of available P and K by AL - method [20], and the content of Zn and Cu by atomic absorption spectrophotometry (AA-7000, Shimadzu, Japan) according to the instructions specified in the ISO 11047 method [21].

Previous extraction of Zn and Cu from the soil was prepared using aqua regia solution (a mixture of HCl and HNO_3 in a ratio of 3:1) as follows: 3 gram of air-dried soil fraction smaller than 2 mm was placed in 250 ml round bottom flask, 21 mL of aqua regia was added to the soil sample, the flask was covered with a watch glass and then allowed to react overnight at room temperature. Afterwards the mixture was heated on hotplate under reflux for 2 h, and after cooling to room temperature the mixture was filtered through quantitative filter paper into 100 ml Erlenmeyer flasks and diluted to the mark with deionized water [22].

2.3 Sampling and Analysis of Plant Material

Leaves and fruits of strawberry were collected at the stage of strawberry commercial maturity. Only ripe fruits and leaves that were fully developed and physiologically active, from the mid-stem of plants were taken.Twenty leaves and fruits from each replication were taken for analysis.

Extraction of Zn and Cu from the plant material was performed as follows: 1 g of dry matter was placed in 100 ml round bottom flask, 10 ml HNO_3 and 4 mL H_2SO_4 was carefully added. The flasks were left for 16 hours at a room temperature and then heated gently on a hot plate for thirty minutes. After cooling to room temperature, the solution was filtered through quantitative filter paper in 50 ml flask and made up with deionized water to the mark [23]. Content of Zn and Cu in plant samples were also measured byatomic absorption spectrophotometry (AA-7000, Shimadzu, Japan).

All experimental measurements were done in triplicates and the results were presented as mean ± standard deviation (means +/- SEM).

2.4 Comparation of Results Cu and Zn Contents in Examined Samples with Legal Regulations

Results of Cu and Zn contents in the soil and plant material were compared with the limit values set down by related legal regulations in Bosnia and Herzegovina. The basic legal regulation used for this part of the study was: ' Rulebook on determination of allowable quantities of harmful and hazardous substances in soils of Federation of Bosnia and Herzegovina and methods for their testing' [24] and 'Rulebook on maximum level for contaminants in food' [25]. As the additional regulation the scientific literature was used [26].

3. RESULTS AND DISCUSSION

3.1 Soil Chemical Properties

The analysis of basic parameters of soil fertility showed that the examined soil had an acid reaction, medium content of OM, with a low content of available P and a high content of available K (Table 2).

Table 1. Pedo-mechanical properties of examined soil horizons

Soil horizon	PSD (%)			P (%)	MS (%)
	Sand	Silt	Clay		
Eg (eluvial horizon)	5.0	78.2	16.7	48.4	62.1
B_{tg} (iluvial horizon)	4.0	68.6	27.3	39.1	69.6

PSD - particle size distribution, P - porosity, MS – microaggregates stability

Table 2. Chemical properties of soil

Parameter	Unit	Measured value	Characteristics of soil
pH (H2O)	pH unit	6.32	Acid
pH (KCl)		5.23	reaction
OM	%	3.09	Moderate supply
Available P	mg/100g	1.05	Very low supply
Available K	mg/100g	30.04	High supply
$CaCO_3$	%	1.23	Very low supply

The content of Zn and Cu in the examined soil was significantly below the limit value prescribed by the related legal regulations in Bosnia and Herzegovina, which indicates that examined soil was suitable for strawberry cultivation and not contaminated by Zn and Cu (Table 3).

In scientific literature were presented large fluctuations in the content of Zn between different soil, from 5 to 770 mg/kg DW [27]. Fluctuations in the content of Cu between different soil were significantly lower and most often in the range of 5 to 50 ppm, although there are studies where the average Cu in agricultural soil were several times higher. The origin of Cu in these soils is mainly of anthropogenic origin or consequence of the excessive use of pesticides that contain copper [28].

Large fluctuations in content of Zn, Cu and other heavy metals between different soils are result the impact of several factors: primarily geological substrate, mechanical composition of soil, sorption capacity, activity of microorganisms, OM content, pH value and specific soil properties such as calcium carbonate levels, clay content etc. [29].

3.2 Dynamics of Zn and Cu in the Soil - Plant System in Strawberry Cultivation

Average values for the content of Zn and Cu in leaves and fruits of strawberries are shown in Table 4.

In this study, the average content of Zn in the leaves and fruits of strawberries was high, but below toxic limit 150 mg/kg DW [9]. These results were significantly higher compared with the results of studies where content of Zn in strawberry varied from 20.37 mg/kg [30] to 37.38 mg/kg DW [31]. Gaweda and Ben [32] found slightly higher values for the Zn content of strawberry in the results of their research; 31 mg/kg in leaves, and 50 mg/kg DW in fruit of strawberries. Unfortunately, in these studies the content of Zn in soil where strawberries were cultivated has not been evaluated, so from results of these studies we could not get a more detailed insight into the dynamics of Zn in the 'soil - plant system' of strawberries on the examined locations, but it is indicative that in these research soil had a higher pH value (5.9 to 7.9) in relation to pH value of soil in our study (pH = 5.23). These data support the fact that soil acidity significantly impact on availability of heavy metal in soil. Namely, the acid reaction of soil contributes to the release of ions of most of heavy metals from adsorption soil complex, especially for Zn, thus making them more readily available to the roots of plant [33]. These observations are compatible with the conclusions of the research of many scientists who have examined the dynamics of Zn in the soil - plant system, or in other agricultural crops [34,35].

Apart from soil pH, OM in soil is also one of the most important soil properties affecting Zn and Cu availability to plants. The OM in soils could increase uptake of Zn and Cu to plant roots primarily because it contributes to the ability of soils for retaining heavy metals in an exchangeable form [36]. Du Laing et al. [37] reported that the dissolved OM in soils can serve as chelates and increase Zn and Cu availability to plants. McCauley et al. [38] also found that the contents of these elements were positively correlated with content of OM in soils.

Table 3. Zn and Cu content in examined soil (DW)

Parameter	Unit	Measured value	Limit value*
Zn	mg/kg	82.06 +/- 14.07	150
Cu	mg/kg	8.45 +/- 2.35	65

** limits for content of Zn and Cu in soils, as prescribed by the B-H legal regulations*

Table 4. Zn and Cu content in leaves and fruits of strawberries (DW)

Parameter tested	Leaves mg/kg	Fruit mg/kg	Toxic limit value*mg/kg
Zn	100.34 +/- 4.61	91.72 +/- 6.32	150
Cu	0.41 +/- 0.11	0.32 +/- 0.18	15

** toxic limit value for plant by Kastori*

Absorption of Zn and Cu by plants as well depends on the mechanical composition of soils. Heavy soils, as a pseudogley due to large amounts of suspended fraction, have a greater ability to retain metallic elements [39]. This fact is one of the possible causes for high content of Zn in the examined soils.

Data presented in Table 4 also showed that the content of Cu in the leaves and fruits of strawberry was very low, which is to be expected if one considers the low value of the Cu content in the examined soil. An additional reason for the low level of Cu accumulation in the leaves and fruits of strawberries is the fact that Cu is one of the elements that have a very low mobility in the plant and the fact that Cu and Zn are antagonists in soil. Namely, these two elements compete for the same membrane carriers in plant cells, which means that the higher presence of one element may a negative influence on the availability of another element [40]. Given the above, in the cultivation of strawberries on the examined site it is recommended to perform additional plant nutrition with copper fertilizers.

4. CONCLUSION

The present results of dynamics of Zn and Cu in the soil - plant system under conditions of intensive strawberry cultivation and their comparison with the findings reported in other related studies suggest that examined soil should not pose any risk to strawberry cultivation.Uptake, translocation and accumulation of Zn in plant was at a satisfactory level in accordance with the plant's needs for this element, which was not the case when the dynamics of Cu was studied. Some of the main reasons for that were: a low Cu content in the examined soil, low mobility of Cu in the plant, and antagonistic relationship between Zn and Cu in soil.Given the above, in the cultivation of strawberries on the examined site it is recommended to perform additional nutrition of strawberries with copper fertilizers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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