Heavy Metals Concentration in Greenhouse Soil Used in Intensive Cucumber Production (*Cucumis sativus* L.)

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Summary

Heavy metal contamination is one of the most important issues regarding the pollution of agricultural soils. Among heavy metals, cadmium (Cd), chromium (Cr) lead (Pb) and nickel (Ni) are of great concern, because they are toxic to organisms at low concentrations. The aim of this study was to evaluate the dynamics of Cr, Cd, Pb and Ni in a greenhouse soil-plant system under intensive cucumber production. The experiment was conducted in a greenhouse located at the Srebrenik municipality, in the north-eastern part of Bosnia and Herzegovina. Flame Atomic Absorption Spectrometry was used to determine the concentrations of Cr, Cd, Pb and Ni in soil and plant samples. The concentration of total Cr and Ni in the examined greenhouse soil was higher than the permissible values established by the legislation in Bosnia and Herzegovina, while concentrations of Cd and Pb did not exceed the permissible values determined by the same legislation. The concentration of available forms of all examined heavy metals in the tested soil as well as in edible parts of the cucumber was low regardless. These results indicate that the assessment of total heavy metals concentration in soils (to the contrary of the assessment of their available forms) is generally deficient in providing reliable information on the contamination of soil by heavy metals, and thus the suitability of such soils for cucumber production.

Key words

Food crops, health, safety, vegetable

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Introduction

Heavy metals are chemical elements with a density of more than 5 g cm^{-3}, and their high concentration in soil can greatly harm plant growth and reproduction (Järup, 2003). Furthermore, the uptake of heavy metals by plants and subsequent accumulation in edible parts of plants poses a potential threat to human health. Among heavy metals, cadmium (Cd), chromium (Cr) lead (Pb) and nickel (Ni) are of great concern because of their ability to negatively affect human health even in small amounts (Jaishankar et al., 2014). Pb has long been recognized as a causative agent of disorders of the central and peripheral nervous systems in humans (Sanders et al., 2009), Cd can cause damage to kidneys and bone structure (Åkesson et al., 2006), Cr has carcinogenic effects (Wang et al., 2017), while nickel exposure results in allergic reactions and respiratory disorders in humans (Rudel et al., 2013).

Cd, Cr, Pb and Ni contamination of soil is a result of various activities such as weathering of the earth's crust, mining, industrial effluents, and sewage discharge (Ming-Ho, 2005). Also, the changes in farming practices characterized by excessive fertilizer and pesticide use have led to soil pollution by hazardous heavy metals, especially in greenhouses where agricultural production is very intensive (Jia et al., 2010). Camelo et al. (1997) pointed out that phosphate fertilizers were an important source of heavy metals, as a result of their production from minerals containing certain amounts of heavy metals, especially Cd.

The presence of toxic heavy metals in soils does not necessarily imply their adverse effects on plants and consequently human health if they do not occur in a form that is easily absorbed by plants. Many scientists support the fact that the available forms of heavy metals in soils play a significant role in assessing the soil pollution by heavy metals (Aydinalp and Marinova, 2003; Nabulo et al., 2010). Thus, the examination of interrelations between the total and available forms of heavy metals may help to understand dynamics of heavy metals in soils and provide useful information on the real contamination of the soil by heavy metals as well as the suitability of these soils for food crops production. In addition, when assessing the usability of a soil for agricultural production in relation to heavy metal pollution, it is important that careful consideration be given to the chemical properties of the soil and their impact on the absorption of heavy metals by plant roots (Tchounwou et al., 2012).

In recent years, areas with greenhouses have rapidly increased across Bosnia and Herzegovina, as well as in Srebrenik region. Unfortunately, there is not much research in Bosnia and Herzegovina related to heavy metals pollution problem in greenhouse soils, especially there is no systematic investigation of the dynamics of Cr, Cd, Pb and Ni in the soil-plant system in intensive cucumber production. Since heavy metals may have many adverse health effects, it is very important to study the magnitude of heavy metal contamination in greenhouse soils, and their impact on vegetable safety, and thus on human health.

The objective of this study was to evaluate the dynamics of hazardous heavy metal (Cr, Cd, Pb and Ni) in the greenhouse soil-plant system in intensive cucumber production (*Cucumis sativus* L. 'Opalit F1').

Material and Methods

Study Area

The experiment was carried out from March to July 2018 in Srebrenik, Bosnia and Herzegovina, in a typical polyethylene-covered greenhouse with natural ventilation and two spans (25 x 9.6) covering an area of 480 m^2. A green knitted shade cloth was used to diffuse light and lower greenhouse temperature during warm days. The area under experiment in greenhouse was divided into three equal plots. The size of each plot was 25 x 4.8 m. According to Soil Taxonomy (FAO, 1998), examined greenhouse soil was classified as Fluvisol, and is characterized by the following physical properties: loam texture, 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.

Soil Sampling and Analysis

A composite soil sample was collected from the study area in March 2018, a few weeks before planting cucumbers and it was made by mixing several subsamples. Each subsample was taken using a clean stainless-steel shovel at a depth of 0 - 30 cm. Homogenised composite soil sample was air-dried at room temperature, crushed and ground using porcelain mortar and pestle, passed through sieves (2 and 1 mm) and then stored until analysis. Soil pH, humus content, content of available forms of phosphorus and potassium, and content of total and available forms of Cr, Pb, Cd and Ni were analysed.

Soil pH in H_2O and 1 M KCl was determined by pH meter according to ISO 10390 method (ISO, 2005), humus content by oxidation with potassium dichromate in the presence of sulphuric acid according to ISO 14235 method (ISO, 1998), available forms of phosphorus and potassium by AL method (Egner et al., 1960), and the total and available forms of Cr, Cd, Pb and Ni were determined by atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan) according to the instructions specified in the ISO 11047 method (ISO, 1998). Average values of three replicates were taken for each heavy metal measurement in a soil sample.

Previous extraction of total heavy metals from the soil sample was carried out as follows: 3 grams of air-dried soil (fraction smaller than 1 mm) was weighed and placed into 250 ml flat bottom flask, after which 28 ml 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 16 h (overnight) at room temperature, and then refluxed on hotplate two hours. The mixture was allowed to cool and filtered through quantitative filter paper into a 100 ml flask, and diluted to the mark with deionized water (ISO, 1995).

The extraction of available forms of heavy metals from the soil sample was performed using EDTA solution according to Trierweiler and Lindsay (1969) as follows: 10 g of air-dried soils was placed in 100 ml plastic bottle, then 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 bottle was shaken for 30 min at 180 rpm in an orbital shaker, and thereafter the extract was filtered through quantitative filter paper into a 25 ml flask and diluted to the mark with deionized water.

Plant Sampling and Analysis

From each examined plot five cucumber plants (whole plant with root) were carefully collected at commercial maturity stage. Leaves, root and fruits of each plant were separated, dried, ground and then stored in small paper bags until measurements.

The concentration of Cr, Cd, Pb and Ni in the plant samples (five average samples of root, leaves, stem and fruit) was determined by atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan) according to the instructions specified in the ISO 11047 method. The average values of three replicates were taken for each heavy metal determination in a plant sample.

Extraction of heavy metals from plant samples 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 was 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 were done in triplicates for each plant sample 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 $P \leq 0.05$ were considered as significantly different.

Results

A summary of selected soil chemical properties is given in Table 1.

Table 1. Basic chemical properties of the experimental greenhouse soil

Parameter	pH H_2O	pH KCl	organic matter (%)	P_2O_5 (mg 100 g^{-1})	K_2O (mg 100 g^{-1})
Value	6.5	5.6	2.81	16.84	80.9

The results of chemical soil analysis showed that examined greenhouse soil had slightly acidic reaction, medium level of organic matter, sufficient supply of phosphorus and high supply of potassium. In accordance with these results the following recommendations were given and carried out identical to all plots: 220 kg/ha superphosphate fertilizer as part of the autumn fertilization and 100 kg ha^{-1} urea and 300 kg ha^{-1} NPK 15:15:15 as part of fertilization during the growing season (amounts of fertilizers were thereafter calculated for each examined soil plot area).

Total heavy metals concentration (Cr, Cd, Pb, Ni) in average soil sample are listed in Table 2.

The concentration of Cr and Ni in the examined greenhouse soil were higher than the permissible value for agricultural soils established by the legislation in Bosnia and Herzegovina (Official Gazette of FBIH, 2009), while the concentration of Cd did not exceed the permissible values prescribed by the same legislation. The presence of Pb in the experimental greenhouse soil was not detected.

Table 2. Total and available heavy metals concentrations of the experimental greenhouse soil (mg kg^{-1} dry mass)

Heavy metal	Total	Available forms	Limit value for total[1]
Cr	128.48	0.21	100
Cd	0.04	0.02	1.5
Pb	17.68	0.51	100
Ni	104.55	2.02	40

[1] limit value prescribed by legislation in Bosnia and Herzegovina

Heavy metals concentration (Cr, Cd, Pb, Ni) in different parts of cucumber plant are presented in Table 3.

Table 3. Heavy metals concentrations in plant samples (mg kg^{-1} dry mass)

Part of plant	Cr	Cd	Pb	Ni
root	20.63 ± 2.93[a]	0.87 ± 0.21[a]	<q. l.	17.57 ± 2.95[a]
stem	2.28 ± 0.91[b]	0.45 ± 0.13[b]	<q. l.	2.73 ± 0.96[b]
leaves	1.81 ± 0.82[b]	0.48 ± 0.21[b]	<q. l.	3.14 ± 1.00[b]
fruit	2.18 ± 1.34[b]	0.02 ± 0.02[c]	<q. l.	3.19 ± 1.81[b]
$Lsd_{0.05}$	1.52	0.16	-	1.77

[1] <q. l. - below instrument quantification limit
The values marked with different letters in the same column indicate significant differences at $P \leq 0.05$

The concentrations of Cr in cucumber fruits were higher in comparison with the permissible value of Cr in food crops (2.3 mg kg^{-1}), recommended by FAO/WHO (2001). On the other hand, the content of Ni and Cd in cucumber fruits did not exceed the permissible values (4 mg kg^{-1} and 0.2 mg kg^{-1} respectively) prescribed by the same regulative, while the presence of Pb in cucumber fruits was not detected. The results of this study also showed that Cr, Ni and Cd accumulated in higher amounts in the roots of cucumber plants, especially Cr and Ni. The Cr and Ni concentration in the roots was several times higher in comparison with concentration of these elements in the other parts of cucumber.

Discussion

In the present study, the total Cr and Ni concentrations in the soil exceeded the limit values prescribed by legislation in Bosnia and Herzegovina (B and H), whereas the concentrations of Pb and Cd were considerably lower. These results are comparable to the results of many scientists who also found a high level of Ni and Cr concentration in soils in the wider area of Srebrenik municipality (Cipurković et al., 2011; Babajić et al, 2017). A detailed analysis of these studies leads to the conclusion that pollution of soil by

Ni and Cr in the examined area is mostly of geogenic origin. Namely, the geological structure at the area of Srebrenik municipality is characterized by sedimentary and magmatic rocks with Cr-enriched magnetite and ilmenite that greatly affect the mineral composition of the soil. In complex formations of parent material in Srebrenik area there are also Ni-rich minerals such as ferromagnesian minerals. The abundance of Ni in rocks generally correlates with those of Mg, Cr, Fe and Co, so in the soils with a high concentration of Cr, high concentration of Ni may be expected, and the preliminary results of this study support this hypothesis. Also, it should be noted that some anthropogenic factors such as intensive fertilization, can further contribute to heavy metal concentration increases in the soil, and this type of fertilization is characteristic of intensive cucumber production. Interestingly, the concentration of Ni in examined greenhouse soil was even more than 100% higher in relation to permissible Ni concentrations in agricultural soils prescribed by regulations in B and H. It is obvious that the prescribed permissible value for Ni in agricultural soils is very strict and that the wide geological diversity in B and H, physical and chemical properties of soils as well as high differences in the mineral composition of the parent material have not been thoroughly considered within above mentioned regulations. The obtained values for the available forms of Cr and Ni in the examined soil support this hypothesis. Namely, these values were very low. Moreover, the amount of available Cr and Ni in examined soil was only 0.16% and 1.9% in relation to total concentration of these elements in the soil, respectively. One of the reasons for the low level of available forms of Ni and Cr in soil is certainly soil pH. The investigated soil was slightly acidic (pH - 6.5), which is not the optimal medium for the release of heavy metals from the soil matrix and their bioavailability for plants. In acidic soil, the availability of heavy metals is much higher. Namely, in acidic soils prevailing is the leaching process, enhancing the release of Ni, Cr and generally heavy metal ions from soil adsorption complex, thus making them more accessible to the plant roots. Furthermore, the plants have evolved different mechanisms to protect themselves from negative effect of toxic heavy metals even when their availability in soil is high. One of these mechanisms is related to limiting of their uptake from the soil through complexion of metals with plant root exudates.

Accordingly, it is evident that the total amount of heavy metals does not provide completely reliable information on their mobility, availability, and toxicity in soils and thus the usability of these soils for agriculture.

The concentration of total and available forms of Cd and Pb in the examined soil was very low, indicating that the lithological structure i.e. the parent material from which examined soil formed is not rich in Pb and Cd ore deposits. In addition, chemical properties of the examined soil characterized by slightly acidic reaction which is not favourable for release and mobility of Cd and Pb additionally contributed to the decrease of available forms of Cd and Pb in soil. Accordingly, the high Cd and Pb concentrations in food crops grown on studied soil were not expected, and the results of analysis of Cd and Pb concentrations in cucumber plants confirm this hypothesis. Moreover, the presence of Pb in any examined parts of cucumber plant was not detected.

The results of this study confirmed the fact that the heavy metals (Cr, Ni and Cd) accumulated in higher amounts in the roots. The concentration of these heavy metals in the above-ground parts of a cucumber was significantly lower. These results suggest that plants possess different mechanisms not only to prevent the entry of heavy metals into root cells, but also to limit translocation of heavy metals from root to other parts of plant. Heavy metal compartmentalization in different intracellular compartments in root cells, or biosynthesis and accumulation of several compounds aimed at metal complexation are certainly some of these mechanisms (Singh et al., 2015).

Conclusions

This study reveals that the assessment of total amounts of Ni and Cr in soils is generally deficient in predicting their toxicity for plants or in providing reliable information on the contamination of soil by these elements, and thus suitability of such soils for agricultural production. The results of this study lead to the conslusion that the concentration of available forms of heavy metals in soils gives more reliable data in assessing soil contamination by heavy metals.

References

Åkesson A., Bjellerup P., Lundh T., Lidfeldt J., Nerbrand C., Samsioe G., Skerfving S., Vahter, M. (2006). Cadmium-Induced Effects on Bone in a Population-Based Study of Women. Environ Health Perspect 114 (6): 830-834. doi: 10.1289/ehp.8763

Aydinalp C., Marinova S. (2003). Distribution and Forms of Heavy Metals in Some Agricultural Soils. Pol J Environ Stud 12 (5): 629-633

Babajić A., Babajić E., Srećković-Batoćanin D., Milovanović D. (2017). Petrographic Characteristics of Mafic Extrusive Rocks along the Southwestern Part of Majevica. Archives for Technical Sciences 16 (1): 1-8. doi: 10.7251/afts.2017.0916.001B

Camelo L. G. L., Miguez S. R., Marbán L. (1997). Heavy Metals Input with Phosphate Fertilizers Used in Argentina. Sci Total Environ 204 (3): 245-250

Cipurković A., Selimbašić V., Tanjić I., Mičević S. Pelemiš D., Čeliković R. (2011). Heavy Metals in Sedimentary Dust in the Industrial City of Lukavac. Eur J Sci Res 54 (3): 347-362

Egnér H., Riehm H., Domingo W. R. (1960). Investigations on the Chemical Soil Analysis as a Basis for the Assessment of the Nutrient status of the Soil. II. Chemical Extraction Methods for Determination of Phosphorus and Potassium. K Lantbr Hogsk Annlr 26: 199-215 (In German)

Food and Agriculture Organization and World Health Organization OF United Nations (2001). FAO/WHO, Report on the Session of the Codex Committee on Food Additives and Contaminants, FAO/WHO Food Standard Programme, Codex Alimentarius Commission, 2–7 July, Geneva, Switzerland.

Food and Agriculture Organization of the United Nations (1998). FAO, World Reference Base for Soil Resources: World Soil Resources Report No. 84. Rome, Italy.

International Organization for Standardization (1995). International Standard ISO 11466, Soil quality - Extraction of Trace Elements Soluble in Aqua Regia. Geneve, Switzerland.

International Organization for Standardization (1998). International Standard ISO 14235, Soil quality - Determination of Organic Carbon in Soil by Sulfochromic Oxidation. Geneva, Switzerland.

International Organization for Standardization (1998). International Standard ISO 11047, Soil quality - Determination of Cadmium, Chromium, Cobalt, Copper, Lead, Manganese, Nickel and Zinc - Flame and Electrothermal Atomic Absorption Spectrometric Methods. Geneva, Switzerland.

International Organization for Standardization (2005). International Standard ISO 10390, Soil quality - Determination of pH. ISO, Geneva, Switzerland.
Jaishankar M., Tseten T., Anbalagan N., Mathew B. B., Beeregowda K. N. (2014). Toxicity, Mechanism and Health Effects of Some Heavy Metals. Interdiscip Toxicol 7(2): 60-72. doi: 10.2478/intox-2014-0009
Järup L. (2003). Hazards of Heavy Metal Contamination. Br Med Bull 68 (1): 167-182. doi: 10.1093/bmb/ldg032
Jia L, Wang W, Li Y, Yang L. (2010). Heavy Metals in Soil and Crops of an Intensively Farmed Area: A Case Study in Yucheng City, Shandong Province, China. Int J Environ Res Public Health 7(2): 395-412. doi:10.3390/ijerph7020395
Lisjak M, Špoljarević M, Agić D, Andrić L, 2009. Plant Physiology Laboratory Manual. Faculty of Agriculture, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, pp. 25-26 (In Croatian)
Ming-Ho Y. (2005). Environmental Toxicology: Biological and Health Effects of Pollutants, Second Edition. CRC Press LLC, Boca Raton, USA, pp. 22-46
Nabulo G., Young S. D., Black C. R. (2010). Assessing Risk to Human Health from Tropical Leafy Vegetables Grown on Contaminated Urban Soils. Sci Total Environ 408 (22): 5338-51. doi: 10.1016/j.scitotenv.2010.06.034
Official Gazette of FBiH (2009). Rulebook on Determination of Allowable Quantities of Harmful and Hazardous Substances in Soils of Federation of Bosnia and Herzegovina and Methods for Their Testing, No 72/09. Sarajevo, Bosnia and Herzegovina (In Bosnian)
Rudel D., Douglas C. D., Huffnagle I. M., Besser J. M., Ingersoll C. G. (2013). Assaying Environmental Nickel Toxicity Using Model Nematodes. PLoS ONE 8 (10): e77079. doi: 10.1371/journal.pone.0077079
Sanders T., Liu Y., Buchner V., Tchounwou, P. B. (2009). Neurotoxic Effects and Biomarkers of Lead Exposure: A Review. REVEH 24 (1): 15-45. doi: 10.1515/REVEH.2009.24.1.15
Singh S., Parihar P., Singh R., Singh V. P., Prasad S. M. (2015). Heavy Metal Tolerance in Plants: Role of Transcriptomics, Proteomics, Metabolomics, and Ionomics. Front Plant Sci 6: 1143. doi: 10.3389/fpls.2015.01143
Tchounwou P. B., Yedjou C. G., Patlolla A. K., Sutton D. J. (2012). Heavy metal toxicity and the environment. EXS 101: 133-64. doi: 10.1007/978-3-7643-8340-4_6
Trierweiler J. E., Lindsay W. L. (1969). EDTA-ammonium carbonate soil test for zinc. Soil Sci Soc Am Proc 39: 49-54. doi: 10.2136/sssaj1969.03615995003300010017x
Wang Y., Su H., Gu Y., Song X., Zhao J. (2017). Carcinogenicity of chromium and chemoprevention: a brief update. Onco Targets Ther 10: 4065-4079. doi: 10.2147/OTT.S139262

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