Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 2, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE ASSESSMENT OF HEAVY METAL BIOACCUMULATION IN PEPPER PLANTS (*Capsicum annuum*) CULTIVATED IN GREEN-HOUSE CONDITIONS, USING CONTAMINATED SOILS FROM THE INDUSTRIAL AREA OF COPȘA MICĂ

Mihaela COSTEA[1, 2], Nicoleta-Olimpia VRÎNCEANU[1], Dumitru-Marian MOTELICĂ[1], Florența PARASCHIV (JAFRI)[1, 2], Costică CIONTU[2]

[1]National Research and Development Institute for Soil Science, Agrochemistry and Environment, 61 Mărăşti Blvd, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: florenta.jafri@doctorat.usamv.ro

Abstract

The study investigated the effects of cadmium, lead, zinc, and copper contamination on the bioaccumulation of heavy metals in pepper plants (Capsicum annuum L.). The experiment was conducted using soil materials contaminated with heavy metals, collected from 24 individual households in the Copșa Mică area, selected to ensure a large range of soil reactions and total heavy metal content. To evaluate the mobility and bioaccessibility of the metals, pepper seedlings (Capsicum annuum L.) were cultivated in green-house conditions. The mobility of metals were assessed by using two extraction methods:extraction with solution NH_4NO_3 (1M) for the easily exchangeable forms and DTPA-$CaCl_2$-TEA, for the bioavailable forms. Experimental data indicated a significant correlation between the cadmium and lead content in soil, in their bioavailable forms, and their content in edible parts of pepper plants. Cadmium demonstrated higher mobility and bioaccumulation compared to lead. The results showed that pepper plants have a relatively low capacity to bioaccumulate zinc and copper but can accumulate cadmium and lead under highly soil contamination conditions.

Key words: *bioaccumulation, heavy metals, soil contamination pepper.*

INTRODUCTION

The contamination of soils with heavy metals is a global issue, particularly in industrialized regions, having a major impact on environmental quality and food safety (Kabata-Pendias, 2011; Alloway, 2013;). Heavy metals such as lead (Pb), cadmium (Cd), and zinc (Zn) are extremely toxic even in low concentrations and can severely affect human health when ingested through food grown in contaminated soils. Nagajyoti et al., 2010, argues that metals are not biodegradable and tend to accumulate in the soil, entering the food chain and eventually reaching human and animal consumers.

Mousavi et al. (2013) and Sabău (2019), presented some causes of anthropogenic soil pollution, including the processing and exploitation of non-ferrous metals, waste and residues, which play an important role in soil pollution. Exhaust gases from road traffic and improper waste disposal practices are also another source of soil pollution, as well as the treatment of soil with fertilizers and pesticides, sludge from wastewater treatment, irrigation with contaminated water, and the burning of fossil fuels, all contributing to soil pollution.

The industrial region of Copșa Mică in Romania is one of the most polluted in Europe, affected by the contamination of soil and groundwater with heavy metals as a result of decades of intense industrial activity. For several decades, Copșa Mică has been a major center of non-ferrous metal processing and the main source of pollution being emissions from industrial platform. This has led to the degradation of surrounding soils and significant accumulation of heavy metals, especially lead and cadmium, in the soil and vegetation.

Yang et al. (2009), Hu et al. (2013) and Jolly et al. (2013), claim that vegetables are edible crops and an essential part of the human diet. They are rich in nutrients necessary for the human body and are an important source of carbohydrates, vitamins, minerals, and fiber. At the same time, they argue that heavy metals can be easily absorbed by the roots of vegetables and can accumulate at high levels in the edible parts of the vegetables, even when heavy metal contents in the soil are at low levels.

According to Ali et al.(2013) and Singh et al. (2015), different plant species have the ability to absorb and accumulate heavy metals through complex transport and cellular retention mechanisms. These processes, are well documented in metal-tolerant species, often used in phytoremediation, such as Indian mustard *(Brassica juncea)* and sunflower *(Helianthus annuus)(Rizwan et al., 2016)*. For example, a study conducted at the National Institute for Laser, Plasma, and Radiation Physics showed that heavy metals such as Pb and Cd are extremely toxic and can cause mutations or cancer, even in small quantities (Achim et al., 2016).

Several researchers (Goyer, 1993; Klaassen et al., 1999; Navas-Acien et al., 2007; Hartley, 2008; Ekong et al., 2016;) considered that heavy metals can persistently accumulate in the body throughout life. They state that chronic exposure to heavy metals, especially cadmium (Cd), can affect the immune system, damage the lungs and liver, negatively impact the development of the nervous system in children, and induce hypertension and cardiovascular diseases.

Capsicum annuum L., known as bell pepper, is an important horticultural crop and is widely consumed globally due to its nutritional value and high content of vitamins and antioxidants. Pepper plants, like other vegetable species, are capable of absorbing heavy metals from the soil and transfer them to different parts of the plant, including the fruit, which can pose a health risk to consumers when grown in contaminated soils (López-Millán et al., 2009).

Bell pepper is one of the plants sensitive to heavy metals, and bioaccumulation in its tissues can vary significantly depending on the type of metal and its concentration in the soil (Maleki et al., 2008; Nagajyoti et al., 2010). Thus, the health risks associated with the consumption of peppers from contaminated areas are significant, as exposure to heavy metals can lead to chronic conditions, including neurological disorders, renal and hepatic dysfunction, as well as carcinogenic effects (Tchounwou et al., 2012).

Given the toxic impact of heavy metals and the lack of effective soil remediation measures in highly contaminated areas, studies on the accumulation capacity of these metals by different crops are essential to assess the risk to food safety (Sharma et al., 2005).

The objective of this study is to evaluate the levels of accumulation of heavy metals (Cu, Cd, Pb, Zn) in bell pepper plants grown in protected spaces, using contaminated soil materials. The study also aims to estimate the concentrations of these metals in edible parts of bell pepper plants (fruits), with the goal of providing essential data on the risks of consumption and supporting the need for soil decontamination measures in the region.

MATERIALS AND METHODS

For this study, 24 soil samples were collected from various rural households located in the industrial area of Copșa Mică. The purpose of this selection was to evaluate the diversity of soil conditions and their impact on the bioaccumulation of heavy metals in vegetables.

The soil samples were analyzed in the Physico-Chemical Analysis Laboratory of the National Institute for Research and Development in Soil Science, Agrochemistry, and Environmental Protection (ICPA Bucharest), to assess the mobility and bioaccessibility of heavy metals (Cu, Zn, Cd, Pb) in the soil using two extractants: NH_4NO_3 (1M), at a soil:solution ratio (w/v) of 1:2 for extracting exchangeable forms, and DTPA-$CaCl_2$-TEA for extracting the potentially available forms of metals.

Each soil material was placed in 3L pots, in which sweet pepper plants (*Capsicum annuum* L.) were cultivated to evaluate the accumulation of heavy metals. The experiment was conducted under greenhouse conditions to control environmental factors. The planting material was obtained from a local producer and planted in May 2023.

The sweet pepper plants were grown in greenhouses (Figure 1) for 10 weeks under controlled humidity and temperature conditions. The development of the sweet pepper plants grown in the 24 soil types, with different levels of heavy metal contamination, is presented in Figure 2.

Figure 1. Experimental setup organized in greenhouses for estimating the bioaccumulation of heavy metals in bell peppers (Vegetation House, ICPA, 2023)

Figure 2. Development of bell pepper plants in the experiment organized in vegetation pots for estimating the bioaccumulation of heavy metals (Vegetation House, ICPA, 2023)

At the end of the experimental period, the edible part (fruit) of the sweet pepper plants, harvested from the pots, was washed, chopped, treated with 10 ml of HNO_3, and then mineralized in the Ethos Easy microwave oven. To determine the concentrations of heavy metals extractable with NH_4NO_3 and DTPA-$CaCl_2$-TEA from the soil and to measure the concentrations of heavy metals in the edible parts of the sweet pepper plants (fruits), both the soil and plant samples extracts were analyzed by atomic absorption spectrometry (AAS) using the iCE 3000 equipment.

For the bioaccumulation analysis in the sweet pepper fruits, both data on the total heavy metal content in the soil and data on their potentially mobile forms were used. The evaluation of correlations between the metal concentrations in the soil and those in the sweet pepper fruits was carried out using power regression charts, considering both total and bioaccessible forms.

RESULTS AND DISCUSSIONS

In the study, correlations between the heavy metal contents (cadmium, lead, zinc, and copper) in the soil and their accumulation in bell pepper plants (*Capsicum annuum* L.) were investigated using logarithmic regression curves of the power type. The main goal was to estimate the bioaccumulation of these metals in bell peppers based on their different forms in the soil (total forms and potentially available forms), considering that the mobility of metals in soil and the plants' ability to translocate them into the fruits can vary considerably.

In Figure 3, logarithmic plots for the power-type regression curves are presented, estimating the stochastic dependence between cadmium content in soil (total forms and potentially available forms) and cadmium content in bell peppers (fruits) cultivated on contaminated soils. The transfer of cadmium from soil to plant is facilitated by the presence of this element in potentially available forms for plants. Numerous conventional extraction methods, universally accepted, are used to estimate the cadmium content present in various forms in the soil. In our study, two extractants, recognized for their ability to extract cadmium in the exchangeable form (extraction with NH_4NO_3) and the potentially available form (extraction with DTPA-$CaCl_2$-TEA buffered solution), were used.

Given the relatively high mobility of cadmium in soil, both the total cadmium content in the soil and the data on cadmium contents in the two potentially mobile forms in the soil were used to estimate the bioaccumulation of this metal in bell pepper fruit. The values of the parameters used varied as follows:

- Cd_{total}: 0.24 mg/kg and 19.27 mg/kg;
- Cd_{DTPA}: 0.20 mg/kg and 8.83 mg/kg;

- Cd_{NH4NO3}: 0.01 mg/kg and 0.15 mg/kg;
- Cd_{plant}: 0.029 mg/kg and 0.106 mg/kg.

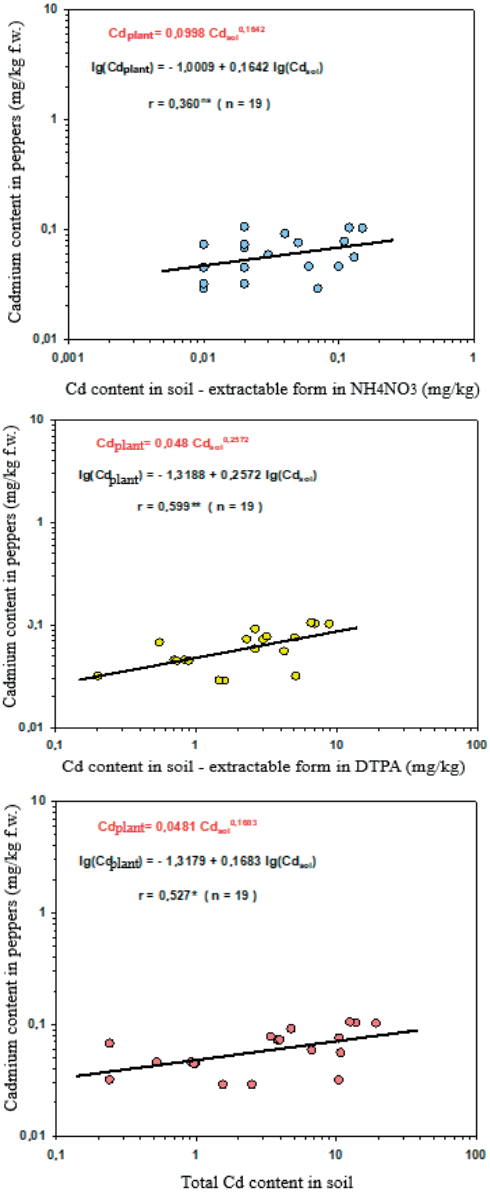

Figure 3. Logarithmic diagrams for power regression curves estimating the stochastic dependence between cadmium concentrations in soil and cadmium content in peppers

The strongest correlation was obtained between the cadmium content extractable in DTPA-$CaCl_2$-TEA buffered solution and the cadmium content in bell peppers (fruits). The form of cadmium extractable in this buffered solution is considered by some authors to be the most accessible for plants, which explains the strong dependence obtained in the greenhouse experiment.

Furthermore, the bioaccumulation of this element in bell peppers can also be estimated based on the total cadmium content in the soil, using power regression curves. The linear correlation coefficient obtained for this stochastic dependence was significantly different from zero, indicating a close correlation between the two studied variables. Using the values for cadmium extractable in NH_4NO_3, considered to be the easily exchangeable form, did not yield satisfactory results in estimating cadmium bioaccumulation in bell peppers edible parts (Figure 3).

According to studies, different plant species have the ability to absorb and accumulate heavy metals through complex transport and cellular retention mechanisms. However, bell peppers are a plant sensitive to heavy metals, and bioaccumulation in tissues can vary depending on the type of metal and its concentration in the soil. For example, a study conducted at the National Institute for Laser, Plasma, and Radiation Physics showed that heavy metals such as Pb and Cd are extremely toxic and can cause mutations or cancer, even in small amounts (Achim et al., 2016). Singh et al. (2012), showed that different vegetable crops grown on soils contaminated with heavy metals demonstrated significant differences in terms of accumulation, absorption, and distribution of metals. The cultivated species also showed remarkable differences in heavy metal concentrations in different parts of the plants. For example, root vegetables like radishes and carrots accumulated smaller amounts of heavy metals, except for zinc, while leafy vegetables such as spinach and mustard accumulated larger amounts of both essential and non-essential metals, except for cadmium and nickel. Potatoes and onions accumulated more cadmium and nickel, while cauliflower and cabbage showed greater accumulation of lead and nickel. In terms of absorption, cauliflower and cabbage absorbed more zinc, lead, and nickel, and mustard absorbed more zinc and cadmium. They concluded that leafy vegetables appear unsafe for soils contaminated with heavy metals, while fruit-type vegetables, such as peas, are more suitable for soils polluted with cadmium, but not for those with nickel and lead.

Literature data indicate bell pepper as having a low rate of translocation of heavy metals to the fruit (Angelova et al., 2009; Morikawa, 2017). Morikawa (2017), analyzing results from a study conducted in 52 individual gardens located in a contaminated area, found that the cadmium content in bell pepper fruits varied between 0.02-0.09 mg/kg fresh weight, with differences depending on the analysed variety. In our experiment, it is notable that for all experimental variants, the cadmium contents in bell peppers (fruits) exceeded the limit value (0.020 mg/kg fresh weight) set for this element by Commission Regulation (EU) 1323/2021 of August 10, 2021.

In Figure 4, logarithmic plots for the power-type regression curves are shown, estimating the stochastic dependence between lead content in the soil (total forms and potentially accessible forms) and lead content in bell peppers (fruits). Lead mobility in the soil is much lower compared to that of cadmium. This also explains the low lead contents in the plant as well as in the potentially accessible forms in the soil.

The values of the parameters used varied as follows:

- Pb_{total}: 19 mg/kg and 668 mg/kg;
- Pb_{DTPA}: 5.6 mg/kg and 134 mg/kg;
- $Pb_{NH_4NO_3}$: 0.02 mg/kg and 0.12 mg/kg;
- Pb_{plant}: 0.010 mg/kg and 0.080 mg/kg.

Based on the correlations obtained in this study, it can be concluded that the bioaccumulation of lead in bell peppers (fruits) can be satisfactorily described using both the total lead content in the soil and the bioavailable lead content (extractable with DTPA-$CaCl_2$-TEA solution).

The linear correlation coefficients obtained for the stochastic dependencies between the lead content in the plant and the total Pb and bioavailable lead contents were significantly different from zero, indicating a strong correlation between the considered parameters.

As with cadmium, the lead extractable in NH_4NO_3 solution did not correlate with the lead content in the plant, excluding this parameter from being used to estimate lead bioaccumulation in bell peppers.

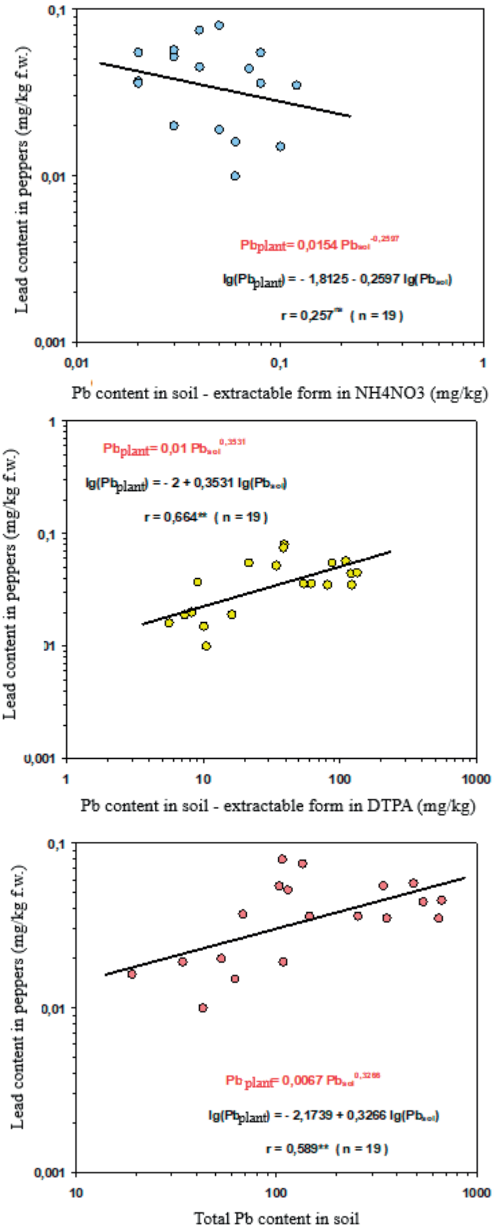

Figure 4. Logarithmic diagrams for power regression curves estimating the stochastic dependence between lead concentrations in soil and lead content in peppers

Regarding the lead content in the plant, it was observed that in none of the experimental variants considered, the maximum level set by Commission Regulation (EU) 1317/2021 of August 9, 2021, was exceeded (Figure 4).

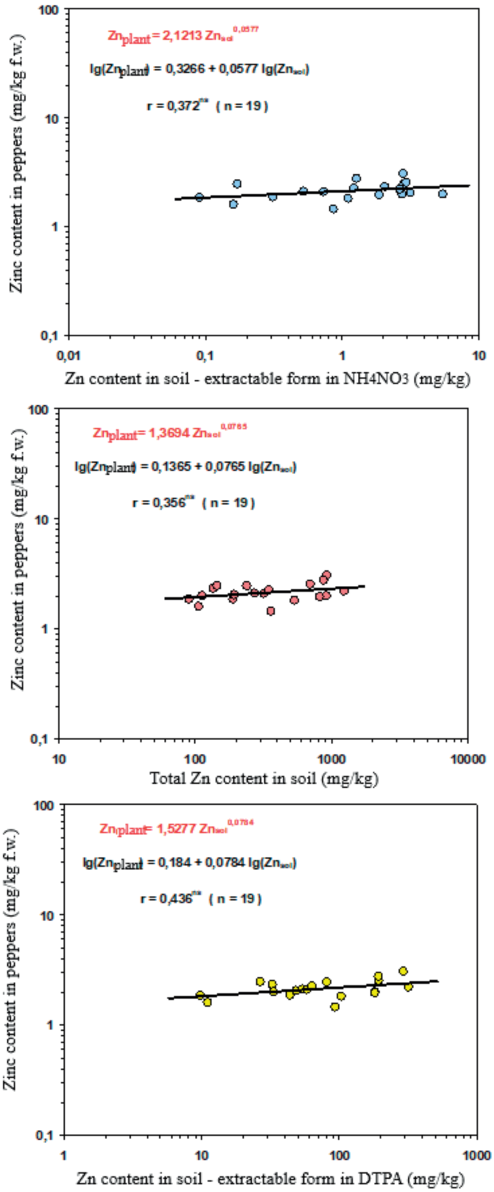

Figure 5. Logarithmic diagrams for power regression curves estimating the stochastic dependence between zinc concentrations in soil and zinc content in peppers

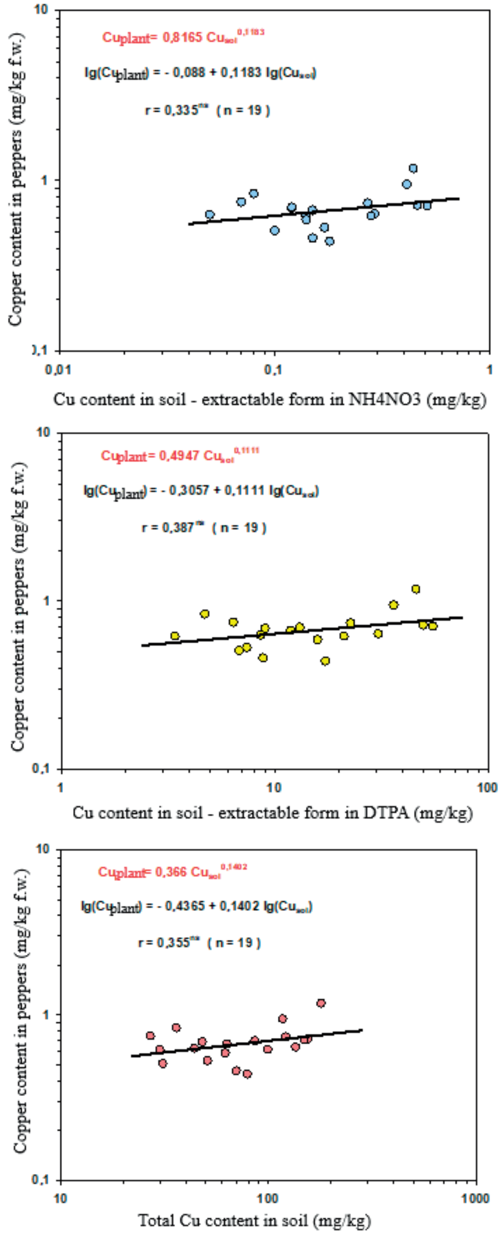

Figure 6. Logarithmic diagrams for power regression curves estimating the stochastic dependence between copper concentrations in soil and copper content in peppers

The reduced potential for lead accumulation observed in bell peppers is also confirmed by the data obtained in this experiment. Thus, bell pepper plants grown on a soil material with a total lead content of 668 mg/kg accumulated reduced quantities of lead in the fruit (0.080 mg/kg fresh weight).

The data collected from the bell pepper experiment, organized in experimental pots using soil materials with different contamination degrees, were also used to parameterize the bioaccumulation models for zinc in bell peppers (fruits). Due to the reduced ability of this vegetable species to accumulate heavy metals, as well as the role of zinc as a micronutrient, the correlations established between the considered parameters had no significantly linear correlation coefficients (Figure 5).

It is noteworthy that peppers, although grown in soils with excessive zinc content (1,323 mg/kg), accumulated very small amounts in the fruit (3.08 mg/kg dry weight). Moreover, although the zinc concentrations in the soil had a wide range of variation, the zinc concentrations determined in peppers (fruit) varied within a very narrow range. The values of the parameters used varied as follows:

- Zn_{total}: 90 mg/kg and 1,232 mg/kg;
- Zn_{DTPA}: 9.7 mg/kg and 318 mg/kg;
- $Zn_{NH_4NO_3}$: 0.09 mg/kg and 5.43 mg/kg;
- Zn_{plant}: 1.46 mg/kg and 3.08 mg/kg.

The logarithmic diagrams for power regression curves estimating the stochastic dependence between copper concentrations in soil and copper content in peppers (fruit) are shown in Figure 6. Since copper is not a major contaminant of the soil materials used for experimentation, and it also plays an essential role in plant development, no strong correlations were established between the parameters considered to describe the accumulation of this element in peppers. Furthermore, the range of variation of the parameters considered was relatively narrow. Thus, the values of the parameters used varied as follows:

- Cu_{total}: 27 mg/kg and 179 mg/kg;
- Cu_{DTPA}: 3.4 mg/kg and 55 mg/kg;
- $Cu_{NH_4NO_3}$: 0.05 mg/kg and 0.51 mg/kg;
- Cu_{plant}: 0.44 mg/kg and 1.18 mg/kg.

For none of the correlations tested, significant linear correlation coefficients different from zero were obtained. Therefore, the data collected during this study do not allow the development of stochastic models for estimating copper bioaccumulation in peppers. Moreover, the obtained stochastic dependencies confirm the ability of these vegetables to reduce the transfer of metals into the fruit.

CONCLUSIONS

The study demonstrated that peppers have a reduced capacity to accumulate heavy metals, being capable of accumulating cadmium and lead in the fruits, but with a low potential for accumulating zinc and copper. The power logarithmic regression models showed significant correlations between the bioaccumulation of cadmium and lead and their bioavailable contents in the soil, especially in the forms extractable with the DTPA-$CaCl_2$-TEA buffered solution. These results are valuable for evaluating the risks of vegetable contamination, particularly in the case of soils contaminated with heavy metals, and for developing monitoring and protection strategies for crops.

The experimental results obtained in our study are supported by literature data (Angelova et al., 2009; Morikawa, 2017 and Lidikova et al., 2021), which mentioned peppers as having a low rate of heavy metal translocation into the fruit, making this vegetable a relatively safe option in terms of heavy metal accumulation in conditions of soil contamination.

REFERENCES

Achim, C., Bercu, M., Bratu, A., & Dumitraș, D. C. (2016). Impactul metalelor grele asupra plantelor. Știință și Tehnică (https://stiintasitehnica.com/impactul-metalelor-grele-asupra-plantelor/)

Ali, H., Khan, E., & Sajad, M.A. (2013). Phytoremediation of heavy metals-concepts and applications. *Chemosphere*, 91(7), 869-881.

Alloway, B. J. (Ed.). (2012). Heavy metals in soils: trace metals and metalloids in soils and their bioavailability (Vol. 22). *Springer Science & Business Media.*

Angelova, V. R., Babrikovb T. D., Ivanova, K. I., 2009. Bioaccumulation and distribution of lead, zinc, and cadmium in crops of Solanaceae family. *Commun. Soil Sci. Plant Anal.,* 40, 2248–2263.

Ekong, E. B., Jaar, B. G., & Weaver, V. M. (2006). Lead-related nephrotoxicity: a review of the epidemiologic evidence. *Kidney international*, 70(12), 2074-2084.

Goyer, R. A. (1993). Lead toxicity: current concerns. *Environmental health perspectives*, 100, 177-187.

Hartley, W., & Lepp, N. W. (2008). Remediation of arsenic contaminated soils by iron-oxide application, evaluated in terms of plant productivity, arsenic and phytotoxic metal uptake. *Science of the Total Environment*, 390(1), 35-44.

Hu, J., Wu, F., Wu, S., Cao, Z., Lin, X., & Wong, M. H. (2013). Bioaccessibility, dietary exposure and human risk assessment of heavy metals from market vegetables in Hong Kong revealed with an in vitro gastrointestinal model. *Chemosphere, 91(4), 455-461.*

Jolly, Y. N., Islam, A., & Akbar, S. (2013). Transfer of metals from soil to vegetables and possible health risk assessment. *SpringerPlus, 2, 1-8.*

Klaassen, C. D., Liu, J., & Choudhuri, S. (1999). Metallothionein: an intracellular protein to protect against cadmium toxicity. *Annual review of pharmacology and toxicology, 39(1), 267-294.*

Lidiková, J., Ceryová, N. , Šnirc, M., Musilová, J., Harangozo, L., Vollmannová, A., Brindza, J., Grygorieva, O. 2021. Heavy Metals Presence in the Soil and Their Content in Selected Varieties of Chili Peppers in Slovakia. *Foods 2021, 10, 1738. https://doi.org/10.3390/ foods1008173*

López-Millán, A. F., Sagardoy, R., Solanas, M., Abadía, A., & Abadía, J. (2009). Cadmium toxicity in tomato (Lycopersicon esculentum) plants grown in hydroponics. *Environmental and experimental botany,* 65(2-3), 376-385.

Maleki, A., & Zarasvand, M. A. (2008). Heavy metals in selected edible vegetables and estimation of their daily intake in Sanandaj, Iran. *Southeast Asian journal of tropical medicine and public health*, 39(2), 335.

Morikawa, C.K. (2017). Reducing Cadmium Accumulation in Fresh Pepper Fruits by Grafting. *The Horticulture Journal* 86 (1): 45–51.

Mousavi, S. M., Bahmanyar, M. A., & Pirdashti, H. (2013). Phytoavailability of some micronutrients (Zn and Cu), heavy metals (Pb, Cd), and yield of rice affected by sewage sludge perennial application. *Communications in soil science and plant analysis*, 44(22), 3246-3258.

Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental chemistry letters*, 8, 199-216.

Navas-Acien, A., Guallar, E., Silbergeld, E. K., & Rothenberg, S. J. (2007). Lead exposure and cardiovascular disease—a systematic review. *Environmental health perspectives, 115(3), 472-482.*

Commission Regulation (EU) 2021/1317 of 9 August 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of lead in certain foodstuffs.

Commission Regulation (EU) 2021/1323 of 10 August 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of cadmium in certain foodstuffs.

Rizwan, M., Ali, S., Adrees, M., Rizvi, H., Zia-ur-Rehman, M., Hannan, F., ... & Ok, Y. S. (2016). Cadmium stress in rice: toxic effects, tolerance mechanisms, and management: a critical review. *Environmental Science and Pollution Research*, 23, 17859-17879.

Sabău, N. C. (2009). Poluarea mediului pedosferic. Editura Universităţii din Oradea.

Sharma, R. K., & Agrawal, M. (2005). Biological effects of heavy metals: an overview. *Journal of environmental Biology*, 26(2), 301-313.

Singh, A., & Prasad, S. M. (2015). Remediation of heavy metal contaminated ecosystem: an overview on technology advancement. *International Journal of Environmental Science and Technology*, 12, 353-366.

Singh, S., Zacharias, M., Kalpana, S., & Mishra, S. (2012). Heavy metals accumulation and distribution pattern in different vegetable crops. *Journal of Environmental Chemistry and Ecotoxicology, 4(10), 170-177.*

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Molecular, clinical and environmental toxicology: volume 3: environmental toxicology, 133-164.*

Yamamoto, F., & Kozlowski, T. T. (1987). Effects of flooding, tilting of stems, and ethrel application on growth, stem anatomy, and ethylene production of Acer platanoides seedlings. *Scandinavian Journal of Forest Research*, 2(1-4), 141-156.

Yang, Y., Zhang, F. S., Li, H. F., & Jiang, R. F. (2009). Accumulation of cadmium in the edible parts of six vegetable species grown in Cd-contaminated soils. *Journal of environmental management*, 90(2), 1117-1122.