STUDIES ON THE INFLUENCE OF HEAVY METALS COPPER AND CADMIUM ON THE DYNAMICS OF PLANT GROWTH AND DEVELOPMENT OF BELL PEPPER (*CAPSICUM ANNUUM L.*) VARIETY *DARIANA BAC*

Gabriel-Alin Iosob[1*], Tina Oana Cristea[1], Maria Prisecaru[2]

[1]Vegetable Research and Development Station Bacău, 220 Calea Bârladului Str., 600388 Bacău, România

[2]Vasile Alecsandri University of Bacău, Faculty of Biology, 157 Mărăşeşti Str., 600115 Bacău, România

Abstract

Pepper plants (Capsicum annuum L.) variety Dariana Bac, patented by Vegetable Research and Development Station Bacău, were grown on peat substrate soil contaminated with different concentrations of Copper (20, 100, 250, 500 mg/kg soil) and Cadmium (1, 3, 5, 10 mg/kg soil). After approximately nine weeks the dynamic of plant growth and development was measured. The total length of a plant (cm), stem length (cm) and root lenght (cm), number of leaves per plant, fresh and dry weight of a plant (g) and dry matter (%). The variety of bell pepper Dariana Bac responded differently to different concentrations of the studied heavy metals. The maximum average of plant and root length was registered on control (30,43 cm respectively 10 cm). The average number of leaves per plant did not differ significantly in any of the variants studied. However, the average fresh weight of a plant decreases with increasing the concentration of heavy metals in the soil, especially in case of the soil treated with Cd in comparison with control. As for the average dry weight of a plant it does not vary depending on the concentration of heavy metals like in case of fresh weight. V_9 (0,122 g) variant treated with 10 mg Cd / kg soil has the lowest dry weight value compared with all tested variants.

Keywords: *Pepper plants, Capsicum annuum L., dynamic of plant growth, heavy metals, Copper and Cadmium*

1. INTRODUCTION

One of the most pressing concerns, as a result of global climate change (Qayyum et al., 2020), is the heavy metal (HM) contamination and the food security and human health (Zheng et al., 2020), the crops are suffering because of biotic and abiotic factors. In present, synthetic products is the problem for urban and agriculture soils pollution. The heavy metal contamination from industrial waste, pesticides, etc. (Qayyum et al., 2020).

Pepper (*Capsicum annuum* L.) is a plant that prefers a warm climate. Among vegetable species, it occupies an important place with numerous uses. The fruits of peppers have a great importance because they can be eaten both fresh and canned. They contain a number of bioactive compounds and essential nutrients, and are also capable of accumulating heavy metals from contaminated soils (Iosob et al., 2019c). High temperatures of air and root zones reduce the yield of bell pepper and increase the incidence of fruit disorders, such as rot blossom-end and hip (Díaz-Pérez and John, 2019). Vegetables are vital for the human diet, that's why the presence in excess of HM in soil is a global problem that threatens plant health, of the fauna and the people to (Iosob et al., 2019a). This study shows the effects of two heavy metals (Cu and Cd) on the dynamics of seedlings plant growth and development of bell pepper (*Capsicum annuum* L.) variety Dariana Bac., patented by Vegetable Research and Development Station Bacău. It's an early variety, with a vegetation period of 120 days until fruiting. The plant has medium vigor. Port 50% erect, 50% horizontal. Color - yellow with green hues at technological maturity and bright red at physiological maturity. Shape - trapezoidal in longitudinal section. Length it is between 9.5-11.5 cm, diameter it is between 7.5-8 cm, number of lobes are 3-4, pulp thickness is 7.5-8.5 mm and average fruit weight is 80-120 g (Iosob et al., 2019c).

Copper (Cu) is one of the essential nutrients for plants, but this metal can't be accumulating excessively in soils and can't become toxic for crops. Studies have shown that excess of Cu inhibits plant growth and seed germination (Iosob et al., 2019b, De Conti et al., 2020) reduced growth of shoots and roots, nutrient imbalance, enhanced degradation of photosynthetic pigments, inhibition of photosynthesis, and alteration of lipid content and fatty acid profiles (Zaouali et al., 2020, De Conti et al., 2020). Humans exposure to high levels of Cu can cause brain and kidney damage, liver cirrhosis and intestinal irritation (Cui et al., 2019).

Cadmium (Cd) determines plant phytotoxicity, therefore soil pollution with Cd is serious and it is due mainly to the use of ores, fertilizers, sewage sludge and pesticides (Iosob et al., 2019b, Li et al., 2018, Ryzhenko et al., 2018). Numerous studies have shown that the toxicity of Cd may have effects on the structure and function of DNA, however, Cd in the plant inhibits seed germination and root growth, but for many plants Cd is easily taken up by the roots, transported to shoots and enter the food chain although it is not a microelement necessary for plant growth (Zhong et al., 2020, Li et al., 2018). Also Cd can cause many diseases to humans, such as emphysema, end-stage renal failure, osteoporosis and cancer (Cui et al., 2019).

2. MATERIAL AND METHODS

The biological material was represented by pepper seedlings (*Capsicum annuum* var. Dariana Bac.), at the age of nine weeks after placing the seeds to germinated in the peat substrate soil. The peat substrate was contaminated with different concentrations of Cu and Cd: V_1 = 20 mg Cu, V_2 = 100 mg Cu, V_3 = 250 mg Cu, V_4 = 500 mg Cu, V_5 = Control - distilled water, V_6 = 1 mg Cd, V_7 = 3 mg Cd, V_8 = 5 mg Cd, V_9 = 10 mg Cd and V_{10} = 500 mg Cu + 10 mg Cd. The values of the solutions of Cd and Cu were corelated with the values presented in Order no. 756/3 November 1997. This order regards the traces of chemical elements in the soil, reported as mg per kg soil. The experiment was achieved in accordance with OECD Protocol 208 (Guidelines for the Testing of Chemicals). For the experiment were used standard solutions of Cu and Cd (1 mg/mL in 5% HNO3).

The ruler and graph paper (figure 1) was used to measure the length of the plant and the roots for each treated variant. Three plants were measured for each variant. The leaves were also counted for each plant measured.



Fig. 1. Measuring the bell pepper seedling (*Capsicum annuum* var. Dariana Bac.)

The thermobalance KEREN MLB 50-3 (figure 2) was used to determine fresh weight (FW), dry weight (DW) and the dry matter in the plant. Each sample was prepared separately. The plant was cut into small pieces and evenly distributed in the aluminum tray. The KERN MLB 50-3 moisture analyser offers 7 different mode types. We use Mode 2 "Dry matter as a percentage (%) as against output value" calculated according to the formula:

$$W(\%) = \frac{\text{residual weight}}{\text{start} - \text{up weight}}\ x\ 100\% \quad (1)$$

If the weighing result of 3 time intervals in succession remains unchanged, drying will be terminated.



Fig. 2. The thermobalance KEREN MLB 50-3 used to determine FW, DW and the dry matter (%) in the bell pepper seedling plant

The experiment was performed in the laboratory of Biotechnologies-In Vitro Cultures from Vegetable Research and Development Station Bacau.

3. RESULTS AND DISCUSSIONS

Following the experiments, it was observed on the substrate treated with a standard solution of Cu that in the variants treated with more than 250 mg Cu pepper seeds did not germinate. At V_2 = 100 mg Cu and V_3 = 250 mg Cu, the seeds germinated but the seedlings died a few days after germination. The only variant where pepper seedlings could be analyzed was V1 where the substrate soil was treated with 20 mg Cu. In the case of variant V_{10} (500 mg Cu + 10 mg Cd) where the substrate peat soil was treated with both the maximum concentration of Cu and Cd the pepper seeds did not germinate.

Analyzing the results regarding the total average size of the bell pepper seedling (root + stem), it can be seen in figure 3 that V_5, the control, is much longer (30,4 cm) than the seedlings grown on the variants treated with heavy metals. Seedlings in V_1 = 20 mg Cu and those in V_8 = 5 mg Cd are equal measuring 26.4 cm. And the smallest seedlings were measured at V_9 where the substrate was treated with 10 mg Cd only 16,1 cm. The average length of the roots of bell pepper seedlings is very important. In the first place, the root is a vegetative organ that has the role of fixing the plant in the soil and absorbing from the soil water and minerals. And in this case, we can observe, that the bell pepper sprouts that grew on the control variant V_5 have the longest roots, on average 10 cm. However, the roots of the plants of V_1 = 20 mg Cu did not have the same size as the plants of V_8 = 5 mg Cd. We can say, however, that plants grown on the substrate treated with 20 mg Cu had a more pronounced development of the stem than of the root. The same can be said of the variants V_7 = 3 mg Cd and V_9 = 10 mg Cd, where the size of the roots is very small compared to the control variant V_5.

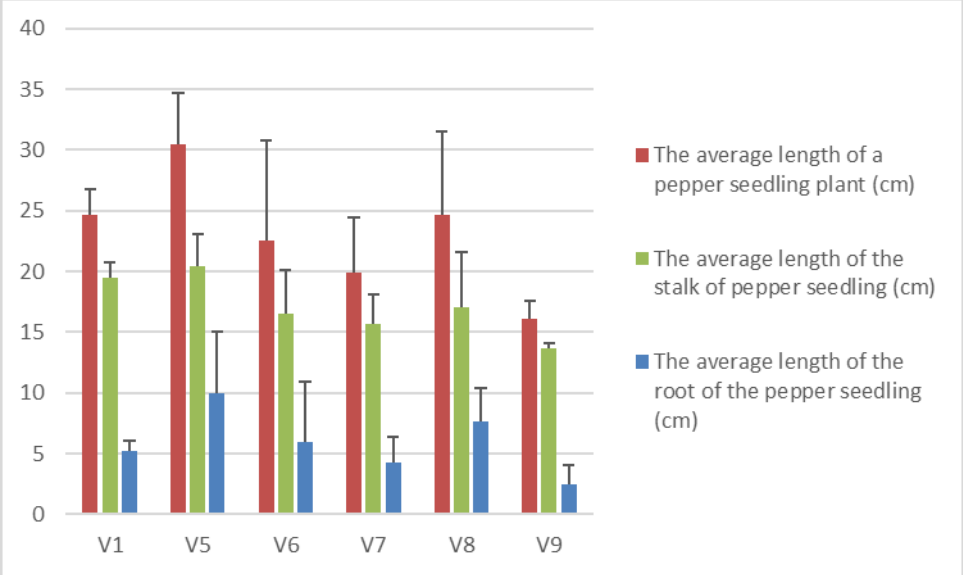

Fig. 3. The average length of bell pepper plant seedlings (cm)

Fulfilling the fundamental function in the process of photosynthesis, also serving in the process of respiration and perspiration, the number of leaves in the bell pepper seedling is very important. In figure 4 we can see that the number of leaves per plant does not differ significantly in the case of pepper seedlings that grew on soil contaminated with 20 mg Cu (V_1), 1 mg Cd (V_6) and 3 mg Cd (V_7) compared with the control variant (V_5). In case of control it can be seen that the number of leaves is almost equal in all the studied plants because the standard error of the average is the smallest. There is a significant decrease in the number of leaves in seedlings grown on substrate V_8 treated with 5 mg Cd and V_9 treated with 10 mg Cd.

As for the FW and DW of the bell pepper seedlings we observe from figure 5 that the plants grown on the untreated substrate (V_5=Control) have the highest FW (4.75 g) and DW (0.43 g). However, this decreases with increasing Cd concentration in the treated soil substrate [1 mg Cd (FW 3,6 g – DW 0,3 g) > 3 mg Cd (FW 3 g – DW 0,2 g) > 5 mg Cd (FW 2,8 g – DW 0,2 g) > 10 mg Cd (FW 1,1 g – DW 0,1 g)]. For plants grown on soil substrate treated with 20 mg Cu (V_1), the FW and DW are relatively smaller than in control (V_5).

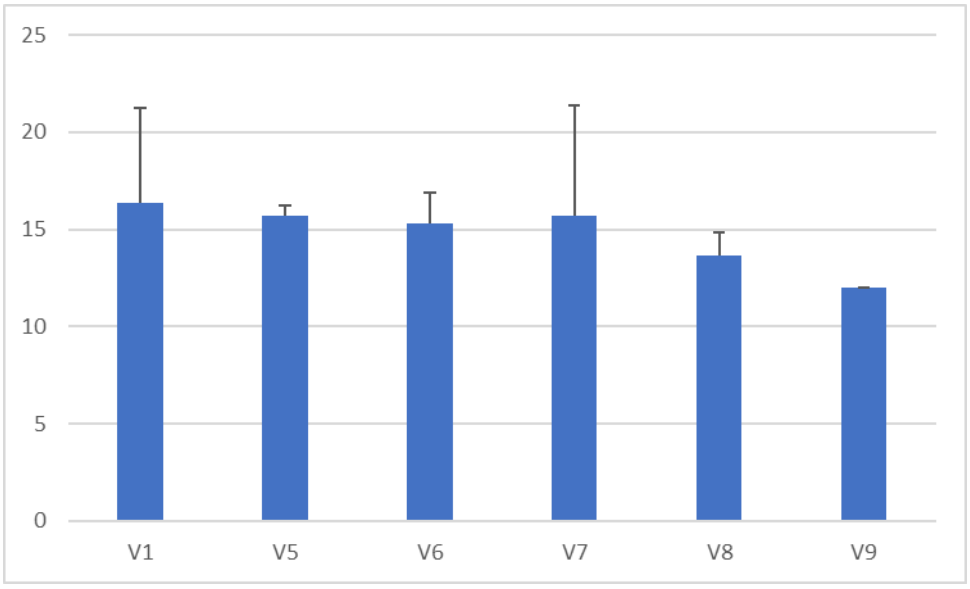

Fig. 4. The average number of leaves on bell pepper seedlings

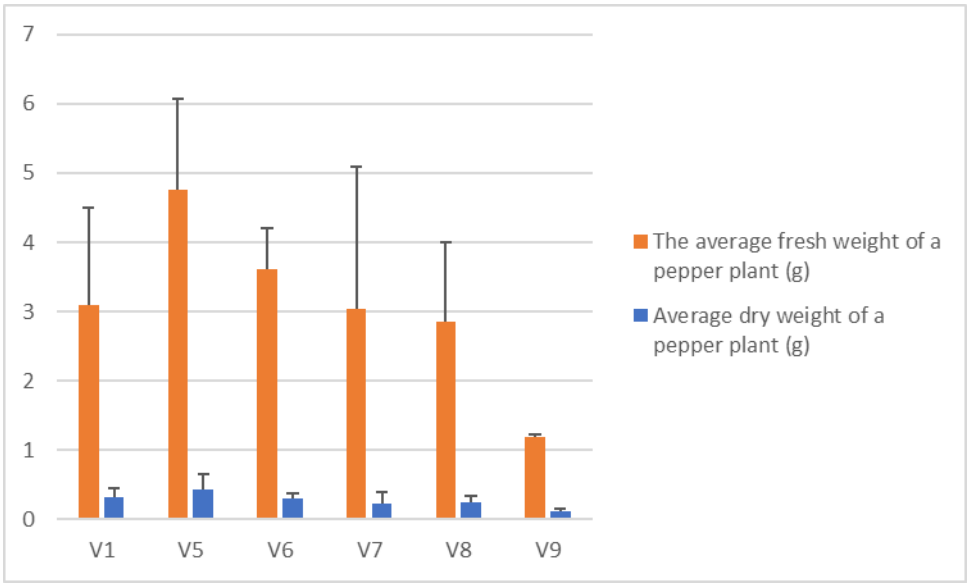

Fig. 5. The average of FW and DW of bell pepper seedling plant (g)

It is observed in figure 6, where the dry matter is calculated as a percentage, that plants that grew on the soil substrate treated with 10 mg Cd (V_9 = 10.3%) have the highest percentage of dry matter followed by bell pepper seedlings that grew on the soil substrate treated with 20 mg Cd (V_1 = 10.1%). The lowest percentage of the dry matter being recorded in bell pepper seedlings on the 3 mg Cd treated variant (V_7 = 7,1%).

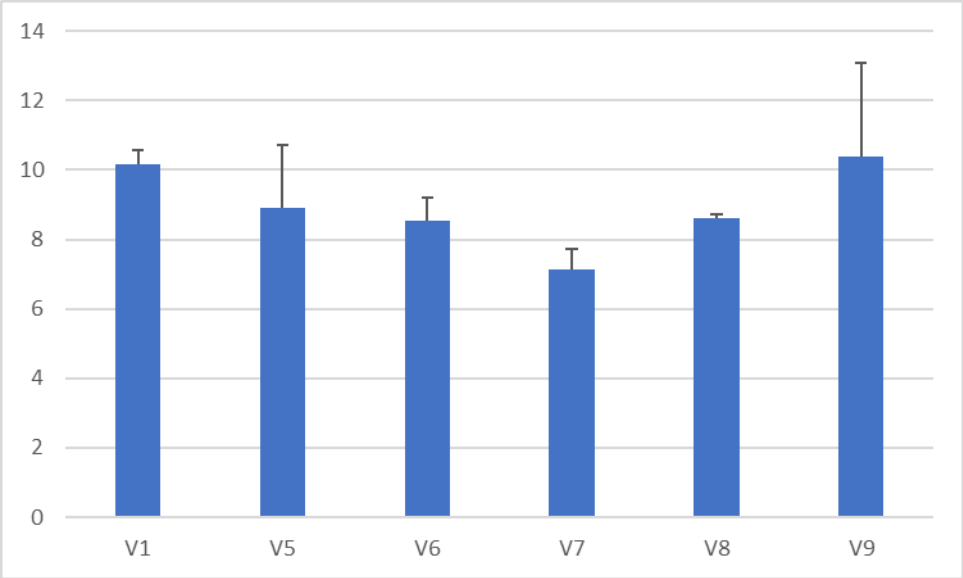

Fig. 6. The average dry matter of bell pepper seedling plant (%)

4. CONCLUSIONS

This study shows the effects of two heavy metals (Cu and Cd) on the dynamics of seedlings plant growth and development of bell pepper (*Capsicum annuum* L.) variety Dariana Bac. The variety Dariana Bac responded differently to different concentrations of the studied heavy metals. It was observed on the substrate treated with a standard solution of Cu that in the variants treated with more than 250 mg Cu pepper seeds did not germinate. At V_2 = 100 mg Cu and V_3 = 250 mg Cu, the seeds germinated but the seedlings died a few days after germination. Also the roots in variants V_7 = 3 mg Cd and V_9 = 10 mg Cd, where very small compared to the control variant V_5. The number of leaves did not vary much but a decrease in their number was observed for the variant V_8 treated with 5 mg Cd and V_9 treated with 10 mg Cd. The FW and DW of the bell pepper seedlings decreases with increasing Cd concentration in the treated soil substrate [1 mg Cd (FW 3,6 g – DW 0,3 g) > 3 mg Cd (FW 3 g – DW 0,2 g) > 5 mg Cd (FW 2,8 g – DW 0,2 g) > 10 mg Cd (FW 1,1 g – DW 0,1 g)]. And the lowest percentage of the dry matter was registred in V_7 soil treated with 3 mg Cd.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1 -1.2-PCCDI-2017-0659 / C. 11 PCCDI/2018 within PNCDI III"

REFERENCES

1. CUI, H., LI, H., ZHANG, S., YI, Q., ZHOU, J., FANG, G. & ZHOU, J. 2019. Bioavailability and mobility of copper and cadmium in polluted soil after phytostabilization using different plants aided by limestone. *Chemosphere,* 242, 125252.
2. DE CONTI, L., CESCO, S., MIMMO, T., PII, Y., VALENTINUZZI, F., B MELO, G. W., CERETTA, C. A., TRENTIN, E., MARQUES, A. C. R. & BRUNETTO, G. 2020. Iron fertilization to enhance tolerance mechanisms to copper toxicity of ryegrass plants used as cover crop in vineyards. *Chemosphere,* 243, 125298.
3. DÍAZ-PÉREZ, J. C. & JOHN, K. S. J. H. 2019. Bell Pepper (Capsicum annum L.) under Colored Shade Nets: Plant Growth and Physiological Responses. 54, 1795-1801.

4. IOSOB, G.-A., NEDEFF, V., PRISECARU, M. & CRISTEA, T. O. 2019a. Extraction and estimation of chlorophyll content in cabbage seedling leaves (Brassica oleracea L. Silviana variety) grown in soil contaminated with different concentrations of Cadmium and Copper. *Studii si Cercetari - Biologie*, 28, 66-70.
5. IOSOB, G.-A., NEDEFF, V., SANDU, I., PRISECARU, M. & CRISTEA, T. O. 2019b. Study of Phytotoxic Effects of Cu2+ and Cd2+ on Seed Germination and Chlorophyll Pigments Content to the Bell Pepper. *Revista de Chimie (Bucuresti),* 70, 1416.
6. IOSOB, G. A., NEDEFF, V., SANDU, I., CRISTEA, T. O., PRISECARU, M. & SANDU, I. G. 2019c. The Effect of Heavy Metals (Copper and Cadmium) on the Germination of Bell Pepper Seeds (Capsicum annuumL. var. Dariana Bac). *Revista de Chimie (Bucuresti),* 70, 3262-3266.
7. LI, S., WANG, M., ZHAO, Z., LI, X., HAN, Y. & CHEN, S. 2018. Alleviation of cadmium phytotoxicity to wheat is associated with Cd re-distribution in soil aggregates as affected by amendments. *RSC advances,* 8, 17426-17434.
8. QAYYUM, S., KHAN, I., MENG, K., ZHAO, Y. & PENG, C. 2020. A review on remediation technologies for heavy metals contaminated soil. *Central Asian Journal of Environmental Science and Technology Innovation,* 1, 21-29.
9. RYZHENKO, N. O., KAVETSKY, S. V. & KAVETSKY, V. M. 2018. Cd, Zn, Cu, Pb, Co, Ni phytotoxicity assessment. *Polish Journal of Soil Science,* 50, 197.
10. ZAOUALI, W., MAHMOUDI, H., SALAH, I. B., MEJRI, F., CASABIANCA, H., HOSNI, K. & OUERGHI, Z. 2020. Copper-induced changes in growth, photosynthesis, antioxidative system activities and lipid metabolism of cilantro (Coriandrum sativum L.). *Biologia,* 75, 367-380.
11. ZHENG, S., WANG, Q., YUAN, Y. & SUN, W. 2020. Human health risk assessment of heavy metals in soil and food crops in the Pearl River Delta urban agglomeration of China. *Food Chemistry,* 316, 126213.
12. ZHONG, M., HUANG, F., LUO, R., LV, Y., ALI, U., SHENG, Z., TANG, S., WEI, X. & HU, P. 2020. The effect of cadmium on the microRNAome, degradome and transcriptome of rice seedlings. *Plant Growth Regulation,* 90, 15-27.