Studii şi Cercetări	Martie 2019	Biologie	28/1	66-70	Universitatea"Vasile Alecsandri" din Bacău

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

Gabriel-Alin Iosob, Valentin Nedeff, Maria Prisecaru, Tina Oana Cristea

Key words*: chlorophyll content, cabbage, Cadmium and Copper toxicity, chlorophyll a, chlorophyll b, carotenoids*

INTRODUCTION

Vegetables are vital for the human diet and, in particular, cabbage it is cultivated for its nutritional properties. "Cabbage head" it is a huge bud that encloses most of the leaves of the plant. It has lower temperature requirements, biological threshold can't be between 5-6°C, the optimum temperature of vegetation is 15-18°C. It also resists at negative temperatures [2, 5, 17].

In addition, besides the ability to absorb the essential nutrients, plants are able to absorb and accumulate heavy metals (HM) [6, 12]. The presence of excess HM is a global problem that threatens plant health, of the fauna and the people to. HM contamination of agricultural soils is the result of urbanization and industrialization. Prolonged application of fertilizers and pesticides resulted in the accumulation of HM in both soil and vegetables [1, 12, 16] .

Cadmium (Cd) and Copper (Cu) are chemical elements that occur naturally in the soil. Yet the toxicity of these HM in culture plants produce effects that not only reduce growth, but also deteriorate the quality of food [4, 6, 8-10, 13]. Cd is one of most toxic HM's. Occurs naturally in soils and plants however, the concentration depends on the bedrock of the soil. Exceedances of acceptable limits in soil and plants are mainly due to anthropogenic activity. The main sources of Cd in the environment are due to industrial processes and phosphate fertilizers used in agriculture [1, 9, 13]. Although the benefits of Cu is recognized in small quantities for normal plant activity. This element is toxic in high concentrations. Also Cu is a micronutrient involved in photosynthesis and breathing and in the culture medium can lead to a decrease in the content of photosynthetic pigments [10, 12, 16].

Chlorophyll is a green pigment, essential in the process of photosynthesis, through this process the transformation of light energy into chemical energy take place and it meets in all the green plants. It is mainly related to the use and evaluation of the quality and freshness of a vegetable [1, 13-15].

MATERIAL AND METHODS

The experiment was conducted in two laboratories Biotechnology - In Vitro Cultures and Plant Physiology from Vegetable Research and Development Station Bacău. In the experiment, it was used a single genotype cabbage (*Brassica oleracea* variety Silviana) certificate by the Research Station. The seeds were put to germinate in pots with peat. All test units benefited from stable laboratory conditions, so that we can evaluate the response of plants to studied HM stress (Cd and Cu). Experimental variants analyzed in this study are represented by different concentrations of Cu and Cd encoded as follows: 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. Values of Cd and Cu solutions have been set in Order no. 756/3 November 1997 with regard to traces of chemical elements in the soil (Table 1).

Test conditions for all variants, including the control sample (CRT) were: temperature between 20-25^{0}C, photoperiod of 14 hours light-10 hours dark and the humidity was moderate. After 23 days after placing cabbage seeds in contact with the treated soil the analysis of photosynthetic pigments from the leaves of the seedlings were carried out.

Evaluation of pigments was carried out after the protocol of Lichtenthaler (1987) [11].

100 mg of fresh plant material it was milled in acetone 80% (v/v). The sample was centrifuged at 3530 rpm, 10 min., with Universal 320 R centrifuge. After collecting the supernatant the absorbance was read at 470, 647 and 663 nm at Boeco Germany S20 spectrophotometer. The results were recorded and the concentrations of chlorophyll *a* (*Chl a*), *b* (*Chl b*) and carotenoids (*Car*) were calculated according to the following equations and expressed in µg/mL fresh weight

Table 1. Reference values for traces of chemical elements in soil (mg / kg dry matter)

Trace elements (Heavy metals)	Normal value	Alert threshold / Types of use		Intervention thresholds / Types of use	
		sensitive	Less sensitive	sensitive sensitive	Less sensitive
Copper (Cu)	20	100	250	200	500
Cadmium (Cd)	1	3	5	5	10

$$Chl.a = 12{,}25 * Abs\,663\,nm - 2{,}79 * Abs\,647\,nm \quad (1)$$

$$Chl.b = 21{,}50 * Abs\,647\,nm - 5{,}10 * Abs\,663\,nm \quad (2)$$

$$Total\ Chl. = 7{,}15 * Abs\,663\,nm - 18{,}71 * Abs\,647\,nm \quad (3)$$

$$Car. = \frac{1000 * Abs\,470\,nm - 1{,}82 * Chl.a - 85{,}02 * Chl.b}{198} \quad (4)$$ [11]

RESULTS AND DISCUSSIONS

Chl a, Chl b and Car they were determined from the fresh leaves of the cabbage seedling after 23 days from placing the seeds in contact with the treated soil. However, plants sprouting on variants V3, V4 and V10 died a few days after sunrise. This demonstrated that a concentration equal to or greater than 250 mg/kg of soil is toxic to the studied cabbage genotype. Recent papers claim that Cu and Cd interfere in the physiological processes of plants, including in the photosynthesis process [3, 10, 16].

Mainly the amount of *Chl a* is higher in the leaves of plants than the amount of *Chl b* [14, 15]. However, in this study there was a very small difference between the amount of *Chl a* and *Chl b* in all analyzed variants. There are no significant differences either in plants grown on soil contaminated with Cu (V_1-V_2) compared to plants grown on soil variants contaminated with Cd (V_6-V_9). Comparing the results in figure 1, the difference to the degree of *Chl a* and *Chl b* in leaves of cabbage seedlings studied under stress conditions and CRT (V_5) they're very small.

Green color of leaves is a general index related to the use and evaluation of the quality and freshness of a plant. And this index is associated with the total content of chlorophyll [1, 7, 13]. After the calculation of total chlorophyll, we can observe, from figure 2, that the greatest amount of chlorophyll it is in the leaves of cabbage seedlings on Variant Two (V_2 = 100 mg Cu), 9.3246 µg/mL, followed by variant eight (V_8 = 5 mg Cd) with a total chlorophyll content of 8.7483 µg/mL and variant nine (V_9 = 10 mg Cd) with 8.6104 µg/mL. CRT (V_5) has a total chlorophyll content of 8.5939 µg/mL.

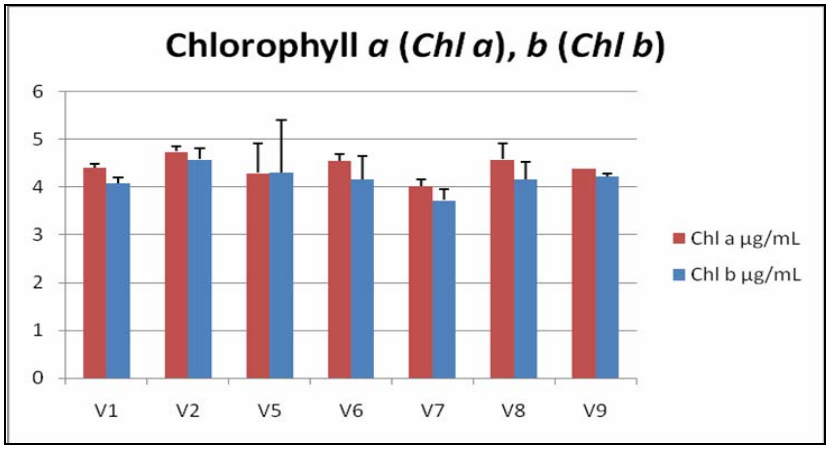

Fig. 1. The amount of *Chl a* and *Chl b* in the cabbage seedlings leaves of *Brassica oleracea* L. variety Silviana after day 23 from the beginning of the experiment

In figure 3 a small amount of *Car* pigments are registered in variant two (V2 = 100 mg Cu) only 0.2284 µg/mL, which is normal because the same variant has the highest chlorophyll content. The highest values of carotenoids pigments are recorded in the variants treated with standard Cd solution (V_7, V_8, V_9) and they're overtaking 0.4000 µg/mL, the highest value being recorded in variant seven (V_7 = 3 mg Cd) with 0.4885 µg/mL.

The coefficient of variation for photosynthetic pigments is represented in Figure 4. A large dispersion is recorded in *Car* pigments in all studied variants, however, it does not exceed 0.45%. The highest variability is recorded in CRT (V_5) for both *Car* pigments and for *Chl a*, *Chl b*.

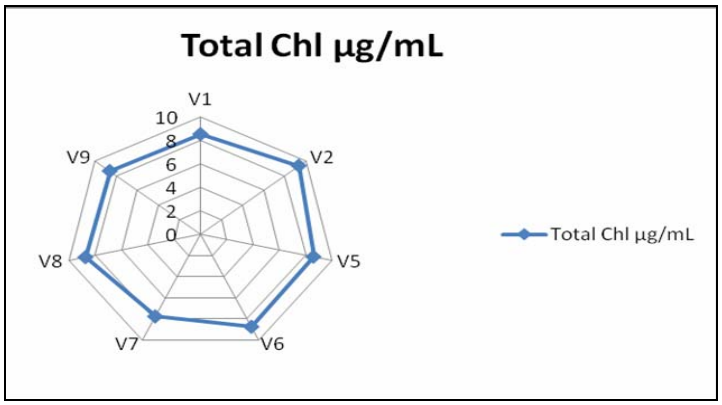

Fig. 2. Total amount of chlorophyll in the leaves of cabbage seedlings *Brassica oleracea* L. variety Silviana after day 23 from the beginning of the experiment

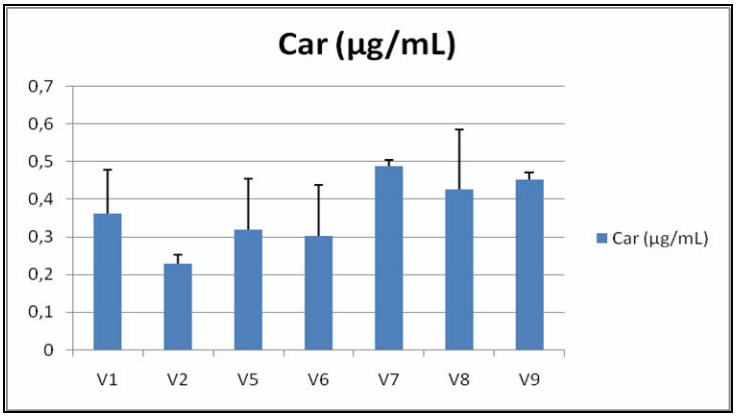

Fig. 3. Total content of carotenoids in the leaves of cabbage seedlings *Brassica oleracea* L. variety Silviana after day 23 from the beginning of the experiment

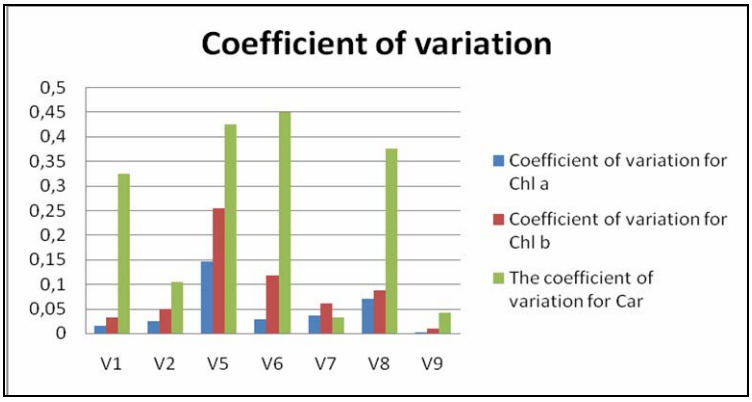

Fig. 4. Coefficient of variation for *Chl a*, *Chl b* and *Car*

CONCLUSIONS

Toxicology study in vegetables to HM exposure and humans health, by consuming contaminated vegetables, it is a serious concern to researchers, especially in recent years. Considering the effects of studied HM (Cu and Cd) on photosynthetic pigments in the leaves of cabbage seedling (*Brassica oleracea* L. variety Silviana), analyzes performed after day 23, showed that there are no big differences between *Chl a* and *Chl b* in none of the studied variants. The largest amount of total chlorophyll is in the plants on variant two (V_2 = 100 mg Cu) and the greatest amount of carotenoids pigments is recorded on variant seven (V_7 = 3 mg Cd). The coefficient of variation for the analyzed photosynthetic pigments does not exceed 0.45%.

ABSTRACT

Vegetables are essential in human diet and the presence of HM in excess it is a global issue that threaten plant health, wildlife and people [4]. This study was conducted under the protocol OECD 208. And values of Cd and Cu solutions were established in Order no. 756/3 November 1997 with regard to traces of chemical elements in the soil. Chlorophyll pigments evaluation was performed following the protocol of Lichtenthaler (1987) [11]. After data analysis the difference observed between the amount of *Chl a* and *Chl b* was very small in all the variants studied.

The largest amount of total chlorophyll and the small amount of carotenoids it was determined variant tow (V_2 = 100 mg Cu). And coefficient of variation for photosynthetic pigments is not greater than 0.45%.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministery of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI /2018, within PNCDI III and presented in frame of FARMRES Conference.

REFERENCES

1. BĄCZEK-KWINTA, R., JUZOŃ, K., BOREK, M., ANTONKIEWICZ, J., 2019 - Photosynthetic response of cabbage in cadmium-spiked soil. Photosynthetica, vol. 57, no. 3, p. 731-739;
2. CALIN, M., 2010 - Guide for growing vegetables in organic farming, Alma Mater Publishing house;
3. CHUNG, I.-M., REKHA, K., VENKIDASAMY, B., THIRUVENGADAM, M., 2019 - Effect of Copper Oxide Nanoparticles on the Physiology, Bioactive Molecules, and Transcriptional Changes in Brassica rapa ssp. rapa Seedlings, Water, Air, & Soil Pollution, vol. 230, vol. 2, doi:10.1007/s11270-019-4084-2;
4. CHIOU W.Y., HSU F.C., 2019 - Copper Toxicity and Prediction Models of Copper Content in Leafy Vegetables. Sustainability, vol 11, no. 22, doi:10.3390/su11226215;
5. CIOFU, R., 2004 - Vegetable treaty, Ceres Publishing house, Bucharest;
6. COSTA, M. B., TAVARES, F. V., MARTINEZ, C. B., COLARES, I. G., MARTINS, C. DE M. G., 2018 - Accumulation and effects of copper on aquatic macrophytes Potamogeton pectinatus L.: Potential application to environmental monitoring and phytoremediation. Ecotoxicology and Environmental Safety, 155, 117–124;
7. DOBRIKOVA, A. G., APOSTOLOVA, E. L., 2019 - Damage and Protection of the Photosynthetic Apparatus Under Cadmium Stress. Cadmium Toxicity and Tolerance in Plants,chapter 11, p. 275 – 298, doi:10.1016/b978-0-12-814864-8.00011-5;
8. GONG, Q., WANG, L., DAI, T., ZHOU, J., KANG, Q., CHEN, H., LI, C., LI, Z., 2019 - Effects of copper on the growth, antioxidant enzymes and photosynthesis of spinach seedlings. Ecotoxicology and Environmental Safety, vol. 171, p. 771–780. doi:10.1016/j.ecoenv.2019.01.016;
9. IOSOB G. A., NEDEFF V., SANDU I., CRISTEA T. O., PRISECARU M., SANDU I. G., 2019 - The Effect of Heavy Metals (Copper and Cadmium) on the Germination of Bell Pepper Seeds (*Capsicum annuum* L. var. Dariana Bac), REV.CHIM.(Bucharest), vol. 70, no. 9, p. 3262 – 3266;
10. IOSOB, G. A., NEDEFF, V., SANDU, I., PRISECARU, M., CRISTEA, T.O., 2019 - Study of Phytotoxic effects of Cu^{2+} and Cd^{2+} on Seed Germination and Chlorophyll Pigments Content to the Bell Pepper, Rev. Chim. (Bucharest), 70, no. 4, p. 1416;
11. KUMARI R., S ASHRAF, GK BAGRI, SK KHATIK, DK BAGRI DL BAGDI, 2018 - Extraction and estimation of chlorophyll content of seed treated lentil crop using DMSO and acetone, Journal of Pharmacognosy and Phytochemistry, vol. 7, no. 3, p. 249-250;
12. LICHTENTHALER, H. K., 1987 - Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Plant Cell Membranes,chapter 34, p. 350–382, doi:10.1016/0076-6879(87)48036-1;
13. MUHAMMAD A., ALI S., RIZWAN M., IBRAHIM M., ABBAS F., FARID M., ZIA-UR-REHMAN M., IRSHAD M. K., BHARWANA S. A., 2015 - The effect of excess copper on

growth and physiology of important food crops: a review, Environ Sci Pollut Res vol. 22, p. 8148–8162;
14. NIKOLIĆ N., KOJIĆ D., PILIPOVIĆ A., PAJEVIĆ S., KRSTIĆ B., ORLOVIĆ M. B. S., 2008 - Responses of hybrid poplar to cadmium stress: photosynthetic characteristics, cadmium and proline accumulation, and antioxidant enzyme activity, Acta Biologica Cracoviensia Series Botanica, vol. 50, no. 2, p 95–103;
15. RAJALAKSHMI .K , N. BANU, 2015 - Extraction and Estimation of Chlorophyll from Medicinal Plants, International Journal of Science and Research (IJSR), Volume 4, Issue 11, p. 209-212;
16. SHAMS M., EKINCI M., TURAN M., DURSUN A., KUL R., YILDIRIM E., 2019 - Growth, nutrient uptake and enzyme activity response of Lettuce (Lactucasativa L.) to excess copper, Environmental Sustainability vol. 2, p 67-73, https://doi.org/10.1007/s42398-019-00051-7;
17. STOIAN L., Practical Guide to the biological culture of vegetables, Vegetable Research and Development Station Bacău, Tipoactiv Publishing house, ISBN 973-87136-2-5.

AUTHORS` ADDRESS

IOSOB GABRIEL-ALIN - Vegetable Research and Development Station Bacău, 220 Calea Bârladului Str., 600388 Bacău and Vasile Alecsandri University of Bacău, Faculty of Engineering, 157 Mărăşeşti Str., 600115 Bacău, România, e-mail: iosob.gabriel@gmail.com;

NEDEFF VALENTIN**,** - Vasile Alecsandri University of Bacău, Faculty of Engineering, 157 Mărăşeşti Str., 600115 Bacău, România and Gheorghe Ionescu Sişeşti Academy of Agricultural and Forest Sciences, 61 Mărăşti Str., 011464 Bucharest, Romania, e-mail: vnedeff@ub.ro;

PRISECARU MARIA - Vasile Alecsandri University of Bacău, Faculty of Biology, 157 Mărăşeşti Str., 600115 Bacău, România, e-mail: prisecaru_maria@yahoo.com;

CRISTEA TINA OANA - Vegetable Research and Development Station Bacău, 220 Calea Bârladului Str., 600388 Bacău, România, e-mail: tinaoana@yahoo.com.