Study of Phytotoxic Effects of Cu^{2+} and Cd^{2+} on Seed Germination and Chlorophyll Pigments Content to the Bell Pepper

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Phytotoxic effects of Cooper and Cadmium have been studied on germination of seeds and the content of chlorophyll pigments.The experiment was completed in accordance with OECD Protocol 208 (Guidelines for the Testing of Chemicals) and four concentration levels for Cu were used (16, 80, 200, 400 mg·kg soil) in the chemical form of CuSO4 and four concentration levels for Cd (0.8, 2.4, 4, 8 mg·kg soil) standard solution for atomic absorbtion standard solution (AAS), Both Cu and Cd have been embedded in the soil.The results show that the effect of inhibiting seed germination of bell pepper is higher in soil contaminated with Cd compared to soils contaminated with Cu. Chlorophyll pigments were extracted from fresh green leaves in an alcoholic extract and quantified using Lichtenthaler's equations (1987), however, the determination of chlorophyll pigments (chlorophyll a, b, carotenes) from bell pepper leaves shows a rather large increase in the coefficients of variation. Therefore, we can conclude that the toxic compounds (Cu and Cd) induce structural and functional changes in the bell pepper photosynthesis mechanism.

Keywords: bell pepper, chlorophyll pigments, phytotoxicity, seed germination, seedling

Among the vegetable species, the bell pepper (*Capsicum annuum L.*) occupies an important place, because the fruit contain a range of bioactive compounds and essential nutrients and they are able to accumulate heavy metals (HM) from contaminated soils [1, 2].

Toxicity of heavy metals includes their binding to essential groups of molecules which are biologically important, such as enzymes which can cause a cellular imbalance [3-6], but because they tend to bioaccumulate in different organs of plants and animals can lead to unwanted toxic effects [1-8]. That's why the environmental pollution with heavy metals it is a big problem in most countries of the world, responsible for the loss of agricultural productivity [9-11].

Cadmium is a divalent cation of heavy metal (Cd^{2+}) which determines plant phytotoxicity [12], therefore soil pollution with Cd is serious and it is due mainly to the use of ores, fertilizers, sewage sludge and pesticides [13]. Numerous studies have shown that the toxicity of Cd may have effects on the structure and function of DNA [3], however, Cd in the plant inhibits seed germination and root growth [14], 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. Most plants are sensitive to low concentrations of Cd, which can lead to symptoms of phytotoxicity, such as inhibition of plant growth, chlorosis, degradation of pigments, the imbalance in the absorption and distribution of macronutrients, micronutrients, and finally death of the plant [12-21].

Copper is one of the metals that the plants need being an essential nutrient but can accumulate excessively in soils [22] where is found in the form of chemical combinations, in particular in the form of sulfides [23] and becomes toxic. The studies have shown that excess of Cu inhibits plant growth and seed germination [24-27], induces the degradation of chlorophylls [28] and interferes with the activity of the photosensitive system [7].

In this paper, we studied the effects of different concentrations of Copper and Cadmium from the soil on the germination process of the seeds and on the concentration of chlorophyll pigments (*chlorophylls a, b* and *carotenes*) on bell pepper, *Capsicum annuum L. var. Dariana Bac*.

Experimental part

Growth conditions and experimental design

Bell pepper seeds (*Capsicum annuum L.var. Dariana Bac*) used for testing were purchased from Vegetable Research and Development Station Bacău,of the batch produced in 2016. The seeds were put in paper bags, sealed and stored in a dry place at a temperature of about 15°C throughout the duration of the experiment. 10 seeds were placed in plastic pots (fig. 1) with 200 g (dry weight) of natural soil originated from the Experimental Polygon of biological agriculture from Vegetable Research and Development Station Bacau, characterized pedologically

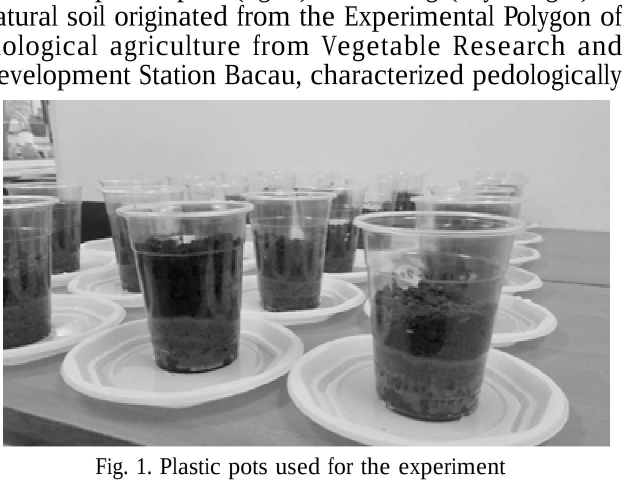

Fig. 1. Plastic pots used for the experiment

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Table 1
REFERENCE VALUES FOR Cd AND Cu

Element	Traces of element	Concentration in soil (mg·kg)	Concentration tested in experiments (mg·800g)	Volume of solution mL	Volume of H_2O for solution mL
Cd	Intervention threshold	10.0	8.00	0.40	1,496.000
		5.0	4.00	0.20	1,498.000
	Alert threshold	3.0	2.40	0.12	1,498.800
	Normal values	1.0	0.80	0.4	1,499.600
Cu ($CuSO_4$)	Intervention threshold	500.0	400.00	20.00	1,300.000
		250.0	200.00	10.00	1,400.000
	Alert threshold	100.0	80.00	4.00	1,460.000
	Normal values	20.0	16.00	0.80	1,492.000

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Before putting the seeds to germinate, the soil was moistened with 150 mL of water or $CuSO_4$ solution (hydrophilic, 98-99% pure) or standard Cd solution (1.000 µg·mL in 5% HNO_3). Different experimental conditions have been taken into consideration: control (CRT) which was made with both natural soil and with standardized soil for flowers, containing eutrophic peat, oligotrophic peat, enriched with complex fertilizers (it has an N content of at least 300 mg·L, P_2O_5 min 100 mg·L, K_2O min 30 g·L and a pH between 6.0 - 7.5) and four test variants. Reference values of Cd and Cu solutions were extracted from Order no. 756 of 3 November 1997 with regard to traces of chemical elements in the soil and were calculated for an amount of 800 g of soil (table 1).

All testing units have benefited from stable laboratory conditions so that we could assess the response of plants to stress of heavy metals (Cd and Cu) on the germination and vegetative stage. The condition for all tested variants including the CRT was: temperature between 20-25^{0}C, photoperiod of 14 h light -10 h of darkness and the humidity was moderate - when the top inch of the soil feels dry. For all treatments, four replicas were prepared and to prevent bias, random assignment of test and control pots were made. After the growing period, which lasted about two months, the plants were collected. From leaves, an alcoholic extract was made (the plant sample was mixed with 15 mL of 95% ethanol) and this mixture was introduced into the quartz cuvette provided with the *Libra S22* spectrophotometer for reading and the results were recorded in the observation sheet.

Seed germination

The seeds were germinated directly into the soil and the number of germinated seeds was recorded on the observation sheet. The observations showed that, bell pepper seeds put to germinate on contaminated soil have sprung after 7 days (on soil treated with $CuSO_4$) respectively 10 days (on the soil treated with standard Cd solution). The observations on germination were accomplished both at the reference values as well as control variants.

Chlorophyll pigments

Chlorophyll pigments were extracted from green leaves (1g) in an alcoholic extract and quantified using Lichtenthaler's equations (1987) [29, 30], after reading the absorbance at λ= 664.1, 648.6 and 470 nm. The results were recorded and concentrations of *chlorophyll a*, *b* and pigments carotenoids have been calculated and expressed in µg·mL fresh weight.

$$C_a = 13.36\, A_{664.2} - 5.19 A_{648.6} \quad (1)$$

$$C_b = 27.43 A_{648.6} - 8.12 A_{664.2} \quad (2)$$

$$C_{a+b} = 5.24 A_{664.2} + 22.24\, A_{648.6} \quad (3)$$

$$C_{x+c} = \frac{100A_{470} - 2.13C_a - 97.64C_b}{209} \quad (4)$$

Results and discussions

Seed germination

The results show that (fig. 2) the seeds sown in the soil treated with $CuSO_4$ needed a shorter period to germinate, only 7 days, compared to germination of the bell pepper seeds sown in the soil treated with standard Cd solution for AAS, which took place three days later. The literature makes reference to the copper ions involved in numerous physiological processes [31]. The ions of cadmium, however, may interfere at molecular levels in the seed germination [32] and all of these could lead to inhibition of plant growth.

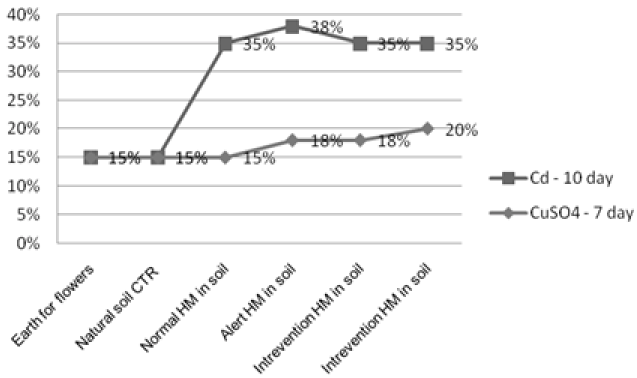

Fig. 2. Germination rate for bell pepper seed in contaminated soil with diferent concentration of heavy metal

In the soil contaminated with the solution of $CuSO_4$, an increase in germination was observed (fig. 3) - on the 12th day from the beginning of the experiment to the seeds on the soil treated with 16 and 200 mg of $CuSO_4$. But on the 13th day, however, an increase in germination rate is observed,compared to the previous day, in soils treated with 80 and 400 mg $CuSO_4$. In the control vessel with flower soil, seed germination rate on the 12th day was the highest, representing 25% from the start of the experimentand until the 14th day.

In the case of bell pepper seeds who have germinated in soil treated with standard solution of Cd it can be noted (fig. 4) a high rate of germination on the 14th day compared to the previous days, less in the version treated with 2.4 mg Cd where the highest germination rate was on day 12 (25%).

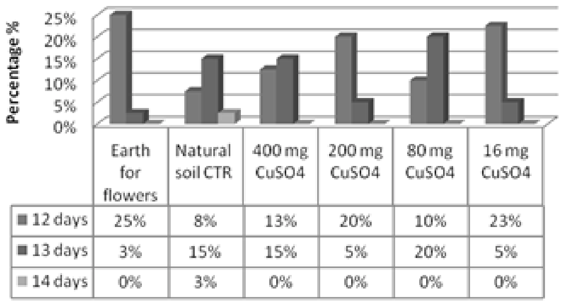

Fig. 3. Percentage of germination per day - soil contaminated with $CuSO_4$ solution

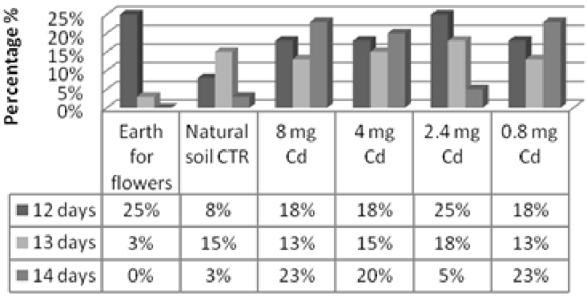

Fig. 4. The percentage germination per days - soil contaminated with standard solution of Cd

The average coefficient of variation in the case of the germination of seeds of bell pepper (*Capsicum annuum L. var. Dariana Bac*) (fig. 5) is quite high in seeds that germinate in the 11th day on the soil treated with Cd and for pepper seeds germinated in soil contaminated with $CuSO_4$ there is an increase in the coefficient of variation on day 14.

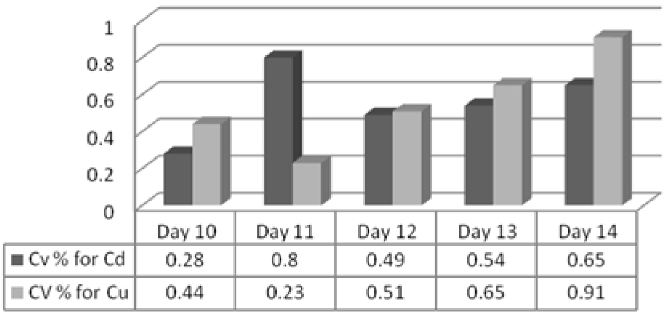

Fig. 5. The average germination of variation coefficient

Chlorophyll pigments

Chlorophyllic pigments *a, b* and carotenoid were determined from bell pepper fresh leaves, because this study considered the plant's defense response to stress conditions, especially because the soil was contaminated with different concentrations of heavy metals (Cd and Cu). Cd induces a decrease in the amount of *chlorophyll a* and *b* by inhibiting the chlorophyll biosynthesis [33]. On the other hand, Cu interferes with the mechanism of photosynthetic biosynthesis and the toxicity of Cu induces the modification of thylakoid membranes and induction of chlorosis as a result of the inhibition of chlorophyll pigments [34].

Therefore, plants grown on contaminated soil, with different concentrations of $CuSO_4$, do not show significant differences on the content of chlorophyll pigments, however, compared to plants grown on natural soil the amount of chlorophyll a registered is lower than the amount of *chlorophyll b*. As for carotenoid pigments, they are found in low amounts in the bell pepper leaves, the lowest value was recorded in plants grown on natural soil (2.524 μg·mL)

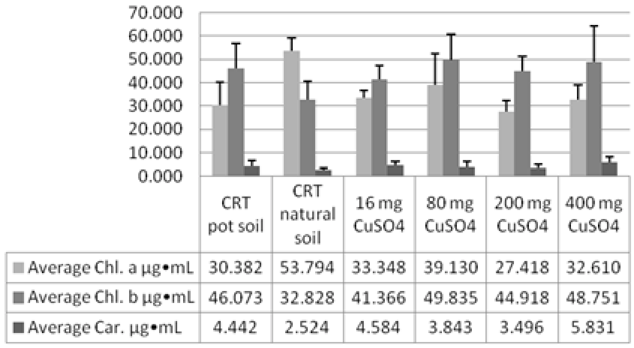

Fig. 6. The average of chlorophyll pigments on plants emerging on $CuSO_4$-treated soil

and the highest value in the leaves of plants grown on soil contaminated with 400 mg $CuSO_4$ (5.831 μg·mL) (fig. 6).

Bell pepper seedlings that grew on Cd-contaminated soil, they do not display significant differences in terms of concentrations of chlorophyll pigments. However, we can see that the mean values for chlorophyll b are also quite high here too. But the highest value is recorded in plants grown on soil treated with 4 mg Cd standard solution (48.144 μg·mL). And the highest value of the carotenoids pigments is recorded from the plants grown on the treated soil with 2.4 mg Cd (5.327 μg·mL) (fig. 7).

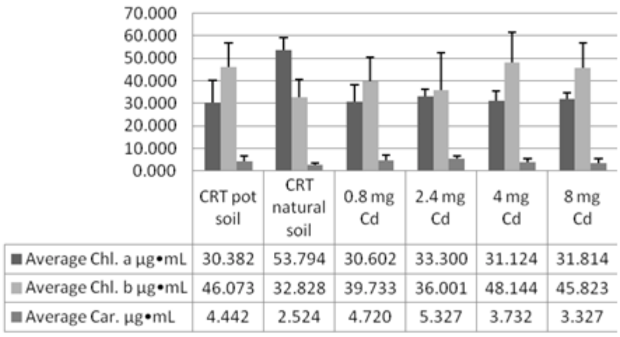

Fig. 7. The average of chlorophyll pigments on plants emerging on Cd-treated soil

Vegetables analyzed by Sahabi and collaborators [35] in Screening for total carotenoids and β-carotene in some widely consumed vegetables in Nigeria, have a relatively high level of the total carotenoid, for example: carrot (*Daucus carrota*) 397.8±2 $\mu g \cdot g^{-1}$, cabbage (*Brassica perkinensis*) - 147.1±2 $\mu g \cdot g^{-1}$, lettuce (*Brassica oleracea*) - 156.7±17.7 $\mu g \cdot g^{-1}$ and red pepper (Capsicum annuum) 33.3±15.7 $\mu g \cdot g^{-1}$. In Romania Costache and collaborators [36] studied chlorophyll pigments from vegetables and in Kaptur F1 pepper have detected a quantity of 45.89 mg·100 g *Chlorophyll a*, 2.52 mg·100g *Chlorophyll b,* 4.78 mg·100g Carotenoids, for Bianca F1 bell pepper, 47.87 mg·100 g *Chlorophyll a*, 63.45 mg·100 g *Chlorophyll b*, 2.59 mg·100g Carotenoidsand for the Maradonna F1 pepper, 127.52 mg·100 g *Chlorophyll a*, 59.00 mg·100g *Chlorophyll b*, 42.83 mg·100 g Carotenoids.

The variation coefficients for chlorophyll pigments on the bell pepper var. *Dariana Bac* seems to be quite high (fig. 8).

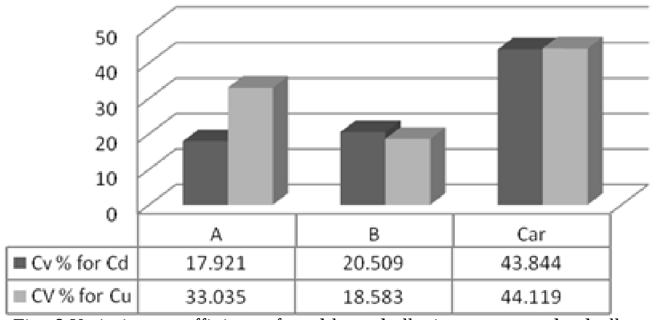

Fig. 8.Variation coefficients for chlorophyll pigments on the bell pepper var. *Dariana Bac*

Conclusions

Due to the results from the literature, we can say the effect of inhibiting germination to the bell pepperis higher in soils contaminated with cadmium compared to copper contaminated soils [31, 32]. This study shows the effects of two heavy metals (Cu and Cd) on seed germination and on the concentration of chlorophyll pigments in the bell pepper leaves (*Capsicum annuum L.* var. *Dariana Bac*).

Both metals can inhibit plant growth, however, the seed germination on $CuSO_4$-treated soil was faster and we can say that the effect of inhibition is higher in the Cd-contaminated soil.

Because this study considered the plant's defense response to stress conditions and biochemical analyzes were made on the leaf of the pepper seedlings, with regard to the amount of chlorophyll pigments, the analyses performed on bell pepper showed a large variety of chlorophyll a and b and also of carotene content and the variation coefficients were high for both heavy metals studied in this paper.

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