

The Effect of Heavy Metals (Copper and Cadmium) on the Germination of Bell Pepper Seeds (*Capsicum annuum* L. var. *Dariana Bac*)

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Bell pepper seeds (Capsicum annuum L. var Dariana Bac), variety patented by Vegetable Research and Development Station Bacau, were germinated on filter paper (Whatman No. 1) moistened with a range of solutions in different concentrations of Copper (20, 100, 250 i 500 mg/kg soil) and Cadmium (1, 3, 5, 10 mg/kg soil). The solutions used are standard solutions of Cu and Cd (1 mg/mL in 5% HNO₃). After 9 days the following indices were calculated: final germination percentage (FGP), mean germination time (MGT), germination index (GI), coefficient of velocity of germination (CVG) and germination rate index (GRI). The variety of bell pepper Dariana Bac responded differently to different concentrations of the studied heavy metals, having an average germination percentage of 76.5 % on variants treated with standard Cd solution and an average germination percentage of only 29.4% on variants tested with Cu standard solution. Also, a decrease in the germination rate was observed with the increase of the concentration of Cu.

Keywords: bell pepper, Copper, Cadmium, fitotoxicity, heavy metal stress, seed germination

Pepper (*Capsicum annuum* L.) is a plant of culture that prefers a warm climate. For pepper seeds to germinate, an optimal temperature of 25-27°C [1-3] is required. Among the vegetable species, it occupies an important place with numerous uses [1, 2]. The fruits of peppers have a great importance because they can be consumed both fresh and preserved [1]. They contain a number of bioactive compounds and essential nutrients and it is also capable of accumulating heavy metals (HM) from contaminated soils [4].

Due to the intensification of various anthropogenic activities in recent decades (urbanization, industrialization, etc.), natural resources, especially soil and water resources, are polluted with various persistent organic and inorganic contaminants [5-22]. Among these, heavy metals (HM) are [4] classified as main soil contaminants [23]. In the recent years, contamination of agricultural land by HM has increased due to anthropogenic activities [23], and as they are easily absorbed by plants [23, 24], can pose a serious threat to human health. However certain HM, such as iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), nickel (Ni), and molybdenum (Mo) are considered to be micronutrients for plants, but also minerals required in the diet of humans and animals [23-25]. On the other hand, some of the HM, such as cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), tin (Sn), silver (Ag), bismuth (Bi), chromium (Cr), are highly toxic to biological systems and they accumulate easily in living organisms [23].

It is known the benefits of copper (Cu) which is an essential element for plant growth and development [4,

23-26]. The largest source of Cu from the soil comes from industrial and agricultural waste [27]. However, the excessive availability of copper generates toxic effects in plants and can lead to a decrease in seed germination [28].

Excess of Cd in soils inhibits seed germination and root growth [29]. In many plants Cd is easily taken over by the root and transported to the shoots, in which case they can penetrate the food chain [4]. Most plants are sensitive to high concentrations of Cd, which can lead to symptoms of phytotoxicity, such as plant growth inhibition, chlorosis, imbalance in absorption and distribution of macronutrients and ultimately plant death [30-33]. However, in different studies, the germination rate and index, root length and dry weight did not show a significant ($P < 0.05$) difference under low Cd concentrations (0 and 50 $\mu\text{mol/L}$) [34].

Because toxicity is not only influenced by concentration, but also on the stage of plant development, the present paper focuses on the effect of heavy metals (copper and cadmium) on the germination of bell pepper seeds at an optimum germination temperature of 25°C. In order to determine if there are any performance differences related to the germination of bell pepper seeds var. *Dariana Bac* in close dependence with the presence of Cu and Cd in the environment, 10 experimental variants were tested. The results were compared with the control variant - V5, in which the seeds were germinated on filter paper moistened only with distilled water.

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Experimental part

Materials and methods

The experiment was conducted in the laboratory of *Biotechnologies-In Vitro Cultures from Vegetable Research and Development Station Bacau*. In the experiment, a single genotype of bell pepper was used (*Capsicum annuum* var. *Dariana Bac*.) patented by *Vegetable Research and Development Station Bacau*. 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 - 9.5-11.5 cm, diameter - 7.5-8 cm, number of lobes - 3-4, pulp thickness - 7.5-8.5 mm, average fruit weight 80-120 g [35].

The experimental variants analyzed in the present research study are represented by different concentrations of Cu and Cd: V1 = 20 mg Cu, V2 = 100 mg Cu, V3 = 250 mg Cu, V4 = 500 mg Cu, V5 = Control - distilled water, V6 = 1 mg Cd, V7 = 3 mg Cd, V8 = 5 mg Cd, V9 = 10 mg Cd and V10 = 500 mg Cu + 10 mg Cd. The values of the solutions of Cd and Cu were correlated with the values established in Order no. 756/3 November 1997 with regard to traces of chemical elements in the soil, reported as mg per kg soil and calculated for 50 g aqueous solution from standard solutions of Cu and Cd (Table 1).

For the experiment standard solutions of Cu and Cd (1 mg/mL in 5% HNO₃) were used. Seed germination was tested in laboratory conditions, on moistened filter paper (Whatman No. 1). In each Petri dish (90 mm in diameter) 30 pepper seeds were placed. The seeds were sown randomly under controlled temperature and light conditions in the Sanyo vegetation chamber (Fig. 1). Seeds were considered germinated when the root was visible (Fig. 2).

About 10 mL of solution of Cu, respectively Cd have been added to ensure the humidity necessary to germinate the seeds of bell pepper. Distilled water was used for the control variant. In this study we also investigated the interaction



Fig. 1. Bell pepper seeds var. *Dariana Bac* in the Sanyo vegetation room

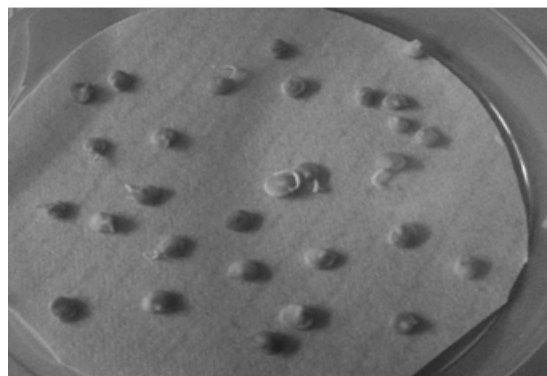


Fig. 2. Germination of bell pepper seeds var. *Dariana Bac*

between Cu and Cd, in order to determine, the effect that these HM can have on the studied bell pepper seeds. In variant 10, above the maximum concentration of Cu (500 mg/kg soil) was added the maximum concentration of Cd (10 mg/kg soil) to observe the synergistic effect of the two heavy metals. Each experimental variant was performed in three repetitions.

After the appearance of the cotyledons, the culture of bell pepper, were passed in photoperiod conditions of 16 h light - 8 h dark, at a temperature of 25°C.

For the calculation of the germination indices, the germinated seeds were counted every two days and the results were noted in the observation book. At the end of the experiment, the following indices were calculated as follows:

Final germination percentage (FGP)

$$FGP = \text{final number of seeds germinated in a seed lot} \times 100 \quad (1)$$

The higher the FGP value is, the higher is the germination of seeds [12, 23, 25-27].

$$MGT = \frac{\sum f * x}{\sum f} \quad (2)$$

where: **f** represents the seeds germinated on the day **x** [12, 23].

Germination index (GI):

$$GI = \frac{\sum T_i N_i}{S} \quad (3)$$

where: **T_i** represents the number of days after the seed has been put into contact with the chemical for analyze, **N_i** represents the number of seeds germinated on the day **i** and **S** represents the total number of seeds per repetition [36-40]. The appearance of the radicle is considered as an index of the germinal faculty [37].

Coefficient of velocity of germination (CVG):

Chemical elements	Traces of elements in soil	Soil concentration (mg/kg)	Concentration tested in experiments (mg/50 mL)	H ₂ O volume for the test solution (mL)
Cu	Normal value	20	1	49.00
	Alert threshold	100	5	45.00
	Intervention threshold	250	12.5	37.50
		500	25	25.00
Cd	Normal value	1	0.05	49.95
	Alert threshold	3	0.15	49.85
	Intervention threshold	5	0.25	49.75
		10	0.5	49.50

Table 1
VARIANTS OF THE Cu AND Cd
SOLUTIONS USED IN THE
EXPERIMENT

$$CVG = 100 * \frac{\sum N_i}{\sum N_i T_i} \quad (4)$$

where: **N_i** is the number of germinated seeds per day, and **T_i** number of days from the beginning of the experiment [36-41].

Germination rate index (GRI):

$$GRI = G1/1 + G2/2 + \dots Gx/x \quad (5)$$

where: **G1** = percentage of germination × 100 on the first day after sowing, **G2** = percentage of germination × 100 on the second day after sowing, **G_x** = percentage of germination × 100 on the day **x** after sowing [41].

Results and discussions

This study shows a significant influence of the HM (Cu and Cd) on the bell pepper seeds (*Capsicum annuum L. var. Dariana Bac*) germination. Figure 3 shows that on the studied variants the highest germination rate is 82% on variant V6 (1 mg Cd/kg soil) and the lowest is 0% on variant V4 (500 mg Cu/kg soil). Compared to V5, which is the control variant and has a FGP value of 74%, variants treated with Cd (V6, V7, V8, V9) have an average germination rate of 76.5%, and seeds treated with solution of Cu (V1, V2, V3, V4) have a lower average percentage of germination rate, only 29.4%. The FGP value decreases with increasing concentration at variants treated with Cu solution. In the case of variant V10 (500 mg Cu/kg soil + 10 mg Cd/kg soil) the presence of the maximum Cd concentration tested, resulted in a slightly increase of germination rate until 4% compared to V4, where the germination was completely inhibited by the presence of Cu in the highest concentration tested. FGP shows that the germination process of the seeds of bell pepper is inhibited by the increased concentrations of Cu. Instead Cd didn't show an

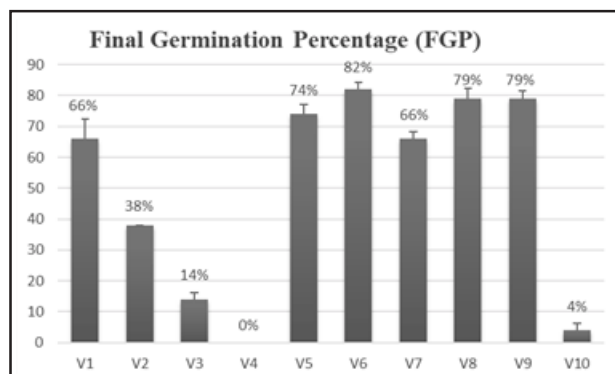


Fig. 3. Final germination percentage of bell pepper seeds (*C. annuum L. var. Dariana Bac*)

inhibitory effect over the germination, moreover the variant with Cd had a slightly increase when compared with the control variant (Fig. 3).

Mean germination time (MGT) it is a measure of the rate of germination and the temporal spread of germination [40]. Thus, it was determined the number of seeds germinated in first day and the total number of seeds germinated on the ninth day, taking in consideration the number of viable seeds germinated. On the second day after the start of the experiment, on none of the experimental variants germinated seeds were registered. This is also due to the fact that pepper seeds require a germination time of 9-11 days at an optimal germination temperature between 25-28°C [1, 2]. On the fourth day the seeds began to germinate both on the control variant V5 and on the variants treated with Cd, and the highest MGT value is on V9 (10 mg Cd/kg soil) - 1.73. Cu has the highest

MGT value in the fourth day on variant V1 (20 mg Cu/kg soil) - 1.37. An increase in the value of MGT was observed on the seeds of bell pepper in the seventh day in three of the variants treated with Cd (V6-3.18, V7-2.33, V8-2.95) but also in variant V2 (100 mg Cu/kg soil) - 1.94, with the highest MGT value registered during the experiment. V5 (control) shows the highest MGT value in the seventh day (2.56). On the ninth day the MGT value is constantly maintained at V9 (10 mg Cd/kg soil) - 1.9, having a value very close to the fourth and seventh days. In the case of bell pepper seeds treated with Cu the value of the MGT index remains high at V1 (20 mg Cu/kg soil) - 1.37. As can be seen from figure 4, in the case of variants with Cu the MGT was higher on the seventh and ninth day as in the case of Cd and control variant. However, the germination

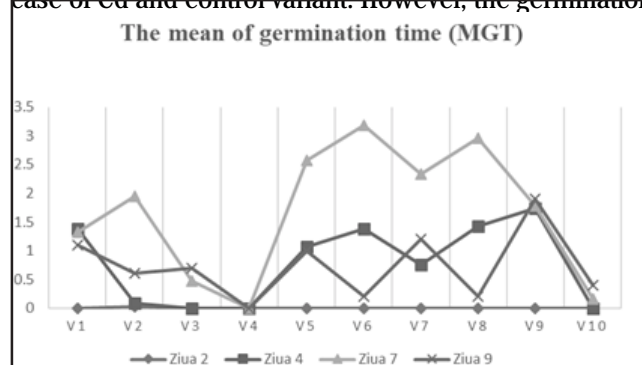


Fig. 4. Mean germination time for bell pepper seeds (*C. annuum L. var. Dariana Bac*)

of bell pepper seeds was much lower on the variants treated with Cu in all four days of observations.

Germination index (GI) reflects the germination speed of the seeds but also the vigor of the seedling by the appearance of the radicle. A low GI expresses a low vitality of bell peppers seed germination [40], and this can be easily seen in the case of the seeds germinated on Cu solution concentrations (Fig. 5). The GI value decreases with increasing concentration. In the case of Cd, the value of GI increases for V6 (1 mg Cd/kg soil) - 4.60, V8 (5 mg Cd/kg soil) - 4.55, and V9 (10 mg Cd/kg soil) - 5.04, but it decreases in the case of the V7 (3 mg Cd/kg soil) - 3.28, compared to GI from variant V5 (control) - 3.94. Seeds treated with Cu standard solution (V1-3.80, V2 -1.57, V3 -0.54 and V4 -0) express a low germination rate as opposed to treatments

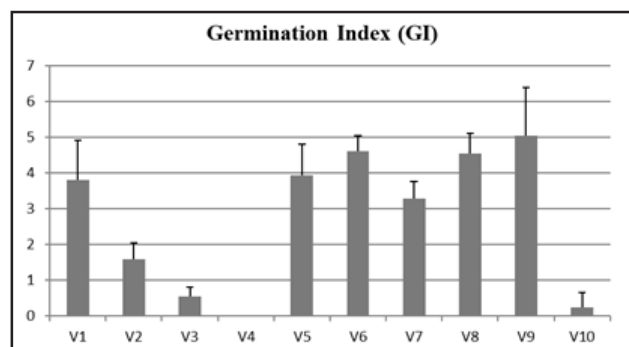


Fig. 5. Bell pepper seeds germination index (*C. annuum L. var. Dariana Bac*)

with Cd standard solution which express a high germination vitality of bell pepper seeds over time.

Coefficient of velocity of germination (CVG) gives us a clue regarding the rapidity of seed germination [30]. The CVG index increases when the number of germinated seeds increases and the time required for germination decreases [42]. Theoretically the highest possible CVG is 100% and this happens if all the seeds tested on one variant

germinate on the same day [41, 42]. Bell pepper seeds did not have a CVG value greater than 50% on any of the tested variants. However, V6 (1 mg Cd/kg soil) has the highest CVG value (6.46%) and is recorded on the seventh day. V9 (10 mg Cd/kg soil) had the highest CVG value recorded on day four (9.43%). This can mean a greater stimulation of germination and a shorter germination time for the bell pepper seeds sprouted on Cd. Seeds germinated on Cu solution have a higher CVG value on V1 (20 mg Cu/kg soil) - 1.34% on the fourth day from the beginning of the experiment and in the seventh day on V2 (100 mg Cu/kg soil) - 2.28%. However, we observed an inhibition for the bell pepper seeds germination tested on Cu standard solution and a longer germination time. The majority of the

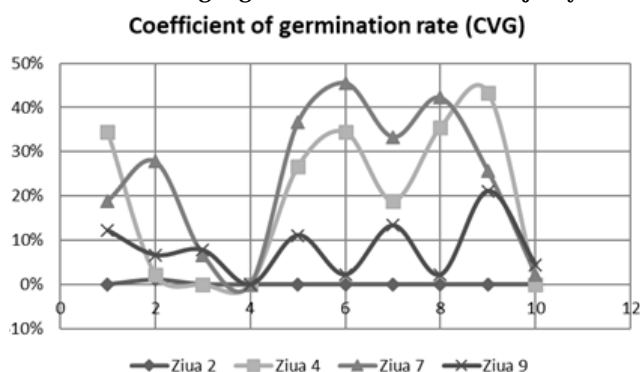


Fig. 6. Coefficient of germination rate for bell pepper seeds (*C. annuum L. var. Dariana Bac*)

seeds germinated on the seventh and ninth day from the beginning of experiment (Fig. 6).

High percentage values of germination rate index (GRI), indicates the higher and faster germination [43] for bell pepper seeds. We observe this in the variants where seeds were treated with Cd standard solution. V9 (10 mg Cd/kg soil) has the highest GRI average percentage (17%) of all analyzed variants and V7 (3 mg Cd/kg soil) has the lowest GRI average percentage (11%) for the germinated seeds on the standard solution of Cd. The average of the GRI index, of all the seeds germinated on the variants of Cd exceeds that of the seeds germinated on the variant V5 (control). However, in the case of Cu, V1 (20 mg Cu/kg soil) has an average GRI percentage equal with V5, but the small GRI percentages of the variants V2 (100 mg Cu/kg soil), V3 (250 mg Cu/kg soil) and V4 (500 mg Cu/kg soil) show that the germination of bell pepper seeds at these concentrations is low and slow (Fig. 7).

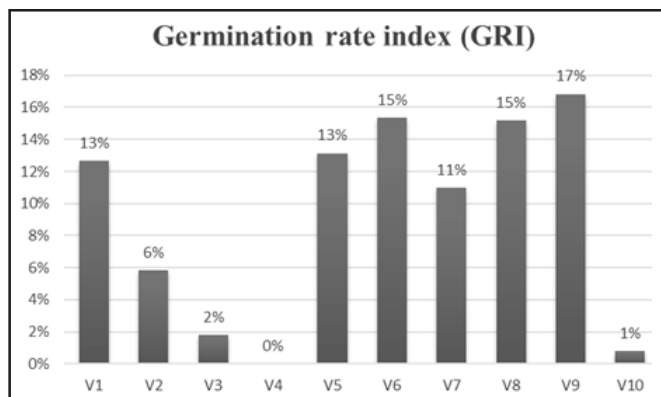


Fig. 7. Germination rate index at bell pepper seeds (*C. annuum L. var. Daiana Bac*).

During the experiments a high rate of appearance of mold and bacteria was observed on the tested variants. The incidence of mold emergence on Cu variants in the case of seeds germinated was much higher than on the

variants tested with Cd, compared to the control variant, where the incidence of molds was zero. Which could mean that Cu, not only inhibits, in high concentrations, the germination of pepper seeds, but also stimulates the appearance of mold. In order to validate this hypothesis, these aspects will be deepened in future studies on the influence of heavy metals on the different stages of germination, growth and development of plants.

Conclusions

The results presented in this paper show the toxic effects of Cu on bell pepper germination but also the stimulatory effects of Cd. As it was observed on the studied variants, that the highest germination rate is 82% on the variant contaminated with Cd solution and the lowest rate of germination is 0% on the variant treated with Cu solution. Compared with V5, which represents the control variant, the variants treated with Cd (V6, V7, V8, V9) have a higher germination percentage, and in the seeds treated with Cu solution (V1, V2, V3, V4) it is much smaller and decreases with the increase of Cu concentration. In the case of V10 (500 mg Cu/kg soil + 10 mg Cd/kg soil) the presence of the maximum Cd concentration tested, resulted in a slightly increase of germination rate until 4% compared to V4, where the germination was completely inhibited by the presence of Cu in the highest concentration tested.

There was an increase in the MGT value in the seeds of bell peppers on the seventh and ninth days on the variants treated with Cd. Also, on the seventh and ninth day, the MGT values also increased in the case of the variants treated with Cu. However, the germination of pepper seeds was much lower on the variants treated with Cu in all four days of observations.

The GI value decreases with increasing concentration in the case of Cu. But the GI values of Cd increase in V6 (1 mg Cd/kg soil), V8 (5 mg Cd/kg soil) and V9 (10 mg Cd/kg soil).

Bell pepper seeds did not have a CVG value greater than 50% on any of the tested variants. However, V6 (1 mg Cd/kg soil) has the highest CVG value and is recorded on the seventh day. V9 (10 mg Cd/kg soil) has the highest CVG value recorded on day four. This express a greater stimulation of germination and a shorter germination time in the seeds of sprouted peppers on Cd.

In the seeds treated with Cd solution we observe the high percentage of GRI values, which indicates the higher and faster germination of bell pepper seeds on the variants treated with Cd compared to the variants treated with Cu.

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