EFFECTS OF COPPER-INDUCED STRESS ON SEED GERMINATION OF MAIZE (*ZEA MAYS* L.)

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Abstract. The existence of heavy metals in polluted soils requires remediation technologies that can solve the problem of contamination in an environmentally friendly way. Plants used in phytoremediation projects can clean the contaminated areas and can become a solution for green approaches to this issue. One of the plants with great potential in phytoremediation is *Zea mays*, a very common crop plant. This experiment aimed to determine the effect of the variation in concentration of copper sulphate on the germination and growth of seeds of *Zea mays*. We wanted to establish which is the highest concentration of copper that seeds of *Zea mays* can tolerate. Seedlings growth investigation and measurements were made after 7 days. The seed germination rate was high for the low concentration and control and decreased dramatically with the increase in concentration. At high concentration the abnormal development of seeds was visible, shoots and roots growing much shorter.

Keywords: copper stress, crop plant, seed germination, *Zea mays*

INTRODUCTION

Copper is a micronutrient that is needed in small amounts by the plants and without it the leaves become twisted and change their colour in dark green. Plants produce phytochelatins when copper is toxic which have the purpose of keeping outside the copper ions that are in excess. It is also involved in processes like oxidation and reduction. Copper is also considered as having a contribution in fruit ripening. The leaves of maize contain 0.0009% dry mass copper (SAPS).

Plants are all the time exposed to different stress factors (temperature, water availability, radiation). In addition, if the plant develops on a heavy metal contaminated soil, it would be severe conditions for the plants. Some of the elements that can be found in this places are essential to the plants in small quantities – micronutrients like Cu, Fe, Mn, Mo, Ni, Zn etc. There can be also elements that are not needed like Pb, Cr, Cd and Ar. All of them can become toxic for the plants in high concentrations (Antreich, 2012).

Former studies (Finch-Savage et al. 2004; Murungu et al., 2004; Msuya and Stefano, 2010; Bakht et al., 2011; Idikut, 2013; Taye et al., 2013; Tian et al., 2014) investigated the effects of different stress factors on maize and analysed the reaction of seeds.

Seed germination may be achieved on optimal conditions of light, temperature and humidity. The requirements for germination varies from species to species.

If maize is able to accumulate heavy metals from contaminated soils, it can become a threat for animals and humans because of the risk of entering the food chain and affecting their health.

In this research, we wanted to establish if copper influences germination of seeds and growth of plants. In the laboratory, the seeds benefit of the same conditions and stable external factors, so the focus will be on the heavy metal stress.

MATERIALS AND METHODS

The seed germination tests took place in the laboratory in order to observe the reaction of seeds to copper stress without having external factors like light intensity, exposition, nutrient availability, microbial activity, temperature. For this research, we chose a crop plant, maize (*Zea mays*) to examine the copper tolerance and the germination ability and viability of seeds. The method used for our tests was rolling six seeds of maize in blotting paper that was put in concentrations of copper from 1 ppm to 100 ppm. The germination of seeds, the length of shoots and roots and dry weight were measured after 7 days.

Nutrient solutions were prepared from the stock solution of 0.2 M $CuSO_4*5H_2O$ per liter in the following concentrations: 1 ppm, 10 ppm, 50 ppm, 100 ppm. For a comparison, distilled water was used for control. From the stock solution of 1000 ppm were pipetted 25 ml in a 250 ml flask that was filled with distilled water to the mark and then adequately homogenized. From the obtained solution were pipetted 50 ml, 10 ml and 1 ml in 100 ml flasks, completed with distilled water and homogenized. We took a random sample of seeds from the package (Fig. 1 a). The selected seeds are cheap, they have a short time to germinate and are large enough to be handled easily in the experiment. Other materials used are illustrated in Fig. 1b. The seeds germinate between two layers of blotting paper. The paper was cut at the proper dimensions and was moistened with distilled water. The seeds were put in rows at approximately 2 - 3 cm from the top with a space of 2 - 3 cm between seeds. Then the blotter paper was rolled taking care that the rolls will not come loose. The germination rolls were labelled and put in the diluted concentrations of copper and the control treatment.

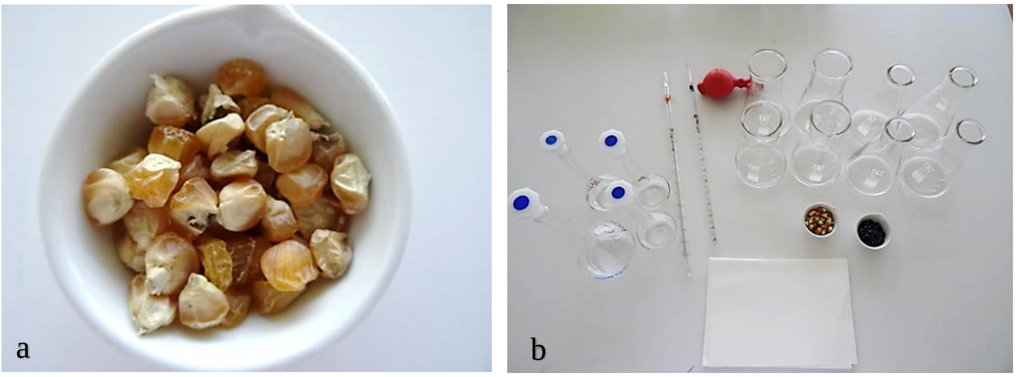

Fig. 1. Tested seeds of *Zea mays* (a) and materials used in the study (b)

Because of the water evaporation and rising concentration of solutes, the solution level was marked on the glass and distilled water was added two times a week. The flasks position was changed periodically so that the rolls would benefit of the same conditions (Fig. 2).

Fig. 2. Germination rolls treated with distilled water for control and graded solutions for Cu: 1ppm, 10 ppm, 50 ppm, 100 ppm

After one week, the rolls were unrolled and we measured the length of roots and shoots, fresh and dry weight and we determined the percentage of germination of seeds.

RESULTS AND DISCUSSION

The seeds were evaluated at 7 days after initiating the experiment. Sprouted seeds can be divided into: normal seedlings (able to develop into plants due to favorable conditions showing root and shoot structures developed properly) and abnormal seedlings (unable to develop further suffering from deficiency, decay or weakness, atrophy in root system or shoot) (Fig. 3).

Fig. 3. *Zea mays* – normal (a, b) and abnormal seedlings (c, d)

The germination percentage (Fig. 4) of the control treatment was close to 70 %, while in the Cu 1 ppm solution only half of the seeds germinated. This happened also in the 50 ppm solution. Seeds germinated best in the 10 ppm solution, with a higher rate than the control. The 100 ppm concentration of Cu seemed to be toxic for the seeds, none of them managed to germinate, as we expected.

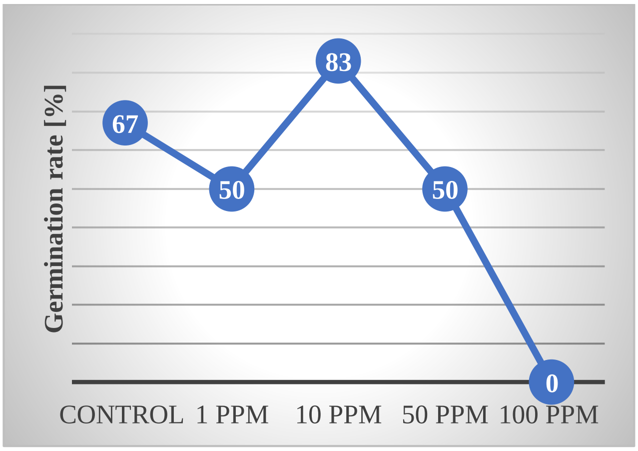

Fig. 4. Germination rates of *Zea mays* seeds

The control roots and shoots were similar to the development in the 1 ppm and 10 ppm. In the case of a normal development of seeds, in all three cases the roots were longer than shoots. The control group seemed to be less developed than the one from the 1 ppm solution, but the measurements revealed that the shoots grew longer than in the 10 ppm solution. There is a supressed root elongation starting from 10 ppm. We observed a decrease in shoot and root length as the concentration becomes higher (Fig. 5).

The 50 ppm concentration showed us extreme conditions for germination and led to abnormal development with nanism problems and a low viability. It was observed that the root length is reduced more than the shoots. This is because the root is responsible for the copper uptake and reduces in dimensions having a smaller surface of absorbtion.

For the highest copper concentration, there were no length and weight measurements because the seeds failed to germinate.

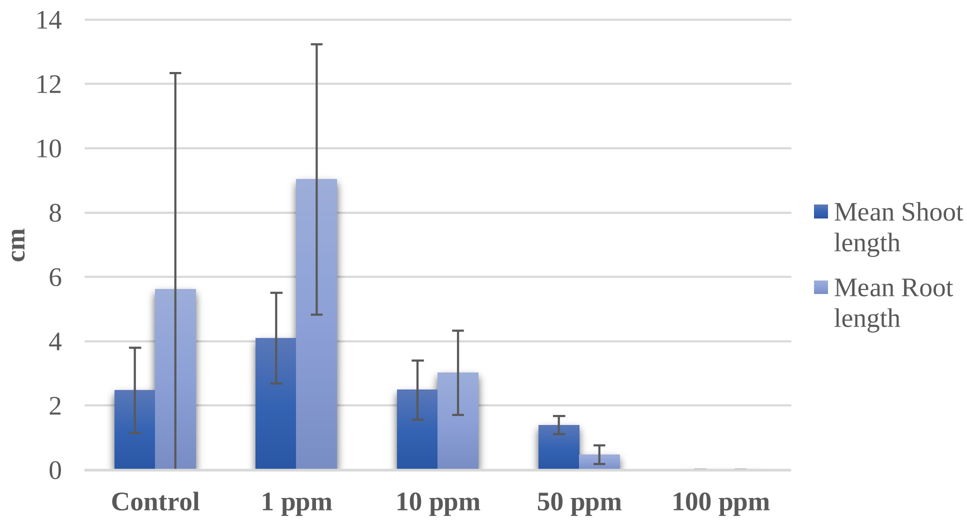

Fig. 5. Shoot / Root measurements of *Zea mays* seedlings (c, d) (*error bars* standard deviation)

The weight measurements showed us that there are no big differences in fresh and dry weight of control, 1 ppm and 10 ppm concentrations, but there is an increase in dry weight in the case of 50 ppm solution. We observed an increase in fresh weight as the concentration decreases. For 1 ppm and 10 ppm, the fresh weight exceeds the weight of the control group (Fig. 6). The seeds from the highest concentration were not weighted because they did not germinate.

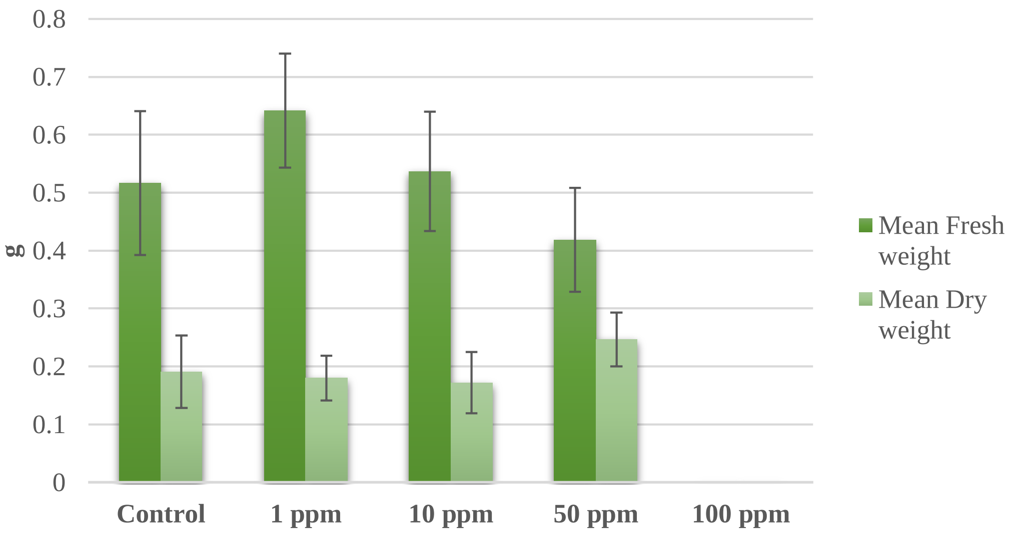

Fig. 6. Fresh and dry weight of maize seedlings (*error bars* standard deviation)

The tolerance index is a measurement that we used to compare the growth of plant roots in the solutions without copper with the roots from the copper solutions at different concentrations. If the value of this index is higher, the plant tolerance to copper is higher (SAPS).

The following formula was used to calculate the copper tolerance index (Humphreys and Nicholls, 1984):

$$Tolerance\ index = \frac{Root\ length\ mean\ in\ metal\ solution}{Root\ length\ mean\ in\ control} \times 100$$

The tolerance at the 1 ppm concentration is extremely high, above 160%, which demonstrates that these plants not only can survive successfully, but prefer a low concentration of Cu to develop better. Even in the case of the of 10 ppm concentration, the tolerance is high, overcoming the threshold of 50%, plants develop with a rate of success also in these conditions. With the increase in concentration of 50 ppm and 100 ppm, tolerance decreases sharply to 8% and a total decrease to 0%, treatment which was toxic for the maize seeds (Fig. 7).

As seen in the presented results, it seems like copper in low concentrations is not affecting the germination rate. A concentration of 1 ppm has no negative effects on the seed germination of maize. The 10 ppm concentration has a higher germination rate than the control which shows that plants need copper for a better development. The seeds tested in 1 ppm and 50 ppm solutions showed similar germination rate, while the control was higher.

Concerning the high dry weight at high concentration while the fresh weight was getting lower, investigations concluded that the development of plant is slower in stressful conditions. The plant makes smaller cells that contain more cell wall tissues. Another interpretation justifies the increase in dry weight at high concentrations due to the uptake of metal ions that bind to the cell wall producing a heavier cell (Krumpholz and Weiszmann, 2013).

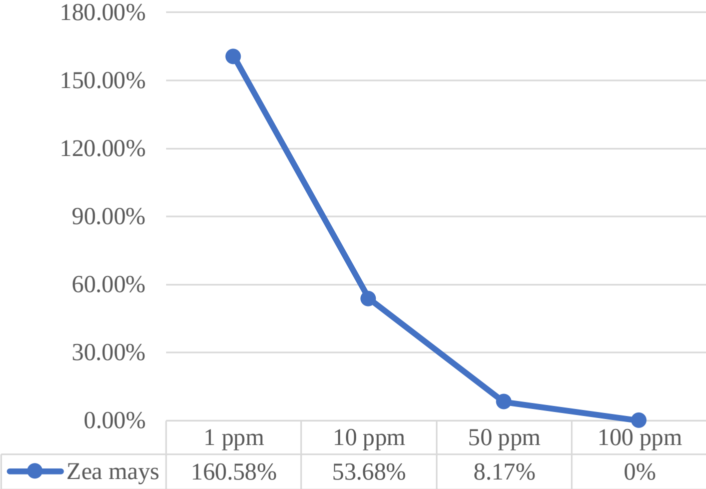

Fig. 7. Copper tolerance of tested seeds

CONCLUSIONS

Heavy metals have an important contribution regarding germination of seeds. Copper, acting as micronutrient, increased the germination rate at its low concentrations which had a stimulating effect on the plants. Copper has a negative effect on plants only at high concentrations: shorter length of shoots and roots, decrease in fresh weight and low germination rate. Copper is toxic at 100 ppm and the seeds did not germinate. In our study we showed that root and shoot elongation at high concentrations is inhibited. Even if some effects of copper-induced stress on growth and germination were positive, considering the high amounts that can be found in a contaminated soil, they are generally toxic. Out test series confirmed that the higher the concentration is, the smaller the roots and shoots develop. For more substantial conclusions, a bigger number of seeds can be tested and also there can be taken more concentrations into consideration because a small number of seeds explain why in low concentration the germination rates are not higher.

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