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COPPER INFLUENCE ON GERMINATION AND GROWTH OF SUNFLOWER (*HELIANTHUS ANNUUS*)

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ABSTRACT. Heavy metal pollution is an important issue worldwide and one of the technologies that can be sustainable in treating the contamination is phytoremediation. One of the plants that is known to tolerate heavy metals is *Helianthus annuus*. It can deal with heavy metals like Zn, Pb, Ni, Cr, Cd, Cu, As, Fe. In this study, our aim was to determine the plant's tolerance to copper and to investigate its influence on the germination of seeds and plant growth. The measurements and collection of data were made one week after establishing the seed germination test. We analysed which was the highest concentration of copper that the seeds of sunflower can tolerate and what is the effect at low and high concentrations. For a low concentration of copper, the germination rate was high, while a high concentration was toxic. We compared the root and shoot lengths and the fresh and dry weight of the plants to determine the effect of copper on the plant's development. Our investigation concluded that the highest seed copper tolerance of 90 % was at 1 ppm concentration and it decreased significantly to 16 % at the concentration of 10 ppm. At the highest concentrations the tolerance was low and the seeds had an abnormal development.

Key words: *germination test, copper stress, Helianthus annuus, phytoremediation*

INTRODUCTION

Zinc (Zn), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni) and chromium (Cr) are some of the most frequent heavy metal pollutants (GWRTAC, 1997; USEPA, 1997; Morel et al., 2006; Duruibe et al., 2007; Wuana and Okieimen, 2011).

A natural tendency to take up heavy metals is characteristic for plants. Some of them are essential mineral nutrients like: Cu, Co, Mn, Ni, Zn, Fe and Mo (Lasat, 2002; White and Brown, 2010; Antreich, 2012).

Copper is fundamental for plant nutrition, even though it is required only in small amounts from 5 to 20 ppm. Concentrations of more than 20 ppm are considered toxic for the plants, while concentrations up to 4 ppm are insufficient.

Copper is a component of plant enzymes which take part in important physiological processes such as photosynthesis, respiration, seed production, metabolism etc. (Bradl, 2005; Kvesitadze et al., 2006).

In soils, copper exists in different forms that are separated between the solution and solid phases. Soil organic matter and Mn and Fe oxides are the elements that influence the distribution of copper among soil constituents (McGrath et al., 1998).

Copper's principal alloys are bronze with tin and brass with zinc. It is used at global scale in electrical industry for wire production. Other applications which can become sources of pollution are: fertilizers, bactericides, fungicides, feed additives, agent for disease control, kitchenware, water systems etc. (Dudka et al., 1996; Xiong, 1998; Peng et al., 2006).

One of the major environmental problems is the heavy metals toxicity and their possibility to enter the food chain. It is very important to establish the bioaccumulation potential of crop plants because of the risks caused by plant's consumption and the threats to the health of humans and animals (Eu et al., 2007; Jadia and Fulekar, 2008).

In this study, we analysed the copper influence on seed germination and growth of sunflower in laboratory conditions which ensures a focus on the heavy metal stress of *Helianthus annuus* and not on the environmental factors that influence directly the development of plants.

MATERIALS AND METHODS

The reaction to copper stress of *Helianthus annuus* was studied using a seed germination test. The method that was chosen is the roll towel test where the seed germinate between paper layers. We tested the germination ability and viability of the seeds, which were taken randomly from the package. They were chosen for the experiment because of their low cost and short germination period. Also, the seeds are big enough to be handled easy during the studies.

In the laboratory, from the stock solution of 0.2 M $CuSO_4$*$5H_2O$ per liter, we prepared diluted series of 1 ppm, 10 ppm, 50 ppm and 100 ppm. For the control, we used distilled water.

Blotting paper was used to prepare the germination test rolls. The paper was soaked in distilled water and eight seeds were placed in rows between two layers of paper. A distance of 2 – 3 cm was left between the seeds and the top of the paper. After rolling and labelling the germination rolls, they were put in glass flasks containing the different concentrations of copper and the distilled water as a control. The level of solutions in the flasks was marked on the glass because of water evaporation and the need to complete the solutes with distilled water to avoid the rise in concentration. The position of flasks was changed from time to time to provide the same conditions for the seeds.

The sunflower seeds were measured after one week. The data collected was the number of germinated seeds, the length of shoots and roots and the fresh and dry weight.

RESULTS AND DISCUSSIONS

Sunflower seeds were evaluated and their development was compared. At low concentrations, the seedlings had a normal development and they managed to develop into plants. The roots and shoots were developing properly because of the favourable factors that stimulated growth (figure 1).

Fig. 1. *Helianthus annuus – normal development*

With the increase in concentration, the toxicity of copper was more obvious because the seedlings had an abnormal development (figure 2). They showed signs of deficiency, atrophy or weakness in their structures.

The abnormal development of sunflower seeds appeared also in the control and the 1 ppm concentration, but at a lower number of seeds than the high concentrations.

Fig. 2. *Seedlings of Helianthus annuus – abnormal development*

For control and the serial copper treatments, the sprouted seeds were registered and we calculated the percentage of germination. All seeds germinated for control, 1 ppm and 10 ppm concentration. The germination rates decreased with the increase in copper concentration to 75 % at 50 ppm and 25 % at 100 ppm. We expected that no seeds will sprout at the highest concentration, but some of the seeds managed to develop a very small root structure (figure 3).

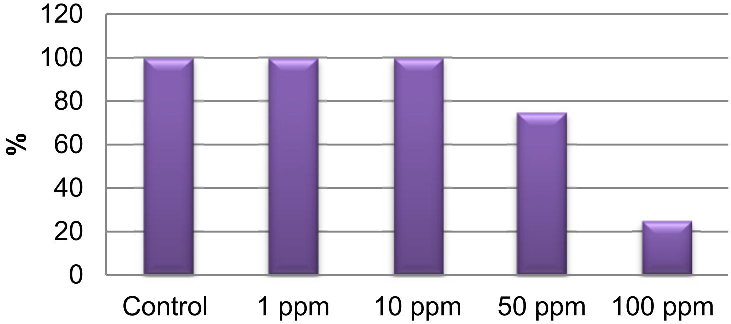

Fig. 3. *Germination rates of Helianthus annuus seeds*

The seedlings from control and the ones from the 1 ppm treatment had a similar development, roots growing longer than the shoots. Roots length decreased dramatically with the increase in concentration. The measurements revealed that the shoots grew longer than the control group in the case of 1 ppm solution. Shoots elongation was supressed starting with 50 ppm concentration.

The higher the concentration increased, the more effects could be noticed on the shoot and root lengths. The concentrations of 50 ppm and 100 ppm were the most toxic ones, leading to abnormal development and a low viability of seeds (figure 4). Surprisingly, some of the seeds from the 100 ppm solution germinated, but did not develop further.

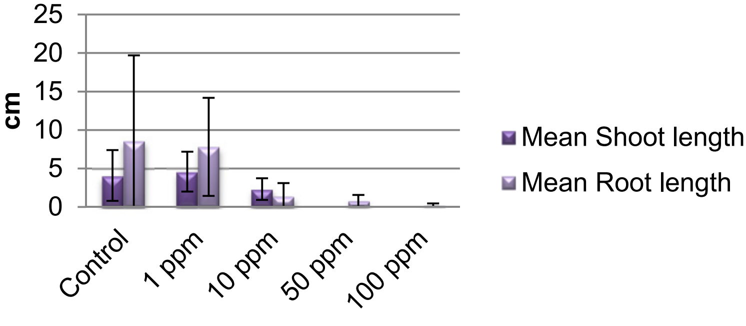

Fig. 4. *Shoot and root measurements of Helianthus annuus seedlings (error bars - standard deviation)*

In order to compare the development of roots in the graded copper solution with the one without copper, the tolerance index is used as a tool of measurement. The higher the value of the index is, the higher the copper tolerance is (SAPS, 2015).

To determine the copper tolerance index we used the following formula (Humphreys and Nicholls, 1984):

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

As a result of the calculations performed, it is observed that the highest copper tolerance of sunflower seeds is at 1 ppm of about 90 %, while it decreases about 5 times to approximately 16 % at the concentration of 10 ppm (figure 5).

Plants have a low tolerance in the case of 50 ppm and 100 ppm concentrations. It is noteworthy that there is a copper tolerance even in the case of the highest concentration.

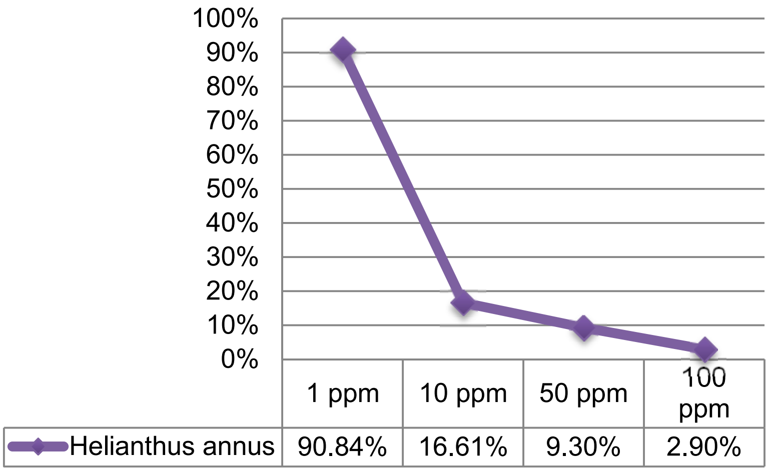

Fig. 5. *Copper tolerance of tested seeds*

The highest mean of fresh weight measured is for 1 ppm concentration, exceeding in value even the control group. As expected, the fresh weight decreases with the increase in concentration (figure 6).

Seedlings were air-dried at room temperature and weighted again. The dry weight keeps the same tendency to loose weight as the concentration becomes higher, except the 100 ppm which increases in dry weight.

Regarding the dry weight that is higher at 100 ppm concentration than the other groups, it can be explained by the slow development of plants in stressful conditions. The cells created are smaller containing more cell wall tissues. Another point of view considers the high dry weight a result of the metal ions uptake that can bind to the cell wall making the cell heavier (Krumpholz and Weiszmann, 2013).

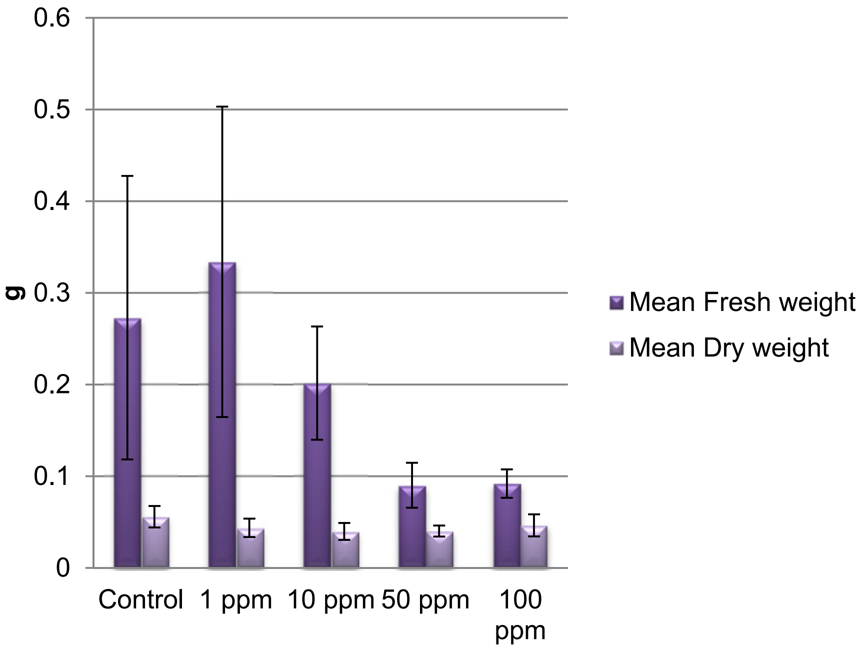

Fig. 6. *Fresh and dry weight of maize seedlings (error bars - standard deviation)*

CONCLUSIONS

Copper effects on sunflower seeds are more visible at high concentrations. The germination rate is not affected by the low concentration. Nanism problems are obvious starting with 10 ppm, but also affect the plants in control and 1 ppm treatment.

Root length reduces in size as the copper concentration becomes higher due to the copper uptake which determines a smaller absorption surface.

Fresh weight is higher for 1 ppm than the control group, which suggests that copper in low amount is important for the better plant development.

Dry weight has the highest value for the highest concentration, 100 ppm. The germination of seeds in 100 ppm solution is surprising, so the copper tolerance of *Helianthus annuus* is high.

Our study proved that shoot and root elongation is inhibited as the concentration increases. The test series that we used to investigate the copper influence on germination and growth of sunflower had positive results, but considering a polluted site that can contain copper in high amounts, it can be extremely toxic for plant's development.

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