THE INFLUENCE OF SOME HEAVY METALS ON SEED GERMINATION AND EARLY SEEDLING GROWTH IN \textit{BRASSICA OLERACEA} L. VAR. \textit{ACEPHALA}

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**Abstract:** The effect of zinc and copper on seed germination and growth in the first ontogenetic stages in the species \textit{Brassica oleracea} L. var. \textit{acephala} was investigated. Copper and zinc was used as sulphate solutions, in four different concentrations: 10 mg/l, 20 mg/l, 40 mg/l and 60 mg/l for copper; 100 mg/l; 200 mg/l; 300 mg/l and 400 mg/l for zinc. We analyzed the following indicators: the percentage of germinated seeds, the mean germination time, the length of the root, the length of the hypocotyl and the tolerance index. The following effects were found: the insignificant modifications of the germination percentage (with little excepts) and the mean germination time; the significant delay of the growth in length of the root and of the hypocotyl; the decrease of the tolerance index. The root was more affected compared to the hypocotyl.

**Keywords:** copper, zinc, tolerance index, ornamental cabbage.

**Introduction**

Among heavy metals, copper and zinc have multiple physiological and metabolical roles being indispensable for the normal growth and development of plants; higher concentrations can be toxic (Wolhouse, 1983; Rout and Das, 2003; Garófalo Chavres et al., 2011). The main symptoms of copper and zinc toxicity are: reduction/inhibition of the germination and of the growth of the root and of the plants; deficiencies of mineral elements; changes in enzyme activity and of the chlorophyll synthesis, chlorosis; changes in physiological processes such as photosynthesis, respiration, etc. (Liu et al., 1996; Ebbs and Kochian 1997; Maksymiec, 1997; Rout and Das, 2003; Wang et al., 2004; Khudsar et al., 2004; Garófalo Chavres et al., 2011; Vassilev et al., 2011).

Romanian legislation provides that the maximum permissible limit for the copper content from soil is 20 mg/kg and for zinc content the maximum permissible limit is 100 mg/kg (Ordin nr. 756/1997 pentru aprobarea Reglementării privind evaluarea poluării mediului). The most important soil pollution sources with zinc and copper are anthropic activities like mining activities, metallurgy, chemical industrial processes; waste processing, industrial waste storage, fossil fuel burning, urban waste burning, overuse of pesticides and fertilizers, etc (Śniek and Nowak, 2005; Yadav, 2010; Vassilev et al., 2011).

Heavy metal pollution is an important environmental problem. One way of removing the excess heavy metals from the soil is phytoremediation. Species of genus \textit{Brassica} (\textit{B. carinata}, \textit{B. campestris}, \textit{B. juncea}, \textit{B. napus}, \textit{B. nigra}, \textit{B. oleracea}) are known to be accumulating heavy metals (copper, zinc, nickel, lead, etc). Studies made show tolerance to some heavy metals and phytoremediation potential (phytoextraction mainly) of these species (Ebbs and Kochian, 1997; Jiang et al., 2000; Purakayastha et al., 2008; Rădulescu et al., 2013).

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Brassica oleracea var. acephala (ornamental cabbage) is a biennial species. In the first year, it has ornamental importance (vegetative phase). It displays a big rosette of glabrous leaves, cereous, undated or fringy, distinct coloured (white/cream, pink, violet, blue-grey up to dark violet). It is utilized to decorate parks, gardens, terraces, etc during autumn (Preda, 1989; Ţelaru, 2007; http://www.missouribotanicalgarden.org). Brassica oleracea var. acephala has big aerial biomass and it accumulates moderate quantities of zinc and copper from the soil (Taghizadeh et al., 2018) and has phytoremediation potential of cadmium from soil (Farahani et al., 2015).

This paper has as purpose the investigation of the zinc and copper influence on the germination and early seedling growth in Brassica oleracea L. var. acephala.

Materials and methods

The biological material was represented by Brassica oleracea L. var. acephala (ornamental cabbage) seeds (standard category; a mix of seeds from distinct coloured leaf breeds) bought from a specialised seed store.

The seeds were disinfected for 5 minutes with 1% sodium hypochlorite solutions and afterward washed several times with distilled water.

For seeds germination sterile Petri dishes with filter paper wetted with distilled water (in the control variant) and zinc sulphate solutions, respectively copper sulphate (in treatment variants) were used. Four concentrations for copper (10 mg/l, 20 mg/l, 40 mg/l and 60 mg/l) and four concentrations for zinc (100 mg/l, 200 mg/l, 300 mg/l and 400 mg/l) were used.

Plates were kept at laboratory condition, at room temperature (21°C-23°C), a photoperiod corresponding to the month of April, 2017. For each variant we used three replications, each replication with 15 seeds. Germinated seeds were counted each 24 hours. After 4 days the root length and hypocotyl length was measured.

In order to estimate the response to the treatments with zinc and copper, the following indicators have been taken into consideration: the germination percentage; the mean germination time; the length of the root (mm); the length of the hypocotyl (mm); the root length / length hypocotyl; the tolerance index.

The mean germination time was calculated according to the formula described by Ellis and Roberts, 1981 (Moradi et al., 2008) and the metal tolerance index (IT) was calculated according to the formula presented by Hakmaoui et al. (2007).

The results presented in figure are expressed as mean value ± standard error (for the germination indicators n = 3; for the morphological indicators n = 30). The data obtained were statistically interpreted. The unifactorial ANOVA test followed by the Tukey test (α = 0.05) was used in order to test the differences between means (Zamfirescu and Zamfirescu, 2008).

Results and discussions

After 2 days from the experiment, the germination percentage was slightly reduced in comparison with the control in 5 treatment variants (concentration of 20 mg/l and 40 mg/l copper, 200 mg/l, 300 mg/l, 400 mg/l zinc ). The lowest germination percentage (by 11.77% against the control) was recorded at the concentration of 300 mg/l zinc. In the other
treatment variants, the germination percentage showed slightly higher values than in the control. The observed changes were statistically insignificant (p > 0.05).

Three days after the experiment was assembled, the germination percentage recorded values: equal to the control (at 40 mg/l copper), lower compared to the control in two treatment variants (20 mg/l copper and 300 mg/l zinc) and higher than the control at the other treatment variants (Fig.1). Statistically, the changes were significant (p < 0.05) at concentrations of 10 mg/l, 20 mg/l and 60 mg/l copper.

After 4 days from the experiment setup, the germination percentage recorded statistically insignificant changes compared to the control (p > 0.05). There was a slight increase in value (with 2.56% - 10.25%) for concentrations of 10 mg/l, 20 mg/l, 60 mg/l copper and 100 mg/l and 300 mg/l zinc and a slight decrease in value (by 7.7%) at the concentration of 20 mg/l of copper. At the concentration of 200 mg/l and 400 mg/l zinc, equal values of the control were recorded (Fig. 1).

![Figure 1. The germination percentage (* - represent significant differences at p < 0.05)](image)

The mean germination time (MGT). Compared with the control, MGT showed a slight increase in value (by 0.43% - 13.02%) in most treatment variants, which was statistically insignificant (p > 0.05) (Fig. 2).

The obtained results suggest that seed germination of *Brassica oleracea* var. *acephala* is tolerant to copper and zinc in the concentrations used in our model experimental. In *Brassica oleracea* var. *capitata*, germination was tolerable at concentrations of 60 mg/l, 100 mg/l and 150 mg/l copper (Stratu et al., 2015). In other species of the Brassicaceae family, germination was tolerable at some of the zinc concentrations used in our model experimental (*Eruca sativa*: Stratu and Costică, 2017) or other zinc and copper concentrations (*Eruca sativa*: Zhi et al., 2015; *Crambe abyssinica*: Hu et al., 2015).

The root length and length of the hypocotyl showed a significant reduction (p < 0.05) compared to the control in all analysed variants (except for the 10 mg/l copper treatment - for the hypocotyl). These indicators were progressively reduced with the increase in the concentration of the metal (Fig. 3).
Compared to the control, the root length was lower with 28.48% - 73.73% for copper concentrations and 59.5% - 79.5% for the zinc concentrations tested. The length of the hypocotyl has been reduced by 13.42% - 30.77% for the copper concentrations and by 40.83% - 49.46% for the zinc concentrations tested.

The root length / hypocotyl length decreased gradually with increasing metal concentration and differed significantly (p < 0.05) from the control in all treatment variants (except for the 10 mg/l treatment variant) (Fig. 4). The results indicate that the unfavourable influence of copper and zinc ions was more pronounced on the root elongation than on the hypocotyl elongation.

The root is the first organ that is in contact with the metal ions from the germination medium and the first organ affected by the unfavourable action of the excess metals.
Similar effects of reducing root growth, hypocotyl, and plantlets in the presence of zinc and copper ions in the growth substrate have also been reported in other species of the Brassicaceae family: *Eruca sativa* (Ozdener and Kutbay, 2009; Al-Qurainy et al., 2010; Stratu and Costică, 2017); *Crambe abyssinica* (Hu et al., 2015); *Arabidopsis thaliana* (Li et al., 2005). In *Alyssum montanum* and *Thlaspi ochroleucum*, Cu$^{2+}$ ions at high concentrations severely reduced the growth of the primary root (Ouzounidou, 1995).

The two heavy metals in the concentrations used in this study do not affect germination but affect growth in the early stages. We consider that the results obtained with germination are due to the structural particularities of seeds and the properties of copper and zinc ions.

Specialty literature mentions that the seed tegument and other tissues around the embryo can play an important role in protecting the embryo from the toxicity of heavy metals; the embryo becomes sensitive to heavy metals at the stage of imbibition and in later stages of growth (Li et al., 2005). At the ornamental cabbage the seed tegument is thin.

The results obtained in this study indicate that heavy metals in the concentrations used in our experimental model constitute a potential stress factor for plant growth. The influence of this stress factor on the incipient growth process varied depending on the parameter studied, the type of metal and the concentrations tested.

The presence of copper and zinc in concentrations that exceed certain values induces oxidative stress by generating reactive oxygen species. At cellular level, they affect proteins, lipids, nucleic acids, increase the lipid peroxidation rate, affect the integrity and membrane permeability, cause metabolic disorders (Maksymiec, 1997; Wang et al., 2004; Jain et al., 2010). Other effects of excessive copper and zinc are: impairment/inhibition of cell division and elongation, induction of chromosomal aberrations (Liu et al., 1996; Maksymiec, 1997; Jiang et al., 2001; Rout and Das 2003; Jain et al., 2010).

The **tolerance index** (IT) showed a gradual decrease in value along with the increase of metal concentration (Fig.5). IT showed the highest value at the concentration of 10 mg/l copper (71.52%), which indicates a high tolerance and low toxicity. At the other copper and zinc concentrations used, IT showed values less than 50%.
Zinc and copper at the concentrations used have a certain degree of phytotoxicity. A gradual reduction in IT was found in *Eruca sativa* at concentrations of 100 mg/l, 150 mg/l and 200 mg/l zinc (Stratu and Costică, 2017). *Brassica oleracea* var. *capitata* showed a moderate tolerance at the concentration of 60 mg/l of copper in growth medium (Stratu et al., 2015).

Plants have physiological mechanisms that help them maintain growth in the presence of potentially toxic metal concentrations. Among the mechanisms involved in heavy metals detoxification and metal stress tolerance presented by Hall (2002) quoted by Rout and Das (2003) include: cellular bonding of metal and cellular exudates, chelation of metals into cytosol by peptides, reduction of metal assimilation, repair of stress-damaged proteins, compartmentalization of metals in vacuole. An important role in the detoxification of heavy metals is represented by glutathione; this chemical compound (is a tripeptide) has antioxidant capacity and is a precursor for the synthesis of phytochelatins, peptides that bind heavy metals (Yadav, 2010).

The results obtained referring to growth in the early ontogenetic stages and the root-based tolerance index indicated that zinc is less toxic than copper; the literature on lower zinc toxicity is confirmed (Woolhouse, 1983). Although the morphological indicators analyzed and the tolerance index showed higher copper values than for zinc, the zinc concentrations tested were much higher than those of copper.

**Conclusions**

*Brassica oleracea* var. *acephala* (ornamental cabbage) has the ability to germinate and grow in the early ontogenetic stages in the presence of copper and zinc in the substrate at the concentrations used. The tolerance levels for copper and zinc was higher at the germination stage.
The growth process in the first ontogenetic stages was affected by presence of both metals, in proportion to the increase in metal concentration.

REFERENCES


Ordin nr. 756/1997 pentru aprobarea Reglementării privind evaluarea poluării mediului.


