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COPPER EFFECT ON SEED GERMINATION AND PLANT SPROUTING OF *ALYSSUM MURALE* SPECIES

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Abstract

The studies aimed at establishing the level of copper concentration in the soil, which Alyssum murale species can tolerate without significantly affecting the percentage of germination and plant sprouting. The experience was conducted in 4 variants with 3 replicates, each replicate having 100 seeds. The humidity of the substrate for sowing was performed with water for the control sample (C) and with $CuSO_4$ solutions for the other variants (it was calculated the required amount of $CuSO_4$ to obtain a contamination with Cu of 20 ppm, 100 ppm, 200 ppm and, respectively, 500 ppm). The influence of copper on seed germination was assessed by the percentage of plant sprouting, germination rate, seedling rate and velocity. The significant decrease in the percentage of normal sprouts and the increase of percentage of dead seeds in variants V3 and V4 suggest the level of supportability of these species. The increase of copper concentration in the germination substrate caused the decrease of the seedling rate and of the velocity of seedling.

Key words*: Alyssum murale, copper, germination, sprouting percentage.*

INTRODUCTION

Pollution has become a major concern in all industrialized countries, at a global level, since every year, millions of tons of toxic pollutants originating from natural sources, but more particularly from anthropogenic sources, are released into the environment. As a result, the research carried out over the years has highlighted the favourable effect that some plants have on the depollution of the environment by absorbing the pollutants and accumulating them in different parts of the plant (Wenzel and Jockwer, 1999; Clemens, 2001; Wierzbicka, 2002; Baranowska-Morek, 2003). Phytoremediation is the use of spontaneous and/or cultivated plant species in order to extract, stabilize and/or neutralize pollutants in soils. Over time, research in phytoremediation showed favorable effect of certain plants species, including ornamental plants, indicating pollution reduction by absorbing pollutants by means of plant roots and accumulating them inside the plant. Lately, phytoremediation has become a well-studied scientific subject, which has led to an interdisciplinary cooperation between biochemists, biologists, soil chemists, agronomists and environmental engineers (Salt et al., 1995).

Copper (Cu) represents one of the first metals that were extracted and used by humans in different activities, thus providing a major contribution to the evolution of human society since ancient times. Research done on the toxicity of this particular metal has highlighted a series of anthropogenic sources of emission of copper in the environment, such as: copper extraction and processing, the agriculture, the electric power industry, the plastics industry, wastewater sludge (animal farms) and steelworks.

In low quantity, copper constitutes the essential element for a proper development in plants, playing an important role in photosynthesis, respiration, lignification and growth, as well as being a constituent of proteins and enzymes.

In excess, copper can become extremely toxic, affecting seed germination and causing a weak root and stem growth, as well as leaf chlorosis and necrosis (Yruela, 2005; Xu et al., 2005; Muhammad A. et al., 2015).

Various studies have reported the germination test as being a basic process of determining the toxicity effects of Cu in numerous plant species. The reduction of the germination percentage as a result of the presence or increased concentrations of copper in the germination environment was highlighted in different species: Sunflower (Pena et al., 2011;

Boroş et al., 2015); *Vicia sativa* L. (Muccifora and Bellani, 2013); *Minuartia hirsuta*, *Silene compacta*, *Alyssum montanum* (Ouzounidou et al., 1994); *Phaseolus vulgaris* L. (Ashagre et al., 2013); *Oryza sativa* L. (Ahsan et. al., 2007; Mahmood et al., 2007)

The results of the studies suggest that Cu toxicity in relation to the seed germination process in different plants can present a remarkable variability of tolerance, both within the genus as well as between different species.

MATERIALS AND METHODS

For the experiment, the biological material used was represented by *Alyssum murale* seeds taken from the collection of the Faculty's Floriculture department, a species that is conserved ex-situ. In the experiment were used containers of 2 kg capacity, containing the substrate made of two parts peat and one part gardening soil, in which 100 seeds were sown per container, three replicates being done for each experimental variant.

Watering was realised with tap water for control sample, and for the other three samples, with solutions of different $CuSO_4$ concentrations.

The necessary quantity of $CuSO_4$ was calculated, in order to obtain certain copper concentrations in the substrate, that represented the maximum admitted limit of Cu - 20 ppm (V_1); the warning threshold - 100 ppm, (V_2); the intervention threshold - 200 ppm, (V_3); exceeding the intervention threshold - 500 ppm (V_4).

During the experiment, the temperature of the vegetation room was constantly maintained at 22 ± 1°C for 8 hours, and at 24 ± 1°C for 16 hours. The seed germination was done under conditions of relative humidity of approximately 80% during the first 3 days and of 87% until the end of the germination period, at a level of light intensity over 8000 lux.

During the entire germination period, daily determinations and observations were made regarding the germination percentage, the number of normal sprouts, the number of abnormal sprouts, the number of dead seeds, the velocity of seedling and the coefficient of velocity (Kotowski, 1962).

RESULTS AND DISCUSSIONS

The observations regarding the influence of copper on the *Alyssum murale* seed germination highlight a decrease of the germination percentage occurring at the same time as an increase in the copper concentration.

By comparing the results obtained in all experimental variants, the influence of copper pollution on *Alyssum murale* seed germination is highlighted by the more accentuated decrease of the germination percentage. The highest germination percentage was obtained in the control variant (93%), and the lowest germination percentage was obtained by the V_4 variant (64%) (Table 1).

Compared with the control variant, for all the other experimental variants, there was a decrease of the germination percentage observed, the highest values being recorded for the V_3 variants, of 20%, and V_4 variants, of 29%.

The determinations regarding the seed germination period have revealed that the *Alyssum murale* seed germination was influenced by the concentration of copper.

Table 1. Influence of copper on the seed germination of the *Alyssum murale* species

Variant	Sowing date	Germination onset date	End of germination date	Total germination*
C	19.03.2014	22.03.2014	26.03. 2014	93
V_1	19.03.2014	22.03.2014	27.03. 2014	88
V_2	19.03.2014	25.03.2014	02.04. 2014	80
V_3	19.03.2014	24.03.2014	03.04. 2014	73
V_4	19.03.2014	29.03.2014	09.04. 2014	64

*The values represent % of normal sprouts

The seed germination began after 3 days in the case of the control variant and V_1, the increase of the copper concentration determining a bigger delay of the onset of germination, the biggest delays being recorded in the case of the V_3 variant (4 days, compared to the control variant) and V_4 variant (4 days, compared to the control variant) (Table 1).

Regarding the influence of copper on the germination period (from the onset until the end), there is a bigger delay observed in the case of the variants that were exposed to higher doses of copper (Table 1).

Figure 1 illustrates that, under conditions of copper contamination, the number of days from

the onset of germination of the *Alyssum murale* seeds, up until its end, ranged between 4 days in the case of the control variant and 12 days in V_4.

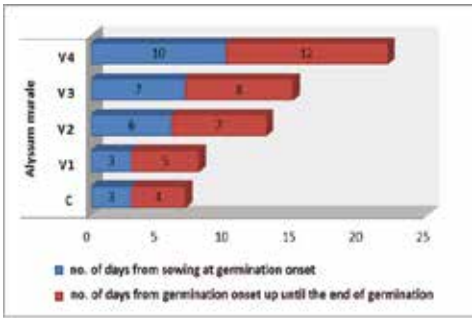

Figure 1. Seed germination duration under conditions of copper contamination (number of days)

The period of time from sowing until the onset of germination was of 3 days, in the case of the seeds originating from the control variant and V_1 variant, of 6 days for the V_2 variant and of 8 days for the V_3 variant.

Regarding the V_4 variant, the results regarding the seed germination were obtained during a period of 12 days.

Compared to the control variant, the biggest delay regarding the number of days from the onset to the end of germination, was observed in the case of the V_4 variant (8 days), followed by the V_3 variant (4 days).

In the case of the variants contaminated with copper, there may be observed an increase in the number of abnormal sprouts, as well as the ratio of dead seeds (Figure 2).

In the case of the *Alyssum murale* species, under conditions of copper contamination, there may be observed an increase in the number of abnormal sprouts, for the variants that were exposed to the highest doses of Cu. The observations made also highlight a change in the number of abnormal sprouts, the differences compared to the control variant being highly significant.

In the case of the control variant, out of the total number of seeds used in determining the germination, 93% have generated normal sprouts, 4% have generated abnormal sprouts and 3% were dead seeds. The increase in the copper concentration has determined an accentuated increase in the percentage of abnormal sprouts and dead seeds (Figure 2).

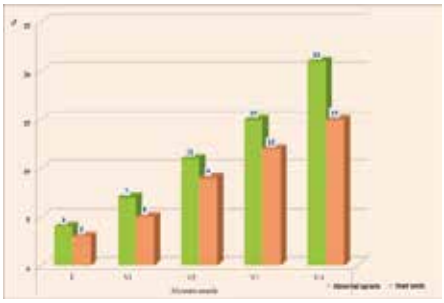

Figure 2. Ratio of abnormal sprouts and dead seeds (%)

Compared to the control variant, the number of abnormal sprouts was much higher in the case of the contaminated variants, the most evident increases in numbers being obtained for the V_4 variant, by 17%, followed by the V_3 variant, with an increase of 11%.

By comparing the number of dead seeds obtained in the case of the contaminated variants with the one obtained by the control variant, the highest increases in numbers can be observed in the case of the variants that had exhibited the highest number of abnormal sprouts as well (12% for V_4 and 9% for V_3).

The results regarding the seedling growth rate (Table 2) indicate the tendency of a decrease in germination percentages, in the case of the variants that show a metal content that represents the intervention threshold and a concentration over the intervention threshold (V_3 and V_4).

Table 2. General characterization of the seedling of the *Alyssum murale* species under exposure to different doses of copper

Variant	Seedling (%)	Seedling duration	Velocity of seedling (%)	Velocity coefficient (%)
C	82.5	3	27.5	4.71
V_1	77.5	3	25.8	3.88
V_2	67.5	6	11.3	2.08
V_3	20.0	7	2.9	1.33
V_4	16.0	10	1.6	0.73

The long time span necessary from sowing until the onset of seedling, correlated with the small number of seedlings obtained in the first days, in the case of the variants exposed to high doses of copper, determine reduced values of velocity and coefficient of velocity.

The velocity (Table 2) registered the lowest

value in the case of the V_4 variant (0.73%) and the highest value in the case of the control variant (4.71%). A similar situation may be observed in the case of the coefficient of the velocity of seedling, where the lowest value is recorded in the case of the V_4 variant (1.6%) and the highest value in the case of the control variant (27.5%).

CONCLUSIONS

The influence of copper pollution on the *Alyssum murale* seed germination has been highlighted by the more accentuated decrease of the germination percentage in the case of the variants with contaminated substrate, the highest values being recorded in the V_3 variant, of 20% and the V_4 variant, of 29%.

Higher concentrations of copper have determined a bigger delay regarding the number of days from the onset until the end of germination, which was more evident in the case of the V_4 variant (8 days) and V_3 variant (4 days).

The development of the sprouts was affected by the increase in the concentration of copper, resulting in a higher number of abnormal sprouts and dead seeds in the case of V_4 and V_3 variants.

The long time span necessary from sowing until the onset of seedling, correlated with the small number of seedlings obtained in the first days, in the case of the variants exposed to high doses of copper, determine reduced values of velocity and coefficient of velocity. The research conducted targeted the practical check of some methodologies for the copper soil decontamination by means of the phytoremediation method, monitoring the influence of copper metal on seed germination of the *Alyssum murale*.

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