COPPER EFFECT ON THE SEEDLING GROWTH AND DEVELOPMENT FOR THE *ALYSSUM MURALE* SPECIES

EFECTUL CUPRULUI ASUPRA CREŞTERII ŞI DEZVOLTĂRII RĂSADURILOR LA SPECIA *ALYSSUM MURALE*

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Abstract. *The aim of the undertaken research was to study the influence of copper on the seedling growth and development for the Alyssum murale species. The experiment was organized in 2 kg soil containers, in 4 variants of 4 repetitions, each repetition having 100 seeds. In order to study the influence of copper on the growth and development of the seedlings, the following doses were used: variant V_1-20ppm , variant V_2 - 100ppm, variant V_3- 200ppm and variant V_4 - 500ppm. The toxicity of copper was determined through biometric determinations: stem height number of leaves, internode length, mean length of the main stem, number and mean length of secondary roots and the photosynthetic pigments content by using the spectrophotometric method. The increase in copper concentration has induced a reduction of the mean plant height, the differences obtained being negative, very significant in variants V_2, V_3 and V_4. The increase in the copper content in the substrate, in variants V_3 and V_4, has determined the decrease in the chlorophyll a content.*
Key words: *Alyssum murale, copper, photosynthetic pigments*

Rezumat. *Scopul cercetărilor efectuate au constat în studierea influenţei cuprului asupra creşterii şi dezvoltării răsadurilor la specia Alyssum murale. În acest sens, a fost luată în studiu una din speciile ornamentale din flora spontană, ce au fost conservate ex-situ (Alyssum murale). Experienţa a fost organizată în în containere cu volumul de 2 kg sol, în 4 variante a câte 4 repetiţii, fiecare repetiţie având câte 100 de seminţe. Pentru studierea influenţei cuprului asupra creşterii şi dezvoltării răsadurilor s-au folosit următoarele doze: varianta V_1- 20ppm, varianta V_2 - 100ppm, varianta V_3- 200ppm, varianta V_4- 500ppm. Toxicitatea cuprului asupra cresterii răsadurilor a fost apreciată prin determinari biometrice ce au cuprins înălţimea tulpinilor şi uniformitatea creşterii şi dezvoltării, numărul de frunze, lungimea internodurilor, lungimea medie a rădăcinii principale, numărul de rădăcini secundare şi lungimea medie a rădăcinilor secundare. De asemenea, pentru a evidenţia influenţa cuprului asupra proceselor fiziologice s-au efectuat determinări privind conţinutului de pigmenţi fotosintetici prin metoda spectofotometrică. Creşterea concentraţiei de cupru a indus o reducere a înălţimii medii a plantelor, diferenţele obţinute fiind*

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negative, foarte semnificative la variantele V_2, V_3 şi V_4. Creşterea conţinutului de cupru în substrat, la variantele V_3 şi V_4 a determinat scăderea conţinutului de clorofilă a.

Cuvinte cheie: *Alyssum murale, cupru,* pigmenţi fotosintetici

INTRODUCTION

In recent years, scientists have begun to generate cost-effective technologies, including the use of micro-organisms or live plants, for cleaning polluting areas (Qui *et al.,* 2006; Kuzovkina *et al.,* 2004). Phytoremediation refers to the natural ability of certain plants to bioaccumulate, degrade or render harmless isolates in soil, water and air, through a natural, biological, physical and chemical way, through the plants actions and processes.

Phytoremediation is an emerging technology that should be taken into consideration for the remediation of contaminated sites for being cost-effective, due to aesthetic benefits and long-term applicability (Boonyapookana *et al.,* 2005).

Research on phytoremediation carried out over time has highlighted the favorable effect of some plant species, including ornamental ones, on the environmental depollution through the absorption of pollutants by roots and their accumulation in the plant.

Some heavy metals, in low doses, are essential micronutrients for plants, but in higher doses, they can cause metabolic disturbances and cause inhibition for most plant species (Sinha, 2005). It is known that in some concentrations, some heavy metals (Cu, Fe, Mn) do not inhibit plant metabolism, but are essential for photosynthesis (Anjali Aggarwal *et al.,* 2012).

Copper (Cu) contributes to several physiological processes in the plant (photosynthesis, respiration, carbohydrate and nitrogen distribution, cell wall metabolism, seed production), including resistance to disease (Kabata-Pendias and Pendias, 2001).

The deficiency of some metals (Cu, Fe, Mn) has a direct impact on the photosynthesis process, but in very high concentrations, these metals become toxic for the cells (Baker and Walker, 1989) and affect photosynthesis in many ways (Gross *et al.,* 1970; Wong and Genter, 1996).

MATERIAL AND METHOD

In order to study the influence of copper on the growth and development of the seedlings, the following doses were used:- 20ppm, variant V_1; 100ppm, variant V_2; 200ppm, variant V_3; 500ppm, variant V_4.

In order to contaminate the substrate, the following stages were followed:

preparing the substrate and contamination of the substrate (the necessary quantity of $CuSO_4$ was calculated in order to obtain a contamination of Cu of 20ppm, 100ppm, 200ppm and 500ppm respectively, from which the solutions were prepared).

The experiment was organized in 2 kg soil containers, in which 100 seeds were sowed, each experimental variant being organized in three repetitions.

The growth of the seedlings was carried out in the SANYO (MLR-351H) germinator, at a temperature of 22±1°C for 8 hours, a relative air humidity of approximately 80% and a luminous intensity of over 8.000 lux.

The influence of copper on the quality of the seedlings was seen at the level of stem height, growth and development uniformity, number of leaves, internode length, mean length of the main stem, number of secondary roots and mean length of secondary roots.

Also, in order to highlight the influence of copper on the physiological processes, determinations have been carried out to measure the photosynthetic pigments content.

The extraction and determination of the assimilatory pigments were carried out in accordance with the Current Protocols in Food Analytical Chemistry (Lichtenthaler and Buchmann, 2001).The tissues of the fresh leaves (0,1 g) were ground in the mortar in acetone (pure solvent) and then centrifuged at 10000 x g for 5 minutes. After centrifugation, the reading of the supernatant was done at the absorbance of 661.6 nm for chlorophyll a (Chl. a), at 644.8 nm for chlorophyll b (Chl. b) and at 470 nm for carotenoids (car.), using the T70 UV/VIS Spectrophotometer PG.

RESULTS AND DISCUSSIONS

Based on the measurements carried out on the variants contaminated with different doses of copper, a tendency of decrease in the average height of the plants is observed, in conjunction with the increase in the copper concentration.

By comparing the results obtained from the contaminated variants with the ones obtained from the control variant it may be noted that the copper dose that represents the maximum admitted value does not affect the quality of the seedlings.

The increase in height of plants from variant V_1 was of 3.2 cm, a value that is very close to the one obtained by the control variant, namely of 3.5 cm (table 1).

The number of leaves per plant varied from 10 leaves in the plants from the control variant to 6 leaves in variants V_3 and V_4.

The highest copper concentrations have induced the decreased in the length of internodes, compared with the plants from the control variant, the values obtained within the two experimental variants (V_3 and V_4) being very reduced.

Table 1

Influence of copper on the *Alyssum murale seedling growth*

Species	*Variant*	*H (cm)*	*No. Lvs.*	*L.i. (cm)*	*L. m. r. (cm)*	*No. s. r.*	*L. s. r. (cm)*
Alyssum murale	*C*	*3.5*	*10*	*0.40*	*4.7*	*12*	*1.51*
	V_1	*3.2*	*10*	*0.34*	*4.5*	*11*	*1.02*
	V_2	*2.3*	*8*	*0.30*	*3.3*	*9*	*0.90*
	V_3	*1.9*	*6*	*0.20*	*3.0*	*7*	*0.75*
	V_4	*1.3*	*6*	*0.20*	*2.3*	*6*	*0.73*

*H – plant height (cm)
No. Lvs. – number of leaves per plant (pieces)
L.i. – length of internodes (cm)
L. m.r.- length of main root (cm)
No. s. r. – number of secondary roots (pieces)
L. s. r. – average length of secondary roots (cm)

The biometric determinations regarding the main root growth in seedlings, under the conditions of contamination with this metal have presented the most accentuated decrease in variant V_4 (2.3).

The copper toxicity in a 500 ppm dose was reflected in the values regarding the number and mean length of secondary roots. Compared with the control variant, the plants from this variant have presented very reduced values of the number and mean length of roots.

The differences compared with the control variant were negative, distinctly significant in plants from variants V_2 and very significant in plants from variants V_3 and V_4.

Table 2

Results regarding the average length of the main root

Species	Variant	L. m.r (cm)	% compared to control variant	± d	Difference significance
Alyssum murale	C	4.7	100.00	0.0	-
	V_1	4.5	95.74	-0.2	-
	V_2	3.3	70.21	-1.4	00
	V_3	3.0	63.83	-1.7	000
	V_4	2.3	48.94	-2.4	000
	DL5% - 0.7 cm DL 1% - 1.0 cm DL 0.1% - 1.6 cm				

The influence of copper toxicity on the average number of roots was reflected by the significance of the difference compared with the control variant, obtained through the statistical interpretation of the results.

Negative differences were obtained compared with the control variant, distinctly significant in variant V_2 and very significant in variants V_3 and V_4.

Table 3

Results regarding the number of roots in the seedlings obtained on substrate contaminated with different Cu doses

Species	Variant	No. roots (pieces)	% compared to control variant	± d	Difference significance
Alyssum murale	C	12	100.00	0.0	-
	V_1	11	91.67	-1.0	-
	V_2	9	75.00	-3.0	00
	V_3	7	58.33	-5.0	000
	V_4	6	50.00	-6.0	000
	DL5% - 1.7 p. DL 1% - 2.4 p. DL 0,1% - 3.6 p.				

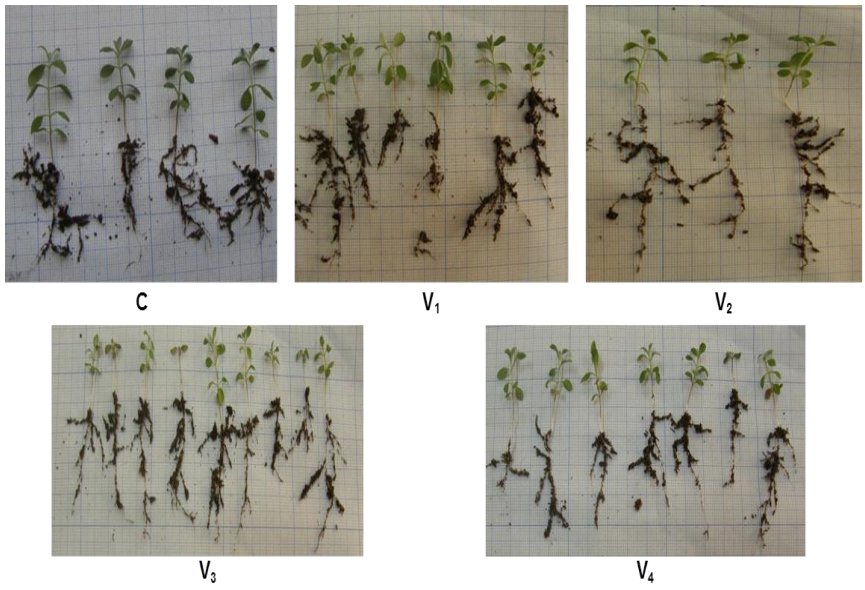

Fig. 1 *Alyssum murale* seedlings obtained on substrate contaminated with different doses of Cu

By comparing the results obtained from the four experimental variants with the ones obtained from the control variant, a slight increase in the content of pigments and the content of chlorophyll a is observed in the variants that contain more reduced doses of Cu.

The total content of chlorophyll pigments has varied between 2.81 mg/g f.w. in the control variant and 3.71 mg/g f.w. in variant V_4.

Compared with the control variant, for the seedlings from the experimental variants that were contaminated with doses that represent the maximum admitted limit (V_1) and the alert threshold (V_2) there were obtained the highest level of chlorophyll a content.

The influence of copper on the increase in chlorophyll a content is highlighted by the values obtained by variants V_1 and V_2, which have registered increases by 0.07 mg/g f.w. and by 0.11 mg/g. f.w. respectively.

The increase in the copper content in the substrate, in variants V_3 and V_4, has determined the decrease in the chlorophyll a content, the results confirming the studies done and indicating that these doses are toxic for the plants.

In these variants there was observed a decrease by 0.11 mg/g f.w. in variant V_3, and by 0.13mg/g.f.w. in variant V_4. The results regarding the chlorophyll b content have indicated normal values that varied between 0.52 mg/g f.w. and 0.62 mg/g f.w.

Under the conditions of contamination with different copper doses, the chlorophyll a/b ratio has presented values that were within theoretical limits.

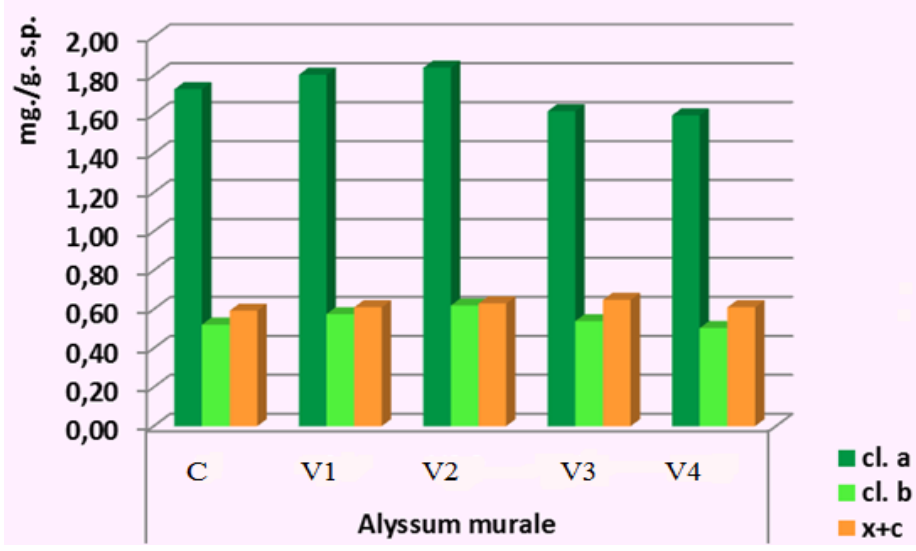

Fig. 2 Content of assimilatory pigments in seedlings obtained on substrate contaminated with different doses of Cu

In seedlings obtained in substrate contaminated with Cu, the chlorophyll a/b ratio has varied between 3.32 mg/g f.w. in the control variant and 2.97 mg/g f.w. in variant V_2.

Analyzing the values obtained by calculating the ratio (Cl. *a*+Cl. *b*)/(c+x) it is noted that the results do not indicate a physiological stress accentuated in the conditions of soil pollution with the doses of copper used in some concentrations.

In the case of this indicator the values have varied between 3.90 mg/g f.w. in variants V_1 and V_2 and 3.33 mg/g f.w. in variant V_3.

Table 4

Content of assimilatory pigments in the seedlings obtained on substrate contaminated with different Cu doses

Species	Variant	Σ	Cl. a/Cl. b	Cl. a+b/ x+c
Alyssum murale	C	2.84	3.32	3.80
	V_1	2.99	3.14	3.90
	V_2	3.09	2.97	3.90
	V_3	2.81	3.00	3.33
	V_4	2.71	3.17	3.44

CONCLUSIONS

The increase in the copper concentration has induced a reduction in the mean plant height, the differences obtained being negative, very significant in variants V_2, V_3 and V_4.

Through the statistical interpretation of the results regarding the number and mean length of secondary roots, negative differences were obtained, very significant in the plants from variants V_3 and V_4.

The copper doses that have represented the maximum permitted limit and the alert threshold have determined a slight increase in the total content of pigments and in the chlorophyll a content.

The increase in the copper content in the substrate, in variants V_3 and V_4, has determined the decrease in the chlorophyll a content, these results indicating a decrease by 0.11 mg/g s.p in variant V_3, and by 0.13mg/g.s.p. in variant V_4.

The chlorophyll a/b ratio and the chlorophyll/carotenoid pigments have presented values that were within theoretical limits, the values obtained in all experimental variants not indicating any physiological stress accentuated in the conditions of soil pollution with the doses of copper used in the study.

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