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TOXICITY OF COPPER ON THE *SINAPIS ALBA* AND *TRITICUM AESTIVUM* PLANTS

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Abstract

In this paper, we studied the effects of soil pollution with copper on the biomass production, the fractal surface of leaves and the elongation of the roots of white mustard (Sinapis alba) and wheat (Triticum aestivum) plants. The soil used in our experiments was polluted with $CuSO_4 \cdot 5H_2O$ solutions in concentrations ranging from 200 mg/kg to 1200 mg/kg. Wheat was the most affected by soil pollution with copper. At the maximum concentration of pollutant in the soil, i.e., 1200 mg/kg, it did not germinate. Compared to white mustard, wheat had a lower plant biomass, i.e., between 28% and 34%, depending on the concentration of soil pollutant. Regarding the length of the roots of the two plants, there is a 50.82% difference between them at the maximum pollutant concentration applied, i.e., (1200 mg/kg), compared to the control sample. The measured fractal surface of the white mustard leaves decreased, as well as the length of the roots, as the copper concentration in the soil increased.

Key words: *toxicity, concentration, biomass, root elongation.*

INTRODUCTION

The presence of copper in the environment is essential for the life and development of many organisms because it is present in many physiological processes, including their respiration or photosynthesis (Hansch and Mendel, 2009).
For the human body, copper is important for the function of many enzymes, in regulating metabolism, heart function, connective tissue and also acts as an antioxidant in regulating immunity (Crandell L. and Mohler N., 2021; Collins J., 2021).
Its existence in high concentrations in the soil can be a consequence of industrial pollution, but it can also result from prolonged intensive fertilisation (Nicholson et al., 2003). Soil pollution with this metal leads to a degradation of the structure and water stability of structural aggregates, which favours soil erosion and compaction (Izydorczyk G. et al., 2021; Cui S. et al., 2021).
High concentrations of copper in plants can restrict their growth, thus having a negative effect on yield and quality (Manivasagaperumal et al., 2011) and can cause disease in humans and animals (Gaetke et al., 2014; Gujre et al., 2021). It is therefore important to maintain optimal copper concentrations in the soil and to limit the absorption thereof by plants if its level in the soil rises excessively (Wyszkowski M., 2017; Żołnowski A. et al., 2013).
A series of hyperaccumulating plants have been used to decontaminate soil contaminated with copper: *Typha orientalis*, *Iris ensata* and *Scirpus radicans* Schk (Usman et al., 2012), *Seriphidium terrae-albae* (Cui S. et al., 2021), *Pinus massoniana* and *P. yunnanensis* (Wang et al., 2019), *Brassica napus and Salix nigra* (Massenet et al., 2021).
Wheat is the most important cereal and the plant that occupies the largest areas in the world. It also represents one of the most important agricultural crops in Romania, with approximately 2 million hectares cultivated every year. It has high biomass yield and develops a strong and rich root system, essential conditions for plants used in soil phytoremediation. It was for this reason that wheat was chosen for this study, to observe from what concentration of copper in the soil these conditions are affected.
White mustard belongs to the same family as rape (*Brassica napus*), the cruciferous family. Rape is one of the plants studied by other researchers and recognised as a hyperaccumulator that can be used in the phytoremediation of soils.

The purpose of this study is to determine the toxicity of copper on germination, root elongation and the morphological development of white mustard (*Sinapis alba*) and wheat plants (*Triticum aestivum*).

MATERIALS AND METHODS

The soil used in the experiments was contaminated with a solution of $CuSO_4 \cdot 5H_2O$ in concentrations from 0 mg/kg to 1200 mg/kg.
The physicochemical characteristics of the soil used in the experiments are shown in Table 1.
Each experimental variant was performed in four repetitions. For each experimental variant, 65 g of soil and 6 seeds of plants were used. The plants used in the experiments were of white mustard and wheat. The plant seeds were purchased from the Bank of Plant Genetic Resources Suceava, Romania.
Pictures of the seeds used in the experiments can be seen in Figures 1-2.

Figure 1. Seeds of *Sinapis alba*

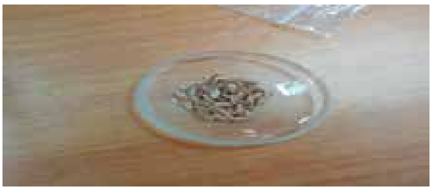

Figure 2. Seeds of *Triticum aestivum*

All experimental variants benefited from the same temperature and humidity conditions, being irrigated every 3 days. The plants sprouted three days after sowing and were harvested after 2 weeks in the case of white mustard and 1 month in the case of wheat.
After harvesting, the plants were separated into morphological parts (roots, stems, leaves) and the following were determined: biomass production, fractal dimension of mustard leaves and length of roots and stems for each experimental variant.

The amount of biomass obtained was determined by weighing the analytical balance of harvested morphological parts.
The fractal dimension is a number that quantifies the degree of irregularity and fragmentation of a geometric structure or object in nature (Zmeskal et al., 2003).
The box-counting method was used to determine the fractal dimension of the mustard leaves. The fractal dimension of binary digital images, i.e., D_b, determined by the box-counting method, measures complex dimensions in the $1 \leq D_b \leq 2$ range by calculating the ratio between the increase in detail and the increase in scale. This is calculated by placing a series of squares with decreasing size s over an image and by measuring, for each square, the number of pixel objects N(s) (Zmeskal et al., 2001). ImageJ1.38 software was used to calculate the fractal dimension.
Root elongation was measured using the Image Tool 3.0 software and the percentage of root elongation inhibition (RI) for each studied plant was calculated with Eq.1 (Oleszczuk, 2008):

$$RI = \frac{A-B}{A} \cdot 100 \quad (1)$$

where: A is the average root length in the control sample; B is the average length of roots under the influence of copper.

RESULTS AND DISCUSSIONS

3 days after sowing, the white mustard and wheat plants sprouted. They can be seen in the Figures 3, 4, 5 and 6.

Figure 3. Emergence of *Sinapis alba* plants under the influence of soil pollution with Cu from the concentration of 0 mg/kg to 1200 mg/kg

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Figure 4. Sprouting of *Triticum aestivum* plant under the influence of soil pollution with Cu from the concentration of 0 mg/kg to 1200 mg/kg

At the end of the growing period, 2 weeks in the case of white mustard and one month in the case of wheat, the 2 types of plants were harvested.

Figure 5. White mustard before harvesting

Figure 6. Wheat before harvesting

Sinapis alba and *Triticum aestivum* plants harvested and separated by morphological parts can be seen in Figures 7 and 8 and the amount of biomass obtained is shown in the Table 2.

0 mg/kg

1200 mg/kg

Figure 7. Separation of harvested white mustard by roots, stems and leaves

As it can be seen, first of all visually, the differences in obtained biomass, at the maximum concentration of soil pollutant, for both white mustard and wheat, are very large compared to the control sample.
At the maximum concentration of the soil pollutant, i.e., 1200 mg/kg, wheat seeds did not germinate.

0 mg/kg

1000 mg/kg

Figure 8. Separation of harvested wheat by roots, stems and leaves

Regarding the amount of biomass harvested, it can be seen that the highest amounts were obtained in the case of white mustard. Wheat was much more affected by the concentration of copper in the soil. To determine the average fractal surface of the white mustard leaves, 30 leaves were measured for each experimental variant.

The fractal surface measured in the ImageJ software for the control sample and the sample with the maximum copper concentration in the soil can be seen in Figure 9.

Results obtained for each experimental variant are presented in Table 3.

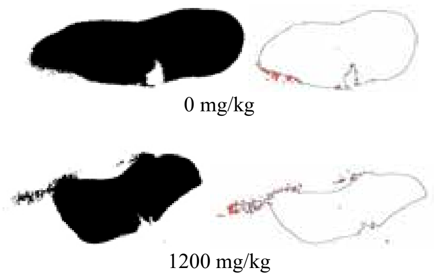


Figure 9. Measured fractal surface

Table 1. Physicochemical properties of the investigated soil

Characteristic parameter	Method of analysis	Analysed soil sample
pH	SR ISO 10390/1999	6.84
Humidity (%)	SR EN 12880/2000	10.13
Granulometry (%)	SR ISO 11465/1997	- sand: 23.60 - dust: 61.40 - clay: 15.00
Total organic carbon (mg/kg dw)	SR ISO 14235/2000 (Walkley-Black Method)	6740
Humus (mg/kg dw)	STAS 7107-1/1976	11793
Total phosphorus (mg/kg dw)	STAS 7184-14/1979	303.5
Total potassium (mg/kg dw)	STAS 7184-18/1980	70
Total nitrogen (mg/kg dw)	SR ISO 11261/2000 (Kjeldahl Method)	594
Nitrate (mg/kg dw)	STAS 7184-7/1987	13.49
Nitrite (mg/kg dw)	STAS 7184-7/1987	0.73
Sulphate (mg/kg dw)	STAS 7184-7/1987	1208
Chloride (mg/kg dw)	STAS 7184-7/1987	39.77

Table 2. Amount of harvested biomass

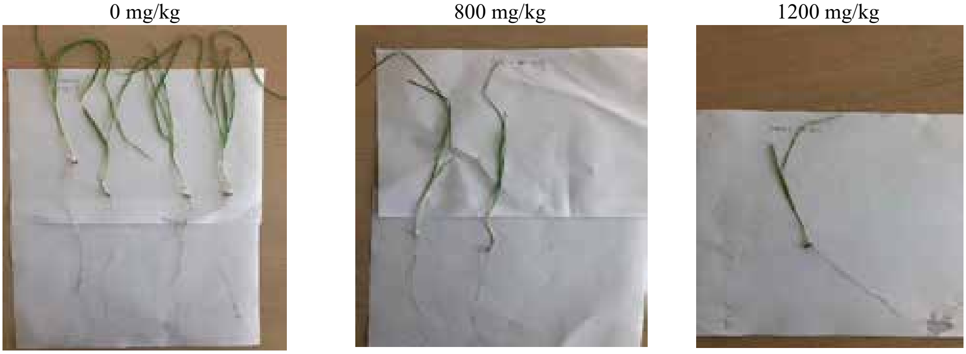
Name of the plant	Concentration of soil pollutant (mg/kg)	Components			Total biomass (g)
		Roots (g)	Stems (g)	Leaves (g)	
Sinapis alba	0	0.609	1.333	1.152	3.094
	200	0.583	1.218	0.952	2.753
	400	0.570	1.197	0.898	2.665
	600	0.565	1.178	0.852	2.595
	800	0.541	1.104	0.822	2.467
	1000	0.536	0.970	0.796	2.302
	1200	0.528	0.790	0.700	2.018
Triticum aestivum	0	0.634	0.733	1.086	2.453
	200	0.635	0.326	0.855	1.816
	400	0.620	0.422	0.763	1.805
	600	0.577	0.571	0.654	1.775
	800	0.546	0.564	0.634	1.771
	1000	0.533	0.527	0.551	1.611
	1200	0	0	0	0

Table 3. The fractal surface of the white mustard leaves

Name of the plant	Concentration of soil pollutant (mg/kg)	Measured fractal surface (m^2)
Sinapis alba	0	0.00187
	200	0.00186
	400	0.00108
	600	0.000925
	800	0.000825
	1000	0.000792
	1200	0.000757

Sinapis alba

0 mg/kg   800 mg/kg   1200 mg/kg



Triticum aestivum

0 mg/kg   800 mg/kg   1000 mg/kg

Figure 10. Harvesting white mustard plants

As shown in Table 1, the soil used in the experiments is a degraded soil, due to the relatively low pH value, high sand and dust content, as well as a low concentration of humus and nutrients.

It is acknowledged that soil characteristics are important for both plant growth and soil metal mobility.

Changing only the agrochemical properties of the soil (soil reaction, humus content, base saturation, granulometric composition) may reduce or increase the heavy metal content of plants several times.

Also, heavy metals are often found in the soil in forms which are less bioavailable to be extracted by the plant's roots. To overcome these shortcomings, various techniques are used that can increase the tolerance of plants to the toxicity of heavy metal ions and can change the conditions in the rhizosphere to favour the extraction of heavy metals, their transport to the roots and subsequent translocation to the aerial parts of the plant (Gavrilescu M., 2022).

For each studied plant, the average root length was determined (Figure 10).

The results obtained can be seen in Figure 11.

The root elongation of studied plants decreased with the increase of the copper concentration in the soil. Thus, the root length decreased in the case of *Triticum aestivum* from 148.30 mm

(0 mg/kg) to 18.70 mm (concentration of 1000 mg/kg) and from 63.70 mm (0 mg/kg) to 40.4 mm (concentration of 1200 mg/kg) in the case of *Sinapis alba.*

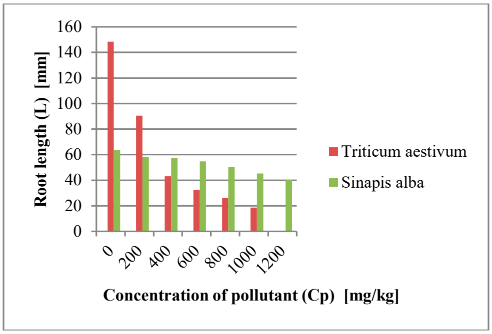


Figure 11. Elongation of wheat and white mustard roots

The correlation between the concentration of pollutant and the inhibition of plant roots under its influence can be seen in the Figures 12 and 13.

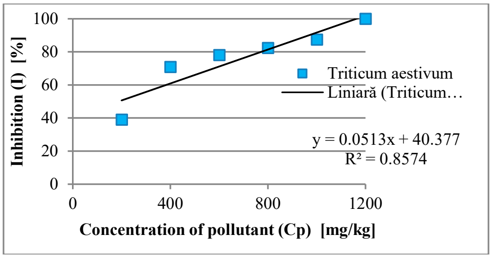


Figure 12. Correlation between the concentration of pollutant (Cp) and inhibition (I) of wheat seeds under the influence of soil pollution with Cu

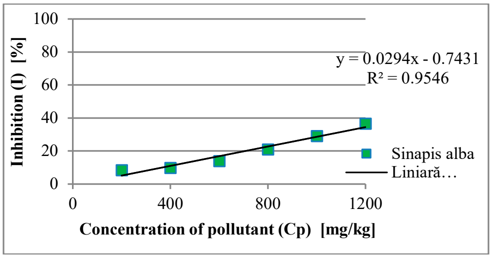


Figure 13. Correlation between the concentration of pollutant (Cp) and inhibition (I) of white mustard seeds under the influence of soil pollution with Cu

CONCLUSIONS

The research conducted and presented in this paper led to the following conclusions:

➢ The plant which was most affected by copper soil pollution was wheat.
➢ There is a biomass difference ranging from 28% to 34%between the two studied plants.
➢ The concentration of copper in the soil also affected the development of white mustard leaves. Their fractal surface area decreased by 59.51% in the case of soil contaminated at a maximum concentration of 1200 mg/kg compared to the unpolluted control sample (0 mg/kg).
➢ The germination of wheat seeds was much lower, decreasing as the applied pollutant concentration was higher. Thus, at a concentration of 1000 mg/kg, only one seed of the 6 seeds used germinated, while at the maximum concentration of 1200 mg/kg, none germinated.
➢ In terms of root length, at the maximum copper concentration in the soil, it decreased by 87.39% in the case of *Triticum aestivum* and by 36.57% in the case of *Sinapis alba* compared to the control sample.

We aim to ascertain in future research whether the two studied plants extracted the metal from the soil and can be used in phytoremediation of copper-polluted soils; also, we wish to determine which of the properties of the soil can contribute to increasing the metal mobility in the soil.

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