

# Response of Two Brassica Species to the Toxic Effect of Different Copper Concentration

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## Abstract

One of the most important challenges in the ecosystem nowadays is the adaptation of plants to damaged environmental factors. Among them, an important attention is paid to the toxic effects of high concentrations of heavy metals (HM). Copper is essentially but highly toxic HM. In the work, first we established, plant's resistance comparison of the two studied Brassica species *Brassica—B. alba*, and *B. napus*—in higher concentrations of copper in the environment, and demonstrated that the two plants were potentially useful for phytoremediation of moderately polluted areas with copper. In plants of the genus *Brassica* grow in a hydroponic culture, experiments showed that the tested species referred to indicator plants. Results show the stability of the studied plants to the toxic effects of excessive copper levels, due to their proline accumulation ability. Studied plants, *B. alba*, and *B. napus*, can be used in selection practice as baselines to generate new plant varieties with increased resistance to heavy metals salts.

## Keywords

*Brassica alba*, *Brassica napus*, Resistance to Copper, Phytoremediation

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## 1. Introduction

Technogenic pollution by heavy metals (HM) is one of the most critical environmental problems [1] [2]. Heavy metals are chemical elements that have a density greater than 5 g/cm<sup>3</sup> and an atomic mass of 40 d [3] [4]. They pose a serious threat, primarily because of their high toxicity [5]. Plants growing on contaminated soil can absorb HM, and accumulate and transfer them to humans and animals in the use of plant products as food. Despite

the fact that many HM, such as copper, zinc, cobalt, and iron, are essential elements, for normal growth and development of the organism at low concentration [6]. In high concentrations, they adversely affect both the plants and the human body. They accumulate in many organs causing various disorders, especially inhibition of growth and yield reduction, inhibition of photosynthesis and respiration, impaired protein synthesis and donor-acceptor relations, inactivation of key metabolic enzymes, the change of water and hormonal status and even the death of the organism [6] [7].

To clean the soil from HM pollution, scientists developed a variety of technologies, one of which is phytoremediation—a method that based on the use of plants to clean up contaminated sites [8] [9]. The most commonly used for this purpose phytoextraction method, which is based on the ability of certain species of plants, absorbs HM from the soil and accumulates them in the aerial organs [10]-[13].

Efficiency of the process Phytoextraction depends on the selection of plant species that not only accumulate HM in the aerial parts, but also produce large amounts of biomass [13]-[15].

Plants grow in contaminated sites, called “phytoremediators”, above all, must be resistant to high concentrations of HM salts [16]-[18]. In addition, they need to grow quickly and produce large amounts of biomass, as well as contain substances that prevent eating them by herbivores to preserve the food chain from toxic heavy metals.

Selection of plant species tolerance to heavy metals is a key element in creating an effective phytoremediation technology. In the literature, there is evidence that the most resistant plants to higher concentrations of heavy metals in the soil are the representatives of the families of the cabbage, cereals and legumes [19].

Copper salts are one of the most toxic metals that contaminate soil. However, currently unknown species of plants have accumulated HM in large quantities, *i.e.* copper hyperaccumulator, although there is evidence as copper remediation in the study of plants *Azollapiñata*, *Brassica juncea* (mustard), *Eichornia crassipes* (water hyacinth), *Helianthus annuus* L. (sunflower), *Hydrocotyle umbellata* L., *Lemna minor* L., *Zygothylus fabago* L. [20].

## 2. Materials and Methods

The work was carried out on two species plants of genus *Brassica*: spring rapeseed *B. napus* (Westar Canadian selection), and wild-type *B. alba* seeds which were obtained from the Baghdad region of Iraq.

Seeds were cultivated in containers with perlite. Aged 10 - 14 days, -3 to 5 plants/1 liter dark plastic containers with a modified Hoagland-Snyder nutrient medium, without EDTA but with iron in the nitrate form (3 mg  $\text{Fe}(\text{NO}_3)_3/\text{liter}$ ). Plants were grown in phytotron chamber, where to maintain a constant temperature 23°C - 25°C daytime and 18°C - 20°C night, photoperiod 12/12 (day/night) hours at 450 lux/m<sup>2</sup>sek illumination intensity. The light source used metal halogen lamps (Philips, Korea) 400 W (2 Lamp each 1.5 m<sup>2</sup>). A complete replacement of the culture medium in the vessels every 5 days was done. In the experiments, 3 - 4 week plants were grown for 5 and 10 days on a nutrient medium containing  $\text{CuSO}_4$  with a concentration of 10, 25, 50, 100 and 150  $\mu\text{Mol}$  (control -0.25  $\mu\text{Mol}$ ). Measurement of fresh biomass of individual plant organs—leaves, roots—performed by standard methods. And we used analytical balances AB54-S (Mettler Toledo, Switzerland).

In the experiments for the determination of proline and content of metals, we used average sample of all the leaves, not counting the cotyledon. All experiments performed in triple biological replicates. The results were statistically processed and expressed as the arithmetic mean and the representativeness of the error standard deviation. The experimental results statistically processed using the program Excel.

### 2.1. The Copper Content in the Plant Material

Determination of the content of copper was performed by Golubkina method [21]. A weighed sample of previously dried plant material (typically 50  $\mu\text{g}$ ) placed in a test tube filled with a mixture of nitric acid (1.6 mL) and hydrochloric acid (0.5 ml) and left overnight. The next day, the sample tubes placed in a preheated to a temperature of 110°C thermostat, incubated for 1 hr then a further 2 h at 180°C. To samples added one drop of concentrated hydrogen peroxide. The next day, decolourised. Transferred into measuring tube and adjusted with distilled water to a certain volume (10 - 15 ml). Measuring the concentration of Cu carried out on atomic absorption spectrophotometer FM 400 (Labist, Russia).

### 2.2. Content of Free Proline

The free proline was determined using the acid ninhydrin reagent method [22]. A weighed sample of fresh plant

leaves tissue (50 mg) transferred to microbiological test tubes; added 10 ml of 100°C distilled water and placed in a boiling water bath. The result is a complete free proline extraction from the plant tissue. To assess the content of proline in 1 ml the obtained extract added 1 ml of glacial acetic acid and 1 ml of ninhydrin reagent (30 ml of acetic acid + 20 ml of 6 M phosphoric acid + 1.25 g ninhydrin) incubated in a boiling water bath for 1 hour and then quickly cooled in ice. The color intensity was determined spectrophotometrically at 520 nm on a spectrophotometer “Thermo Fisher Scientific, Genesys 20” (USA).

### 3. Results

#### 3.1. Effect of Copper on the Growing of Plants

By the end of the experiment, control plant variant *B. napus* accumulated relatively large biomass, whereas the growth of *B. alba* was slower.

However, the inhibitory effect of excessive concentrations of copper was very strong in the fresh plant biomass accumulation *B. napus*. Already starting with the lowest concentration of 10  $\mu\text{Mol}$   $\text{CuSO}_4$  reduction was after 5 days of exposure about 20% and after 10 days of exposure about 30% of control. At a concentration of 100 - 150  $\mu\text{Mol}$  copper in the medium, reduction of plants biomass in *B. napus* was 68% and 76% after 5 days, 71% and 77% after 10 days of exposure than plants growing in standard copper content (0.25  $\mu\text{Mol}$ ). In contrast, the growth of plants *B. alba* at high concentrations of copper in the media affected weakly than *B. napus*, maximal reduction was 64% of controls. There was even more obvious difference between the growths of two species. Inhibitory effect is strongly manifested in the accumulation of fresh plant biomass *B. napus*, but throughout the range of  $\text{CuSO}_4$  concentrations used—from 10 to 150  $\mu\text{Mol}$ —*B. alba* were less affected when compared to *B. napus* (Table 1). So there are some differences in stability between species for the benefit of *B. alba*.

#### 3.2. Copper Accumulation in Plant Tissues

One of the most important parameters that determine the phytoremediation potential of culture is accumulated heavy metals in aerial plant organs [23]. Therefore, in our experiment, it was necessary to assess the ability of the studied plants in the accumulation of copper in plants organs (leaves and roots). founded that when growth in medium contain standard concentration of  $\text{CuSO}_4$  (0.25  $\mu\text{Mol}$ ), copper concentrations in the organs of the two young plants species studied almost identical, being in the range of 21 - 30  $\mu\text{g/g}$  dry weight 5 - 10 days exposure in the roots of *B. alba* and 20 - 24  $\mu\text{g/g}$  dry weight 5 - 10 days exposure in the roots of *B. napus* (Table 2) and 7 - 10  $\mu\text{g/g}$  dry weight 5 - 10 days exposure in the leaves of *B. alba* and 7 - 11  $\mu\text{g/g}$  dry weight 5 - 10 days exposure in the leaves of *B. napus* (Table 3).

However, introducing a large excess of  $\text{CuSO}_4$  in the nutrient solution resulted in the accumulation of copper in studied plant at high concentrations. Brassica plant species studied did not differ in the content of copper in the roots of control options (Table 2). Increasing the concentration of  $\text{CuSO}_4$  in the medium resulted in a strong accumulation of copper in the roots of plants of all two species studied of *Brassica*. It was seen that for all used variants of excess copper in the environment its accumulation in the roots of *B. alba* exceed the data obtained for plant *B. napus*. At the maximum concentration of  $\text{CuSO}_4$  in the medium (150  $\mu\text{Mol}$ ) excess over control values for plants *B. napus* was 61-folds, after 5 days and 98-folds after 10 days, whereas *B. alba* 92-folds after 5 days and 114-folds after 10 days. The copper content in leaves of control options studied *Brassica* species was quite close (Table 3).

**Table 1.**  $\text{CuSO}_4$  effect on growth of young plants. Fresh biomass (g).

Variants	Exposure - 5 days		Exposure - 10 days	
	<i>B. alba</i>	<i>B. napus</i>	<i>B. alba</i>	<i>B. napus</i>
0.25 $\mu\text{Mol}$ .	1.45 $\pm$ 0.18	1.86 $\pm$ 0.20	2.72 $\pm$ 0.20	3.71 $\pm$ 0.25
10 $\mu\text{Mol}$ .	1.22 $\pm$ 0.18	1.52 $\pm$ 0.18	1.91 $\pm$ 0.16	2.51 $\pm$ 0.24
25 $\mu\text{Mol}$ .	0.93 $\pm$ 0.16	1.17 $\pm$ 0.15	1.79 $\pm$ 0.17	2.09 $\pm$ 0.20
50 $\mu\text{Mol}$ .	0.85 $\pm$ 0.15	0.82 $\pm$ 0.16	1.43 $\pm$ 0.16	1.35 $\pm$ 0.19
100 $\mu\text{Mol}$ .	0.72 $\pm$ 0.12	0.60 $\pm$ 0.13	1.12 $\pm$ 0.13	1.07 $\pm$ 0.13
150 $\mu\text{Mol}$ .	0.61 $\pm$ 0.08	0.44 $\pm$ 0.10	0.99 $\pm$ 0.11	0.85 $\pm$ 0.12

**Table 2.** Cu content in the roots.  $\mu\text{g/g}$  dry wt.

Variants	Exposure - 5 days		Exposure - 10 days	
	<i>B. alba</i>	<i>B. napus</i>	<i>B. alba</i>	<i>B. napus</i>
0.25 $\mu\text{Mol}$ .	21 $\pm$ 0.9	20 $\pm$ 0.9	30 $\pm$ 0.9	24 $\pm$ 0.9
10 $\mu\text{Mol}$ .	200 $\pm$ 1.8	197 $\pm$ 2.5	240 $\pm$ 1.5	297 $\pm$ 2.0
25 $\mu\text{Mol}$ .	340 $\pm$ 5.0	280 $\pm$ 5.0	540 $\pm$ 4.0	580 $\pm$ 5.0
50 $\mu\text{Mol}$ .	709 $\pm$ 9.0	578 $\pm$ 7.0	1196 $\pm$ 9.0	781 $\pm$ 8.0
100 $\mu\text{Mol}$ .	1484 $\pm$ 10	991 $\pm$ 10	2313 $\pm$ 10	1527 $\pm$ 11
150 $\mu\text{Mol}$ .	1942 $\pm$ 11	1231 $\pm$ 10	3422 $\pm$ 11	2362 $\pm$ 12

**Table 3.** Cu content in the leaves.  $\mu\text{g/g}$  dry wt.

Variants	Exposure - 5 days		Exposure - 10 days	
	<i>B. alba</i>	<i>B. napus</i>	<i>B. alba</i>	<i>B. napus</i>
0.25 $\mu\text{Mol}$ .	7 $\pm$ 0.4	7 $\pm$ 0.5	10 $\pm$ 0.3	11 $\pm$ 0.6
10 $\mu\text{Mol}$ .	12 $\pm$ 0.3	13 $\pm$ 0.8	17 $\pm$ 0.4	20 $\pm$ 0.6
25 $\mu\text{Mol}$ .	19 $\pm$ 0.4	22 $\pm$ 0.6	24 $\pm$ 0.5	26 $\pm$ 0.8
50 $\mu\text{Mol}$ .	26 $\pm$ 0.5	30 $\pm$ 1.0	51 $\pm$ 0.7	37 $\pm$ 1.0
100 $\mu\text{Mol}$ .	40 $\pm$ 0.8	44 $\pm$ 2.0	63 $\pm$ 0.9	59 $\pm$ 1.5
150 $\mu\text{Mol}$ .	46 $\pm$ 1.0	49 $\pm$ 1.5	76 $\pm$ 1.0	80 $\pm$ 2.0

In addition, there were no significant differences in the concentration of the copper accumulated in the leaves of *B. napus* with increasing concentration of  $\text{CuSO}_4$  in the medium up to 150  $\mu\text{Mol}$  after 5 days of exposure, but after 10 days of exposure differences in accumulation significantly observed especially in concentration 50  $\mu\text{Mol}$  in *B. alba*. This difference manifested both in absolute terms and in relation to the concentration in the leaves of control plants of the same species. Particularly evident dominance *B. alba* in the calculation with respect to the values for the control group of the same species. Thus, the maximum concentration of copper accumulated in the leaves than the corresponding values of the control plants variant *B. alba* 5.1, 6.3 and 7.6 times at 50, 100 and 150  $\mu\text{Mol}$  respectively after 10 days exposure, while in *B. napus* 3, 5 and 7 times at 50, 100 and 150  $\mu\text{Mol}$  respectively after 10 days exposure. Thus, the increased resistance of plants *B. alba* and *B. napus* to excess  $\text{CuSO}_4$  content in the medium was not associated with the restriction of the absorption of copper ions by roots and translocation in their escape from the plants of these species (Table 2 and Table 3). On the contrary, it was realized under conditions of slightly higher copper accumulation in the roots and leaves of the plant *B. alba* in comparison with plant *B. napus*.

### 3.3. Effect of Copper on the Content of Free Proline

In the study of the possible reasons for the differences in the resistance to excessive concentrations of copper in the medium between the plants, *Brassica* species conducted to study the influence of stress factors on the proline content in relation to its known function as a chemical chaperone. It shown that, the level of proline in the leaves of plants species studied equal. Proline concentration in the control variant plants *B. alba* and *B. napus* was close 0.8 - 1.0  $\mu\text{Mol/g}$  wet mass of leaves (Table 4). Increased concentrations of  $\text{CuSO}_4$  in the medium caused a strong accumulation of proline in leaves of the studied *Brassica* species. Even at entering 10  $\mu\text{Mol}$   $\text{CuSO}_4$  in the medium concentration of proline in leaves was increased to 4.3 - 6 times after 5 days and 5 - 7 after 10 days in relation to the values of the control options for the respective species. Increasing copper concentrations, results significantly in increasing prolin concentration in leaves of *B. alba* significantly which exceeded that of *B. napus*. The maximum accumulation of proline to 27.9  $\mu\text{Mol/g}$  wet mass of leaves was found in the leaves of plants *B.*

**Table 4.** Proline content in leaves of 2 *Brassica* species ( $\mu\text{Mol/g}$  wet mass 5 and 10 days).

Variants	Exposure - 5 days		Exposure - 10 days	
	<i>B. alba</i>	<i>B. napus</i>	<i>B. alba</i>	<i>B. napus</i>
0.25 $\mu\text{Mol}$ .	0.8	0.8	0.8	1.0
10 $\mu\text{Mol}$ .	4.8	3.5	5.9	4.8
25 $\mu\text{Mol}$ .	5.4	5.1	7.7	6.2
50 $\mu\text{Mol}$ .	7.3	6.7	10.3	8.8
100 $\mu\text{Mol}$ .	11.5	8.5	14.4	14.7
150 $\mu\text{Mol}$ .	21.4	11.5	27.9	18.7

*alba*, at 150  $\mu\text{M}$   $\text{CuSO}_4$  after 10 days of exposure (Table 4). As a result, the maximum proline content exceeded the value of control options of the respective species of plants *B. alba* about 33 times of the control, whereas *B. napus* about 19 times from control.

These results indicate a significant role of proline in plants adaptation of *B. alba* and *B. napus* to toxic effect of high concentrations  $\text{CuSO}_4$  in a nutrient medium

#### 4. Discussion

Copper is one of the most HM as essential elements in trace amount, which is necessary for the life of all living organisms. Studies have shown that plants grown in Cu-contaminated soil usually accumulate high concentration of copper in their tissues. As a result, a number of physiological and toxicological responses take place in plants depending on the concentration of copper [24] [25].

Small number of known plant species, at which the level of copper accumulation in aerial organs exceed 1000 mg per 1 g dry weight [26], which is a determinant to classify them as hyperaccumulators of copper. Although none of the species examined in the present study can be attributed to this small group, however, in terms of phytoremediation, this is not a definitive barrier to the use of plants of the genus *Brassica*.

Many studies show that different species of the genus *Brassica* have the ability to accumulate Cu in tissues with substantial concentrations [27]. Different species of *Brassica* showed variation for biomass accumulation and concentration of copper. *B. alba* were less than *Brassica napus* in the production of large biomass of shoot as well as having a high concentration of copper in shoots and roots. Previously reported that *B. juncea* shows good accumulation of several heavy metals, including copper [27] [28]. Fargasova reported that *B. alba* have the ability to accumulate many heavy metals, including copper in the root and in greater numbers than in the shoot of plants [29]. Rossi also demonstrated that the roots of *Brassica napus* accumulated several-fold more copper than the aerial organs, which is in accord with the present results [30].

In this study, it was found that, although the accumulation of aboveground biomass in plants *B. alba* lagged behind *Brassica napus*, copper accumulation in the leaves of plants of this species considerably exceeded plant *Brassica napus*. Moreover, *B. alba* showed greater resistance to the excess copper at the stage young plants grow in comparison with *Brassica napus*. The ability to grow plants with a significant amount of biomass and accumulation of copper in large amounts in the roots and shoots of *B. alba* showed fairly good resistance to high concentrations of  $\text{CuSO}_4$ , making it promising for further research in respect to phytoremediation [29].

#### 5. Conclusion

The results of this study allow us to consider *B. alba* as a promising species for phytoremediation of moderately contaminated areas as *B. alba* accumulates copper in the aerial organs (76  $\mu\text{g/g}$  dry wt.) at a relatively higher biomass accumulation (about 1 g/plant) comparing with *Brassica napus* 80  $\mu\text{g/g}$  dry wt. for the accumulation of copper with 0.85 g biomass. From all the above results, we confidently recommend *B. alba* for phytoremediate in contaminated copper territory. However, a more detailed study should be performed for the ability of this type of plant to grow well in the experimental soil culture. Also further genetic study is important to determine the

genes that involve in stress tolerance.

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