# ORIGINAL PAPER COPPER TOLERANCE AND BIOACCUMULATION IN SEEDLINGS OF WHITE MUSTARD (SINAPIS ALBA L.)

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Abstract. Seedlings of Sinapis alba L. species (white/yellow mustard) were grown in nutrient solution containing different concentrations 50, 100, 250 and 500 mg/kg Cu for 10 days. Seedlings were measured and weighed at the end of the experiment. Leaf pigment concentration (chlorophylls a and b and carotenoids), tissular copper concentration and Biological Accumulation Coefficients (BAC) were determined.

Copper content in Sinapis alba L. tissues reached 74.54 - 171.20 mg/kg dry mass, with highest values at high ambient concentration. BAC reached 1.49 at 50 mg/kg and was subunitary at higher concentrations, between 0.75 - 0.34. Shoot length and biomass emphasize a significant decrease at high ambient copper levels, while photosynthetic pigment concentrations did not decrease.

These results indicate a limited tolerance of white mustard to high copper concentrations and a limited bioaccumulation potential of this metal.

Keywords: white/yellow mustard, copper, bioaccumulation, pigments.

### **1. INTRODUCTION**

Soil pollution with heavy metals is a growing problem worldwide, with negative effects on agricultural output, animal and human health. Finding convenient means of remediation is a research priority. Another one is exploiting valuable minerals at low concentrations (subeconomic ores).

Phytoaccumulation (metal bioconcentration in plants) is a possible solution to both (through phytoprospection, phytostabilization, phytoextraction, phytomining) [1, 2].

While heavy metals are toxic to all organisms, some plant species are able to limit this toxicity by various means. These include root exclusion, root sequestration, or metal translocation and accumulation of metal ions throughout the plant, including aboveground organs.

Only a few species are metal-accumulating, while almost 0.2% is hyperaccumulating and such abilities often depend on local soil conditions or other pedo-climatic factors. Among metal accumulator vegetals, many species belong to the Brassicaceae family (*Thalaspi* sp., *Alyssum* sp., *Arabidopsis halleri*, *Streptanthus polygaloides*, *Brassica juncaea* etc.) [1, 25].

*Sinapis alba* L. species (white/yellow mustard) is a popular condiment plant, native to Central and Northern Europe, is widely cultivated in temperate areas throughout the world. It

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is a native of Europe, being cultivated for centuries in European countries. It is a mediumsized herb, with pinnate leaves and bright yellow flowers [3].

Mustard seeds also have various medicinal applications (expectorant, analgesic, stimulant and antimicrobial) [4]. Other plant organs can be used for forage or as source of lignocellulosic materials [5].

Copper is an essential microelement in plants. It is a component of plastocyanin; it has a role in binding various components of the photosynthetic system and plays a crucial role in electron transport in photo-system 2. Copper also, helps maintaining the normal functional structure of chromosomes [6].

However, copper is also an increasing source of pollution and phytotoxicity, due to its many uses in industry and agriculture (copper-based pesticides). Copper excess stimulates pigment synthesis, but inhibits leaf development. A higher amount of this metal could generate destructuring of thylacoid structure. It also affects DNA conformation, limiting cell division. It enhances lipid peroxidation and induces abnormal leaf senescence. Also, copper inhibits elongation growth of plant organs [6].

Tolerance to copper involves various mechanisms, such as limiting uptake, efflux stimulation, chelating or storage (accumulation) in vacuoles, apoplast or in specialized cells (mostly epidermal cells and trichomes) [7].

The purpose of this paper was to present an experiment able to determine the effect of different copper (II) solutions concentrations on mustard plant growth and and to determine the phytoaccumulation potential of white mustard for this metal.

### 2. MATERIALS AND METHODS

#### 2.1. MATERIALS

Romanian commercial mustard seeds were used for plant cultivation.

For seedling germination and growth, Knop's nutrient solution was used, in two versions: unamended and amended with  $CuSO_4$  solution to final copper concentrations of 50, 100, 250 and 500 mg/kg [8, 9].

For leaf pigment analysis, mortar and pestle, 80% acetone solution, NH<sub>4</sub>Cl, CaCl<sub>2</sub> and a WPA S106 UV-vis spectrophotometer were used.

Sample mineralization was done employing concentrated  $HNO_{3}$ , 30%  $H_2O_2$  solution and an electric oven. For copper content determination, a High-Resolution Continuum Source Atomic Absorption Spectrometer ContrAA700, Analytik Jena AG, Germany, with atomizer in acetylene flame technique (FL-AAS) was used [18-23, 28].

### 2.2. METHODS

Mustard seeds were germinated, left for 10 days to grow in basic nutrient solution and transferred to solutions amended with copper, as mentioned above. After another 10 days, samples were taken for analysis.

Analyses included measurement of shoot length and plant biomass (over 10 seedlings from each batch), to evaluate heavy metal effect on plant growth.

For the same reason, the amount of foliar pigments was determined. 0.1 g leaf material was grounded in 10 mL acetone solution, filtered and spectrophotometric absorbance was determined at specific wavelengths 470 nm, 647 nm, 663 nm. By using the equations of

Lichtenthaler and Buschmann [10], the concentrations of chlorophylls *a* and *b* and carotenoids (xantophyll and carotin), were calculated.

For copper determination, seedlings were oven dried (3 days, 80°C). 0.25 g of plant sample were digested for one day in 5 mL concentrated HNO<sub>3</sub> and boiled (1 hour, 150°C). After adding 2 mL H<sub>2</sub>O<sub>2</sub>, then samples were boiled again (2 hours, 150°C). Solutions were diluted to 50 mL (adding 2% NH<sub>4</sub>Cl and 0.5% CaCl<sub>2</sub> to prevent interference) and analyzed by AAS standard procedure at copper specific wavelength 357 nm [11, 18-23].

Average values, standard deviations and Pearson's correlation coefficients between pairs of determined parameters were calculated (values close to -1 and 1 indicate strong negative or positive correlations) [12].

Biological accumulation coefficients (BAC) were calculated as a ratio of metal concentration in shoots versus nutrient solution [13, 14].

# **3. RESULTS AND DISCUSSION**

## 3.1. RESULTS

Fig. 1 shows the average copper concentration in mustard plants at 50, 100, 250 and, respectively, 500 mg/kg ambient copper levels in hydroponic solution and Fig. 2. emphasize the effect of different copper concentrations in nutrient solutions on white mustard seedling growth. Table 1 shows average biological accumulation coefficients (BAC) for plants growing at the selected copper levels.



Figure 1. Copper concentration in dry mustard shoot tissues at selected concentrations in hydroponic solution (mg/kg; average values).

 Table 1. Biological Accumulation Coefficients (BAC) of mustard seedlings at studied copper concentrations.

Copper in nutrient solution [mg/kg]	50	100	250	500
BAC	1.49	0.75	0.66	0.34





Figure 2. Effect of different copper concentrations in nutrient solutions on white mustard seedling growth.





Figure 3. Average shoot length (cm; A) and wet biomass (g; B) of mustard seedlings at the end of the experiment.

Fig. 3 (A and B) shows shoot length and total (wet) biomass at the end of the experimental period, for plants grown in selected nutrient solutions (average values). Fig. 4 presents the average concentrations of foliar pigments (chlorophylls and carotenoids).

Correlations between parameters are shown in Table 2.



Figure 4. Concentration of foliar chlorophyll *a*, chlorophyll *b* and xantophyll+carotin [µg/g] in mustard seedlings grown at selected copper levels.

Correlation	Cu in solution	Cu in plant tissue	Chl a	Chl b	X+C	Shoot length	Biomass
Cu in solution	1	0.88	0.78	0.73	0.57	-0.92	-0.87
Cu in plant tissue		1	0.75	0.62	0.89	-0.94	-0.97
Chl a			1	0.98	0.62	-0.57	-0.57
Chl b				1	0.45	-0.46	-0.44
X+C					1	-0.72	-0.81
Shoot length						1	0.99
Biomass							1

 Table 2. Correlation coefficients between analyzed parameters.

### 3.2. DISCUSSIONS

Assessing the usefulness of a certain species for phytoextraction involves two aspects: tolerance to high heavy metal levels and ability to uptake and concentrate metal in plant tissues.

Concerning bioaccumulative abilities, several factors should be considered. Average copper content in plant tissue – in the "standard reference plant" – is around 10 mg/kg [15]. The lowest copper contents in mustard seedlings, in this experiment, were higher than 74 mg/kg (at 50 and 100 mg/kg Cu in nutrient solution; Fig. 1).

It should be noted that most soils in Romania contain 0-120 mg/kg copper, 20 mg/kg is the legally-defined reference threshold for sensitive soils, while 100 mg/kg is the alert thershold [16], values similar to those in the first two batches.

However, mustard seedlings cannot be considered as hyperaccumulative, since the minimum threshold is usually considered to be 1,000 mg/kg (alternatively, 300 mg/kg) [1, 15], while the maximum average metal content (at 500 mg/kg) was only 171.2 mg/kg dry mass (Fig. 1).

The final factor is the BAC. A value constantly above 1 indicates an accumulating plant, valuable for phytoextractive purposes. This requirement is fulfilled only at the lowest ambient concentrations (1.49 at 50 mg/kg; Table 1), while BAC decreases at higher concentrations. Overall, mustard seedlings can only be considered as copper "moderate accumulators" [17]. This still indicates a potential for phytostabilization – remediation of polluted soils through metal sequestration, depending on copper concentration in root tissue [13, 14, 25-29].

Regarding tolerance, results show that copper concentrations of 250 mg/kg and higher hindered proper plant growth, differences in shoot length and biomass being statistically significant and strongly correlated to copper levels (Fig. 3, Table 2).

Photosynthetic pigments followed a different pattern. Both chlorophylls showed a decrease at 100 mg/kg, and an increase at 500 mg/kg. Carotenoids were more strongly (positively) correlated to ambient copper, but with lower differences among samples (290 - 326  $\mu$ g/g). This is consistent with data fund in literature [6] and indicates a toxic effect on the photosynthetic apparatus of studied seedlings.

### 4. CONCLUSIONS

The general conclusions of this study are presented below.

While copper concentrations in plant tissues were above the average standard for all studied seedlings, BAC values were higher than 1 only at 50 mg/kg and decreased at higher concentration, indicating a limited potential for phytoextraction.

At concentrations 250 and 500 mg/kg Cu, seedlings exhibited a significantly lower growth, in terms of length and biomass than at 50-100 mg/kg, while pigment variations indicated issues concerning the development the photosynthetic apparatus.

The obtained experimental results emphasize that *Sinapis alba* L. species could be valuable for phytoextraction/phytostabilization purposes only on soils with copper contents below or close to the alert threshold.

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