

# Phytotoxic Effect and Bioaccumulation of Chromium in White Mustard (*Sinapis alba* L.) Seedlings

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*The chromium bioaccumulation potential in Sinapis alba L. was studied in experimental hydroponic conditions. Mustard seedlings were grown in nutrient solution containing 50, 100, 250 and 500 ppm Cr. After 10 days, seedlings were analyzed for shoot length, biomass, leaf pigment (chlorophylls and carotenoids) and tissular Cr concentration. The Biological Accumulation Coefficients (BAC) were also determined. Cr content in S. alba dry mass reached 415-2,064 ppm, depending on ambient Cr concentration. BAC ranged between 8.30 to 4.13, with a peak at 100 ppm and lowest values at higher concentrations. Shoot length, biomass and pigment concentration values showed an average decrease at high Cr concentrations (mostly, at 500 ppm), without a definite statistical significance. Results indicate that S. alba is tolerant to high Cr levels and has hyperaccumulative abilities.*

**Keywords:** phytoaccumulation, Brassicaceae, copper, chromium, zinc, manganese

Soil pollution involving heavy metals causes an increasing concern worldwide. Heavy metal pollution is a consequence of industrial activities and can lead to significant drops in agricultural productivity and health hazards.

Another important issue regarding heavy metals in soil is the possible exploitation of subeconomic ores.

For both problems, phytoaccumulation, with its two main applications – phytoremediation and phytomining – is a possible solution [1, 2].

While heavy metals are toxic to all plants (especially at root level), some species have developed mechanisms for limiting their effect. Such strategies range from exclusion of metal ions from the root system, or sequestration at root level and exclusion from aboveground organs, to accumulation and even hyperaccumulation in both aboveground and underground organs.

Not many species are metal accumulators and even fewer are hyperaccumulators (around 0.2% of known species). This is the reason why screening local native vegetation for such species and searching for strategies to enhance their potential is of great importance.

Many members of the Brassicaceae family have such abilities (*Thalaspis* sp., *Alyssum* sp., *Arabidopsis halleri*, *Streptanthus polygaloides*, *Brassica juncea* etc.) [1].

*Sinapis alba* L. (white/yellow mustard) is a member of this family common crop in many temperate areas of the world. It is a native of Europe, being cultivated for centuries in Central and Northern European countries. A medium-sized herbaceous species, it has pinnate leaves and bright yellow flowers [3].

Besides being a popular condiment, mustard seeds also have medicinal properties (expectorant, analgesic, stimulant and antimicrobial, useful in digestive and respiratory illnesses) [4]. Other parts of the plant can be used as forage or as a lignocellulosic raw material [5].

Chromium is a metal commonly used in industry, for specific alloys. Another important use is for the production of catalysts (such as copper chromites) used in chemical reactions involving hydrocarbons or in pollution treatments [6].

Chromium concentration in soils might vary to up to 2,000 ppm. However, normal values are, usually, below 50 ppm. For instance, data collected in common garden soils in Western Romania showed a Cr content of 20-25 ppm [7], values also found in local soils in Constanta (unpublished data).

The purpose of the current research was to assess the effect of different concentrations of chromium on mustard plants and to determine the phytoaccumulation potential of this metal.

## Experimental part

White mustard seedlings (10 days from germination) were grown hydroponically in Knop's solution amended with  $K_2Cr_2O_7$  [8, 9]. Seedlings were grown in solutions containing 50, 100, 250 and 500 ppm Cr, respectively.

After another 10 days, seedlings were taken for analysis.

To assess the effect of chromium concentration on plant growth, at least 10 seedlings were weighed and had their shoot length measured for each concentration.

Leaf pigment concentration is another indicator of plant health. 0.1 g of mustard leaves were taken for analysis and grounded in 10 mL acetone (80%). The extract was filtered and analyzed with a WPA S106 spectrophotometer, at 470, 647, 663 nm wavelengths.

For determining the actual concentrations of chlorophylls a and b and carotenoids (total xanthophyll and carotin), the trichromatic equations of Lichtenthaler and Buschmann were used [10].

For Cr content determination, seedlings were cut in small pieces and oven dried for three days at 80°C. 0.25 g dry material were taken for analysis for each sample, digested in 5 mL of concentrated  $HNO_3$  overnight and boiled at 150°C for one hour, then 2 mL of  $H_2O_2$  were added and samples were boiled 2 h at 150°C. Solutions were diluted at 50 mL (with an addition of 2%  $NH_4Cl$  and 0.5%  $CaCl_2$ ) [11, 12] and analyzed with an atomic absorption spectrometer (HR-CS ContrAA700, Analytik Jena AG, with acetylene-nitrous oxide flame) at 357 nm wavelength.

Average values and standard deviations were calculated using MS Excel software. Pearson's correlation coefficients

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were also calculated, in order to assess correlations between various measured values. Values close to -1 and 1 indicate strong negative, respectively positive correlations [13].

Biological accumulation coefficients (BAC) for chromium were calculated as a ratio of metal concentration in aboveground organs (shoots) to that in the nutrient solution [14-17].

## Results and discussions

Figure 1 shows the average concentration of chromium in mustard plants at the four different Cr levels in the hydroponic nutrient solution, while biological accumulation coefficient (BAC) values are shown in table 1.

Figure 3 (A, B) shows the average stem length and (wet) biomass at the end of the experiment. Chlorophylls and carotenoid pigment concentrations are presented in figure 4.

The correlation coefficients are shown in table 2.

The first standard to compare these data is the so-called *standard reference plant*, an average of various elements concentration in worldwide flora. For chromium, this standard is believed to contain around 1.5 ppm [18], almost 300 times below the lowest value in this experiment. From

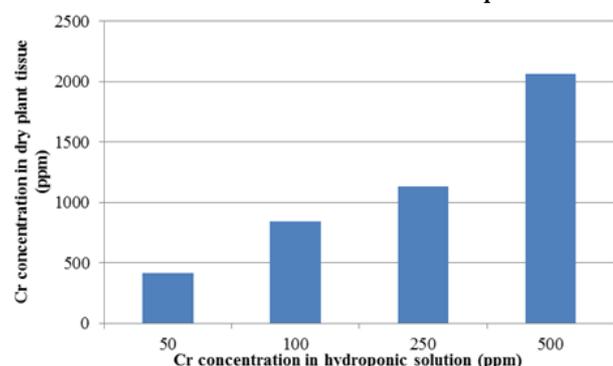


Fig. 1. Metal concentration in dry plant shoot tissue at studied concentrations in nutrient solution (ppm; average values)



Fig. 2. Comparison of white mustard seedlings shoot length after growth at different Cr concentrations (50 ppm, 500 ppm)

Cr in nutrient solution (ppm)	50	100	250	500
BAC	8.298	8.44	4.528	4.128

**Table 1**  
BIOLOGICAL ACCUMULATION  
COEFFICIENTS OF Cr AT  
STUDIED CONCENTRATIONS

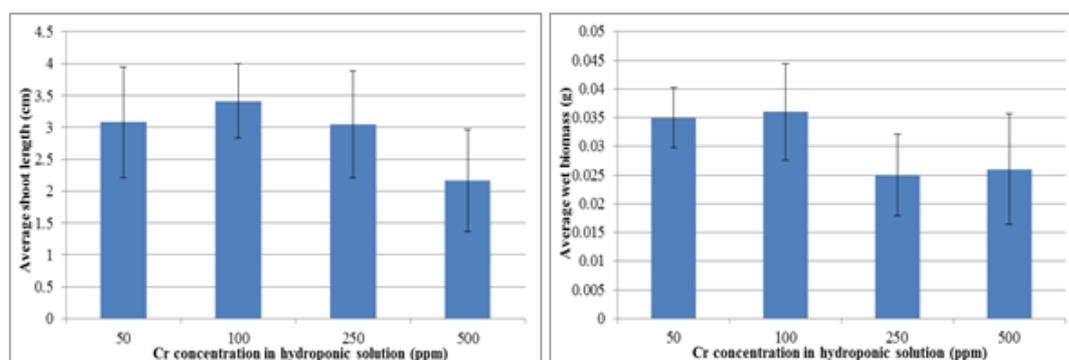


Fig. 3. Average plant shoot length (cm; A) and wet biomass (g; B) at the end of the experiment

this point of view, mustard seedlings analyzed can be considered chromium hyperaccumulators.

Another way to define a hyperaccumulator is the ability to accumulate the selected metal above a specific threshold (1,000 ppm for Cr, for most authors, 300 ppm proposed in recent studies) [1, 18]. Since studied seedlings, accumulated up to 2,064 ppm, they also fit this definition (fig. 1).

However, such definitions do not take into consideration the adaptation of a specific plant species to particular substrate conditions. Some only show hyperaccumulative traits on metal-rich soils [1]. Thus, the BAC (indicating a high shoot : soil metal concentration ratio) is another crucial value to consider. Plants with a BAC above 1 have a phytoextraction potential, since they translocate the metal in aboveground, harvestable organs. Plants with  $BAC < 1$  are only useful as phytostabilizers. While the BAC itself is not a key character, coupled with high metal tolerance and an exhibition of accumulation traits at high concentrations indicate valuable species [18].

BAC values for *S. alba* seedlings at studied concentrations ranged from 8.30 to 4.13, with a maximum at 100 ppm Cr in nutrient solution and a significant decrease at higher concentrations (table 1). These indicate a high Cr accumulation and translocation (BAC above 2 is rarely found in plants growing on normal soils) [14-17, 19]. However, it should be noted that the BAC calculated in hydroponic experiments has not the same significance as BAC on soils, since in the latter case, metal concentration is calculated in dry mass.

Concerning the phytotoxic effect of high chromium concentrations, the amount and ratio of leaf pigments is known to be a good indicator of plant health and phytoremediation efficiency. A phytotoxic effect is commonly associated with a significant drop in chlorophyll and carotenoid concentration [20].

Such an effect was not found in the current investigation. Chlorophyll *a* had a 483-572  $\mu\text{g/g}$  concentration while chlorophyll *b*, between 843-998  $\mu\text{g/g}$ . Maximum concentrations were found at 100 ppm. While lower values were found at the highest Cr concentration, the drop did not exceed 15% from the maximum value. Similar results were determined for total carotin and xanthophylls (139-173  $\mu\text{g/g}$ ; fig. 4).

The chlorophyll *a* : chlorophyll *b* ratio was constant (0.573 similar to results found in literature). For chlorophyll *a* : carotenoid ratios, values can be significantly influenced by toxicity [18], however, in the current experiment, all ratios were similar (3.2-3.9). While a certain phytotoxic effect cannot be excluded, the selected Cr concentrations

Correlation	Cr in solution	Cr in plant tissue	Chl a	Chl b	X+C	Shoot length	Biomass
Cr in solution	1	0.99	-0.86	-0.86	-0.64	-0.91	-0.81
Cr in plant tissue	-	1	-0.80	-0.80	-0.55	-0.86	-0.74
Chl a	-	-	1	1	-0.45	0.99	0.63
Chl b	-	-	-	1	-0.45	0.99	0.63
X+C	-	-	-	-	1	0.47	0.97
Shoot length	-	-	-	-	-	1	0.66
Biomass	-	-	-	-	-	-	1

**Table 2**  
CORRELATION  
COEFFICIENTS  
BETWEEN DIFFERENT  
DETERMINED VALUES

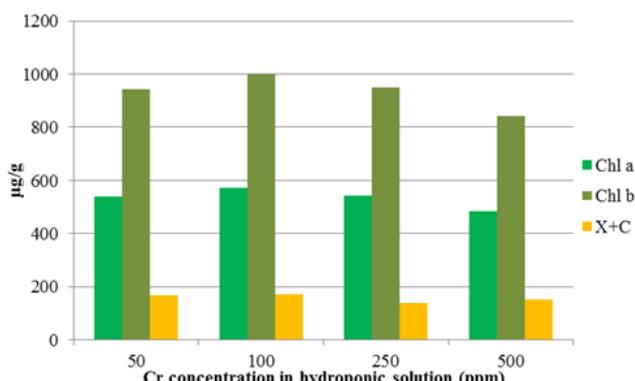


Fig. 4. Leaf pigment concentration – chlorophyll a, chlorophyll b, xanthophyll+carotin (µg/g) at studied Cr concentrations

did not interfere with normal pigment synthesis in mustard seedlings.

Regarding plant growth, differences between average shoot length and biomasses at different Cr concentrations were found, with lower values at 250 and 500 ppm (fig. 2, 3). While there was a significant negative correlation between chromium content in nutrient solution/plant tissue and shoot length, biomass, as well as chlorophylls concentration (correlation coefficient < -0.80; table 2), data showed that this drop in length and biomass was not statistically significant, due to large variation among individual seedlings (fig. 3).

## Conclusions

*Sinapis alba* seedlings grown in nutrient solution amended with excess Cr shown a remarkable tolerance and bioaccumulation potential.

Bioaccumulation coefficients ranged between 8.30 to 4.13, with a maximum at 100 ppm and a decline at higher values, while the Cr content in plant tissue reached 415-2,064 ppm. Thus, the analyzed plants can be easily considered as potential chromium hyperaccumulators.

While Cr concentrations above 100 ppm in the nutrient solution led to a decrease in leaf pigment concentration, shoot length and biomass, these variations could not be considered as statistically significant. This might indicate a high tolerance to Cr, at least regarding overall productivity and pigment synthesis mechanisms.

These preliminary results show that white mustard, a common crop, might have a significant potential for chromium phytoextraction and open the way for a new research direction, regarding potential practical applications (phytoremediation of heavy metals polluted soils or phytomining).

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Manuscript received: 14.03.2016