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PHYTOTOXIC EFFECTS OF HEXAVALENT CHROMIUM ON RAPESEED PLANTS

FITOTOKSYCZNOŚĆ CHROMU SZEŚCIOWARTOŚCIOWEGO DLA ROŚLIN RZEPAKU

Abstract: Rapeseed (*Brassica napus* L. *subsp. napus*) plants were exposed to six different concentrations (12, 24, 60, 120, 240, 480 μmol dm^{-3}) of $K_2Cr_2O_7$ for 7 days. Dry mass of shoots and roots decreased rapidly with increasing external Cr(VI) concentration. Application of Cr(VI) concentrations ≥ 120 μmol dm^{-3} caused that leaves were strongly chlorotic and some of them even desiccated. Roots of these plants where subtile and brownish. Notable decrease in chlorophyll content was observed already at the lowest (12 μmol dm^{-3}) used concentration. Content of soluble proteins in leaves decreased rapidly within the studied concentration range, whereby the lowest protein content was observed after application of 240 μmol dm^{-3} Cr(VI). Lipid peroxidation expressed as a content of malondialdehyde in leaves was notable already after application of 12 μmol dm^{-3} Cr(VI). At lower applied Cr(VI) concentrations (12÷120 μmol dm^{-3}) the bioaccumulation factors related to Cr accumulation in roots were higher then those determined for shoots. Treatment with higher Cr(VI) concentrations (240 and 480 μmol dm^{-3}) had an opposite effect and BAFs for the shoots exceeded those determined for the roots. The portion of Cr allocated in shoots related to the total Cr amount accumulated by plant ranged from 23.3% (12 μmol dm^{-3}) to 94.7% (480 μmol dm^{-3}). In the case of higher applied external Cr(VI) concentrations (120÷480 μmol dm^{-3}) the defence mechanisms of plants were evidently impaired and uncontrolled Cr translocation within the plant occurred.

Keywords: bioaccumulation and translocation factors, chlorophyll content, chromium, lipid peroxidation, rapeseed

Chromium is a toxic carcinogen and it is released into the soil mainly from leather tanning, textile, carpet and electroplating industries [1]. It occurs in soil mainly in two oxidation states, as Cr(III) or Cr(VI) ions. Chromium(VI) remains stable for several months in the soil without changing its oxidation state. In fact, oxidative behaviour of the chromium in soils is of ecological significance, since Cr(VI) is found to be more toxic to plants and animals than Cr(III) [2]. Chromium is bioaccumulated by plants and its accumulation is biomagnified at different trophic levels through the food chain [3].

Symptoms of Cr phytotoxicity include decrease of seed germination, reduction of root growth, induction of leaf chlorosis, reduction of biomass, induction of biochemical changes and it was demonstrated that chromium inhibit photosynthetic and mitochondrial electron transport in higher plants [4]. Further, plants growing in chromium-stressed environment face a potential risk from *reactive oxygen species* (ROS) like superoxide, hydroxyl radicals and hydrogen peroxide. Their presence cause oxidative damage to the biomolecules such as lipids, proteins and nucleic acids [5]. In the plants, metal induced lipid peroxidation has been reported [6], which profoundly alters the structure of membranes and consequently modifies their enzymatic and transport activities. Plants have developed complex defence mechanisms by which they mediate the deleterious effects of the ROS. Such defence systems involve both enzymatic and non-enzymatic antioxidants [7]. The enzymatic

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protective mechanism operates by sequential and simultaneous action of a number of antioxidant enzymes such as catalase, superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase and glutathione reductase [7].

Rapeseed (*Brassica napus* L. *subsp. napus*) is known to be able to accumulate substantial amounts of metals; moreover, this plant has a high biomass, various genotypes are easily available and the plant belongs to Brassicaceae family [8] that has received considerable attention [9] based on the capacity of these plants to uptake and accumulate Cr and other heavy metals in amounts higher than those of other plant species [10].

The aim of this study is to investigate the effect of Cr(VI) on plant biomass as well as Cr accumulation and translocation in plant organs of hydroponically cultivated *B. napus* plants (cv. Verona). In addition, content of soluble proteins, *malondialdehyde* (MDA), chlorophyll *a* and *b* as well as carotenoids in leaves was determined.

Material and methods

For experiments $K_2Cr_2O_7$ of analytical purity was used (Slavus, Bratislava). For cultivation of experimental plants the seeds of *Brassica napus,* cv. Verona (Centre for Research of Crop Production, Research Institute of Crop Production, Piešťany) were used. Three-weeks old rapeseed plants were exposed in hydroponics for seven days in controlled conditions (mean air temperature: 25 ±0.5ºC, relative air humidity: 80% and photosynthetic active radiation: 80 μmol m^{-2} s^{-1}): control variant in Hoagland solution and metal-treated variants in Hoagland solution containing 12, 24, 60, 120, 240 and 480 μmol dm^{-3} $K_2Cr_2O_7$. Then the length and dry mass of shoots and roots were determined. Flame *atomic absorption spectroscopy* (AAS Perkin Elmer Model 1100, USA) was used for determination of Cr content in shoots and roots of rapeseed plants. Protein content in leaves was determined spectrophotometrically (Genesys 6, Thermo Scientific, USA) according to Bradford [11]. Content of malondialdehyde in leaves was determined spectrophotometrically as a content of *thiobarbituric acid reactive substances* (TBARS) according to method described in [12]. Photosynthetic pigments were determined spectrophotometrically after extraction into 80% acetone [13].

Results and discussion

Shoot and root dry mass as well as length of both plant organs of rapeseed plants treated with Cr(VI) are summarized in Table 1. Significant reduction of dry biomass (both plant organs) was observed after application of concentrations higher than 60 μmol dm^{-3}. Shoot length was affected by applied concentrations of Cr(VI) to a greater extent this of root. General response of decreased root growth due to Cr toxicity could be due to inhibition of root cell division/ root elongation or to the extension of cell cycle in the roots. The reduction in plant height might be mainly due to the reduced root growth and consequent lesser nutrients and water transport to the above parts of the plant. In addition to this, Cr transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoots contributing to the reduction in plant height. The overall adverse effect of Cr on growth and development of plants could be serious impairment of uptake of mineral nutrients and water leading to deficiency in the shoot [4].

Chlorosis was notable already after application of the lowest Cr(VI) concentration (12 μmol dm^{-3}). Concentrations of Cr(VI) higher than 60 μmol dm^{-3} caused that leaves of plants were strongly chlorotic, some of them even desiccated and fell off (240 and 480 μmol dm^{-3}). Roots of plants treated with two highest concentrations were subtile and brownish. Content of chlorophyll *a* and carotenoids (Fig. 1) was affected by Cr(VI) to a greater extent than that of chlorophyll *b*. Significant decrease of chlorophyll *b* was observed after application of 60 μmol dm^{-3} Cr. The decrease in the chlorophyll *a*/*b* ratio caused by Cr indicates that Cr toxicity possibly reduces size of the peripheral part of the antenna complex [14]. The decrease in chlorophyll *b* due to Cr could be due to the destabilization and degradation of the proteins of the peripheral part. The inactivation of enzymes involved in the chlorophyll biosynthetic pathway could also contribute to the general reduction in chlorophyll content in most plants under Cr stress. It could be assumed that chromium toxicity is not located at the level of δ-aminolevulinic acid synthesis, but, probably at the δ-aminolevulinic acid dehydratase (ALAD activity which was more severely affected during chlorophyll biosynthesis). Finally, impaired chlorophyll biosynthesis results in reduced total chlorophyll content [4].

Table 1

Shoot and root length and dry mass of rapeseed plants treated with different concentrations of Cr(VI). Mean ± S.E., n = 5. Means followed by different letters are significantly different at the 0.05 probability level

c [μmol dm^{-3}]	Shoot dry mass [mg]	Root dry mass [mg]	Shoot length [cm]	Root length [cm]
0	417.3 ± 43.7^{a}	29.8 ± 2.9^{a}	21.2 ± 1.2^{a}	16.1 ± 1.2^{a}
12	424.8 ± 64.1^{a}	22.5 ± 3.0^{b}	20.4 ± 0.5^{a}	16.8 ± 1.6^{a}
24	378.5 ± 33.4^{a}	22.3 ± 2.6^{b}	18.3 ± 1.1^{b}	16.6 ± 1.0^{a}
60	247.8 ± 26.7^{b}	17.3 ± 2.1^{c}	17.0 ± 1.3^{bc}	$17.2. \pm 0.8^{a}$
120	213.3 ± 28.2^{bc}	16.0 ± 1.8^{c}	17.7 ± 0.9^{bc}	16.1 ± 1.4^{a}
240	168.0 ± 13.0^{c}	13.3 ± 2.2^{c}	16.9 ± 0.3^{c}	16.2 ± 0.7^{a}
480	111.0 ± 9.2^{d}	7.5 ± 1.7^{d}	16.4 ± 1.1^{c}	16.5 ± 0.8^{a}

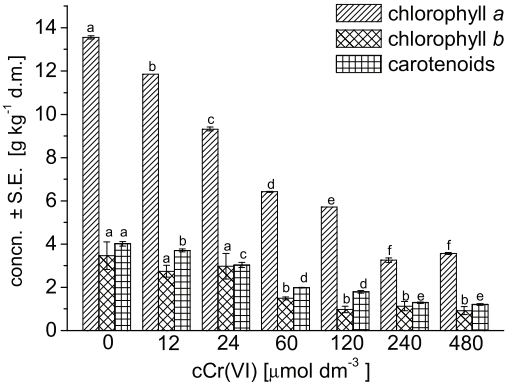

Fig. 1. Effect of applied Cr(VI) concentration on chlorophyll *a*, chlorophyll *b* and carotenoids concentration in leaves of rapeseed plants. Mean ± S.E., n = 3. Means followed by different letters are significantly different at the 0.05 probability level; d.m. - dry mass

Application of Cr(VI) concentrations higher than 24 µmol dm^{-3} caused strong decrease of protein content in leaves (Table 2). Degradation of proteins in rapeseed plants by Cr result in the inhibition of *nitrate reductase* activity (NR), whereby the correlation between NR activity and proteins has been well documented in plants [15]. A decline in amino acid cysteine may result in the degradation of sulphate-reducing enzymes leading to toxic effects [16].

Heavy metal toxicity is considered to induce the production of reactive oxygen species and may result in significant damage to cellular constituents. Membrane lipids and proteins are especially sensitive to attack by free radicals and they are considered to be reliable indicators of oxidative stress in plants [17, 18]. Content of proteins and malondialdehyde (MDA) in leaves of rapeseed plants is presented in Table 2.

Chromium induced oxidative stress in rapeseed plants was evident from the increased lipid peroxidation (content of MDA) in leaves, which was notable already after application of 12 µmol dm^{-3} Cr(VI). This finding is in agreement with the results of other studies carried out with plants cultivated in hydroponics [19, 20].

Table 2

Protein and MDA concentrations in leaves of rapeseed plants treated with different concentrations of Cr(VI). Mean ± S.E., n = 3. Means followed by different letters are significantly different at the 0.05 probability level; d.m. - dry mass

Cr(VI) conc. [µmol dm^{-3}]	**Protein conc. [g kg^{-1} d.m.]**	**MDA conc. [mmol kg^{-1} d.m.]**
0	45.0 ± 2.7^{a}	1.89 ± 0.03^{a}
12	37.4 ± 6.1^{ab}	1.94 ± 0.05^{a}
24	36.4 ± 4.00^{ab}	2.18 ± 0.07^{b}
60	31.1 ± 2.2^{bc}	2.19 ± 0.02^{bc}
120	23.3 ± 2.9^{cd}	2.32 ± 0.04^{cd}
240	17.5 ± 2.00^{d}	2.40 ± 0.06^{d}
480	18.7 ± 3.7^{d}	2.82 ± 0.03^{c}

Table 3

Chromium concentrations in shoots and roots of rapeseed plants treated with different concentrations of Cr(VI), corresponding values of bioaccumulation (BAF) and translocation (TF) factors and fraction of accumulated Cr allocated in shoots related to the total amount of Cr accumulated by plants (in [%]); d.m. - dry mass

c [µmol dm^{-3}]	**Cr conc. [mg kg^{-1} d.m.] BAF**		**TF [% Cr in shoot]**
	Shoot	**Root**	
0	0.3	6	-
12	36 **29.1**	2259 **1810.3**	0.016 **23.3**
24	92 **36.9**	2463 **986.9**	0.037 **38.8**
60	508 **81.4**	2462 **394.4**	0.206 **74.7**
120	1740 **139.4**	2587 **207.3**	0.673 **90.0**
240	3161 **126.7**	2615 **102.3**	1.209 **93.9**
480	3931 **78.8**	3251 **65.2**	1.209 **94.7**

Chromium content in shoots and roots of rapeseed plants treated with different metal concentrations, the corresponding *bioaccumulation* (BAF) and *translocation* (TF) *factors* as well as the fraction of accumulated Cr allocated in shoots related to the total amount of Cr accumulated by plants are summarized in Table 3.

Bioaccumulation factor (BAF) express the ratio of the metal concentration in the biological material [μmol or μg g^{-1} dry mass] to the metal concentration in external solution in [μmol or μg dm^{-3}]. In the concentration range 12÷120 μmol dm^{-3} chromium, BAF values of roots were higher than those of shoots. After application of 240 and 480 μmol dm^{-3} of Cr the BAFs determined for shoots exceeding those for roots reflect more effective mobility of Cr in the plants (Table 3). The translocation factor corresponds to the ratio of accumulated Cr amount in shoots and roots. Translocation factor values higher than 1 correspond to higher Cr concentration [mg kg^{-1} dry mass] in the shoots than in the roots. However, the total Cr content occurring in individual plant organs is affected not only by Cr concentrations in shoots and roots but also by actual dry mass of these plant organs. While after application of 12 and 24 μmol dm^{-3} Cr(VI) the higher portion of Cr (76.7 and 61.2%) was accumulated in roots, treatment with higher Cr(VI) concentrations (60÷480 μmol dm^{-3}) had an opposite effect and the majority of Cr accumulated by rapeseed plants (74.7 to 94.7%) was allocated in shoots (Table 3).

Chromium uptake by plants is mainly non-specific, probably as a result of uptake of essential nutrients and water [21]. It has been reported that translocation of chromium from roots to shoots was inhibited in the presence of toxic levels of the metals [22]. Rapeseed appeared to accumulate Cr mainly in the root system, whereas the shoots content is relatively low. The reason of the high accumulation in roots of the plants could be because Cr is immobilized in the vacuoles of the root cells, thus is less toxic, which may be a natural toxicity response of the plant [23]. The dependence of accumulated Cr content in shoots on the applied concentration of Cr(VI) showed practically linear increase up to application of 240 μmol dm^{-3} Cr(VI). On the other hand, root tissue was gradually saturated by the studied metal. In the case of higher applied external Cr(VI) concentrations (120÷480 μmol dm^{-3}) the defence mechanisms of plants were evidently impaired and uncontrolled Cr translocation within the plant occurred. Considerably higher biomass of shoots (more than one order) in comparison with that of roots as well as higher Cr concentration in the shoots than in the roots observed after treatment with 240 and 480 μmol dm^{-3} Cr significantly contributed to very high values of the fraction of accumulated Cr allocated in the shoots related to the total amount of Cr accumulated by plants.

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