Vol. 15, No. 3 2008

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PHYTOTOXICITY OF CHROMIUM AND NICKEL

FITOTOKSYCZNOŚĆ CHROMU I NIKLU

Summary: The dry and fresh biomass, water content, photosynthetic pigments production and metal accumulation in roots and shoots of mustard (*Sinapis alba* L.) seedlings was evaluated in laboratory experiments with three types of washing wastewaters from cutlery production line with high content of Cr and Ni. All tested washing waters reduced root dry mass, whereas the dry mass of shoots was either not affected or it increased. The effect of tested washing waters was stronger on fresh mass production than on dry mass production. This indicates problems in water reception and translocation. The adverse effect on photosynthetic pigments production increased only slowly with remaining washing wastewater concentration. Almost all Chl a/b ratios were the same as for the control and this indicated no significant differences in the reduction of either *a* or *b* chlorophylls. In opposite to chlorophylls carotenoids content was in the presence of tested washing wastewaters increased and overreached their content in a control or their concentration was on the same level as in the control. As the ratio of Chl(a+b)/Car was lower than that for the control for almost all tested samples, a stronger reduction in chlorophylls than in carotenoids was confirmed. While the accumulation of Cr was higher in the roots, Ni was distributed equally through the whole plant seedlings. Cr uptake in the roots and shoots was in average about 1.7 and 7.3 times, respectively, lower than that of Ni. Nickel percentage uptake from washing waters in the roots and shoots was nearly equal and range from 10.2 to 15.8%. These determined adverse effects of washing wastewaters from this cutlery production line classified them as too dangerous to be spread on open-land soil.

Keywords: phytotoxicity, cutlery washing wastewaters, chromium, nickel, mustard *Sinapis alba* L.

Vascular plants are very effective in the recognition and prediction of metal stress in the environment. Through their ability to accumulate toxic substances, they indicate the presence of toxins in the environment even when their concentration is very low [1]. While considering the toxicity of heavy metals, a distinction should be made between elements essential to plants, and those which have no proven beneficial biochemical effects [2].

Phytotoxicity assessment plays an important role in environmental monitoring and risk assessment of metal-contaminated places. Because, only a few guidelines are available for the assessment of heavy metal phytotoxicity [3] quality-controlled toxicity data using standardized methods are actually quite rarely reported in the literature. Efroymson

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et al. [4] developed toxicological benchmarks for screening the effects of contaminants that have the potential to arouse concern. This included the effect of certain heavy metals on terrestrial plants, and they reviewed phytotoxicity data derived from experiments conducted in nutrient culture and spiked soils. Phytotoxicity tests generally use toxicological endpoints such as root growth, shoot length, biomass production and germination percent. However, physiological responses of plants to toxic metals are not only growth and production inhibition, but also changes in intensity of various physiological parameters [5]. There are not standardized yet, *eg* mainly photosynthetic activity, chlorophyll fluorescence and some enzymes activity in plant tissues [6]. Also, up to now relationships between metal toxicity and metal tissue concentration have been poorly characterized.

Due to their wide industrial use, chromium (Cr) and nickel (Ni) are considered as serious environmental pollutants. Contamination of soil and water by Cr and Ni are of particular recent concern. In contrast to other toxic trace metals, such as Cd, Pb, Hg and Al, Cr has received little attention. The impact of Cr contamination on the physiology of plants depends on the metallic species responsible for its mobilization, uptake and toxicity in the plant system [7]. Cr toxicity in plants is observed at multiple levels, from reduced yield, to effects on leaf and root growth, through to the inhibition of enzymatic activities and mutagenesis [8]. Chromium is found throughout the environment, including air, water and soil. Detailed reviews on the critical assessment of Cr in the environment published Kotas and Stasicka [9]. While Cr is not considered an essential element for plant nutrition [10], Ni is classified an essential trace element [11]; and although it is found everywhere in the environment, it usually occurs only in trace amounts. It is required for the enzymatic break down of urea by urease and the liberation of nitrogen into a usable form for plants, and also for iron absorption. Seeds need it in order to germinate. If nickel is deficient, plants may fail to produce viable seeds. However, nickel is considered as phytotoxic at a level of 100 mg $\cdot$ dm^{-3} or higher.

This article describes the phytotoxicity of wastewaters from washing reservoirs of a cutlery production line to terrestrial plant seedlings of mustard (*Sinapis alba* L.). The following parameters: fresh and dry mass production, water content, photosynthetic pigments content and metal accumulation were studied. All contamination of tested washing wastewaters came from heavy metals (Ni and Cr) and non-polar extractable compounds (NEC). Because tested wastewaters were previously classified without verification as dangerous and were sunk as hazardous liquid wastes, requirement to analyze justness of such classification under the new legislative for waste management (Waste Law No. 223/2001, Slovak Republic) was appeared.

Materials and methods

Mustard (*Sinapis alba* L.) seeds were germinated in Petri dishes with a 17 cm diameter and filter paper and plastic net underneath. Washing wastewaters were used in ten varying concentrations (from 1 to 250 cm^3) and tap water (80 mg Ca $\cdot$ dm^{-3}, 27 mg Mg $\cdot$ dm^{-3}; pH = 7.3 ± 0.05) was used for their dilution. In each Petri dish 50 healthy looking seeds of similar size were spread on plastic net and flushed with 50 cm^3 of tested wastewaters. Normal tap water was used as the control. The covered Petri dishes were placed in a dark thermostat (t = 25°C; air humidity 80%). After 72 h, Petri dishes with germinated seeds were transferred from the thermostat into the laboratory box with a day-light cycle and a constant temperature 23 ± 1°C. The dishes were shielded from direct sunlight and cultivation lasted for the next 7 days. The shoots were not in direct contact with wastewater solutions. After 10 days growth (3 + 7) the plants were divided into roots and shoots and the fresh mass was immediately weighed. The plant material was then dried in a drying-chamber (t = 80°C) to a constant weight. The water content of the plants was determined on the base of fresh and dry mass under Drazic and Mihailovic [12] equation:

$$WC = (FM - DM)/DM$$

where: WC - water content; FM - fresh mass, DM - dry mass; in $g \cdot g^{-1}$ dry mass of seedling part.

Pigment content of chlorophyll a, b and total carotenoids were determined in 1 g of fresh shoot mass after their extraction in 96% ethanol and they were spectrophotometrically measured at 665, 649 and 470 nm wavelength. The pigment amount was calculated using the following equations [13]:

$$\text{Chl } \underline{a} = 13.95\ (A_{665}) - 6.88\ (A_{649})$$

$$\text{Chl } \underline{b} = 24.96\ (A_{649}) - 7.32\ (A_{665})$$

$$\text{Car} = [1000\ (A_{470}) - 2.05\ (\text{Chl } \underline{a}) - 114.8\ (\text{Chl b})]/245$$

where: Chl a - chlorophyll a, Chl b - chlorophyll b, Car - carotenoids; in $\mu g \cdot mg^{-1}$ DM, DM - dry mass.

For determination of metal accumulation plant samples (including control plants) were taken after 10 days (3+7) of exposure. The seedlings were removed from tested wastewaters and washed with acidified deionized water (at pH 4 with HCl) and then with deionized water to remove excess metals at the surface of plants. Different plant parts (roots, shoots) were separated manually and dried at 80°C for 24 h. Dried shoot and root tissues were ground to a fine powder using a porcelain mortar. Then, aliquots (30 mg of roots; 350 mg of shoots) were placed in separate glass tube, and 2 cm^3 of concentrated HNO_3/H_2O_2 (4/1, v/v) was added to each tube. After 24 h till 3 cm^3 of HNO_3/H_2O_2 was added in the tube and each tube was separately heated in sealed teflon container at 160°C for 2 h in oven. After cooling, the digested solution was filtered through Whatman (no. 1) filter and the filtrate was diluted to 25 cm^3 with deionized water. This final solution was analyzed for Ni and Cr concentration. A blank tube with no dry plant matter added was HNO_3/H_2O_2 (4/1, v/v) treated in a similar fashion. The total concentration of Cr and Ni in the extracts of both plant parts were analyzed by ET-AAS (Cr) and F-AAS (Ni) (AAS; Varian, spectr. AA, Australia, GTA 110, with Zeeman 220 background correction). The instrument was zeroed with 1% HNO_3 blanks. High level fortified standard for trace elements (NWRI Canada) was used as a certified stock solution. Cr and Ni solutions were prepared from 1 $g \cdot dm^{-3}$ stock solutions (MERCK, Darmstadt, Germany). All the concentrations were reported on a dry mass basis for both plant tissues. The concentrations reported are mean values from triplicate analyses of each sample after reduction of control.

The tested samples comprised three different wastewaters from washing reservoirs from a cutlery production line mainly polluted by heavy metals (Cr and Ni), non-polar extractable compounds (NEC; residues of oils and waxes from polishing of stainless steel cutlery) and detergents (used for cutlery degreasing). Heavy metal content in wastewaters is required to be liquidated as hazardous liquid waste. The total metal and non-extractable organic compounds (NEC) contents are shown in Table 1.

Table 1

Composition of tested washing wastewaters from a cutlery production line

Sample	Cr [$mg \cdot dm^{-3}$]	Ni [$mg \cdot dm^{-3}$]	NEC[1] [$mg \cdot dm^{-3}$]
R1	41.6	50.2	1.78
R2	18.8	6.52	2.24
R3	0.3	0.26	6.49

[1] NEC - non-polar extractable compounds

The first two reservoirs (R1, R2) collected wastewaters from degreasing baths, where the cutleries are degreased from residual oils and furniture creams (POLO TITAN produced by TRIUMPH Partizánske, Slovakia - mixture of tensides, alkali and water), while the third reservoir (R3) collected waters from the cutlery washing pool. Samples consistence was liquid with black clusters, grey colored, inodorous. Analytical results for combined sample from all three reservoirs (volume 20 dm^3) and selected parameters are shown in Table 2. Because most dangerous contaminants in these waters were Cr and Ni the adverse effects were related to these heavy metals.

Table 2

Mean values [$mg \cdot dm^{-3}$] of selected parameters in combined sample of washing waste-waters from a cutlery production line (mixture from three reservoirs; volume 20 dm^3)

Parameter	Content [$mg \cdot dm^{-3}$]	Parameter	Content [$mg \cdot dm^{-3}$]
pH	7.84	Fe	32.18
NEC[1]	3.38	NH_4^+	2.16
DOC[2]	4500	NO_3^-	1.4
COD_{Cr}[3]	18 204	NO_2^-	0.21
Cr_{total}	18.7	PO_4^{3-}	0.89
Ni	19.1	Anionactive tensides[4]	1 087

[1] NEC - non-polar extractable compounds; [2] DOC - dissolved organic carbon, [3] COD - chemical oxygen demand - oxidability with $K_2Cr_2O_7$; [4] anionactive tensides re-count to sodium dodecylsulfonate

Herein, the estimation of harmful compounds satisfied the demands of Supplement No. 14 Regulation of Ministry of the Environment, Slovak Republic No 283/2001 "Threshold indicator" values for non hazardous waterwaste leachate". It indicates that all determined parameters in the combined sample of washing wastewaters from a cutlery production line, except DOC and COD_{Cr} and anionactive tensides, are lower than those introduced in Supplement No. 14, and they fall within the required limits.

All phytotoxicity tests were carried out in triplicate and included a control. Quality control data were considered acceptable to control charts and other established criteria. ADSTAT 2.0 software was used for statistical evaluation. A T-test was used to assess the significant difference between control and other treatments ($P \leq 0.05$). Data was expressed as the average ± standard deviation (SD).

An analysis of regression between metal tissues concentrations and the dry weights of plants (shoots and roots) was also carried out. Analytical precision of the method was improved by including triplicate samples. Reproducibility was within ± 5%. Analytical data quality of Cr and Ni were ensured through repeated analysis (n = 3) of EPA quality control samples in water and the results were found to be within ± 3.15% of certified value. For plants, recoveries of metal from the plant tissues were found to be 99% as determined by digesting four samples each from untreated plant with known amount of metal. The blanks were run all the time.

Results and discussion

The main prerequisite for a higher yield in plants is an increase in biomass production in terms of dry mass. Tested washing wastewaters influenced the dry and fresh mass production of both parts of the *S. alba* seedlings (Fig. 1). With increasing wastewater concentration, a reduction in root dry mass was observed for all washing-waters, whereas the dry mass of shoots in the majority of applied concentrations was either not affected, or it increased.

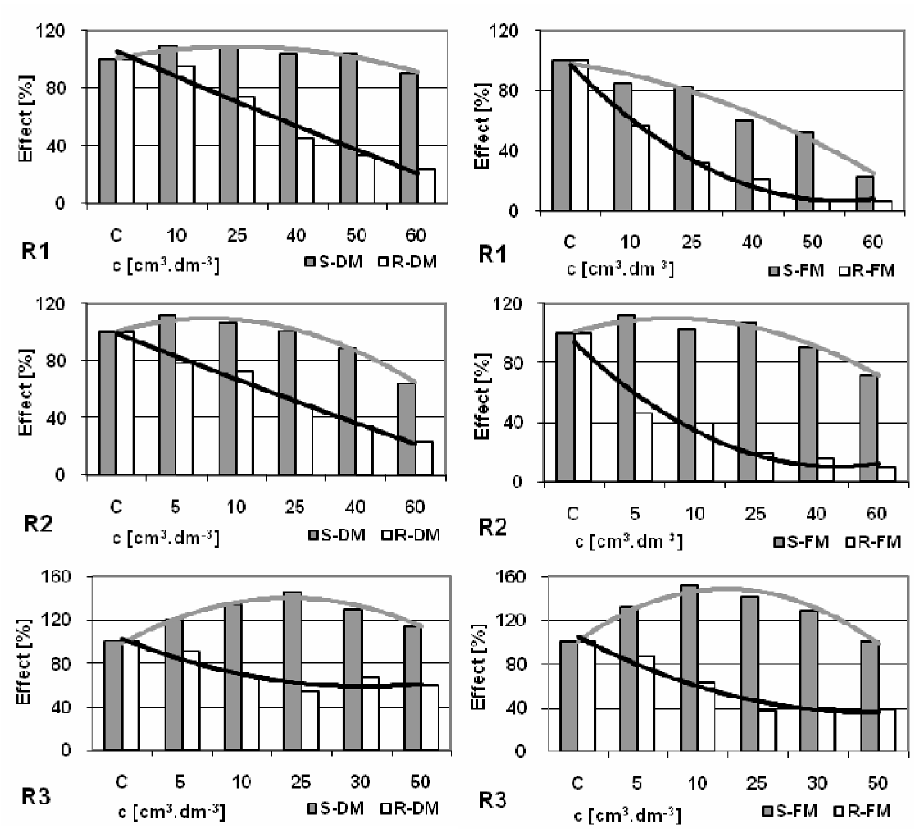

Fig. 1. Dry mass (DM) and fresh mass (FM) production [%] and their polynomic trend lines after 10 days growth in the presence of tested washing wastewaters (S - shoot; R - root; C - control) (mean of 3 determinations and a standard deviation of 6% or less)

The washing water from reservoir R3 showed the strongest stimulatory effect mainly on shoot growth - 140% more than that on the control, even in a concentration of 25 $cm^3 \cdot dm^{-3}$. A reduction in shoot dry mass was determined only for wastewater from the R1 and R2 reservoirs in concentrations of higher than 60 and 40 $cm^3 \cdot dm^{-3}$, respectively (Fig. 1. DM). The effect of tested washing waters was stronger on fresh mass than on dry mass production (Fig. l. FM). This indicates problems in water reception and translocation. Root fresh mass production was again observed to be reduced more strongly than that in shoots. Only washing water from the R1 reservoir seriously reduced the production of shoot fresh mass. The weakest inhibitory effect on both, roots and shoots, was observed in washing water from the R3 reservoir.

The effects of metals on plant processes during its early growth and development culminates in the reduction in yield and total dry mass. This is a consequence of poor production, translocation and apportioning of assimilates to the economic parts of the plant. The negative effect on the yield and dry mass of plants is essentially a general indirect effect caused by heavy-metal stress, and particularly by the presence of Cr [7, 14]. The overall adverse effect of many metals such as Cr and Ni, on the growth and development of plants may be a serious impairment of the uptake of mineral nutrients and water which leads to a deficiency in the shoot [15]. When comparison of washing wastewaters from a cutlery production line effect on biomass production was done to Cr and Ni effects on plants these results agree with those introduced by many authors [8]. Results conformable with ours for washing wastewaters reported for the cabbage (*Brassica oleacera* L.) Pandey and Sharma [14], where the root dry mass was diminished by a nickel excess, and Bennicelli et al. [7] for the water fern *Azolla caroliniana* with its biomass reduction in the presence of chromium. It was found that dry matter production in *Vallisneria spiralis* L. was severely affected by Cr(VI) concentrations above 2.5 $\mu g \cdot cm^{-3}$ in a nutrient medium [16] and that indicates at least a 10 times higher value than that for the adverse effects of washing wastewaters on *S. alba* dry mass production. Supporting the experiments with young seedling herein, a distinct reduction in dry biomass was also reported by Hanus and Tomas [17] for the flowering stage of *S. alba* when Cr(VI) was introduced at rates of 200 or 400 $mg \cdot kg^{-1}$ soil along with N, P, K and S fertilizers.

The overall adverse effect of Cr and Ni on the growth and development of plants may be serious impairment of the uptake of mineral nutrients and water, which leads to deficiency in the shoots. Wilting of various crops and plant species due to Cr toxicity has been reported [18], but little information is available on the exact effect of Cr and Ni on water relationships in higher plants. Water content was reduced very rapidly in comparison with that in control seedlings, and this occurred mainly in the roots where water content varied with tested water concentrations (Fig. 2). Water content in the shoots was not significantly reduced in the presence of tested wastewaters. It can be concluded that tested washing wastewaters with Cr and Ni inhibited water absorption by the root, but not water translocation into the upper seedlings parts. These results agree with the Chatterjee and Chatterjee [19] conclusion, that excess Cr decreases the water potential and transpiration rates and increases diffusive resistance and relative water content in leaves of cauliflowers. However, Barcelo et al. [20] observed a decrease in leaf water potential in a Cr treated bean plant. Decreased turgor and plasmolysis was also observed in epidermal and cortical cells of bush bean plants exposed to Cr, because toxic levels of Cr decreased tracheary vessel diameter, thereby reducing longitudinal water movement [21].

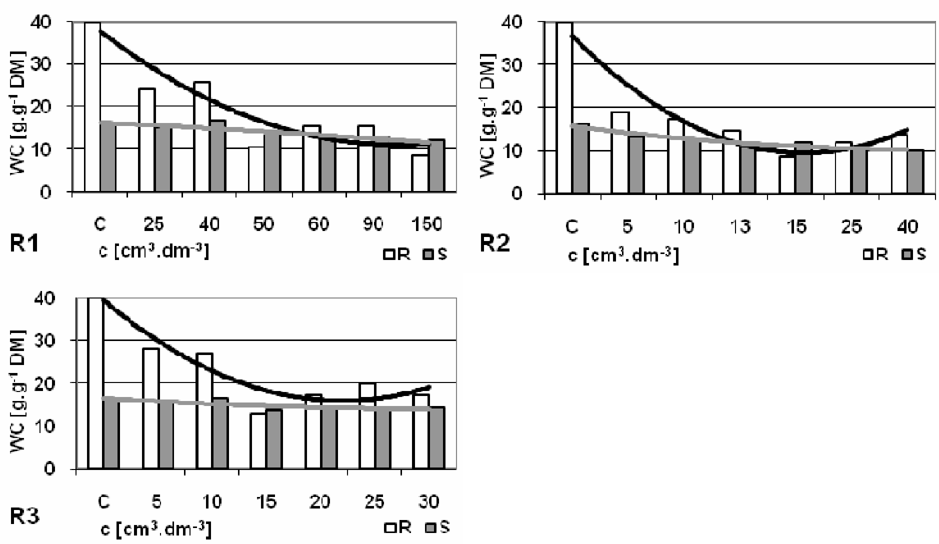

Fig. 2. Water content [g · g^{-1} DM] in roots and shoots of *S. alba* seedlings and their polynomic trend lines after 10 days growth in the presence of tested washing wastewaters from cutlery production line (S - shoot; R - root; C - control)

Table 3

Photosynthetic pigments content [μg · mg^{-1} DM] in *S. alba* shoots for various concentrations of wastewaters from cutlery production line after 10 days growth)

R1				R2				R3			
c [cm^3 · dm^{-3}]	Chl a	Chl b	Car	C [cm^3 · dm^{-3}]	Chl a	Chl b	Car	c [cm^3 · dm^{-3}]	Chl a	Chl b	Car
C[1]	3.20	1.19	0.60	C[1]	3.20	1.19	0.60	C[1]	3.20	1.19	0.60
5	3.00	0.99	0.67	5	3.10	1.15	0.60	10	3.26	1.15	0.59
10	2.98	1.13	0.73	10	3.00	1.12	0.75	25	3.17	1.17	0.60
15	2.14	0.73	0.72	15	2.88	1.04	0.78	40	3.04	1.20	0.68
25	1.86	0.65	0.66	25	2.75	0.92	0.80	50	2.72	1.09	0.69
40	1.38	0.60	0.52	30	2.46	0.80	0.77	60	1.95	1.04	0.71
60	1.50	0.48	0.44	50	1.63	0.68	0.59	250	1.47	0.84	0.61

[1] C - control; DM - dry mass; Chl a - chlorophyll a; Chl b - chlorophyll b; Car - carotenoids

The photosynthetic pigments' levels in *S. alba* seedling shoots for various concentrations of tested wastewaters from washing reservoirs of cutlery line production are introduced in Table 3 and as percentage of control in Figure 3. The strongest inhibitory effect on all photosynthetic pigments production had waste-water from degreasing baths (reservoir R1) and the weakest from washing pool (reservoir R3). The rank order of inhibition could be arranged as follows: R1 > R2 > R3. Base on statistical evaluation as well as from polynomic trends lines it is evident that no significant differences of Chl a and Chl b contents were determined for R1 and R2 reservoirs. For R3 reservoir were significant differences for above-mentioned pigments observed only as the washing wastewater concentration overreach 40 $cm^3 \cdot dm^{-3}$.

Introduced results are partly in agreement with conclusions from experiments with fly [22] and tannery wastes [23, 24] with high metal content, including Cr and Ni, when plants could tolerate elevated metals levels. Herein tested washing wastewaters used in lower concentrations have any or very low adverse effects on chlorophylls production. However, increasing concentration of washing waters decreased chlorophylls content analogous to higher concentrations of sludge and tannery wastes [23]. The decrease in the concentration of Chl a and Chl b also introduced Vazquez et al. [21] and Chatterjee and Chatterjee [19] after exposure to metal pollutants. The decrease in chlorophyll concentration may be the result of an inhibited photosynthetic electron transport [25] and decomposition of the chloroplast membrane with metal excess [26]. The adverse effects of washing wastewaters from cutlery production line with heavy metals in excess tested on *S. alba* seedlings may be due by interference of these metals in the formation of chlorophyll either through the direct inhibition of an enzymatic step or through the induced Fe deficiency as introduced Van Assche and Clijsters [27] in cauliflower. Identical to Brown et al. [11] conclusions stunting of growth and changes in photosynthetic pigments production, as two major symptoms of Ni toxicity in plants, were also confirmed in our study.

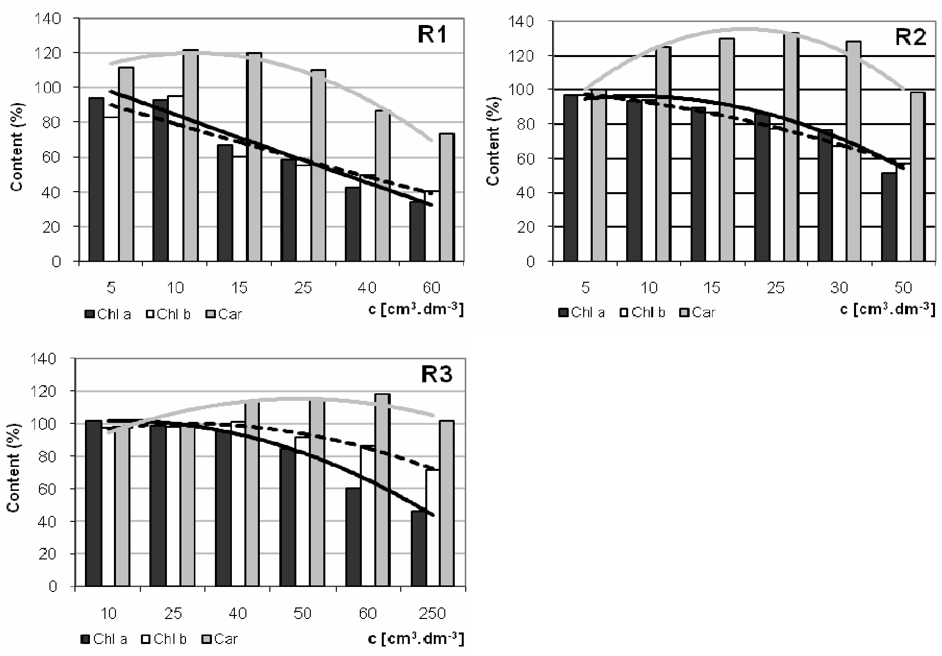

Fig. 3. Photosynthetic pigments production [%] and their polynomic trend lines after 10 days growth in the presence of tested washing wastewaters (Chl a - chlorophyll a; Chl b - chlorophyll b; Car - carotenoids) (pigments content in control is consider as 100%; mean of 3 determinations and a standard deviation of 6% or less)

As opposed to chlorophylls, carotenoid content increased in the presence of tested washing wastewaters and their concentration was at the same level or exceeded that of the control (Fig. 3). The strongest stimulation of carotenoid production was observed in the presence of water from reservoir R2 where carotenoid content in wastewater with concentrations of 15 and 25 $cm^3 \cdot dm^{-3}$ reached 130 and 133%, respectively. Carotenoids, which are non-enzymatic antioxidants, are a part of photosynthetic pigments. They play an important role in the protection of chlorophyll pigments under stress conditions. An increase in carotenoid content is considered to be a plant defense strategy in the reduction of metal stress [23]. Siddaramaiah et al. [28] reported that increased carotenoid content was observed in heavy-metal rich industrial effluent exposed *Capsicum annum.* Results here agreed with their findings. Similar to other heavy metals, high concentrations of Cr can induce a degradation of carotenoids in plants [29]. However, an increase in carotenoids under Cr treatment, comparable with that in this study, was reported for *Vallisneria spiralis* and other aquatic plants [16]. This increase in carotenoid content may act as an antioxidant to scavenge ROS (*Reactive Oxygen Species*) generated as a result of Cr and Ni toxicity.

For intact and fully functional green tissues the typical pigment ratios are more conclusive than the individual pigment values. Retarded or blocked greening (chlorophyll formation) leads to higher a/b ratios. Stress and senescence show a chlorophyll decline and they usually produce either normal values for Chl a/b of around 3 or much lower values as chlorophyll breakdown finishes. During continuous stress, such as that caused by heavy metal exposure, the weight ratio of chlorophylls to carotenoids Chl(a+b)/Car usually shows lower values in region of 4 or 3.5, and they can be even lower when the chlorophyll Chl(a+b) content declines. Resulting pigment ratios are presented in Figure 4. From this figure it is evident that in almost all cases Chl a/b ratio was the same as in the control and this indicates no significant differences in the reduction of both chlorophylls. Total chlorophyll content Chl(a+b) from washing wastewaters from the R1 reservoir was reduced linearly with increased concentrations; while in the water samples from the R2 and R3 reservoirs a reduction was observed only when the concentration reached 25 and 50 $cm^3 \cdot dm^{-3}$, respectively. Based on the observed results it can be concluded that in the presence of R1 and R2 washing wastewaters there was conformation of mainly a stress reaction on chlorophylls. The Chl a/b ratio was slightly increased in comparison with that of the control. Samples from the R3 reservoir indicated that no stress occurred until a concentration of 50 $cm^3 \cdot dm^{-3}$ was reached; while in higher concentrations chlorophylls breakdown was observed (the Chl a/b ration fell under 2). The ratio Chl(a+b)/Car for all tested samples was lower than that for the control. This indicates a stronger reduction in chlorophyll production compared with that of carotenoids. One exception was observed only in low concentrations of water (10 and 25 $cm^3 \cdot dm^{-3}$) from the R3 reservoir when the Chl(a+b)/Car ratio was nearly the same as that for the control. The same situation was confirmed when a sample of wastewater from the R2 reservoir was applied in a concentration of 5 $cm^3 \cdot dm^{-3}$. In these cases the Chl(a+b)/Car pigment ratio exhibited a value of about 7.3 which is normal for fully green plant tissue growing in poor light conditions, such as under shady-leafed trees conditions.

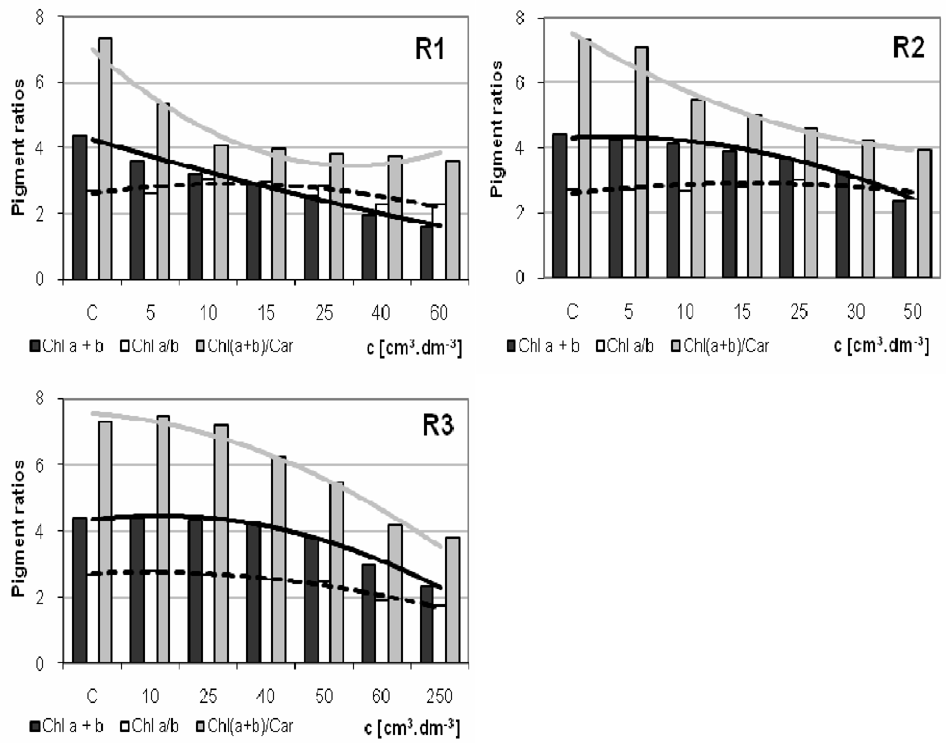

Fig. 4. Photosynthetic pigment ratios in *Sinapis alba* seedlings shoots and their polynomic trend lines after 10 days growth in the presence of tested washing wastewaters (Chl a - chlorophyll a; Chl b - chlorophyll b; Car - carotenoids)

Prasad [30] determined that heavy metals usually decreased the total Chl and Chl a/b ratio in higher plants. The decrease in the Chl a/b ratio by Cr indicates that Cr toxicity possibly reduces the size of the peripheral part of the antenna complex [31]. The decrease in Chl b in presence of Cr may be due to the destabilization and degradation of proteins in the periphery. The deactivation of enzymes involved in the chlorophyll biosynthetic pathway could also contribute to the general reduction in chlorophyll content in most plants which are under Cr stress. Carotenoids were generally less affected by heavy metals, resulting in lower Chl(a+b)/Car ratio in higher plant and this agrees with results obtained during this testing of washing wastewaters on *S. alba* seedlings. Krupa et al. [32] investigated the relative changes in the content of Chl(a+b) and total carotenoids in the first leaves of rye seedlings treated with Cd, Pb, Ni and Zn, and they concluded that the determination of Chl and total carotenoids appears to be a reliable marker of heavy metal toxicity in higher plants. As described Vajpayee et al. [16] the substitution *in vivo* of the central atom of Chl magnesium by heavy metals (Hg, Cd, Cu, Ni, Zn, Pb, Cr) is an important mechanism in the control of damage caused in metal stressed plants. This substitution prevents photosynthetic light-harvesting in the affected chlorophyll molecules, resulting in the break-down of photosynthesis. However, the extent of thus damage varies with light intensity. In low-intensity light irradiation all the central atoms of Chl are accessible to heavy metals, and heavy metal-chlorophylls are formed. Some of these are much more stable in irradiation than Mg-Chl. Consequently, plants remain green even when they are dead. In high intensity light production, almost all Chl decays, showing that under such conditions most of the Chls are inaccessible to heavy metal ions. The experimental results for photosynthetic pigment production herein fully agree with those of Chandra and Garg [33], who worked with *Limnanthemum cristatum* Griseb. In both cases, chromium caused a slight reduction in chlorophyll and almost no change in carotenoids.

The results from the uptake of Cr and Ni from washing wastewaters from cutlery production line in the roots and shoots of *S. alba* seedlings are introduced in Table 4. While the accumulation of Cr was higher in the roots, Ni was distributed equally through the whole plant seedlings. However, Prasad [30] observed that Cr is accumulated rather in the shoots (stems and leaves) than in the roots and rhizomes of plants, our results are in agreement with those introduced by Carry et al. [34] who observed chromium accumulation mainly in the roots. Ni accumulates uniformly in roots and shoots [30] and this is in good agreement with our results. Cr was from tested washing waters accumulated in both plant parts in lower amount than Ni, and its percentage uptake range from 6.8 to 8.7 and 1 to 2.5% for roots and shoots, respectively. Cr percentage uptake in the roots and shoots was in average about 1.7 and 7.3 times, respectively, lower than that of Ni. Ni percentage uptake from washing waters in the roots to shoots was nearly equal and range from 10.2 to 15.8%.

Table 4

Uptake of nickel and chromium from washing wastewaters after 10 days growth into the roots and shoots of *Sinapis alba* seedlings

Roots						
Reservoir	**Initial conc. in the wastewater [mg · dm^{-3}]**		**Cr**		**Ni**	
	Cr	**Ni**	**Tissue conc. [mg · g^{-1} DM]**	**% uptake**	**Tissue conc. [mg · g^{-1} DM]**	**% uptake**
R1	0.376	0.377	0.0258	6.8	0.0595	15.8
R2	0.312	0.130	0.0213	6.9	0.0148	11.4
R3	0.15	0.013	0.0130	8.7	0.0013	10.2
Shoots						
Reservoir	**Initial conc. in the wastewater [mg · dm^{-3}]**		**Cr**		**Ni**	
	Cr	**Ni**	**Tissue conc. [mg · g^{-1} DM]**	**% uptake**	**Tissue conc. [mg · g^{-1} DM]**	**% uptake**
R1	0.520	0.628	0.0051	0.98	0.0873	13.9
R2	0.282	0.098	0.0041	1.45	0.0103	10.5
R3	0.15	0.013	0.0038	2.5	0.0014	10.4

DM - dry mass; all the values are means of triplicates after reduction of control; standard deviation 6% or less

Singh et al. [23] described differences in the metal accumulation in the different parts of plants and suggested on different cellular mechanism of bioaccumulation and translocation of metals. The high accumulation of metals (Cr, Fe, Zn and Mn) particularly in the root tissues of *H. annuus* may be due to complexation of metals with the sulfhydryl groups resulting into less translocation of metals to upper part of the plant, which vary from one metal to another. Similar conclusions can be done from our study of Cr accumulation from washing wastewaters to roots and shoots of *S. alba* seedlings. Cr translocation from roots to shoots of *S. alba* was in our study minimum as well as that introduced for cauliflower by Chatterjee and Chatterjee [19]. It appears that translocation of Cr from roots to tops was inhibited in the presence of toxic levels of the metal. As suggested earlier [35] both Cr(VI) and Cr(III) salts hinder the translocation of the element from roots to tops of cauliflower. Cr mainly moved in the xylem of the plants [36] and the maximum quantity of element contaminant was always contained in roots and a minimum in the vegetative and reproductive organs [37, 38]. These results correspond with Cr accumulation in the roots and shoots of *S. alba* seedlings herein. The reason of the high accumulation in roots of the plants could be that Cr is immobilized in the vacuoles of the root cells, thus rendering it less toxic, which may be a natural toxicity response of the plant [39].

However, Ni is in very low concentrations micronutrient for plants [11] toxic amounts of this element can occur in many environments. Plants containing more than 100 mg · dm^{-3} Ni develop symptoms of toxicity. The resistance to this metal and its translocation through plant depends on plant species. While some plants are introduced as Ni hyperaccumulators other are very sensitive and introduced as non-accumulators [40]. In the cytoplasm, high levels of free nickel generally avoid removal of the metal ions to the vacuoles and the formation of complexes with organic acids [41]. If the nickel level remains high, it inevitably binds organic macromolecules and denatures them. Furthermore, nickel can replace iron, zinc and magnesium due to the chemical affinity with those elements, interfering with their metabolism [42]. Ni is transported to underground plant parts by the oxygen atoms either as metal complexes of organic acids or as hydrated cations [43]. While Barman et al. [44] introduced its higher accumulation in the roots of *Cyperus difformis* L. and *Chenopodium ambrosiodes* L., Pandey and Sharma [14] observed in *Brassica oleracea* L. plants higher nickel accumulation in shoots. This statement confirmed results obtained during accumulation tests with washing wastewater herein. In previous study with Ni accumulation in *S. alba* roots and shoots Fargašová [45] and Fargašová and Beinrohr [46] confirmed higher Ni accumulation in the shoots than in the roots when Ni concentration in the shoots was about twice as high as that in roots. This was not confirmed during our study when Ni accumulation in the shoots was equal to that in the roots and no significant differences were confirmed in accumulated metal amounts from medium.

Conclusions

It is concluded from present study that washing wastewaters from cutlery production line are quite toxic to plants and they reduced biomass and photosynthetic pigment production and influence water and metal translocation through the plant. Because of this study high toxicity of the presented wastewaters from the metal surface finishing was confirmed and justness of their liquidation as hazardous wastes by legally assigned persons was confirmed.

Acknowledgement

This study was supported by grant VEGA 1/4361/07.

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FITOTOKSYCZNOŚĆ CHROMU I NIKLU

Streszczenie: W warunkach laboratoryjnych badano zawartość wody, produkcję pigmentów fotosyntetyzujących oraz akumulację metali w suchej i świeżej biomasie korzeni i pędów gorczycy (*Sinapis alba* L.). Rozpatrywano wpływ trzech rodzajów wód opadowych (o dużym stężeniu Cr i Ni), pochodzących z linii produkcyjnej sztućców. Ścieki zmniejszały suchą masę korzeni, natomiast sucha masa pędów albo pozostawała niezmieniona, albo wzrastała. Wpływ ścieków był większy w przypadku świeżej masy niż suchej. Wskazuje to na trudności z przyswajaniem i transportem wody. Wpływ wód odpadowych na pigmenty fotosyntetyzujące był niewielki, nastąpił jednak pewien wzrost ich wytwarzania. Prawie wszystkie stosunki Chl a/b były takie same jak dla kontroli, co wskazuje na brak statystycznie istotnych różnic w redukcji chlorofilu a lub b. W przeciwieństwie do chlorofili zawartość karotenoidów w obecności wód odpadowych rosła, przekraczając ich zawartości w kontroli lub ich stężenie nie ulegało zmianom. Stosunek Chl(a + b) / Car był mniejszy niż zawartość w próbkach kontrolnych dla prawie wszystkich badanych próbek, co potwierdza większe zmniejszenie zawartości chlorofili niż karotenoidów. Nagromadzenie Cr było większe w korzeniach, a Ni był równo rozłożony w sadzonkach roślin. Pobieranie Cr w korzeniach i pędach było średnio 1,7 i 7,3 razy większe niż, odpowiednio, Ni. Pobieranie Ni z wód odpadowych przez korzenie i pędy było niemal równe i wahało się w zakresie od 10,2 do 15,8%. Negatywne wpływy wód odpadowych, pochodzących z linii produkcyjnej sztućców, czyni niemożliwym bezpośrednie ich wylewanie do gleby.

Słowa kluczowe: fitotoksyczność, oczyszczanie ścieków z produkcji sztućców, chrom, nikiel, gorczyca *Sinapis alba* L.