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PHYSIOLOGICAL RESPONSE OF TWO *Brassica napus* L. CULTIVARS TO NICKEL TREATMENT

WPŁYW NIKLU NA REAKCJE FIZJOLOGICZNE DWÓCH ODMIAN *Brassica napus* L.

Abstract: Adverse effect of nickel on hydroponically cultivated plants of two *Brasssica napus* L. cultivars (Verona and Viking) was investigated. Dry mass of shoots and roots as well as some biochemical characteristics (concentration of photosynthetic pigments, TBARS and proteins) of plant leaves were determined. In addition, the content of nickel in plant organs was estimated. Visible symptoms of Ni toxicity were notable already at the lowest applied concentration (6 μmol · dm^{-3}). Higher applied Ni concentrations (24, 60 and 120 μmol · dm^{-3}) resulted in moderate to strong toxic effects on plants of both studied cultivars. After application of 6 and 12 μmol · dm^{-3} Ni shoot dry mass of cv. Viking was substantial lower than that of cv. Verona. Decrease of root dry mass after treatment with 6, 12 and 120 μmol · dm^{-3} Ni was similar for both cultivars. Strong decrease in content of photosynthetic pigments was observed after application of 120 μmol · dm^{-3}. Comparing to the control, the content of these pigments in leaves of plants dropped under 50% (both cultivars). The highest applied Ni concentration 120 μmol · dm^{-3} caused that protein content in leaves dropped by 39% (cv. Verona) and 37% (cv. Viking) comparing to the control plants. After application of 120 μmol · dm^{-3} Ni the content of malondialdehyde in leaves was 2.64- (Viking) and 2.31- (Verona) times higher than that of control. Nickel amounts accumulated in roots of plants were higher than those in shoots. Accumulated Ni amounts in roots of cv. Verona plants were 1.3- (120 μmol · dm^{-3}) to 1.9- (6 μmol · dm^{-3}) times lower than those of cv. Viking plants, whereas metal amounts accumulated in shoots of cv. Verona plants were 1.2- (120 μmol · dm^{-3}) to 1.8- (6 μmol · dm^{-3}) times lower than those of cv. Viking plants.

Keywords: bioaccumulation, nickel, chlorophyll, protein, malondialdehyde, rapeseed

Brassica napus L. (rapeseed) is an important crop for edible oil production [1]. Recent efforts have focused on the use of rapeseed oil to produce bio-fuels and rapeseed is currently the third most important crop after soybean and corn for biodiesel production [2]. *B. napus* is known to be able to accumulate substantial amounts of metals, moreover, this plant is high in biomass and various genotypes are available. Rapeseed belongs to *Brassicaceae* family and it is known that this family includes several hyperaccumulator species [3].

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Nickel is twenty-second most abundant element of the earth's crust and it occurs in igneous rocks as a free metal, or together with iron. Ni is released into the environment mainly from anthropogenic activities, such as metal mining, smelting, industrial and municipal wastes and fertilizer applications [4]. Concentration of this metal in polluted soil may be 20- to 30-fold higher than the overall range (10-1000 mg · kg^{-1}) found in natural soil [5]. Nickel is essential for higher plants in low concentrations and it is up-taken by plants mainly through root system via passive diffusion (cation transport system) and active transport, using the magnesium ion transport system, or by high-affinity nickel transport proteins [6]. Similarly to other micronutrients nickel becomes toxic to plants when applied in excess. The common visible symptoms of Ni toxicity include growth inhibition, chlorosis, necrosis and wilting. Disturbance of mineral nutrition, photosynthesis, water relations, respiration as well as nitrogen metabolism have been reported for plants submitted to Ni stress [7-9]. Nowadays, attention of researchers has been focused on Ni-induced oxidative stress. It has been found, that despite relatively low redox potential, nickel may induce formation of reactive oxygen species (ROS), which are responsible for oxidation of macromolecules in plant tissues [8]. Impairment of membrane function, its decreased thickness and enhanced leakiness have been attributed to structural changes associated with modifications of membrane lipid composition and lipid peroxidation [10, 11]. Plants have developed several antioxidant mechanisms, both enzymatic and non-enzymatic, to prevent the damage caused by the overproduction of ROS. Concerning non-enzymatic mechanisms, the accumulation of soluble proline is recognized as having an important protective function against heavy metal stress, being reported to act as a radical scavenger or involved in metal chelation [12]. Glutathione, cysteine and ascorbic acid can also directly interact with and detoxify oxygen free radicals [13]. In addition, the lipid-soluble antioxidants carotenoids play a multitude of functions in plant metabolism including oxidative stress tolerance [14]. Enzymatic mechanisms include enzymes such as superoxid dismutase (SOD), catalase (CAT), peroxidase (POD) [15].

Aim of this paper is to investigate the adverse effects of Ni on plants of two *B. napus* cultivars Verona and Viking. Dry mass of shoots and roots as well as some biochemical characteristics (concentrations of photosynthetic pigments, TBARS and proteins) of plant leaves were determined. In addition, the content of nickel in plant organs was estimated.

Material and methods

Analytical reagent-grade chemicals purchased from Centralchem (Bratislava) were used for the preparation of all solutions. Freshly distilled water was used in all experiments. For Ni treatments $NiCl_2 \cdot 6H_2O$ was used. The seeds of *B. napus* (cv. Verona and cv. Viking) were purchased from Slovak Centrum of Agricultural Production, Research Institute of Plant Production in Piestany, Slovakia.

Seeds of rapeseed, cv. Verona and Viking were sown into the soil and after 7 days the seedlings were transferred into Hoagland hydroponic solution and cultivated 14 days at controlled conditions (photoperiod 16 h light/8 h dark; irradiation: 80 μmol · m^{-2} · s^{-1} PAR; mean air temperature: 25°C). Thereafter they were grown in Hoagland solution containing Ni (0, 6, 12, 24, 60 and 120 μmol · dm^{-3}). The response of plants to Ni treatment was evaluated 7 d after metal application. For each experiment six plants were used.

Concentrations of assimilation pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) in plant leaves were determined spectrophotometrically (Chl *a* at 663.2 nm, Chl *b* at 646.8 nm, and Cars at 470.0 nm) after extraction into 80% (v/v) acetone (Genesys 6, Thermo Scientific, USA) according to Lichtenthaler [16]. Concentration of malondialdehyde (MDA; main product of lipid peroxidation) was determined spectrophotometrically (Genesys 6, Thermo Scientific, USA) as a content of thiobarbituric acid reactive substances (TBARS) in rapeseed leaves according to method described in detail in [17]. Protein concentration in rapeseed leaves was determined spectrophotometrically (Genesys 6, Thermo Scientific, USA) according to Bradford [18] using Bradford reagent; the exact procedure is described in [17]. Dried plant samples were heated in concentrated HNO_3 in the oven at 160°C for 6 h, then diluted with redistilled water and metal contents were determined using the flame atomic absorption spectrometry method (AAS Perkin-Elmer Model 1100, at 232.0 nm with deuterium background correction). Standard reference Ni stock solution (1 g · dm^{-3}, Merck, Germany) and the certified standard reference materials NCS DC 73350 Poplar Leaves (China) and NCS DC 733 49 Bush Branches and Leaves (China) were used to quality assurance of the results. The detection limit for Ni was 30 μg · dm^{-3}. The precision of Ni determination (n = 3) expressed by relative standard deviation varied in the range from 1 to 3%.

The results were evaluated by the multifactorial ANOVA algorithm ($p \leq 0.05$) after verification of normality and homogeneity of the variance. The multiple comparisons of means were based on the method of Tukey contrast.

Results and discussion

For all experiments two cultivars of rapeseed (cv. Viking and cv. Verona) were used. Three weeks old plants were treated with different concentrations (6, 12, 24, 60 and 120 μmol · dm^{-3}) of Ni for seven days. After this time period production characteristics of plants as well as content of photosynthetic pigments, TBARS and proteins in leaves were evaluated. Moreover, Ni content in roots and shoots of plants was estimated.

Visible symptoms of Ni toxicity on plants of both cultivars were notable already at the lowest applied metal concentration (6 μmol · dm^{-3}), some leaves were mildly chlorotic. Application of higher nickel concentrations (24, 60 and 120 μmol · dm^{-3}) resulted in moderate to strong toxic effects on plants (both studied cultivars). After application of 24 μmol · dm^{-3} Ni leaves of plants were chlorotic to a great extent. Furthermore, in case of cv. Verona plants, tips of some leaves were wilted and roots brownish. Two highest applied concentrations of nickel (60 and 120 μmol · dm^{-3}) caused that leaves of both cultivars were strongly chlorotic, wilted and some even desiccated (only for 120 μmol · dm^{-3}). Roots were brownish, small and growth of these plants was stunt. Inhibitory effect of Ni on plant growth might be due to Ni-caused alternations of fundamental metabolic processes, *eg* photosynthesis and transport of photoassimilates from leaves [19]. It has been proposed that also H_2O_2 plays an important role in the inhibition of growth of heavy metal-stressed plants [6].

Figure 1 presents dependence of dry mass of plant organs of both studied cultivars on the applied Ni concentration. It is obvious that dry mass of plant organs decreased with increasing Ni concentration in external solution. Our results are consistent with earlier studies of various crops which have shown that higher levels of trace elements, including

Ni, cause a significant reduction in dry mass and essential metabolites [20-23]. Figure 1 also shows some considerable differences between studied cultivars. After application of concentrations 6 and 12 μmol·dm^{-3} shoot dry mass of cv. Viking was substantial lower than that of control. On the other hand, dry mass of shoots of cv. Verona plants treated with 12 μmol · dm^{-3} Ni was not significantly different from that of the control plants.

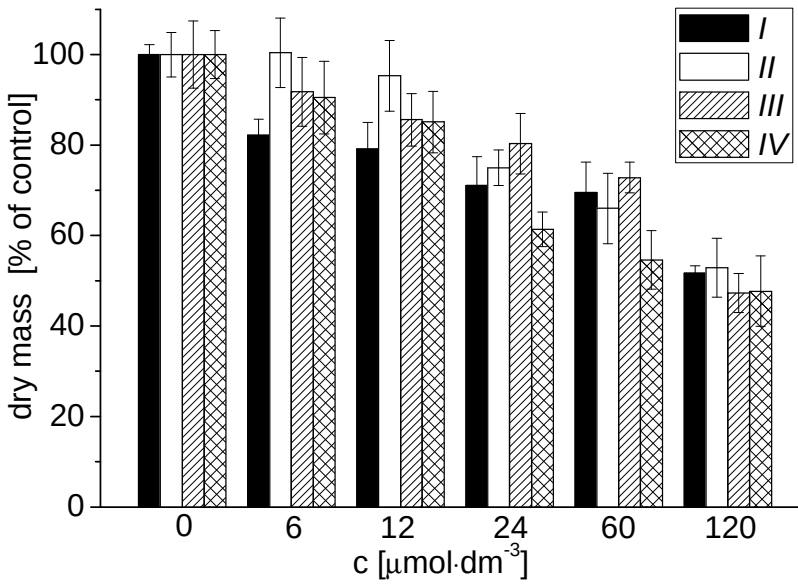

Fig. 1. Dependence of dry mass of plant organs of two *B. napus* cultivars on applied Ni concentrations. *I* - shoots, cv. Viking; *II* - shoots, cv. Verona; *III* - roots, cv. Viking; *IV* - roots, Verona

Application of 60 μmol · dm^{-3} Ni resulted in slight decrease of shoot dry mass of Verona in comparison to that of Viking. Decrease of root dry mass after application of 6, 12 and 120 μmol · dm^{-3} Ni was similar for both cultivars in comparison to the control. Nickel concentrations 24 and 60 μmol · dm^{-3} caused that roots dry mass of cv. Verona plants was significantly lower than that of Viking. Plant growth differences among rapeseed cultivars treated with various Ni concentrations were confirmed also in experiments of Ali et al [24]. Variability among cultivars to nickel stress may have been due to differences in accumulation or distribution of Ni in shoots and roots [25].

Concentration of chlorophyll *a* and *b* as well as concentration of carotenoids in leaves of plants of two studied *B. napus* cultivars are summarized in Table 1. Significant concentration decrease of all three photosynthetic pigments with increasing external nickel concentration was evident for both cultivars, although plants of Verona seem to be more sensitive to Ni treatment. After application of the lowest studied Ni concentration (6 μmol · dm^{-3}) the concentration of chlorophyll *a* and *b* as well as concentration of carotenoids in leaves of cv. Verona plants was about 80, 57 and 80% of that of control, respectively. In case of Viking it was as follows: 90, 81 and 87% of that of control, respectively. Strong decrease in concentration of pigments was observed after application of

120 μmol · dm^{-3} Ni. Comparing to the control, concentration of all photosynthetic pigments in leaves of plants dropped under 50% (both cultivars). Plants of cultivar Viking seem to be little less sensitive, especially in case of chlorophyll *b*, concentration of which dropped by about 60% comparing to the control, while in case of cv. Verona plants the decline was about 87% in comparison to the control. Concentration of chlorophyll *a* and of carotenoids was at the highest applied Ni concentration (120 μmol · dm^{-3}) similar for both cultivars. Several authors reported decreased chlorophyll concentration in the leaves of plants treated with Ni [26-28] suggesting that such chlorosis could result from both Fe and Mg deficiency and the inhibition of chlorophyll synthesis [27].

Table 1

Concentration of chlorophyll *a* and *b* as well as carotenoids in leaves of plants of two *B. napus* cultivars treated with different Ni concentrations. Mean ± S.E., *n* = 3. Data followed by different letters are significantly different at the 0.05 probability level; d.m. - dry mass, S.E. - standard error

cultivar	Ni conc. [μmol · dm^{-3}]	chl *a* conc. [g · kg^{-1} d.m.]	chl *b* conc. [g · kg^{-1} d.m.]	carot. conc. [g · kg^{-1} d.m.]
VIKING	0	15.3 ± 0.6a	4.7 ± 0.2a	4.5 ± 0.2a
	6	13.7 ± 0.4b	3.8 ± 0.2b	3.9 ± 0.1b
	12	11.6 ± 0.4c	3.1 ± 0.1c	3.5 ± 0.1b
	24	10.3 ± 0.4c	2.6 ± 0.2de	2.8 ± 0.2c
	60	8.8 ± 0.4d	2.2 ± 0.2ed	2.2 ± 0.2d
	120	5.4 ± 0.4e	1.9 ± 0.1d	1.6 ± 0.1e
VERONA	0	14.8 ±0.5a	4.6 ± 0.2a	4.0 ± 0.3a
	6	11.8 ± 0.5b	2.6 ± 0.2bc	3.2 ± 0.2b
	12	10.6 ± 0.7bc	2.8 ± 0.3ab	2.9 ± 0.2b
	24	9.7 ± 0.7c	3.0 ± 0.3ab	2.6 ± 0.2d
	60	7.3 ± 0.6d	0.9± 0.1d	1.7 ± 0.2c
	120	4.8 ± 0.5e	0.6 ± 0.1cd	1.3 ± 0.1c

Table 2 summarizes concentrations of proteins and TBARS in leaves of plants of studied rapeseed cultivars treated with Ni. Decrease of protein concentration in leaves with increasing Ni concentration in external solution was observed for both cultivars. These findings agree with earlier studies carried out with pea [29], *Cunonia macrophylla* [30] and sunflower [31]. The highest applied Ni concentration 120 μmol · dm^{-3} caused the decrease of protein concentration in leaves by 39% (cv. Verona) and 37% (cv. Viking) in comparison to the control plants. After application of 6 μmol · dm^{-3} Ni was the decrease only about 6% (cv. Verona) and 5% (cv. Viking). Plants of cultivar Verona appear to be more sensitive to the nickel stress, although the difference between cultivars in the decrease of protein levels is not remarkable. The decreasing protein concentration in leaves could be assign to ROS acting. Osman et al [32] showed that high levels of Ni can result in a delay in the protein biosynthesis essential for plant growth. In this study, nickel stress caused a 20% reduction of the amino acid pool in *Scenedesmus obliquus* and *Nitzschia perminuta*.

Concentration of malodialdehyde (MDA; main product of lipid peroxidation) in leaves was determined as a content of thiobarbituric acid reactive substances (TBARS). A rapid increase of TBARS in leaves with increasing Ni concentration in external solution was observed (comparing to the control). These results are in good accordance with previous studies with wheat [33] and rapeseed [28]. The difference between cultivars was evident at two highest applied metal concentrations (60 and 120 μmol · dm^{-3}) and the lipid

peroxidation was more pronounced in plants of cv. Viking. After application of 120 μmol · dm^{-3} Ni the concentration of TBARS in leaves was 2.64- (Viking) and 2.31-times (Verona) higher than that of control. After application of the lowest Ni concentration (6 μmol · dm^{-3}) the TBARS level in leaves was not significantly different (both cultivars) from the control. It has been reported that stimulation of lipoxygenase activity under stress conditions reflects higher lipolytic activity in membranes and oxidation of membrane-bound fatty acids by causing propagation of lipid peroxidation [34].

Table 2

Concentration of protein as well as concentration of TBARS in leaves of plants of two *B. napus* cultivars treated with different Ni concentrations. Mean ± S.E., n = 3. Data followed by different letters are significantly different at the 0.05 probability level; d.m. - dry mass, S.E. - standard error

Ni conc. [μmol · dm^{-3}]	**protein conc. [g · kg^{-1} d.m.]**		**TBARS conc. [mmol · kg^{-1} d.m.]**	
	VIKING	**VERONA**	**VIKING**	**VERONA**
0	79.9 ± 3.4a	73.5 ± 2.5a	1.42 ± 0.04a	1.35 ± 0.05a
6	76.1 ± 2.9ab	69.1 ± 2.8ab	1.45 ± 0.04a	1.37 ± 0.04a
12	71.6 ± 1.3bc	62.3 ± 2.2bc	2.01 ± 0.09b	1.78 ± 0.05b
24	67.0 ± 2.5cd	59.4 ± 3.1c	2.18 ± 0.10b	2.07 ± 0.05c
60	64.0 ± 1.5d	46.2 ± 1.5d	3.01 ± 0.10c	2.61 ± 0.04d
120	50.3 ± 2.2e	44.8 ± 2.4d	3.75 ± 0.16d	3.12 ± 0.06e

Bioaccumulation factors (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}]. Figure 2 presents dependence of BAF values related to Ni accumulation in roots (Fig. 2A) and shoots (Fig. 2B) of plants of two *B. napus* cultivars on applied Ni concentration. Higher BAF values estimated for shoots reflect more effective mobility of Ni in the plants. At two lowest applied Ni concentrations 6 and 12 μmol · dm^{-3} the root BAFs of cv. Viking were 1.6- and 1.9-times higher than those of cv. Verona. With further external Ni concentration increase of the differences were less pronounced. For shoot BAFs, the differences between cultivars were more notable in concentration range 6-24 μmol · dm^{-3}. BAF values of shoots of cv. Viking plants were 1.6- (24 μmol · dm^{-3}) to 2-times (6 μmol · dm^{-3}) times higher than those of cv. Verona.

In general, nickel amounts accumulated in roots of plants were higher than those of shoots, although concentrations of Ni translocated into shoots were considerably high. These findings are supported by previous experiments with wheat [33] and *Solanum nigrum* L. [35] plants. Dependence of accumulated amount of Ni in roots on the applied metal concentration showed quasi-parabolic course with gradual saturation of tissue by metal (both studied cultivars). In case of shoots (cv. Verona as well as cv. Viking), the linear increase of accumulated metal with increasing external Ni concentration was observed. Nevertheless, some differences between cultivars were obvious. In the studied metal concentration range 6 to 120 μmol · dm^{-3} the amount of nickel accumulated in roots ranged from 272 mg · kg^{-1} d.m. (6 μmol · dm^{-3}) to 2123 mg · kg^{-1} d.m. (120 μmol · dm^{-3}) for Viking and from 144 mg · kg^{-1} d.m. (6 μmol · dm^{-3}) to 1589 mg · kg^{-1} d.m. (120 μmol · dm^{-3}) for Verona. In case of shoots the accumulated Ni varied from 20 mg · kg^{-1} d.m. to 341 mg · kg^{-1} d.m. for Viking and from 11 mg · kg^{-1} d.m. to 284 mg · kg^{-1} d.m. for Verona.

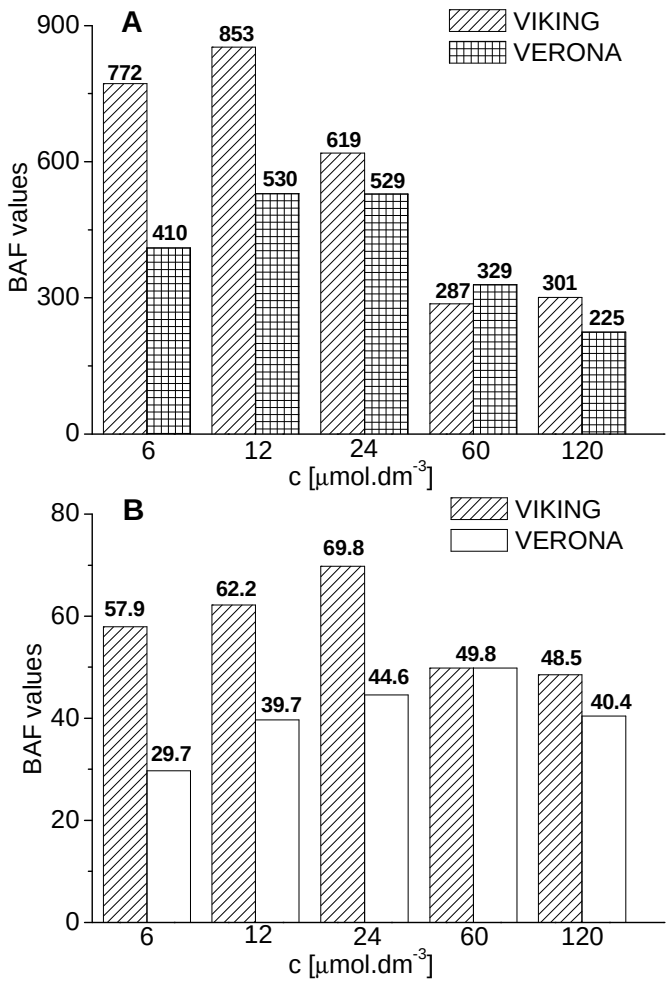

Fig. 2. Dependence of BAF values related to Ni accumulation in roots (A) and shoots (B) of plants of two *B. napus* cultivars on applied Ni concentration

Accumulated Ni amounts in roots of cv. Verona plants were 1.3- (120 µmol · dm^{-3}) to 1.9- (6 µmol · dm^{-3}) times lower than those of cv. Viking plants, whereas metal amounts accumulated in shoots of cv. Verona plants were 1.2- (120 µmol · dm^{-3}) to 1.8- (6 µmol · dm^{-3}) times lower than those of cv. Viking plants. Differences in accumulation of Ni in plant organs among six cultivars of *B. napus* were confirmed in experiments of Ali et al [24]. It was also suggested that cultivars having low shoot/root Ni ratio had better ability to retain Ni in the roots, possibly by binding and sequestering it in the vacuoles [36], which might have contributed to the tolerance to Ni. Interspecies differences in accumulation and translocation of Ni were confirmed by Bosiacki and Wojciechowska [37] in their experiments with ornamental plant species *Tagetes erecta* L., *Helianthus annus* L. and *Amaranthus caudatus* L.

Table 3

Translocation factor (TF) values as well as the portion from the total accumulated Ni amount by the plant occurring in the shoots of plants of two *B. napus* cultivars treated with $NiCl_2 \cdot 6\ H_2O$

Ni conc. [μmol · dm^{-3}]	*TF*		% of Ni in shoots	
	VIKING	VERONA	VIKING	VERONA
6	0.075	0.072	55.1	54.8
12	0.073	0.075	55.4	55.6
24	0.113	0.084	64.9	60.7
60	0.174	0.151	75.4	73.4
120	0.161	0.179	76.4	74.9

Translocation factors (*TF*) as well as the portion from the total accumulated metal amount by the plant occurring in the shoots are summarized in Table 3. The *TF* factors correspond to the ratio of accumulated Ni amount in shoots and roots. Calculated values of translocation factor were < 1 in whole studied concentration range (6-120 μmol · dm^{-3}), which suggests less effective translocation of metal to above ground parts of plants. *TF* values of plants of both cultivars were similar, although some differences are notable. The portion from the total accumulated amount of metal by the plant occurring in the shoots (which also depends on the actual dry mass of plant organs) was well above 50% (both cultivars) in whole studied Ni concentration range (6-120 μmol · dm^{-3}). After application of 60 and 120 μmol · dm^{-3} Ni was the portion of metal occurring in shoots above 70%. The ability of rapeseed plants to translocate substantial amounts of metal into shoots was observed in our previous hydroponic experiments with cultivar Verona, where even lower applied Cd concentrations (6-24 μmol · dm^{-3}) caused that the portions from the total accumulated metal amount by the plant occurring in the aboveground parts were relatively high, up to 53% [17]. In case of Cr(VI) (concentration range 12 to 120 μmol · dm^{-3}), it varied from 23% (12 μmol · dm^{-3}) to 90% (120 μmol · dm^{-3}) [38]. On the other hand, translocation of mercury into shoots of rapeseed plants (cv. Verona) was found to be relatively poor. In concentration range from 6 to 60 μmol · dm^{-3} Hg the portion from the total accumulated amount of metal by the plant occurring in the shoots reached only 5.8% (6 μmol · dm^{-3}) to 8.7% (60 μmol · dm^{-3}) [17].

Conclusions

Even though the differences between rapeseed cultivars Viking and Verona were moderate, it seems that cultivar Viking was more tolerant to the nickel treatment. The fact, that substantial amounts of studied metal are translocated in aboveground parts of plants suggest that *Brassica napus* L. species with many new cultivars is utilizable in field of phytoremediation, as a good accumulator of nickel and other metals.

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WPŁYW NIKLU NA REAKCJE FIZJOLOGICZNE DWÓCH ODMIAN *Brassica napus* L.

Abstrakt: Zbadano niekorzystny wpływ niklu na dwie odmiany hydroponicznej, uprawnej rośliny *Brassica napus* L. (Werona i Viking). Określono suchą masę pędów i korzeni, a także niektóre właściwości biochemiczne (stężenie barwników fotosyntetycznych, TBARS i białek) liści roślin. Ponadto dokonano oceny stężenia niklu w organach roślin. Objawy zatrucia Ni było zauważalne już przy najniższym zastosowanym stężeniu (6 μmol · dm^{-3}). Wyższe zastosowane stężenia Ni (24, 60 i 120 μmol · dm^{-3}) dały od umiarkowanych do silnych efektów toksyczności dla roślin obu badanych odmian. Po zastosowaniu 6 i 12 μmol · dm^{-3} Ni sucha masa odmiany Viking była znacznie mniejsza niż odmiany Werona. Spadek suchej masy korzeni po wprowadzeniu 6, 12 i 120 μmol · dm^{-3} Ni był podobny dla obu odmian. Po zastosowaniu 120 μmol · dm^{-3} zaobserwowano silny spadek zawartości barwników fotosyntetycznych. W porównaniu do kontroli ilość tych pigmentów w liściach roślin spadła poniżej 50% (obie odmiany). Największe zastosowane stężenie Ni 120 μmol · dm^{-3} spowodowało, że zawartość białka w liściach spadła o 39% (odmiana Werona) i 37% (odmiana Viking) w porównaniu z roślinami kontrolnymi. Po wprowadzeniu 120 μmol · dm^{-3} Ni zawartość dialdehydu malonowego w liściach była 2,64 razy większa (odmiana Viking) i 2,31 razy większa (odmiana Verona) niż w przypadku kontroli. Stężenia Ni w korzeniach roślin były wyższe niż w pędach. Stężenie Ni w korzeniach odmiany Werona było od 1,3 (120 μmol · dm^{-3}) do 1,9 (6 μmol · dm^{-3}) razy mniejsze niż w odmianie Viking, natomiast ilość metali zgromadzonych w pędach odmiany Werona była od 1,2 (120 μmol · dm^{-3}) do 1,8 (6 μmol · dm^{-3}) razy mniejsza niż w odmianie Viking.

Słowa kluczowe: bioakumulacja, nikiel, chlorofil, białko, dialdehyd malonowy, rzepak