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REACTION OF THE SUNFLOWER (*Helianthus annuus* L.) TO NICKEL CONDITIONED BY THE WAY OF METAL PENETRATION

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Abstract. The purpose of this paper was to determine the sunflower (*Helianthus annuus* L.) reaction to nickel depending on the metal concentration and penetration way, i.e. through roots or leaves. Nickel was introduced into the nutritional solution (intraroot application) at amounts: 0 (control), 35, 100 or 200 µM, while intraleaf application was performed by spraying plants with water (control) or nickel containing solution at the concentration of 5 or 10 µM. Plants treated with nickel intraroot than intraleaf were characterized by a considerably higher metal content in roots and lower in leaves. Independently of the penetration way increasing metal concentrations caused a significant decrease of the parameters of physiological root activity, i.e. the root volume, total and active adsorption surface and 1 cm^3 root active surface, in that a greater decrease of the root parameters was shown on intraroot than intraleaf application of the metal. Environment contamination with nickel caused also a chlorophyll concentration decrease in leaves, in that intraleaf nickel application, in contrast to intraroot, resulted in a higher decrease of chlorophyll *b* than *a*. Older than younger leaves showed a higher susceptibility to nickel applied intraroot, whereas roots were resistant to nickel applied intraleaf. The content of $S\text{-}SO_4$ in the sunflower depended on the nickel concentration and penetration way – intraleaf nickel application caused significant $S\text{-}SO_4$ increase in leaves and roots, whereas intraroot application resulted in $S\text{-}SO_4$ content increase especially in roots.

Key words: chlorophyll, nickel, physiological parameters of root, sunflower, $S\text{-}SO_4$

INTRODUCTION

A general and intensive anthropological activity causes a progressive contamination of the natural environment with heavy metals. One of them is nickel whose content in soil, water and atmosphere has considerably increased [Krasowski et al. 1994, Äyräs et al. 1997, Gorlach and Gambuś 1997, Terelak et al. 1997, Barcan and Kovnatsky 1998,

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Jasiewicz et al. 1998, Barałkiewicz and Siepak 1999, Jensen et al. 2000]. The concentration and penetration way of metals into plants is of great importance in the resistance of the particular plant organs to them [Arvik and Zimdahl 1974, Zimdahl and Koeppe 1978, Salim et al. 1993, Krämer et al. 1997, Ye et al. 1997, Reid et al. 2003, Robinson et al. 2003, Watson et al. 2003, Marques et al. 2004]. Studies of plant response to toxic concentration of trace metal elements concern largely the consequences and protection mechanisms induced by metal penetration through the root system. There are however relatively few papers concerning the problem when metal penetrates the plant through leaves. For this reason the purpose of our studies was to determine the reaction of sunflower – a plant tolerant to nickel in dependence on the metal concentration and penetration way, i.e. through roots and leaves.

MATERIALS AND METHODS

Plant material and vegetation conditions. The study object was common sunflower (*Helianthus annuus* L.). An experiment was carried out by the method of water cultures. The plants were grown in 1 dm^3 glass jars with doubly concentrated Hoagland nutritional solution supplemented with 2 $cm^3 \cdot dm^{-3}$ of 1% A-Z solution containing essential elements; pH of the nutrient medium was established at 6.5. Plant vegetation was conducted in phytotrone at 14/10 h photoperiod and 90 $W \cdot m^{-2}$ light intensity corresponding to irradiance intensity 405 μmol(quantum)$\cdot m^{-2} \cdot s^{-1}$ PAR, day/ night temperature range 25/17°C and relative air humidity about 75%. The experiment was differentiated with regard to the concentration and penetration way of nickel. Through root nickel was introduced at amounts: 0 (control), 35, 100 or 200 μM. Its intraleaf application was done by spraying the plants with 10 cm^3 of distilled water (control) or a solution containing nickel at 5 or 10 μM concentration. In all experiment variants nickel was used in the form of $NiSO_4 \cdot 7H_2O$. The experiment covered 10 replicates in each series and 3 time replicates, and the values plotted in the diagrams are the means of the results obtained from successive replicates. The experimental nickel dose was taken according to the standards of admissible nickel concentration in arable soils, ground waters and free air [Monitor Polski 1986, Kabata-Pendias et al. 1993, Gorlach et al. 1994, Galler 1992, Emsley 1997, Kabata-Pendias and Pendias 1999, Macioszczyk and Dobrzyński 2002]. Independently of the penetration way nickel was applied to plants in two equal portions – the first at the stage of 4 proper leaves, the second three days later. After the metal application the plants were growing over three weeks. During vegetation the plant appearance was observed, paying particular attention to morphological symptoms occurring in plants as a result of nickel contamination of the environment.

Determination of the total and active adsorption surface of the root system. On the day of finishing the experiment we determined the physiological parameters of roots: the volume, total and active adsorption surface and 1cm^3 root active surface by the method of Sabinin and Kołosov [Basławska and Trubieckowa 1964]. The volume of the roots taken out of the solution, washed in distilled water and thoroughly and delicately dried with blotting paper was measured immersing them in a graduated measuring cylinder of an appropriate volume partially filled with water. The roots were then delicately dried with blotting paper and successively put into two measuring cylinders (in the first for 3 min, and for 2 min in the other) containing methylene blue at 0.0064 mg/dm^3 concentration of a tenfold greater volume than that of the roots. From each cylinder 10 cm^3 of solution were taken with a pipette and transferred into a 100 cm^3 measuring flask and filling it up to the mark. The permeability of both solutions was determined with a photocolorimeter KF-2 using a red filter and 50 mm cuvettes. The dilution of the solutions was read from an analytical curve which was plotted on graph paper, giving the standard solution concentrations of the methylene blue (0.00032 mg/cm^3; 0.00016 mg/cm^3; 0.00008 mg/cm^3) on X-axis, and permeability % on Y-axis.

The root physiological parameters were calculated using the following formulae:

$[C_o - (C_1 \cdot 10)]\ 10V \cdot 1.1$ for total adsorption surface,

$[C_o - (C_2 \cdot 10)]\ 10V \cdot 1.1$ for active adsorption surface,

$[C_o - (C_2 \cdot 10)]\ 10V \cdot 1.1 / V$ for 1 cm^3 root active surface, where C_o is the concentration of methylene blue before root immersion (0.0064 mg/cm^3), C_1 is the concentration of methylene blue after taking the roots out of the first cylinder (in mg/cm^3), and C_2 is the concentration of methylene blue after taking the roots out of the other cylinder (in mg/dm^3), V is the root volume (in cm^3), 10V is the solution volume in the cylinders (in cm^3), 10 is the tenfold dilution, 1.1 is the surface in m^2 covered by 1mg of methylene blue at dye molecules packing in the monomolecular layer (m^2/mg).

Analysis of chlorophyll content. The content of chlorophyll *a* and *b* was determined by the method described by Lichtenthaler and Wellburn [1983]. In the nickel treated plants via roots the chlorophyll content was determined in the 2nd and 5th leaf pair from underneath (lower and upper leaves, respectively), while in nickel treated ones via leaves in the 5th leaf pair. For analysis the material was taken with a 9 mm cork borer, and then the weight of seven taken discs was determined. The dyes were extracted by grinding the tissue with 4–5 cm^3 of 80% acetone. The homogenate was quantitatively deposited into a funnel with a filter connected to a vacuum pump, washing it with small solvent portions. The obtained extract was transferred into measuring flasks and made up with acetone to the final 25 cm^3 volume. Next, extinction was read from the Perkin-Elmer Corporation Lambda E2150 spectrophotometer UV/Vis at two wavelengths λ = 663 nm (for chlorophyll *a*) and λ = 645 nm (for chlorophyll *b*). The concentration of the individual dyes was calculated from the following formulae:

$C_{chl\ a} = [(12,7 \cdot E_{663} - 2,69 \cdot E_{645}) \times 25] / (m \cdot 1000)$,

$C_{chl\ b} = [(22,9 \cdot E_{645} - 2,69 \cdot E_{663}) \cdot 25] / (m \cdot 1000)$, where E is the extinction at the given wavelength, m is the sample mass (7 discs).

Analysis of sulphate sulphur. $S\text{-}SO_4$ content was determined in dry material of lower and upper leaves (2nd and 5th leaf pair from underneath, respectively), stems and roots by the nephelometric method [Ostrowska et al. 1991], in that sulphur sulphate content in the leaves of nickel sprayed plants was determined only in the 5th leaf pair. Air-dry mass of 0.5-1.0 was poured into a container, flooded with 100 cm^3 of 2% CH_3COOH and shaken for 15 min. Next, 1 g of active carbon was added and the contents was shaken again for 5 min and filtered through a hard filter. 25 cm^3 of the filtrate were poured into 50 cm^3 measuring flask, adding 5 cm^3 of 25% HNO_3 and 4 cm^3 mixture of ice-cold acetic acid and o-phosphoric acid at 1:3 ratio and introducing then 1 g of

$BaCl_2$ in crystals, making up the contents to the mark. The flask contents was mixed by turning its bottom up and down 3 times and leaving it for 10 min; the mixing procedure was repeated turning the flask bottom down and up 10 times and leaving the contents for about 1–1.5 hr. After a repeated tenfold mixing of the flask contents colorimetric reading was done with Lambda E2150 spectrophotometer UV/Vis using a filter S-48 (λ = 480 nm). The percentage content of $S\text{-}SO_4$ was calculated on the basis of the reading from the standard curve (µg S:1000).

Nickel content. The content of nickel in the dry mass of roots, stems and lower and upper leaves (2^{nd} and 5^{th} leaf pair from underneath, respectively) was determined after initial dry mineralization of the plant material by atomic absorption spectrophotometry (AAS). Measurements were conducted with a Philips apparatus, model PU 9100X.

Statistical analysis of the results. The results of the particular determinations were statistically analyzed by ANOVA programme, Statistica 5.0. Variation analysis was carried out by estimating arithmetic means and the least Tukey's significant difference for mean pairs (LSD) at significance level P = 0.05. The results are the mean of the measurements made in 3 independent replicates. The numerical data given in the diagrams were rounded up for the sake of clarity.

RESULTS AND DISCUSSION

The aim of the studies was to determine the response of the plant tolerant to nickel depending on the penetration way of this metal, i.e. via roots or leaves. The experimental plants was chosen on the basis of the study results of Gorlach and Gambuś [1992], who found that the sunflower belongs to plants resistant to a medium-high metal concentration. Also Spiak's studies [1996] confirmed that of six plant species studied (sunflower, millet, field pea, phacelia, alfalfa and horse bean) sunflower appeared most resistant to increasing nickel doses in the growth medium in the range from 40–120 mg Ni/kg. Increasing nickel concentrations: 35, 100 or 200 µM applied in our studies intraroot, as well as 5 and 10 µM applied intraleaf resulted in a significant Ni content increase in roots, stems and leaves, in that a considerably higher content of the metal was found in roots and lower in leaves of the plants treated with nickel through roots than leaves (fig. 1). Moreover, it was shown that in the plants applied nickel via roots the upper leaves contained a higher concentration of the metal than lower ones, which indicates a great mobility of nickel translocating from the physiological base to the youngest plant organs (fig. 1).

Regardless of the way of nickel penetration into plants, the highest studied concentrations of the metal caused conspicuous phytotoxicity symptoms in sunflower. The observed changes in the appearance of sunflower and symptoms of its damages were specific. Leaves of the plants treated with nickel intraroot showed symptoms suggesting iron deficiency, and the chlorosis starting from the base of young leaves gradually developed in the whole leaf blade. Similar nickel toxicity symptoms were observed also by other researchers, among other things in such species as: cucumber, bean, maize, wheat, radish and duckweed [Vergnano and Hunter 1952, Hunter and Vergnano 1953,

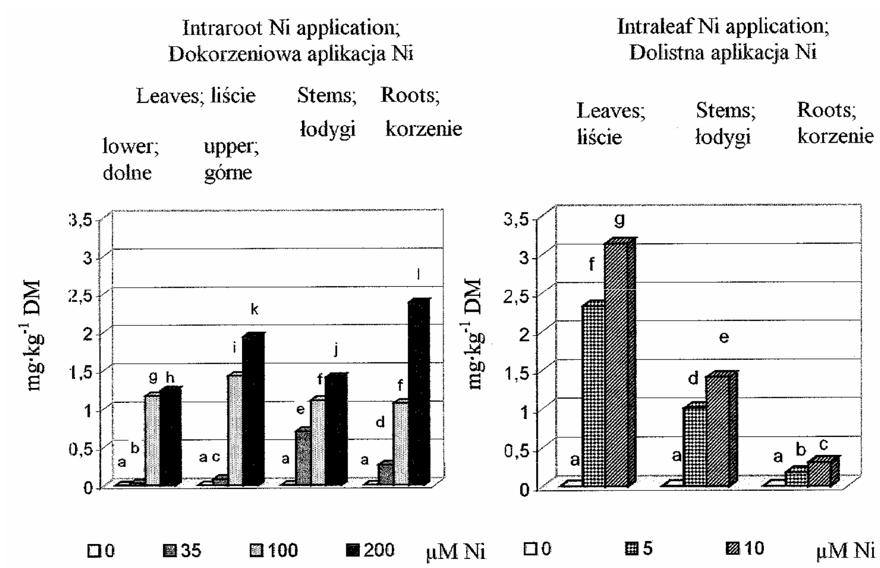

Fig. 1. Nickel content in sunflower plants in relation to concentration of nickel applied by roots or foliar spray; For each of two ways of metal penetration means in columns marked with the same letter are not significantly different at P = 0.05

Rys. 1. Zawartość niklu w roślinach słonecznika w zależności od koncentracji niklu aplikowanego przez korzenie lub liście; Dla każdego ze sposobów wnikania metalu do rośliny średnie oznaczone tą samą literą nie różnią się statystycznie przy poziomie istotności P = 0,05.

Murashige and Skoog 1962, Szkolnik 1980, Taylor and Allison 1981, Sarosiek and Wożakowska-Natkaniec 1993, Spiak 1993, Szymańska and Molas 1994]. However, as a result of nickel penetration through leaves a distinct reduction of the leaf blade surface was observed. Furthermore, at the tips of the younger leaves and at the blade base of older ones damages were seen, and after spraying them with a solution of 10 µM Ni concentration leaf necrosis was observed. These symptoms, characteristic of iron deficiency, caused by excess nickel are an example of secondary antagonism between these elements. From the literature data it appears that interaction between nickel and iron consists largely in excluding the latter from physiological fractions [Crooke 1956, Mishra and Kar 1974, Szymańska and Molas 1994, Szymańska and Matraszek 1996, Kabata-Pendias and Pendias 1999].

Sunflower contamination with nickel, independently of the metal penetration, caused also changes in the architecture of the root system, in that more distinct changes were observed after intraroot application. They were indicated by elongative growth inhibition, a very weak development of lateral roots, density decrease of root hairs and characteristic 'tufty' roots, in that the latter change occurred only after intraroot nickel application. These changes were probably caused among other things by inhibition of mitosis in the apical root meristem, decreased wall plasticity of the cells formed after

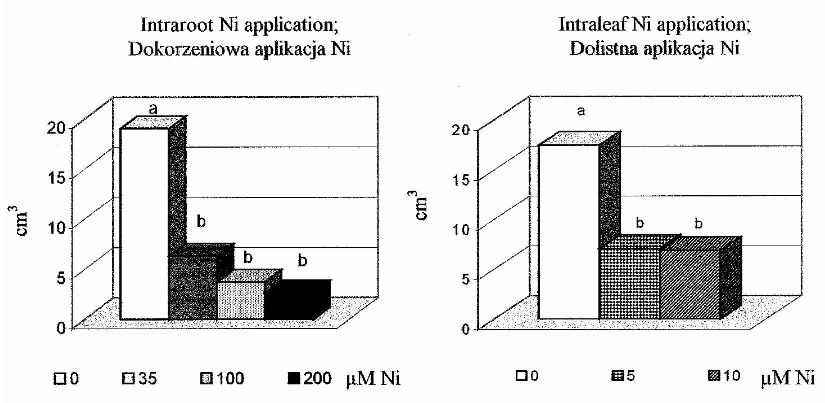

Fig. 2. Total volume of sunflower roots in relation to concentration of nickel applied by roots or foliar spray; For each of two ways of metal penetration means in columns marked with the same letter are not significantly different at P = 0.05

Rys. 2. Objętość korzeni słonecznika w zależności od koncentracji niklu aplikowanego przez korzenie lub liście; Dla każdego ze sposobów wnikania metalu do rośliny średnie oznaczone tą samą literą nie różnią się statystycznie przy poziomie istotności P = 0,05.

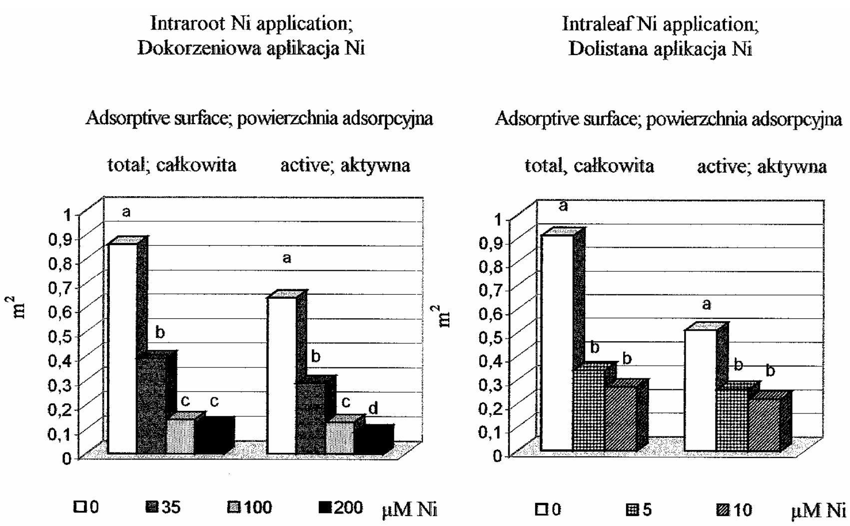

Fig 3. Total and active adsorptive surface of sunflower roots in relation to concentration of nickel applied by roots or foliar spray; For each of two ways of metal penetration means in columns marked with the same letter are not significantly different at P = 0.05

Fig. 3. Ogólna i aktywna powierzchnia adsorpcyjna korzeni słonecznika w zależności od koncentracji niklu aplikowanego przez korzenie lub liście; Dla każdego ze sposobów wnikania metalu do rośliny średnie oznaczone tą samą literą nie różnią się statystycznie przy poziomie istotności P = 0,05.

mitotic divisions and membrane permeability with simultaneously growing blockade for water [Moya et al. 1993, Punz and Sieghardt 1993, Obroucheva et al. 2001, Seregin et al. 2003]. These disturbances in our studies resulted in a statistically significant decrease in the general volume and changes of the physiological activity of roots, i.e. their total and active adsorption surface. The above changes were more distinct after intraroot than intraleaf metal application (figs. 2 and 3). Nickel at 35 μM concentration caused a significant increase, and at 200 μM a statistically proved decrease of the active 1 cm^3 root surface of sunflower was recorded (fig. 4).

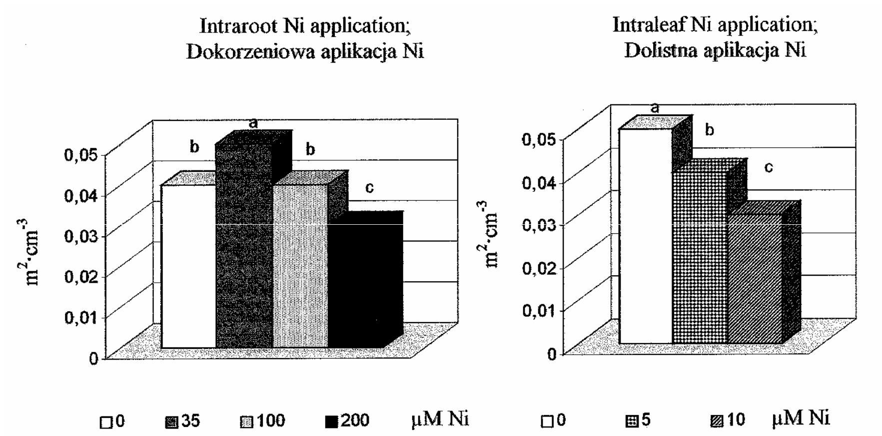

Fig. 4. 1 cm^3 active surface of sunflower roots in relation to concentration of nickel applied by roots or foliar spray; For each of two ways of metal penetration means in columns marked with the same letter are not significantly different at P = 0.05

Rys. 4. Powierzchnia aktywna 1cm^3 korzeni słonecznika w zależności od koncentracji niklu aplikowanego przez korzenie lub liście; Dla każdego ze sposobów wnikania metalu do rośliny średnie oznaczone tą samą literą nie różnią się statystycznie przy poziomie istotności P = 0,05.

The physiological activity of the aboveground plant parts was estimated from chlorophyll content – a dye considered as plant vitality indicator. In control a higher chlorophyll content was found in the lower than upper leaves (fig. 5). A reverse relationship was shown in conditions of environment contamination with nickel. With increasing nickel dose both in the nutritional solution and air a significant concentration decrease of both analyzed chlorophyll forms occurred, in that a considerably greater decrease was observed at intraleaf than intraroot metal application. The chlorophyll content decrease in the presence of nickel could be caused both by intensified chlorophyllase action in the plants treated with the metal and sensitivity of other enzymes of porphyrins synthesis pathway as well as by synthesis inhibition of delta-aminolevulinic acid dehydratase (ALAD, E.C.4.2.1.24) – an enzyme participating in synthesis of assimilation dyes [Stiborova et al. 1986, Drążkiewicz 1994, Kączkowski 1992]. Chlorophyll content decrease in plant exposed to nickel can also result from decreased action of ironporphyrin enzymes – catalase and peroxydase, or from decreased availability of iron needed for chlorophyll synthesis [Pandey and Sharma 2002]. The obtained results indicate that the content of chlorophyll *a* and *b* after intraroot application decreased more in lower than upper leaves. Moreover, it was found that intraleaf nickel application caused, in contrast to intraroot, a greater content decrease of chlorophyll *b* than *a* (fig. 5). Krupa et al. [1993], studying the influence of nickel on the photosynthetic apparatus of bean found a greater decrease of chlorophyll *b* than *a*. However, earlier studies of the authors of this paper on maize, lettuce, mustard, cucumber and spinach plants growing in the presence of nickel showed a more distinct decrease of chlorophyll *a* than *b* [Szymańska and Molas 1994, Matraszek 1998]. A greater content decrease of chlorophyll *a* than *b* caused by the presence of nickel in the nutritional medium of plants can result from the fact that this metal enhances the action of chlorophyllase more in relation to chlorophyll *a* than *b* [Tolgyessy et al. 1993, Abdelbasset et al. 1995, Ouzounidou 1995].

The participation of sulphur in the resistance mechanism of plants to unfavourable environment conditions is well known [Leustek and Saito 1999, Scherer 2001, Abrol and Ahmad 2003]. Greater amounts of sulphate sulphur in roots were found in plants with intraroot than intraleaf application of nickel, in that, regardless of the metal penetration way, $S\text{-}SO_4$ concentration generally increased with nickel contamination increase of the environment (fig. 6). However, in leaves of plants treated with nickel intraroot, in contrast to intraleaf application, the content of this sulphur fraction decreased with nickel

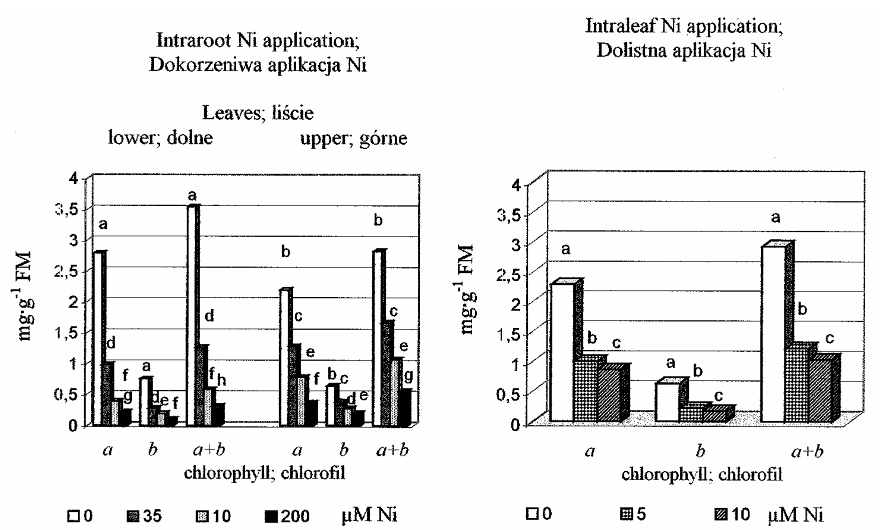

Fig. 5. Chlorophyll content in sunflower leaves in relation to concentration of nickel applied by roots or foliar spray; For each of two ways of metal penetration means in columns marked with the same letter are not significantly different at P = 0.05

Rys. 5. Zawartość chlorofilu w liściach słonecznika w zależności od koncentracji niklu aplikowanego przez korzenie lub liście; Dla każdego ze sposobów wnikania metalu do rośliny średnie oznaczone tą samą literą nie różnią się statystycznie przy poziomie istotności P = 0,05.

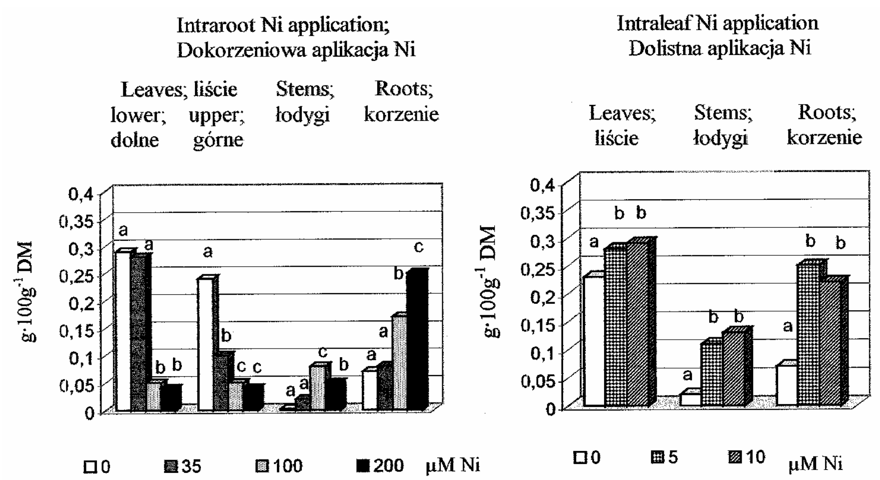

Fig. 6. $S-SO_4$ content in sunflower plants in relation to concentration of nickel applied by roots or foliar spray; For each of two ways of metal penetration means in columns marked with the same letter are not significantly different at P = 0.05

Rys. 6. Zawartość $S-SO_4$ w roślinach słonecznika w zależności od koncentracji niklu aplikowanego przez korzenie lub liście; Dla każdego ze sposobów wnikania metalu do rośliny średnie oznaczone tą samą literą nie różnią się statystycznie przy poziomie istotności P = 0,05.

concentration increase in the environment. In leaves directly threatened with metal contamination a significant $S-SO_4$ content increase was found. In stems a higher $S-SO_4$ content was found after intraleaf nickel application in comparison with intraroot one, and the content of sulphates generally increased with increasing metal dose in the environment (fig. 6). This points to controlled change of sulphur metabolism and translocation of this element. Sulphur accumulated under nickel stress undergoes reduction including synthesis of glutathione – a tripeptide conditioning tolerance of plants and its increased transport into roots. Sulphur in a mineral form however is accumulated in roots [Rennenberg 1982, Tukendorf and Rauser 1990, May et al. 1998, Foyer et al. 2001, Hartmann et al. 2004, Kopriva and Rennenberg 2004]. No significant differences in the content of the analyzed sulphur fraction resulting from the used nickel dose were found either in stems or roots of the plants treated with the metal intraleaf (fig. 6).

CONCLUSIONS

Sunflower responses to nickel were characterized by a certain specificity despite their common features regardless of its penetration way. Increasing nickel contaminations: 35, 100 or 200 µM (intraroot application) and 5 or 10 µM (intraleaf application) were found to effect:

1. A significant decrease of physiological activity parameters of sunflower roots such as their total volume, general and active adsorption surface and active 1 cm^3 root surface, which was greater after intraroot than intraleaf nickel adsorption.

2. A significant, deepening concentration decrease of the particular chlorophyll forms in sunflower leaves, in that intraleaf nickel application caused a greater decrease of chlorophyll *b* than *a* in contrast to intraroot application.

3. A significant nickel content increase in the examined sunflower organs with increasing metal dose, in that considerably higher content of the element was found in root and lower in leaves of the plants treated with nickel intraroot than intraleaf. Furthermore, in plants treated with nickel intraroot the upper leaves contained more of this element than the lower ones, which indicates a high mobility of the studied metal being transferred from the physiological base into youngest plant organs.

$S\text{-}SO_4$ concentration in the examined plant organs differed in relation to the metal penetration way. Nickel contamination of the nutritional medium caused a significant increase of the sulphur fraction content in roots and stems, and a significant concentration decrease of sulphides in leaves. Intraleaf nickel application, however, caused $S\text{-}SO_4$ increase in all organs. Taking into consideration the obtained results and the fact that increasing sulphur content in the biomass is one of the factors affecting the tolerance of plants to heavy metals, it can be found that older leaves than young ones are more resistant to nickel applied intraroot, whereas roots are more resistant to the metal when applied intraleaf.

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REAKCJA SŁONECZNIKA (*HELIANTHUUS ANNUUS* L.) NA NIKIEL UWARUNKOWANA DROGĄ WNIKANIA METALU

Streszczenie. Celem pracy było określenie reakcji słonecznika (*Helianthuus annuus* L.) na nikiel, w zależności od stężenia i drogi wnikania metalu, tj. przez korzeń lub liść. Nikiel wprowadzano do pożywki (aplikacja dokorzeniowa) w ilościach: 0 (kontrola), 35, 100 lub 200 µM, natomiast aplikację dolistną wykonano, opryskując rośliny wodą (kontrola) lub roztworem zawierającym nikiel w stężeniu 5 lub 10 µM. Znacznie wyższą zawartością niklu w korzeniach i niższą w liściach charakteryzowały się rośliny traktowane niklem dokorzeniowo niż dolistnie. Niezależnie od sposobu wnikania, wzrastające stężenia niklu wpłynęły na istotny spadek parametrów fizjologicznej aktywności korzeni, tj. objętości, ogólnej i aktywnej powierzchni adsorpcyjnej oraz powierzchni 1 cm^3, przy czym większy spadek wskaźników korzeni wykazano po dokorzeniowej niż dolistnej aplikacji metalu. Skażenie środowiska niklem powodowało również spadek koncentracji chlorofilu w liściach, przy czym dolistna aplikacja niklu, w przeciwieństwie do aplikacji dokorzeniowej wpłynęła na większy spadek chlorofilu b niż a. Większą wrażliwością na nikiel aplikowany dokorzeniowo mają liście starsze w porównaniu z liściami młodymi, natomiast organami odpornymi na nikiel aplikowany dolistnie są korzenie. Zawartość $S\text{-}SO_4$ w słoneczniku zależała od drogi wnikania i stężenia niklu – aplikacja dolistna wpłynęła wyraźnie na wzrost koncentracji $S\text{-}SO_4$ w liściach i korzeniach, natomiast aplikacja dokorzeniowa na zwiększenie zawartości $S\text{-}SO_4$ szczególnie w korzeniach.

Słowa kluczowe: nikiel, fizjologiczne wskaźniki korzenia, chlorofil, słonecznik, $S\text{-}SO_4$

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