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THE SYNTHESIS OF COMPOUNDS RICH IN -SH GROUPS IN PLANTS OF SELECTED *Silene vulgaris* ECOTYPES DEPENDING ON NICKEL DOSE

SYNTEZA ZWIĄZKÓW BOGATYCH W GRUPY -SH W ROŚLINACH WYBRANYCH EKOTYPÓW *Silene vulgaris* W ZALEŻNOŚCI OD DAWKI NIKLU

Abstract: The main goal of the research was to determine the influence of increasing nickel doses (0, 30, 60, 90 mg · kg^1) on the content of -SH groups in plants of selected *Silene vulgaris* ecotypes originating from other habitats.

In the experiment we used *Silene vulgaris* seeds from calamine regions of Upper Silesia (an area next to the *Szopienice* foundry in Katowice), a location connected with serpentinite deposits exploitation (Wiry landmass) and natural ecotype seeds from an area uncontaminated by heavy metals (Gajkow near Wrocław). The analyzed plant material had -SH groups but there were clearly more in the above-ground parts of the selected *S. vulgaris* ecotypes. Only in the case of Gajkow ecotype *S. vulgaris*, from a location without nickel, the number of thiol groups was clearly higher in roots. Chemical analyses of plant material showed that along with the increase of nickel dose, the –SH group concentration also increased in the shoots of *Silene vulgaris* of all ecotypes. However, the concentration was the highest with the dose of 60 and 90 μM Ni for Gajkow ecotype plants from an area with a naturally low content of heavy metals. On the other hand, the smallest number of thiol groups deposited in shoots was found in Wiry ecotype plants from a habitat rich in nickel (serpentinite spoil tip).

Keywords: *Silene vulgaris*, ecotype, nickel, -SH groups, phytochelatins

Introduction

Nickel belongs to a group of heavy metals forming Earth's crust. Its occurrence is strictly connected with alkaline igneous rocks and sedimentary clay rocks [1]. Nickel content in Earth's soils varies strongly between 3 and 1000 mg · kg^{-1} [1]. The mean content of this element, calculated for Polish soils, is 9 mg · kg^{-1} [1], but serpentinite soils (naturally rich in nickel compounds) in Lower Silesia visibly surpass this value.

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Studies conducted by Zolnierz [2] in the selected serpentinite massifs have shown concentrations exceeding $3000 \text{ mg} \cdot \text{kg}^{-1\cdot}$.

Nickel is a necessary nutrient for plants. However, the demand for this element is small and is only 0.1–5 mg [1, 3–5]. Nickel is an important micro-element ensuring proper growth and development of plants by activating the enzymes of numerous metabolic processes [3–5]. Nickel is a part of the active site of urease, an enzyme hydrolyzing urea, and thus plays a significant role in providing urea for plants and takes part in the metabolism of urea derivatives and in binding atmospheric nitrogen by soil bacteria. In plants nickel is also a part of hydrogenases and nickel deficiencies lead to leaf tip necrosis and urea accumulation in cells [3–5].

Nickel belongs to transition elements and its bioavailability for plants, particularly on contaminated soils, is big and increases along with its increased concentrations in the environment [3–5]. Nickel absorbed by a plant moves from roots to above-ground parts. Nickel ions are very rarely transported in a plant in a free form. After entering the symplast they are almost instantaneously complexed by low molecular ligands (chelatine ligands) [3–5]. Among the essential nickel ion chelates there are organic acids (citric acid, malic acid, malonic acid, oxalic acid), amino acids (*eg* histidine, which forms coordination complexes with Ni ions), metallothioneins, low molecular mass proteins rich in cysteine and phytochelatins (metallothiol, low molecular polypeptides) [3–5]. The above-mentioned compounds enable easy transport of nickel in the plant and decrease its toxicity, which is important when the intake of this metal is too big [3–5]. The mechanism of nickel's toxic influence on plants has not been fully explained yet.

The toxic influence of nickel is related, for instance, to the occurrence of chlorosis connected with excluding iron from physiological functions. The excess of nickel also stops the functioning of enzymes containing iron, *eg* catalase and peroxidase and, after excessing critical concentration, disrupts photosynthesis [3–5] due to the influence on the transport of electrons and the decrease in chlorophyll.

Researchers are now interested in plants which adapted to life in highly contaminated areas or areas naturally rich in heavy metals, *eg* post-industrial areas, post-mining areas or metal-rich soils [6–10]. In Poland areas where surface soils are naturally rich in nickel compounds are limited to serpentinite soils particular for Lower Silesia and formed of serpentinite rocks [2]. Serpentinite rock habitats are characterized by many unfavorable properties (high and potentially toxic concentrations of nickel, chromium and magnesium; low content of phosphorus, potassium and nitrogen; low humidity; highly alkaline pH) and, thus, have unique vegetation [2].

Post-mining and post-industrial serpentinite locations (spoil tips, quarries) of Lower Silesia are characterized by low biodiversity and dispersion and *Silene vulgaris* is one of the few taxa on the floristic content list [6–8].

The species is a good indicator of heavy metal contamination in soils and its presence was determined both on areas naturally rich in heavy metals and on locations where human influence caused the heavy metals contamination [8–12].

In literature one may find descriptions of this species' unique adaptation capabilities leading to the emergence of separate ecotypes adapted to extremely unfavorable habitat conditions. Apart from *Silene vulgaris* ecotypes resistant to lead and zinc, there are also ones tolerating the excess of cadmium and copper [8–12].

Comparative studies conducted by Koszelnik-Leszek and Bielecki [13] have shown that *Silene vulgaris* ecotypes originating from different habitats (with high and naturally low heavy metal contents in soil) differed from one another in morphological features – plant height, leaf lobe width – and a nickel experiment has shown different reactions of chosen ecotypes to the metal. Serpentinite ecotype plants, as compared to other ecotypes, accumulated more nickel.

One of the most important parts of plant's defense against heavy metal poisoning is the synthesis of phytochelatins, as it is believed that the synthesis of these compounds is one of the key detoxification mechanisms [3–5, 13–20]. Phytochelatins, which are structurally derivative of glutathione, are formed on the basis of a particular dipeptide – Glu-Cys (2–11 times repetition) and their synthesis occurs enzymatically and is activated by metal ions, among others Cd^{+2}, Pb^{+2}, Cu^{+2}. Due to numerous thiol groups (-SH) of cysteine residue they are capable of successfully bond and deactivate ions of many heavy metals [13–20].

That is why the aim of this paper is to determine the level of glutathione and phytochelatins through counting thiol groups in the selected ecotypes of *Silene vulgaris* growing in the conditions of raised soil nickel levels.

The conducted studies will allow us to determine if the selected *Silene vulgaris* ecotypes have a clear increase in synthesized phytochelatins and glutathione along with the increased levels of soil nickel and which of the selected ecotypes have a higher or smaller predilection for these compounds.

Materials and methods

The plants of three *Silene vulgaris* ecotypes were the study material. The *Gajkow* ecotype comes from a habitat with naturally low nickel contents near Gajkow village located to the south-east of Wroclaw [13]. The *Szopienice* ecotype grows in an area located 250 m from pollution emitter, a foundry in Katowice (Huta Metali Niezelaznych *"Szopienice"*) in Upper Silesia [18]. The *Wiry* ecotype grows on a small spoil tip connected with serpentinite rock exploitation in Lower Silesia, near Wiry village located at the western base of Sleza Mountain [13].

Pot experiment

The seeds of three *Silene vulgaris* were planted in pots filled with autoclaved, moist garden soil. After two weeks the plants of each ecotype were to separate pots. The plants were grown in glasshouse conditions with sunlight exposure. After 8–9 weeks a part of the plants of each ecotype were studied biometrically and the other part had their roots cleaned thoroughly and planted in pots filled with Hoagland and Arnon nutrient solution [21] (0.5 dm^3). After the plants acclimatized themselves, nickel sulfate was added to the nutrient solution so that the concentration of nickel reached 0, 30, 60, and 90 μM (each dose was applied in 4 repetitions). The experiment lasted two weeks. In that time, the nutrient solution was regularly aerated. After seven days the nutrient solution was exchanged for a new one and nickel sulfate was again added in the same doses. After two weeks the experiment was finished, the plants gathered and preserved for further chemical analyses. The compounds rich in -SH groups were marked in the sample materials using Wojcik and Tukiendorf method [16] and the technique of High-performance liquid chromatography (HPLC). The number of -SH groups was measured from standard curve for glutathione.

Results and discussion

Phytochelatins are of particular interest in research into plant tolerance levels. It is believed that the synthesis of these compounds is one of the essential heavy metals detoxification mechanisms in plants [13–20].

The structure of phytochelatins is formed by numerous thiol groups (-SH) bonding heavy metals and thus limiting their negative impact on the plant [13–20].

The essence and definition of plant tolerance to heavy metals is the ability to sustain heavy metal ion homeostasis on cellular and tissue level [20]. Currently it is believed that sustaining heavy metal ion homeostasis in plants is connected with complexing leaf and root cells in cytosol. In case of the phytochelatin complex (PCS) – metal is transported to the vacuole, where it is separated from other cellular organelles [13–20].

In the analyzed plant material (under- and above-ground parts of selected *S. vulgaris* ecotypes – Fig. 1) the study revealed the presence of -SH groups but they were clearly more numerous in the above-ground parts (Fig. 1). Only in the case of the *Gajkow* ecotype from a nickel free location (control) the number of thiol groups was clearly higher than in the roots (Fig. 1).

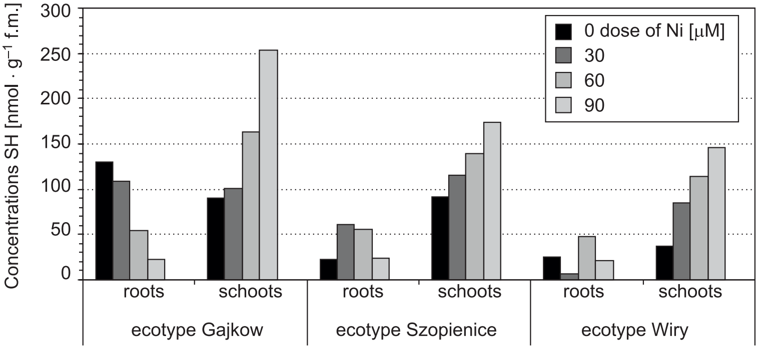

Fig. 1. The concentration of -SH groups in roots and shoots in *Silene vulgaris* ecotypes (e.)

Chemical analyses of plant material has shown that along with the increase of nickel dose the concentration of -SH groups in the shoots of *Silene vulgaris* also increased in all ecotypes. However, the most groups were detected (mainly in the dose of 60 and 90 μM Ni) in the *Gajkow* ecotype coming from a location with naturally low heavy metal content. What is more, the smallest amount of thiol groups was deposited in the shoots of *Wiry* ecotype originating from a habitat rich in nickel (a serpentinite spoil tip).

Earlier studies by the author [13] conducted on the same ecotypes and in the same experiment conditions with increasing nickel doses have shown that the *Wiry* ecotype accumulated more nickel in the above-ground parts than the *Gajkow* ecotype, which, in the same experiment conditions, accumulated the least of it.

The previous [13] and current results obtained by the author may suggest that, as it is implied by Seth et al [19], in stress conditions caused by hydroponic cultivation with the addition of nickel, phytochelatins take part in metal detoxification in plants with low tolerance to metals (*Gajkow* ecotype). On the other hand, in plants with higher tolerance to metals (*Wiry* ecotype), the role of phytochelatins in detoxification in high-stress conditions seems to have a smaller impact on their tolerance despite the induction of phytochelatins synthesis [12]. According to De Knecht et al [12], though, the amount of phytochelatins does not always indicate the tolerance to metals. Analyses of selected ecotypes' roots (Fig. 1) have shown that only in the case of the *Gajkow* ecotype the number of thiol groups regularly decreased along with nickel dose. In the cases of other ecotypes there was no clear dependence.

A clear increase in the number of thiol groups in the shoots of experimental plants as compared to the roots was determined by Wojcik and Tukiendorf [16] because after 14 days of hydroponic cultivation of *Arabidopsis* with cadmium dose they observed a clear increase in phytochelatins in above-ground parts in comparison to the roots (particularly with higher doses).

Summing up the thiol group number in roots and shoots (Fig. 2a–d) it has been determined that, despite the nickel dose, the natural *Gajkow* ecotype deposited the most of them and the *Wiry* ecotype from the serpentinite spoil tip – the least.

However, the natural ecotype plants grown in control conditions had the number of thiol groups comparable to that of plants from locations with 30 and 60 μM nickel doses (Fig. 2a–c). It is important to remember that the nutrient solution itself (control location) is a source of easily available metal forms. With the highest dose – 90 μM Ni – the analyzed parts of the natural ecotype of *Silene vulgaris* have shown a visible increase in -SH groups.

In the cases of other ecotypes from locations with higher metal concentrations (Szopienice and Wiry) it was determined that the phytochelatins content in plants from control locations was lower in comparison to their concentrations in plants growing in raised nickel conditions (Fig. 2a–d). In these ecotypes it was also observed that with lower nickel doses – 30 and 60 μM (Fig. 2b, 2c) – the content of thiol groups was balanced (within the ecotype) but with a higher dose – 90 μM (Fig. 2d) – it was clearly higher than in the other nickel combinations. The increasing number of phytochelatins (usually) increasing along with the nickel dose confirm the research by Gawel et al [17] where the authors note that phytochelatins concentration reflects the current level of heavy metal availability in the environment.

The recorded lower content of thiol groups (phytochelatins and glutathione) in *Silene vulgaris* of the *Wiry* and *Szopienice* ecotypes as compared to the *Gajkow* ecotype (Fig. 2a–b) may be connected with their habitats of origin, respectively one rich and one

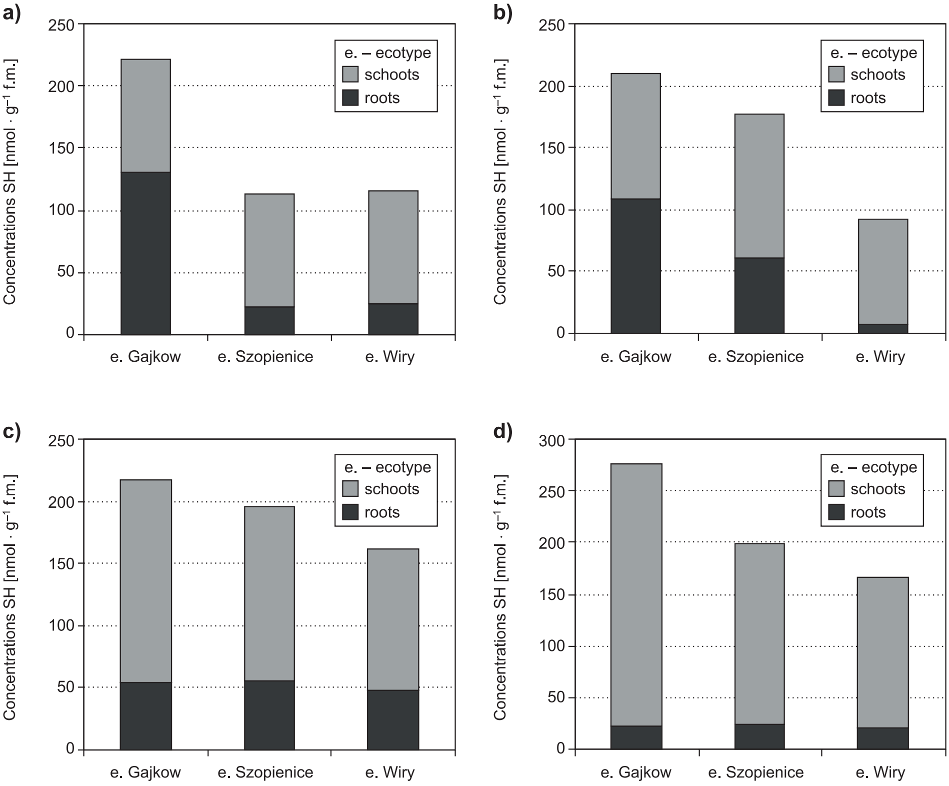

Fig. 2. The concentration of -SH groups in selected *Silene vulgaris* ecotypes depending on nickel dose: a) control, b) 30 μM Ni, c) 60 μM Ni, d) 90 μM Ni

contaminated with heavy metals. And so, in both cases, the results may reflect the adaptation to growth in conditions with higher metal contents and, consequently, weaker reaction to nickel and other metals in the nutrient solution manifesting in lower levels of synthesized phytochelatins and glutathione.

Conclusions

One of the most important elements of response to stress caused by heavy metals is the synthesis of phytochelatins and glutathione resulting in higher concentrations of thiol groups in plant material. The concentration of phytochelatins reflects the current level of heavy metal availability in the surrounding environment and determines the physiological stress level in plants. Therefore, the obtained results are a contribution to further research into these compounds which may be used as, for instance, bio-indicators of heavy metal contamination in plants.

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References

[1] Kabata-Pendias A. Trace Elements in Soils and Plants. Fourth Edition. Boca Raton-London-New York: CRC Press Taylor Francis Group; 2011. http://www.petronet.ir/documents/10180/2323242/Trace_Elements_in_Soils_and_Plants.

[2] Żołnierz L. Zbiorowiska trawiaste wystepujące na Dolnoślaskich serpentynitach-wybrane aspekty ekologii. [Grassland on serpentines in Lower Silesia (SW Poland) some aspects of their ecology]. Zesz Nauk Uniwersytetu Przyrodniczego we Wrocławiu, Rolnictwo 2007; 555: 231 pp. http://www.dbc.wroc.pl/Content/4255/Zolnierz_Ludwik_ZNUP557_Rozpr249_DBC_link.pdf.

[3] Chen C, Huang D, Liu J. Functions and toxicity of nickel in plants: Recent advances and future prospects. Clean 2009;37(4-5): 304-313. DOI: 10.1002/clen.200800199.

[4] Fabiano CC, Tezotto T, Favarin JL, Polacco JC, Mazzafera P. Essentiality of nickel in plants: a role in plant stresses. Front Plant Sci. 2015;6:754. http://dx.doi.org/10.3389/fpls.2015.00754.

[5] Yusuf M, Fariduddin Q, Hayat S, Ahmad A. Nickel: an overview of uptake, essentiality and toxicity in plants. Bull Environ Contamin Toxicol. 2001;86:1-17. DOI: 10.1007/s00128-010-0171-1.

[6] Kasowska D, Koszelnik-Leszek A. Ecological features of spontaneous vascular flora of serpentine post-mining sites in Lower Silesia. Archiv Environ Protect. 2014;40(2):33-52. DOI: 10.2478/aep-2014-0014.

[7] Koszelnik-Leszek A. Accumulation of heavy metals by *Silene vulgaris* occurring on serpentine waste dump in Grochów (Lower Silesia). Ecol Chem Eng A. 2012;19(4-5):411-420. DOI: 10.2428/ecea.2012.19(04)043.

[8] Wierzbicka M, Panufnik D. The adaptation of *Silene vulgaris* to growth on a calamine waste heap (S. Poland). Environ Pollut. 1998;101:415-426. DOI: 10.1016/S0269-7491(98)00012-8.

[9] Bratteler M, Widmer A, Baltisberger M, Edwards PJ. Genetic architecture of assiociated with habitat adaptation in *Silene vulgaris* (Caryophyllaceae). Bull Geobotanical Institute ETH. 2002;68: 95-103. http://www.srcosmos.gr/srcosmos/showpub.aspx?aa=6024.

[10] Nadgórska-Socha A, Kandziora-Ciupa M, Ciepał R, Walasek K. Effects of Zn, Cd, Pb on Physiological Response of *Silene vulgaris* Plants from Selected Populations. Pol J Environ Stud. 2001;20(3):599-604. http://www.pjoes.com/pdf/20.3/Pol.J.Environ.Stud.Vol.20.No.3.599-604.pdf

[11] Harmens H, Den Hartog PR, Wilma M, Bookum T, Verkleij JAC. Increased zinc tolerance in *Silene vulgaris* (Moench) Garcke in not due to increased production of phytochelatins. Plant Physiol. 1993;103: 1305-1309. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC159120/pdf/1031305.pdf

[12] De Knecht JA, Van Baren J, Ten Bookum WM ,Wong Fong Sang WF, Koevoets PLM, Schat H, et al. Synthesis and degradation of phytochelatins in cadmium-sensitive and cadmium tolerant *Silene vulgaris*. Plant Sci. 1995;106: 9-18. DOI: 10.1016/0168-9452(95)04066-4.

[13] Koszelnik-Leszek A, Bielecki K. Response of selected *Silene vulgaris* ecotypes to nickel. Pol J Environ Stud. 2013;22(6):1741-1747. http://www.pjoes.com/pdf/22.6/Pol.J.Environ.Stud.Vol.22.No.6.1741-1747.pdf.

[14] Cobbett CS. Phytochelatins and their roles in heavy metal detoxification. Plant Physiol. 2000;123:825-832. DOI: http://dx.doi.org/10.1104/pp.123.3.825.

[15] Schat H, Liugany M, Vooijs R, Hartley-Whitaker J, Bleeker PM. The role of phytochelations in constitutive and adaptive heavy metal tolerance in hyperaccumulator and nonhyperaccumulator metallophytes. J Experimen Bot. 2002;53(379):2381-2392. DOI: 10.1093/jxb/erf107.

[16] Wójcik M, Tukiendorf A. Phytochelatin synthesis and cadmium localization in wild type of *Arabidopsis thaliana.* Plant Growth Regulation. 2004;44:71-80. DOI: 10.1007/s10725-004-15929.

[17] Gawel JE, Hemond HF. Biomonitoring for metal contamination near two Superfund sites in Woburn, Massachusetts, using phytochelatins. Environ Pollut. 2004;131(1):125-135. http://dx.doi.org/10.1016/j.envpol.2004.01.012.

[18] Kandziora M, Heflik M, Nadgórska-Socha A, Ciepał R**.** Synteza zwiazków bogatych w grupy -SH jako odpowiedź na podwyższone steżenie metali ciežkich u roślin *Silene vulgaris* (*Caryophyllaceae*) [The synthesis of the compounds rich in -SH groups as an answer to the increased heavy metals concentration in *Silene vulgaris* (*Caryophyllaceae*)]. Ochr Środ Zasob Natur. 2007;33:69-72. http://www.ios.edu.pl/pol/wydawnictwa/nr33.pdf.

[19] Seth CS, Chaturvedi PK, Misra V. The role of phytochelatins and antioxidants in tolerance to Cd accumulation in *Brassica juncea* L. Ecotox Environ Safe. 2008;71:76-85. DOI: 10.1016/j.ecoenv.2007.10.030.
[20] Olko A. Fizjologiczne aspekty tolerancji roślin na metale ciężkie [Physiological aspects of plant heavy metal tolerance]. Kosmos. 2009;58:221-228. Kosmos.icm.edu.pl/PDF/2009/221.pdf.
[21] Hoagland DR, Arnon DI. The water culture method for growing plants without soil. Berkeley: Calif Agricultural Exp Station, University of California: 1950: 31 pp. https://archive.org/details/waterculture me3450hoag.

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Abstrakt: Głównym celem badań było określenie wpływu wzrastających dawek niklu (0, 30, 60, 90 mg · kg^{-1}) na zawartość grup -SH w roślinach wybranych ekotypów *Silene vulgaris* pochodzących z odmiennych siedlisk.

W eksperymencie wykorzystano nasiona *Silene vulgaris* pochodzące z obszarów galmanowych Górnego Śląska (obszar przyległy do huty „Szopienice" w Katowicach), związanych z eksploatacją złóż serpentynitowych (zwałowisko w Wirach) oraz nasiona ekotypu naturalnego, z terenu nie zanieczyszczonego metalami ciężkimi (Gajków koło Wrocławia).

W analizowanym materiale roślinnym, stwierdzono obecność grup -SH z tym jednak, że wyraźnie więcej było ich w częściach nadziemnych wybranych ekotypów *S. vulgaris.* Jedynie w przypadku roślin *S. vulgaris* ekotypu Gajków pochodzącego z obiektu bez niklu, liczba grup tiolowych była wyraźnie wyższa w korzeniach.

Analizy chemiczne materiału roślinnego wykazały również, że wraz ze wzrostem dawki niklu wzrastała koncentracja grup -SH w łodygach *Silene vulgaris* wszystkich ekotypów z tym że najwięcej ich zliczono przy dawce 60 i 90 mg Ni, dla roślin ekotypu Gajków pochodzącego z obszaru o naturalnie niskiej zawartości metali ciężkich. Natomiast wyraźnie najmniej grup tiolowych zliczono w częściach nadziemnych *S. vulgaris* ekotypu Wiry pochodzącego z siedliska zasobnego w nikiel (hałda serpentynitowa).

Słowa kluczowe: *Silene vulgaris*, ekotyp, nikiel, grupy -SH, fitochelatyny