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Original Research

Response of Selected *Silene vulgaris* Ecotypes to Nickel

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Abstract

The main aim of our research was to determine the response of *Silene vulgaris* ecotypes, occurring in different habitats, to increased nickel concentration. We used *S. vulgaris* seeds originating from Zn-Pb ore areas of Upper Silesia (the area adjacent to the "Szopienice" ironworks in Katowice), a serpentine dump in Wiry, as well as seeds of natural ecotype collected from an area not contaminated with heavy metals (Gajków near Wrocław). Laboratory experiments covered comparative analysis of select *S. vulgaris* ecotypes. In the course of pot experiments, it was possible to state that *S. vulgaris* ecotypes differ in morphological features (plant height, leaf shape). The other aim of research was determination response of select ecotypes to the presence of nickel in the substrate. Is *Silene vulgaris* originating from a serpentine dump characterized by a higher tolerance to elevated nickel content in a growth medium in comparison to other ecotypes. Studies with nickel (0, 30, 60, 90 mg·kg^{-1}) have shown that, according to the increase in nickel dose, there was an increase in its concentration in above-ground parts of *Silene vulgaris* ecotypes. Plants from serpentine dump accumulated considerably higher amounts of Ni than the remaining ecotypes. However, applied nickel did not influence magnesium content in above-ground parts of the examined *Silene vulgaris*. According to the increase in Ni dose, a decrease of Fe in *Silene vulgaris* shoots was observed and that phenomenon was most evident in plants of serpentine ecotype.

Keywords: *Silene vulgaris*, ecotype, nickel, dump, serpentine rocks

Introduction

One assumption of evolutional ecology is habitat variability of organisms. In the case of plants, this methodological assumption is expressed by ecotype variability of species [1]. An ecotype means populations within the frames of a particular species that are evolutionally adapted to specific environmental conditions. Ecotypes occurring in various habitats differ from one another in morphological traits (e.g. shape, size, or leaf color) [1, 2].

Bladder campion *Silene vulgaris* (Moench) Garcke is a perennial plant from the *Caryophyllaceae* family [3, 4]. It commonly occurs in Europe, North Africa and Asia, as well as in both Americas. Within Poland the genus Silene can be found on meadows, fields, and in forests [5-7], as well as on serpentine dumps, Zn-Pb ore areas, or other post-mining areas [5-7]. The exceptional adaptation abilities of this species [8] has led to the occurrence of independent ecotypes adapted to extremely unfavorable habitat conditions. In addition to *Silene vulgaris* ecotypes resistant to lead [5, 9, 10], there are also known ones tolerating excessive amounts of cadmium, zinc, copper [10-12], arsenic, and cobalt [13].

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However, *Silene vulgaris* resistance to nickel has not yet been explained. This phenomenon is usually described as an example of non-specific co-tolerance of *S. vulgaris* to different metals [13-15].

Populations of plants growing on metalliferous (and, secondarily, enriched with heavy metals) areas (dumps, heaps, etc.) have developed two strategies for growth and development in these unfavorable conditions. One of them is avoidance of metals uptake, the other is based on elimination of harmful effect of metals (tolerance). Avoidance can be achieved by immobilizing metals in the soil through excretion of root exudates, modifying soil pH, changes in the transport activity in the plasma-membrane of root cells, active efflux from roots, storing the metals in cell walls, and apoplast. Tolerance may be affected in different ways including cytosolic sequestration and enhanced efflux from cytosol to inactive compartment (vacuole, apoplast). Cytosolic sequestration is achieved through the synthesis of metal-binding compounds like amino acids, organic acids, and (poly)peptides, or thiol compounds such as glutathione, metallothioneins, and phytochelatins. Also, transmembrane metal transporters have major role in the compartmentalization of excessive metals in cells, organs, or tissue [16-19]. Plants occurring on metal reach soils can be divided into four categories: excluders, accumulators, indicators, and hyperaccumulators. Excluders restrict heavy metals transport to the shoot and accumulate them in the roots. Accumulators can translocate and accumulate high metal levels in above-ground plant parts without toxicity symptoms. Hyperaccumulators accumulate over 100-fold more metals in shoots than normal plants. Indicators are characterized by an almost linear relationship between metal content in the plant and concentration of the same element in the soil [20].

Serpentine rocks are unique in Poland, as they are limited to the Lower Silesia region [21]. These rocks are characterized by specific maternal formations of soils, affecting vegetation growing on them [21-23]. These metamorphic rocks are formed nearly exclusively from the minerals serpentine group, occupying the largest areas in the periphery of the Owl Mountains in the region of Sobótka and Jordanów (Gogołów-Jordanów massif), as well as near Ząbkowice Śląskie (Grochowa-Braszowice massif) [21, 23, 24]. Specific chemistry of serpentine rocks, originating from the transformation of ultrabasic deep-sea rocks, determines the formation of peculiar soils. They are rich in magnesium and poor in calcium (low ratio Ca:Mg), also relatively rich in Ni, Cr, and Co [25-28] to a degree exceeding the values (sometimes toxic for plants) recorded for other soils of Lower Silesia and Poland [6, 23, 24, 29]. Other limiting factor for plant growth on serpentine soils is macronutrient deficiency. Also, physical properties of the soil and the restricted water supply have harmful effect on the growth of plants. Thus, a serpentine environment is extremely different from a metal-enriched one. A crucial factor for plant survival on some serpentine soils is heavy metal tolerance, especially to Ni. One particular strategy to cope with high levels of metals shown by serpentine plants is hyperaccumulation. Serpentine plants also exhibit adaptation to water deficiency (e.g. xeromorphism), low nutrient availability, and a low calcium-magnesium ratio [20, 28].

The aim of our research was to determine the differences in morphological features of seeds and plants between three *Silene vulgaris* populations originating from sites with normal and elevated Ni levels in soil, as well as nickel accumulation capabilities of ecotypes.

Materials and Methods

Research materials were seeds obtained in 2010 from most representative plants belonging to three ecotypes of *S. vulgaris*. Ecotype "Gajków" was from a natural habitat near the town of Gajków in southwest of Wrocław [29]. Ecotype "Szopienice.250" covers an area 250 meters from the pollution emitter, i.e. Szopienice steelworks of non-ferrous metals in Katowice in Upper Silesia [7]. This site was covered with 80% vegetation, where *S. vulgaris* was the dominant species (30% all species), creating distinct patches. Ecotype "Wiry" grows on a small dump (connected with exploitation of serpentine rocks) near the small town of Wiry, not far from the western foot of Ślęża Mountain [6]. Vegetation covering this site was poor and dispersed, covering about 35% of the area. However, *Silene vulgaris* dominated and created more or less even patches.

The measurements of 25 randomly selected seeds (each ecotype of *Silene vulgaris*), were done in the widest and the narrowest site of a seed, using Axioskop 2 Plus microscope, at magnification 10x and Axio Vision 2.0 program.

Thousand seed weight (TSW) was determined by weighing 50 randomly selected seeds of each ecotype in 5 replications. Then the resulting weight value was averaged and recalculated over 1000 seeds. The obtained results were subjected to statistical analysis according to the STATISTICA program. We determined standard deviation and the least significant difference (LSD) at significance level 0.05 and there were distinguished homogenous groups.

A germination test was performed with surface-sterilized (3% H_2O_2) seeds. Three layers of filter paper were placed on a Petri dish and moistened with water. Twenty-five seeds of each ecotype were placed on each plate with the lid on, and then incubated in the dark at 23±2ºC. During two weeks germinated seeds were counted daily. Triplicate sets were performed for each ecotype.

Pot Experiment

Three *S. vulgaris* ecotypes were sown separately in pots filled with autoclaved garden soil. After two weeks three plants from each ecotype were planted in separate pots. The plants were cultivated in greenhouse conditions with availabile sunlight. After 8-9 weeks, part of plants from each ecotype were subjected to biometric examination, while the remaining part of plants (after thorough root cleaning) were placed in pots filled with 0.5 dm^3 Hoagland and Arnon nutrient solution [30]. After a two-day acclimation period, nickel sulphate was added to nutrient solution to achieve

Table 1. Thousand-seed weight of selected Silene vulgaris ecotypes.

Ecotype	1000 seed weight [g]
Gajków	0.8236^a
Szopienice.250	0.5268^b
Wiry	0.4624^b
$LSD_{0.05}$	0.0655

Letters denote homogenous group

nickel concentration equal to 0, 30, 60, and 90 µM (each dose was applied in four replications). The experiment was conducted for two weeks and during that time the nutrient was regularly aerated. After seven days the nutrient solution was changed into a new one and nickel sulphate was added (in the same doses).Two weeks later the plants were harvested and after drying underwent chemical analyses. The above-ground parts of selected *Silene vulgaris* ecotypes were hand crushed and ground. Plant material was subjected to dry mineralization (three replications). The content of Ni, Fe, and Mg was determined according to the AAS technique using a Spectra 220 Fast Sequential apparatus. The results were subjected to statistical analysis.

Biometric measurements of ecotype leaf blades were done following the methods by Kim and Park [31], which consists of measuring the following parameters: leaf blade length and width in the middle of its length, and in its widest point. Approximately 60 leaf blades of each type underwent that measurement. The obtained results were subjected to statistical analysis.

Results and Discussion

Determination of thousand-seed weight allowed us to indicate differences between the examined *Silene vulgaris* ecotypes in terms of seeds (Table 1). The mentioned TSW values differ from one to another. TSW value for the ecotype "Gajków" is significantly different from "Szopienice.250" and "Wiry." TSW of "Szopienice.250" and serpentine ecotype does not significantly differ and form a homogenous group.

Measurement results of seeds from three ecotypes of *Silene vulgaris* are shown in Table 2. It is possible to state that seed sizes of select ecotypes evidently differ from one to another. The seeds of natural population were larger than the seeds of ecotypes living in the regions contaminated with heavy metals. The "Gajków" seeds are definitely larger than the remaining genotype seeds. Their mean length was 1.48 mm, width 1.22 mm. Mean length values of "Wiry" and "Szopienice.250" seeds were 1.22 mm and 1.25 mm, respectively, while their mean width was 1.02 mm and 1.05 mm. Therefore, it is possible to state that the size of *Silene vulgaris* seeds growing in areas with higher concentrations of heavy metals in the substrate are only slightly different from one other.

Table 2. Plant height, leaf blade, and seed size of select ecotypes of *Silene vulgaris*.

Examined trait	Ecotype	Mean	Min.	Max.	Standard deviation	Variation coefficient [%]
Seed length [µm]	Gajków	1480	710	1750	237	16.0
	Szopienice. 250	1250	780	1520	163	13.0
	Wiry	1220	1070	1440	90.9	7.45
Seed width [µm]	Gajków	1220	816	1560	147	12.0
	Szopienice. 250	1050	837	1400	117	11.1
	Wiry	1030	850	1230	87.0	8.47
Plant height [mm]	Gajków	174	104	249	32.2	18.5
	Szopienice.250	148	39.0	200	39.7	27.1
	Wiry	129	52.0	214	40.5	31.4
Length of leaf blade [mm]	Gajków	22.30	15.0	40.0	5.12	22.9
	Szopienice.250	27.62	16.0	38.0	5.05	18.3
	Wiry	21.71	9.0	30.0	4.51	20.8
Maximum width of leaves [mm]	Gajków	9.19	4.0	17.0	3.77	41.0
	Szopienice.250	8.82	3.5	14.0	2.76	31.3
	Wiry	5.29	2.5	10.0	2.18	41.1
Leaf width in the middle of their length [mm]	Gajków	8.63	4.0	15.0	3.48	40.3
	Szopienice.250	7.88	3.5	13.0	2.32	29.4
	Wiry	4.68	1.5	9.0	1.91	40.9

The seeds of the natural population Gajków have a typical size, not exceeding the length range from 1.25 to 1.5 mm and the width range of 1-1.25 mm [3]. The length of seeds originating from the areas contaminated with heavy metals did not exceed the lower limit of typical length range, while seed width values could be found below lower values of width range of model seeds width for *S. vulgaris*.

The seeds of select ecotypes of *Silene vulgaris* germinated intensively between the third and the fifth days after sowing (Fig. 1). During that time, the highest number of germinated seeds belonged to serpentine ecotype. The rates of germination, expressed in percentage of germinated seeds, were similar for Szopienice.250 and Gajków ecotypes.

After the eight-week pot experiment we performed length measurements of 20 plants of each ecotype (Table 2). The highest plants were grown from the natural ecotype seeds, whose mean height was 174 mm. The plants germinated from seed ecotypes living in the vicinity of the steelworks were lower and their average height was 148 mm. The shortest plants were obtained from the seeds of serpentine ecotype, and their mean height was below 130 mm. The values of variation coefficient were higher for ecotypes from areas contaminated with heavy metals in comparison to the values of variation coefficient for the natural ecotype.

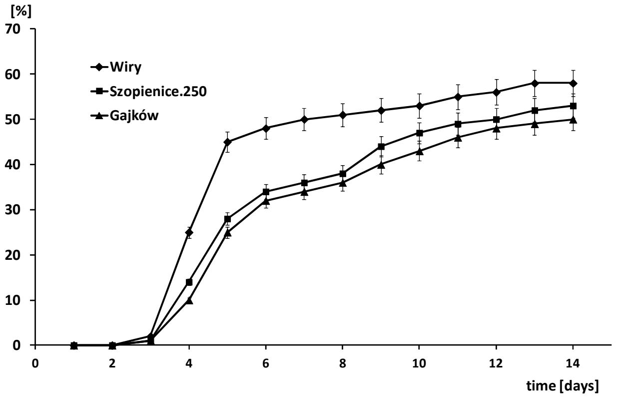

Fig. 1. Seed germination of *Silene vulgaris* ecotypes (±SE).

The results of biometric measurements of leaf blades of select *Silene vulgaris* ecotypes are shown in Table 2. Analyzing the results, it was possible to state that the longest leaves were produced by *Silene vulgaris* plants grown from the seeds originating from Upper Silesia. Average leaf length Szopienice.250 genotype was about 28 mm. Mean length of leaf blades of the remaining ecotypes – serpentine and natural ones – were similar to each other and were about 22 mm. Considering the values of leaf blade widths, it was recorded that the widest leaves were produced by plants grown from Gajków seeds genotype. Another observation dealt with the fact that in the case of plants from Gajków and Katowice, mean values of leaf blade widths varied about 4 mm. The narrowest leaves were produced by serpentine ecotype, as their mean width amounted to slightly more than 5 mm, while the narrowest leaves of that ecotype were, merely, of 1.5 mm.

Populations of *Silene vulgaris*, growing on soils rich in metal, create the forms of characteristic habit. They are small, dwarf plants of smaller leaves as compared to natural forms. The latter ones are of an upright habit, with long stem and their leaves are considerably larger and wider [5, 6, 32, 33]. Poorer growth of *S. vulgaris* plants occurring in areas contaminated with heavy metals can be explained by energy costs for the maintenance of heavy metals tolerance mechanisms, i.e. synthesis of, for instance, metallothioneins and phytochelatins [12]. The decrease in leaf blade surfaces in plants growing in dry habitats is connected with preventing excessive water loss from plants. Leaf blades can often undergo considerable reduction. The diminished transpiration surface often results in alteration in the size of the whole organism. Such a phenomenon takes place in the case of production of smaller plant cells, which, as a consequence, leads to dwarf forms of plants [32]. Scanned leaves (exemplary) of three ecotypes of *Silene vulgaris*, arranged in order from the oldest to the youngest, from left to right, are shown in Fig. 2. A comparative study conducted by Wierzbicka and Panufnik [5], regarding *Silene vulgaris* ecotype, coming from Zn-Pb ore dump from the region of

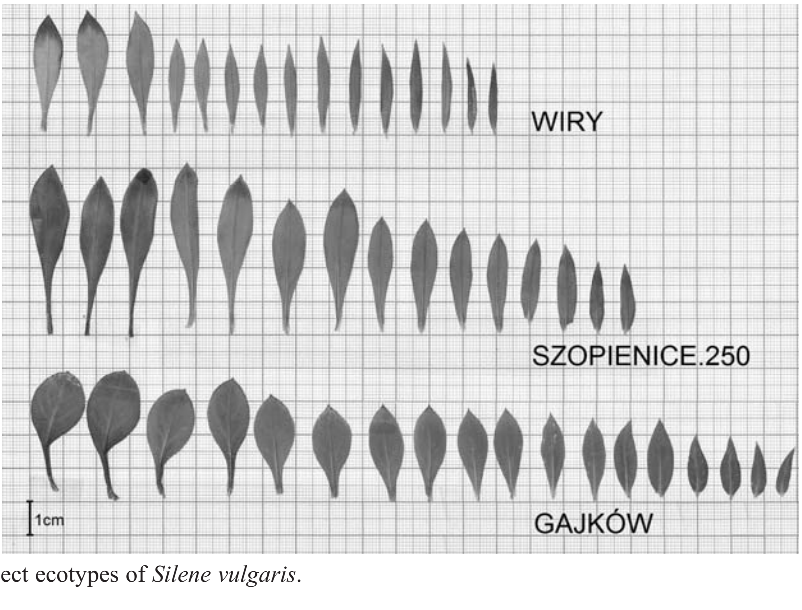

Fig. 2. Leaf shapes of select ecotypes of *Silene vulgaris*.

Olkusz, involving the natural population, proved that plants of the mentioned population feature a different general morphological habit, as well as small, narrow leaves. The leaves of population growing in areas contaminated with heavy metals were smaller, but thicker than those of the natural population. As far as plant development was concerned, the dump population is characterized by faster development and reached its generative stage much faster than the natural population.

Results and observation presented in this work are similar to the results reported by Wierzbicka and Panufnik [5], i.e. the plants of serpentine and Szopienice.250 ecotypes, resembling Zn-Pb ore area ecotype described by the authors mentioned above, were lower with narrow, lance-shaped leaves. An investigation of the Zn-Pb ore area, covered by *Silene vulgaris*, shows that alterations in the appearance of plants growing on the areas contaminated with heavy metals reflect a type "r" life strategy [33]. Species following the "r" strategy feature poorer development of vegetative parts, enter their generative stage earlier, and produce a considerable amount of small seeds characterized by poor germination. They are typical pioneer species colonizing open areas, free from competitors. Populations realizing this type of life strategy characterize great reproductive effort and small body size. They occur on unpredicted habitats and, therefore, are exposed to considerably unfavorable changes in environmental conditions [34]. The model described above fits *Silene vulgaris* populations originating from areas contaminated with heavy metals, i.e. Wiry and Katowice.

Chemical analyses allowed comparing responses of the examined populations of *Silene vulgaris* to increased nickel content in the nutrient solution. Nickel content in above-ground parts of the examined plants was shown in Fig. 3. Analyzing the obtained results, it is possible to state that nickel concentration in above-ground parts of plants increased according to the increase in nickel dose. At 30 μM Ni concentrations the value of nickel content in the plants significantly increased, and it was similar for all ecotypes. In the case of higher doses, 60 μM Ni and 90 μM Ni, significant differences in the amount of accumulated nickel in particular ecotypes became evident. The plants of serpentine ecotype accumulated in their stems and leaves the highest quantity of nickel, i.e. 317 mg Ni·kg^{-1} dry matter, at a dose of 90 μM Ni. At the same level of nickel in the nutrient solution, the plants of Szopienice.250 ecotype accumulated 283 mg Ni·kg^{-1} in their above-ground parts, while the plants of natural ecotype accumulated 265 mg Ni·kg^{-1} in the examined parts. The serpentine population showed the highest predilection for nickel. The *Silene vulgaris* population originating from the natural habitat accumulated significantly lower amounts of the examined metal and, therefore, it can be supposed that has reduced nickel accumulation capacity.

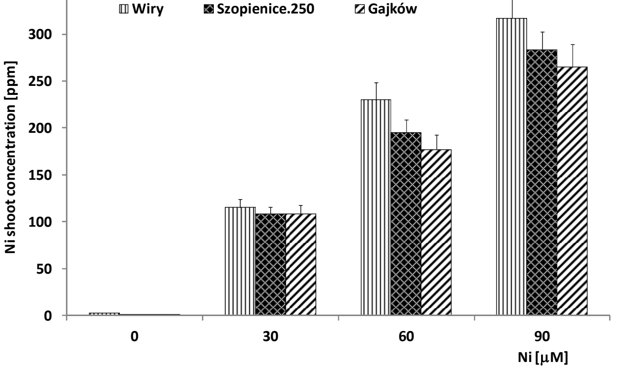

Fig. 3. Nickel content in above-ground parts of selected *Silene vulgaris* ecotypes after exposure to increasing Ni concentrations (±SE).

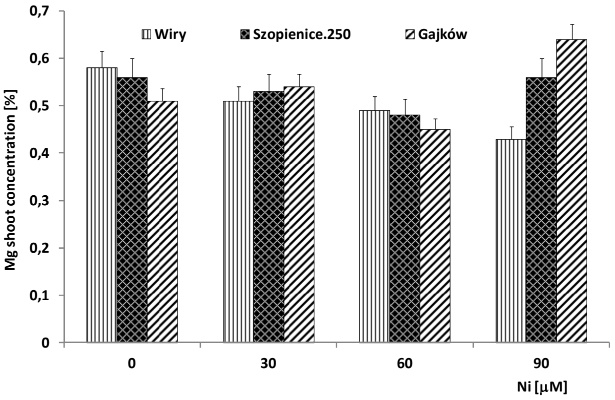

Fig. 4. Magnesium content in above-ground parts of selected *Silene vulgaris* ecotypes after exposure to increasing Ni concentrations (±SE).

Serpentine habitats, because of their specificity, are occupied by atypical vegetation. The role of nickel in their formation has not been explained so far and it varies among different areas [35, 36]. The literature has described populations of species tolerating nickel, as well as the plants of serpentine habitats that are resistant to toxic effects of nickel, but not forming nickel-tolerant ecotypes [15]. In Lower Silesian serpentine plant populations, in certain species, there can be found different tolerance to nickel, which may indicate the role of this metal as a significant factor of selection leading to the emergence of local ecotypes [35].

Fig. 4 shows magnesium content in above-ground parts of selected *S. vulgaris* ecotypes according to the nickel dose. The content of magnesium in stems and leaves of selected *S. vulgaris* ecotypes ranged 0.4-0.5% d.m. Among the examined populations of *S. vulgaris* there was not recorded a significant difference in the content of that metal in above-ground parts of plants, although at the highest dose of nickel (90 mg Ni), accumulation of this macroelement increased in the plants of Szopienice.250 and natural populations. Dzida and Jarosz [37] reported that optimal magnesium content in plant leaves should be from 0.35% to 0.8% Mg d.m. Research conducted with the plants cultivated on soils rich in nickel (serpentine soils) demonstrated that magnesium content in dry matter of above-ground parts of those plants was in the range 0.4-0.6% [23]. Magnesium and nickel, two main elements of serpentine complex, become involved in interactions in which magnesium reduces the toxic effects of nickel [38-40].

Iron contents in dry matter of above-ground parts of select ecotype *S. vulgaris* plants, according to applied nick-

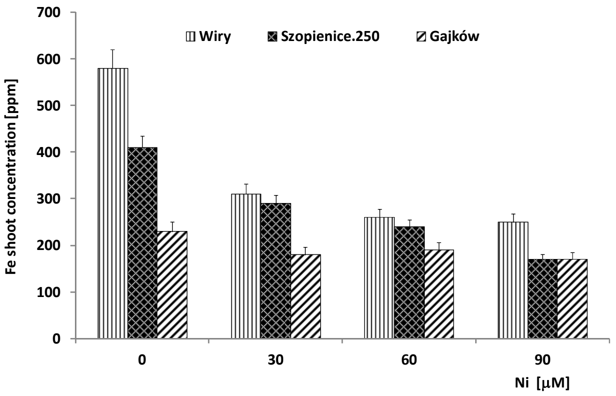

Fig. 5. Iron content in above-ground parts of selected *Silene vulgaris* ecotypes after exposure to increasing Ni concentrations (±SE).

el dosage, were shown in Fig. 5. Analysis of plant material shows that above-ground parts of control object serpentine population *S. vulgaris* contained the highest quantities of Fe in comparison to the remaining populations. According to the increase in nickel doses, the amount of iron in stems and leaves of *S. vulgaris* ecotypes decreased and that phenomenon was most evident in the plants of serpentine ecotype. The content of iron in the plants of natural population was lower than in the remaining ecotypes, but it maintained nearly the same level in the course of introduced nickel dosage.

It should be noted that ecotypes from the areas contaminated with heavy metals accumulated considerable quantities of nickel in their above-ground organs. Therefore, it can be assumed as possible that those plants accumulate higher amounts of nickel at the expense of reduced uptake of iron. In plant mineral nutrition the phenomenon of ion antagonism often occurs, which is expressed by inhibiting the uptake of some particular ions through excessive uptake of other ions. It can result from ion competition for carriers or for the carrier binding site in processes of their uptake. Nickel has a similar character to some elements that are necessary for appropriate functioning of plant organisms, such as Fe. Nickel competitiveness increases according to the increase in its concentration. It can inhibit iron absorption and cause its deficit in plants [40, 41].

Conclusions

S. vulgaris ecotypes differ in morphological features (plant height, leaf shape). Ecotype Szopienice.250 developed the longest leaves, while serpentine ecotype is characterized by the narrowest leaves. The plants of serpentine and Zn-Pb ore populations produce smaller and lighter seeds in comparison to the seeds of the natural population. Plants from serpentine dump accumulated considerably higher amounts of Ni than the remaining ecotypes. The increase in Ni dose, decrease of Fe content in *S. vulgaris* shoots, and that phenomenon was most evident in plants of serpentine ecotype. However, applied nickel did not affect the content of magnesium in above-ground parts of examined *Silene vulgaris*.

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