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PHYSIOLOGICAL RESPONSES OF NONMETALLICOLOUS AND SERPENTINE *SILENE VULGARIS* ECOTYPES CULTIVATED IN DIFFERENT SOILS

Silene vulgaris ecotypes Wiry and Gajków, originating from a serpentine heap and a natural site, respectively, were cultivated from seeds on two substrates. The former was a serpentine heap located in Wiry (Poland, Lower Silesia), the latter – natural soil located in Gajków (Poland, Lower Silesia). The growth of both ecotypes on the Wiry soil was strongly inhibited. The Wiry ecotype grown on the serpentine heap accumulated more macro-, micro-nutrients and heavy metals (Ni, Co, Cr) than ecotype Gajków. Enzyme pyrogallol peroxidase was more active in the leaves of the Wiry ecotype grown only on Wiry soil. Ecotype Gajków, grown on the serpentine heap, was characterized by higher non-protein thiol, total polyphenol and anthocyanin content. The results obtained in the study indicated heterogeneous responses between ecotypes, depending on the applied substrate, while parallel studies of tolerant and sensitive populations made possible the study of the taxon's tolerance mechanisms to heavy metals.

1. INTRODUCTION

In soils with high metal concentrations, important evolutionary processes occur. Those processes lead to genetic and physiological adaptations which enable organisms to colonize extreme environments. The majority of plant species growing on these sites display tolerance to high metal concentrations due to the limited transport of metals to, for instance, above-ground parts [1]. In Poland, sites naturally rich in metals are limited to southern areas of the country. Metal deposits occur in the Sudetes and the pre-Sudetes geological block, the Świętokrzyskie Mountains, the Carpathian Mountains, the Upper Silesian Coal Basin and its north-eastern periphery [2].

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The Sudetes Mountain range and its foothills are one of the most mineral resource rich regions of Poland due to its structure and geological history. Serpentines undoubtedly belong to the most interesting rock formations present in Poland. The particular chemistry of serpentines, formed of transformed ultra-alkaline plutonic rocks, causes the formation of unique habitats characterized by numerous negative properties resulting from, among others, potentially toxic concentrations of heavy metals such as nickel, chrome, or cobalt. This, in turn, creates a distinctive and often poor type of vegetation [2, 3]. One of the taxa on this shortlist of species is *Silene vulgaris* [3–5]. *Silene vulgaris* (Moench) Garcke is a perennial plant from the *Caryophyllaceae* family. The species is commonly found in Europe, Northern Africa, Asia, and both Americas. In Poland, the species occurs on grasslands, fields, and forests but it can also be found in synanthropic plant communities as an element of ruderal habitats such as serpentine or calamine heaps or other post-mining areas. *Silene vulgaris* is a bioindicator of heavy metal contamination and its presence was recorded in areas both naturally rich in heavy metals and contaminated by human activity. Literature provides examples of the species' unique adaptation capabilities leading to the formation of separate ecotypes adapted to extreme habitat conditions. Apart from *Silene vulgaris* ecotypes immune to lead and zinc, there are also ones able to tolerate an abundance of copper and nickel [3–5].

This species is a metallophyte and a good bioindicator of heavy metal pollution in soils [3–5]. Its presence was found in areas naturally rich in heavy metals and areas with secondary metal contamination (anthropogenic influence) [35]. Descriptions of the unique adaptation properties of the species which lead to the formation of separate ecotypes adapted to unfavorable habitat conditions have been published [3–5].

Elevated concentrations of heavy metals in the environment cause the disturbance of numerous physiological processes occurring in plants due to oxidative stress [5–9]. Oxidative stress occurs when there is an over-production of oxygen radicals in plant cells. Plants are sedentary organisms and cannot avoid stressors. Therefore, they had to evolve a efficient system of defense against reactive oxygen species (ROS). Plants are protected from the harmful effects of ROS by non-enzymatic oxidizing agents, which are low-particle chemical substances such as glutathione, tocopherol, carotenoids, flavonoids, or ascorbate. Plants are also protected by a highly specialized enzymatic system, including enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), ascorbate peroxide (APX), or glutathione reductase (GR) [5, 10–16].

The conducted experiment aimed to analyze the response of two *Silene vulgaris* ecotypes – the nonmetallicolous ecotype Gajków and the serpentine ecotype Wiry, cultivated on serpentine substrate originating from a serpentine heap and on the soil naturally low in metal content. A pot growth test conducted in a growth chamber attempted to determine the influence of experimental soils on the growth and heavy metal accumulating properties of test plants. Additionally, the experiment searched for indicators of stress caused by heavy metals. The activity of pyrogallol peroxidase, polyphenol contents, anthocyanin, and non-protein-thiol levels were studied in the selected ecotypes of *Silene vulgaris.*

2. MATERIALS AND METHODS

Silene vulgaris seeds of ecotypes Wiry were collected from plants living on a serpentine heap in location Wiry (51°058.010″N, 17°186.402″E Poland, Lower Silesia), while seeds of ecotype Gajków were taken from plants grown on natural soil in location Gajków (50°835.520″N, 16°631092″E, Poland, Lower Silesia). Seeds of each ecotype were sown on two substrates of the serpentine heap located in Wiry and natural soil located in Gajków. Experimental pots were filled with 1 kg of substrate. 50 seeds of selected *Silene vulgaris* ecotype were sown in the pots. The number of germinated plants varied between 40 and 45. Each variant was repeated four times. Plants were grown for 9 weeks in a growth chamber and watered daily for optimal soil moisture.

For analysis, bulk soil samples were air-dried, ground, and fractions below 2 mm were separated by sieving. The measured chemical properties of soil were as follows: pH determined potentiometrically in a 1:2.5 suspension soil–1 M KCl; available forms of P, K [17], Mg [18], micro-elements, and heavy metals [19] using colorimetry and atomic absorption spectrometry. At the end of the experiment, aerial biomass of plant parts was harvested, rinsed in deionized water, and dried at 50 °C. Samples were ground and digested in a muffle furnace. The resulting ash was dissolved in 1 M HNO_3. The concentrations of magnesium, micronutrients and heavy metals were determined by atomic absorption spectrometry. Other parts of collected plants were subjected to the following analyses: activity of pyrogallol peroxidase [20], total polyphenol [21] anthocyanin [22], total non-protein thiol content [23].

3. RESULTS AND DISCUSSION

The serpentine soil used was characterized by high heavy metal content (Table 1). The concentrations of Ni, Cr, Co, and Zn (mobile forms) exceeded mean values for these elements for Poland and Central Europe.

The values were, however, typical of Polish serpentine occurrences [2, 3]. The analysis also showed that for Poland, the heap material used in the experiment was characterized by a particularly low content of phosphorus and potassium while having very high concentrations of magnesium. pH equal to 7.8 is typical of serpentine habitats in Lower Silesia [2, 3]. The soil from Gajków was characterized by a low content of the selected macroelements and heavy metals.

Environmental variability is one of the main foundations of evolutionary ecology. In the case of plants, this methodological assumption expresses itself in the ecotypical diversity of species, manifested in morphological diversity (depending on the habitat), e.g., growth form, shape, size of leaf color [3–5].

Table 1

Mineral content in soil types examined

Item	Soil type	
	Gajków	Wiry
pH	7.22±0.28	7.75±0.35
Mg, mg/100 g	9.52±0.25	80.2±4.1
P, mg/100 g	3.04±0.12	0.77±0.07
K, mg/kg	5.88±0.46	2.85±0.16
Zn, mg/kg	36.8±0.21	350±28
Cu, mg/kg	22.3±0.13	19.7±0.11
Ni, mg/kg	6.25±0.43	273±18
Cr, mg/kg	3.34±0.17	12.7±0.09
Co, mg/kg	3.19±0.24	24.9±1.9

Data are means±SD, $n = 5$.

Silene vulgaris of both ecotypes growing on soils with naturally low heavy metal content (Gajków soil) were taller (Fig. 1) than plants of the same ecotypes cultivated on serpentine soil.

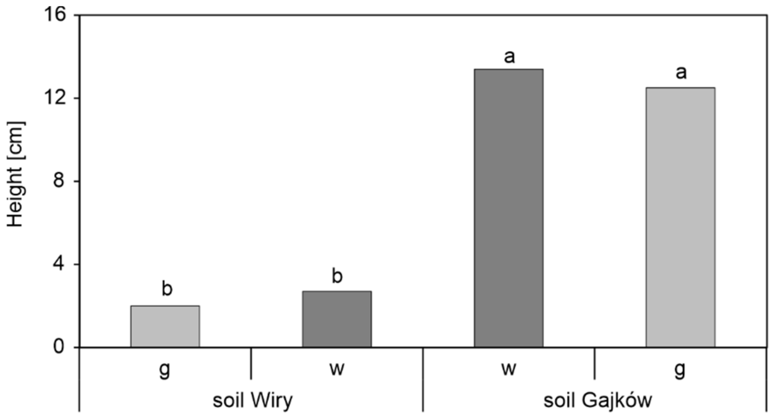

Fig. 1. Height of *Silene vulgaris* plants; w – ecotype Wiry, g – ecotype Gajków. Different letters denote significant difference between variants ($p < 0.05$)

The cost of developing tolerance and using large amounts of energy to detoxify metals in symplast manifest themselves in lower growth rate, lower biomass, dwarfism, limited shoot growth, and smaller flowers in tolerant ecotypes as compared to the sensitive ones, which was established for, among others, *Agrostis capillaris*, *Silene maritima*, *Quercus coccifera*, *Potentilla detomasii* and *Rumex acetosella* [3–5, 24]. *Silene vulgaris* of the serpentine ecotype was higher than Gajków type on both the soil rich in heavy metals and the soil with naturally low content thereof. Similar results were obtained by Wierzbicka and Panufik [4] in studies of selected types of cultivated *Silene vulgaris*.

A varied reaction of both ecotypes was also noticeable on the level of accumulated macroelements, particularly in the experiment conducted on serpentine soil, because the above-ground parts of *Silene vulgaris* of the Wiry ecotype (serpentine heap) contained more macroelements in comparison to the Gajków ecotype (Table 2).

Table 2

Mineral composition of *Silene vulgaris* ecotypes grown on different soil

Element	Ecotype/soil			
	w/Ws	g/Ws	w/Gs	g/Gs
N, g/kg	38.4±2.6	10.2±0.7	16.8±0.7	15.2±0.5
P, g/kg	1.42±0.09	0.88±0.08	5.18±0.31	3.42±0.28
K, g/kg	55.8±3.4	15.4±0.8	59.1±2.9	54.2±3.1
Mg, g/kg	16.0±0.07	12.7±0.06	2.40±0.10	3.50±0.12
Ca, mg/kg	12.8±0.09	4.90±0.38	7.05±0.41	4.03±0.29
Zn, mg/kg	49.9±3.1	23.3±1.4	41.3±1.9	37.9±1.7
Cu, mg/kg	7.23±0.38	3.96±0.23	6.16±0.36	4.75±0.28
Ni, mg/kg	45.6±4.1	25.9±1.9	4.45±0.38	3.47±0.29
Cr, mg/kg	7.52±0.69	4.82±0.38	5.70±0.47	4.47±0.36
Co, mg/kg	6.05±0.51	4.43±0.38	4.10±0,38	3.40±0.29

w – ecotype Wiry, g – ecotype Gajków, Ws – soil Wiry, Gs – soil Gajków; data are means±SD, $n = 5$.

The ecotype originating from the area naturally poor in metals experienced a disturbance in the accumulation and distribution of macroelements caused by factors unfavorable to plant growth present in the serpentine soils. It is assumed that the serpentine ecotype of *Silene vulgaris* is better adapted. As is mentioned by some authors [25–27], populations of plants with high tolerance to heavy metals are less susceptible to nutrient defects, have smaller nutrition requirements, and have distinctive mineral economy adapted to metals compared to sensitive populations.

It was determined that in *Silene vulgaris* there is a connection between the capability to accumulate phosphorus and reaching a high tolerance level to lead. In plants treated with lead, there was a significantly higher level of phosphorus in tissues as in the control group. It seems that phosphorus availability probably supports the processes connected with the accumulation and detoxification of lead in cell walls [25].

In the case of calcium, the *Silene vulgaris* tolerant to lead had a distinctive economy of this element, namely a high status of calcium in tissues and a high tolerance to calcium deficits [25]. Krupa et al. [26] observed that an elevated level of copper and cadmium in the environment leads to lower levels of calcium in plant tissues.

The higher magnesium content in experimental plants cultivated on serpentine soil in comparison to the concentration of Mg in plants collected from experimental soil with low metal content (Gajków) results from serpentine waste's richness in magnesium. A comparison of magnesium content in both *Silene vulgaris* populations growing on serpentine soils showed that the serpentine ecotype accumulated more macroelements. It is probably a consequence of this plant's adaptation to growth in toxic conditions of high magnesium content in the environment [27].

The serpentine ecotype cultivated on soil from the serpentine heap accumulated more heavy metals than the Gajków ecotype cultivated on the same soil (rich in metal) (Table 2). This may mean that the *Silene vulgaris* from the serpentine heap is adapted to typical "serpentine metals" – nickel, chromium, and cobalt. In the experiment with soil poor in metal, *Silene vulgaris* plants of both ecotypes accumulated similar (low) amounts of metals in the analyzed parts; and in that case, the Wiry ecotype contained a bit more. The serpentine ecotype is better adapted to extreme conditions resulting from metal accumulation in the environment.

A similar relation between metal content in the plant and the level of tolerance was determined in the serpentine population of *Silene paradoxa* (nickel-tolerant), which, in an experiment with increasing doses of $NiSO_4$, accumulated more nickel (a clearly demonstrable fact with higher concentrations of $NiSO_4$) in comparison to the remaining populations of the species – copper-tolerant and non-metal-tolerant [24]. The serpentine ecotype of *Silene vulgaris* cultivated in hydroponics with an addition of nickel accumulated significantly more nickel than the other ecotypes of the species used in the experiment – natural and calamine ones [3].

During the whole vegetation period, plants are susceptible to numerous biotic and abiotic environmental factors causing oxidative stress. The stress occurs in the cells of plants submitted to salinity, drought, low temperatures, herbicides, pathogen attacks, or high concentrations of heavy metals [6–9, 20, 21]. Heavy metals (Cu, Zn, Cd, Hg, Pb, Al, Cr, Ni) generate reactive oxygen species (ROS) and oxygen radicals (FR), which leads to oxidative stress – a state in which more ROS and FR emerge than the plant can metabolize. Consequently, ROS and FR cause lipid peroxidation, which, in turn, leads to damage to cell membranes and membranes within cells; nucleic acid structure, which may cause mutagenesis; protein peroxidation leading to changes in structure and their inactivation; the inactivation of the photosynthetic electron transport chain. Oxidative stress caused by heavy metals was observed in, e.g., in *Triticum aestivum*, *Phaseolus aureus*, *Helianthus annuus*, *Nicotiana tabacum*, *Arabidopsis thaliana*, *Malva silvestris*, *Brassica juncea*, *Pinus sylvestris* [5–13, 20, 21, 24]. Evolution developed numerous enzymatic and non-enzymatic defense mechanisms aimed at keeping the ROS low and harmless for cells. The activation of antioxidative enzymes in plants under the influence of heavy metals increases remains the same or lowers depending on the species, the type of ion of its concentration, and exposition time [5].

One of the most important mechanisms of plants protection against heavy metals is binding metal ions to non-protein thiols (NPT). This group of compounds includes sulfur-rich oligopeptides called phytochelatins, as well as glutathione (GSH), homoglutathione, or free cysteine [10]. Apart from being a substrate for the synthesis of phytochelatins, GSH itself has been reported to act as a heavy metal chelator. It is also a very important low-molecular antioxidant participating in glutathione-ascorbate cycle, which plays a key role in the regulation of H_2O_2 content in plant cells.

Thiols (–SH) play various roles in biological systems. The study determined the presence of non-protein thiols in the leaves of the selected ecotypes of *S. vulgaris*, though their presence was higher in plants cultivated on serpentine soils (Fig. 2). An analysis of NPT content in *S. vulgaris* leaves from serpentine soils showed that in the Gajków ecotype plants, it is higher than in the one in the Wiry ecotype leaves.

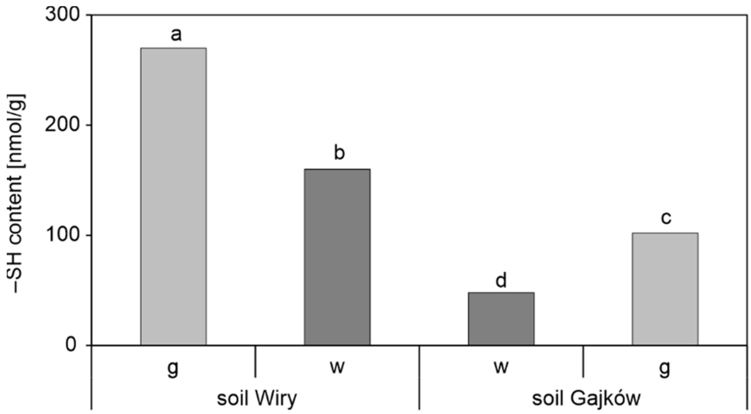

Fig. 2. Non-protein –SH group content in *Silene vulgaris* leaves; w – ecotype Wiry, g – ecotype Gajków. Different letters denote significant difference between variants ($p < 0.05$)

The function of phytochelatins in plants is connected with mechanisms of heavy metal tolerance. It is believed that phytochelatins partake in the detoxification of heavy metal ions primarily in a short exposition of plants to high concentrations of heavy metals, especially in plants with low tolerance [10–12]. However, as was reported by Knecht et al. [11], the number of phytochelatins does not always correspond with tolerance to metals.

In cases of chronic stress, consisting of long exposition to smaller doses of heavy metals, the role of phytochelatins in metal ion detoxification is also negligible, as was stated by Seth et al. [12]. It is probably the result of the high energy costs of phytochelatins synthesis. In the case of tolerance to cadmium, the content of phytochelatins in *Silene vulgaris* from the population tolerant to Cd was lower when compared to the content in plants from the sensitive population [11]. Similarly, increased tolerance to zinc in the same species was not correlated with increased production of phytochelatins.

The results showing a lower content of thiols in *Silene vulgaris* of the Wiry ecotype as compared to the Gajków ecotype may be connected with their original habitat, respectively rich and poor in heavy metals. Consequently, tolerance to the growth in conditions of higher metal content may be decisive. Due to its common occurrence in plant tissues, peroxidase is an enzyme regarded as an indicator of plant reaction to stress factors [5].

Heavy metal ions may increase, decrease or have no influence on the activity of peroxidase in plants, depending on species, plant part, or particular metal [5, 13, 14, 24].

Our research showed that pyrogallol peroxidase was more active in the leaves of the Wiry ecotype, cultivated on serpentine soil, as compared to Gajków growing on the same soil, or to the leaves of both experimental ecotypes cultivated on Gajków soil – characterized by low heavy metal content. In the case of the latter, the activity of pyrogallol peroxidase was on a similar level (Fig. 3).

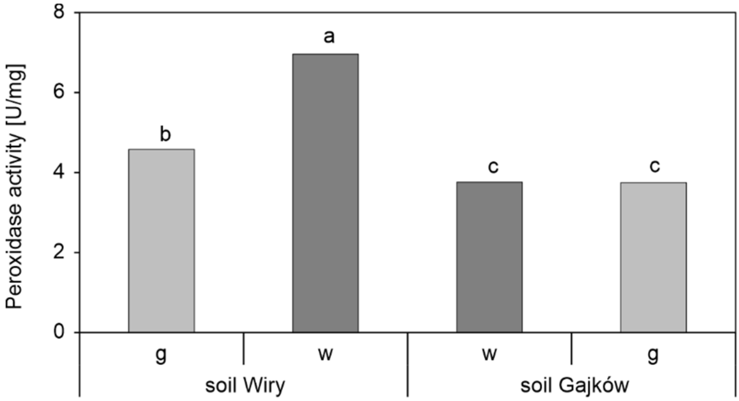

Fig. 3. Pyrogallol peroxidase activity in *Silene vulgaris* leaves; w – ecotype Wiry, g – ecotype Gajków. Different letters denote significant difference between variants ($p < 0.05$)

The study by Źróbek-Sokolnik et al. [14] noticed that Cu ions in concentrations of 500 μM caused a permanent increase, while Cu ions in the concentration of 10 μM a decrease of guaiacol peroxidase activity in suspension-cultured tobacco cells of *Nicotiana tabacum*. The presence of Cd (500 μM) caused an initial decrease, short increase, and decrease of the enzyme activity below the one observed for control.

In a *Silene paradoxa* population, characterized by a high tolerance level to nickel, studies revealed the phenomenon of limited inhibiting properties of this metal to peroxisomal enzymes. The inhibition of these enzymes activity induced by the presence of metal caused oxidative stress in plants susceptible to nickel [24].

In a study of the influence of Pb on the activity of selected antioxidants in cultivated plant tissue such as horsegram (*Macrotyloma uniflorum*) and bengalgram (*Cicer arietinum*), Reddy et al. [6] found a higher activity of pyrogallol peroxidase in *Macrotyloma uniflorum*, a species considered to be tolerant to lead, as opposed to the sensitive *Cicer arietinum.* Słomka et al. [7] found that in long-term influence of heavy metals on plants, the activity of peroxidase in *Viola tricolor* of metalliferous population was lower than in same species plants of the non-metalliferous population. Varying results concerning peroxidase in *Vaccinium myrtillus* leaves in polluted and non-polluted areas were obtained by Kandziara-Ciupa et al. [8], as the highest and lowest activity of the enzyme was observed in plants originating from polluted areas.

Phenols have anti-oxidative properties due to their high tendency to bond with both heavy metals and free radicals. These secondary metabolites in plants are divided into several groups depending on their structure. Some of them are widespread in the Plantae kingdom, while others are specific for family, or even species [5, 15]. Our research showed that the total polyphenol (Fig. 4) content was higher in *Silene vulgaris* plants of both ecotypes cultivated on serpentine soil – rich in heavy metals. Heavy metals are strong ROS generators, and the accumulation of phenol metabolites usually grows in response to the surplus of metal [15].

However, *Silene vulgaris* leaves of the Gajków ecotype cultivated on soils rich with or free of heavy metals contained more total polyphenols. In her studies conducted on several ecotypes of *Silene vulgaris*, Muszyńska et al. [5] showed that in experimental plants under stressor influence (e.g., heavy metals), the leaves of non-metalliferous ecotype accumulated far more total secondary metabolites and selected phenol groups than the metalliferous ecotypes.

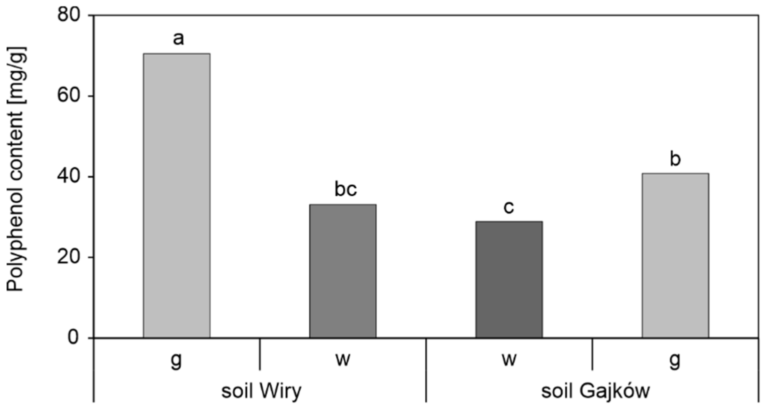

Fig. 4. Total polyphenols content in Silene vulgaris leaves; w – ecotype Wiry, g – ecotype Gajków. Different letters denote significant difference between variants ($p < 0.05$)

In the course of the experiment, it was noticed that there is a visible anthocyanin coloring of lamina and stems of the Gajków ecotype plants from the areas with naturally low metal content and cultivated on serpentine soil (Fig. 5). This effect was not observed in the Wiry serpentine ecotype cultivated on the same soil, nor in plants of both ecotypes on low metal content soil. An increased anthocyanin synthesis may be connected with their role in nullifying the toxic influence of residual elements on plants. According to Hale et al. [16] these compounds mat directly protect cells from the harmful impact of metal ions through forming lasting compounds.

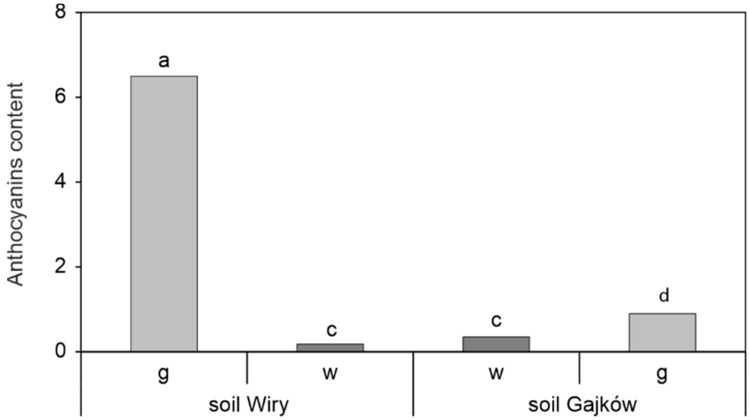

Fig. 5. Anthocyanins content in *Silene vulgaris* leaves; w – ecotype Wiry, g – ecotype Gajków. Different letters denote significant difference between variants ($p < 0.05$)

A clear disproportion between the levels of accumulated anthocyanins (Fig. 5) in the studied ecotypes, particularly those cultivated on serpentine soil. An increased accumulation of anthocyanins in the Gajków ecotype may be connected with the lowered resistance of the "natural" ecotype to heavy metals in soil, which be the first indicator of insufficient detoxification of heavy metals caused by disturbed plant physiology [16, 22]. It may also be a result of disturbed water economy and/or water deficit in a plant [22].

4. CONCLUSIONS

One of the premises of evolutionary ecology is the environmental changeability of organisms, which manifests itself in the eco typical diversity of species. The unique properties of metallicolous Silene vulgaris ecotypes are confirmed by many studies. Growth in an environment contaminated by heavy metals is reflected in the condition of the plants. It results, among others, from physiological disturbances caused by the abundance of metal. One of the symptoms of the toxic influence of heavy metals on plants is the occurrence of oxidative stress in their cells. The process is a result of generating reactive oxygen species (ROS) and free radicals (FR). However, plants have developed numerous enzymatic and non-enzymatic defense mechanisms aimed at keeping ROS at a low, harmless level. The results obtained in the study indicated heterogeneous responses between ecotypes, depending on the applied substrate, while parallel studies of tolerant and sensitive populations allowed the study of the taxon's tolerance mechanisms to heavy metals.

REFERENCES

[1] ERNST W.H.O., *Bioavailability of heavy metals and decontamination of soils by plants*, Appl. Geochem., 1996, 11, 163–167. DOI: 10.1016/0883-2927(95)00040-2.

[2] CABAŁA J., BADERA J., *Heavy metals in Poland. Geology, mining history*, [In:] M. Wierzbicka (Ed.), *Ecotoxicology. Plants, soils, metals*, Wyd. UW, Warsaw 2015, 137–162 (in Polish).

[3] KOSZELNIK-LESZEK A., BIELECKI K., *Response of selected Silene vulgaris ecotypes to nickel*, Pol. J. Environ. Stud., 2013, 22, 6, 1741–1747.

[4] WIERZBICKA M., PANUFNIK D., *The adaptation of Silene vulgaris to the growth on a calamine waste heap (S. Poland)*, Environ. Pollut., 1998, 101, 415–426. DOI: 10.1016/s0269-7491(98)00012-8.

[5] MUSZYŃSKA E., LABUDDA M., RÓŻAŃSKA E., HANUS-FAJERSKA E., KOSZELNIK-LESZEK A., *Structural, physiological and genetic diversification of Silene vulgaris ecotypes from heavy metal-contaminated areas and their synchronous in vitro cultivation*, Planta, 2019, 249, 1761–1778. DOI: 10.1007/s00425-019-03123-4.

[6] REDDY A.M., KUMAR S.G., JYOTHSNAKUMARI G., THIMMANAIK S., SUDHAKAR C., *Lead induced changes in antioxidant metabolism of horsegram (Macrotyloma uniflorum (Lam.) Verdc.) and bengalgram (Cicer arietinum L.)*, Chemosphere, 2005, 60 (1), 97–104. DOI: 10.1016/j.chemosphere.2004.11.092.

[7] SŁOMKA A., LIBIK-KONIECZNY M., KUTA E., MISZALSKI Z., *Metalliferous and non-metalliferous populations of Viola tricolor represent similar mode of antioxidative response*, J. Plant. Physiol., 2008, 165 (15), 1610–1619. DOI: 10.1016/j.jplph.2007.11.004.

[8] KANDZIORA-CIUPA M., CIEPAŁ R., NADGÓRSKA-SOCHA A., BARCZYK G., *A comparative study of heavy metal accumulation and antioxidant responses in Vaccinium myrtillus L. leaves in polluted and non-polluted areas*, Environ. Sci. Pollut. Res. Int., 2013, 20 (7), 4920–4932. DOI: 10.1007/s11356-012-1461-4.

[9] MALINOWSKA K., SMOLIK B., *Physiological and biochemical activity of spring wheat (Triticum vulgare) under the conditions of stress caused by cadmium*, Environ. Prot. Eng., 2011, 37, 1, 73–81.

[10] GUPTA D.K., TOHOYAMA H., JOHO M., *Changes in the levels of phytochelatins and related metal-binding peptides in chickpea seedlings exposed to arsenic and different heavy metal ions*, J. Plant. Res., 2004, 117, 253–256. DOI: 10.1007/s10265-004-0152-8.

[11] DE KNECHT J.A., VAN BAREN J., TEN BOOKUM W.M.,WONG FONG SANG W.F., KOEVOETS P.L.M., SCHAT H., *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.

[12] SETH C.S., CHATURVEDI P.K., 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.

[13] PUKACKA S., PUKACKI P., *Seasonal changes in antioxidant level in Scots pine (Pinus sylvestris L.) needles exposed to industrial pollution. II. Enzymatic scavenders activities*, Acta. Physiol. Plant. (Poland), 2000, 4, 457–464. DOI: 10.1007/s11738-000-0089-z.

[14] ŹRÓBEK-SOKOLNIK A., GÓRSKA K., GÓRECKI R.J., *The activity of antioxidant enzymes in suspension cultured tobacco cells treated with heavy metals*, Pol. J. Nat. Sci., 2007, 22 (4), 704–713. DOI: 10.2478/v10020-007-0060-1.

[15] KOVÁČIK J., KLEJDUSB B., HEDBAVNY J., ZOŃ J., *Significance of phenols in cadmium and nickel uptake*, J. Plant. Physiol., 2011, 168, 576–584. DOI: 10.1016/j.jplph.2010.09.011.

[16] HALE K.L., TUFAN H.A., PICKERING I.J., GEORGE G.N., TERRY N., PILON M., PILON-SMITHS E.A.H., *Anthocyanins facilitate tungsten accumulation in Brassica*, Physiol. Plant., 2002, 116, 351–358. DOI: 10.1034/j.1399-3054.2002.1160310.x.

[17] EGNER H., RIEHM H., DOMINGO W.R., *Untersuchungen über die Chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung*, Kungliga Lantbrukshögskolans Annaler, 1960, 26, 199–215.

[18] SCHACHTSCHABEL P., *Die Bestim mungdes Manganversorgungsgrades von Böden und seine Beziehung zum Auftreten der Dörrfleckenkrankheitbei Hafer*, Zeitsch. Pflanz. Düng, Bod., 1957, 78 (123), 147.

[19] RINKIS G.J., *Methods of fast colorimetric determination of microelements in biologic species*, Izd. Akad. Nauk Latv. SSR, Riga 1963 (in Latvian).

[20] KNÖRZER O.C., DURNER J., BOGER P., *Alterations in the antioxidative system of suspension-cultured soybean cells (Glycine max) induced by oxidative stress*, Physiol. Plant., 1996, 97, 388–396. DOI: 10.1034/j.1399-3054.1996.970225.x.

[21] JULKUNEN-TIITTO R., *Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics*, J. Agric. Food Chem., 1985, 33 (2), 213–217. DOI: 10.1021/jf00062a013.

[22] POLITYCKA B., GOLCZ A., *Content of chloroplast pigments and anthocyanins in the leaves of Ocimum basilicum L. depending on nitrogen doses*, Folia Hortic. Ann., 2004, 16 (1), 23–29.

[23] MAAS F.M., DE KOK L.J., PETERS J.L., KUIPER P.J.C., *A comparative study of the effects of* H_2S *and* SO_2 *fumigation on the growth and accumulation of sulfate and sulfhydryl compounds in Trifolium pratense L., Glycine max MERR and Phaseolus vulgaris L.*, J. Exp. Bot., 1987, 38, 1459–1469. DOI: 10.1093/jxb/38.9.1459.

[24] GONELLI C., GALARDI F., GABBRIELLI R., *Nickel and copper tolerance and toxicity in three Tuscan population of Silene paradoxa*, Physiol. Plant, 2001, 113, 507–514. DOI: 10.1034/j.1399-3054.2001.1130409.x.

[25] ANTOSIEWICZ D.M., *The relationship between constitutional and inducible Pb-tolerance and tolerance to mineral deficits in Biscutella laevigata and Silene inflata*, Environ. Exp. Bot., 1995, 35, 55–69. DOI: 10.1016/0098-8472(94)00026-2.

[26] KRUPA Z., SIEDLECKA A., SKÓRZYŃSKA-POLIT E., MAKSYMIEC W., *Heavy metal interactions with plant* nutrients, [In:] M.N.V Prasad, K. Strzałka (Eds.), *Physiology and biochemistry of metal toxicity and tolerance in plants*, Kluwer Academic Publishers, Dordrecht 2002, 287–301. DOI: 10.1007/978-94-017-2660-3_11.

[27] PROCTOR J., MCGOWAN I.D., *Influence of magnesium on nickel toxicity*, Nature, 1976, 260, 134. DOI: 10.1038/260134a0.