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HEAVY METALS BIOACCUMULATION AND PHYSIOLOGICAL RESPONSES TO HEAVY METAL STRESS IN POPULATIONS OF *Silene vulgaris* Moench (Garcke) FROM HEAVY METAL CONTAMINATED SITES

BIOAKUMULACJA METALI CIĘŻKICH I ODPOWIEDŹ FIZJOLOGICZNA ROŚLIN *Silene vulgaris* Moench (Garcke) Z TERENÓW ZANIECZYSZCZONYCH METALAMI CIĘŻKIMI

Abstract: In this study we have evaluated the heavy metals accumulation in the leaves of *Silene vulgaris* and its influence on glutathione, free amino acids and anthocyanins concentrations.

Glutathione is a tripeptide (γ-Glu-Cys-Gly) which is involved in many metabolic processes of a plant cell. It is related to the sequestration of xenobiotics and heavy metals and it is also an essential component of the antioxidant system, which keeps reactive oxygen species under control.

The accumulation of anthocyanin pigments in the leaves can be induced by environmental and anthropogenic stressors, such as pollution, osmotic stress, and nutrients deficiency. The positive role of anthocyanins as well as amino acids and organic acids in metal sequestration is suggested.

Samples of *Silene vulgaris* leaves were collected in May and July 2003–2005 from the heavy metals contaminated sites – from the nearest vicinity of non-ferrous metal smelter Szopienice at the distance of 50, 250, 450 m, from zinc wastes heap in Katowice and from a former calamine site in Dąbrowa Górnicza (the South of Poland). In the previous study we noted that the nearest vicinity of non-ferrous metal smelter Szopienice was more polluted than the other areas. The highest Zn, Cd and Pb accumulation in the leaves of *Silene vulgaris* was noted in the plants collected in the nearest vicinity of the non-ferrous metal smelter Szopienice. The free amino acids concentrations have differed in the vegetative season. It was higher in the most polluted sites (50 and 250 m) in July. Higher content of the reduced form of glutathione was estimated in the plant leaves collected in the distance of 250 m from the emitter in July than at the beginning of the study. Also the highest anthocyanins accumulation in *Silene vulgaris* leaves was noticed in July and in plants from the most polluted area (250 m).

The estimations, especially glutathione content, seem to be promising in ecophysiological research connected with heavy metals stress in plants growing in the postindustrial areas.

Keywords: heavy metals, *Silene vulgaris*, glutathione, amino acids, anthocyanins

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The metallurgical processing of zinc, lead, copper and iron is responsible for considerable amounts of heavy metals introduced into environment (especially plant emissions, postindustrial dumps) [1–5]. Metallophyte – *Silene vulgaris* – is a specific plant to the calamine areas and it is a pioneer plant in the nearest vicinity of non-ferrous metal smelter [6–11]. Such plants are tolerant of high heavy metals concentration and have high heavy metals accumulation ability [7, 11–13]. Several plant species such as *Silene vulgaris*, *Cardaminopsis arenosa*, *Plantago laceolata* have been shown to be good bioindicators of soil contamination with lead and zinc [3, 6]. Many investigations were undertaken to determine plants defense against heavy metals. The investigators looked for good stress and defense indicators. Glutathione is involved in the cellular defense against the toxic influence of xenobiotics as well as metal cations [14, 15] The positive role of anthocyanins next to amino acids and organic acids in metal sequestration is suggested [16, 17]. Anthocyanins are associated with the enhanced tolerance to effects of chilling and freezing, to heavy metal contamination, to dessication and to wounding [18].

The aim of this work was to determine the heavy metal bioaccumulation in leaves of *Silene vulgaris* and to define the responses (glutathione, free amino acid and anthocyanin content) of *Silene vulgaris* to environmental metals exposure.

Material and methods

The investigation was carried out in May and July in 2003–2005. The leaves from *Silene vulgaris* (Moench) Garcke plants (from 20 individuals) were collected in the nearest vicinity of non-ferrous plant Szopienice at the distances of 50, 250, 450 m, on the zinc smelter spoil heap in Katowice Wełnowiec (H) and from the former calamine site in Dąbrowa Górnicza (C). In order to determine the heavy metal concentration, the plant material was washed in tap and in distilled water, dried at 105 ^{o}C to a constant weight and ground to fine powder, then mineralized and dissolved in 10 % HNO_3. After filtration Zn, Pb and Cd contents were measured using flame Atomic Absorption Spectrometry (AAS) [19]. The quality of the analytical procedure was controlled by using the samples of the reference material in each series of analysis. (Certified Reference material CTA-OTL-1 Oriental Tobacco Leaves). Anthocyanins was estimated according to method of Fuleki and Francis [20]; Giusti and Wrolstad [21] by extracting the pigment in methanol/HCl (99/1, v/v). Glutathione (reduced and oxidative forms) was extracted from plants by homogenizing plant material (0.5 g) at 4 ^{o}C in ice cold 5 % trichloroacetic acid with the addition of Na-phosphate buffer (pH 7.5) and then centrifuged at 11000 × g for 10 min and determined according to method of Anderson [22] and Nishimoto et al [23]. The supernatant was used for GSH and GSSG derminations by the DTNB-GSSG reductase recycling procedure. GSSG was determined after GSH had been removed by 2-vinylpyridine derivatizations. Changes in the absorbance of the reaction mixtures were measured at λ = 412 nm. Free amino acids were extracted from plants by homogenizing plant material (0.5 g) at 4 ^{o}C in 80 % acetone and determined with the usage of ninhydrin method of Keller [24]. All plant samples were carried out in six replications. The data was processed using the software

Statistica to compute significant statistical differences between samples ($p < 0.05$) according to Tukey's multiple range test and to compute Pearson's correlation coefficient.

Results and discussion

The highest bioaccumulation of the investigated metals was found in the nearest vicinity of non-ferrous plant "Szopienice". Cd (50, 250 m) and Zn (all investigated areas) were above the toxic level, Pb amounts accumulated in *Silene vulgaris* leaves were in the toxicity range [25] (Table 1). In the earlier study in these stands the declined plant biomass was noticed. However, the highest metal fitoextraction was found for plants in the distance of 450 m [13].

Anthocyanins can be formed as a reaction to lots of adverse environmental conditions. At nutrient shortage, such as N- and P-deficiency, a surplus of carbohydrates can be stored as anthocyanins [26]. The highest concentration of anthocyanins was found in the plant leaves collected in the distance of 250 m from the emitter in July (Fig. 1). Anthocyanins can be associated with enhanced resistance to the heavy metals contamination. The changes of plant colours (due to increased anthocyanins concentration) may be the first indication of insufficient detoxification of metals resulting in deregulation of a plant physiology. However these symptoms may be not necessarily related to a surplus but also to the shortage of metals [27]. Under strong light (in environmental condition in July in our study), however, the anthocyanins serve as a useful optical filter diverting excessive high energy quanta away from an already saturated photosynthetic electron transport chain. Chloroplasts irradiated with light that has first passed through a red filter have been shown to generate fewer superoxide radicals, thereby reducing the propensity for structural damage to the photosystems.

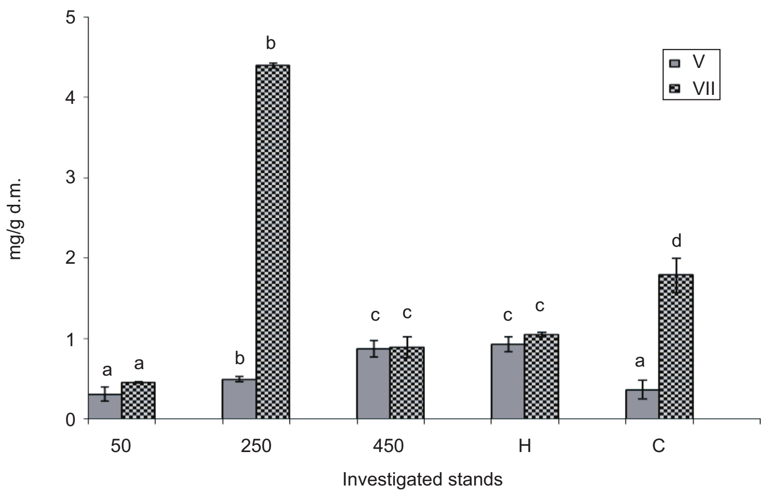

Fig. 1. Mean anthocyanins concentration in leaves of *Silene vulgaris*. Values with the same letter (for the month) are statistically the same for $p < 0.05$

Table 1

Mean heavy metals concentration in *Silene vulgaris* leaves. Values with the same letter (in the columns) are statistically the same for $p < 0.05$

Metal / Site	Zn				Pb				Cd			
	May	SD	July	SD	May	SD	July	SD	May	SD	July	SD
50 m	3800.1a	145.5	4183a	125.6	241.5a	34.3	260.2a	28.3	38.5a	1.50	40.23a	3.5
250 m	3627.2a	241.7	3959.1a	119.5	112.5b	16.1	60.6b	6.6	31.51b	1.70	26.42b	1.5
450 m	1549.3b	180.5	2590b	130.1	25.2c	6.6	18.5c	2.5	4.1c	1.90	6.32c	1.4
H	2482.2c	102.7	2888.5c	148.2	29.9c	14.1	21.42c	4.9	4.3c	1.02	2.1d	0.8
C	846.1d	72.1	806.4d	34.8	35.23c	2.3	13.95d	2.5	7.5d	1.80	5.15e	0.4

Most likely elevated concentration of anthocyanins does not indicate a high surplus of heavy metals. Gould emphasized [28], that anthocyanins offer multifaceted, versatile and effective protection to a plant under stress.

Higher concentrations of free amino acids were found in May rather than in July, in plant leaves, where smaller metal bioaccumulation was noted. However the free amino acid content increased in the plant leaves collected in most polluted site in the distance of 50 m from the emitter during vegetative season – in July (Fig. 2). Amino acids and particularly phytochelatins and glutathione play an important role in metal binding [17]. Lesko and Sarcadi showed that cadmium treatment at higher concentration caused the highest accumulation of total amino acid content in the shoots and roots of wheat seedlings [29]. Positive correlation between metal concentration and free amino acid content was found in our study (Correlation coefficient were 0.8 for Zn, 0.9 for Cd and 0.7 for Pb). Heavy metals treatment modified free amino acid composition and concentration. It may be suggested that proline is involved in detoxification of heavy metals [14, 17]. Metal tolerant populations of *Deschampsia* and *Silene* have been shown to have higher constitutive content of proline as compared with nontolerant counter-parts. *Allysum,* when exposed to nickel produce histidine, proportionally to an applied Ni dose [32]. The further research is needed to find out that free amino acid could be indicator of metal stress in laboratory and in the environmental conditions.

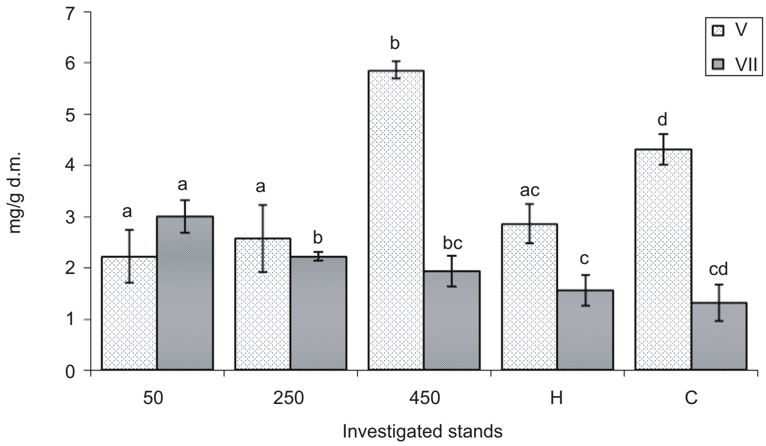

Fig. 2. Mean free amino acids content in *Silene vulgaris* leaves in May and July. Values with the same letter (for the month) are statistically the same for $p < 0.05$

The higher concentrations of the reduced form of glutathione were observed in the leaves of *Silene vulgaris* in July rather than in May. The increased concentration of glutathione in plant tissues in the distance of 250 m suggested a positive role of this tripeptide in plant metal defense (Figs. 3, 4). However, strong positive correlation was noted only with Zn (correlation coefficient was 0.84). The increased content of GSSG forms was observed in May. The increased GSSG level facilitates formation of mixed disulfides (protein gluthationation), which induces changes in redox status of thiols

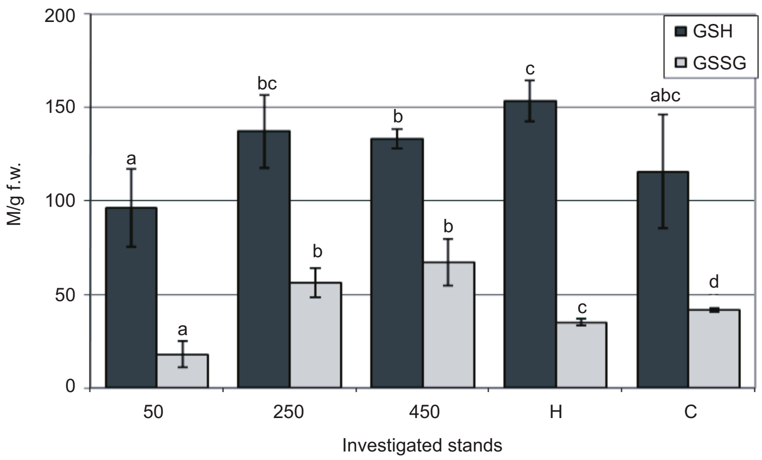

Fig. 3. Mean glutathione content in leaves of *Silene vulgaris* in May. Values with the same letter (for the glutathione form) are statistically the same for $p < 0.05$

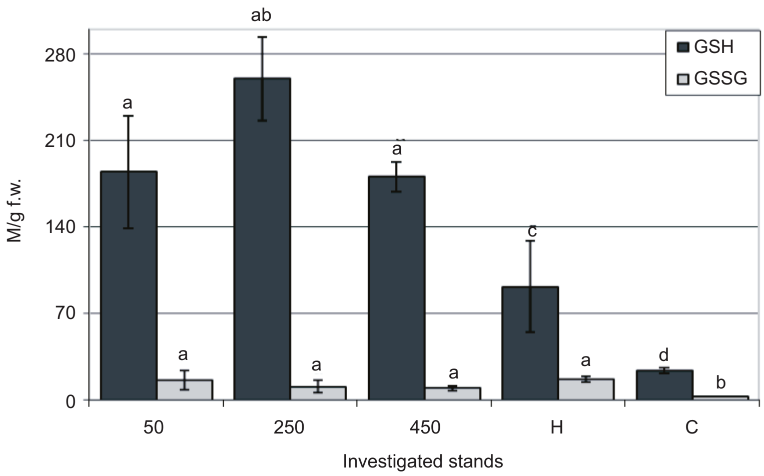

Fig. 4. Mean glutathione content in leaves of *Silene vulgaris* in July. Values with the same letter (for the glutathione form) are statistically the same for $p < 0.05$

[30]. Bruns et al pointed that the intracellular sequestration of heavy metals by GSH is favored in mosses treated with Cd [31]. Glutathione is related to the sequestration of xenobiotics and heavy metals and is also an essential component of the cellular antioxidative defense system, which keeps reactive oxygen species (ROS) under control [15]. Glutathione could be suitable candidate as a stress marker. Strong evidence has indicated that the elevated GHS concentration is connected with the plant ability to metal induced oxidative stress [32, 33].

Conclusions

Zinc was bioaccumulated in the highest amount in *Silene vulgaris* leaves on all investigated stands. The highest bioaccumulation of all investigated metals (Zn, Cd and Pb) was noticed for *Silene vulgaris* leaves collected in the distance of 50 and 250 m from the smelter.

Positive correlation between heavy metal concentrations and free amino acid contents in *Silene vulgaris* leaves was found. It is very likely that free amino acids were involved in plant heavy metals defense. The problem needs further investigations.

The anthocyanins content increase in July in the plant leaves from the most polluted area could be an effective protection to the plant under stress, however not only heavy metals.

The glutathione content was the highest in the plant leaves collected from the distance of 250 m from the smelter, where high levels of heavy metal concentrations in plant leaves was noted. It seems to be promising in further investigations.

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BIOAKUMULACJA METALI CIĘŻKICH I ODPOWIEDŹ FIZJOLOGICZNA ROŚLIN *Silene vulgaris* Moench (Garcke) Z TERENÓW ZANIECZYSZCZONYCH METALAMI CIĘŻKIMI

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Abstrakt: Przeprowadzono badania nad akumulacją Zn, Pb i Cd w liściach *Silene vulgaris* i wpływie tych metali na zawartość glutationu, wolnych aminokwasów i antocyjanów. Glutation jest tripeptydem (γ-Glu-Cys-Gly), który jest zaangażowany w obronę przed ksenobiotykami i metalami ciężkimi i jest podstawowym komponentem komórkowej antyoksydacyjnej obrony, utrzymującym reaktywne formy tlenu (ROS) pod kontrolą. Akumulacja barwników antocyjanowych może być powodowana przez środowiskowe i antropogenne czynniki stresowe, takie jak zanieczyszczenie, stres osmotyczny, niedobór składników odżywczych. Sugeruje się pozytywną rolę antocyjan obok, wolnych aminokwasów i kwasów organicznych w oddzielaniu metali ciężkich.

Liście roślin *Silene vulgaris* zbierano w maju i lipcu 2003–2005 z terenów zanieczyszczonych metalami ciężkimi (z najbliższego sąsiedztwa Huty Metali Nieżelaznych „Szopienice" w odległości 50, 250, 450 m, z hałdy pocynkowej w Katowicach Wełnowcu oraz terenu po eksploatacji galmanu w Dąbrowie Górniczej). W poprzednich badaniach wykazano największe zanieczyszczenie metalami ciężkich na terenie najbliżej położonym przy Hucie Metali Nieżelaznych „Szopienice". Największą akumulację Zn, Cd, Pb stwierdzono w liściach roślin *Silene vulgaris* zbieranych w najbliższym sąsiedztwie emitora (50, 250 m). Stężenie wolnych aminokwasów różniło się podczas sezonu wegetacyjnego. Zawartości były większe na najbardziej zanieczyszczonym terenie w lipcu niż na początku badań. Większą zawartość zredukowanej formy glutationu oznaczono w liściach roślin zbieranych w odległości 250 m od emitora w lipcu niż na początku badań. Zanotowano także największą akumulację antocyjanów w liściach roślin w lipcu i u roślin z najbardziej zanieczyszczonego terenu (250 m). Szczególnie oznaczanie zawartości glutationu wydaje się być obiecujące w ekofizjologicznych badaniach nad stresem wywoływanym przez metale ciężkie u roślin na terenach poprzemysłowych

Słowa kluczowe: metale ciężkie, *Silene vulgaris,* glutation, wolne aminokwasy, antocyjany