Polish J. of Environ. Stud. Vol. 20, No. 3 (2011), 599-604

Original Research

Effects of Zn, Cd, Pb on Physiological Response of *Silene vulgaris* Plants from Selected Populations

Aleksandra Nadgórska-Socha*, Marta Kandziora-Ciupa,
Ryszard Ciepał, Katarzyna Walasek

Department of Ecology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland

Received: 19 July 2010
Accepted: 4 January 2011

Abstract

In *Silene vulgaris* plants (Szopienice population, Heap population, Calamine population) grown on substrate with the addition of Cd, Zn, and Pb, accumulation of heavy metals and antioxidant stress parameters were compared. The highest accumulation of the studied elements was noticed in leaves of Heap population. There was also shown different sensitivity of antioxidant system in *Silene vulgaris* leaves in response to the studied heavy metals. The highest concentration of non protein thiols and proline was shown in the plant leaves of Calamine population grown on substrate with the addition of Cd and a mixture of metals. In the plant leaves of Szopienice population grown on substrate with the addition of Zn and the substrate with the mixture of metals in the case of Heap population plants, we noticed the growth of guaiacol peroxidase activity.

Keywords: *Silene vulgaris*, heavy metals, oxidative stress

Introduction

Industrial development results in environmental degradation. Increased heavy metal concentrations noted in soils, plants, and animals tissues are one of its effects. Plants show a great ability to adapt their metabolism to rapid changes in the environment, such as elevated heavy metals concentrations [1, 2]. Plant species resistant to the elevated concentrations in soil have evolved. Metallophytes are plants characterized by a natural tolerance to high concentrations of metals. One of them is *Silene vulgaris* (Moench) Garcke, which has been proven to be a good bioindicator of heavy metals soil contamination, especially with zinc and lead [3-6].

The elevated concentration of metals caused functional disorders for many physiological processes in plants, perhaps from the oxidative action of metals. Upon exposure to heavy metals, plants often synthesize a set of diverse metabolites that accumulate to concentrations in the milimolar range, particularly specific aminoacids (such as proline and histidine, and different thiols such as glutathione and phytochelatins (PC) [7, 8]. It has been shown that changes in the above-mentioned antioxidant levels were a successful response to metal toxicity. Changes of different antioxidative enzymes such as guaiacol peroxidase (POD), superoxide dismutase (SOD), and catalase CAT were observed [8, 9]. The aim of the study was to compare the accumulation of heavy metals in leaves of *Silene vulgaris* whose seeds came from the nearest surrounding of the Nonferrous Smelting Plant "Szopienice" (Szopienice population), the heap of Metallurgical Plant Silesia (Heap population), and the former calamine site in Dąbrowa Górnicza (Calamine population) on the substrate with the addition of Cd (100 mg/kg), Zn (6,000 mg/kg), and Pb (1,000 mg/kg) separately and with a mixture of them. Looking for indicators of stress caused by heavy metals, we studied the activity of guaiacol peroxidase, proline content, and non-protein thiol level in leaves of the investigated species *Silene vulgaris*.

---

*e-mail: aleksandra.nadgorska-socha@us.edu.pl

Table 1. The concentrations of heavy metals in the available fractions of soils extracted with $CaCl_2$ in different variants of contaminated substrate in mg·kg^{-1}; average ± SD.

Substrate version / Element	control	Cd	Zn	Pb	Mixture of metals
Cd	0.17±0.1 a	1.33±0.8 b			4.76±0.3 c
Zn	3.4 ±1.5 a		361.1±13.3 b		379.0±28.4 b
Pb	1.12±0.2 a			1.55±0.4 a	3.6±0.9 b

a,b,c – different letters denote significant differences between metal concentrations in contamination variant ($p<0.05$).

Experimental Procedures

Seeds of *Silene vulgaris* populations were collected from heavily polluted sites in the vicinity of the Szopienice former non-ferrous smelting plant in Katowice (Szopienice population), the zinc wastes heap located in Katowice (Heap population), and at a former calamine site in Dąbrowa Górnicza (where in the 19th century Zn and Pb ores were exploited) (Calamine population). The seeds collecting area was located in southern Poland. The seeds were germinated and grown (25 plants per pot, 5 replicates) in commercial peat-based substrate (organic matter content 30%, pH 5.5-6.5- $CaCl_2$). The substrate was sieved through a 2 mm sieve. The substrate had been amended one month previously with Zn (6,000 mg·kg^{-1}, Cd (100 mg·kg^{-1}) and Pb (1,000 mg·kg^{-1}) separately and with mixtures of them. Substrate without heavy metals was established as control. The heavy metals were all added to the substrate as a nitrate salt.

After 90 days of cultivation the leaves of ten individuals were taken for analysis. In order to determine the heavy metal concentration, the plant material was washed in tap and distilled water, dried at 105ºC to constant weight and ground to fine powder. Then subsamples (0.2 g) of finely ground plant tissue were digested with 4 ml of HNO_3 in 120ºC and diluted to 10 ml with redistilled water. Next, the wet mineralization and dilution metal contents (Zn, Pb, and Pb) were measured with flame absorption spectrometry (Unicam 939 Solar). The quality of analytical procedure was controlled by using samples of the reference material in each series of analysis (Certified Reference material CTA-OTL-1 Oriental Tobacco Leaves). Heavy metals contents were extracted using 0.01 M $CaCl_2$ – a potentially bioavailable fraction. The contents of metals were measured in the filtered extracts [10, 11].

Guaiacol peroxidase POD (E.C. 1.11.1.7) activity was measured according to Fang and Kao [12]. For the purposes of enzyme extraction the leaf tissues were homogenized with 100 mM sodium phosphate buffer (pH 6.8) for POD chilled pestle and mortar. The homogenate was centrifuged at 12,000 × g for 20 minutes, and obtained supernatant was used for determination of enzymes activity. The whole extraction procedure was carried out at 4ºC. Guaiacol was used as a substrate for POD activity. The increase in absorbance was recorded in the spectrophotometer at 470 nm and a unit of peroxidase activity was expressed as a change in absorbance per minute and per gram fresh weight of tissue [12]. The acid-ninhidryn, method was used to determine the proline content. Plant material (0.5 g) was homogenized in 10 ml of sulfosalicylic acid (3 g per 100 ml) and the homogenate was filtered through Whatman No. 2 filter paper. The reaction mixture containing 2 ml of homogenate, 2 ml acid ninhydrin, and 2 ml of glacial acetic acid was incubated at 100ºC for 1 h. The reaction mixture was placed on ice and extracted with 4 ml toluene. The absorbance was read at 520 mn using toluene for a blank. The proline content expressed as μmol proline g^{-1} fresh weight was calculated as described by Bates et al. [13]. The total content of the non-protein –SH groups was determined according to Mass et al. [14]. The plant material was homogenized in mortar with the addition of 5 vol/g mixture of the composition 2% 5-sulphosalicylic acid, 1 mM Na4-EDTA, and 0.15% sodium ascorbate. The extract was centrifuged at 20,000 × g for 10 min. Absorbance was read at wavelength λ=415 nm 1 min after the addition of DTNB. The number of the non-protein –SH groups was calculated from the standard curve made with the use of L-cysteine. Five replications for each analysis were done. The data were processed using Statistica software (version 9.1) to compute significant statistical differences between samples ($p<0.05$) according to Tukey's multiple range test, and to compute Pearson´s correlation coefficients.

Results

The contents of $CaCl_2$-extracted Zn, Cd, and Pb were low compared to the doses added to the substrate. The concentrations in potentially bioavailable fraction of substrate were higher in substrate where mixture of metals (Zn, Pb, Cd) had been amended. Zn concentrations were on a comparable level in both amended substrata (Table 1). Zn was more extractable than Cd. 6-6.31% of the added Zn was $CaCl_2$-extracted compared with Cd, which was extracted in 1.33-4.76%. Potentially bioavailable Pb contents made up below 1% of the dose added to the substrate (Table 1). The mean heavy metal concentrations in *Silene vulgaris* leaves (in mg/kg d.w.) were found in descending order, to be Zn, Pb, Cd, respectively (Table 2). Concentrations of Zn ranged from 1,273 to 3,737 mg·kg^{-1} d.w. (heap population). The Zn concentrations (except Szopienice plants) were higher in the plants growing on substrate with a mixture of metals. The lower concentration of Pb was found in leaves of Szopienice population plants. The higher concentration of about 107

Table 2. Concentrations of Cd, Zn, and Pb in leaves of *Silene vulgaris* plants from select populations; average ± SD.

Population	Calamine	Szopienice	Heap
Substrate version			
Amended with Cd	11.7±0.9 b	2.6±0.9 b	5.5±1.3 b
Amended with mixture of metals	40.6±1.4 c	23.1±5.0 c	39.2±4.7 c
Control	0.14±0.05 a	0.12 ±0.05 a	0.2±0.07 a
Amended with Zn	2,082.5±287.8 b	1,866.3±383.7 b	1,272.7±362.7 b
Amended with mixture of metals	2,627.1±35.4 c	1,596.7±306.4 b	3,736.5±494.3 c
Control	57.1±19.1a	77.11±11.1 a	71.3±3.6 a
Amended with Pb	41.94±8.5 c	23.22±7.03 b	69.05±12.11 b
Amended with mixture of metals	23.71±3.11 b	26.73±4.11 b	106.75±5.61 c
Control	3.71±0.33 a	1.32±0.67 a	3.03±0.87 a

a,b,c – different letters denote significant differences between metal concentrations in one population ($p<0.05$).

mg·kg^{-1} was estimated for Heap plants. The cadmium concentrations ranged from 11.7 mg·kg^{-1} d.w. (Calamine plant population on substrate with Cd) to 41 mg·kg^{-1} d.w. (Calamine plant population on substrate with a mixture of metals). The content of Cd stated in *Silene vulgaris* leaves of all the studied populations was much higher in plants grown on the substrate with the mixture of metals (Table 2). We detected some differences in the concentration of non-protein thiols. An increase of –SH groups amounts in response to Cd, Zn, and Pb, and mixture of metals was observed in leaves of Heap population to Cd, Pb, and mixture of metals in leaves of Szopienice population to Cd and mixture of metals also in leaves of Calamine population. The highest thiols concentration was noted in leaves of plants of the Calamine population, grown on substrate with metals mixture (Table 3). A positive correlation between Cd and thiol concentrations was found (correlation coefficient = 0.64). There was shown an increase of proline concentration in leaves of Calamine population in response to Pb, Cd, and mixture of metals, and in Heap population in response to Pb and Cd. Contrary results were obtained for the Szopienice population plants, where lower or similar proline concentrations in leaves of these plants grown on the soil polluted with heavy metals in relation to control plants was found (Table 3). Positive correlation between Cd and proline concentration was found (correlation coefficient = 0.96). The increase of guaiacol peroxidase activity was shown in leaves of the Szopienice population on the substrate with Zn addition, and in leaves of Heap population on the substrate with a mixture of metals (Table 3). Positive correlation between Zn concentration in leaves of the studied populations and peroxidase activity (correlation coefficient = 0.75) was shown.

Discussion

The found Zn and Cd contents in *Silene vulgaris* leaves were above the levels recognized as toxic for plants (100-400 mg·kg^{-1} d.w. Zn and 5-30 mg·kg^{-1} d.w. for Cd) [15]. The noticed Pb concentration in *Silene vulgaris* leaves grown on the substrate with Pb addition and a combination of metals were in the range recognized as a toxic (30-300 mg·kg^{-1} d.w.) [15, 16]. The highest Zn content (leaves from Heap population on the substrate with mixture of metals) accounted for about 38% of concentrations found at hyperaccumulators (>10,000 mg·kg^{-1} d.w.), similarly 10% in the case of Pb (>1,000 mg·kg^{-1} d. w.), and about 40% for Cd (>100 mg·kg^{-1} d.w.) [17, 18]. Relatively low bioavailability of the studied heavy metals, especially Pb, could be connected with high content of organic matter (30%). In heavy metal-polluted soil with high content of organic matter there was high activity of soil dehydrogenase. High content of organic substance can show that they are changed into biologically inactive forms [19]. Metal ions can be complexed by organic matter, decreasing their availability to plants [20]. Similarly, low Zn extraction 2.2-8.8% and higher Cd (11.3-37.2%) after extraction of metals from the soil with the use of ammonium acetate was observed by Pongrac et al. [9]. The obtained Zn concentrations in *S. vulgaris* leaves were higher for Heap population (on the substrate with mixture of metals) and for Calamine population (on the substrate with the addition of Zn and mixture of metals) and on a similar level (Szopienice population) in comparison to concentrations noticed in leaves collected directly from the natural habitat of the plants (806-2,889 mg·kg^{-1} d.w.). Also, Cd concentration at *Silene vulgaris* populations grown on the substrate with combination of metals was higher than concentrations of the element noticed for the plants in terrain (2.1-6.3 mg·kg^{-1} d.w.). Pb concentration at Calamine and Szopienice populations in comparison to plants in their natural habitat was on the comparable level (14-35 mg·kg^{-1} d.w.) [6]. The obtained results confirm the statement of Ernst et al. [21], that heavy metal-resistant ecotypes are mostly resistant to those heavy metals that are in surplus in their natural environment. However, Ernst et al. [21] noticed seedlings deaths of some *Silene vulgaris* populations on the soil coming directly from the terrain. The researchers showed that enhanced

Table 3. Proline, non-protein-SH group contents and peroxidase activity in *Silene vulgaris* leaves from selected populations grown on heavy metal-contaminated substrata; average ± SD.

Population	Substrate version	Proline content [μmol·g^{-1} f.w]	Non-protein SH group content [nmol –SH g^{-1} f.w.]	Peroxidase activity [Δ A min^{-1}·g^{-1}]
Calamine	Cd	0.35±0.02 c	154.9±12.5 b	0.26±0.08 ac
	Zn	0.12±0.02 b	107.1±10.5 a	0.36±0.13 a
	Pb	0.41±0.04 d	111.2±7.0 a	0.42±0.08 ab
	Mixture of metals	0.44±0.03 d	227.6±14.03 c	0.43±0.02 ab
	Control	0.23±0.01 a	125.2±5.8 a	0.72±0.1 d
Heap	Cd	0.20±0.002 b	150.2±7.1 c	0.36±0.09 a
	Zn	0.14±0.02 a	126.6±7.6 b	0.41±0.06 a
	Pb	0.36±0.03 c	130.8±11.6 b	0.35±0.09 a
	Mixture of metals	0.10±0.03 a	123.0±0.8 b	0.63±0.08 b
	Control	0.13±0.002 a	98.5±2.1 a	0.35±0.05 a
Szopienice	Cd	0.12±0.01 b	121.7±11.3 b	0.21±0.07 a
	Zn	0.09±0.01 a	76.4±5.8 a	0.72±0.17c
	Pb	0.25±0.03 d	127.5±4.7 b	0.49±0.03 b
	Mixture of metals	0.16±0.02 c	120.5±2.7 b	0.18±0.09 a
	Control	0.25±0.02 d	75.4±5.5 a	0.41±0.08 b

a,b,c – different letters denote significant differences between metal concentrations in one population ($p<0.05$).

metal concentration in the seedlings can be a sensitive and early indicator of metal-exposed plants. In the following studies, with the doses of heavy metals applied, we did not observe early seedling death.

Tolerance toward metals correlates well with the level of non-protein content thiols that include not only glutathione but also thiol rich peptides known as phytochelatins and other –SH rich compounds [22, 23]. In these studies there was observed an increase of non-protein -SH group content in leaves of the all studied *Silene vulgaris* populations on the substrate with Cd and a combination of metals. In the presence of increased Zn content in the substrate, the increase of thiol content was only noticed in leaves of Heap population. Pongrac et al. [9] noticed an increase in non-protein thiols in *Thlaspi praecox* grown in Cd-amended substrate, which was not noticed on the substrate with Zn. There was not also shown an increase of thiol concentration along with the increase of Cd and Zn in the substrate in leaves of *T. caerulescens*. The increased concentration of glutathione was noticed in leaves of the Szopienice population in natural habitat in comparison to concentrations of glutathione observed in leaves of Calamine and Heap populations [6]. Hawrylak and Szymańska [22] showed that selenate contributed to a larger accumulation of non-protein –SH groups in the shoots than in the roots of spinach and tomato plants. However, studies of De la Rosa et al. [24] showed that in leaves of tumbleweed (*Salsola kali*), the production of thiols decreased at the highest Cd concentration tested.

In general, proline has three major functions, namely: metal binding, antioxidant defense, and signaling [7]. An increase in proline levels as responses of plants to heavy metal stress was noted by many investigators [7, 25-27]. These studies showed an increased proline accumulation in leaves of Calamine and Heap populations (on the substrate with the addition of Cd and Pb) in comparison to the control plants. There was not noticed an increase of proline concentration in leaves of the Szopienice population grown on the substrate polluted with heavy metals (Table 3). As it results from the observation of this population, the plants were characterized by the thickest leaf blade, which was confirmed in natural conditions of the plant occurrence (Nadgórska-Socha 2006, data not published). Proline accumulation in plants under Cd stress is induced by a Cd-imposed decrease of the plant water potential and the functional significance of this accumulation would lie in its contribution to water balance maintenance [26]. The results obtained by Schat et al. [25] demonstrated that metal-induced proline accumulation depends on the development of metal-induced water deficit in the leaves. The metal-tolerant ecotype used in this study has smaller, thicker, and waxier leaves than the nontolerant one. Despite the fact that in the metal-tolerant ecotype the constitutive proline concentration in the leaves was 5 to 6 times higher than in the nontolerant ecotype, the results obtained by Schat et al. [25] showed that adaptive tolerance to Cu, Cd, and Zn is associated with a strongly decreased tendency to accumulate proline upon heavy metal exposure.

In these studies the increased guaiacol peroxidase activity in relation to the control plants was only shown in leaves of *Silene vulgaris* grown on the substrate with Zn (Szopienice), and with a mixture of metals (Heap population). Stress intensity may cause an increase or decrease in the oxidative metabolism [27]. Pongrac et al. [9] demonstrated no effect of added Cd and Zn on guaicol peroxidase activity in *Thlaspi praecox* and *T. caerulescens* leaves. Reducing the need for antioxidative enzyme activity may be accomplished by preventing ROS from forming rather than removing them, as demonstrated in nickel involved tolerance metabolism in *Alyssum argenteum* [9]. In studies on peroxidase activity in plant leaves coming from polluted areas, we observed an increase of enzyme activity in comparison to peroxidase activity in plant leaves collected from potentially clean areas [29, 30]. Results obtained by Pukacka and Pukacki [29] indicated that the sensitivity of the radical scavenging system in Scots pine needles differs among populations grown in the vicinity of industrial objects. The study of Źlóbek-Sokolnik et al. [31] noticed that Cu ions in concentrations of 500 µM caused a permanent increase and Cu ions in concentration of 10 µM a decrease of guaiacol peroxidase activity in suspension cultured tobacco cells 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 the conducted studies we found a number of accumulations in leaves of *Silene vulgaris* Zn>Pb>Cd. The highest concentrations of the studied elements were noticed in leaves of plants of Heap population. The studied metals were accumulated in higher amounts in plants growing on the ground polluted by a mixture of metals. Obtained results indicated that the sensitivity of the free radical scavenging system in *Silene vulgaris* leaves differs among populations, especially in proline content.

References

1. SAWICKA-KAPUSTA K. The responses of plants to sulphur dioxide and heavy metals in the environment- bioindication. Wiadomości Ekologiczne **XXXVI**, (3), 95, **1990** [In Polish].
2. MAKSYMIEC W. Signaling responses in plants to heavy metals stress. Acta Physiologiae Plantarum **29**, 177, **2007**.
3. GRODZIŃSKA K., SZAREK-ŁUKASZEWSKA G. Zinc-lead mine spoils near Olkusz - past, present and future. Kosmos. Problemy nauk przyrodniczych **51**, (2), 127, **2002**. [In Polish]
4. WIERZBICKA M., ROSTAŃSKI A. Microevolutionary changes in ecotypes of calamine waste heap near Olkusz, Poland: A Review. Acta Biologica Cracoviensia Series Botanica **44**, 7, **2002**.
5. KOSZELNIK LESZEK A. Content of selected heavy metals in soil and Silene vulgaris in serpentine spoil. Roczniki Gleboznawcze **LVIII**, 1-2, 63, **2007** [In Polish].
6. NADGÓRSKA-SOCHA A., CIEPAŁ R, KANDZIORA M., KAFEL A. Heavy metals Bioaccumulation and physiological responses to heavy metal stress in population of Silene vulgaris Moench (Garcke). Ecological Chemistry and Engineering **16**, (4), 389, **2009**.
7. SHARMA S. S., DIETZ K. J. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. Journal of Experimental Botany **57**, 711, **2006**.
8. SHAMSI I. K., WEI K., ZHANG G. P., JILANI G. H., HASSAN M. Interactive effects of cadmium and aluminium on growth and antioxidative enzymes in soybean. Biologia Plantarum **52**, 165, **2008**.
9. PONGRAC P. ZHAO F-J., RAZINGER J., ZRIMEC A. REGVAR M. Physiological responses to Cd and Zn in two Cd/Zn hyperaccumulating *Thlaspi* species. Environmental and Experimental Botany **66**, 479, **2009**.
10. OSTROWSKA A., GAWLIŃSKI S., SZCZUBIAŁKA Z. Methods of analysis and evaluation of properties of soils and plants. Catalogue of the Environmental Protection Institute, Warsaw, Poland, pp. 113-115, 241-244, **1991** [In Polish].
11. BOUWMAN L., BLOEM J., RÖMKENS P., BOON G., VANGRONSVELD J. Beneficial effects of the growth of metal tolerant grass on biological and chemical parameters in copper - and zinc contaminated sandy soils. Minerva Biotechnology **13**, 19, **2001**.
12. FANG W.C, KAO C. Enhanced peroxidase activity in rice leaves in response to excess iron, copper and zinc. Plant Science **158**, 71, **2000**.
13. BATES L., WALDREN R., TEARE D. Rapid determination of free proline for water-stress studies. Plant Soil **39**, 205, **1973**.
14. MASS F., DE KOK L., PETERS J., KUIPER P. A comparative study on 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., *Phaseolus vulgaris* L. Journal of Experimental Botany **38**, 1459, **1987**.
15. KABATA-PENDIAS A., Pendias H. Biochemistry of trace metals. PWN. Warszawa, pp. 364, **1999** [In Polish].
16. ALLOWAY B. J., AYRES D. Chemical basis of environment al pollution. PWN. Warszawa, pp. 423, **1999** [In Polish].
17. SALT D. E., SMITH R. D., RASKIN I. Phytoremediation. Annu.Rev. Plant Molecular Biology **49**, 643, **1998**.
18. WÓJCIK M. Phytoremediation- a way of cleaning the environment. KOSMOS. Problemy Nauk Biologicznych **49**, (1-2), 135, **2000** [In Polish].
19. CIARKOWSKA K., GAMBUŚ F. Dehydrogenase activity In soils contaminated by heavy metals on the area of Olkusz. Zeszyty Problemowe Postępów Nauk Rolniczych **501**, 79, **2004** [In Polish].
20. KABATA-PENDIAS A. Soil – plant transfer of trace elements – an environmental issue. Geoderma **122**, 143, **2004**.
21. ERNST W. H. O., NELISSEN H. J. M., TEN BOKUM W. M. Combination toxicology of metal-enriched soils: physiological responses of a Zn- and Cd-resistant ecotype of *Silene vulgaris* on polymetallic soils. Environmental and Experimental Botany **43**, 55, **2000**.
22. HAWRYLAK B., SZYMAŃSKA M. Selenium as a sulphydrylic group inductor in plants. Cellular and Molecular Biology Letters **9**, 329, **2004**.
23. MISHRA S., TRIPATHI R. D., SRIVASTAVA S., DWIVEDI S., TRIVEDI P. K., DHANKHER O.P., KHARE A. Thiol metabolism play significant role during cadmium detoxification by *Ceratophyllum demersum* L. Bioresource Technology **100**, 2155, **2009**.
24. DE LA ROSA G., MARTINEZ-MARTINEZ A., PELAYO H., PERALTA-VIDEA J. R., SANCHES-SALCIDO B.,

GARDEA-TORESDEY J.L. Production of low molecular weights thiols as a response to cadmium uptake by tumbleweed (*Salsola kali*). Plant Physiology and Biochemistry **43**, 491, **2005**.

25. SCHAT H., SHARMA S., VOOIJS R. Heavy metal-induced accumulation of free proline in metal tolerant and nontolerant ecotype of *Silene vulgaris*. Physiologia Plantarum **101**, 477, **1997**.
26. TEKLIĆ T., HANCOCK J. T., ENGLER M, PARADIKOVIĆ S., CESAR V., LEPEDUŠ H., ŠTOLFA I., BEŠLO D. Antioxidative responses in radish (*Raphanus sativus* L.) plants stressed by copper and lead in nutrient solution and soil. Acta Biologica Cracoviensia **50/2**, 79, **2008**.
27. ZENGIN F., MUNZUROGLU O. Effect of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. Acta Biologica Cracoviensia **47/2**, 157, **2005**.
28. BAYCU G., TOLUNAY D., ÖZDEN H., GUENENEBAKAN S. Ecophysiological and seasonal variations in Cd, Pb, Zn and Ni concentrations in the leaves of urban deciduous trees in Istanbul. Environmental Pollution **143**, 545, **2006**.
29. 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 physiologiae plantarum **4**, 457, **2000**.
30. KAFEL A., NADGÓRSKA-SOCHA A., GOSPODAREK J., BABCZYŃSKA A., SKOWRONEK M., KANDZIORA M, ROZPĘDEK K. The effect of infestation by *Aphis fabae* on the antioxidant response and heavy metal content of *Philadelphus coronarius* plants. Science of Total Environment **408**, 1111, **2010**.
31. ŹRÓBEK-SOKOLNIK A., GÓRSKA K., GÓRECKI R. J. The activity of antioxidant enzymes in suspension cultured tobacco cells treated with heavy metals. Polish Journal of Natural Science **22**, (4), 704, **2007**.