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Robinia pseudoacacia and *Melandrium album* in trace elements biomonitoring and air pollution tolerance index study

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Abstract The accumulation efficiency of selected trace elements in the leaves of *Melandrium album* and *Robinia pseudoacacia* grown on heavy metal contaminated sites in comparison with a non-contaminated one was evaluated. The study was undertaken to calculate air pollution tolerance index and to determine the contents of selected metabolites: glutathione, non-protein thiols, ascorbic acid, chlorophyll and the activity of antioxidant enzymes: guaiacol peroxidase and superoxide dismutase. Such estimations can be useful in better understanding of plants defense strategies and potential to grow in contaminated environments. The results in the most contaminated site revealed higher contents of metals in *M. album* leaves, especially Zn, Cd and Pb (3.4, 6 and 2.3 times higher, respectively) in comparison with the *R. pseudoacacia*. Better accumulation capacity found in *M. album* was shown by metal accumulation index values. The plants could be used as indicators of Zn, Cd (both species) and Pb (*M. album*) in the soil. Glutathione content (in both species) and peroxidase activity (in *M. album*), general markers of heavy metals contamination, were increased in contaminated sites. In most cases in contaminated areas *R. pseudoacacia* had decreased ascorbic acid and chlorophyll levels. Opposite tendency was recorded in *M. album* leaves, where similar or higher contents of the above-mentioned metabolites were found. In our study, *M. album* and *R. pseudoacacia* proved to be sensitive species with the air pollution tolerance index lower than 11 and can be recommended as bioindicators.

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Abbreviations

POD	Guaiacol peroxidase
SOD	Superoxide dismutase
GSHt	Glutathione total
NP-SH	Non-protein thiols
APTI	Air Pollution Tolerance Index
MAI	Metal Accumulation Index

Introduction

The environments of human populations are dominated with urban areas having elevated levels of heavy metals in the air and soil caused by emissions from industrial plants and automobile exhaust. Such contamination with trace elements is a worldwide problem, resulting in bioaccumulation in the food chain and posing a direct threat to wildlife and human health (Simon et al. 2011, 2014; Bini et al. 2012; Hu et al. 2014).

Plants are significant components in urban ecosystems where they transfer elements from the abiotic to biotic environment. Due to this role, they may be useful as biological indicators of air and soil pollution, not only because of their uptake of pollutants but also due to their wide range. Various reports confirm the biomonitoring potential of indigenous plants, especially ruderals—which first colonize disturbed lands (Kovács 1992; Massa et al. 2010; Nadgórska-Socha et al. 2013). One example is *Melandrium album* from the Caryophyllaceae family, found in heavy polluted sites in southern Poland (Silesia) in Miasteczko Śląskie, used in the determination of the vegetation pattern for heavy metal accumulation at a mine tailings at

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Gyöngyösoroszi, Hungary, and in Olkusz (southern Poland) (Tamás and Kovács 2005; Stefanowicz et al. 2012; Nadgórska-Socha et al. 2013). Literature reports often mention the use of plant leaves in environmental evaluations. Urban trees and shrubs, which play an important role in filtering ambient air by removing heavy metals and adsorbing particulate matter, are often used for biomonitoring in industrial and urban areas. Several species of trees are noted: *Robinia pseudoacacia, Acer pseudoplatanus, A. negundo, Populus alba, P. tomentosa, Betula pendula, Tilia cordata, Aesculus hippocastanum, Salix* sp., *Quercus ilex, Q. robur, Platanus orientalis, Celtis occidentalis* and *Pinus nigra* (Baycu et al. 2006; Liu et al. 2007; Samecka-Cymerman et al. 2009; Sawidis et al. 2011; Serbula et al. 2012; Ugolini et al. 2013; Hu et al. 2014; Simon et al. 2014; Tzvetkova and Petkova 2015). *Robinia pseudoacacia* from the Fabaceae family is a tree species commonly planted in urban environments, and it is usually found along roadsides in industrial and agricultural areas and urban parks, even with high levels of pollution. In addition to high metal accumulation by its leaves, this plant flourishes in the industrial areas because of its adaptability to dryness, fast growth and ability to fix nitrogen (Samecka-Cymerman et al. 2009; Serbula et al. 2012; Tzvetkova and Petkova 2015).

An excess of metals in the environment causes functional disorders of many physiological processes in plants due to oxidative action of metals. Recent experimental research (Baycu et al. 2006; Yadav 2010; Seth et al. 2012; Nadgórska-Socha et al. 2013; Viehweger 2014) has shown different plants to have strong antioxidant systems involving high glutathione (GSH) levels that are either required or at least beneficial for metal tolerance. But apart from non-enzymatic antioxidants (such as glutathione, ascorbate and carotenoids), field studies have also reported the antioxidative enzyme activity of plants in response toward heavy metals, especially peroxidase activity which reflects the total phytotoxic effects (Baycu et al. 2006; Seth et al. 2012; Nadgórska-Socha et al. 2013). In polluted areas toxic levels of heavy metals cause a reduction in chlorophyll content in plants (Baycu et al. 2006). Thus, the estimation of the above-mentioned plant ecophysiological responses in a field study may be useful in pollution biomonitoring as well as in verifying the effect of metal contamination on plant physiology. Efficient plant species can be used to protect vulnerable areas in urban settings to decrease human exposure to anthropogenic pollutants (Hu et al. 2014). Biomonitoring based on plant leaves has recently been recognized as a thrust area in the field of particulate matter science (Simon et al. 2014; Rai and Singh 2015). Plants exposed to environmental pollutants e.g., heavy metals absorb, accumulate and integrate these pollutants into their systems (Enete et al. 2013). The

determination of resistance or susceptibility to different air pollutants can be associated and understood by analyzing chlorophyll content, ascorbic acid content, relative water content (RWC) and leaf extract pH. A combination of these parameters, air pollution tolerance index (APTI), seems to be more suitable for ecophysiological research than individual indicators, since plants show different responses to different pollutants (Ogunkunle et al. 2015). Air pollution tolerance index is one approach that may be employed in assessing urban biodiversity (Enete et al. 2013).

The present study attempts to determine the usefulness of *Melandrium album* and *Robinia pseudoacacia* leaves for evaluation of magnitude of the environmental contamination with selected metals and metal accumulation efficiency in an urban industrial area, in comparison with a potentially non-contaminated rural area on the outskirts of a nature reserve area (in the provinces of Śląsk and Małopolska in southern Poland). The research was carried out in mid-June 2012. We also estimated physiological and biochemical responses such as: the contents of glutathione, ascorbic acid, chlorophyll, as well as antioxidant defense enzymes activity (guaiacol peroxidase and superoxide dismutase), relative water content and pH of the leaves, which may reveal general condition of the plants. The evaluations and the calculated APTI contribute to scientific knowledge about the potential of this species to grow in contaminated environments.

Materials and methods

Study area

Sampling was carried out in mid-June 2012 in four heavily contaminated sites (vicinity of the zinc smelter Miasteczko Śląskie (C1); phytocoenoses on the sides of main roads with heavy traffic in the city centers of Dąbrowa Górnicza (C2) and Katowice (C3) power plant Jaworzno III in Jaworzno (C4)) and one clean site at the outskirts of the Pazurek Nature Reserve (NC). All of the sites were situated in southern provinces of Śląsk and Małopolska in Poland (Table 1).

Soil and plant sampling

Three composite leaf samples from the *Robinia pseudoacacia* L. and *Melandrium album* (Mill.) Garcke were collected from each site. The leaves from *R. pseudoacacia* were collected from trees of about the same age from all the sites, taken randomly from the lower foliage, as well as samples from *M. album*, and taken in ice to the laboratory for analysis. Soil samples were collected from the same sites. After removing any surface litter, three composite

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Table 1 Site description

Abbreviation	Sites	GPS latitude, longitude
NC	The direct vicinity of Pazurek Nature Reserve in Jaroszowiec (control site)	50°20′22.57″N; 19°37′13.39″E
C1	The direct vicinity of Zinc Plant Miasteczko Śląskie	50°31′22.655″N; 18°56′8.699″E
C2	Królowa Jadwiga street (roadside, main road with high traffic in the center of the city of Dąbrowa Górnicza)	50°19′33.36″N; 19°12′13.87″E
C3	Katowice, Silesian Insurgents' park, direct vicinity of main road with high traffic	50°15′47.74″N; 19°1′29.47″E
C4	Direct vicinity of the "Jaworzno III" power plant in Jaworzno (active since 1979)	50°12′14.111″N; 19°11′51.792″E

soil samples were sampled from a depth of 0–15 cm at each site.

Analysis of metal concentration in soil and plant samples

Soil samples were sieved through a 2 mm screen, air-dried and used for pH, heavy metal and organic matter estimation. The metal content of the soil was estimated according to the method by Ostrowska et al. (1991) and previously described in detail (Nadgórska-Socha et al. 2013). Metals were extracted from the air-dried samples of soil using 2 M HNO_3 (HNO_3 extracted elements). Soil pH was measured in a 1:2.5 soil to water weight ratio. Organic matter content (expressed in %) was measured following the method by Ostrowska et al. (1991). The HNO_3-extractable fraction was obtained by shaking a sample (10 g) with 100 ml of 2 M HNO_3 for 1 h. Metals were extracted from air-dried samples of soil using 0.01 M $CaCl_2$. The bioavailable fraction (potentially bioavailable elements) was obtained by shaking a sample (1:10) with 0.01 M $CaCl_2$ for 2 h (Wójcik et al. 2014). Finally, the content of metals was measured in the filtered extracts using flame absorption spectrometry (Thermo Scientific iCE 3500).

A soil pollution index (SPI) was calculated for each locality according to the equation given below (Sanka et al. 1995; Diatta et al. 2003) and was based on limit values as reported in the regulations of the Ministry of Environment (2002). The limit values (permissible concentration) of heavy metal contents in soils were: Zn—300 mg kg^{-1}, Pb—100 mg kg^{-1} Cd—4 mg kg^{-1}.

$$\text{SPI} = (1/n) \sum_{i=1}^{n} 100 \, \text{VS/LS}$$

n—number of elements, VS—content of an element in the soil, in mg kg^{-1}, LS—limit value for an element in the soil, in mg kg^{-1}.

Half of the leaves were used for heavy metal determination, where they were washed thoroughly with tap water to remove any substrate and dust deposits and then rinsed with deionized water, and then oven-dried at 105 °C. Dry weight subsamples 0.25 g were wet digested in HNO_3 at 120 °C and then diluted to 25 ml with deionized water (Lin et al. 2008). Trace elements (Cd, Pb, Zn, Cu, Fe and Mn) were estimated by flame absorption spectrometry (Thermo Scientific iCE 3500). For assurance of the quality of substrate analysis, the procedures were performed on blank samples and on certified reference materials.

To assess the overall performance of the plants in terms of metal accumulation, we calculated a Metal Accumulation Index (MAI) according to the following calculation:

$$\text{MAI} = (1/N) \sum_{j=1}^{N} Ij$$

where N is the total number of metals analyzed

$$Ij = x/\delta x$$

Ij is the sub-index for variable j, obtained by dividing the mean value (x) of each metal by its standard deviation δx (Liu et al. 2007; Hu et al. 2014).

Analysis of the biochemical parameters of the plants

Crushed plant parts were homogenized in a 100 mM phosphate buffer (pH 6.8) for the analysis of POD activity (1:7 ratio) and centrifuged at 12,000g for 20 min. The entire procedure was carried out at 4 °C. The guaiacol peroxidase activity was measured at 470 nm according to Fang and Kao (2000), with guaiacol as the substrate. The POD activity was measured in a reaction mixture (3 ml) containing a 50 mM phosphate buffer (pH 5.8), 1.6 μl H_2O_2, 1.5 μl guaiacol and 0.2 ml enzyme extract. The activity was calculated using the extinction coefficient (26 mM^{-1} cm^{-1}) for tetra-guaiacol and was expressed in μM tetra-guaiacol min^{-1} g f w^{-1}. The analysis of superoxide dismutase (SOD) was performed in a buffer with 3 mM $MgSO_4$, 1 mM dithiotreitol (DTT), 3 mM EDTA (1:5 ratio) and centrifuged at 12,000g for 20 min. The entire procedure was carried out at 4 °C. The reaction was measured spectrophotometrically at 560 nm according to Beauchamp and Fridovich (1971). One unit of SOD was defined as the amount of enzyme activity that was able to inhibit the photoreduction of nitroblue tetrazolium (NBT) to blue formazan by 50 %. The protein content was measured following the method of Bradford (1976). To detect the

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glutathione concentration, plant leaves were homogenized in TCA (trichloroacetic acid, 5 g per 100 ml) and 0.125 mM phosphate buffer (pH 6.3) with 6.3 mM EDTA and then were centrifuged at 10,000g for 10 min at 4 °C. The linear changes in the absorbance of the reaction mixtures were measured at 412 nm, and GSHt was expressed as μM GSH g^{-1} fresh weight. To measure the content of non-protein thiols, the plant material was homogenized in a 5 ml/g mixture containing 5-sulfosalicylic acid (2 g per 100 ml) and 1 mM EDTA and sodium ascorbate (0.15 g per 100 ml). The samples were centrifuged at 20,000g for 10 min at 4 °C. Then 0.5 ml of liquid supernatant, 0.5 ml of a 1 M sodium phosphate buffer (pH 8.0) and 100 μl of 10 mM 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) were put into test tubes. The absorbance at 415 nm was read 1 min after the addition of DTNB (Anderson 1985). The number of non-protein SH groups was established based on a curve prepared using L-cysteine and expressed as nM–SH g^{-1} fresh weight (Mass et al. 1987).

The relative leaf water content (RWC) was analyzed. After recording the fresh weight (FW) the leaves were immersed in water overnight, blotted dry and weighed to determine the turgid weight (TW). The leaves were then dried overnight in an oven at 70 °C and re-weighed to obtain the dry weight (DW). The leaf RWC (%) was determined and calculated by the formula given by Pathak et al. (2011).

$$RWC\,(\%) = [(FW - DW/TW - DW)] \times 100$$

The total chlorophyll content in fresh leaves (mg g^{-1} fresh weight) was determined following the method of Arnon (1949), whereby crushed leaves were extracted in 80 % acetone, and the absorbance was taken at 645, 663 and 652 nm. The pH of the leaves was determined with a pH meter by homogenizing 5 g f.w. of leaves in 10 ml of deionized water. The ascorbic acid content of leaf sample was determined with the use of a spectrophotometer. Ascorbic acid content was calculated by the formula given by Keller and Schwanger (1977).

$$Ascorbic\,acid\,(mg \times g^{-1}f.w.) = \frac{(E_o - E_s - E_t)V}{W \times 100} \times 100$$

where V is the volume of the extract, W is the weight of the leaf sample (g), and E_o, E_s and E_t are optical densities of a blank sample, plant sample and sample with ascorbic acid, respectively.

Ascorbic acid content, leaf extract pH, total chlorophyll content and relative water content were taken into the following expression:

$$APTI\,(Air\,Pollution\,Tolerance\,Index) = \frac{A(T + P) + R}{10}$$

where A is the ascorbic acid content in mg g^{-1} f.w., T the total chlorophyll in mg g^{-1} f.w., P the pH of leaf extract

and R the relative water content, in percentage (Prajapati and Tripathi 2008).

Data analysis

After testing the data for normality, the differences in soil and plant variables between the different sites were tested using one-way analysis of variance (ANOVA) according to Statistica version 10 package, StatSoft, Inc. Significant statistical differences were established using Tukey's test, at $p < 0.05$. We also calculated the Pearson's correlation coefficient for assessing the relationship between estimated metal concentrations and pH and organic matter content as well as between the metal concentrations and biochemical parameters in the leaves.

Results and discussion

Heavy metals in the soils and plants

One-way ANOVA revealed significant differences in leaves heavy metal concentrations (except Cu) between the investigated stands, as well as in metal concentrations, pH and organic matter in soil (Table 2). The concentrations of Cd, Pb, Zn, Fe, Mn and Cu in the soil of each site are given in Table 3. The most contaminated site was C1 (vicinity of the zinc smelter). The highest concentrations of heavy metals (HNO$_3$ extracted) as well as the lowest organic matter content and pH higher than 7 (Table 3) were found. However, the bioavailability of metals in the most contaminated site C1 was relatively low (0.83, 4.58 and 0.12 %, respectively, for Zn, Cd and Mn extracted with HNO$_3$). The highest concentrations of bioavailable metals were recorded in site C4. This fact can be connected with the lowest pH value. The low soil pH value of site C4 was related to the vicinity of a pine forest, similar to the NC site. The pH values were negatively correlated with bioavailable soil concentrations of Zn and Mn (Table 4). Bioavailable Mn, Zn (CaCl$_2$ extracted) and Fe (HNO$_3$ extracted) were positively correlated with organic matter content. Also Pb, Mn and Zn concentrations (HNO$_3$ extracted) were positively correlated with pH (Table 4). According to the calculated SPI values the most contaminated site was also C1 (Table 3). The Zn, Pb and Cd content (extracted with HNO$_3$) in soil from sites C1 and C4 were at a comparable level to those found by Kandziora-Ciupa et al. (2013) (soil from the immediate vicinity of the same zinc smelter and power plant). The levels of heavy metals in the most polluted stand C1 were lower than the total concentration found in waste deposits in Piekary Śląskie, Brzeziny and Bolesław (Wójcik et al. 2014). In the case of high traffic sites such as C2 the content of Zn, Cd,

Table 2 Effect of site on heavy metal levels in soil and in the investigated species, as well as on pH, organic matter content, physiological and biochemical parameters in plant leaves (one-way ANOVA)

Variable	d	F	p
R. pseudoacacia			
Chlorophyll	4	73.43	0.000000
Ascorbic acid	4	100.72	0.000000
pH	4	24.98817	0.000000
RWC	4	68.03572	0.000000
APTI	4	252.16	0.000000
GSHt	4	270.50	0.000000
NP–SH	4	409.70	0.000000
POD	4	8.28	0.000413
SOD	4	292.83	0.000000
Zn	4	74.43	0.000000
Pb	4	28.62	0.000000
Cd	4	80.63	0.000000
Fe	4	18.26	0.000002
Mn	4	51.13	0.000000
Cu	4	1.25	0.323104
M. album			
Chlorophyll	4	18.68	0.000002
Ascorbic acid	4	18.45	0.000002
pH	4	62.58397	0.000000
RWC	4	27.11584	0.000000
APTI	4	65.35	0.000000
GSHt	4	278.12	0.000000
NP–SH	4	15.39	0.000007
POD	4	293.36	0.000000
SOD	4	147.83	0.000000
Zn	4	219.99	0.000000
Pb	4	959.69	0.000000
Cd	4	1660.21	0.000000
Fe	4	25.44	0.000000
Mn	4	368.31	0.000000
Cu	4	21.09	0.000001
Soil			
pH	4	26235.56	0.000000
Organic matter	4	2849.89	0.000000
Zn ($CaCl_2$)	4	59.93	0.000000
Cd ($CaCl_2$)	4	41.10	0.000000
Mn ($CaCl_2$)	4	28.16	0.000000
Zn (HNO_3)	4	255.75	0.000000
Pb (HNO_3)	4	265.30	0.000000
Cd (HNO_3)	4	1364.68	0.000000
Fe (HNO_3)	4	25.85	0.000000
Mn (HNO_3)	4	53.65	0.000000
Cu (HNO_3)	4	99.75	0.000000

Pb was higher than in the study by Hu et al. (2014) at, respectively: 5.26–11.27; 0.48–2.75; 2.15–15.9 mg kg^{-1}. The soil contamination with Zn and Pb in Pisa (Italy) (Bretzel et al. 2014) was at a comparable level to the soil collected in C2 (high traffic).

The bioavailability of the investigated metals was low (Table 3). This finding is connected with the high pH value (C1, C2 and C3) or higher organic matter content (24.6 % in C4, Table 3). Low bioavailability is characteristic for soils from areas connected with Pb/Zn mining, with high organic matter content as well as pH value (Lei et al. 2010; Banásowá et al. 2006; Ciarkowska et al. 2014).

The analysis of metal content in the foliage of *R. pseudoacacia* and *M. album* indicated that heavy metals were accumulated in higher amounts in *M. album* (Table 5). The highest Zn (253.6 mg kg^{-1}), Cd (18.4 mg kg^{-1}) and Pb (160.2 mg kg^{-1}) levels were recorded in site C1. However, the highest Fe (195.9, 212.6 mg kg^{-1}) level was found in the leaves of *M. album* collected in sites C2 and C3. The highest Mn level was found in C2 (78.8 mg kg^{-1}, Table 5). Also, in *R. pseudoacacia* the highest Zn and Cd were found in the foliage collected in site C1 (109.6, 3.0 mg kg^{-1}, respectively). The highest Mn and Pb concentrations were recorded in *R. pseudoacacia* leaves from site C4.

A comparison of the obtained concentrations of heavy metals in the studied species to the contents observed by other investigators at similar contaminated areas indicates the suitability of the examined plants as bioindicators. The observed ranges of examined metal concentrations correspond the level of ecological and health risk. Analysis of the metal content in the leaves of *R. pseudoacacia* and *M. album* revealed that the leaves of *M. album* were generally richer in metals, especially Zn, Cd and Pb. We obtained positive correlation coefficients between Cd in the leaves of both species and Cd in the soil ($CaCl_2$ and HNO_3 extracted), Zn in the leaves of both species and Zn in the soil (HNO_3 extracted), and between Pb in the leaves of *M. album* and Pb in the soil (HNO_3 extracted) (Tables 6, 7).

Melandrium album is very often indicated as the species colonizing post-mining areas, e.g., it was observed at the abandoned fields on the fertile sandy soils of a former coke factory in north-east France (Dazy et al. 2009). The species *M. album* was often an abundant therophyte (hemicryptophyte) in the vascular flora on spoil heaps located in Piekary Śląskie and Bytom (southern Poland) (Skubała 2011). *M. album* also colonized serpentinite mining dumps in Lower Silesia (southwestern Poland) (Kasowska and Koszelnik-Leszek 2014). However, information concerning its accumulation capabilities is rather rare. In our study the accumulated amounts of heavy metals were higher than those indicated by Evangelou et al. (2012). *M. album* syn.

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Table 3 Soil properties: heavy metals content (mean ± SD, extraction with HNO_3 and $CaCl_2$) [mg kg^{-1}]

Soil property	Site				
	NC	C1	C2	C3	C4
pH	4.97 ± 0.01 a	7.44 ± 0.01 b	7.13 ± 0.02 c	7.89 ± 0.01 d	4.74 ± 0.01 e
Organic matter content	7.48 ± 0.2 a	5.71 ± 0.3 b	9.58 ± 0.1 c	5.11 ± 0.2 b	24.64 ± 0.2 d
Cd ($CaCl_2$)	0.43 ± 0.02 a	1.45 ± 0.2 b	0.17 ± 0.04 a	0.15 ± 0.01 a	1.24 ± 0.1 b
Mn ($CaCl_2$)	2.48 ± 0.1 b	0.31 ± 0.2 a	nd	0.54 ± 0.1 a	13.48 ± 1.3 c
Zn ($CaCl_2$)	18.38 ± 1.2 a	14.77 ± 1.6 a	1.11 ± 0.3 b	2.11 ± 0.6 b	47.59 ± 5.1 c
Mn (HNO_3)	21.76 ± 1.7 a	250.64 ± 2.9 b	151.53 ± 10.3 d	167.24 ± 10.5 d	127.44 ± 7.2 e
Cu (HNO_3)	3.49 ± 0.4 a	33.75 ± 0.2 b	7.69 ± 0.7 a	19.47 ± 3.1 c	21.38 ± 3.9 c
Fe (HNO_3)	390.66 ± 39.1 a	1143.32 ± 76.9 c	319.43 ± 29.6 a	789.66 ± 32.1 b	1682.12 ± 89.1 d
Cd (HNO_3)	1.31 ± 0.2 a	31.67 ± 0.8 b	3.12 ± 0.1 a	8.40 ± 0.2 c	7.56 ± 0.7 c
Zn (HNO_3)	50.23 ± 2.8 a	1787.40 ± 121.5 b	159.78 ± 6.1 c	861.86 ± 25.4 d	422.94 ± 24.9 e
Pb(HNO_3)	75.76 ± 5.6 a	513.50 ± 2.1 b	117.02 ± 6.8 a	460.82 ± 27.1 b	326.14 ± 54.6 c
Soil Pollution index (SPI)	108.12	2750.21	276.69	1225.57	720.63

Mean pH value and organic matter content [%]. Different letters in the same row denote significant differences between particular parameters ($p < 0.05$)

nd not detectable

Table 4 Correlation coefficients between the concentrations of particular metal concentrations in the soil (after $CaCl_2$ and HNO_3 extraction) and pH value and organic matter content (values with * are statistically significant. $p < 0.05$)

	$CaCl_2$ extracted metals			HNO_3 extracted metals					
	Cd	Mn	Zn	Pb	Mn	Cu	Fe	Cd	Zn
Organic matter content	0.39	0.89*	0.84*	−0.1	−0.18	0.04	0.63*	−0.22	−0.1
pH	−0.24	−0.68*	−0.76*	0.49*	0.71*	0.37	−0.24	0.39	0.71*

Silene alba, grew spontaneously in soil from a military shooting range in Switzerland, where in its shoots it accumulated Sb, Cd, Pb, Cu and Zn at, respectively: ∼0.1, 4, 2, 11 and ∼75 mg kg^{-1}. In a study by Escarré et al. (2011) on vegetation in heavily contaminated sites with potential toxic metals (Zn, Pb, Cd, As and Tl) at a former mine in the district of Les Malines 40 km north of Montpellier, France, *Silene latifolia* (also known as *S. alba* or *M. album*) showed hyperaccumulation capacities for Tl. *S. latifolia* grown in the surroundings of the Mónica mine close to the village of Bustarviejo Madrid (Spain) accumulated Cd, Zn, Cu, Mn and Fe in the following ranges, respectively: 0.48–13.6, 21.3–440, 4.36–9.66, 27–155.9 and 52.6–399.8 mg kg^{-1} Moreno-Jiménez et al. (2009).

The average Zn, Pb, Cd, Fe, Mn and Cu concentrations in *R. pseudoacacia* leaves on contaminated sites in our study were, respectively: 84, 14, 1.0, 93.1, 16 and 13 mg kg^{-1}. The obtained mean concentrations of Cd, Zn, Cu and Fe in the foliage of *R. pseudoacacia* were higher than the mean contents (0.62 Cd; 11.2 Cu, 92 Fe, 146 Mn, 25 Pb and 68 Zn) in the leaves of this species in Oleśnica town in the study by Samecka-Cymerman et al. (2009).

Average levels of Zn (32.4), Pb (10.1) and Cd (0.38) in the leaves of *R. pseudoacacia* within the city of Istanbul (Baycu et al. 2006) were lower than the concentrations found in *R. pseudoacacia* foliage in this study (Table 5). The heavy metal content in the investigated plants is in accordance with the findings of Gwóźdź (2013) and Stryjewska (2014). We considered the concentrations found in the foliage of the investigated species as toxic or sufficient (normal) concentrations. The Pb, Cd and Zn concentrations obtained in the leaves of *M. album* from C1 and C3 and from C1 (only Zn) in *R. pseudoacacia* leaves were at toxic concentrations (30–300, 5–30, 100–400 mg kg^{-1}, respectively). Generally, Mn levels in the investigated species were below (*R. pseudoacacia*) or at a normal (*M. album*) concentration threshold (30–300 mg kg^{-1}). Moreover, the obtained Fe and Cu concentrations were in normal concentration ranges of Fe (5–250 mg kg^{-1}) and Cu (5–30 mg kg^{-1}). Also, Zn concentrations—found only for *R. pseudoacacia*—were in normal concentration ranges (27–150 mg kg^{-1}) (Kabata-Pendias 2001).

Melandrium album had a higher value of metal accumulation index (MAI) than *R. pseudoacacia*. Mean MAI in

Table 5 Heavy metals content in the leaves of investigated plants in mg kg^{-1} (mean ± SD) and MAI values

Species	Site	Metal							MAI
		Mn	Fe	Cu	Pb	Cd	Zn		
Robinia pseudoacacia	NC	21.43 ± 1.3 c	78.28 ± 7.2 a	12.07 ± 0.7 a	2.79 ± 1.1 a	0.11 ± 0.1 a	70.06 ± 3.4 a		11.51
	C1	12.99 ± 1.2 a	73.19 ± 7.5 a	13.11 ± 0.4 a	17.69 ± 3.1 c	3.01 ± 0.6 b	109.64 ± 3.5 c		12.89
	C2	15.99 ± 1.9 b	86.84 ± 13.7 ab	12.50 ± 0.7 a	8.11 ± 3.9 b	0.31 ± 0.1 a	62.85 ± 5.4 a		7.98
	C3	13.96 ± 1.2 ab	106.86 ± 4.9 b	12.40 ± 2.3 a	3.57 ± 1.2 a	0.59 ± 0.1 a	80.16 ± 5.3 b		10.36
	C4	21.91 ± 0.3 c	105.32 ± 1.3 b	13.53 ± 0.1 a	27.93 ± 8.4 d	0.22 ± 0.2 a	82.94 ± 5.1 b		13.06
Melandrium album	NC	51.09 ± 2.7 d	183.48 ± 18.4 d	9.68 ± 1.1 b	6.89 ± 1.0 b	0.10 ± 0.04 a	84.76 ± 19.1 b		8.35
	C1	35.99 ± 0.9 e	147.60 ± 7.7 c	12.37 ± 1.1 a	160.22 ± 9.3 e	18.39 ± 0.95 c	253.57 ± 10.4 e		26.4
	C2	78.78 ± 2.7 f	195.92 ± 11.5 de	11.07 ± 1.7 a	11.09 ± 0.6 b	0.90 ± 0.02a	127.94 ± 4.1c		21.80
	C3	43.42 ± 0.7 g	212.59 ± 12.4 e	6.45 ± 1.3 c	44.49 ± 5.0 f	2.45 ± 0.1 b	185.61 ± 4.8 d		25.46
	C4	42.40 ± 1.7 g	156.21 ± 5.9 c	12.53 ± 0.2 a	7.03 ± 0.3 b	0.18 ± 0.03 a	113.09 ± 1.6 c		19.66

The different letters denote significant differences between particular metal concentrations ($p < 0.05$)

R. pseudoacacia could be arranged in a descending order C4 > C1 > NC > C3 > C2 and, respectively, in *M. album* C1 > C3 > C2 > C4 > NC. The MAI values for investigated species are summarized in Table 5. The highest MAI value was found in *M. album* (26.4) collected in site C1, the lowest was found in *R. pseudoacacia* collected in site C2 (7.98). The MAI values for *M. album* (a plant growing low to the ground) were greater than those obtained for *R. pseudoacacia* (a tree growing high above the ground). It indicates that herbaceous plants such as *M. album* show high heavy metal accumulation while growing in contaminated environments. This fact was consistent with the study of Hu et al. (2014) where the shrubs had higher MAI values than the investigated trees. The authors suggested that plants with higher MAI values should be used as barriers between contaminated and non-contaminated areas.

Biochemical status of plants

One-way ANOVA revealed significant differences in biochemical and physiological parameters in the plant leaves between the investigated sites (Table 2). Metabolites such as non-protein thiols, glutathione, ascorbic acid, as well as the activity of POD and SOD involved in response to heavy metal stress, were determined in the leaves of *M. album* and *R. pseudoacacia* to verify the influence of contamination on the physiology of the species between the varied contaminated environments.

A higher POD activity was recorded in the leaves of *M. album* and *R. pseudoacacia* from the contaminated areas in comparison with the activity found in the species from NC site (Table 8). An elevated POD activity in the leaves of the investigated plants in our study was recorded at contaminated sites. We found positive correlations between Pb and POD activity in *R. pseudoacacia* leaves and between Pb, Cd and Zn in *M. album* leaves (Tables 9, 10). In Baycu et al. (2006), some differences in POD activity were detected in the leaves of the trees, among these *R. pseudoacacia*, between the control and urban site samples within Istanbul. Only in the tree—*Populus nigra* in the urban spring samples did POD activity show an increased tendency; however, in urban autumn samples an increase and decrease were recorded. Indeed the increases in peroxidase activity were a response to the increases in peroxides, in the distribution of the plasma membrane by lipid peroxidation and in the ROS produced by heavy metals accumulation, and therefore, POD activity may be a suitable indicator of metal stress (MacFarlane and Burchett 2001; Baycu et al. 2006). In the case of SOD activity in the leaves of the *R. pseudoacacia*, similar trends of POD activity were found. A significant increase in the activity of this enzyme in the leaves of above-mentioned species at

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Table 6 Correlation coefficients between the concentrations of particular metal concentrations in the soil (after $CaCl_2$ and HNO_3) and in the leaves of *R. pseudoacacia* (values with * are statistically significant. $p < 0.05$)

| | $CaCl_2$ extracted metals | | | HNO_3 extracted metals | | | | | |
	Cd	Mn	Zn	Pb	Mn	Cu	Fe	Cd	Zn
Mn	0.06	0.63*	0.65*	−0.56*	−0.76*	−0.49*	0.14	−0.55*	−0.74*
Fe	0.19	0.40*	0.20	0.18	−0.07	0.01	0.31	−0.38	0.17
Cu	0.36	0.3	0.31	0.22	0.22	0.32	0.42*	0.21	0.22
Pb	0.70*	0.62*	0.69*	0.32	0.29	0.52*	0.72*	0.38	0.33
Cd	0.56*	−0.33	−0.14	0.65*	0.73*	0.76*	0.22	0.95*	0.64*
Zn	0.74*	0.01	0.19	0.81*	0.66*	0.89*	0.55*	0.93*	0.67*

Table 7 Correlation coefficients between the concentrations of particular metal concentrations in the soil (after $CaCl_2$ and HNO_3) and in the leaves of *M. album* (values with * are statistically significant. $p < 0.05$)

| | $CaCl_2$ extracted metals | | | HNO_3 extracted metals | | | | | |
	Cd	Mn	Zn	Pb	Mn	Cu	Fe	Cd	Zn
Pb	0.55*	−0.36	−0.18	0.72*	0.76*	0.80*	0.23	0.97*	0.69*
Mn	−0.60*	−0.26	−0.42*	−0.76*	−0.24	−0.68*	−0.61*	−0.60*	−0.40*
Cu	0.61*	0.36	0.49*	−0.06	0.19	0.27	0.36	0.31	0.04
Fe	−0.86*	−0.40*	−0.61*	−0.27	−0.25	−0.53*	−0.56*	−0.61*	−0.19
Cd	0.60*	−0.33	−0.13	0.66*	0.74*	0.78*	0.23	0.97*	0.64*
Zn	0.38	−0.39	−0.31	0.82*	0.87*	0.82*	0.23	0.87*	0.85*

contaminated sites was found (Table 8). A dose-related effect was observed for SOD activity in herbaceous plant in a study by Dazy et al. (2009) at a former coke factory in France. Elevated SOD and POD activity was found by Guo et al. (2007) during a study of barley, along with the accumulation of Al, Cu and Cd.

We also detected in both investigated species higher contents of glutathione (GSHt) and non-protein thiols (NP-SH) (with the exception of *M. album* in C1, C2 and C4) in the leaves of investigated species in the contaminated areas in comparison with the non-contaminated one (Table 8). The highest content of GSHt was recorded in the leaves of *M. album* and *R. pseudoacacia* in site C1 (743.2 and 312.5 μM GSH g^{-1} f.w., respectively). In *R. pseudoacacia* leaves from C1, the highest content of NP–SH was found (328.7 nM—SH g^{-1} f.w.) (Table 8). A positive correlation between non-protein thiols and Cu, Pb, Cd, Zn in the leaves of *R. pseudoacacia* and Fe in *M. album* was found (Tables 9, 10). Moreover, a positive correlation was found between GSHt and Cd, Zn in the leaves of *R. pseudoacacia* and Pb, Cd, Zn in *M. album*. In the leaves of dandelions grown on sediments from the main roads of the inner city of Pisa, the non-protein thiols were lower or were at a comparable level (Bretzel et al. 2014). In a previous study in the plants *P. lanceolata* and *C. arenosa* from metalliferous sites in the vicinity of smelters and areas connected with former mining activities, lower or comparable level of NP–SH was detected in comparison with plants from the control area (Nadgórska-Socha et al. 2013). On the other hand, in *Vaccinium myrtillus*, upon analysis of the antioxidant response of this plant in contaminated sites

(immediate vicinity of zinc and iron smelters, and a power plant), the highest concentration of NP–SH was observed in the leaves of *V. myrtillus* in the most contaminated site—the immediate vicinity of the zinc smelter in Miasteczko Śląskie (Kandziora-Ciupa et al. 2013). This fact is in agreement with findings on non-protein thiols in the leaves of *R. pseudoacacia*. It was confirmed that GSH participates and plays a fundamental role in many detoxification processes of xenobiotics and heavy metals. In this regard, it plays a central role in metal chelation (by itself or as a precursor for PCs), as well as through its antioxidant capacities (Yadav 2010; Seth et al. 2012). In that study, in *P. lanceolata* and *C. arenosa* leaves from metalliferous stands as well as in *V. myrtillus* from the most contaminated site, higher levels of GSHt were generally recorded and positive correlations between GSHt and metals such as Pb (*P. lanceolata*) and Zn and Cd (*C. arenosa*) were found (Nadgórska-Socha et al. 2013; Kandziora-Ciupa et al. 2013).

In the case of ascorbic acid content, we observed a decreasing tendency in contaminated sites in the foliage of *R. pseudoacacia* (except C3), opposite to the leaves of *M. album*, where in the most contaminated stand C1 the highest ascorbic acid content was found (Table 8). However, a positive correlation between Pb, Cd, Zn and Cu and ascorbic acid content was only found in *M. album* leaves (Table 10). In the study of Rai and Panda (2014), the ascorbic acid content was lower in the leaves of plant at a contaminated site than those of the control site. The variations in contents in the above-mentioned metabolite were found in the foliage of trees across the exposed locations

Table 8 Biochemical parameters in investigated plants (mean ± SD)

Species	Analysis site	Total chlorophyll content (mg g⁻¹ f.w.)	Ascorbic acid content (mg g⁻¹ f.w.)	pH	RWC (%)	APTI	GSHt content (μM GSH g⁻¹ f.w.)	NP–SH content (nM –SH g⁻¹ f.w.)	POD activity (μM tetra-guaiacol min⁻¹ g f.w.⁻¹)	SOD activity (U)
Robinia pseudoacacia	NC	2.1 ± 0.12 e	3.7 ± 0.1 b	6.1 ± 0.08 a	82.5 ± 1.6 c	11.3 ± 0.1 d	26.4 ± 5.7 a	90.9 ± 5.9 b	0.05 ± 0.002 a	65.2 ± 4.3 a
	C1	1.4 ± 0.07 c	1.5 ± 0.4 a	6.2 ± 0.06 a	73.7 ± 1.5 b	8.5 ± 0.3 a	312.5 ± 22.7 d	328.7 ± 13.9 e	0.077 ± 0.003 a	119.0 ± 1.0 b
	C2	2.2 ± 0.03 e	1.9 ± 0.1 a	6.4 ± 0.05 c	65.7 ± 1.9 a	8.2 ± 0.2 a	252.9 ± 17.0 cd	206.9 ± 16.2 d	0.088 ± 0.001 ab	337.7 ± 1.2 e
	C3	1.5 ± 0.15 c	3.6 ± 0.2 b	6.1 ± 0.05 a	74.3 ± 1.4 b	10.2 ± 0.2 c	220.1 ± 17.1 c	220.2 ± 9.2 d	0.079 ± 0.003 ab	204.0 ± 16.8 d
	C4	1.8 ± 0.02 d	2.2 ± 0.3 a	6.1 ± 0.07 a	74.31 ± 1.6 b	9.1 ± 0.2 b	70.9 ± 3.0 b	327.9 ± 4.1 e	0.096 ± 0.007 ab	192.3 ± 24.4 d
Melandrium album	NC	0.7 ± 0.01 a	1.9 ± 0.2 a	6.6 ± 0.05 b	82.39 ± 1.3 c	9.6 ± 0.1 b	57.7 ± 18.4 b	48.3 ± 17.1 a	0.12 ± 0.02 b	154.7 ± 8.0 c
	C1	0.7 ± 0.07 a	3.2 ± 0.1 b	6.6 ± 0.04 b	71.92 ± 1.7 b	9.5 ± 0.1 b	743.2 ± 68.5 f	45.1 ± 13.2 a	0.62 ± 0.01 e	44.4 ± 1.0a
	C2	0.8 ± 0.04 a	1.9 ± 0.6 a	6.99 ± 0.07 c	65.94 ± 1.3 a	8.1 ± 0.5 a	177.3 ± 20.8 c	62.2 ± 14.1 ab	0.35 ± 0.01 d	216.6 ± 28.0d
	C3	0.9 ± 0.04 ab	1.3 ± 0.3 a	6.9 ± 0.03 c	62.04 ± 2.2 a	7.2 ± 0.3 a	284.1 ± 15.1 e	144.8 ± 22 c	0.21 ± 0.04 c	62.0 ± 3.0 a
	C4	1.0 ± 0.09 b	1.9 ± 0.3 a	6.5 ± 0.06 b	76.47 ± 1.9 b	9.1 ± 0.3 b	206.1 ± 24.1 c	43.5 ± 3.9 a	0.20 ± 0.01 c	149.9 ± 5.0c

Different letters in the same row denote statistical difference at $p < 0.05$

for each tree species, and higher ascorbic acid content was found at the university in Ilorin (north central Nigeria) than in the control site (nearby village) (Ogunkunle et al. 2015). Increased levels of ascorbic acid content favors pollution tolerance, which may be due to the defense mechanism of the respective plants (Rai and Panda 2014). The total chlorophyll content differed significantly between the investigated species. Generally the depletion of total chlorophyll content was observed in *R. pseudoacacia* leaves (with the exception of site C2, Table 8). An opposite tendency was observed for *M. album* where a higher or comparable level of the green pigment was found in the contaminated stands in comparison with the non-contaminated stand. This is consistent with the results of Ogunkunle et al. (2015). The high chlorophyll content recorded at the staff residence showed that *Acacia nilotica*, *Prosopis africana* and *Vitellaria paradoxa* exhibited tolerance to the pollution in the area, as a higher chlorophyll content may favor tolerance to pollutants. The pH of the leaf extract ranged from 6.06 to 6.99 (Table 8). A higher pH is known to improve tolerance to air pollution (Pathak et al. 2011; Ogunkunle et al. 2015). Lower RWC values were recorded in the leaves of both species in the contaminated sites. The pH values of the leaf extracts of *R. pseudoacacia* were generally at the comparable level in plants collected at contaminated sites (except C2) in comparison with the site NC, and the *M. album* pH extracts were only higher in two of the contaminated sites—C2 and C3 (Table 8).

The use of a single parameter in ecophysiological research may not provide a clear picture of the pollution induced changes that may occur in plants. Therefore, an air pollution tolerance index based on combined values of ascorbic acid, relative water content, total chlorophyll and pH of leaf extract has been used for plant tolerance evaluation (Pathak et al. 2011; Rai and Panda 2014; Ogunkunle et al. 2015). Higher APTI values were calculated for *R. pseudoacacia* than for *M. album*. The mean APTI values for all investigated sites were 9.4 and 8.7 for *R. pseudoacacia* and *M. album*, respectively (Table 8). In these two species, the APTI values were also higher in the NC site (except C1 and C4 in *M. album*) in comparison with the contaminated sites. These findings are in agreement with the results of Rai and Panda (2014), who observed higher APTI values at the control site compared to the contaminated site. In APTI evaluation studies, varied plant species are selected (available and often widespread on study areas trees, shrubs and herbaceous plants). The researchers emphasize that the sensitivity and response of plants to pollutants vary. Plant species that are more sensitive (lower APTI values) act as bioindicators of air pollution while tolerant species according to APTI can be useful for green belt development (Sharma et al. 2013; Enete et al. 2013; Padmavathi et al. 2013; Ogunkunle et al. 2015; Rai and

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Table 9 Correlation coefficients between the concentrations of particular metal concentrations and metabolites in *R. pseudoacacia* (values with * are statistically significant. $p < 0.05$)

	Total chlorophyll	Ascorbic acid	pH	RWC	APTI	GSHt	NP–SH	POD	SOD
Mn	0.44*	0.26	−0.24	0.46*	0.41*	−0.90*	−0.27	0.37	0.03
Fe	−0.17	0.25	−0.28	−0.14	0.06	−0.20	0.20	−0.09	−0.13
Cu	−0.29	−0.32	0.01	−0.12	−0.18	0.02	0.42*	−0.09	0.07
Pb	−0.29	−0.59*	0.01	−0.17	−0.47*	−0.02	0.79*	0.44*	0.03
Cd	−0.65*	−0.53*	0.05	−0.09	−0.41*	0.66*	0.53*	0.01	−0.27
Zn	−0.83*	−0.39	−0.27	0.11	−0.25	0.42*	0.67*	0.11	−0.45*

Table 10 Correlation coefficients between the concentrations of particular metal concentrations and metabolites in *M. album* (values with * are statistically significant. $p < 0.05$)

	Total chlorophyll	Ascorbic acid	pH	RWC	APTI	GSHt	NP–SH	POD	SOD
Fe	−0.04	−0.76*	0.77*	−0.51*	−0.78*	−0.49*	0.71*	−0.54*	0.17
Cd	−0.43*	0.79*	−0.31	−0.09	0.33	0.96*	−0.17	0.87*	−0.68*
Pb	−0.41*	0.73*	−0.27	−0.17	0.24	0.96*	−0.06	0.85*	−0.76*
Mn	−0.28	−0.28	0.72*	−0.24	−0.32	−0.51*	−0.04	−0.61*	0.81*
Cu	0.04	0.42*	−0.49*	0.41*	0.53*	0.32	−0.61*	0.27	0.18
Zn	−0.25	0.52*	−0.02	−0.48*	−0.1	0.93*	0.23	0.64*	−0.78*

Singh 2015). According to Padmavathi et al. (2013) the investigated plants in our study belong to the sensitive species (APTI < 11) and can be recommended as bioindicators.

Conclusion

Our results indicate that the investigated species *R. pseudoacacia* and *M. album* can grow in metal polluted areas and that the contamination alters its physiological parameters. The investigated species varied in studied heavy metal accumulation capability. The highest content of the examined metal, also shown in the metal accumulation index, was found in *M. album*—a plant growing low to the ground in comparison with the higher growing *R. pseudoacacia*. The actual metal transfer and bioavailability in the environment as estimated in the studied species could be used in ecological and health risk assessment. Consequently, this study confirms the possibility of using *M. album* as an indicator of soil pollution from Zn, Cd, Pb and the use of *R. pseudoacacia* as an indicator of soil pollution from Zn and Cd. The general markers of heavy metal contamination in the two investigated species were POD activity and GSHt content. In particular *M. album* can act as a tolerant biomarker plant with high Pb, Zn and Cd accumulation levels in leaves, with significantly higher increases in the above-mentioned parameters as well as lower phytotoxic effect on chlorophyll biosynthesis than *R. pseudoacacia*. However, *R. pseudoacacia* may also be thought of as a sensitive biomarker species for contaminated area according to its APTI calculation. The APTI values evaluated with the four biochemical parameters in plant leaves can be used as forecast of air quality. Plants respond differently to the contamination; hence, the different indices obtained for the examined species. The investigated plants found in the contaminated stands had rather lower APTI index values and can be indicative of contamination.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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