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.

Keywords Heavy metals • Air pollution tolerance index • Antioxidants

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...
Biomonitoring based on plant leaves has recently been recognized as a thrust area in the field of 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).

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 to 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 samples from the same site were collected from the lower foliage and taken to the laboratory in ice.
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 HNO₃ (HNO₃ 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 HNO₃-extractable fraction was obtained by shaking a sample (10 g) with 100 ml of 2 M HNO₃ for 1 h. Metals were extracted from air-dried samples of soil using 0.01 M CaCl₂. The bioavailable fraction (potentially bioavailable elements) was obtained by shaking a sample (1:10) with 0.01 M CaCl₂ 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. 

\[
\text{SPI} = \frac{1}{n} \sum_{i=1}^{n} \frac{100 \text{ VS}}{\text{LS}}
\]

where \( n \) is the number of elements, \( \text{VS} \) — content of an element in the soil, in mg kg⁻¹, \( \text{LS} \) — 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 HNO₃ 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:

\[
\text{MAI} = \left( \frac{1}{N} \right) \sum_{j=1}^{N} I_j
\]

where \( N \) is the total number of metals analyzed

\( I_j \) is the sub-index for variable \( j \), obtained by dividing the mean value (\( x \)) of each metal by its standard deviation \( \delta x \) (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 H₂O₂, 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 MgSO₄, 1 mM dithiothreitol (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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**Table 1 Site description**

| Abbreviation | Sites | GPS latitude, longitude |
|--------------|------|-------------------------|
| NC           | The direct vicinity of Pazurek Nature Reserve in Jaroszowice (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 Górnica) | 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′14.11″N; 19°11′51.792″E |
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 GSH 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).

\[
RWC(\%) = \frac{([FW - DW/TW - DW]) \times 100}{100}
\]

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).

\[
Ascorbic\ acid\ (mg \times g^{-1} f.w.) = \frac{(E_o - E_s - E_i)V}{W \times 100} \times 100
\]

where \(V\) is the volume of the extract, \(W\) is the weight of the leaf sample (g), and \(E_o, E_s\) and \(E_i\) 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 (HNO₃ 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 HNO₃). 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 (CaCl₂ extracted) and Fe (HNO₃ extracted) were positively correlated with organic matter content. Also Pb, Mn and Zn concentrations (HNO₃ 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 HNO₃) 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, Brzegi and Bolesław (Wójcik et al. 2014). In the case of high traffic sites such as C2 the content of Zn, Cd,
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; Banašów 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 Piekarz Śląskie and Bytom (southern Poland) (Skubala 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.

| 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 |
| GSH | 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 |
| GSH | 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 |
Table 3 Soil properties: heavy metals content (mean ± SD, extraction with HNO₃ and CaCl₂) [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 (CaCl₂)         |        | 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 (CaCl₂)         |        | 2.48 ± 0.1 b  | 0.31 ± 0.2 a  | nd         | 0.54 ± 0.1 a  | 13.48 ± 1.3 c |
| Zn (CaCl₂)         |        | 18.38 ± 1.2 a | 14.77 ± 1.6 a | 11.1 ± 0.3 b | 2.11 ± 0.6 b  | 47.59 ± 5.1 c |
| Mn (HNO₃)          |        | 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 (HNO₃)          |        | 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 (HNO₃)          |        | 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 (HNO₃)          |        | 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 (HNO₃)          |        | 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(HNO₃)           |        | 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 CaCl₂ and HNO₃ extraction) and pH value and organic matter content (values with * are statistically significant. p < 0.05)

| CaCl₂ extracted metals | HNO₃ extracted metals |
|------------------------|------------------------|
| Organic matter content | Cd                     |
|                        | 0.39                   |
|                        | 0.89*                  |
|                        | 0.84*                  |
| pH                     | -0.1                   |
|                        | -0.18                  |
|                        | 0.04                   |
|                        | 0.63*                  |
|                        | -0.22                  |
|                        | -0.1                   |
|                        | 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 Escarre 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. Silene 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 Gwoźdź (2013) and Stryjewskas (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
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 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.

### Table 5

| Species          | Site  | Metal | Mn (± SD) | Fe (± SD) | Cu (± SD) | Pb (± SD) | Cd (± SD) | Zn (± SD) | MAI      |
|------------------|-------|-------|-----------|-----------|-----------|-----------|-----------|-----------|----------|
| *Robinia pseudoacacia* | NC    | Mn     | 21.43 ± 1.3c | 78.28 ± 7.2a | 12.90 ± 0.7a | 42.40 ± 1.7g | 156.21 ± 4.59 | 113.33 ± 1.6c |
|                  | C1    | Cd     | 12.90 ± 0.7a | 78.28 ± 7.2a | 12.90 ± 0.7a | 42.40 ± 1.7g | 156.21 ± 4.59 | 113.33 ± 1.6c |
|                  | C2    | Cu     | 13.96 ± 4.9b | 106.86 ± 13.7d | 13.53 ± 4.1b | 43.42 ± 0.7g | 156.21 ± 4.59 | 113.33 ± 1.6c |
|                  | C3    | Pb     | 13.96 ± 4.9b | 106.86 ± 13.7d | 13.53 ± 4.1b | 43.42 ± 0.7g | 156.21 ± 4.59 | 113.33 ± 1.6c |
|                  | C4    | Pb     | 13.96 ± 4.9b | 106.86 ± 13.7d | 13.53 ± 4.1b | 43.42 ± 0.7g | 156.21 ± 4.59 | 113.33 ± 1.6c |
| *Melandrium album*  | NC    | Mn     | 31.64 ± 1.3e | 78.28 ± 7.2a | 12.90 ± 0.7a | 42.40 ± 1.7g | 156.21 ± 4.59 | 113.33 ± 1.6c |
|                  | C1    | Cd     | 12.90 ± 0.7a | 78.28 ± 7.2a | 12.90 ± 0.7a | 42.40 ± 1.7g | 156.21 ± 4.59 | 113.33 ± 1.6c |
|                  | C2    | Cu     | 13.96 ± 4.9b | 106.86 ± 13.7d | 13.53 ± 4.1b | 43.42 ± 0.7g | 156.21 ± 4.59 | 113.33 ± 1.6c |
|                  | C3    | Pb     | 13.96 ± 4.9b | 106.86 ± 13.7d | 13.53 ± 4.1b | 43.42 ± 0.7g | 156.21 ± 4.59 | 113.33 ± 1.6c |
|                  | C4    | Pb     | 13.96 ± 4.9b | 106.86 ± 13.7d | 13.53 ± 4.1b | 43.42 ± 0.7g | 156.21 ± 4.59 | 113.33 ± 1.6c |

The different letters denote significant differences between particular metal concentrations ($p < 0.05$).
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 (GSH) and non-protein thiols (NP–SH) (with the exception of M. album) in the leaves of investigated species in the contaminated areas in comparison with the non-contaminated one (Table 8). The highest content of GSH 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 GSH 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 (Nadgorska-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 Miaszczko 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 GSH were generally recorded and positive correlations between GSH and metals such as Pb (P. lanceolata) and Zn and Cd (C. arenosa) were found (Nadgorska-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.
Biochemical parameters in investigated plants (mean ± SD)

| Species          | Ascorbic acid (mg g⁻¹ f.w.) | pH | RWC (%) | APTI | GSHt content (µM GSH g⁻¹ f.w.) | POδ activity (U f. g⁻¹ f.w.) | SOD activity (U g⁻¹ f.w.) |
|------------------|----------------------------|----|---------|------|-------------------------------|-----------------------------|---------------------------|
| R. pseudoacacia  | 2.1 ± 0.12 e                | 3.7 ± 0.1 b | 64.2 ± 0.2 c | 0.86 ± 0.04 d | 1.3 ± 0.3 a | 1.6 ± 0.2 a |
| M. album         | 1.4 ± 0.07 c                | 1.9 ± 0.04 a | 74.3 ± 0.2 b | 0.07 ± 0.04 b | 2.0 ± 0.1 b | 0.08 ± 0.01 b |
| C1               | 0.7 ± 0.02 c                | 1.9 ± 0.04 b | 62.0 ± 0.2 c | 6.5 ± 0.06 b | 1.9 ± 0.03 a |
| C2               | 0.8 ± 0.04 b                | 1.3 ± 0.02 a | 74.5 ± 0.2 b | 0.07 ± 0.04 b | 2.0 ± 0.1 b | 0.08 ± 0.01 b |
| C3               | 0.9 ± 0.04 b                | 1.3 ± 0.02 a | 74.5 ± 0.2 b | 0.07 ± 0.04 b | 2.0 ± 0.1 b | 0.08 ± 0.01 b |
| C4               | 1.0 ± 0.09 b                | 1.9 ± 0.03 a | 74.5 ± 0.2 b | 0.07 ± 0.04 b | 2.0 ± 0.1 b | 0.08 ± 0.01 b |

Different letters in the same row denote statistical difference at p < 0.05.
Zn – Cd – Pb – Cu – Fe

In particular contamination in the two investigated species were POD from Zn and Cd. The general markers of heavy metal species (APTI) investigated plants in our study belong to the sensitive group in comparison with the higher growing R. pseudacacia. 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. pseudocacacia 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. pseudocacacia. However, R. pseudocacacia 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.

**Conclusion**

Our results indicate that the investigated species R. pseudocacacia 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 parameters. The investigated species varied in studied 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. pseudocacacia. However, R. pseudocacacia 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.

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**Compliance with ethical standards**

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

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

| Metal | 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* | 0.37 | 0.07 |
| 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)

| Metal | 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* |
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