

Original Research

Bioaccumulation of Heavy Metals in Selected Organs of Black Locust (*Robinia pseudoacacia*) and their Potential Use as Air Contamination Bioindicators

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Abstract

We analyzed concentrations of Cd, Cu, Fe, Pb, and Zn in bark, leaves, young shoots, pericarp, and seeds of *Robinia pseudoacacia* from several sites (three industrial cities and two rural villages) in southern Poland. The objective of the study was to determine the possible usefulness of various organs and tissues of this species for biomonitoring. The analyses showed that bark and leaves are organs that may serve as good bioindicators. The lowest concentrations of Cd, Pb, and Zn were found in one of the industrial cities in the area. On the other hand, the concentrations of Cd, Pb, and Zn were similar in the rural areas when compared to the concentrations observed in the cities. The high level of metal contamination of air in rural areas may be a result of the long-range transport of emissions. Also, the use of bark and leaves of *R. pseudoacacia* enables us to estimate the level of contamination by Fe and Cu of areas in the vicinity of railway tracks. On the basis of the data presented in this paper and the data from references, a conclusion can be drawn that the bark and leaves of *R. pseudoacacia* are good bioindicators of metal contamination of air in towns and

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cities with different traffic intensities and within the surroundings of industrial plants and railway tracks. *R. pseudoacacia* was also used for the first time to assess the long-range transport of emissions.

Keywords: biomonitoring, *Robinia pseudoacacia*, plant organs, traffic emissions, long-range transport

Introduction

Since the beginning of the industrial revolution contamination of the environment by toxic metals has increased dramatically worldwide. Ferrous and non-ferrous smelting and mining, road traffic, combustion of fossil fuels, and waste incineration are major contributory factors in the rise of Cd, Cu, Pb, Zn, and Fe levels in soil, water, and air. All those metals may have a negative effect on plants, animals, and human beings [1]. Thus monitoring becomes a necessary tool for maintaining the concentrations of metals in the biosphere at a safe level.

Biomonitoring is a technique based on analysis of organisms or biomaterials to obtain data on environmental contamination, which is also a relatively cost-effective method and can be successfully applied worldwide [2]. This technique seems to be particularly useful in air monitoring. So far the vast majority of measurements of metal concentration in the atmosphere have been carried out using lichens and mosses [3-14]. However, in polluted areas higher plants seem to be more suitable as ecological indicators of air contamination, since lichens and mosses are often missing from such environments [15-16].

Various species of herbaceous plants [17-23] and trees [16, 24-30] have been used to monitor concentrations of metals in the air. In urban environments, however, trees seem to be more suitable as ecological indicators because crowns and bark are very efficient at trapping atmospheric particles [16, 31-33]. So far most studies have been performed on coniferous tree species from the genus *Pinus*, e.g. *P. sylvestris* [34-38], *P. halepensis* [15], or *P. nigra* [39]. Investigations also have been carried out on deciduous trees, among which *Aesculus hippocastanum* [40-42], *Salix* spp. [43-45], *Populus* spp. [39-40, 43], *Quercus* spp. [24-25, 39], and *Fraxinus* spp. [40, 46-47] have mostly been chosen.

When compared to other deciduous or coniferous trees there is still a dearth of data on the use of *Robinia pseudoacacia* in the determination of metal levels in the air, although it has various positive features as a bioindicator. Black locust (*Robinia pseudoacacia* L.) is a deciduous tree species native to North America [48], nevertheless, it occurs in Europe, Asia, Australia, Africa, and also in parts of South America [32, 49-51], where it is planted for ornamental purposes and to stabilize dry soil as it can survive in a wide temperature range and grows in almost any type of soil [49 and literature therein, 52]. This species is mainly planted along roadsides in both urban and rural areas as it is resistant to environmental pollution [32, 53-54]. Moreover, sampling, identification, and cultivation of *R. pseudoacacia* is easy and inexpensive [32, 49].

Few papers on *R. pseudoacacia* as a bioindicator have been published so far. Most of them report on metal

emissions in Turkey and concern industrial, urban, and rural areas [39-40, 49, 55-57]. As far as the authors are concerned there are four papers that present data from other parts of the world, namely Poland [32], Macedonia [58], Serbia [59], and Iran [60]. All these articles present results of studies on *R. pseudoacacia* as a bioindicator of direct industrial and/or road traffic emissions. To the authors' knowledge there have been no studies on how this tree species could be used as an indicator of long-range atmospheric transport of metal contaminants.

Various tree parts such as leaves, bark, and shoots as well as tree rings are used for bioindication purposes. Predominantly leaves have been examined in *R. pseudoacacia* [32, 39-40, 43, 49, 55-56, 58-60], whereas concentrations of metals in bark [32], shoots [57, 59-60], or flowers [58] have seldom been investigated. The suitability of other organs or tissues of *R. pseudoacacia* for bioindication purposes has not been examined so far. However, investigations have shown that metals can be taken up by leaves and translocated to different plant organs. For example, foliar spray of Zn, Cu, and Fe is commonly used as a form of fertilization and results in the distribution of metals to different plant parts [61-62]. Moreover, it was found that cadmium applied as a solution on the leaves of various wheat species was taken up and translocated to the remainder of shoots as well as to the roots [63]. Similar results were obtained with dicotyledonous plants. The uptake of Cd by the tips of leaves and the transport of the metal to petioles and stipules were observed in *Pisum sativum* [64]. Moreover, experiments conducted on this plant species in a filtered air growth cabinet showed that atmospheric Pb and Cd can contribute to metal concentration in pods (Cd, Pb) and peas (Pb) [65 and literature therein].

The objectives of the present study were to assess *R. pseudoacacia* as a potential bioindicator of the contamination of air with metals (Cd, Cu, Fe, Pb, Zn) resulting from direct industrial and road traffic emissions as well as long-range atmospheric transport. Also, the usefulness of selected organs and tissues for bioindication was determined. For this reason we examined metal accumulation (Cd, Cu, Fe, Pb, Zn) in bark, leaves, young shoots, pericarps, and seeds of *R. pseudoacacia*.

Materials and Methods

Study Area

The present study was carried out in two areas: the industrial area of the Upper Silesian Industrial Region (USIR) and a rural area in the vicinity of Kraków – both of which are in southern Poland (Fig. 1).

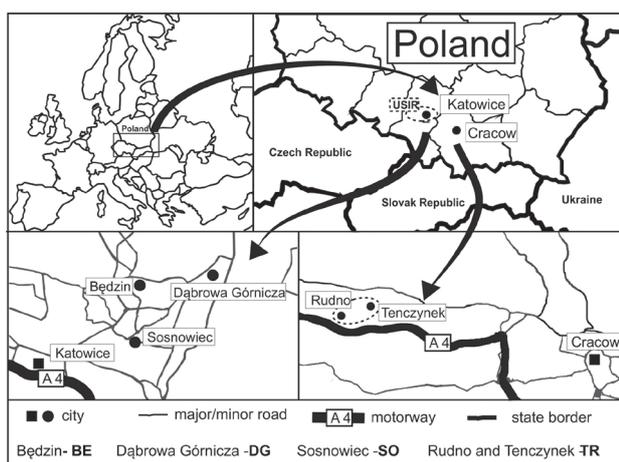


Fig. 1. Location of the study area.

Field sampling was conducted in the cities of Będzin, Dąbrowa Górnicza, and Sosnowiec in the USIR – a region that has been the site of intensive coal mining as well as Zn/Pb and Fe smelting since the 19th century. Power plants, smelters, coal mines, and chemical plants are situated within the cities. Moreover, chemical and food industries, coke-oven batteries as well as machine and electrical engineering also account for emissions of metal contaminants in the region [66].

Rudno and Tenczynek were the second sampling site. They are villages situated close to Kraków, one of the largest Polish cities. Rudno is located in the Tenczynek Natural Landscape Park, whereas Tenczynek is situated in the park’s vicinity and both villages are holiday resorts. Although Tenczynek and Rudno are located in a non-industrial area, they are surrounded by a number of industrial plants, e.g., the Alwernia chemical plant (8 km), Trzebinia oil refinery (11 km), the Boleslaw Mine and Metallurgical Plant in Bukowno (lead and zinc smelter, 19 km), the Jaworzno III power plant (21 km), and Dwory Chemical Company (22 km). In addition, the A4 motorway runs south of the site.

The USIR was chosen as a sampling site because it is an area with direct industrial and road traffic pollution emissions, whereas Tenczynek and Rudno were chosen as villages exposed to long-range atmospheric transport of contaminants.

Sample Collection

Samples were collected in the USIR in September 2007 and 2008 from Będzin (BD), Dąbrowa Górnicza (DG), and Sosnowiec (SO), and from the villages Tenczynek and Rudno (both TR). Collection of samples was carried out in September in order to determine the concentration of metals that had been accumulating for the whole vegetative period. Samples were taken in each city (BD, DG, SO) at five sampling sites (e.g., BD1, BD2, BD3, BD4, BD5) and in both villages (TR) at four sampling sites (TR1, TR2, TR3, TR4) for a total of 19 sites altogether (Table 1). Each sampling site consisted of a clump of at least five trees. In each case black locust (*Robinia pseudoacacia* L.) seed pods were taken from all trees in the clump and subsequently divided into seeds (S), pericarps (P), bark (B), leaves (L), and young shoots (SH).

The tree trunks were cleaned with a brush at a height of 1.3 m above the ground in order to remove the deposited dust particles. Then bark samples of 2-3 mm thickness were collected. Four to five young greenish shoots and 10-15 bunches of pods were collected up to a height of 3.5 m from all sides of the crown. Leaves (500-800 g of fresh weight) were taken up to 3.5 m from all sides of the crown as well as from its centre.

Sample Preparation and Analysis

In the laboratory the collected plant material was rinsed several times with tap water and then with distilled water in order to remove current contaminants as per [18, 42, 60]. The material was subsequently air-dried and pods were divided into seeds and pericarps. The air-dried samples were ground in a mill. The plant tissue was then dried in an oven at 105°C. In order to analyze the concentration of metals for each sampling site three subsamples were prepared from the dried plant tissue. The subsamples were subsequently dry-ashed at 460°C for 24 h. The ash was dissolved in 10% HNO₃ and filtered (filter paper with pore diameter of 3-5 μm) [67]. In the resulting solution the concentrations of Cd, Cu, Fe, Pb, and Zn were determined using of flame atomic absorption spectrophotometry (Unicam 939 Solar). The total number of analyzed subsamples of all tree organs and tissues from all sampling sites amounted to 285.

Table 1. Categories of sampling site locations distinguished on the basis of metal emission sources (industry, traffic, and railway).

Categories of sampling site locations		Sampling sites			
		Będzin (BD)	Dąbrowa Górnicza (DG)	Sosnowiec (SO)	Tenczynek and Rudno (TR)
Industry and heavy traffic	IH	BD3	DG3, DG4	SO1, SO2, SO4	
Railway	RW	BD2	DG2	SO5	TR1
Industry and low traffic	IL	BD1, BD4, BD5	DG1, DG5	SO3	
Long-range atmospheric transport	LR				TR2, TR3, TR4

Table 2. Metal concentrations (mg kg⁻¹) in organs and tissues (bark, leaves, seeds, pericarps, shoots) of *Robinia pseudoacacia* according to the categories of sampling site locations (IH, RW, IL, LR).

Element	Organ or tissue	Categories of sampling site locations			
		IH	RW	IL	LR
Cd	bark	2.44±0.17 (2.52) a	2.19±0.12 (2.27) a	2.21±0.10 (2.20) a	2.63±0.14 (2.74)
	leaves	2.20±0.07 (2.26) a.AB	1.95±0.16 (1.94) ab.AB	1.74±0.13 (1.83) ab.B	2.40±0.19 (2.74) A
	seeds	1.21±0.09 (1.28) b.B	1.54±0.16 (1.49) ab.AB	1.45±0.13 (1.28) bc.AB	1.89±0.21 (1.67) A
	pericarps	1.39±0.13 (1.28) b	1.31±0.16 (1.06) b	1.25±0.07 (1.28) c	2.08±0.28 (2.50)
	shoots	1.53±0.10 (1.49) b	1.79±0.17 (1.66) ab	1.41±0.11 (1.28) bc	1.76±0.22 (1.67)
Fe	bark	211.5±22.07 (207.9) a.BC	558.6±127.72 (354.9) a.AB	211.2±33.97 (151.0) a.C	600.3±88.42 (496.7) a.A
	leaves	59.7±3.75 (54.9) b.B	95.8±10.71 (85.4) ab.A	58.1±5.71 (56.3) b.B	53.8±7.03 (61.9) bc.AB
	seeds	66.7±3.62 (63.9) b.AB	72.0±1.96 (85.4) b.A	58.7±1.19 (58.0) b.B	62.3±6.01 (62.0) ab.AB
	pericarps	55.9±1.81 (52.4) b.A	61.6±7.35 (57.9) b.A	41.3±2.96 (36.8) c.B	20.3±7.32 (8.31) bc.B
	shoots	53.8±2.18 (52.4) b.A	59.3±7.67 (61.1) b.A	43.5±2.64 (41.4) bc.AB	6.58±0.82 (8.32) c.B
Pb	bark	29.4±1.98 (29.1) a.AB	22.1±4.26 (22.1) B	24.3±3.04 (25.8) a.AB	34.3±3.44 (36.5) a.A
	leaves	20.6±2.81 (18.7) ab	27.3±4.21 (31.2)	16.1±2.03 (13.9) ab	16.6±4.18 (8.33) ab
	seeds	10.8±1.21 (9.16) b	11.1±2.13 (8.31)	10.3±1.61 (8.31) bc	15.1±3.72 (8.33) b
	pericarps	15.0±1.87 (14.0) b.AB	19.8±3.72 (14.0) A	9.26±1.78 (6.24) c.B	27.1±6.01 (33.2) ab.A
	shoots	18.4±2.24 (18.7) b	15.9±2.58 (13.7)	16.9±2.16 (15.3) ab	10.2±1.40 (8.32) b
Cu	bark	9.98±0.19 (9.65) ab.A	10.7±0.56 (11.8) a.A	9.81±0.48 (9.64) a.A	6.83±0.62 (6.83) ab.B
	leaves	6.78±0.65 (6.24) c.A	7.86±0.36 (8.30) b.A	6.77±0.61 (8.29) b.A	3.71±0.28 (3.53) c.B
	seeds	10.9±0.18 (10.6) a.B	11.8±0.38 (11.6) a.AB	11.5±0.26 (11.3) a.AB	12.3±0.33 (11.9) a.A
	pericarps	5.48±0.66 (4.16) c	4.79±0.55 (4.16) b	4.47±0.52 (4.16) b	4.28±0.15 (4.16) bc
	shoots	7.15±0.73 (8.71) bc	6.67±0.73 (6.65) b	6.97±0.54 (8.31) b	4.77±0.60 (4.16) bc
Zn	bark	59.0±5.53 (54.7) a.A	35.8±4.74 (41.6) bc.B	47.0±4.96 (52.0) a.AB	52.0±5.41 (53.1) ab.AB
	leaves	50.8±4.70 (50.7) a	42.8±4.22 (42.5) ab	37.8±4.12 (35.0) a	52.4±5.48 (46.8) ab
	seeds	124.9±35.08 (64.5) a	86.6±22.55 (57.9) a	86.4±13.64 (65.0) a	73.6±9.26 (90.2) a
	pericarps	15.4±1.89 (16.6) b.A	13.8±2.29 (11.7) c.AB	7.45±0.64 (7.98) b.B	24.8±1.36 (23.3) c.A
	shoots	107.2±34.20 (61.2) a.A	43.5±6.50 (41.5) ab.AB	40.3±5.10 (38.0) a.AB	35.4±5.88 (27.3) bc.B

Values are means ±SE ($n = 7-18$). Numbers in brackets are medians. Medians followed by the same letter are not significantly different from each other using the Kruskal-Wallis non-parametric analysis of variance and *post hoc* multiple comparison test ($p < 0.05$).

a-d. Medians are significantly different for each metal in columns (among organs).

A-B. Medians are significantly different for each organ in rows (among the categories of sampling site location).

IH – industry and heavy traffic. RW – railway. IL – industry and low traffic. LR – long-range atmospheric transport.

All elements were determined against standards (BDH Chemicals Ltd, pro analysi quality) and blanks were prepared in 0.5 M nitric acid. Blanks and standards contained the same matrix as the samples. The results were calculated on a dry-weight basis.

Statistical Analysis

On the basis of metal emission sources (industry, traffic, and railway) four categories of sampling site locations

were distinguished: 1) close to industrial plants and heavy traffic (IH), 2) close to the railway track (RW), 3) close to industrial plants and low traffic (IL), and 4) far from industry and traffic but exposed to long-range atmospheric transport (LR). Each of the 19 sampling sites was assigned to one of the location categories as shown in Table 1.

We conducted normality tests (chi square or Kolmogorov-Smirnov with the Lilliefors correction) of the measurements of five metal concentrations (Cd, Cu, Fe, Pb, and Zn) in particular plant organs (B, L, S, P, SH). The

tests showed that the distribution of most of the variables was far from normal, thus a non-parametric Kruskal-Wallis ANOVA test was run, and *post hoc* multiple comparison tests (when necessary) were applied to detect significantly different levels of concentrations of particular metals in plant organs and tissues. In other cases a parametric ANOVA test was applied. Correlations between metal concentrations as well as between plant organs were tested through Spearman's ρ coefficient. Cluster analysis using average linkage between groups and Pearson's correlation as a measure interval was applied [68] in order to compare element concentrations in organs and tissues. Statistical analysis was performed using Statistica version 9.0 software (StatSoft Inc, Tulsa, OK, USA).

Results

Effects of Sampling Site Locations on Metal Concentrations in Organs and Tissues of *Robinia pseudoacacia*

Cadmium

Statistically significant differences in the concentrations of Cd were found in leaves and seeds (Table 2, numbers in rows). At the sites subsumed under the LR category the concentration of this metal in leaves was higher when compared to the sampling sites belonging to the other categories, although statistically significant differences were identified only between the LR and IL category. In the case of seeds, statistically significant differences occurred between the LR and IH categories. The least amount of Cd was found in leaves from the IL category as well as in seeds from the IH category. However, significant differences were only observed between the IH and IL for leaves, in which the Cd concentration in the IL category was substantially lower.

Iron

Statistically significant differences in the concentration of Fe between the categories were found for each *R. pseudoacacia* organ investigated (Table 2). The highest amount of Fe in leaves, seeds, shoots, and pericarps was observed in the samples of the RW category. On the other hand the lowest amount of Fe was found in shoots and pericarps of the LR category.

Lead

The highest concentration of Pb was found in the bark of trees belonging to the LR category, whereas the lowest was in the bark of trees from the RW category with differences being statistically significant (Table 2). Compared to the other categories a considerably higher concentration of Pb was also found in the pericarps from the LR category. The amount of Pb in the leaves, seeds, and shoots did not differ substantially among the categories.

Copper

The lowest concentration of Cu was observed in the bark of trees from the LR category, while in the bark of trees from the RW category the concentration of Cu was at its highest and the differences were statistically significant (Table 2). A similar tendency was observed in leaves. The highest amount of Cu in seeds was found in the LR category and the lowest in the IH category. No significant differences in the concentration of this metal were observed among the categories in shoots and pericarps.

Zinc

The highest amount of Zn was found in bark and shoots from the IH category (Table 2), while the highest concentration of Zn in pericarps was observed in the LR category. However, the amount of Zn from the latter category did not vary considerably from the amount in the IH category. No statistically significant differences of Zn concentrations in leaves and seeds were found among the categories.

The data provided above (Table 2) indicate that the sampling sites in the LR are more heavily contaminated with Cd and Pb when compared to the sampling sites from the other categories, including the IH category. On the other hand, the highest concentration of Zn in most of the organs of *R. pseudoacacia* can be found in the IH category. The highest amount of Fe and Cu occurred in the RW category, which can be attributed to the vicinity of railway tracks. Since one of the effects of abrasion of railway tracks and traction are metallic Fe and Cu, it can be assumed that the accumulation of those forms of the elements occurs mainly in bark and leaves. Therefore, Fig. 2 shows the concentrations of Fe and Cu in the above-mentioned organs from the different sampling sites subsumed under the RW category. The highest amount of Fe ($1,208 \text{ mg kg}^{-1}$) in the bark of *R. pseudoacacia* was found at the sampling site in Tenczynek and Rudno (TR1), which is situated approximately 2 m below the tracks of a frequently used railway line. The lowest concentration of Fe (202 mg kg^{-1}) was detected in Sosnowiec (SO5), where the sampling site was located at the same level of a rarely used railway line leading to an industrial plant. Moderate concentrations were found in the bark of *R. pseudoacacia* at the sampling sites in Dąbrowa Górnicza and Będzin situated at the same level as the tracks of a frequented railway line. The concentration of Fe in leaves exhibited a tendency similar to the concentration of this element in bark. Nevertheless, such significant differences in the concentrations of Fe in leaves were not detected among the sampling sites as it was observed in bark. A similar concentration relationship to that of Fe was observed with Cu in the bark of *R. pseudoacacia* (Fig. 2).

Comparison of Metal Concentrations in Different Plant Parts

In order to evaluate the usefulness of particular organs for bioindication purposes, we compared mean metals'

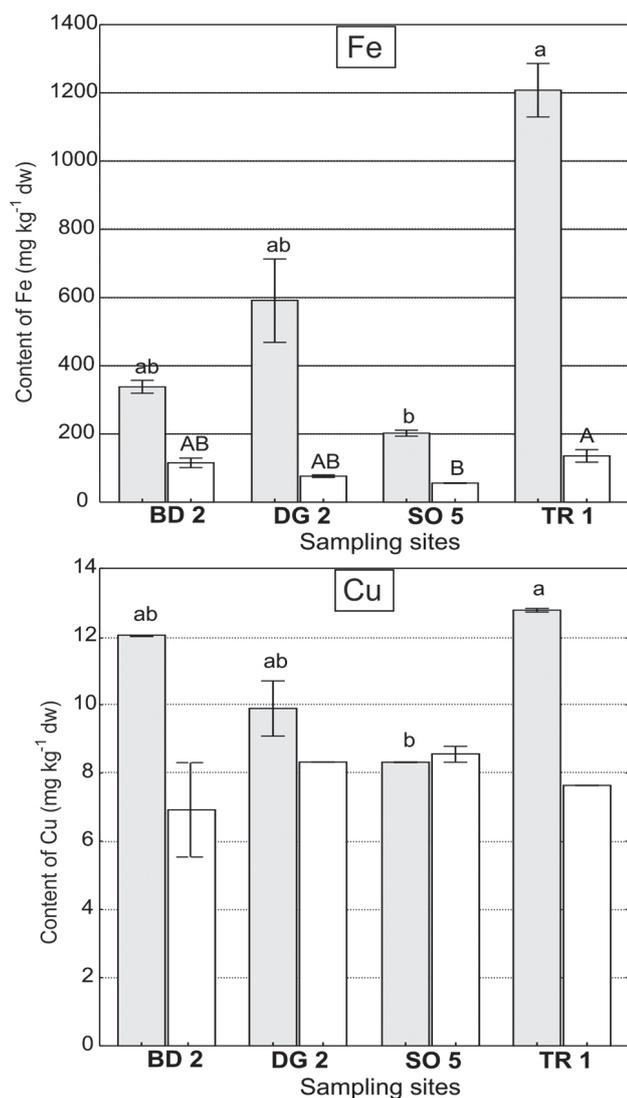


Fig. 2. Concentrations (mg kg⁻¹) of Fe and Cu in bark (grey bars) and leaves (white bars) of *Robinia pseudoacacia* collected at the sampling sites situated in the vicinity of the railway tracks. Values are means \pm SE. Values followed by the same letter (a-b, for bark and A-B, for leaves) are not significantly different from each other using ANOVA and *post hoc* test LSD ($p < 0.05$). BD – Będzin, DG – Dąbrowa Górnicza, SO – Sosnowiec, TR – Tenczynek and Rudno.

concentrations in all organs for all categories (Fig. 3). The highest amounts of Cd, Pb, and Fe were found in bark, whereas the highest concentrations of Cu and Zn were observed in seeds. Apart from Zn in shoots and pericarp, no significant differences in the amount of the metals were detected (Fig. 3).

Correlation between Element Concentrations in Organs and Tissues of *Robinia pseudoacacia*

Table 3 shows Spearman's rank correlation coefficients between element concentrations in each organ and tissue as well as between various organs and tissues of *R. pseudoacacia*. The largest number of statistically significant correlations were found between bark and leaves,

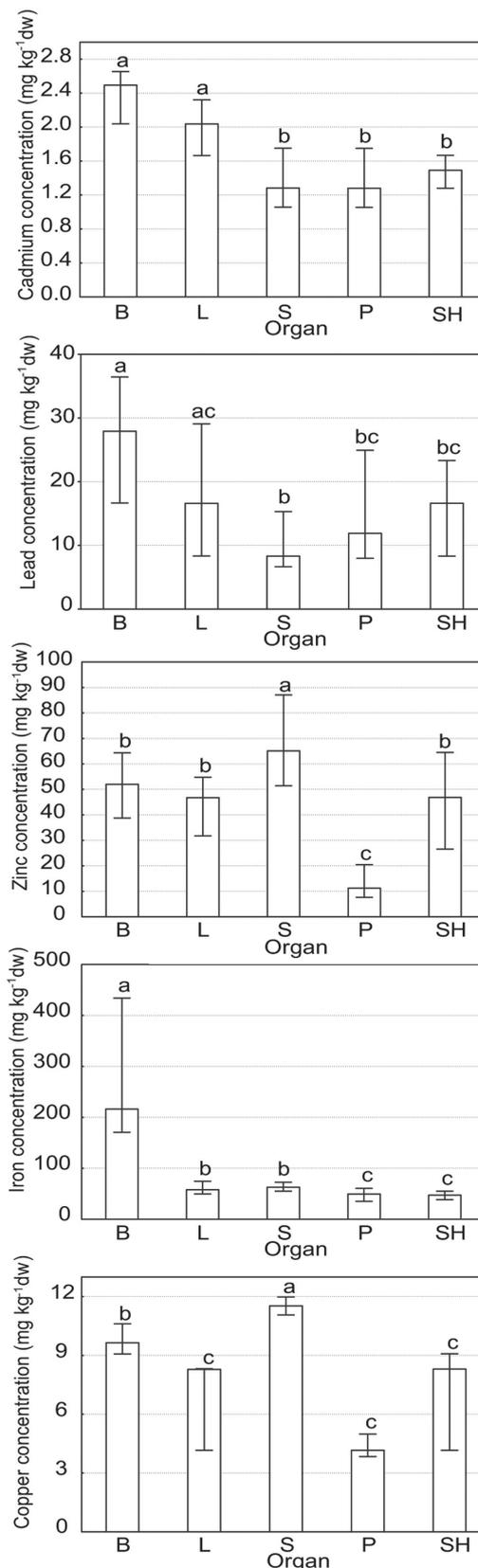


Fig. 3. Comparison of metal concentrations (mg kg⁻¹) among *Robinia pseudoacacia* organs and tissues. Values are medians \pm quartiles ($n = 49-57$). Medians followed by the same letter are not significantly different from each other using the Kruskal-Wallis non-parametric analysis of variance and *post hoc* multiple comparison test ($p < 0.05$). B – bark, L – leaves, S – seeds, P – pericarps, SH – young shoots.

Table 3. Spearman's rank correlation coefficients between the element concentrations in each organ or tissue and between various organs and tissues of *Robinia pseudoacacia* (n = 49-57).

	bark					leaves					seeds					pericarp					shoots					
	Cd	Fe	Pb	Cu	Zn	Cd	Fe	Pb	Cu	Zn	Cd	Fe	Pb	Cu	Zn	Cd	Fe	Pb	Cu	Zn	Cd	Fe	Pb	Cu	Zn	
bark	Cd	1.00																								
	Fe	0.21	1.00																							
	Pb	0.16	0.02	1.00																						
	Cu	0.14	0.04	-0.41*	1.00																					
	Zn	0.57*	-0.06	0.40*	-0.01	1.00																				
leaves	Cd	0.15	0.28*	0.12	0.03	1.00																				
	Fe	0.00	0.29*	-0.22	0.27*	-0.14	1.00																			
	Pb	0.42*	-0.03	-0.01	0.08	0.17	0.06	1.00																		
	Cu	-0.29*	-0.22	-0.21	0.03	-0.17	-0.10	0.03	1.00																	
	Zn	-0.33*	0.13	0.16	0.05	0.43*	0.26	0.08	-0.19	1.00																
seeds	Cd	-0.01	0.22	0.26	-0.34*	0.00	0.09	0.12	-0.09	0.12	1.00															
	Fe	-0.19	0.07	-0.05	0.01	-0.34*	0.11	0.40*	-0.02	0.11	-0.13	1.00														
	Pb	0.31*	0.27	0.30*	-0.22	0.40*	0.00	0.19	0.27*	0.06	0.09	0.08	1.00													
	Cu	-0.24	0.30*	-0.22	-0.26	-0.45*	0.08	-0.02	-0.12	0.10	-0.23	0.13	0.35*	0.01	1.00											
	Zn	0.21	0.19	0.15	0.09	0.29*	0.25	0.17	0.20	0.04	0.40*	0.24	0.14	0.35*	0.10	1.00										
pericarp	Cd	-0.04	0.29*	0.12	-0.20	0.01	0.24	-0.21	0.20	-0.02	-0.05	0.14	0.07	0.22	0.31*	0.52*	1.00									
	Fe	0.09	0.08	-0.18	0.45*	0.10	-0.22	0.40*	0.09	0.02	0.14	-0.10	0.24	0.05	-0.44*	-0.06	-0.29*	1.00								
	Pb	0.13	0.28*	0.10	-0.10	-0.06	0.25	0.27*	0.05	-0.15	0.30*	0.01	0.37	0.01	0.12	0.05	0.11	0.06	1.00							
	Cu	-0.29*	0.36*	-0.03	-0.06	-0.11	0.11	0.02	-0.34*	0.14	-0.07	0.00	0.18	-0.01	0.40*	0.09	0.19	0.10	-0.01	1.00						
	Zn	0.48*	0.53*	0.25	-0.14	0.27	0.50*	0.14	0.16	-0.48*	0.61*	0.12	0.30*	0.26	0.07	0.3	0.33*	0.00	0.48*	0.04	1.00					
shoots	Cd	0.01	0.08	-0.18	0.03	-0.07	0.02	0.10	0.19	0.01	0.00	0.01	-0.05	-0.11	0.19	0.13	0.17	-0.06	0.33*	0.01	0.17	1.00				
	Fe	-0.10	-0.04	-0.10	-0.30*	-0.04	-0.23	-0.05	-0.16	0.45*	-0.07	-0.10	0.32*	-0.16	-0.28	-0.08	-0.12	0.73*	-0.19	0.12	-0.31	-0.18	1.00			
	Pb	-0.12	-0.20	-0.10	-0.04	-0.21	-0.03	-0.14	-0.14	-0.11	-0.11	-0.05	-0.17	-0.10	-0.39*	-0.03	-0.04	-0.16	-0.21	-0.16	-0.16	-0.03	0.34*	1.00		
	Cu	-0.18	-0.09	-0.17	-0.13	-0.20	-0.07	-0.10	-0.11	0.45*	-0.21	0.14	-0.03	-0.11	-0.15	0.33*	0.17	-0.01	-0.15	0.12	-0.25	0.32*	0.24	0.12	1.00	
	Zn	0.22	-0.04	-0.23	-0.09	0.34*	-0.20	-0.06	0.28*	0.24	0.49*	0.02	0.08	-0.15	-0.24	0.53*	0.12	0.11	-0.09	0.00	0.33*	0.11	0.25	0.09	0.25	1.00

* Statistically significant coefficients (p<0.05).

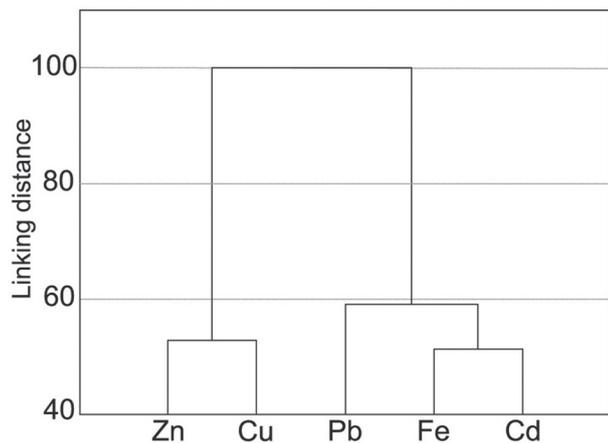


Fig. 4. Cluster analysis dendrogram concerning element concentrations in organs and tissues according to average linkage between the groups and Pearson's correlation as a measure of interval.

bark and seeds, bark and pericarp, leaves and pericarp, and seeds and pericarp. Although we found that the Cd concentration in *R. pseudoacacia* is positively correlated with the concentrations of Zn and Pb, it is negatively correlated with the concentration of Cu apart from the positive interaction of Cd-Cu in shoots. Predominantly positive correlations were observed between Fe and other elements. The Pb concentration was positively correlated with the Zn and Cd concentrations, but it was negatively correlated with the Cu concentration. The Cu concentration was negatively correlated with the Cd, Zn, and Pb concentrations. The amount of Zn correlated positively with the amount of Cu and Cd, whereas it correlated negatively with the amount of Fe and Cu.

A dendrogram showing similarities among concentrations of analyzed metals in organs and tissues is presented in Fig. 4. The cluster analysis revealed three

clusters, of which one embraced the two microelements Cu and Zn, the second Pb, and the third Cd and Fe.

Comparison of Element Concentrations between Cities

The highest concentration of Cd was found in Tenczynek and Rudno regardless of whether the concentration of this metal was analyzed in bark (Table 4A) or leaves (Table 4B). This high concentration of Cd in an area situated far from industrial plants can be attributed to long-range transport. The highest Fe concentrations in bark were detected in Tenczynek and Rudno (691.1 mg kg^{-1}), and they differed in a statistically significant manner from the concentrations in other examined areas ($185.4\text{--}257.6 \text{ mg kg}^{-1}$; Table 4A). The highest concentrations of Cu were observed in Będzin and Dąbrowa Górnicza, whereas the lowest concentrations were found in Tenczynek and Rudno (Table 4). The lowest concentrations of Pb and Zn were detected in Będzin, whereas no statistically significant differences in the concentrations of both elements were observed among the other examined areas.

Discussion

The results presented in this paper suggest that bark could be a very good bioindicator of air contamination with Cd, Pb, and Fe, which correlates with the highest concentrations of these elements in bark when compared to other organs and tissues. The research conducted by Samecka-Cymerman et al. [32] in the town of Oleśnica (Poland), in which the main air contamination source is traffic, indicated that the concentrations of Cd, Pb, and Fe in the bark of *R. pseudoacacia* amounted to 0.8 , 58 , and 106 mg kg^{-1} , respectively. Higher concentrations of Cd and Fe in the bark of *R. pseudoacacia*, which were

Table 4. Comparison of element concentrations between the cities in *Robinia pseudoacacia* organs and tissues, (A) in bark (B) in leaves.

Sampling area	Element ($\text{mg kg}^{-1} \text{ dw}$)				
	Cd	Fe	Pb	Cu	Zn
(A)					
BD	2.01 ± 0.10 (2.04) b	253.6 ± 34.05 (257.6) b	19.3 ± 2.53 (16.6) b	10.7 ± 0.43 (9.65) a	36.6 ± 3.77 (41.5) b
DG	2.34 ± 0.10 (2.50) ab	269.4 ± 57.93 (207.6) b	27.1 ± 2.39 (29.0) ab	9.38 ± 0.42 (9.65) ab	57.1 ± 2.04 (54.7) a
SO	2.48 ± 0.20 (2.62) ab	183.9 ± 6.44 (185.4) b	35.278 (36.3) a	9.69 ± 0.29 (9.65) ab	60.7 ± 6.84 (64.4) a
TR	2.60 ± 0.11 (2.50) a	752.1 ± 104.1 (691.1) a	27.4 ± 4.43 (34.8) ab	8.32 ± 0.90 (7.64) b	41.6 ± 6.73 (43.8) ab
(B)					
BD	1.87 ± 0.08 (1.84) b	63.5 ± 7.67 (52.3)	11.3 ± 1.29 (9.97) b	7.15 ± 0.58 (8.29) ab	29.4 ± 2.91 (24.9) b
DG	1.93 ± 0.13 (2.20) ab	70.6 ± 4.66 (68.7)	25.6 ± 2.87 (26.9) a	8.59 ± 0.39 (8.69) a	52.1 ± 2.87 (49.4) a
SO	2.13 ± 0.09 (2.20) ab	61.2 ± 4.30 (54.9)	23.4 ± 3.24 (20.7) a	5.86 ± 0.56 (4.16) bc	54.2 ± 4.86 (54.7) a
TR	2.41 ± 0.17 (2.62) a	74.2 ± 12.52 (61.9)	21.2 ± 3.95 (16.6) ab	4.69 ± 0.55 (3.85) c	49.8 ± 4.26 (43.4) a

Values are means \pm SE. For (A) and (B) $n = 12$. Numbers in brackets are medians. Medians followed by the same letter are not significantly different from each other using the Kruskal-Wallis non-parametric analysis of variance and *post hoc* multiple comparison test ($p < 0.05$). BD – Będzin, DG – Dąbrowa Górnicza, SO – Sosnowiec, TR – Tenczynek and Rudno.

found in categories IH and IL, when compared to the results presented by Samecka-Cymerman et al. [32], indicate that industry is a vital factor when it comes to air contamination by these elements. The highest concentration of Pb in the bark of *R. pseudoacacia* was observed in plants growing in countryside areas (category LR) and did not vary significantly from the concentration of Pb in the bark in urban areas (categories IH and IL), in which *R. pseudoacacia* was exposed to industrial and traffic contamination. Similar concentrations of this metal in the bark of *Fraxinus pensylvanica* were found by Fujiwara et al. [47] in Buenos Aires as well as by Faggi et al. [69] in Montevideo, both in heavily trafficked areas. Slightly lower concentrations of Pb than those provided for both South American cities were observed by Samecka-Cymerman et al. [32] for *R. pseudoacacia* in the town of Oleśnica. Based on the data presented above, a conclusion can be drawn that in the case of categories IH and IL the Pb concentration in air comes from traffic and industry, on the other hand in category LR mainly from long-range transport of industrial emissions. These results suggest that *R. pseudoacacia* bark might be used for biomonitoring the long-range transport of industrial emissions.

Similar to bark, leaves seem to be good bioindicators of Cd and Pb as the accumulation of these elements is only slightly lower when compared to the accumulation found in bark. This correlation has been observed regardless of the category. Samecka-Cymerman et al. [32] obtained similar results and stated that the bark of *R. pseudoacacia* is a good bioindicator of long-term cumulative traffic pollution, whereas leaves are good indicators of short-term seasonal accumulation trends. The leaves of *R. pseudoacacia* were successfully used by other authors as well [39-40, 43, 49, 55, 59].

Data presented in this paper show significantly lower concentrations of Cu and Zn in bark and leaves when compared to seeds. In the seeds of broad bean (*Vicia faba* var major), the concentrations of Cu and Zn [70, 71] were similar to the results presented in this paper. The above data suggest that both Cd and Zn as microelements, due to their physiological functions, are stored in seeds and that air contamination is not one of their sources. Therefore, the above results could suggest little potential for the use of seeds as an ecological indicator of air contamination with Cu and Zn. This does not, however, exclude the possibility of applying other organs of *R. pseudoacacia* (bark and leaves in particular) in determining the level of air contamination with Cu and Zn. The usefulness of the chestnut leaves for biomonitoring Cu in areas highly contaminated with these elements was demonstrated by Aničić et al. [42].

Data on the use of shoots for air biomonitoring is scarce, and we found only a few publications, including two concerning shoots of *R. pseudoacacia* [59, 60] and another one concerning the shoots of *Populus alba* [72]. In their research on the concentrations of Cu, Pb, and Zn in leaves and shoots of *R. pseudoacacia*, Serbula et al. [59] found higher concentrations of Cu and Zn in shoots than in

leaves. Monfared et al. [60] also observed higher concentrations of Cd and Pb in shoots of *R. pseudoacacia* when compared to leaves. However, typically lower concentrations of Cu, Fe, Pb, and Zn in shoots in comparison with the leaves of *Populus alba* were observed by Madejón et al. [72]. In this paper significantly lower concentrations in the shoots when compared to the leaves of *R. pseudoacacia* were found in the case of Cd and Fe. Taking into account the data presented above and the low number of articles on the use of shoots for biomonitoring, it remains to be seen whether this organ could be used for determining air contamination.

As pericarp had not been used for air biomonitoring until recently, an attempt has been made in this paper to test the usefulness of this tissue for determining the concentrations of metals in air. As is evident from the data presented in Table 2 and Fig. 3, no significant differences in the concentrations of the examined elements in pericarp were observed, the only exception being zinc. Thus although it is suggested that, like seeds and shoots, pericarp does not seem to be a good bioindicator, further investigations are needed.

Data on the concentrations of Cd, Cu, Mn, Ni, Pb, and Zn in leaves and flowers of *R. pseudoacacia* suggest that flowers of *R. pseudoacacia* are better bioindicators of Pb and Zn when compared to leaves [58]. However, the concentrations of both metals in the flowers and leaves of *R. pseudoacacia* found by Gjorgieva et al. [58] are considerably higher than the concentrations of these metals observed by the authors of this paper in all the examined organs and tissues. Because of inconsistent results it seems that conducting further research in order to determine the usefulness of flowers of *R. pseudoacacia* as a bioindicator of metal contamination of air is necessary.

On the basis of the conducted research it was possible to determine the differences in contamination of the examined areas. The lowest concentrations of Cd, Pb, and Zn were found in Będzin (BD). Although the town is a part of the USIR, many industrial plants situated there have been closed since the 1990s. The results presented in this paper seem to show that *R. pseudoacacia* could be a good bioindicator of air contamination and made it possible to demonstrate improvement in Będzin air quality. On the other hand, in Tenczynek and Rudno, which are agricultural areas, the concentrations of Cd, Pb, and Zn were similar to those found in the cities with still functioning industry – Dąbrowa Górnicza and Sosnowiec – which can be linked to long-range transport of industrial emissions.

As a result of conducted cluster analysis, three groups of elements were distinguished. The first one consisted of Zn and Cu, as these elements fulfill important physiological functions and are mainly accumulated in seeds. Copper and zinc are microelements and therefore it is tempting to suggest that they were predominantly taken up by roots and to a much lower extent absorbed from the air. The second cluster consists of Pb, and the third cluster consists of Fe and Cd. These two clusters are located on the one big branch of the dendrogram. The fact that both Pb and Cd (elements that have no physiological functions) are in

the one big cluster could suggest that their accumulation in the organs and tissues of *R. pseudoacacia* was mainly connected with the absorption of these elements from the air. Also, a microelement (Fe) was found in the same cluster as the toxic element Cd. This shows that it could come predominantly from the air and may be indicative of high concentrations of this element in the examined area, in particular in the vicinity of railway tracks.

Conclusions

The results presented in this paper and the data from references suggest that *R. pseudoacacia* may be a good bioindicator of metal contamination of air. Of the examined organs, bark and leaves appeared to be most useful for bioindication, while pericarp, seeds, and shoots appeared less effective. *R. pseudoacacia* might be used to examine the long-range transport of emissions, the effect of railway tracks, and the level of metal contamination of air in cities. However, more investigations in Europe and other parts of the world are necessary. Among the examined cities, Będzin was found to be the least contaminated area, which corresponds with the fact that many industrial plants in this city are out of operation. Interestingly, the villages Tenczynek and Rudno, which are agricultural areas, were found to be contaminated with metals to a similar extent as the cities, which is likely to be connected with the long-range transport of emissions.

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