

Original Research

Biomonitoring of Air Pollution by Trace Elements Using Italian Ryegrass (*Lolium multiflorum* L. 'Lema')

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Abstract

Results of investigations and assessment of air pollution by cadmium, lead, and arsenic using Italian ryegrass are presented in this paper. The experiment was carried out in the 2011 growing season in Poznań city and surroundings areas. *Lolium multiflorum* L. 'Lema' exhibits several properties useful for active biomonitoring of air pollution. Plants were exposed at sites varying in environmental characteristics. High cadmium and lead concentrations in leaves were noted in plants exposed within the city area. Canonical variate analysis illustrated variability in concentrations of elements in certain exposure series. The highest arsenic concentrations were observed in the first exposure series, while the highest lead concentrations were observed during the second series. Comparison of trace element concentrations at exposure sites to the control site revealed that comparable levels occurred in the city sites and the Agro-ecological Landscape Park. This was an effect of high cadmium and lead levels at city sites, and arsenic at the rural site. The lowest level of measured trace elements was observed at an exposure site located 15 km from Poznań in a rural area.

Keywords: trace elements, air pollution, bioimmonitoring, Italian ryegrass

Introduction

Trace elements present in the atmosphere cannot be transported as far as gaseous pollution. The range of translocation depends on the scale of emission, type and amount of substrate, and the size of particulates [1]. Biological indications of levels and concentrations of pollution support the observations of changes caused by human activities in certain areas. Trace elements are emit-

ted in the form of gases, aerosols, or solid particles. Two types of aerosols have been defined. Primary aerosols are directly emitted to the atmosphere from the earth surface, while secondary aerosols are created during chemical reactions, and include a gaseous component, primarily aerosol and water vapour [2]. The persistence of aerosols in the air, which consist of trace elements, is a function of the particle size. The smallest particles (0.001-0.08 µm diameter) survive less than 1 hour, which is connected with their coagulation in larger particles. Medium particles with diameter of 0.08-0.1 µm can function 4-40 days. The biggest fractions

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(more than 1 μm diameter) occur for a relatively short time, from 1 minute to 24 hours, which is due to fast deposition at the nearest surface [3]. Trace elements and organic compounds, which are emitted in aerosol forms, usually settle on the basis of dry and wet deposition. Both can cause changes in surface water quality, as well as problems with development of living organisms [4].

Trace elements are natural components of the Earth crust, having a specific gravity of more than 5 $\text{g}\cdot\text{cm}^{-3}$ and atomic mass more than 50 [5]. They are also stable and can accumulate in the environment. The most frequent in the atmosphere are cadmium (Cd), lead (Pb), mercury (Hg), nickel (Ni), and arsenic (As).

Air quality can be monitored by direct measurements of air pollution, using special models or with the aid of plant indicators. Physical and chemical methods provide information about emission, transmission in the atmosphere, and concentrations near the surface, as well as level of deposition. The obtained data can be used for assessment of human and environmental risks. This kind of monitoring provides information about potential effects on the environment, but it is not possible to evaluate the real frequency and magnitude of this effect [6]. There are of course very important data obtained during physical and chemical monitoring about the level of air pollution. However, this system is relatively expensive and there is a possibility of error, when very low concentrations occur. Biological methods can fill this gap, due to their relatively low costs and widespread use in many inaccessible places.

Biomonitoring is a process in which living organisms or their parts are used to acquire quantitative information about environmental quality [7]. The most important is the possibility to observe and measure changes in morphology and anatomy [8]. Resistant species can accumulate pollutants in organs without visible symptoms. Knowledge about possibilities of accumulation combined with simple methods of sampling and measurement protocols can significantly accelerate the whole process [9]. Mosses and lichens have been found to be good trace element bioindicators due to their capacity for sorption, especially for metal ions. Both groups absorb water and soluble substances (including pollutants) through the whole body surface [10]. Higher plants are used as bioindicators in heavily industrialized areas, where lichens and mosses do not occur or are present in insufficient amounts. Bioindicator plants have an ability to accumulate trace elements present in aerosols, as well as the capacity to adsorb trace elements through the root system [11]. One of the most popular plants used for evaluation of air quality due to pollution by trace elements is Italian ryegrass (*Lolium multiflorum* L.). This is an annual species characterized by very fast growth after sowing. Fast root growth causes fast nutrient uptake as well as easy trace element adsorption [12]. The aims of the study were as follows:

- (i) To examine the possibility of using Italian ryegrass in Wielkopolska region conditions.
- (ii) To compare the obtained results of trace elements concentrations in plants between exposure sites and series.

Materials and Methods

Plant Material

Italian ryegrass (*Lolium multiflorum* ssp. *italicum* L.) cultivar 'Lema' was chosen for the present investigations. Seeds of plants were obtained from Hohenheim University in Stuttgart, Germany. These plants are characterized by relatively fast growth in a short period.

Experimental Design

The experiment was carried out during the 2011 growing season. The investigation schedule was provided according to the standardized method of the German Engineering Association [13]. Similar amounts of seeds were sown into 5 L pots filled with a standard mixture of peat and sand. Plants were watered with deionized water to avoid the additional application of trace elements. Moreover, plants were fertilized (according to their needs) during growth in the greenhouse. The last fertilizing was at least one day before transport to the exposure site. Whenever plants reached 8-10 cm height, and also one day before exposure, they were cut to 4 cm. After six weeks of cultivation in greenhouse conditions pots with plants were transported to exposure sites. Five sites were selected for these investigations, in Poznań and surrounding areas. Sites varied in air quality characteristics – there were two city sites (Nos. 1 and 2), one site in a suburban area (No. 4), one site in an agricultural area (No. 5), and one site in the Agro-ecological Landscape Park (No. 3) (Fig. 1). Sites were selected on the basis of former information about potential air pollution contamination. Plants were exposed for 28 ± 1 days. Four exposure series were carried out in the year 2011: 16.05-12.06, 13.06-10.07, 11.07-07.08, and 08.08-04.09. Five plants were exposed at every site. A similar set of plants (5 pots) was cultivated in greenhouse conditions and treated as a control (site No. 0). Continuous water supply was conducted through glass fibre wicks placed in pots and in specially constructed water reservoirs with volume ca. 8 L. Hence it was not necessary to check and water plants every week. Construction made it

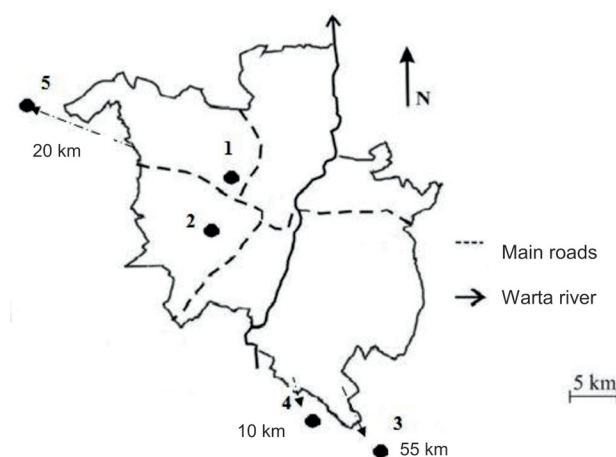


Fig. 1. Location of exposure sites in Poznań and surrounding areas.

possible to locate plants at 130 cm height above ground at every site, so we obtained comparable results of plant response to air pollution by trace elements. At each site five plants were arranged in the square: one plant in the middle with two meters diagonal.

Trace Element Concentration Measurements in Plants

Lead (Pb), cadmium (Cd), and arsenic (As) concentrations in leaves were measured after every exposure series. For this purpose leaves were dried and 0.5 g of dry weight was placed in 9 mL ultrapure HNO₃. Samples were mineralized in MARS 5 Digestion Microwave System (CEM Corporation, USA). The whole process was divided into three phases: reaching certain parameters, maintaining the parameters (pressure 300 PSI, temperature 175°C) for 15 minutes, and cooling for the next 15 minutes. The digested samples were quantitatively transferred into 10 mL volumetric flasks, and the final volume adjusted to the mark with deionized water. Three elements (Pb, Cd, As) were determined in each prepared sample using an inductively coupled plasma mass spectrometer (ICP-MS) equipped with a dynamic reaction cell (Elan DRC II, Perkin Elmer, Canada). An ICP-MS spectrometer equipped with a Meinhard concentric nebulizer, cyclonic spray chamber, Pt cones, and quadrupole mass analyzer were used for this study. Typical instrument operating conditions for the ICP-MS spectrometer were: RF power 1150 W, plasma Ar flow rate 15 L/min, nebulizer Ar flow rate 0.98 L/min, and auxiliary Ar flow rate 1.2 L/min. While tuning the ICP-MS, compromise conditions for maximum signal intensity of the analyte (²⁴Mg⁺, ¹¹⁵In⁺, ²³⁸U⁺) and minimum ratio of oxide (¹⁴⁰Ce¹⁶O⁺/¹⁴⁰Ce < 3%) and doubly charged ions (¹²⁸Ba²⁺/¹²⁸Ba⁺ < 3%) were found. The proper conditions of ICP-MS operation were checked using a solution containing Mg, In, and U at concentrations of 1 µg·L⁻¹, and Ba concentration of 10 µg·L⁻¹ (Smart Tune Solution – Elan DRC II/plus, Atomic Spectroscopy Standard, PerkinElmer Pure). Calibration curves were established using aqueous standards of relevant elements (Pb, Cd, As). The standard solutions were prepared using 10 mg·L⁻¹ Multi-element ICP-MS Calibration Std 3 (Atomic Spectroscopy Standard, PerkinElmer, Canada). The isotopes of Sc⁴⁵ and Rh¹⁰³ were prepared from appropriate solutions with a concentration of 1000 mg·L⁻¹ (Merck, Germany). All standards were prepared daily after subsequent appropriate dilution with high-purity deionized water (Millipore, USA). The internal standards correction, scandium and rhodium at the concentration of 1 µg·L⁻¹, allowed us to correct for matrix-induced variation and instrumental drift.

The trueness of the analytical results was assessed by the use of certified reference materials for Pb, Cd, and As, in water reference material NIST 1643e.

Statistical Analysis

Results were analyzed using one-way analysis of variance with exposure site as the treatment factor. Tukey's test

Table 1. One-way analysis of variance of trace element concentrations in four exposure series with exposure site as treatment factor.

Exposure series	Pb	Cd	As
1 st	4.31**	3.241*	4.46**
2 nd	16.99***	2.32ns	385.37***
3 rd	9.26***	5.6**	60.77***
4 th	45.22***	7.93***	8.45***

*** $\alpha \leq 0.001$, ** $\alpha \leq 0.01$, * $\alpha \leq 0.05$, ns – not significant

was used to analyze the differences between measured parameters [14]. For this purpose STATISTICA 9.1 software was employed.

Additionally, comparison between the exposure site and series effects for all trace elements was presented in the space of canonical variates [15]. Evaluation of experimental series effects in comparison to the mean value of all series effects and comparison of trace element accumulation for exposure site effects to the control site were determined with the aid of canonical variate analysis [16]. The Mahalanobis procedure was used for multidimensional distances [17-19].

Results and Discussion

Biomonitoring is a common tool for assessing air pollution. For trace element pollution mostly mosses and lichens were previously used. However, some investigations revealed the usefulness of higher plants for investigating levels of select trace elements [20]. *Lolium* sp. has been considered as a potential bioindicator due to its possibility to be located in many places, as well as its fast growth. Moreover, *Lolium multiflorum* ssp. *italicum* cv. 'Lema' was recognized as a good biomonitor of trace elements, sulphur, fluoride, and organic pollution in urban centres of Europe, due to fast growth and reliable information in a short period [21, 22]. This plant species was also used in our experiment.

One-way analysis of variance revealed a highly significant ($\alpha \leq 0.001$) effect of exposure site to lead (Pb) concentrations in ryegrass leaves during the 2nd to 4th exposure series (Table 1). There were no statistically significant differences ($\alpha = 0.05$) between exposure sites, although values were always higher than control plants in the first and third exposure series. The highest levels of Pb concentration in the second exposure series were observed at sites located in the city area. Moreover, the highest values throughout the entire experimental period were observed during this series. In the next series again a higher level was observed at city sites (site Nos. 1 and 2); however, high values (statistically significantly different at level $\alpha = 0.05$) were also recorded at the site located in the Agro-ecological Landscape Park (site No. 3). During the last series the highest levels of Pb concentration in leaves were noted in one of the city sites (site No. 1), and in the rural area (site No. 5) (Fig. 2). The latter

one might be associated with the increase of agro-technical works during the late summer season, and high emissions of Pb from diesel engines of agricultural machines.

The level of Pb in green fodder is usually in the range 0.4–2.5 ppm in areas not polluted directly [23], and our results indicate that all sites were not highly polluted. This is in agreement with the investigations of Rodriguez et al. [22], where much higher mean values of Pb concentrations in *L. multiflorum* leaves were noted even at a low vehicular traffic site. Moreover, our results are comparable to results obtained in the cities of Stuttgart and Klagenfurt during a biomonitoring experiment in 2001 undertaken in several European countries using the same bioindicator [21]. Our results are at a comparable level in the city during the second and fourth exposure series to results obtained using a lichen biomonitoring system in Ankara, Turkey [24].

Moreover, we noted almost 20-fold lower values than in investigations of trace element concentrations in tree samples collected from an urban zone in Guangzhou city, China [25]. The results obtained in our investigations were even 100-fold lower than concentrations noted in mosses collected from an industrial area of southern Poland [26]. This means that our region is not polluted and the method is sensitive to low concentrations and useful to determine differences in areas with lower levels of pollution. Comparing our results to European Union law for the Pb food limit values of leafy vegetables ($0.3 \text{ mg} \cdot \text{kg}^{-1} \text{ FW}$) [27] none of the exposure sites and series exceeded this value.

The highest influence of the exposure site factor for cadmium (Cd) concentrations in leaves was noted in the

last exposure site (Table 1). There were no differences between Cd concentrations in exposure sites during the 1st exposure series. Only the 5th exposure site differed statistically ($\alpha=0.05$) from the control site. There were also no differences between sites during the second exposure series. However, we did notice some tendencies. The highest level was observed at city sites (No. 1 and 2). Higher values were again observed at these sites during the next two series. However, a high level was also recorded at site No. 3 (located in the Agro-ecological Landscape Park) and in the agricultural area (site No. 5) during the third series.

During the last series the Cd concentrations excluding city sites were more or less at a comparable level (Fig. 3). Normally, cadmium concentrations vary between 0.05 and 2 ppm, and a toxic effect in plant tissues is noticed when concentrations exceed 5 ppm [23]. In our investigations we noticed higher than normal concentrations, but they did not reach toxic values. The main source of cadmium is the metallurgical industry, which does not occur in our experimental area. Hence, values in our plants were not as high as in mosses collected in industrial areas in southern Poland [26]. Our results were at a comparable level as in plant samples collected in urban monitoring stations in Italy [28]. Moreover, values of Cd concentrations in the city area were comparable to levels obtained in *Elephantopus mollis* collected in the summer season around a coal thermal power plant in Candiota, Brazil [29]. On the other hand, the results of leaf Cd accumulation obtained in our investigations were at least twice as high as in *L. multiflorum* leaves collected from urban sites in a biomonitoring experiment of European

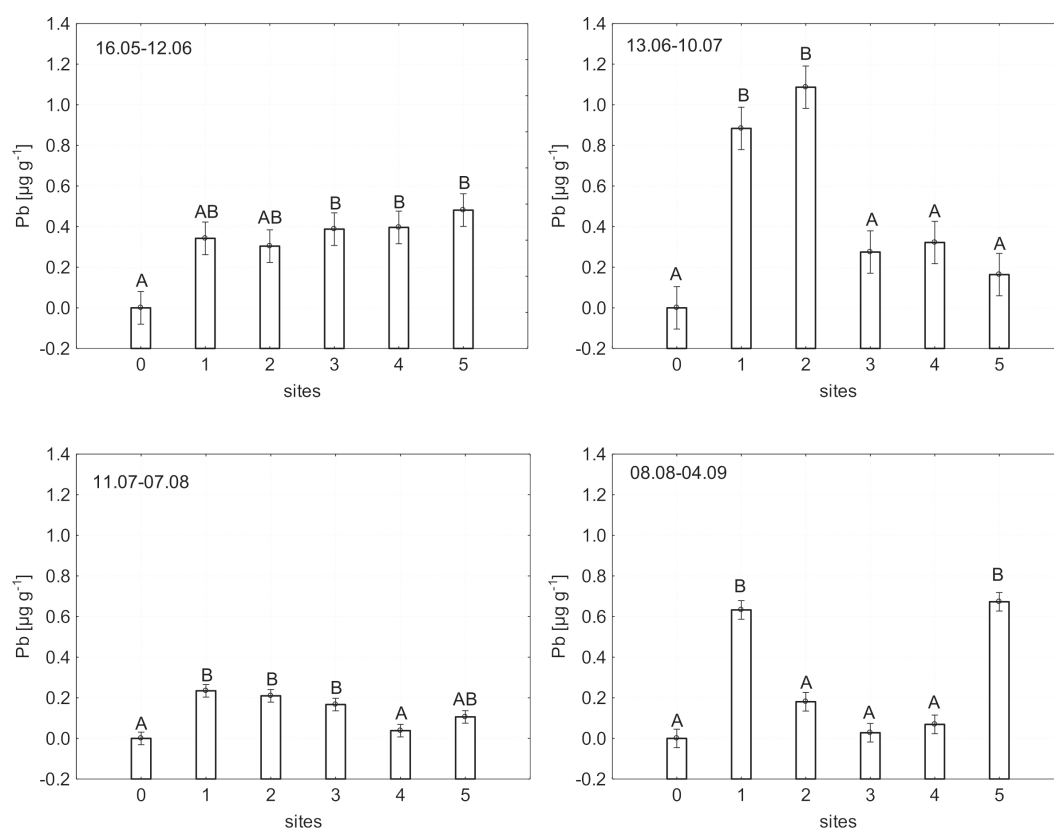


Fig. 2. Mean \pm SE (standard error) Pb concentrations in Italian ryegrass leaves during four exposure series in chosen sites (Site No. 0 – control site; Nos. 1–5 – exposure sites). Letters denote significant differences between means at $\alpha=0.05$.

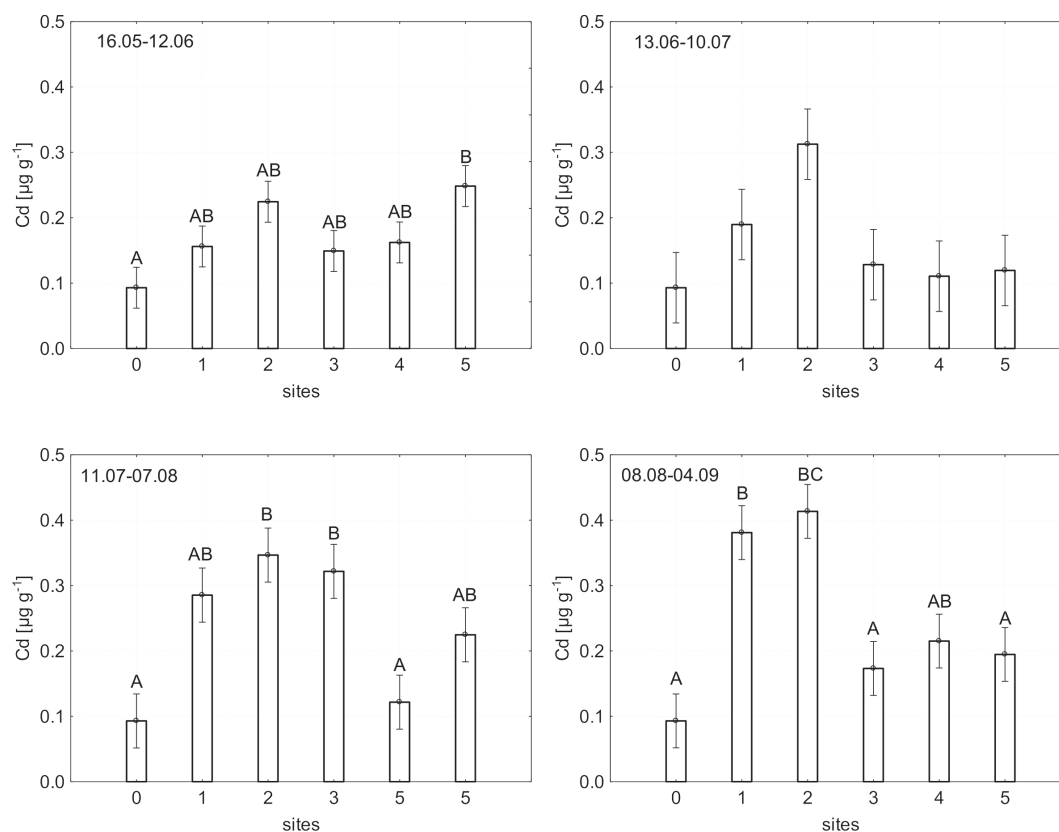


Fig. 3. Mean \pm SE Cd concentrations in Italian ryegrass leaves during four exposure series in chosen sites (Site No. 0 – control site; Nos. 1-5 – exposure sites). Letters denote significant differences between means at $\alpha=0.05$.

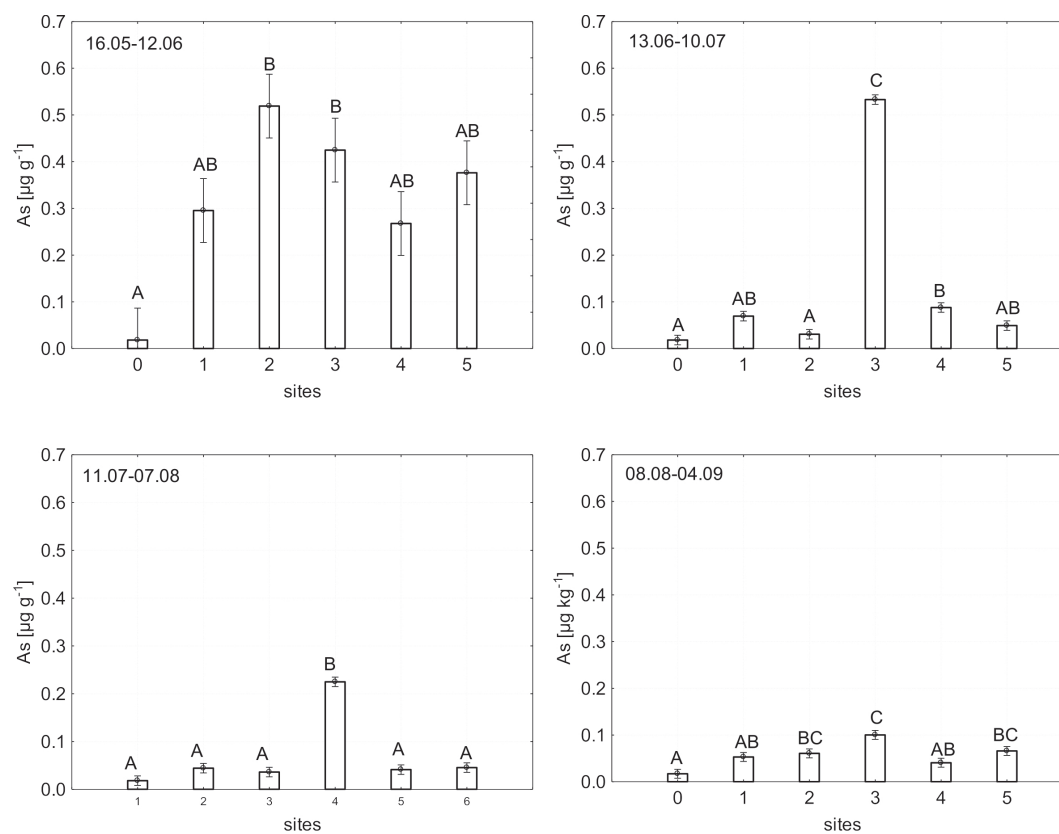


Fig. 4. Mean \pm SE As concentrations in Italian ryegrass leaves during four exposure series in chosen sites (Site No. 0 – control site; Nos. 1-5 – exposure sites). Letters denote significant differences between means at $\alpha=0.05$.

Table 2. Evaluation of experimental series effects (the mean value of trace elements for the exposure site) in comparison to mean value of all series effects.

Exposure series	Trace elements			
	Pb [$\mu\text{g}\cdot\text{g}^{-1}$]	Cd [$\mu\text{g}\cdot\text{g}^{-1}$]	As [$\mu\text{g}\cdot\text{g}^{-1}$]	Δ
1 st	0.028	-0.033*	0.182**	5.41**
2 nd	0.164**	-0.046**	-0.013	2.04**
3 rd	-0.165**	0.032*	-0.077**	2.35**
4 th	-0.027	0.047**	-0.092**	1.62**

** $\alpha \leq 0.01$, * $\alpha \leq 0.05$

Δ – Mahalanobis distances between term effects and mean value of all term effects

Table 3. Comparison of trace element accumulation for exposure site effects to control site (the mean value for all investigation series).

Exposure site	Trace elements			
	Pb [$\mu\text{g}\cdot\text{g}^{-1}$]	Cd [$\mu\text{g}\cdot\text{g}^{-1}$]	As [$\mu\text{g}\cdot\text{g}^{-1}$]	Δ
1	0.523**	0.144**	0.066**	11.17**
2	0.445**	0.215**	0.112**	11.16**
3	0.214**	0.084**	0.271**	13.42**
4	0.206**	0.043*	0.060**	2.09**
5	0.356**	0.087**	0.084**	5.8**

Δ – Mahalanobis distances between exposure site and control site. * and ** – significance level

cities [21]. The higher cadmium concentrations might be connected with emissions from combustion of motor fuels in cars and trucks and possible emissions to air resulting from particles from tire wear, as well as from pharmaceutical, metal plating and battery industries located in Poznań, and stationary fossil fuel combustion [8, 30-32].

Levels of arsenic (As) concentrations were highly influenced by exposure site factor during the 2nd to 4th series (Table 1). The highest levels during the first exposure series

were noted at one of the city sites (No. 2) and at the Agro-ecological Landscape Park (No. 3). In the second to fourth series the highest level was noted in plants exposed in the Agro-ecological Landscape Park (site No. 3) (Fig. 4). As concentrations in unpolluted areas range between 3 and 1,500 ppb [23]. This means that our results did not exceed normal values. Peak values of our results (especially at site No. 3) were at comparable levels as values in mosses collected at urban monitoring stations in Italy [29]. In most exposure series As accumulations in all exposure sites (excluding site No. 3) were at least twice as low as values noted in *L. multiflorum* collected in the biomonitoring experiment in European cities [21]. The Agro-ecological Landscape Park (site No. 3) is partly located in an agricultural area, and some arsenic could be emitted from pesticides treated in the field [32], although this type of chemical is already banned. However, arsenic is very accumulative, so the effect can be observed even several years after application.

Canonical variate analysis illustrated variability in element concentrations in certain exposure series. The highest arsenic concentrations were observed in the first exposure series, cadmium during the 4th exposure series, and lead during the second series. Overall, the highest trace element concentrations during the third and fourth exposure series were at a comparable level, while in the first they were higher (Table 2, Fig. 5). Higher arsenic levels in the first exposure series during May and June might be connected with emissions together with particles transported from the soil, which is not yet covered by plants. High lead concentrations

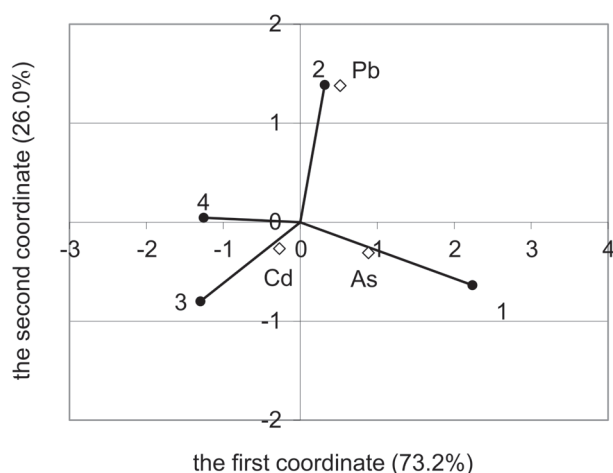


Fig. 5. Position of selected exposure series (1-4) differing in levels of trace elements in the space of the first two canonical variates and spacing of the trace elements in the dual space of canonical variates (values of canonical coordinates multiplied by 10).

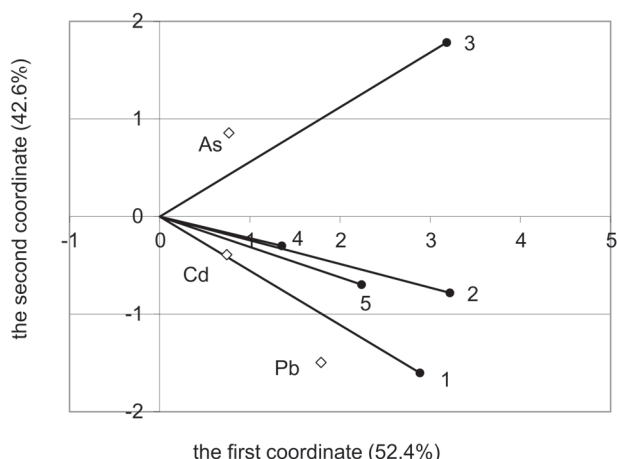


Fig. 6. Position of select exposure sites (1-5) in comparison to control site differing in levels of trace elements in the space of the first two canonical variates and spacing of the trace elements in the dual space of canonical variates (values of canonical coordinates multiplied by 10).

during the second series might be connected with fossil fuel combustion during June and July, when people are leaving for their holidays. Comparison of trace element concentrations at exposure sites to the control site revealed that comparable levels occurred in the city sites (Nos. 1 and 2) and the Agro-ecological Landscape Park (No. 3) (Fig. 6). This was an effect of high cadmium and lead levels at city sites, and arsenic at the rural site (Table 3). The lowest level of measured trace elements was observed at the exposure site located 10 km from Poznań (No. 4) in a suburban area (Table 3, Fig. 6). Previously, transport sources have been recognized as one of the major sources of particle-bound trace metals [33]; hence here we noted higher concentrations in urban sites.

Conclusions

Our investigations confirmed the possibility of using *Lolium multiflorum* ssp. *italicum* cv. Lema for a biomonitoring system in the Wielkopolska region, where relatively low trace element concentrations occur in the atmosphere. The experiment revealed differences between exposure sites and series. Higher Pb and Cd concentrations were noted in plants exposed at city sites, which might be connected with higher traffic, electroplating and pharmaceutical industries, and stationary fossil fuel combustion. Higher arsenic accumulation was noted in the Agro-ecological Landscape Park, which might be connected with the use of pesticides for plant protection in previous years. Overall, results were comparable to other European cities for most of the measured trace elements; only Cd was twice as high.

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