www.czasopisma.pan.pl www.journals.pan.pl

ACTA BIOLOGICA CRACOVIENSIA Series Botanica 60/1: 35–44, 2018
DOI: 10.24425/118043

POLSKA AKADEMIA NAUK
ODDZIAŁ W KRAKOWIE

RELATIONS BETWEEN PHOTOSYNTHETIC PIGMENTS, MACRO-ELEMENT CONTENTS AND SELECTED TRACE ELEMENTS ACCUMULATED IN *LOLIUM MULTIFLORUM* L. EXPOSED TO AMBIENT AIR CONDITIONS

KLAUDIA BOROWIAK[1]*, ANNA BUDKA[2], ANETTA HANĆ[3], DARIUSZ KAYZER[2],
MARTA LISIAK[1], JANINA ZBIERSKA[1], DANUTA BARAŁKIEWICZ[3], DONATA IWANIUK[1]
AND NATALIA ŁOPATKA[1]

[1]*Department of Ecology and Environmental Protection,*
Poznan University of Life Sciences, Piątkowska 94C, 60-649 Poznań, Poland
[2]*Department of Mathematical and Statistical Methods,*
Poznan University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland
[3]*Department of Trace Element Analysis by Spectroscopy Method, Faculty of Chemistry,*
Adam Mickiewicz University in Poznan, Umultowska 89B, 61-614 Poznań, Poland

Received October 25, 2017; revision accepted January 11, 2018

The major aim of the study was to identify the relationships of photosynthetic pigments with elemental contents of plants exposed to various ambient air conditions. *Lolium multiflorum* L. plants were exposed at five sites varying in environmental characteristics, including potential air pollution levels. The effect of air pollution by trace elements on plants was examined. Selected trace elements (Pb, Cd, As, Ni, Cr), some macro-elements as well as chlorophyll content were measured after each of four series. The graphical visualization revealed groups of sites with similar response of elements and chlorophyll contents. Sites located outside the city were grouped into one, and two urban sites were grouped into another. The trace element contents were relatively low and, excluding Ni and As, did not reach toxic levels in dry mass of leaves. However, some relations could be noted, which indicates the sensitivity of the photosynthetic process even at low levels of trace elements in ambient air. Chlorophyll *b* was found to be more sensitive to most of the analyzed trace elements than chlorophyll *a*. The results revealed chlorophylls, K and Na as indicators of plant stress caused by trace elements present in ambient air, even at relatively low levels.

Keywords: air pollution; elements; bioindicators; Italian ryegrass, chlorophylls, PCA, correlations

INTRODUCTION

Recently, air pollution by particulate matter (PM) has been increasing or remaining at a stable level in many European countries. The main source of PM is fossil fuel combustion, including traffic, household and industry. Hence, higher contents are usually noted in urban and suburban areas, in comparison to rural areas (Budka et al., 2015). PM is also a main donor of other compounds and elements, such as trace elements or polycyclic aromatic hydrocarbons. The high demand for food production and plant materials has led to intensification of agriculture and further increases in levels of pesticide and fertilizer application. These compounds increase the quantity and quality of the yield, but also include a number of various elements, such as Zn, Cd, Cr, Cu, Hg, Ni, Mo, Pb, U, and V (Alloway and Ayres, 1997; Gambuś and Wieczorek, 2012). Developing civilization and industry have led to the development of transport, which is one of the sources of trace elements. Transport itself and the whole infrastructure are the source of many trace elements, such as As, Hg, Se, Ag, Ba, Cd, Pb, and Co (Pulles et al., 2012; Wang et al., 2006).

Trace elements are absorbed by plants in the same way as other compounds that are necessary for their proper functioning. The effect of trace elements on plants is related to their contents in plant tissue. A toxic effect is observed when a certain

---

* Corresponding author, email: klaudine@up.poznan.pl

PL ISSN 0001-5296

concentration in their tissue is exceeded. A standard level occurs when the content does not cause negative effects. The possibility of accumulation and high tolerance to trace elements in the environment are necessary features for bioindicators. There are some trace elements with a dual role (Ociepa-Kubicka and Ociepa, 2012). These elements are necessary in some contents, while exceedance results in a toxic effect, as in the case of nickel and chromium (Gorlach and Mazur, 2001).

Trace elements are recognized as factors inhibiting assimilation and synthesis of pigments: chlorophyll *a*, chlorophyll *b* and carotenoids. The most sensitive is the process of creation of porphobilinogen molecules with the aid of dehydratase of delta-aminolevulinic acid (ALAD). It has been found that plants exposed to heavy metals show a decreased level of chlorophyll due to lower activity of ALAD (Stobart et al., 1985; Vajpayee et al., 2000). This enzyme consists of cysteine in the active centre, which readily joins with heavy metals and oxidizes. Moreover, trace elements also limit magnesium and iron absorption, which are necessary for chlorophyll synthesis, or trace elements can replace magnesium in the chlorophyll molecule (Burzyński, 1985).

Plants respond to trace elements by changes in the functioning of metabolic systems and enzyme inhibition (Kabata-Pendias, 2010), and as a result a decrease of photosynthetic pigment content can be noted (Budka et al., 2014; Dubey and Pandey, 2011; Karakoti et al., 2014; Muradoglu et al., 2015).

The major aim of the study was to identify the relationship of photosynthetic pigments with elemental contents of the plants exposed to various ambient air conditions.

MATERIALS AND METHODS

PLANT MATERIAL AND EXPERIMENTAL DESIGN

The experiment was carried out during the growing season of the years 2012 and 2013. The investigation schedule was provided according to the standardized method of the German Engineering Association (VDI 3957). Similar amounts of seeds of *Lolium multiflorum* ssp. *italicum* var. 'Lema' were sown into 5 L pots filled with a standard mixture of soil and peat. The plants were watered with deionized water to avoid additional application of heavy metals. Moreover, they were fertilized while growing in the greenhouse; the last fertilization was at least one day before transport to the exposure site. Every time the plants reached 8–10 cm in height, they were cut to 4 cm, also one day before each exposure series. After six weeks of cultivation in the greenhouse conditions, pots with plants were transferred to exposure sites. Five sites were selected for the present investigations and located in the city of Poznań and surrounding areas. The sites varied in the air quality characteristics – there were two city sites (no. 1 and 2), one site in a suburban area (no. 4), one site representing an agriculture area (no. 5) and one site located in a landscape park (no. 3) (Fig. 1). The plants were exposed for 28 ± 1 days. Four exposure series were carried out during the growing season in the following periods: in 2012, 14.05–10.06, 11.06–08.07, 09.07–05.08, and 06.08–02.09; and in 2013, 13.05–09.06, 10.06–07.07, 08.07–04.08, and 05.08–01.09. Five pots with plants were exposed at each site. Similar sets of plants were cultivated in greenhouse conditions with very low possibility of heavy metal air pollution. A continuous water supply was provided through glass fiber wicks placed in the pots, using specially constructed water reservoirs with a volume of ca. 8 L. The design made it possible to locate the plants at 130 cm height at every site, so we obtained comparable results of plant response to air pollution by heavy metals.

The results of elements and chlorophylls were related to basic meteorological parameters and particulate matter concentrations (PM 10) were measured at one of the experimental sites (the city) by Provincial Environmental Agency.

SAMPLE PREPARATION

After collection, the samples of plants were dried, milled and sieved, then the samples underwent digestion in a microwave oven, model Mars (CEM Corporation, North California, USA). The samples for digestion were prepared as follows: 0.5 g of the sample was transferred to the digestion vessels and 9 mL of 65% nitric acid (Merck, Germany) were added to each vessel. The microwave oven heating program proceeded in steps: (1) ramp time of 15 min to reach 1200 W, (2) hold time of 15 min at 1200 W, and (3) cooling for 20 min. The temperature during the digestion process was 175°C. The digested samples were quantitatively transferred into volumetric flasks. The digests were adjusted to 10 mL with deionized water. In parallel, the procedural blanks, including the same reagents as the samples, were prepared and digested in the same way as the samples in each digestion run.

ANALYTICAL PROCEDURE FOR ELEMENTS AND CHLOROPHYLLS

An Elan DRC II ICP-MS instrument (Perkin Elmer SCIEX, Ontario, Canada) was used to determine As, Ca, Cd, Cr, Mg, Ni and Pb levels. An ICP-MS

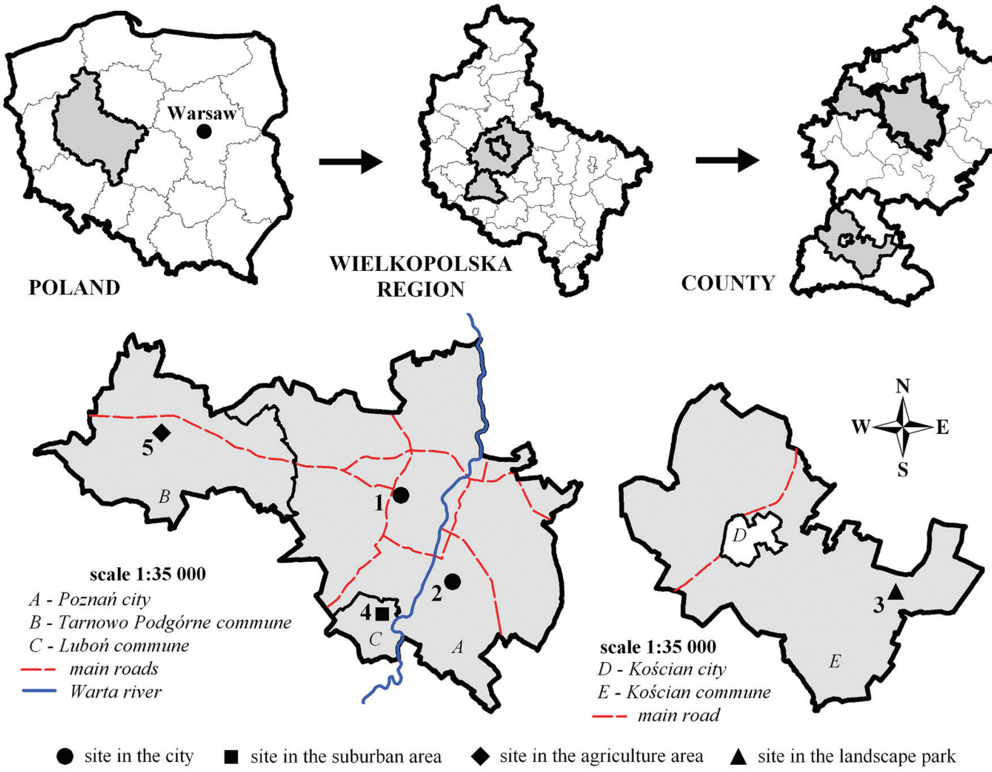

Fig. 1. Location of exposure sites.

spectrometer equipped with a Meinhard concentric nebulizer, cyclonic spray chamber, Pt cones and quadrupole mass analyzer were used for this study. Whilst tuning the ICP-MS, compromise conditions for maximum signal intensity of the analyte ($^{24}Mg^{+}$, $^{115}In^{+}$, $^{238}U^{+}$) and minimum ratio of oxide ($^{140}Ce^{16}O^{+}/140Ce < 3\%$) and doubly charged ions ($^{128}Ba^{2+}/^{128}Ba^{+} < 3\%$) were found. The proper working conditions of ICP-MS working were checked by using a solution containing Mg, In, and U at a concentration of 1 mg L^{-1} and Ba at a concentration of 10 mg L^{-1} (Smart Tune Solution e Elan DRC II/plus, Atomic Spectroscopy Standard, Perkin Elmer Pure). Deionized water was used throughout the experiment.

Elements such as Na and K were determined in each prepared sample using the flame atomic emission spectrophotometry (FAES) technique (AAnalyst 200; PerkinElmer SCIEX, Ontario, Canada) (Quality control, Supplementary material S1).

Chlorophyll *a* and *b* in dry weight content were measured after every exposure series, with extraction using the DMSO method (Hiscox and Israelstam, 1978).

STATISTICAL ANALYSIS

Empirical results of the levels of the investigated elements and chlorophyll were analyzed applying the method of multivariate two-way analysis of variance. The hypotheses about factors taken into consideration (exposure site and series) were tested with the aid of Lawley-Hotelling test statistics (Lejeune and Caliński, 2000). If the hypotheses of no effect of particular factors of the classification were rejected, the tools of repeated measures analysis of variance were applied. Let N denote the number of all observations for each element or type of chlorophyll of the i-th exposure site ($i = 0,\ldots,5$; 0 denote the results for the greenhouse) in the j-th series ($j = 1,\ldots,4$) and k-th replication ($k = 1,\ldots,5$) for one year ($N = 120$).

Let us assume the structure of a model for sample y_{ijk} in the form:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + e_{ijk},$$

where: y_{ijk} is the content of the respective element or the particular chlorophyll in *L. multiflorum* leaves; μ is the grand mean; α_i is the i-th exposure site effect; β_j is the j-th exposure series effect; γ_{ij} is the i,j-th effect of the site × series interaction; e_{ijk} is the random error.

Let

$$\gamma = [\hat{y}_{0;1}, \ldots, \hat{y}_{0;4}, \ldots, \hat{y}_{5;1}, \ldots, \hat{y}_{5;4}]'$$

denote the vector of mean values for the respective sites and series.

The comparison of the content of the respective element or the particular chlorophyll in a certain exposure series was analyzed on the basis of linear functions between the entries of the vector γ (Seber, 1980). Contrasts $\mathbf{c}'\gamma$, where $\mathbf{c} = [-1, 1, 0, \ldots, 0]'$, compare the content of the analyzed trace elements for the first site with the control in the first series. Tukey's test (multiple comparison procedure) was used for comparing the mean analyzed elements under different exposure sites in the particular series and splitting up the set of the mean values into homogeneous groups.

Two heatmaps and cluster analyses were here performed – the first one analyzed similarities between element and chlorophyll contents to the experimental sites, the second one investigated the correlations between variables. The first one presents the mean values of the measured parameters at a certain site throughout all experimental series. Afterwards a cluster analysis was performed to group similar sites in regard to all the analyzed parameters. Euclidean distance measures and Ward hierarchical clustering were used to determine the dendrogram. The Euclidean distance measure can designate a similar structure in interactions between the analyzed parameters. In the second heatmap the Pearson's correlation matrix was designated for the contents of elements and chlorophylls. Afterwards, a cluster analysis was performed, in order to arrange the analyzed elements and chlorophylls in groups, with the highest degree of association within each group and the lowest degree of association between different groups.

For evaluation of effect-response associations between the analyzed parameters, simple correlation analysis was performed as well as principal component analysis (PCA). PCA was performed to determine interactions between independent variables (correlations between levels of elements and chlorophylls) and it was based on selection of the components as a linear combination of variables.

Statistical analyses were performed using the R computational platform (R Core, 2014). The following packages were used: 'agricole' v. 1.2–3, 'devtols' v.1.7.0, 'ggplot2' v.1.0.0, 'gplots' v. 2.14.1, 'graphics' v.3.1.1, 'pgirmess' v. 1.5.9, and 'prabclus' v. 2.2–4.

RESULTS

ACCUMULATION OF TRACE ELEMENTS

The hypotheses about two factors and their interaction taken into consideration (series and site) were rejected with the Lawley-Hotelling trace test statistic L and approximation F for $p < 0.01$ (Table 1). Cd accumulation in *L. multiflorum* leaves was higher in the plants exposed to ambient air conditions in all series and sites, excluding 1st series site no. 4. The highest level (above 0.6 µg g^{-1}) was observed in the plants exposed in the third series of 2012 at sites no. 1 and 3, and in 2013 in the fourth series at site no. 1. In 2013 relatively low levels were noted in the first three series at almost all sites (significantly different than in the fourth series), while in 2012 series no. 1 and no. 2 were characterized by higher levels of Cd content in leaves. This was especially valid for sites no. 1–3. Pb contents in leaves of the exposed plants were higher in comparison to the control in all series and sites. Higher levels were noted in the 2012 growing season. Regarding the 2012 season, relatively low levels (significantly different at the level $\alpha = 0.05$) were observed in plants exposed in the third series at site no. 3, while the highest levels were observed at sites no. 2 and 3. In 2013 lower levels (significantly different) were noted at site no. 5 in all exposure series. Moreover, the third series was

TABLE 1. MANOVA of all parameters.

Sources of variability	Degrees of freedom	Hotelling-Lawley statistic	Approx. F statistic	Numerator degrees of freedom	Denominator degrees of freedom	Empirical significance level
Series	3	27.0	57.5	39	248	< 0.001
Site	5	39.0	49.5	65	412	< 0.001
Series: Site	15	41.3	17.0	195	1068	< 0.001
Residuals	96					

characterized by elevated content in the plants exposed at site no. 2, and the lowest content of Pb in the plants exposed at sites no. 3–5. Arsenic content in leaves was higher in the exposed plants, although there were no differences between sites and series in both years, excluding site no. 3 in the first two series of 2012. In the case of nickel, contents in leaves of the plants exposed to ambient air conditions were not always higher than in the control plants. The highest contents were noted in the plants exposed in 2013 in the last two series at almost every site. An elevated content was also recorded in the plants exposed in 2012 in the second series at site no. 5, and in the third series in sites no. 1–3. Cr content was higher in the plants exposed to ambient air conditions in comparison to the plants in all exposure sites and series. The highest levels were observed in the 2013 season in the third and fourth series. It was not valid for the third series of the plants exposed at sites no. 1 and 2. In 2012 an elevated level was recorded in the second series at site no. 5, and in the third series at sites no. 1–3 (Supplementary table S2).

CONTENT OF MAJOR ELEMENTS AND CHLOROPHYLLS

The contents of elements varied among sites and series. Na contents in leaves were several times lower in 2013 in comparison to 2012. Moreover, in 2013 there was not such variation of Na as in 2012. In 2012 the highest (significantly different) level was recorded in the control plants in all series. There were higher (not always significantly) contents of Na at site no. 1 and 2, excluding the second series. Considering 2013, some variations in Na level can be observed in the first and second series. Potassium content was significantly higher in the control plants in 2013, while in 2012 the control plants did not always exhibit higher levels. Moreover, there was not such high variation in K level. However, we can observe some relations, such as a lower level of K at sites no. 4 and 5. Considering exposure sites in 2013, the highest level was noted in the plants exposed at site no. 2. Magnesium content varied between sites and series. Calcium content in leaves was several times higher in 2013. Moreover, there was high variability in series and years. The control plants showed a significantly different level in comparison to the exposed plants in 2013 (Supplementary table S3).

A decrease of chlorophyll content in comparison to the control plants was noted at almost every site and exposure series, excluding the last series of the 2012 season. A larger decrease was observed in 2013, especially in the third series, when higher accumulation of some elements was also noted (Supplementary figure S4).

VISUALIZATION OF RELATIONS BETWEEN PARAMETERS

The Ward hierarchical clustering with mean values of all parameters revealed formation of two groups. The first one included three sites located in suburban, agriculture and landscape park area. The second group consists of two urban sites. In the first case most of the analyzed parameters were lower than in the second, excluding As, which was the highest, while the second group was characterized by higher levels of elements. Chlorophyll *a* and *b* varied between these two sites, the highest was noted for site no. 2, the lowest for site no. 1. In the following year the same three sites were grouped, while the urban sites were treated separately, especially site no. 2, where the highest values were recorded for almost every element. Similarly as for 2012, the highest chlorophylls *a* and *b* were found at site no. 2, and the lowest at site no. 1 (Fig. 2).

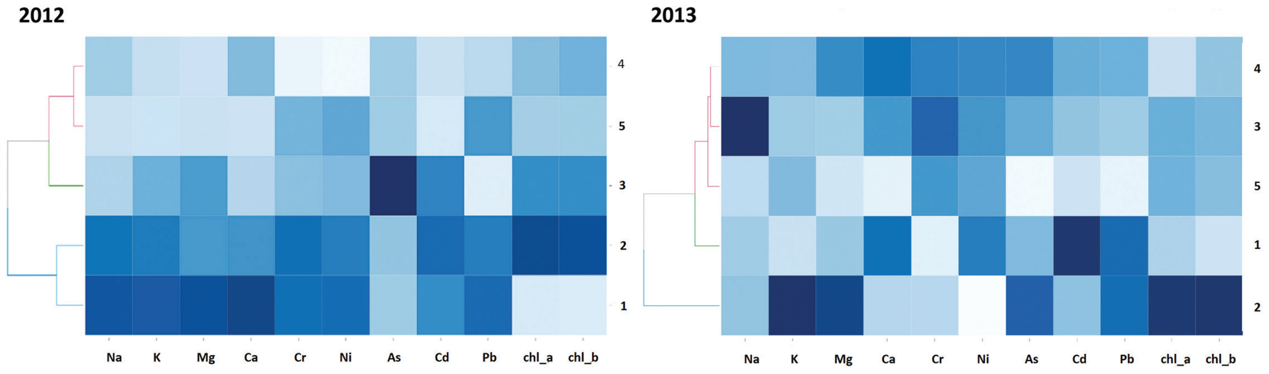

Fig. 2. Similarities between sites based on mean values of parameters throughout the series (2012 and 2013).

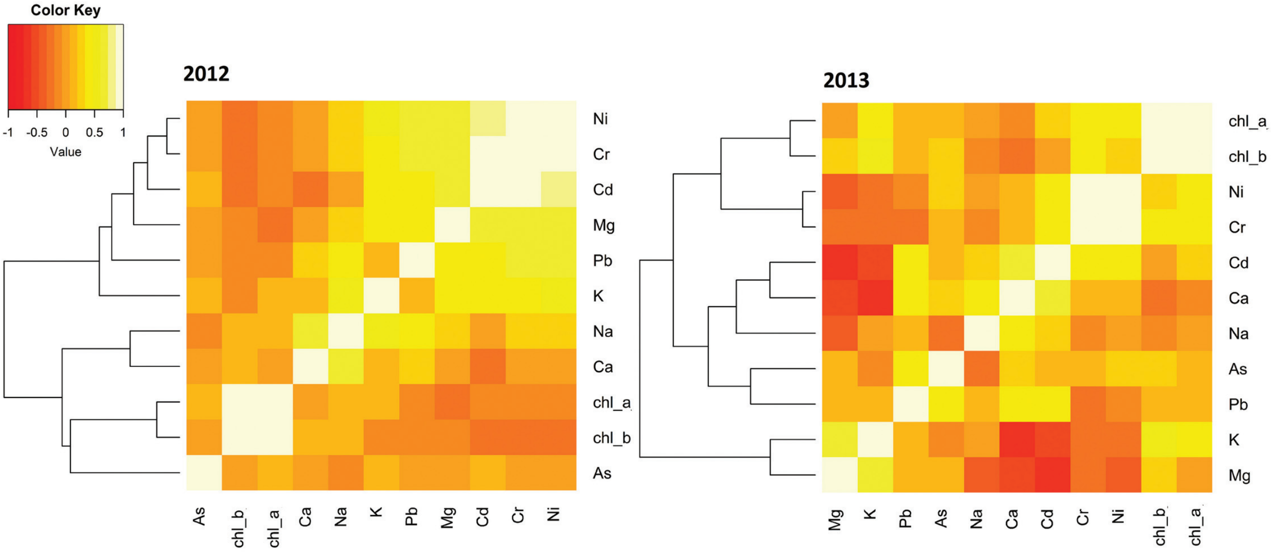

Fig. 3. Correlations between analyzed parameters and cluster analysis (2012 and 2013).

Based on Ward hierarchical clustering and Euclidean's distance, the groups with the highest internal similarities were assigned. In both years three groups were determined. Ni and Cr revealed high similarities in both years, although in the first year of the experiment they were combined in the same group with Cd, Pb, K, and Mg, and in the second with chlorophylls. However, the type of correlation among all these parameters varies. Magnesium revealed various positions in grouping in both years. Chlorophyll content was negatively related to trace elements. This was especially valid for chlorophyll *b* and all trace elements in 2012, while in 2013 for Ni and Cd (Fig. 3).

Graphical data presentation by principal component analysis explained a significant part of data variability – over 60% of variability in both experimental years. Strong negative correlations between chlorophylls and heavy metals were observed for both years. Magnesium showed negative relationships with chlorophyll levels and positive relationships with trace elements in 2012, while opposite relations were observed in the next year. Potassium and sodium revealed positive associations with chlorophyll levels in both years, while Ca showed a positive association or lack of association with chlorophylls. Moreover, the control site was found in a separate position to exposure sites. Exposure site no. 2 revealed the closest relation to the control one and also the nearest to average levels of each parameter. The rest of the exposure sites revealed similar tendencies (Fig. 4).

DISCUSSION

Traffic, industry and households emit huge amounts of air pollution, including trace elements, which can accumulate in living organisms. Plants were found to be indicators of environmental quality. This is also true for here presented species, Italian ryegrass, which we examined a year before and positive results were obtained (Borowiak et al., 2014; Budka et al., 2014). Our experiment revealed differences in accumulation of trace elements present in ambient air by the exposed plants. Although most accumulated elements did not reach toxic levels, we can see variability among sites and series, which may relate to various environmental conditions, such as land use structure and in consequence emission of some pollutants. Moreover, temporal differences may be connected with meteorological conditions, as well as with particulate matter, which is a donor of many other pollutants, including trace elements. There was variability in PM10 concentrations during the experiment. Higher PM10 concentrations in certain series were noted in 2012. Moreover, rain can also affect final accumulation, and the highest sum of precipitation was noted in the second series of 2012, while the highest temperature was recorded in the third and fourth series of 2013 (Table 2). These data were related to some accumulated trace elements – such as higher Pb and Cd accumulation in the third series of 2012 together with higher PM10 concentration. Meanwhile, Cr and Ni were

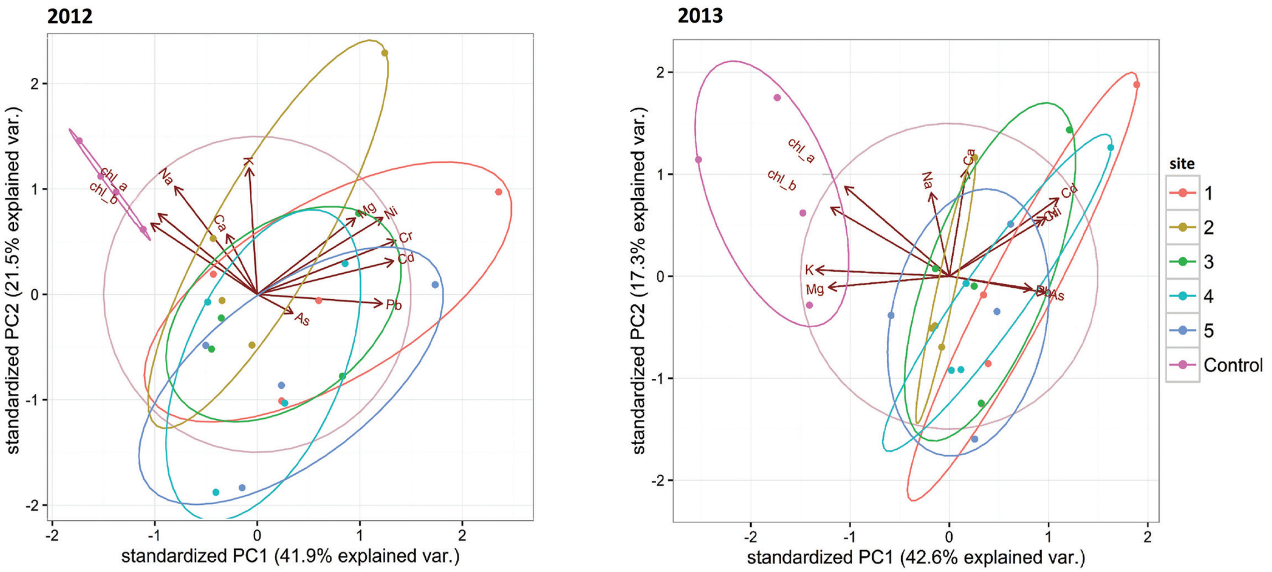

Fig. 4. PCA of analyzed parameters in 2012 (a) and 2013 (b) growing season for certain exposure sites.

TABLE 2. Mean values of meteorological parameters and particulate matter concentrations in ambient air for series in 2012 and 2013 measured at one of urban sites by Provincial Environment Agency.

Series	Temperature [°C]	Precipitation [mm]	UVB radiation [W m^{-2}]	Particulate matter PM10 [µg m^{-3}]
		2012		
1	17.9	22.5	226	19.8
2	21.5	140.8	211	20.2
3	22.0	52.7	214	21.2
4	21.2	30.4	178	18.6
		2013		
1	18.1	63.4	186	20.4
2	21.1	98.6	214	15.9
3	23.6	35.2	243	15.6
4	22.5	30.6	205	17.2

the highest in the last two series of 2013, together with the lowest precipitation, which may suggest that dust was not washed out from the leaves and also had sufficient time for absorption by plants.

The photosynthesis of higher plants was found to be sensitive to the occurrence of heavy metals in the environment (Tanyolac et al., 2007). The process of photosynthesis is usually disturbed by damage of the photosynthetic apparatus. Some heavy metals are recognized as necessary for proper plant functioning and a certain concentration in the environment is accepted by plants. However, exceeding this level can cause a negative effect. This is true for nickel, toxic levels of which can cause disturbances of photosynthesis (Ali et al., 2009), and as a result chlorosis can be noted

(Pandey and Sharma, 2002). Nickel at very low contents can play an important role as part of the active metallocenter of the hexamer enzyme urease (Gerandas et al., 1999). This rapidly uptaken metal was found to be an inhibitor of the Calvin cycle and chlorophyll biosynthesis (Van Assche and Clijsters, 1990), so negative effects can be noted relatively fast after absorption. In our experiment the Ni level in plants did not reach a toxic level (10 μg g^{-1} d.m.), but a negative association with chlorophyll was noted. Similar results were observed by Dubey and Pandey (2011), who tested the effect of Ni on *Vigna mungo*. In their experiment with *Triticum aestivum* L. Rao and Sresty (2000) and Shafeeq et al. (2012) found that chlorophyll content was affected by heavy metals due to peroxidation of chlorophyll membranes. Moreover, lipid peroxidation can increase due to the creation of reactive oxygen species (ROS). Nickel was also found to influence nutrient levels in plant tissue, such as Ca, Mg, Fe, K, and Na of wheat (Ouzounidou et al., 2006), as well as accumulation of cations of Ca^{2+}, K^{+}, and Na^{+} in mung bean (Ahmad et al., 2007). The same observation was made in the present experiment; however, Ca revealed various response in particular years.

As it was previously noted, Cd also affects photosynthesis, which can be observed as a decrease of chlorophyll content. De Oliveira et al. (1994) noted a decrease of both chlorophylls in soybean plants, but chlorophyll *a* revealed higher sensitivity to Cd. A similar relation was also noted by Muradoglu et al. (2015) in an experiment with strawberries affected by various Cd contents. In our experiment relations between elements and chlorophylls revealed higher sensitivity of chlorophyll *b* to Cd. The decrease of chlorophyll content caused by Cd may be related to the inhibitory effect on enzymes responsible for pigment biosynthesis (Qian et al., 2009).

A negative effect of heavy metals occurring in the ambient air has also been noted in lower plants, such as lichens. These plants are well-known bioindicators of trace elements. Moreover, the investigations conducted by Yildiz et al. (2011) and Karakoti et al. (2014) revealed a decrease of all chlorophylls related to heavy metals. Karakoti et al. (2014) also suggested investigating the effect of trace elements on other biomonitors in relation to photosynthetic pigments. Moreover, Yildiz et al. (2011) noted a larger decrease of chlorophyll *a* than chlorophyll *b*. In our experiment a higher negative correlation was noted for chlorophyll *b*, especially for Cd and Ni. However, Singh et al. (2012) found a larger decrease of chlorophyll *b* than chlorophyll *a* in *Vigna mungo* L. seedlings affected by a combination of Ni and Pb, which might suggest higher sensitivity of this chlorophyll form for simultaneous influence of trace elements. Ewais (1997) noted a larger negative effect on chlorophylls, when contents of Pb and Ni were recorded on three weed species. A stronger negative effect of Cd than Pb was also recorded by Zengin and Munzuroglu (2005) in an experiment with *Phaseolus vulgaris*. In our experiment Pb accumulation in leaves was much lower than the toxic level, and relations with chlorophylls were more neutral. However, in the experiment conducted a year before presented here we found a negative relation between Pb and chlorophylls (Budka et al., 2014). Cenkci et al. (2010) recorded a larger decrease of chlorophyll *b* than chlorophyll *a* in *Brassica rapa* L. affected by Pb. Moreover, as it was previously found, Pb also affects Mg, which is an important element of chlorophylls (Burzyński, 1985), but in our experiment the contents were probably too low to negatively affect (replace) Mg.

Chromium reached toxic levels in the second year of the experiment. Its toxicity was previously recorded in various ways, such as reduced yield, decreased leaf and root growth, and inhibition of enzymatic activity (Shanker et al., 2005). The relations between parameters revealed the opposite position of Cr to all chlorophyll forms. This was already observed in other experiments, such as with *Oryza sativa* (Hadif et al., 2015), *Sinapis alba* (Fargašova and Molnárová, 2010), *Brassica napus* (Najafian et al., 2012) and *Tetraselmis* sp. (Nainggolan et al., 2015). Moreover, a stronger negative relationship was also observed with chlorophyll *b* than with chlorophyll *a*. As Shanker et al. (2005) suggested, the chlorophyll *b* decrease may be associated with destabilization and degradation of the proteins of the peripheral part, as well as – the same as with other trace elements – the distortion of biosynthesis of pigments due to inactivation of enzymes. Chromium was also found to be an inhibitor of uptake of other elements, such as Mg and K (Shanker et al., 2005), which is also in agreement with our results.

Arsenic did not reach toxic levels of accumulation in the present experiment, but we could note differences between exposure sites. Moreover, the associations with chlorophylls were mainly negative. However, these relations are not so clear as in the case of other examined trace elements. The literature also provides various information about the effect of arsenic on chlorophyll content. On the one hand, there is information about the negative effect of arsenic on photosynthesis, as well as on chlorophyll biosynthesis (Bandaru et al., 2016; Choudhury et al., 2011; Rahman et al., 2007; Shaibur et al., 2006), although Sushhant and Ghosh (2010) observed a positive effect of arsenic on chlorophylls in onions, and Miteva and

Merakchiyska (2002) observed no effect in bean plants. In the case of our experiment the weak relations might be associated with relatively low contents of arsenic in plant tissue.

CONCLUSIONS

Trace elements did not reach toxic values of accumulation in the exposed *Lolium multiflorum* (excluding Cr and As values in some series and sites). However, some interesting relations were noted. Moreover, differences between sites and years were also found, which indicates the important role of seasonal changes and the potential source of trace elements in the ambient air. Sites located outside the city were grouped into one, and two urban sites were grouped into another. The analysis of the results revealed negative relations between chlorophylls and trace elements, while magnesium was found in various associations with heavy metals and chlorophylls. K and Na revealed the same tendencies as chlorophylls, while Ca showed a similar relation as chlorophylls or lack of association. Chlorophyll *b* was found to be more sensitive to most of the analyzed trace elements. Based on the obtained results, we can conclude that it is already possible to observe some relations between the analyzed parameters, which indicate the high sensitivity of photosynthesis in plants exposed to air pollution and the possibility to treat chlorophylls, K and Na as indicators of stress caused by trace elements, even when present in low concentrations in ambient air.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the support of project grant No.: N 0540/B/P01/2011/40 from the National Science Centre to publish this work. We would also like to thank the Adam Mickiewicz University Botanical Garden and the Institute for Agricultural and Forest Environment of Polish Academy of Sciences, Research Station in Turew for their provision of space for exposure sites.

REFERENCES

AHMAD MS, HUSSAIN M, SADDIQ R, and ALVI AK. 2007. Mung Bean: a nickel indicator, accumulator or excluder. *Bulletin of Environmental Contamination and Toxicology* 78: 319–324.

ALI MA, ASHRAF M, and ATHAR HR. 2009. Influence of nickel stress on growth and some important physiological/biochemical attributes in some diverse canola (*Brassica napus* L.) cultivars. *Journal of Hazardous Materials* 172: 964–969.

ALLOWAY BJ, and AYRES DC. 1997. *Chemical principles of environmental pollution, second edition*. Chapman and Hall, USA.

BANDARU V, DAUGHTRY CS, CODLING EE, HANSEN DJ, WHITE-HANSEN S, and GREEN CE. 2016. Evaluating leaf and canopy reflectance of stressed rice plants to monitor arsenic contamination. *International Journal of Environmental Research and Public Health* 13: 606.

BOROWIAK K, ZBIERSKA J, BARAŁKIEWICZ D, HANĆ A, BUDKA A, KAYZER D, and KAWALA A. 2014. Biomonitoring of air pollution by trace elements using Italian ryegrass (*Lolium multiflorum* L. 'Lema'). *Polish Journal of Environmental Studies* 23(3): 681–688.

BUDKA A, BOROWIAK K, KAYZER D, HANĆ A, ZBIERSKA J, BARAŁKIEWICZ D, WOLNA-MARUWKA A, and LISIAK M. 2015. Nickel and chromium concentrations in Italian ryegrass exposed to ambient air in urban, suburban and rural areas. *Atmospheric Pollution Research* 6: 1123–1131.

BUDKA A, KAYZER D, ZBIERSKA J, BOROWIAK K, BARAŁKIEWICZ D, and HANĆ A. 2014. Canonical variate analysis of chlorophyll content in plants exposed to different lead concentrations in ambient air conditions. *Civil and Environmental Engineering Reports* 14 (3): 15–26.

BURZYŃSKI M. 1985. Influence of lead on the chlorophyll content and on initial steps of its synthesis in greening cucumber seedlings. *Acta Societatis Botanicorum Poloniae* 54: 95–105.

CENKCI S, CIĞERCI IH, YILDIZ M, ÖZAY C, BOZDAĞ A, and TERZI H. 2010. Lead contamination reduces chlorohyll biosynthesis and genomic template stability in *Brasicca rapa* L. *Environmental and Exerimental Botany* 57: 467–473.

CHOUDHURY B, CHOWDHURY S, and BISWAS A. 2011. Regulation of growth and metabolism in rice (*Oryza sativa* L.) by arsenic and its possible reversal by phosphate. *Journal of Plant Interactions* 6: 15–24.

DE OLIVERIA JA, OLIVA MA, and CAMBRAIA J. 1994. Effects of cadmium on chlorophyll contents and on peroxidase activity in soybean. *Brasilian Journal of Plant Physiology* 6(2): 97–101.

DUBEY D, and PANDEY A. 2011. Effect of nickel (Ni) on chlorophyll, lipid peroxidation and antioxidant enzymes activities in black gram (*Vigna mungo*) leaves. *International Journal of Sciences and Nature* 2(2): 395–401.

EWAIS EA. 1997. Effects of cadmium, nickel and lead on growth, chlorophyll content and proteins of weeds. *Biologia Plantarum* 39(3): 403–410.

FARGAŠOVA A, and MOLNÁROVÁ M. 2010. Assessment of Cr and Ni phytotoxicity from cutlery-washing waste-waters using biomass and chlorophyll production tests on mustard *Sinapis alba* L. seedlings. *Environmental Science and Pollution Research* 17(1): 187–194.

GAMBUŚ F, and WIECZOREK J. 2012. Pollution of Fertilizers with Heavy Metals. *Towarzystwo Chemii i Inżynierii Ekologicznej* 19: 353–360.

GERANDAS J, POLACCO JC, FREYERMUTK SK, and SATTELMACHER B. 1999. Significance of nickel for plant growth and metabolism. *Journal of Plant Nutrition and Soil Science* 162: 241–256.

GORLACH E, and MAZUR T. 2001. *Agricultural chemistry*. Wyd. PWN, Warszawa. (in Polish)

Hadif WM, Rahim SA, Sahid I, Rahman A, and Ibrahima I. 2015. Influence of chromium metal on chlorophyll content in leaves of paddy *Oryza sativa* L. *International Journal of Chemical Sciences* 13(3): 1238–1252.

Hiscox JD, and Israelstam GF. 1978. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany* 57: 1332–1334.

Kabata-Pendias A. 2010. *Trace elements in soils and plants, fourth edition*. CRC Press Inc., Boca Raton.

Karakoti N, Bajpai R, Upreti DK, Mishra GK, Srivastava A, and Nayaka S. 2014. Effect of metal content on chlorophyll fluorescence and chlorophyll degradation in lichen *Pyxine cocoes* (Sw.) Nyl.: a case study from Uttar Pradesh, India. *Environmental Earth Science* 71: 2177–2183.

Lejeune M, and Caliński T. 2000. Canonical analysis applied to multivariate analysis of variance. *Journal of Multivariate Analyses* 72: 100–119.

Miteva E, and Merakchiyska M. 2002. Response of chloroplasts and photosynthetic metabolism on bean plants to excess arsenic in soil. *Bulgarian Journal of Agricultural Science* 8: 151–156.

Muradoglu F, Gundogdu M, Ercisli S, Encu T, Balta F, Jaafar HZE, and Zia-Ul-Haq M. 2015. Cadmium toxicity affects chlorophyll a and b content, antioxidant enzyme activities and mineral nutrient accumulation in strawberry. *Biological Resources* 48(1): 11.

Nainggolan EM, Aryandari R, Fadholi HP, Nughoro AP, and Suyono EA. 2015. Effects of chromium on chlorophyll-a, carbohydrate and protein contents in *Tetraselmis* s. (Butchar 1959). The 3rd International Conference on Biological Science 2013, KnE Life Sciences, 556.

Najafian M, Kafilzadeh F, Azad HN, and Tahery Y. 2012. Toxicity of chromium (Cr^{6+}) on growth, ions and some biochemical parameters of *Brassica napus* L. *American-Eurasian Journal of Agricultural and Environmental Sciences* 12(2): 237–242.

Ociepa-Kubicka A, and Ociepa E. 2012. Toxic effect of heavy metals on plants, animals and human. *Inżynieria i Ochrona Środowiska* 15: 169–180. (In Polish)

Ouzounidou G, Moustakas M, Symeonisdis L, and Karataglis S. 2006. Response of wheat seedlings to Ni stress: effects supplemental calcium. *Archives of Environmental Contamination Toxicology* 50(3): 346–352.

Pandey N, and Sharma CP. 2002. Effect of heavy metals Co^{+2}, Ni^{+2} and Cd^{+2} on growth and metabolism of cabbage. *Plant Science* 163: 753–758.

Pulles T, Denier van der Gon H, Appelman W, and Verheul M. 2012. Emission factors for heavy metals from diesel and petrol used in European vehicles. *Atmospheric Environment* 61: 641–651.

Qian H, Li J, Sun L, Chen W, Sheng GD, Liu W, and Fu Z. 2009. Combined effect of copper and cadmium on *Chlorella vulgaris* growth and photosynthesis-related gene transcription. *Aquatic Toxicology* 94: 56–61.

R Core Team, 2014. *A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/ (accessed 21.07.2016).

Rahman M, Hasegawa H, Rahman M, Islam M, Miah M, and Tasmen A. 2007. Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. *Chemosphere* 67: 1072–1079.

Rao KVM, and Sresty TVS. 2000. Antioxidative parameters in the seedlings of pigeon pea (*Cajanus cajan*) in response to Zn and Ni stress. *Plant Science* 157: 113–128.

Seber GAF. 1980. *The linear hypothesis: A general theory*. Charles Griffin, London, 117.

Shafeeq A, Ali Butt Y, and Muhammad S. 2012. Response of nickel pollution on physiological and biochemical attributes of wheat (*Triticum aestivum* L.) var. Bhakar-02. *Pakistan Journal of Botany* 44: 111–116.

Shaibur MR, Kitajima N, Sugawara R, Kondo T, Huq SMI, and Kawai S. 2006. Physiological and mineralogical properties of arsenic-induced chlorosis in rice seedlings grown hydroponically. *Soil Science and Plant Nutrition* 52: 691–700.

Shanker AK, Cervantes C, Loza-Tavera H, and Avudainayagam S. 2005. Chromium toxicity in plants. *Environment International* 31: 739–753.

Singh G, Agnihotri RK, Sharma Reshma R, and Ahmad M. 2012. Effect of lead and nickel toxicity on chlorophyll and proline content of urd (*Vigna mungo* L.) seedlings. *International Journal of Plant Physiology and Biochemistry* 4(6): 136–141.

Stobart AK, Griffiths WT, Ameen-Bukhari I, and Sherwood RP. 1985. The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. *Physiologia Plantarum* 63: 293–298.

Sushhant KS, and Ghosh AK. 2010. Effect of arsenic on photosynthesis, growth and its accumulation in the tissues of *Allium cepa* (onion). *International Journal of Environmental Engineering and Management* 1(1): 39–50.

Tanyolac D, Ekmekci Y, and Unalan S. 2007. Changes in photochemical and antioxidant enzyme activities in maize (*Zea mays* L.) leaves exposed to excess copper. *Chemosphere* 67: 89–98.

Vajpayee P, Tripati RD, Rai UN, Ali MB, and Singh SN. 2000. Chromium accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content of *Nymphaea alba*. *Chemosphere* 41: 1075–1082.

Van Assche F, and Clijsters H. 1990. Effects of metal on enzyme activity in plants. *Plant Cell and Environment* 13: 195–206.

Wang Y, Huang K, Li T, Mi H, Luo H, and Tai J. 2006. Emissions of fuel metals content from a diesel vehicle engine. *Atmospheric Environment* 37: 4637.

Yildiz A, Aksoy A, Akbulut G, Demirezen D, Islek C, Altuner EM, and Duman F. 2011. Correlation between chlorophyll degradation and the amount of heavy metals found in *Pseudevernia furfucaea* in Kayseri (Turkey). *Ekoloji* 20(78): 82–88.

Zengin FK, and Munzuroglu O. 2005. Effect of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. *Acta Biologica Cracoviensia Series Botanica* 47(2): 157–164.