Journal of Elementology ISSN 1644-2296

Parzych A., Zduńczyk A., Astel A. 2016. *Epiphytic lichens as bioindicators of air pollution by heavy metals in an urban area (northern Poland).* J. Elem., 21(3): 781-795. DOI: 10.5601/jelem.2016.21.1.861

EPIPHYTIC LICHENS AS BIOINDICATORS OF AIR POLLUTION BY HEAVY METALS IN AN URBAN AREA (NORTHERN POLAND)

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Abstract

The capability of accumulating lead (Pb), zinc (Zn), nickel (Ni), cooper (Cu), manganese (Mn) and iron (Fe) by tree lichen species was compared. Samples of lichens were taken in the autumn of 2013, from *Betula pendula* Roth, *Fraxinus excelsior* L., *Acer platanoides* L., *Acer Pseudoplatanus* L., *Populus* sp. trees and other broad-leaved trees growing within the city limits of Słupsk. The sampling stations were located in housing estates, green urban parks, near streets with heavy traffic and close to industrial plants. The aim of the study was to evaluate the pollution of the municipal environment of Słupsk with selected heavy metals using for this purpose thalli of three lichen species: *Hypogymnia physodes*, *Parmelia sulcata* and *Xanthoria parietina*, and to compare their accumulative properties. The heavy metal content in lichens was determined by atomic absorption spectrometry. The highest concentration of heavy metals was discovered in lichens collected in the city centre, while the lowest concentrations were found in parks and green areas. The largest quantities of Ni and Pb were accumulated by the lichen species *H. physodes*, most Zn and Fe accumulated in the lichen *X. parietina*, whereas Mn accumulated in similar amounts in all the tested species. A strong direct proportional relationship between the content of Zn and Fe, Fe and Cu, Zn and Cu in thalli of lichens was revealed. Some similarity between the Pb-Cu and Pb-Fe correlations was found as well as that of Pb-Mn in thalli of *P. sulcata* and *X. parietina*, along with a series of statistically significant differences between the examined species of lichen related to pH and the volume of accumulated heavy metals in thalli. The most significant differences in the content of heavy metals in thalli assessed in series of pairwise comparisons including (i) *H. physodes* vs *P. sulcata,* (ii) *P. sulcata* vs *X. parietina* and (iii) *H. physodes* vs *X. parietina* were found for (i') Pb, Cu and Fe, (ii') Ni and (iii') Pb, Ni, Cu and Fe, respectively.

Keywords: accumulation, biomonitoring, *Hypogymnia physodes, Parmelia sulcata, Xanthoria parietina.*

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INTRODUCTION

Pollution of the environment is one of the many problems in the contemporary world. Intensive development of industry, transport and urbanization causes changes in the spatial distribution of environmental pollution, chainging from a local to the global phenomenon. Emission of heavy metals leads to their increased concentrations in different ecosystems. As constituents of particulate matter and aerosols, they are widely transported in the atmosphere for longer distances, leading to contamination of places remote from sources of emission (TAINIO et al. 2010). Certain heavy metals, as natural constituents of ecosystems, are necessary in traceable quantities to maintain proper functions of many living organisms. However, their excessive concentrations in the environment can be noxious. In higher concentrations, they disturb the functioning of ecosystems, posing a threat to plants, animals and humans (NAGAJYOTI et al. 2010). Urban areas are ecosystems affected by strong man-made impact, mostly due to housing and transport. Pollution of urban environments with substances of various origin, including heavy metals, is one of the most important symptoms of anthropogenic pressure in such areas (YANG et al. 2001). The level of pollution in cities depends on the presence, size and character of industrial areas, traffic intensity, density of population and waste utilization facilities. The harmful impact of pollutants on the human body is very difficult to trace and therefore it is necessary to use analytic bioindicators with different levels of sensitivity. Because lichens are able to accumulate elements and airborne particles from rain and dry deposition, they can be effectively used as biomonitors of air pollution. The term "lichens" is widely used in the current literature despite the fact that lichens belong to fungi and many mycologists and lichenologists include them in the fungal system (PRINTZEN 2010). Lichens accumulate pollutants in thalli proportionally to atmospheric concentrations of SO_2, N_xO_y and especially to heavy metals present in atmospheric dust (CONTI et al. 2004, BIAŁOŃSKA, DAYAN 2005, DZUBAJ et al. 2008, KŁOS et al. 2008). Unlike higher plants, metabolism of lichens is active in winter, which is when the level of air pollution is higher than in summer. Lichens are among the best indicators of the sanitary state of air. Their growth and development depend on the humidity of a habitat, type of a substratum, sun exposure and other factors. Observations of indicator species facilitate a quick response to potential threat, counteracting it or warning against adverse and possibly irreparable changes in the environment (CONTI et al. 2004). The lichen species *Xanthoria parietina* is common in urban areas and belongs to the most popular lichen species in European biomonitoring programmes, along other species such as *Hypogymnia physodes* and *Parmelia sulcata* (NIMIS et al. 2001). *Xanthoria parietina* has a large contact surface for atmospheric pollutants and is able to accumulate high amounts of heavy metals in polluted areas (SCERBO et al. 2002, KŁOS 2007). Bioindication studies involving lichens provide much information on the qualitative status of the environment and are extremely useful in evaluating long-term effects of biomonitoring programmes.

The aim of the study was to evaluate the urban environment pollution of Słupsk with selected heavy metals using the thalli of tree lichens *Hypogymnia physodes*, *Parmelia sulcata* and *Xanthoria parietina*, and to compare their accumulative properties.

MATERIAL AND METHODS

Research site

The research area is situated in the northern part of Poland (54°28′N, 17°02′E), and covers 43.15 km^2. Owing to the proximity of the Baltic Sea (17 km), the area has the marine climate. The weather is mild and humid, without big temperature fluctuations, but with relatively heavy and changeable cloudiness and substantial precipitation evenly distributed throughout the year. Słupsk is located in a river valley, and the land depression makes the air of the city stagnant. The average annual air temperature was 5.18°C. January is the coldest month (-7.54°C), and July is the warmest one (18.43°C). The annual rainfall is 993.5 mm, and the humidity reaches 88.73%. The highest humidity has been recorded in November (96.94%), and the lowest one appears in July (76.44%) (Damps et al. 2013). The population of Słupsk is around 96 650 people. The city is dominated by footwear and motor industries as well as furniture, varnish and plastic production. Various species of lichen have been identified in the city area. Inventory studies held showed the presence of 86 species within the city limits in the years 1975-1977 and 115 species in 2001-2004 (Izydorek, Zduńczyk 2007).

Sampling procedure

Lichen samples for physical and chemical tests were taken in the autumn of 2013, at sampling stations within the city limits. The stations were located in housing estates, municipal parks, near busy streets and close to industrial enterprises (Figure 1). In most cases, lichens were collected from tree trunks at breast height (1.3 m above the ground). A single station included one or a few trees growing close to one another within the radius of about 10 m. In most cases, groups of trees represented the same species of phorophyte. Samples of lichen weighing 3 g each were taken with a wooden spatula from trees of *Betula pendula* Roth, *Fraxinus excelsior* L., *Acer platanoides* L., *Acer Pseudoplatanus* L., *Populus* sp. or other broad-leaved trees. From each station, two or three species of lichen were taken. Thalli of *H. physodes* originated from 10 stations, *P. sulcata* from 19 stations, and *X. parietina* from 18 stations located within the city limits.

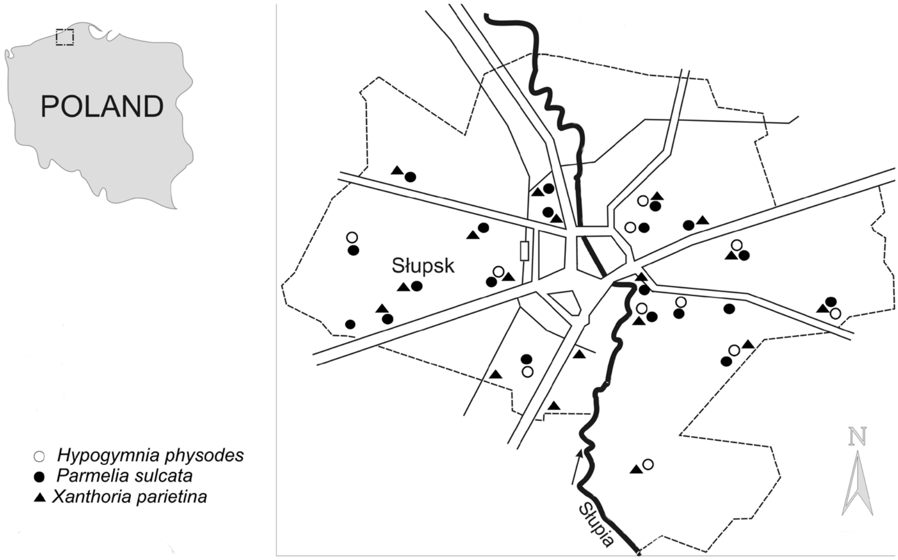

Fig. 1. Distribution of lichen research stations in Słupsk

Research methods

After being transported to the laboratory, lichen samples were carefully cleaned with plastic tweezers to remove pieces of bark. The experimental lichen material was dried in paper bags at 65°C for 24 h to constant mass and homogenized in a laboratory grinder (A11, IKA, Germany). Prior to that, the samples had not been washed to avoid losing particles trapped on the lichen surface and because washing can unpredictably alter the elemental composition of lichens (Bettinelli et al. 1996). Until analyses, the samples were kept in leak-proof, airtight polyethylene containers. For all lichen samples, pH was measured with a pH-meter CPI 551 (Elmetron, Poland), in 1:10 weight ratio water solution. In order to determine the content of heavy metals, the lichen samples were mineralized in a mixture of 65% HNO_3 acid and 30% H_2O_2 in a closed system. The concentration of heavy metals in plants was determined by atomic absorption spectrometry (AAS) on an Aanalyst 300 (Perkin Elmer, USA). The analyses were performed in oxy-acetylene flame. The tests were run using original standards (Merck KGaA, 1 g 1000 ml^{-1}).

Statistical analysis

Data distribution was examined by the Shapiro-Wilk test at $p = 0.05$. For discussion of the results, mean, minimum and maximum values as well as standard deviations, correlation coefficient of the Spearman ranges and coefficients of variance were determined. In order to identify the factors determining the heavy metal content in the lichens, Principal Components Analysis (PCA) was performed as a method of factor extraction. Comparison of the significance of statistical variation in the lead (Pb), zinc (Zn), nickel (Ni), copper (Cu), manganese (Mn) and iron (Fe) content in thalli of *H. physodes*, *P. sulcata* and *X. parietina* was achieved by the non-parametric *U* Mann - Whitney test.

RESULTS AND DISCUSSION

The species of lichen selected for physical and chemical tests appeared in nearly the whole area of Słupsk, except its central part. The water solutions of the tested lichen thalli yielded different pH levels. The lowest pH was characteristic for the thalli of *H. physodes* (4.8), while the highest one was determined for *X. parietina* (7.1) – Table 1. The pH index showed little variantion within the research stations, with the coefficient of variation from 1.9% (*X. parietina*) to 2.9% (*H. physodes*). The results show that the response of thalli is probably a species-specific trait, although we cannot exlude some influence of the pH of the bark of the trees they populate. According to Marmor and Randlane (2007), pH of the tree bark in most cases ranges from 3.0 to 5.5, depending on a tree species.

Table 1

Median, coefficient of variation (CV) and pH range values of studied lichens

Species	Median	Range	CV (%)
Hypogymnia physodes $n = 30$	4.8	4.6-4.9	2.9
Parmelia sulcata $n = 57$	6.3	6.1-6.5	2.1
Xanthoria parietina $n = 54$	7.1	6.9-7.3	1.9

Lichen absorbs most of the vital substances from the atmosphere. They are capable of collecting various particles from the air, including heavy metals (Richardson 1995). The presence of heavy metals in the atmosphere depends largely on the deposition of particulate matter originating from industries, motor transport and heat and power generation, which does not undergo rapid migration or change. The accumulation and absorption of airborne substances by lichen depends substantially on the level of air humidity, and tends to increase with an elevated air humidity (Kar et al. 2014).

The heavy metal content in the lichen thalli in Słupsk was diverse and depended on a lichen species as well as the location of a research stations. The biggest quantities of Pb were found in thalli of *H. physodes* (2.5-8.9 mg kg^{-1}), slightly less Pb was detected in *P. sulcata* (1.6-8.7 mg kg^{-1}), and the lowest Pb content was in *X. parietinia* (2.2-7.0 mg kg^{-1}) – Figure 2, which was most probably because of the species-specific differences in the structure of

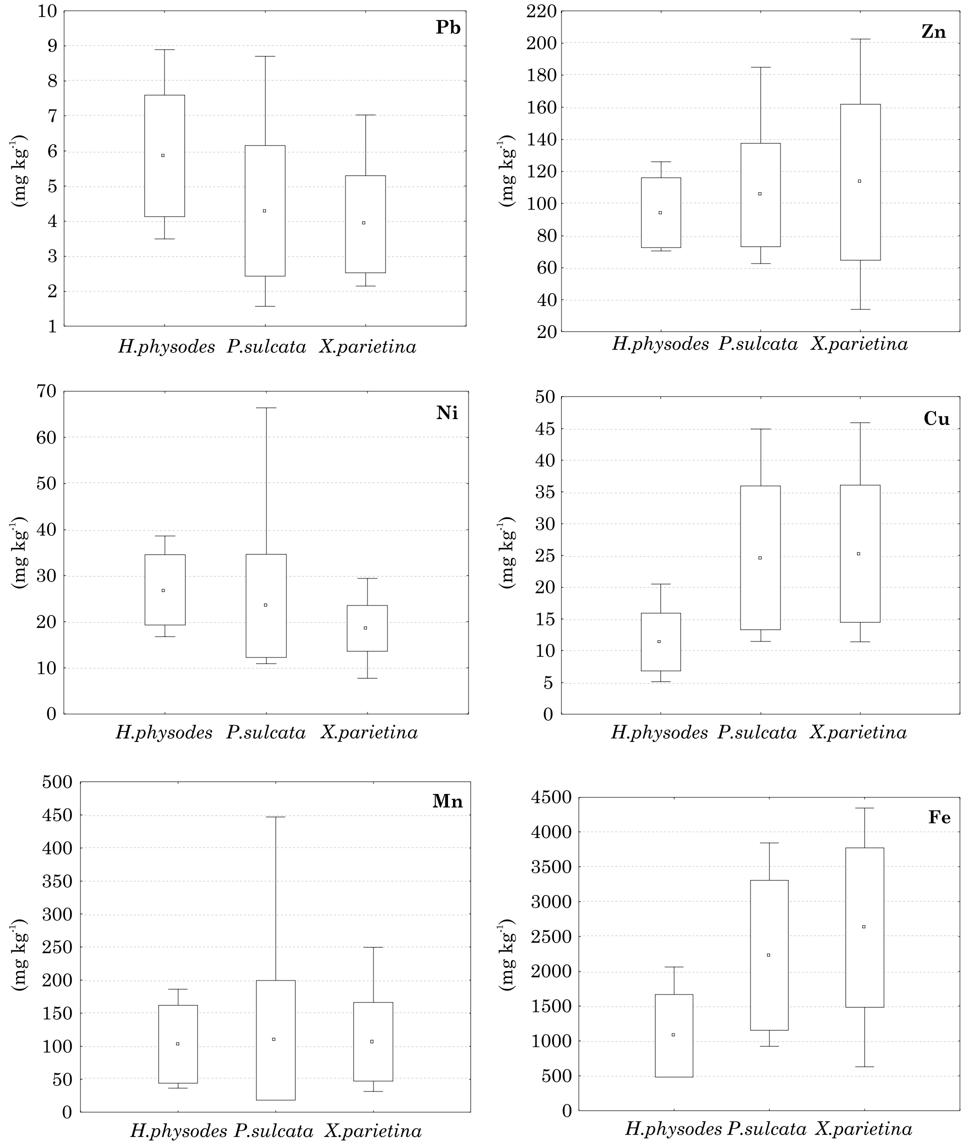

Fig. 2. The content of heavy metals in lichens, point (mean value), rectangle (standard deviation), bars (minimum-maximum)

thalli. Among the investigated species, *H. physodes* has the most corrugated thalli, and therefore presents the largest contact surface with the atmospheric air. Hence, dust and pollutants are arrested more easily (KŁOS 2007). The biggest quantities of Pb were found at the research stations situated in the city centre and in the vicinity of streets characterized by heavy traffic, while the smallest Pb content was determineed in lichens from the outskirts of the city and in the vicinity of green parks. According to Damps et al. (2013), from 73 to 95% of Pb in plants is of atmospheric origin. An average admissible concentration of Pb in the air, according to the World Health Organization (WHO) and the European Union, is 0.5 μg m^{-3}. This amount is also the limit value in Poland. According to Damps et al. (2013), an average concentration of particulate matter suspended within the area of Słupsk during the years preceding our research was: PM10 = 25-28 μg m^{-3} and PM2.5 = 19 μg m^{-3}, including the concentration of Pb of about 0.01-0.02 μg m^{-3}. In Poland, the admissible Pb concentration per hour must not exceed 5 μg m^{-3}. The main sources of Pb in Słupsk are: atmospheric particulate matter, combustion of fuels, mainly for heating homes in residential estates with detached houses, as well as vehicle combustion engines. Much more Pb was discovered in the samples of lichen collected in other Polish cities, e.g. in Kielce near street street junctions (*H. physodes*, 27.6-55.6 mg kg^{-1}, Jóźwiak 2012), in Wrocław (*H. physodes*, 9-23 mg kg^{-1}, Bojko et al. 2004), but also in the Stabrowskie Coniferous Forests (*H. physodes*, 18.2 mg kg^{-1}, Kłos et al. 2008). Slightly higher Pb pollution was discovered in other European and Asian cities, e.g. in Burnham Beeches in the United Kingdom (*P. sulcata*, 30.0 mg kg^{-1}, Purvis et al. 2008), in Körfez in Turkey (*X. parietina*, 46 mg kg^{-1}, Demiray et al. 2012) and in Košice in Slovakia (*X. parietina*, 34.3 mg kg^{-1}, Dzubaj et al. 2008). In Italian cities, the Pb content in lichen thalli was in the range of 3.9 mg kg^{-1} and 18.8 mg kg^{-1} (Bargagli et al. 2002). These results indicate similar or higher air Pb contamination than in Słupsk.

Zinc was determined at the level of 70.5 and 126.1 mg kg^{-1}, 62.7 and 184.9 mg kg^{-1}, and between 34.0 and 201.6 mg kg^{-1} in thalli of *H. physodes*, *P. sulcata* and *X. parietina*, respectively (Figure 2). The variation in the Zn content was substantial, reaching 23.1% in *H. physodes*, 31.5% in *P. sulcata* and 42.9% in *X. parietina*, and apparently resulted from the different location of the research stations. The Zn contamination originates mainly from immigratory sources as well as dispersed ones such as combustion of solid fuels. During combustion, Zn is released into the atmosphere together with the finest (<2,5 mm) fraction of particulate matter (Linak, Miller 2000). About 1.5-2.0% of zinc comes from the abrasion of automobile tires, which is confirmed by an increased Zn content in the thalli of lichens collected close to busy streets. In addition, the production of paints and varnishes made with addition of Zn and Pb compounds, which have been manufactured for years in Słupsk, is another source of Zn and Pb. A substantially higher concentration of Zn was found in the thalli of *H. physodes* in Opole (166 mg kg^{-1}, Kłos et al. 2008), and similarly, in *P. sulcata* in Świętokrzyski National Park – 121 mg kg^{-1} (Sawicka-Kapusta et al. 2010) and in Gorczański National Park – 83.3-175.3 mg kg^{-1} (Czarnota 1995). Slightly lower concentrations of Zn were found in lichen in Borecka Primeval Forest (55 mg kg^{-1}, Białońska, Dayan 2005), growing outside the impact of anthropogenic factors. A higher concentration of Zn was found in thalli of *X. parietina* in some Turkish cities (255 mg kg^{-1}, Demiray et al. 2012).

The examined lichen species showed accumulation of Ni. The Ni content varied between 16.3 and 39.8 mg kg^{-1} (H. physodes), 11.5 and 44.9 mg kg^{-1} (*P. sulcata*), as well as 11.1 and 45.9 mg kg^{-1} in thalii of *X. parietina* (Figure 2). The values of the coefficients of variation for the Ni concentration in lichens were 28.3%, 53.1% and 26.7%, respectively, and resulted from the location of the research stations. The largest quantities of Ni were found in proximity of busy streets. The contamination of the atmospheric air with Ni is closely connected with emissions from the metallurgical industry and combustion of liquid fuels. The Ni content in particulate matter suspended over Słupsk was found to be quite diverse and ranged from 6.8 to 11.5 μg m^3 in the period directly preceding the research (DAMPS et al. 2013). According to TARHANEN et al. (1999), the sorption from the atmospheric precipitation in lichens is most effective at pH = 5. A similar observation was made by HAAS et al. (1998) in the case of uranium absorption by *Peltigera membranacea*. Analogous relationships were indicated in a study on *X. parietina* in Pisa Province, Italy (0.01-9.54 mg kg^{-1}, SCERBO et al. 2002) and in an urban area of Dilovasi (Kocaeli Province along the İzmit Bay) in Turkey (from 2.7 to 10.2 mg kg^{-1}, DEMIRAY et al. 2012).

The Cu content ranged from 5.1 to 20.5 mg kg^{-1} in *H. physodes*, from 11.1 to 45.9 mg kg^{-1} in *X. parietina* and from 11.4 to 44.9 mg kg^{-1} in *P. sulcata* (Figure 2). The coefficient of variability for the Cu content was 41.7%, 43.0% and 46.1%, respectively. In air, Cu is most often connected with particulate matter, but much copper is also found in a water-soluble form or in forms bound to Fe and Mn or to organic substances. With quite a high coefficient of bio-accumulation for Cu and mostly an anthropogenic origin of this pollutant, there is a threat that copper can contaminate local biota. The most significant sources of the environmental contamination with Cu compounds are: production of pesticides, herbicides and fertilizers as well as the presence of Cu compounds in car brakes. Although there are plants in Słupsk manufacturing footwear, furniture, automobile glass, the main contributor to the emission of particulate matter is the urban heat power engineering system (DAMPS et al. 2013). The Cu content in thalli of *H. physodes* collected in Kielce (Poland) was on average 27.5 mg kg^{-1} (JÓŹWIAK 2012), in Körfez (Turkey) it varied between 8.0 and 26.0 mg kg^{-1} (DEMIRAY et al. 2012), while in Košice (Slovakia) it ranged between 9.2 and 68.5 mg kg^{-1} in *X. parietina* (DZUBAJ et al. 2008).

The Mn content was from 31.6 to 249.9 mg kg^{-1} in *X. parietina*, from 35.5 to 446.9 mg kg^{-1} in *P. sulcata* and from 36.6 to 186.6 mg kg^{-1} in *H. physodes* (Figure 2). The variation in the Mn concentrations between the research stations was 55.9%, 83.2% and 32.1% for *X. parietina*, *P. sulcata* and *H. physodes*, respectively. The level of the Mn geochemical background in thalli of *H. physodes* is 22 to 149 mg kg^{-1} (BENNET 1995). The presence of Mn in thalli is directly proportional to its content in the air, although around 50% of airborne Mn is carried out by natural dust. In urban agglomerations, around 50% of Mn is of natural origin. In comparison to Słupsk, a slightly lower Mn content was found in non-urbanized areas of Poland and Bulgaria Its concentration in *H. physodes* collected in Gorczański National Park varied within the range of 22 and 175 mg kg^{-1} (CZARNOTA 1995), in Świętokrzyski National Park it was around 99 mg kg^{-1} (GAŁUSZKA 2005), while a study completed in Vitosha Mountain Natural Park in Bulgaria revealed the Mn content at the level of 1001.0 mg kg^{-1} (CULICOV, YURUKOVA 2006). In contrast, similar Mn concentrations were found in several studies conducted in Polish urban areas. In Wrocław, for example, the concentration of Mn in *Hypogymnia physodes* after a month-long exposure ranged between 45.0 and 95.0 mg kg^{-1} (BOJKO et al. 2004). KRAWCZYK et al. (2004), in research conducted 2 km inland from the Baltic Sea, reported the Mn content in *H. physodes* equal 43.0 mg kg^{-1}. A slightly higher Mn content in thalli of *X. parietina* was discovered in Slovakia, where it was within the range of 59.0 to 1684 mg kg^{-1} (DZUBAJ et al. 2008). In Italian cities of Tuscany and in a small village of Sappada, the Mn content was found to be from 65.5 mg kg^{-1} to 70.0 mg kg^{-1} and 299.0 mg kg^{-1}, respectively (BARGAGLI et al. 2002). The research done by DEMIRBAS (2004) in Trabzon (Turkey) showed that the average Mn content in thalli of *P. sulcata* was 219.5 mg kg^{-1}, and in X. parietina – 126.4 mg kg^{-1}. EĞILLI et al. (2003) reported the Mn content in lichen thalli samples collected in Turkish cities such as Demirköy and İğneada close to the average values found in Słupsk (101.0 mg kg^{-1} - 121.0 mg kg^{-1}).

Iron is an element that undergoes considerable bioaccumulation, as it is broadly used in various branches of industry. The main sources of Fe introduced directly to the environment are: emission from metallurgical factories and coal combustion. The tested species of lichen appeared to differ in their ability to accumulate Fe. The highest accumulation of this element was found in *X. parietina* (633.6-4345.0 mg kg^{-1}), slightly lower in *P. sulcata* (925.5-3844.7 mg kg^{-1}), while the lowest one was detected in *H. physodes* (384.0-2063.7 mg kg^{-1}) – Figure 2. The values of the coefficients of variability of Fe concentrations in lichen were 43.5%, 48.3% and 56.3%, respectively for the above species, and depended on the distribution of the research stations. The level of the Fe geochemical background in thalli of *H. physodes* is 480 to 1710 mg kg^{-1} (BENNET 1995). KRAWCZYK et al. (2004) showed that in areas close to the Baltic Sea lichens accumulate substantially higher quantities of Fe than higher plants. The largest concentration of Fe in Słupsk was found in the vicinity of busy streets. In Poland, thalli of *H. physodes* contained from 670 to 2442 mg kg^{-1} of Fe in Gorczański National Park, (CZARNOTA 1995), and 815 mg kg^{-1} in Świętokrzyski National Park (GAŁUSZKA 2005). In the city of Demirköy in Turkey, the content of Fe was 880 mg kg^{-1} (EĞILLI et al. 2003), and in the city of Zapolyarnij (Russia) it reached 1475 mg kg^{-1} (ERMAKOVA et al. 2004), while and in thalli of *X. parietina* in Slovakia it varied between 931 and 15248 mg kg^{-1} (DZUBAJ et al. 2008). In Cerje (Serbia), thalli of *P. sulcata* accumulated around 810.4 mg Fe kg^{-1} (STEMKOVIĆ 2013).

According to DEMIRBAS (2004), the diversity in the content of elements in lichen thalli proves that it accumulation of elements is a species-dependent trait and may be affected by mutual impact of various elements. Our results are in agreement with those of DEMIRBAS (2004), who demonstrated that out of the three lichen species exposed to the same urban environment thalli of *P. sulcata* accumulated the largest quantity of Mn, *X. parietina* had the highest Cu content while the lowest concentration of Pb was found in *X. parietina*. The research by BOJKO et al. (2004) showed metal loads up to about 90 mg kg^{-1} (Mn, Zn), 700 mg kg^{-1} (Fe), 20 mg kg^{-1} (Pb) and 14 mg kg^{-1} (Cu) in lichen had a crucial impact on the processes of degradation, particularly on the decomposition of chlorophyll.

The accumulation of heavy metals by lichens most probably depends on the origin and the form of pollutants, as well as on the size of their particles in the atmosphere.

The concentrations of Pb, Zn, Ni, Cu, Mn and Fe in thalli of lichens were referred to the scale described by BARGAGLI and NIMIS (2002) – Table 2. The

Table 2

Scale for the interpretation of heavy metal concentrations in lichens (mg kg^{-1})

Air pollution	Zn	Cu	Ni	Pb
Very low	<35	<9	<1.5	<10
Low	65	15	3.0	25
Moderate	95	25	5.0	55
High	135	40	7.0	95
Very high	>135	>40	>7.0	>95

results suggest that the content of the tested elements in Słupsk is mostly between very low (Pb), low and moderate (Cu), moderate (Zn) and high (Ni).

The PCA was applied in order to identify factors determining the chemical composition of the tested lichens. The content of Pb, Zn, Ni, Cu, Mn and Fe in *Hypogymnia physodes, Parmelia sulcata* and *Xanthoria parietina* was used in the calculations. By applying the analysis of principal components, two independent factors were distinguished that explained 60% of variability (variance) of the chemical composition of lichens in Słupsk (Table 3). For data interpretation, only those values of factor loading which exceeded 0.5 were used. The results clearly differentiate sources of heavy metals in Słupsk. Factor 1 comprised Zn, Cu and Fe and indicated their common origin from the same local sources of pollution, such as production of paints and varnishes, or chemicals used for plant protection, brake lining and combustion of coal in households. Factor 2 explained 19% of total variance and comprised of Pb and Ni, indicating that the main source of airborne Pb and Ni in Słupsk is the precipitation of atmospheric particulate matter, transportation, communication and heating power engineering (DAMPS et al. 2013).

Based on the heavy metal content in lichen thalli, correlation coefficients were established for the Spearman ranges (Table 4). In all the species,

Table 3

Results of PCA based on results of metal content in thalli of lichens (n = 141)

Metals	Factor PC1	Factor PC2
Pb	0.19	**0.79**
Zn	**0.86**	0.03
Ni	-0.05	**0.56**
Cu	**0.85**	0.03
Mn	0.35	-0.48
Fe	**0.92**	-0.01
Eigenvalues	2.49	1.18
Explained variance (%)	41	19
	60	

Note: factor loading higher than 0.5 are in bold

Table 4

Correlation coefficients (Spearman) between each pair of variables in lichens, significant correlation values are in bold

Metals	Pb	Zn	Ni	Cu	Mn	Fe
	Hypogymnia physodes (n = 30, $p < 0.05$, $r_{crit.}$ = 0.31)					
Pb	-	**-0.37**	-0.08	-0.09	-0.15	0.05
Zn	**-0.37**	-	0.24	**0.42**	**0.34**	**0.51**
Ni	-0.08	0.24	-	0.18	-0.00	0.06
Cu	-0.09	**0.42**	0.18	-	-0.03	**0.75**
Mn	-0.15	**0.34**	-0.00	-0.03	-	-0.16
Fe	0.05	**0.51**	0.06	**0.75**	-0.16	-
Parmelia sulcata (n = 57, $p < 0.05$, $r_{crit.}$ = 0.24)						
Pb	-	0.14	-0.17	**0.56**	**-0.38**	**0.31**
Zn	0.14	-	0.02	**0.66***	0.10	**0.68***
Ni	-0.17	0.02	-	-0.10	-0.06	0.11
Cu	**0.56**	**0.66***	-0.10	-	-0.21	**0.80***
Mn	**-0.38**	0.10	-0.06	-0.21	-	-0.16
Fe	**0.31**	**0.68***	0.11	**0.80***	-0.16	-
Xanthoria parietina (n = 54, $p < 0.05$, $r_{crit.}$ = 0.25)						
Pb	-	**0.65***	**0.66***	**0.50**	**0.41**	**0.56**
Zn	**0.65***	-	**0.39**	**0.46**	**0.74***	**0.83***
Ni	**0.66***	**0.39**	-	0.20	**0.30**	**0.31**
Cu	**0.50**	**0.46**	0.20	-	**0.61***	**0.69***
Mn	**0.41**	**0.74***	**0.30**	**0.61***	-	**0.79***
Fe	**0.56**	**0.83***	**0.31**	**0.69***	**0.79***	-

* $p < 0.01$, $r_{crit.}$ – critical values of Spearman's correlation coefficient referred to Ramsey (1989)

strong positive correlations between Zn and Fe (r = 0.51 in *H. physodes*, r = 0.68 in *P. sulcata*, r = 0.83 in *X. parietina*), Fe and Cu (r = 0.75, r = 0.80 and r = 0.69) were found and positive correlation coefficients were derived for Zn and Cu (r = 0.42, r = 0.66, r = 0.46, respectively). Similar correlations were found by STAMENKOVIĆ et al. (2013) in thalii of *P. sulcata* and by LUDENIUS et al. (2010) in thalii of *H. physodes*. The results also demonstrate a similarity between the species in terms of Pb-Cu and Pb-Fe (positive correlation coefficients) as well as Pb-Mn (positive and negative correlation coefficients) in thalli of *P. sulcata* and *X. parietina*. The presence of a series of positive, statistically reliable correlation coefficients between the contents of Mn, Pb, Zn and Fe in thalli *X. parietina* is confirmed by DZUBAJ et al. (2008) and DEMIRAY et al. (2012). By comparing the relationships between the distribution of heavy metal content in the tested species, statistically valid correlations between the Pb and Zn content and between Zn and Mn were demonstrated in *H. physodes* and *X. parietina*. In addition, statistically verifiable positive correlation coefficients were obtained only in *X. parietina* for the relationships: Pb-Ni (r = 0.66), Zn-Ni (r = 0.39), Ni-Mn (r = 0.30), Ni-Fe (r = 0.31), Cu-Mn (r = 0.61) and Mn-Fe (r = 0.79) – Table 4. Reliable positive correlations between Zn-Ni and Pb-Ni can be supported by SCERBO et al. (2002). The current literature results suggest that lichens prefer nitrophyllic habitats as well as areas which are moderately polluted by dust (NIMIS et al. 2001). The diversity of the correlation coefficients determined for *H. physodes, P. sulcata* and *X. parietina* mainly results from interspecific differences and ionic interactions.

Having submitted the results for particular lichen species to the nonparametric U Mann Whitney test, a series of statistically valid differences were shown in the quantities of accumulated heavy metals and pH of their water solutions. The lichens showed species-characteristic pH values. Statistically significant differences were revealed in the accumulation of: Pb, Cu and Fe by *H. physodes* and *P. sulcata*, Ni by *P. sulcata* and *X. parietina* and Pb, Ni, Cu and Fe by *H. physodes* and *X. parietina* (Table 5). The series of signifi-

Table 5

The significance of variation of pH and heavy metal concentration in the tested lichens (*U* Mann-Whitney's test)

	Hypogymnia physodes – Parmelia sulcata	*Parmelia sulcata - Xanthoria parietina*	*Hypogymnia physodes - Xanthoria parietina*
pH	+++	++	+++
Pb	++	–	++
Zn	–	–	–
Ni	–	+	++
Cu	+++	–	+++
Mn	–	–	–
Fe	++	–	+++

The significance level: +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$, – no differences cant differences shown in the accumulation of the above elements indicates that further research on *H. physodes, P. sulcata* and *X. parietina* should be conducted, including tests on the morphological traits of their thalli.

CONCLUSIONS

An analysis of the content of heavy metals in thalli of lichens collected in Słupsk was performed to evaluate the quality of air in Słupsk. It was determined that the most severe pollution appeared in the city centre, and the lowest pollution was found in the city outskirts and within the city parks. The level of Pb was determined as very low, Cu – low and moderate, Mn – low, Fe – elevated in the city centre, Zn – moderate, and Ni – very high. Out of the three species, the largest quantities of Ni and Pb were accumulated by the thalli of *H. physodes*, Zn and Fe – *X. parietina*, and Mn was accumulated at the same level by all three species.

By applying the method of PCA, two independent factors were separated explaining 60% of variance of chemical composition of lichen at the territory of Słupsk. In the tested species strong positive correlation coefficients were found between Zn and Fe, Fe and Cu and Zn and Cu. Moreover, a similarity in relations of Pb-Cu, Pb-Fe and Pb-Mn in thalii of *P. sulcata* and *X. parietina* were revealed. Several statistically significant differences were demonstrated in the accumulation of metals as well as in pH between the three lichen species. The most important differences were found in the accumulation of Pb, Cu and Fe in thalli of *H. physodes* and *P. sulcata*, Ni in *P. sulcata* and *X. parietina* as well as in Pb, Ni, Cu and Fe by *H. physodes* and *X. parietina.*

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