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ASSESSMENT OF AIR POLLUTANTS IN AN URBAN AGGLOMERATION IN POLAND MADE BY THE BIOMONITORING OF TREES*

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Abstract

In 1970-2000, the state of the natural environment in many regions of Poland was unsatisfactory. This situation has been improving since the integration with the European Union, mainly owing to the reduction of emissions of sulphur and nitrogen oxides. The present study included three tree species: rowan (*Sorbus aucuparia* L. em. Hedl.), small-leaved lime (*Tilia cordata* Mill.), and aspen (*Populus tremula* L.). The trees grew in a pollution free environment (Huszlew) and in an urban agglomeration (Lublin). The aim of this research was to make comparative observations of the structure of leaves in relation to the content of lead, zinc, copper, nitrogen and sulphur determined in these organs. The N/S ratio in the leaves was accepted as an indicator of proper metabolic processes. Observations of leaves were made with using light microscopy and the scanning electron microscopy (SEM) technique. The content of heavy metals in leaves was determined by atomic absorption spectrometry (AAS) after dry mineralization. Total sulphur was determined by the turbidimetric method, while nitrogen by the Kjeldahl method after mineralization in sulphuric acid. When influenced by polluted air, the epidermis of the examined leaves was composed of smaller cells with higher stomatal density per unit area and had a lower number of open pores compared to the epidermis of the leaves developed in a clean environment. Among the species investigated, aspen was characterized by the highest ability to bioaccumulate heavy metals in its leaves. Among the elements determined only the Zn content exceeded the permissible limit, whereas Pb and Cu were found to occur in the range of values

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considered to be optimal. The dominant share of lead in suspended dust caused changes in the epidermis of leaves, which led to disorders in plant water relations.

Keywords: environment, heavy metals, nitrogen, sulphur, morphology, anatomy, leaves, *Populus tremula* L., *Sorbus aucuparia* L., *Tilia cordata* Mill.

INTRODUCTION

The progressive chemical degradation of the atmosphere and soil is particularly evident in urban agglomerations, where it occurs as a result of anthropogenic activity, increasing traffic, and the effects of municipal sewage and waste (Kołodziej et al. 2012). This process is also intensified near manufacturing facilities as well as in agriculturally used soils under due to, for example, misused pesticides and mineral fertilizers (Savci 2012). The accumulation of copper, zinc and lead in cultivated plants is an undesired phenomenon, since it leads to their inclusion in the food chain of humans and animals (Medyńska et al. 2009).

Following the introduction of environmental protection regulations in line with the law in effect in the European Union countries, since 1990, Poland has seen a gradual decrease in emissions of pollutants such as sulphur dioxide and nitrogen oxide as well as dust. The largest reduction has been recorded in sulphur dioxide emissions, which decreased from 1511 thousand tonnes in 2000 to 974 thousand tonnes in 2010 (GUS 2012).

According to a zone classification map prepared according to the assessment of air quality in The Province of Lublin (region) in 2004, the city of Lublin lies in a zone where the permissible limit is exceeded only by concentrations of suspended dust (PM10) (Żelazny 2013) .

Plants are universal research material owing to their high ability to absorb and store elements. In a polluted environment, they can accumulate and tolerate in their tissues high levels of heavy metals, including Pb and Zn, which disturb their proper metabolism, although plants frequently do not reveal any morphological symptoms (Shi et al. 2011).

The aim of this study was to evaluate the anthropogenic effects of air pollutants on the basis of the accumulation of nitrogen, sulphur, zinc, copper and lead in leaves of three tree species growing in a pollution free environment and within an urban agglomeration, as well as to show and compare changes in the leaf structure.

MATERIAL AND METHODS

Location of research

The study, which was conducted in 2011-2012, included three tree species: rowan (*Sorbus aucuparia* L. em. Hedl.), small-leaved lime (*Tilia cordata* Mill.) and aspen (*Populus tremula* L.). The plant material was collected in the second 10-day period of August from trees growing in a pollution free environment (a forest near the village of Huszlew, the Province of Masovia N52°9′28.08″ E22°51′18.94″) and in an urban agglomeration (Lublin, Głęboka and Akademicka Streets N51°14′30.38″ E22°32′37.92″) – Figure 1.

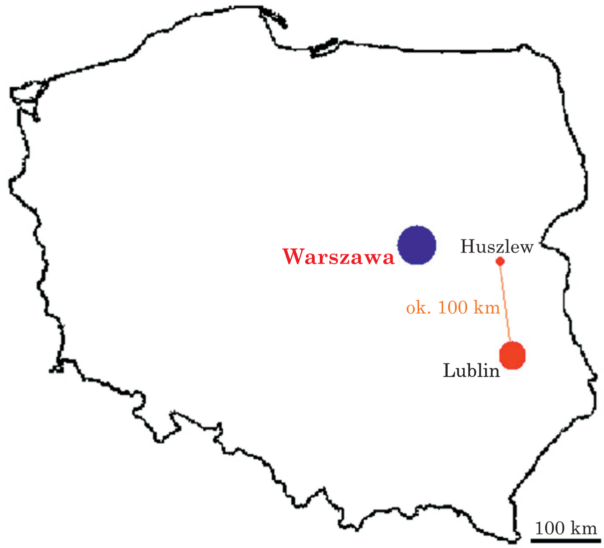


Fig. 1. Location of the study area

Three trees of each species, located 5-10 meters from a roadway, were chosen. From the bottom layer of each tree crown (1/3 of crown length), 5 samples of 20 leaves each were collected. From the sampled leaves, one combined sample was prepared for each species,. One half of a combined sample was submitted to laboratory analyses unwashed, while the other half was first washed.

Microscopic observations

Preliminary examination of the leaves was performed by light microscopy. From fresh material, hand-cut cross-sections were made from the central part of 10 leaves of each species. The sections were mounted in glycerol-gelatin. The thickness of the leaf blade, midrib and vascular bundle, the diameter of the largest vessels as well as the height of the epidermal and palisade parenchyma cells were measured. The measurements were made in 15 replicates.

The micromorphology of the leaf epidermal surface was viewed by scanning electron microscopy. Leaf blade pieces were fixed in 4% glutaraldehyde for 6 hours at room temperature and in 0.1 M phosphate buffer with a pH of 7.0 at 4°C for 48 hours. The fixed plant specimens were dehydrated in acetone series, critical-point dried in liquid CO_2. and coated with gold in an EMITECH K550X sputter coater. Observations of the epidermal surface and measurements of the stomata as well as photographic documentation were made in a TESCAN VEGA II LMU scanning electron microscope (SEM).

Chemical laboratory analysis of the plant material

The assessment of air pollution in the urban agglomeration was performed based on the concentration of heavy metals (Pb, Cu, Zn) as well as of S and N in the leaves of the investigated tree species compared to samples collected from the natural environment (Huszlew). For laboratory analysis, the leaves were prepared in two series: leaves washed with distilled water for 1 minute and unwashed leaves. Having been dried, ground and dry mineralized, the plant material was dissolved in HNO_3 (OSTROWSKA et al. 1991) and the content of Cu, Zn and Pb was determined in the solution by atomic absorption spectrometry (AAS) using a HITACHI Z-8200. Total sulphur was determined by the turbidimetric method of BUTTERS, CHENERY (1959) while nitrogen was determined after mineralization in sulphuric acid by the Kjeldahl method (WIERCIŃSKI 1999).

Statistical analysis

The analysis of the significance of differences relating to the studied traits in the structure of leaves and the content and relationships of the elements were analyzed statistically using the integrated software package Statistica statistical and analytical 6.0. Univariate and multivariate analysis of variance (Anova) and the Tukey's multiple comparison tests were performed. Statistical inference was tested at the significance level $a = 0.05$.

RESULTS

Micromorphology of surface epidermal cell

SEM observations revealed differences in the micromorphology of the leaf epidermis of the trees growing in the urban agglomeration compared to the epidermis of the leaf blades originating from a clean environment. Irregularly shaped and different-sized fine deposits were seen on the adaxial surface of the leaves of *Sorbus aucuparia* growing in the polluted environment (Figure 2*A-B*). The outer walls of the epidermal cells formed distinctly thicker

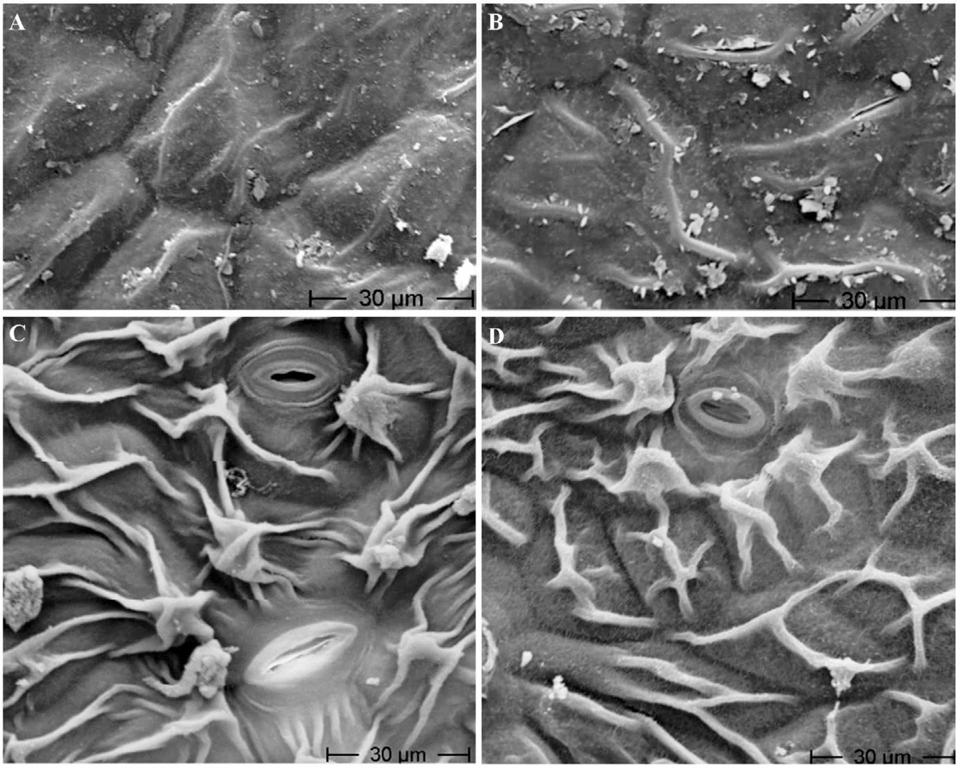


Fig. 2. Fragments of adxial (A, B) and abaxial (C, D) leaf epidermis surface of *Sorbus aucuparia* from Huszlew – unpolluted environment (A, C) and Lublin – urban agglomeration (B, D)

bands of cuticle running through the central part of the cell outline. Longitudinal cracks in the cuticle occurred at some places on these cuticular projections. In turn, the abaxial epidermis of the leaves of this species had smaller cells and a rough surface between the strands of cuticle, which formed a simpler arrangement compared to the cuticle in the leaves of this species growing in the clean environment (Figure 2A-D).

The abaxial epidermal surface in the leaves of *Tilia cordata* growing in the urban environment was irregular and rugged. It had a disrupted arrangement of longitudinal cuticular striae, which formed bands around the pores in the leaf epidermis of the lime trees growing in the urban environment (Figure 3A, B).

The abaxial epidermis in *Populus tremula* leaves originating from Lublin was composed of much smaller cells than the epidermal cells in the control. In turn, the stomata were mostly closed compared to the epidermal surface in the leaves formed by the trees of this species growing in the clean environment (Figure 3C, D).

The number of stomata on the abaxial surface of the leaf epidermis in the lime, rowan and aspen trees growing in Lublin was higher by 11, 12 and

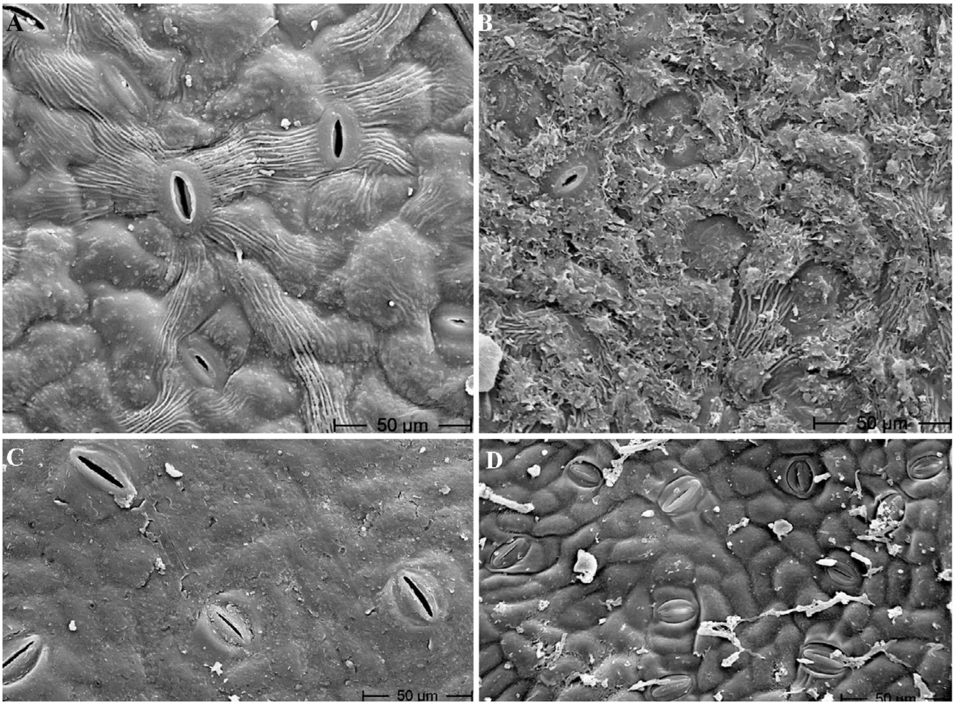


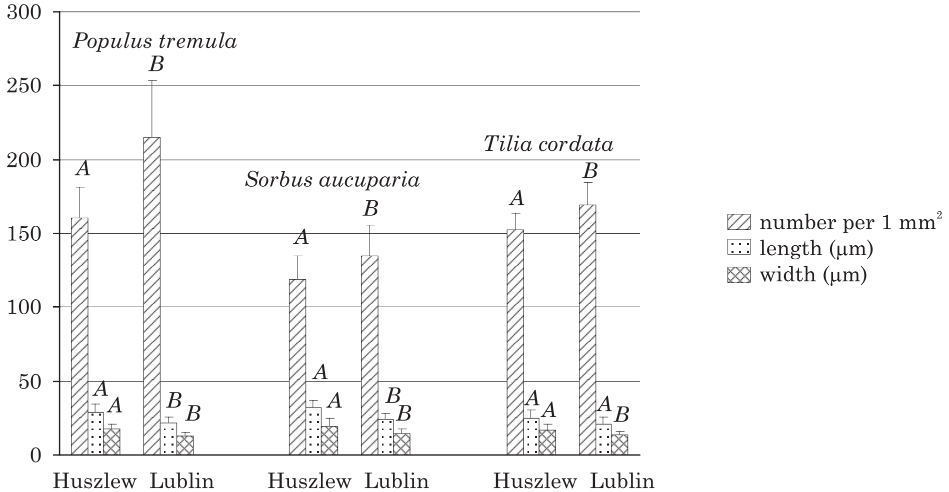
Fig. 3. Fragments of abaxial leaf epidermis surface of *Tilia cordata* (A, B) and *Populus tremula* (C, D) from Huszlew – unpolluted environment (A, C) and Lublin – urban agglomeration (B, D)

34%, respectively, than the number of stomata in the epidermis of the leaves of the taxa in question from the clean environment. Their size in the leaf epidermis of the trees growing in Lublin was much smaller than the dimensions of the stomata in control trees (Figure 4). In most stomata, the aperture between the cuticular ledges was closed or its width was smaller compared to the stomata in the control (Figures 2, 3).

Anatomical changes

As far as their anatomical structure is concerned, the examined leaves of the trees growing in Lublin were found to show inhibition of leaf blade thickness ranging from 5 to 13% and of the midrib thickness from 1 to 12% as well as a decrease in the size of the epidermal cells, with a simultaneous reduction in the height of the palisade parenchyma cells compared to the leaves collected from control trees. The height of the vascular bundle in the midrib was also found to be reduced by 8 to 14%. The diameter of the largest metaxylem vessels in the specimens sampled from the urban agglomeration ranged 14-35 µm, whereas in the control it was 16-38 µm (Table 1).

Among the examined trees from the unpolluted environment and urban agglomeration, larger changes in the anatomy of leaves (leaf blade thickness



A, B means followed by the same letter are not significantly different between pollution degrees in Huszlew (unpolluted environment) and Lublin (urban agglomeration) at $p \leq 0.05$

Fig. 4. Number and size of stomata in the leaf epidermis of the trees growing in a clean environment and in an urban environment

and nerve root, the amount of epidermal cells and palisade parenchyma as well as vessel diameter) were found in *Populus tremula*. These differences were statistically proven.

In the leaf blades of *Tilia cordata*, significant changes were found in the amount of epidermal cells and palisade parenchyma, but in *Sorbus aucuparia* only the changes in the thickness of the nerve root were statistically proven.

Content of elements

The leaf content of Zn, Cu and Pb in the studied tree species growing in the urban agglomeration was within the range of average values for natural environments. Only the zinc content in *Populus tremula* leaves was three times higher than its content in the unpolluted area. A significantly higher concentration of Pb and an elevated level of Zn were found in the samples of unwashed leaves from all the tree species investigated, which shows that atmospheric dust is significantly polluted with these metals (Table 2).

The nitrogen content in the leaves of the three taxa studied was in the range of the lower values considered to be optimal, but the values obtained in the natural environment were significantly higher than in the urban agglomeration area.

In contrast, the leaf sulphur content in *Populus tremula* and *Sorbus aucuparia* was higher in the urban agglomeration than in the natural environment. The N/S ratio in the leaves of the trees growing in Lublin was below

Table 1

Anatomical characteristics of some traits of the leaves of the studied trees ($\bar{x}$ ±SD, μm)

Trait		*Populus tremula*		*Sorbus aucuparia*		*Tilia cordata*	
		Huszlew	Lublin	Huszlew	Lublin	Huszlew	Lublin
Thickness of	leaf blade	169.9±17.33^{A}	147.1±19.79^{B}	160.1±24.10^{A}	152.3±22.54^{A}	420.5±66.17^{A}	399.5±62.80^{A}
	midrib	428.1±58.92^{A}	386.1±50.88^{B}	837.3±100.3^{A}	736.1±89.90^{B}	1315.0±176.5^{A}	1300.1±132.4^{A}
Height of	abaxial epidermal cells	21.53±3.701^{A}	16.20±2.732^{B}	20.67±3.722^{A}	18.20±2.183^{A}	25.07±3.243^{A}	20.27±4.532^{B}
	palisade cells	90.27±9.003^{A}	73.20±11.26^{B}	47.93±10.20^{A}	32.07±6.352^{A}	45.40±9.101^{A}	34.20±5.481^{B}
	vascular bundle	826.1±49.50^{A}	717.1±84.14^{B}	239.5±20.29^{A}	224.1±25.44^{A}	239.5±20.3^{A}	224.1±25.44^{A}
Diameter of	the largest metaxylem vessels	38.07±8.572^{A}	35.07±6.41^{A}	16.07±3.112^{A}	14.07±2.401^{A}	26.13±5.112^{A}	25.07±6.463^{A}

$^{A, B}$ means followed by the same letter are not significantly different between Huszlew unpolluted environment) and Lublin (urban agglomeration) at $p \leq 0.05$,
SD – standard deviation

Table 2

The content of elements in washed (I) and unwashed (II) leaves of tree trees species from an unpolluted (Huszlew) and unpolluted (Lublin) environment

Elements		Huszlew		Lublin	
		I	II	I	II
Populus tremula					
Zn	(mg kg^{-1})	24.00±2.70^{B}	25.13±3.26^{b}	69.00±2.65^{A}	78.67±4.51^{a}
Cu	(mg kg^{-1})	2.132±0.25^{B}	2.604±1.10^{b}	6.431±1.27^{A}	6.971±2.80^{a}
Pb	(mg kg^{-1})	0.164±0.02^{B}	0.201±0.03^{b}	0.263±0.01^{A}	0.342±0.04^{a}
N	(g kg^{-1})	18.23±1.15^{A}	18.57±1.21^{a}	15.43±1.72^{A}	16.00±1.97^{a}
S	(g kg^{-1})	1.632±0.06^{B}	1.772±0.06^{b}	1.873±0.12^{A}	2.014±0.12^{a}
N/S		11.19±1.05^{A}	10.52±0.79^{a}	8.294±1.14^{B}	8.002±1.33^{b}
Sorbus aucuparia					
Zn	(mg kg^{-1})	11.80±1.40^{A}	12.49±1.16^{a}	12.20±1.05^{A}	13.00±1.30^{a}
Cu	(mg kg^{-1})	5.734±1.04^{B}	6.201±0.90^{b}	11.03±1.52^{A}	11.70±2.20^{a}
Pb	(mg kg^{-1})	0.223±0.03^{B}	0.383±0.10^{b}	0.344±0.04^{A}	0.461±0.05^{a}
N	(g kg^{-1})	15.27±0.93^{A}	15.47±0.91^{a}	12.50±1.30^{B}	12.60±1.50^{b}
S	(g kg^{-1})	1.172±0.06^{A}	1.334±0.15^{b}	1.532±0.06^{B}	1.603±0.10^{a}
N/S		13.13±1.47^{B}	11.74±1.94^{a}	8.141±0.60^{A}	7.861±0.45^{b}
Tilia cordata					
Zn	mg kg^{-1})	13.90±1.80^{B}	25.03±6.43^{b}	28.10±2.20^{A}	30.83±4.15^{a}
Cu	mg kg^{-1})	4.003±0.70^{B}	4.573±0.91^{b}	8.932±0.57^{A}	9.603±0.90^{a}
Pb	mg kg^{-1})	0.053±0.02^{B}	0.093±0.03^{b}	0.273±0.05^{A}	0.372±0.06^{a}
N	(g kg^{-1})	15.97±0.59^{A}	16.37±0.61^{a}	13.30±1.61^{A}	14.00±1.90^{a}
S	(g kg^{-1})	1.334±0.06^{A}	1.463±0.13^{a}	1.373±0.06^{A}	1.472±0.06^{a}
N/S		11.98±0.42^{A}	11.27±0.74^{a}	9.77±1.55 ^{B}	9.531±0.99^{b}

$^{A, B; a, b}$ means followed by the same small letter are not significantly different ($p \leq 0.05$) between unwashed and those followed by the same capital letter between washed leaves of trees from an unpolluted (Huszlew) and polluted (Lublin – urban agglomeration) environment. SD – standard deviation.

12:1, and it was more dependent on the low nitrogen content than on the elevated content of sulphur in this area. In the natural environment, its value was significantly higher owing to a higher N content in the soil formed from forest leaf litter and on account of lower sulphur emissions in this area (Table 2).

DISCUSSION

The influence of air gaseous pollutants and heavy metals on the metabolism of plants

The long-term effects of an increased concentration of airborne pollutants include their accumulation in tissues of the photosynthetic organs and possible poisoning of plants. Sulphur dioxide and nitrogen oxides have a particularly adverse effect on changes in the relationships of soil micronutrient content and availability versus the chemical composition of leaves (Niewęgłowska-Guzik 1995, Rennenberg et al. 1996). Gaseous compounds of nitrogen (NO, NO_2) and sulphur (SO_2) can penetrate directly through the assimilatory organs, while ammonium and nitrate nitrogen (NH_4^+ and NO_3^-) are taken up by the root system. Plants absorb different gases from the air and act as a biofilter of airborne gaseous pollutants. For instance, research proves that a 500-meter-wide urban green belt reduces the concentration of sulphur dioxide three times and up to 75% of the concentration of nitrogen oxides (Nowak et al. 2006). An excessive SO_2 concentration in the air gradually destroys the parenchyma layer of leaves, which causes inhibited photosynthesis, chlorophyll degradation as well as disturbances in transpiration and respiration (Wang et al. 2011).

A classification of the agglomeration of Lublin according to the health protection criteria in terms of pollutants such as sulphur dioxide, nitrogen dioxide, lead and carbon oxide performed in 2011 placed the city to be in the cleanest class. The annual assessment of plant protection also excluded excess of the permissible limits for nitrogen oxides and sulphur dioxide (Żelazny 2013). This entails the absence of harmful effects of SO_2 on the sulphur/nitrogen balance in plants in the Lublin agglomeration. Low emissions of air pollutans, which are primarily generated by vehicle traffic as well as by local coal-fired boiler plants and central heating units, have a significant influence on the condition of atmospheric air in the Lublin region.

Morphological and anatomical changes under the influence of leaf pollutants

The examination of the leaves showed morphological and anatomical changes in the leaf blades of the three tree species from the area of Lublin. A decrease in leaf blade thickness with a simultaneous reduction in the size of the epidermal and mesophyll cells in the leaves of the investigated trees from the urban agglomeration, are a result of the deposition and storage of heavy metal ions in the leaves, water stress, cell division inhibition and stomatal closure (Pourkhabbaz et al. 2010, Stevovic et al. 2013). The formation of smaller stomata in the leaf epidermis of the trees growing in Lublin was compensated by their larger number. An increase in the number of stomata per unit area was indicated as one of the defence mechanisms against toxicant induced stress (Stolarska et al. 2007, Azmat et al. 2009). On the other hand, the aperture size between the cuticular ledges in the stomata was probably attributable to the increased permeability of protein-lipid membranes caused by the presence of heavy metals (YANG et al. 2004).

Stomata are blocked by dust particles (chemically neutral) as a result of their mechanical deposition on the leaf surface, which leads to an increased leaf temperature and disrupts the metabolic processes in the leaves, including the photosynthesis. On the other hand, chemically active dust components have a toxic effect on the leaf's physiological processes and on soil chemistry (PRAJAPATI 2012).

The smaller epidermal cells and mostly closed stomata, as found in the present study, can result from disturbances in the physiological processes, photosynthesis and water balance, which occur for example under the influence of air pollutants (WERYSZKO-CHMIELEWSKA, CHWIL 2005, ZHAO et al. 2011). Despite the higher frequency of stomata, the transpiration rate is lower. This is due to the impeded water transport in the plant caused by Pb^{2+} ions, which contribute to a reduction in the number of xylem vessels, and to the reduced stomatal opening, which is confirmed by the observations of the epidermis of the leaves sampled from the urban agglomeration area (CHWIL 2001, KOSOBRUKHOV et al. 2004). Such leaves tend to close the guard cells in the presence of lead. Water stress that occurs under such conditions slows down the rate of photosynthesis in the consequence of less effective enzymatic action in the carboxylation process and also reduces electron transport, photophosphorylation and total chlorophyll content (BARCELÓ, POSCHENRIEDER 1990). Dust pollutants and trace elements which they contain affect plants directly by depositing on the leaf surface and blocking the stomata, as well as indirectly by causing changes in soil chemistry through their deposition on the soil. A well-developed wax layer on the surface of tree leaves is a biological protective barrier which, by binding heavy metals from dust fall, reduces their penetration into the leaf tissues through the leaves themselves (MISHRA, PANDEY 2011).

The content of heavy metals and relationship elements as indicators of the degree of pollution of air

Lead and zinc belong to elements with a medium level of accumulation, but zinc is characterized by a higher rate of bioaccumulation (the ratio of the content of an element in the plant to its concentration in the soil) than lead (YOON et al. 2006). An airborne lead concentration near urban agglomerations can exceed 20.000 ng m^{-3}, while its natural content is 0.5-10 ng m^{-3}. The optimal lead content in tree leaves is 1.5-7.8 mg Pb kg^{-1} DW, but in polluted areas it can be several times higher (POLKOWSKA et al. 2001).

Research shows poor translocation of lead in the plant; even with its increased content in the soil, it accumulates primarily in the roots and only a small amount of lead travels to the plant's aerial parts (POURRUT et al. 2011). The differences found between the washed and unwashed leaf samples collected from the investigated trees are an indicator of the elevated lead and zinc content in the air. Compared to lead, a higher zinc content in the examined leaves, as found in the present study, results from the greater mobility of this element in the plant. This means that some of the total amount of zinc found in the leaves comes from the atmosphere, while a significant share originates from the soil, since the accumulation of metals in fallen leaves causes a secondary increase in the contamination of topsoil with these metals (Kabała et al. 2010).

CONCLUSIONS

1. Leaves of the examined species of trees (rowan, small-leaved lime and aspen) growing in an urban agglomeration, as compared to an unpolluted area, have thinner blades, a reduced size of the epidermal cells and palisade parenchyma. Furthermore, they form a higher number of of smaller stomata.

2. Pollutants also cause abnormal cuticular ornamentation, a lower midrib thickness as well as a lower height of the vascular bundle and the diameter of the largest xylem vessels.

3. Changes in the morphological and anatomical leaf blades structure of trees from the urban agglomeration are the consequence of particulate matter (PM 10) deposition together with a substantial share of Zn and Pb.

4. The results reported above are consistent with the evaluation included in the report on the condition of the natural environment in the Province of Lublin. The content of the elements determined in the leaves of the bioindicator plants was in the range of values considered to be natural.

5. Among the analyzed species of trees, aspen accumulated the most while rowan gathered the least of the analyzed elements. Aspen was characterized by an increased ability to accumulate zinc, since the leaf Zn content determined in the Lublin agglomeration exceeded three-fold the natural range. The content of the other elements was on a similar level for all the three trees species, both from the urban agglomeration and from the forest, being in the optimal range for plants.

6. The range of the content of these elements determined in the leaves did not implicate excessive air pollution with heavy metals, sulphur oxides (VI) or nitrogen oxides (III). The higher leaf Pb content shows the dominant share of this metal in suspended dust.

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