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CONCENTRATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN *Hypogymnia physodes* (L.)*Nyl.* THALLI AND CHANGES TO MORPHOLOGICAL STRUCTURE

KUMULACJA WIELOPIERŚCIENIOWYCH WĘGLOWODORÓW AROMATYCZNYCH W PLECHACH *Hypogymnia physodes* (L.)*Nyl.* I ZMIANY W ICH BUDOWIE MORFOLOGICZNEJ

Abstract: Apart from widely known anthropogenic pollutants as SO_2, NO_x, CO_2, CO, there are another dangerous substances emitted to the air named polycyclic aromatic hydrocarbons (PAH). In the air they occur in a form of vapours and aerosols deposited on dust particles of 10 μm (PM 10) and 2.5 μm (PM 2.5) in diameter. In cities, the air polluted by gases and atmospheric particulate was analysed using special automatic or semi-automatic equipment or analytic procedures. That is why a powerful development of bioanalytical techniques based on using organisms as bioindicators is observed in recent years. The lichens are the most frequently used organisms in bioindication. The purpose of this research is to evaluate air pollution by PAHs in urban agglomeration with the use of *Hypogymnia physodes (L.)Nyl.* The research was performed in two hundred thousand occupants in south-east Poland in 2004-2007. The lichens placed on tree branches of 30 cm on 4 crossroads, and the 3 branches were put in each research point. Before starting the exposition, the "O" sample had been collected that had been stored in a closed container before chemical analysis. The exposition period lasted for 3 months. Then PAHs were determined in collected lichens. The analysis was performed with high performance liquid chromatography (HPLC), LiChrosper (TM) column 100 RP - 18, UV detector; $\lambda = 254$ nm. The concentration was expressed in mg/kg of dry mass that is after deducting PAH value determined in "O" sample. The analysis of obtained results showed diverse concentration of the pollution in the analysed crossroads depending on the road traffic density and season. PAH concentrations were determined from 0.61 $mg \cdot kg^{-1}$ d.m. in the 1st quarter of 2004 to 2.56 $mg \cdot kg^{-1}$ d.m. in the 1st quarter of 2006, and from 0.48 $mg \cdot kg^{-1}$ d.m. in the 4th quarter of 2004 to 2.22 $mg \cdot kg^{-1}$ d.m. in the 4th quarter of 2006. Meteorological conditions influence the concentration of PAHs in lichens. The atmospheric precipitation contributed to the decrease of PAHs concentration in the air by scavenging the pollution with atmospheric particulate. The regression line amounted to $y = 1.91759 - 0.00674 \cdot x$, at the confidence interval equal to $p = 0.0308$. A relation between the PAH concentration and air relative humidity turned out to be the most essential correlation. This relation indicates that the concentration of PAHs in the lichens increases with an increase of humidity. The line regression amounted to $y = -1.04196 + 0.02897 \cdot x$, at the confidence interval equal to $p = 0.0505$.

Keywords: air pollution, bioindicators, lichens, morphological change

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Introduction

The powerful development of industry all over the world took place in the beginning of the 19th century. Non-ferrous and ferrous metallurgy, steelworks, and armaments industry have been quickly constructed. Those large industrial centres have influenced fast urban development and high population density in cities. Not advanced technological processes at that time and usage of the coal with high sulfur content as energy source were the reasons for exacerbating the air quality. Additionally the households using coal for heating purposes contributed to that process. Apart from widely known anthropogenic pollutants as SO_2, NO_x, CO_2 and CO, there are another dangerous substances emitted to the air named *polycyclic aromatic hydrocarbons* (PAH). In the air they occur in a form of vapours and aerosols deposited on dust particles of 10 µm (PM 10) and 2.5 µm (PM 2.5) in diameter. Polycyclic aromatic hydrocarbons penetrate the human body by gas exchange (respiratory system), with food and through the body skin. Penetrability of organisms by polycyclic aromatic hydrocarbons results from lipophilicity of PAHs. The epidemiological research on the carcinogenic influence of PAHs on the human organism showed close relation between benzo[a]pyrene concentration in the air and lung cancer disease. It is claimed that such tissues and organs as epithelium, bone marrow, reproductive system, and lymphatic system are particularly susceptible to PAHs. Because of high toxicity and carcinogenicity of PAHs, Directive 2004/107/EC of 15 December 2004 relating to arsenic, cadmium, mercury, nickel and polycyclic aromatic hydrocarbons in ambient air assumes that a target value for PAH concentration in ambient air shall not exceed 1 $ng \cdot m^{-3}$. In cities, the air polluted by gases and atmospheric particulate is analysed using special automatic or semi-automatic equipment or analytic procedures. However this traditional approach based on measuring equipment and chemical analysis does not provide information about an impact of pollutants on organisms. That is why a powerful development of bioanalytical techniques based on using organisms as bioindicators is observed in recent years. The lichens are the most frequently used organisms in bioindication.

The earliest biomonitoring research with the use of lichens, aimed at providing a theoretical basis and developing research methods, dates back to the 1960s [1]. At present, these organisms are used for bioindication purposes in various regions of the world: Holland [2], Finland [3], Argentine [4-6], the USA [7], Germany [8], Italy [9, 10], Poland [11-15], Chile [16], Portugal [17], Slovenia [18] and Israel [19].

Together with the development of analytical procedures, the transplantation method has been used more and more frequently [9, 13, 15, 20-23]. Experimental investigations into lichen transplantation, *ie* lichen removal from low-pollution areas (primeval forests or rural areas with preserved natural landscape) into sites where lichens do not occur in natural clusters (lichen-free zones) or their populations are considerably limited due to anthropogenic influences, began in the late 19th century. At that time, studies mostly relied on morphological, macroscopic observation and monitoring of thallus depletion [1]. The removal of living thalluses into polluted areas, their exposition to pollutant activity on sites selected on account of emission sources, together with a subsequent chemical composition analysis enable the assessment of the extent of air pollution for the purposes of human health protection. Consequently, transplantation techniques, while requiring ongoing methodological upgrade, have become a significant part of biomonitoring (Fig. 1), as they offer some of the best solutions for long-term environmental management.

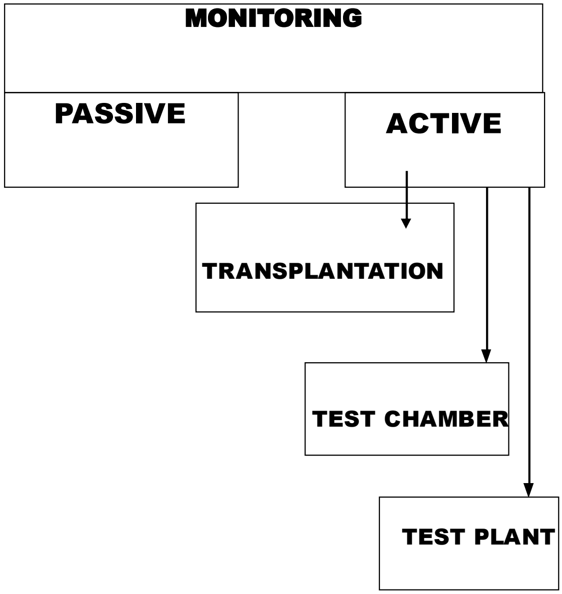

Fig. 1. Place of transplantation in biomonitoring [23]

The research based on lichens relating to air pollution performed so far mainly focused on determining the concentration of heavy metals in the air in industry centres [12, 24], around urban agglomerations [11] along transportation routes outside cities [5], in the countryside [6, 10] and in the protected areas [13]. However the highest amounts of pollutants that have a significant contribution to human health are emitted in urban agglomerations.

Lichen transplantation methods

Transplantation methods rely on a range of techniques and purpose-built structures in order to optimise lichen thallus exposition. A commonly used method is to place nylon mesh with lichens inside. Samples exposed in this manner have been transplanted in Argentina. Nylon mesh or bags were suspended from the height of 3 m AGL (*above ground level*) [5] (Fig. 2).

This method was also used in Argentine and Italy in the years 2002 and 2004, but the exposition height was altered and the samples were placed at 1.5÷2.0 m AGL [5, 6, 10].

Another method, used in Germany [25], relies on spreading PVC-coated wire mesh, fastened to trees at 1.5÷2.0 m AGL. Lichen sample transplants are also exposed with the use of PVC cords placed at 2.5÷3.0 m AGL. This method has been applied in Israel [26]. Lichens can also be threaded along nylon lines which are then arranged at the intervals of 3 cm on a transverse board. Separated with transparent PVC tubes, they are placed at 3 m AGL. This method has been used in Norway and north-eastern Sweden (Fig. 3).

Fig. 2. Nylon bags with lichen samples [5]

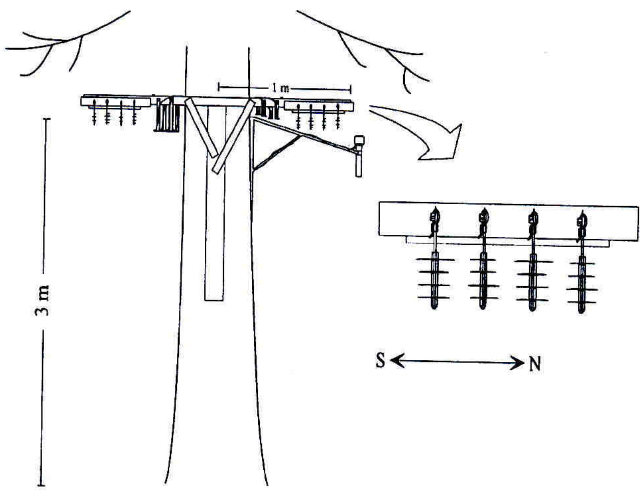

Fig. 3. Lichens threaded on nylon strings [27]

Specially sheltered, wooden panels to which lichens are fastened are likewise constructed and placed along tree trunks. The placement height is ca 1.60 m AGL [28] (Fig. 4).

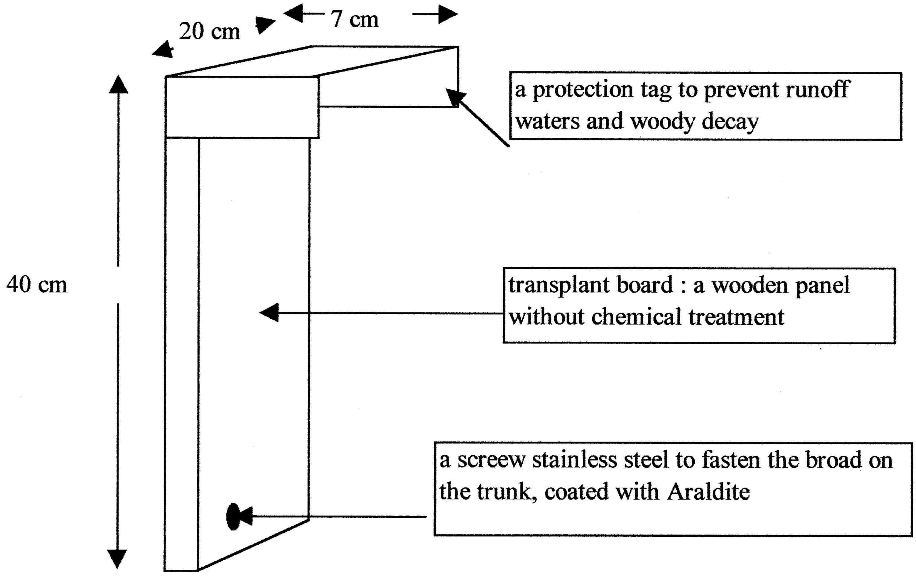

Fig. 4. Wooden panels with lichen [28]

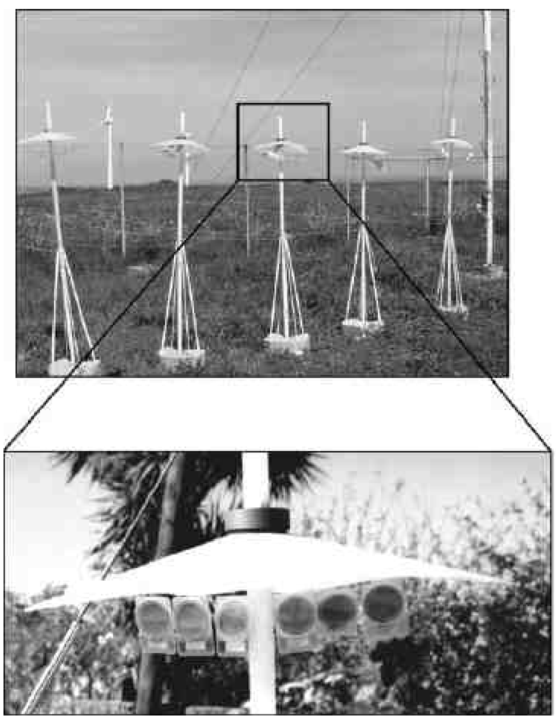

Fig. 5. Petri dishes with lichens roofed and placed on the stands [29]

Polyamide mesh can also be used if mesh space size does not provide an obstacle for pollutant deposition. In Portugal, mesh of porosity of 61µm is used [17]. Freitas et al [29] have used Petri dishes of 47 mm in diameter, which were placed on purpose-sheltered stands at 1.5 m AGL (Fig. 5).

In Poland, exposition methods underwent several changes over the years 1969-2008. The first transplants were bark discs, cut out together with the lichen, installed directly in tree trunks; later, in order to eliminate the possible influence of the base, small boards were used, with bark discs fixed to them [30]. The boards were placed at heights ranging from 2 m AGL to 8 m AGL [31, 32]. Another transplantation method, proposed by Sawicka-Kapusta and Zakrzewska [33] in Poland (after Jeran et al [21]), consists in direct placement of twigs overgrown with lichen thallus of the *Hypogymnia physodes* on tree trunks at the height of ca 2.0 m AGL (Fig. 6). This method is still used in Poland for the implementation of a measurement programme under the Natural Environment Integrated Monitoring System.

Fig. 6. Branches covered with lichen thallus, put in places of exposure on the tree trunks (photo M. Jóźwiak)

The detailed methodology for sample collection for transplantation purposes requires determination of natural conditions, lichen collection sites, meteorological and soil parameters, tree species or rock types from where the lichens originate, as well as exposition AGL height. In order to minimise the influence of field conditions, the lichens are to be collected at the height of 1÷2.5 m along the entire site perimeter [2].

A wide variety is also to be observed in transplant sample size. It may be expressed in area units [2] or in wet weight [5]. More often, however, the weight units are used because this facilitates ensuring uniformity of all samples. The weight is expressed as wet, its value given at the moment of transplantation (6.0 g) - Carreras, Pignata [5] and González, Pignata [27], or as dry matter of the sample - 30÷40 g [10] or 100 mg [17], dried at the temperature of 35ºC prior to mineralisation.

Sloof [2] has transplanted samples of area of ca 9 cm^2. M.C. Freitas [29] has placed them on Petri dishes of 47 mm diameter; samples of area of ca 4 cm^2 have been used by Conti [10]. Transplantation which occurs with the use of epiphytic lichen thalluses determines the length of twigs with lichens fastened to their surfaces. The total twig length, including the thallus, reaches 30 cm [11, 12, 33]. Transplanted lichens are separated from tree branch or tree trunk bark, or are transplanted jointly [12] and separated from the original base only after exposition.

Similarly, the number of samples in a particular area as well as their placement sites may vary. The height at which the samples are suspended ranges from 1.5÷2 m (Poland, Slovenia), 2 m (Italy), 3 m (Argentine), to 3.5 m (Holland). Another difference in research methodology is the duration of thallus exposition to polluted air, ranging from one to twelve months. From an analysis of the available literature, it follows that the most common are periods of six months (the hot and cold half-year). Yet, in areas of considerable air pollution (the vicinity of foundries, refineries, metallurgical plants, cement plants, thermal power plants, or large urban agglomerations), shorter (four- or three-month-long) exposition periods are common.

In the studied cases, the various methodologies of sample transplantation have shown shared features, as well as accentuating major differences and their significance for the studies. In each case, care has been taken to transplant lichen from low-pollution areas. Transplants exposed on the south-western Atlantic coast have been removed by A. C. Freitas [29] from the unpolluted areas of northern Portugal (Baiáo). Thalluses for bioindication studies of air pollution in the industrial area of Cordoba, obtained by Carreras and Pignata [5], have been collected in a low-pollution area situated 70 km away from investigation sites. Similarly, González and Pignata [27] have collected their "O" sample material to the north-west of Cordoba, in the vicinity of La Calera. Conti et al [10] transplanted lichens collected in the Abruzzo National Park (central Italy) and removed them to the city centre and industrial areas. The "O" lichen samples collected by Godinho et al [17] have been taken from central Portugal's rural and unspoilt areas in the vicinity of the town of Tomar. Studies in Slovenia [18] have been conducted with the use of lichens collected in the unpolluted area of Rogla (Pohorje Gory). Dutch studies [2] have consisted in transplanting thalluses from the clean north-western part of Holland into areas of both low and high pollution. In the case of the studies by Budka et al [11] and Bialonska [12], the lichen thallus collection area for bioindication studies has been north-eastern Poland: an area situated away from any metropolis or emission sources - the Borecka Primeval Forest. The Borecka Primeval Forest has also been used for thallus collection for the "O" sample and subsequent transplantation in the Swietokrzyskie National Park by Sawicka-Kapusta et al [13, 33] and in the city of Kielce [15, 24, 34].

Species used in the transplantation method

Transplantation in selected areas enables the observation of specific morphological changes as well as the chemical analysis of lichen thalluses exposed to specific pollution types. The pollution may originate from urban areas, expressways, industrial estates, thermal power plants, chemical plants and refineries. Bioindicator transplants are most frequently filamentous or foliose, which results from their greater sensitivity in comparison with the fruticose and the crustose. In the world, the following species are most commonly used for research purposes:

- *Hypogymnia physodes* (L.) *Nyl*. - transplanted in urban areas of south-eastern Poland (city of Kielce) and national parks (Swietokrzyskie National Park), Krakow Industrial Region [11, 12, 15, 33]; in Slovenia - transplanted in the vicinity of chemical plants and metalworks [18], as well as in the central Ural Mts. around mines [35],
- *Usnea amblyoclada* (*Mill. Arg.*)*Zahlbr*. - in Argentine, transplanted around the industrial agglomeration of Cordoba in the vicinity of a cement plant where hazardous waste is processed [5],
- *Evernia prunastri* (L.) Ach. - transplanted in Italy to three anthropogenically modified areas: urban (Cassino), rural (S. Elia Fiumerapido) and industrial (Piemont S. Germano) [10] as well as in Portugal [17],
- *Parmelia caperata* (L.) Ach., 1803 - transplanted in the industrial city of Sines, in Portugal [17],
- *Parmelia sulcata Taylor*. - in Holland, Germany and Belgium, transplanted in industrial areas: the agglomeration of Rotterdam, the Ruhr and the German-Belgian industrial regions [2],
- *Punctelia subrudecta* (*Nyl.*) *Krog.* - in Argentine, transplanted in the vicinity of a power plant in the city of Cordoba [27],
- *Ramalina lacera* (*With.*) *J.R.Laundon.* - in Israel, transplanted around fossil-fuel power stations [36],
- *Ramalina ecklonii* (*Sprengel*) *G.Meyer & Flotow* - in Argentine, transplanted along communications routes of various traffic density [4],
- *Ramalina duriaei* (*De Not.*) *Bagl.* - in Israel, transplanted along expressways near residential areas [36],
- *Pseudevernia furfuracea* (L.) Zopf. - in Italy and Switzerland [37],
- *Nephroma antarcticum* (*Jacq.*)*Nyl.* - in Patagonia, transplanted in two cities: San Carlos de Bariloche, a tourist destination, and Villa Regina, a city surrounded with farmland in the province of Río Negro [6].

All the above lichens rank high in the lichenoindication scale, thus fulfilling the requirements for bioindicators.

There are lots of studies in which the transplantation of lichens to evaluate the air pollution level in cities caused by heavy metals is used, whereas there are no reports describing the pollution by polycyclic aromatic hydrocarbons using lichens.

Materials and methods

The study was performed in urban agglomeration in 2004-2007. Based on road traffic density, 4 crossroads were selected for the research area regarding PAHs. *Hypogymnia physodes* lichen was carried from Borecka Primeval Forest (North-eastern Poland) that is recognized as non-polluted reference area for Poland. The lichens placed on tree branches of 30 cm in length were moved to designed areas, and the 3 branches were put in each research point. Before starting the exposition, the “O” sample had been collected that had been stored in a closed container before chemical analysis. The exposition period lasted for 3 months. Such cycle was established after the 6-month trial of lichen exposition in the city with 2-month exposition times that was carried out in 2003. 2-month exposition period was too short and did not reflect meteorological changes and human activity (cold season). Then PAHs were determined in collected lichens. The analysis was performed with *high performance liquid chromatography* (HPLC), LiChrosper (TM) column 100 RP - 18, UV detector; λ = 254 nm. The concentration was expressed in mg/kg of dry mass that is after deducting PAH value determined in "O" sample. After exposition the lichens were tested using FEI QUANTA 200 *scanning electron microscope* with digital image processing. Sections of the lichens showing colour changes (blackened, whitened and browned areas) and of control sample (without colour changes) were prepared for tests (Figs 7 and 8).

Fig. 7. Morphological changes of *Hypogymnia physodes* thalli after exposed on the crossroads (M.A. Jóźwiak)

Fig. 8. Natural thalli structure *Hypogymnia physodes* (M.A. Jóźwiak)

Research area

The city of Kielce is located in Central-Eastern Poland and has 202,000 inhabitants. The orography of the area occupied by the city (109.45 km^2) is versatile, and dense urban development in particular in the city centre and in the housing estates together with minimized green areas contribute to heat balance of the city, temperature differences, atmospheric precipitation and cloudiness. Annual course of wind activity in the Kielce city indicates two phases: autumn-winter phase with intensified winds from the South and from the South-East (25.4%) and spring-summer phase with winds from the North (7.4%). The city is located in the zone of medium and low windiness. The strongest winds occur in the winter and in the beginning of the summer (11÷15 m·s^{-1} and more than 15 m·s^{-1}) what results from high baric gradients and increased atmospheric circulation in those seasons. Daily changes of wind speed are modified by turbulence, convection and buildings. In the city the streets that are parallel to the main wind directions are aired to the highest extent, while in the streets that are perpendicular to the main wind directions so-called "wind shadow" is formed favouring accumulation of pollutants. The most unfavourable conditions for ventilation are found in the city centre. An atmospheric silence reaching 15.5% in a year in the city of Kielce is an important indicator of airing of the city. It can be observed mostly in the autumn with a maximum in October when a relative humidity reaches over 70%. Hollows of polluted air are observed due to so many windless days. Additionally this phenomenon intensifies inversion fogs that are observed in the city mostly in October. What is more the city of Kielce is located in a zone of relatively unfavourable conditions for vertical air exchange. An atmospheric instability state is observed during 23 to 29% of hours in a year. A stable dynamic balance found mainly in the winter is also unfavourable to the airing of the city. The winter time is a period of increased activity of inhabitants connected with heating season. During the summer an increased level of temperature compared to surrounding areas and decreased humidity cause the city of Kielce to be an urban island of heat. Under those unfavourable meteorological conditions the pollutants are released to the air from local emitters located inside the city: industrial plants, municipal heat power plant, housing estate power plants, and fugitive emission. A road transportation is considered a significant emitter, too. State, regional and local roads run across the city in total having 384 km in length. The road traffic density in the main crossroads varies from 2328 vehicles per hour to 4241 vehicles per hour in the morning rush hours while it amounts from 2412 vehicles per hour to 5590 vehicles per hour in the afternoon rush hours [38]. These areas are ranked among so-called line emission sources where the motor vehicles are the main source of emission. A combustion of diesel oil and gasoline is one of the substantial sources of emission of PAHs in the urban conditions [39]. The sources of these dangerous compounds in the crossroads include also: rubbing off the rubber tires when braking, car linings and asphalt consisting fractions of polycyclic aromatic hydrocarbons. A car tire can lose even up to 2 kg as a result of rubbing off during use. In case of truck tires, the losing of weight may even reach 12 kg. Approximately 9% of rubbed off rubber is released to the air in a form of dust. Currently tires contain twice as much or more PAHs as a limit value defined in the project of UE Directive (10 $mg \cdot kg^{-1}$). The winter time is especially dangerous because of increased fuel consumption at low air temperatures. The city of Kielce was categorized as zone III - class "C" by the Regional Environmental Protection Inspectorate in which the level of atmospheric particulate concentration was exceeded adding up a tolerance margin.

Results and discusion

The air pollution in the city of Kielce by dust particles of 10 µm in diameter (PM 10) calculated based on data from Regional Environmental Protection Inspectorate in Kielce shows that average concentration of PM 10 dust in the city in 2004-2007 amounted to 23.6 $\mu g \cdot m^{-3}$, while the highest amounts were obtained in cold months with average of 33.0 $\mu g \cdot m^{-3}$ in the 1st quarter and varied from 29.6 $\mu g \cdot m^{-3}$ in February to 35.7 $\mu g \cdot m^{-3}$ in January, and with average of 28.8 $\mu g \cdot m^{-3}$ in the 4th quarter ranging from 24.8 $\mu g \cdot m^{-3}$ in October to 31.4 $\mu g \cdot m^{-3}$ in December. Significantly lower values of the concentration of atmospheric particulate were noted in warm months. The distribution of average annual values of PM 10 dust concentration in the crossroads in the city of Kielce in 2004-2007 is shown in the Figure 9.

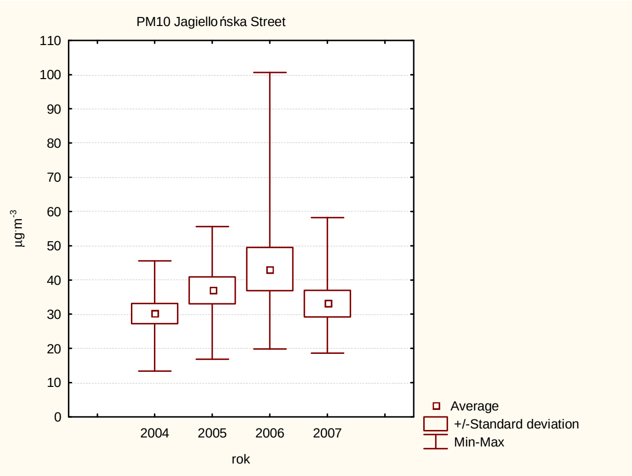

Fig. 9. Average yearly dust PM10 concentration on the crossroads in Kielce in the years 2004-2007

The concentration of dust in the air depended on many factors in which apart from the emission, the main role was played by meteorological conditions (air temperature, atmospheric precipitation, air relative humidity, and wind speed). The atmospheric precipitation was the factor limiting the dust concentration in the air (Fig. 10), and also the increase of the temperature decreased the level of dust concentration whereas the decrease of the temperature contributed to the increase of the dust concentration (Fig. 11), and air relative humidity was a stimulating factor (Fig. 12).

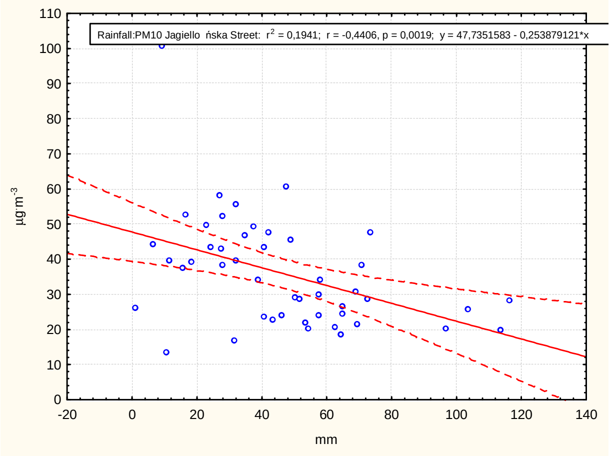

Fig. 10. Regression line suggesting a correlation between PM 10 and precipitation on the crossroads in Kielce

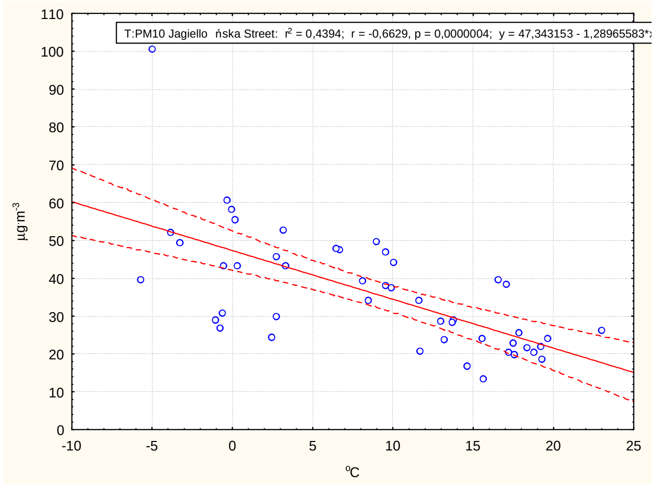

Fig. 11. Regression line suggesting a correlation between PM 10 and temperature on the crossroads in Kielce

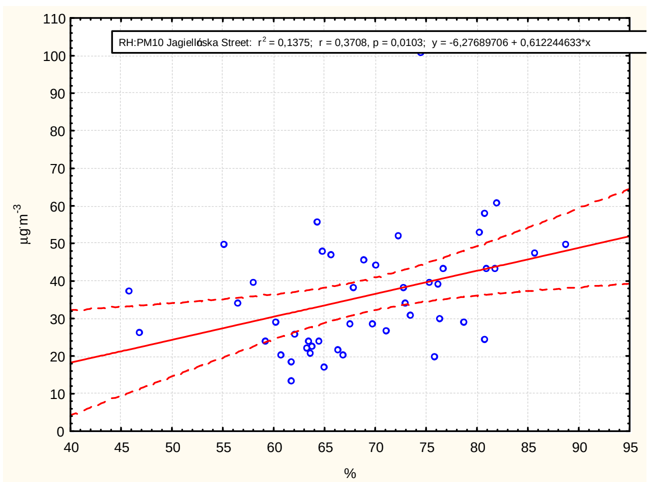

Fig. 12. Regression line suggesting a correlation between PM 10 and humidity on the crossroads in Kielce

Accumulation of PAHs in the *Hypogymnia physodes* (L.)*Nyl.* lichen thalli

The average concentration of PAHs in 4 main crossroads in the city of Kielce in 2004-2007 amounted to 0.94 $mg \cdot kg^{-1}$ d.m. with the year 2006 singled out (Fig. 13).

The analysis of obtained results showed diverse concentration of the pollution in the analysed crossroads depending on the road traffic density and season. The highest concentrations were found in the crossroads No. I and III (Fig. 14).

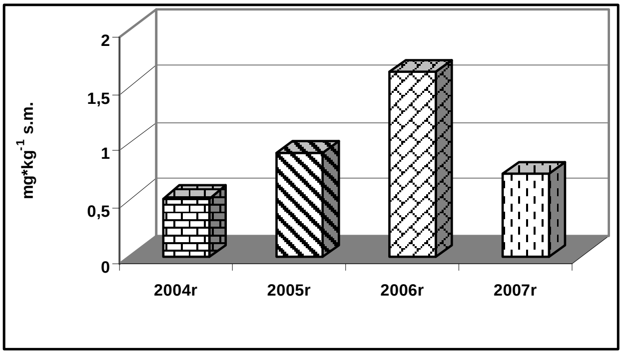

Fig. 13. Average yearly PAH concentration in lichens exposed on the crossroads in Kielce in the years 2004-2007

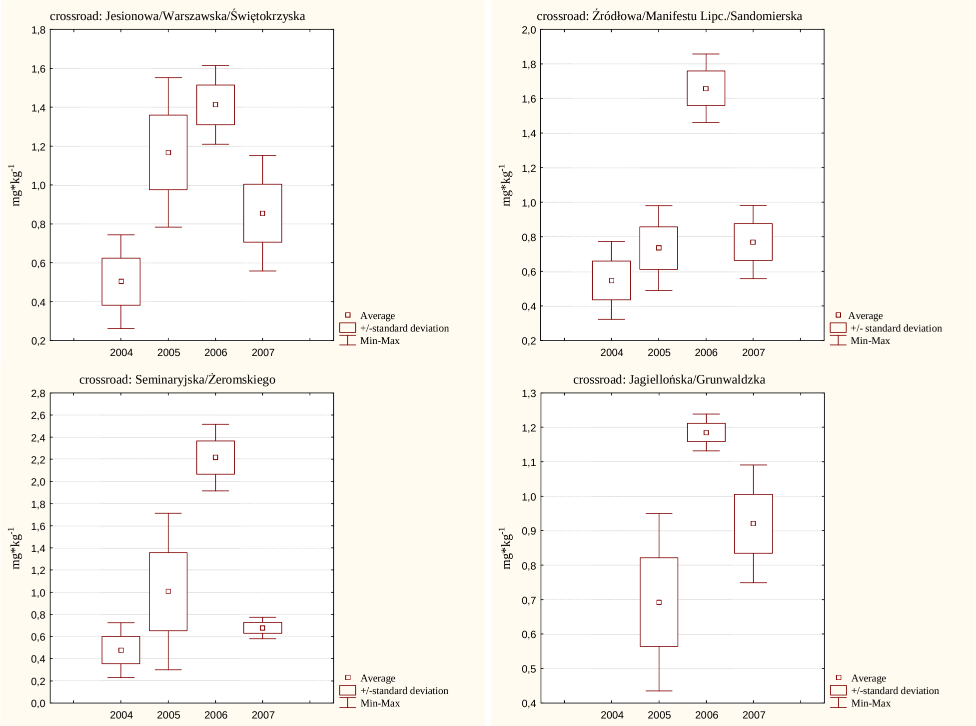

Fig. 14. Average yearly PAH concentration in lichens exposed at selected crossroads in Kielce in the years 2004-2007

The highest accumulation of polycyclic aromatic hydrocarbons in the lichen thalli was observed in the 1st and 4th quarter of the year. The highest variations were reported in the crossroad No. III that reached from 0.61 mg·kg^{-1} d.m. in the 1st quarter of 2004 to 2.56 mg·kg^{-1} d.m. in the 1st quarter of 2006, and from 0.48 mg·kg^{-1} d.m. in the 4th quarter of 2004 to 2.22 mg·kg^{-1} d.m. in the 4th quarter of 2006. This crossroad is located in the city centre between 3-floor buildings, and because of no planted greenery in those areas, the accumulation of pollutants during high road traffic density was observed. Furthermore in 2006 in relation to renovation of the parallel road, the traffic was concentrated in the tested crossroad what caused accumulation of PAHs in the air to be the highest in all quarters in 2006 and throughout the research period (Table 1).

Table 1

PAH concentrations in *Hypogymnia physodes* lichen thalluses, exposed on the crossroads in Kielce in the years 2004-2007

Place and exposition time	**I quarter**	**II quarter**	**III quarter**	**IV quarter**
	[mg·kg^{-1} d.m.]			
	2004			
Crossroad I*	0.73	0.27	0.32	0.69
Crossroad II**	0.71	0.29	0.43	0.76
Crossroad III***	0.61	0.26	0.28	0.76
Crossroad IV ****	bd	bd	bd	bd
	2005			
Crossroad I	1.69	0.95	0.82	1.21
Crossroad II	0.86	0.55	0.51	1.02
Crossroad III	1.96	0.46	0.48	1.12
Crossroad IV	0.87	0.42	0.53	0.95
	2006			
Crossroad I	1.68	1.25	1.26	1.46
Crossroad II	1.91	1.55	1.46	1.72
Crossroad III	2.56	2.13	1.85	2.32
Crossroad IV	1.15	1.24	1.22	1.13
	2007			
Crossroad I	1.22	0.65	0.58	0.97
Crossroad II	1.01	0.64	0.55	0.88
Crossroad III	0.78	0.58	0.61	0.74
Crossroad IV	1.12	0.81	0.75	1.00

*Crossroad I - traffic density: morning rush hour - 4241 cars/hr; afternoon rush hour - 5590 cars/hr.
**Crossroad II - traffic density: morning rush hour - 3287 cars/hr; afternoon rush hour - 3350 cars/hr.
***Crossroad III - traffic density: morning rush hour - 4207 cars/hr; afternoon rush hour - 4241 cars/hr.
****Crossroad IV - traffic density: morning rush hour - 3474 cars/hr; afternoon rush hour - 3537 cars/hr.

The tests of PAH concentration in the soil near the main communication routes revealed that the concentration of PAHs varied from 0.028 mg·kg^{-1} d.m. to 5.656 mg·kg^{-1} d.m. depending on the distance from the road [40]. The highest concentrations were found at the border of the road. Then one must suppose that also in the city of Kielce there is an increased soil contamination by polycyclic aromatic hydrocarbons at the crossroads.

Meteorological conditions influence the concentration of PAHs in lichens. The atmospheric precipitation contributed to the decrease of PAHs concentration in the air by scavenging the pollution with atmospheric particulate. The regression line for the crossroad No. I amounted to $y = 1.91759 - 0.00674 \cdot x$, at the confidence interval equal to $p = 0.0308$ (Fig. 15). A relation between the PAH concentration and air relative humidity turned out to be the most essential correlation. This relation indicates that the concentration of PAHs in the lichens increases with an increase of humidity (Fig. 16). The line regression for the crossroad No. I amounted to y = –1.04196 + 0.02897 · x, at the confidence interval equal to p = 0.0505.

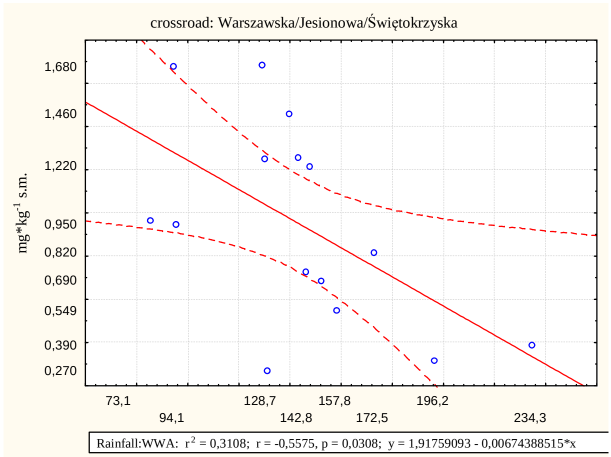

Fig. 15. Dependency of PAH concentration in transplanted lichens on atmospheric precipitation in Kielce

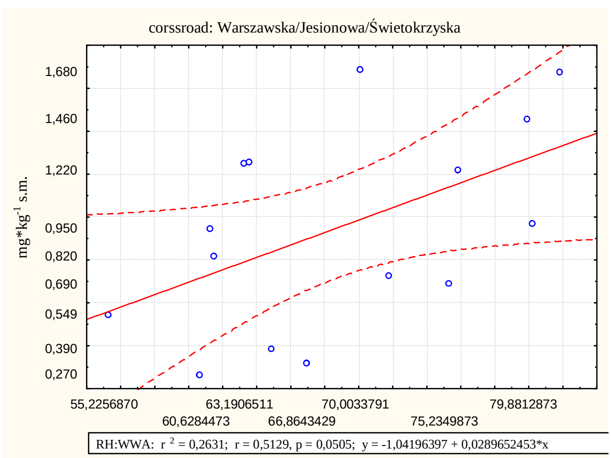

Fig. 16. Dependency of PAH concentration in transplanted lichens on humidity (RH) in Kielce

The analysis of changes to the structure of *Hypogymnia physodes* (L.) *Nyl*. lichens in the image from scanning electron microscope

A scanning electron microscope is a valuable tool for the research of cells and subcellular structures in the lichen thalli. It enables to observe a morphology of cells and alleged tissue thalli [41-44]. The use of scanning electron microscope for the research of lichen damage exposed under unfavourable conditions allowed to detect changes in the lichens that limit or make it impossible for the organism to function.

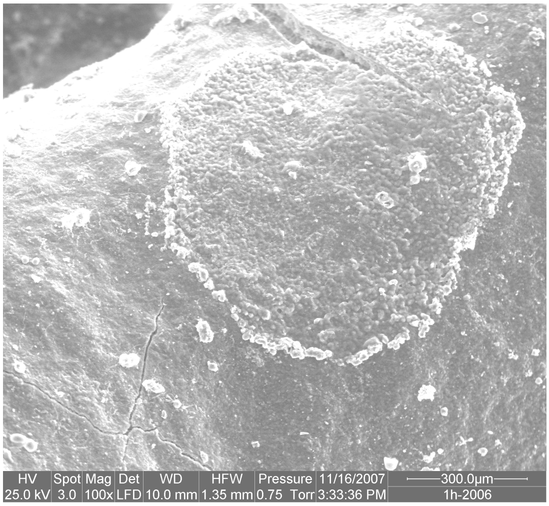

Fig. 17. Large-area pollution accumulation on *Hypogymnia physodes* (L.), *Nyl.* thallus surface, exposed on the crossroads in the third quarter of 2006 (magnified 100x) (M.A. Jozwiak)

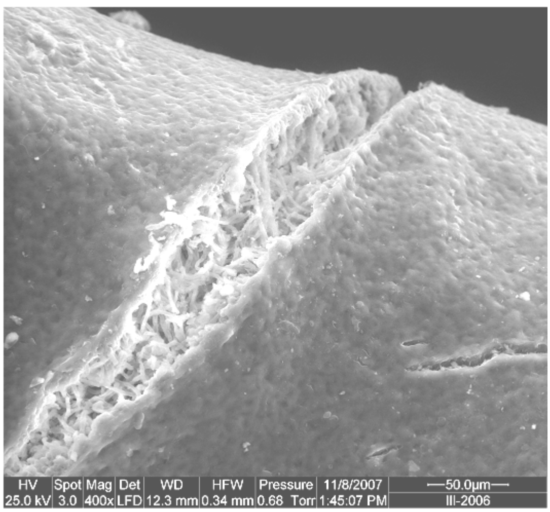

Fig. 18. Fissured pseudocyphelia on the *Hypogymnia physodes* (L.), *Nyl.* thallus surface, control group (magnified 400x) (M.A. Jozwiak)

After 3-month exposition of *Hypogymnia physodes* lichen in the crossroads, pollutants accumulated in various lichen parts and in particular concentrated around widely open chinks of pseudocyphellae (Fig. 17) what was not observed in the lichens in the control sample (Fig. 18). Pseudocyphellae chinks are the areas where gas exchange occurs naturally but at the same time under the conditions of polluted air, the pollutants penetrate into the lichens through these chinks. No separated algae layer in the structure of upper cortex was another observed change. (Fig. 19). In the structure of not damaged thalli, the algal layer is evidently located under the cortex of the mycelium (Fig. 20), and algal cells have circular shape (Fig. 21) what is visible in the cross-section of "O" control sample (Fig. 22). Changes like deformation of algal cells covered with pollutants can be visible at higher magnification (Figs 22 and 23).

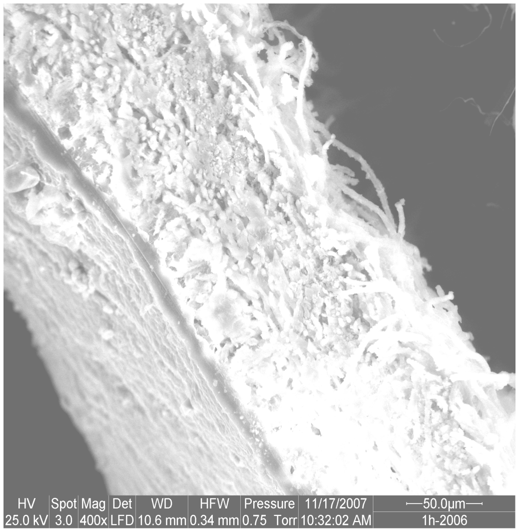

Fig. 19. *Hypogymnia physodes* (L.), *Nyl.* thallus cross-section with no gonidial zone, exposed on the crossroads in the third quarter of 2006 (magnified 400x) (M.A. Jozwiak)

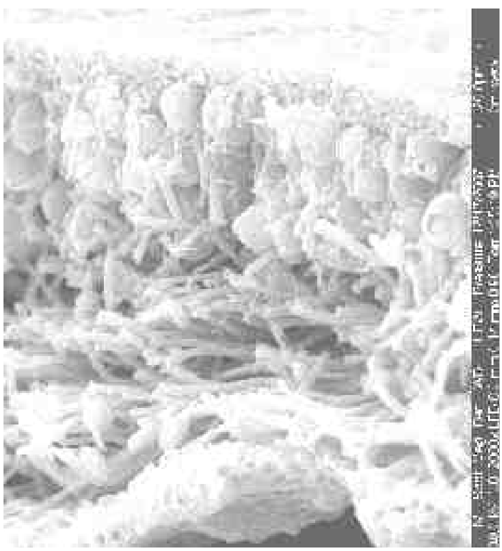

Fig. 20. *Hypogymnia physodes* (L.), *Nyl.* thallus layered structure, control group (magnified 2000x) (M.A. Jozwiak)

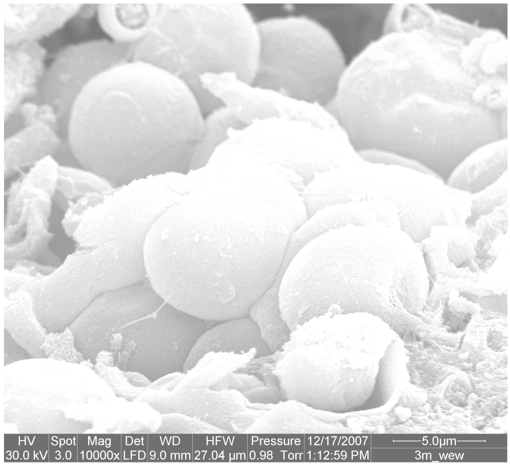

Fig. 21. Coccoid cells of *Trebouxia* sp. from the algae zone of the *Hypogymnia physodes* (L.), *Nyl.* thallus, control group (magnified 10 000x) (M.A. Jozwiak)

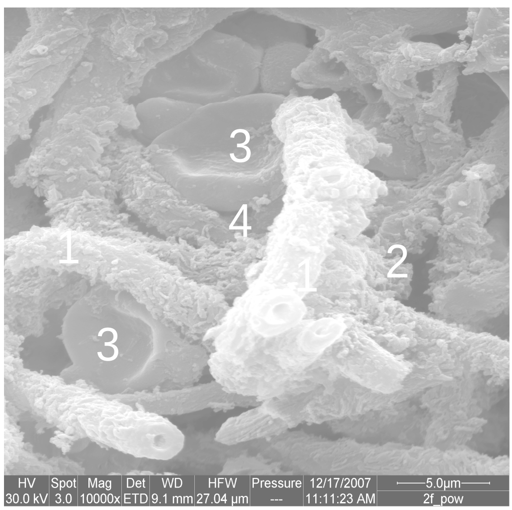

Fig. 22. Deformations of algae and mycelia inside the *Hypogymnia physodes* (L.), *Nyl.* lichen, exposed on the crossroad (magnified 10 000x) (M.A. Jozwiak), 1 - pollution on the surface of hyphae; 2 - pollution in the intercellular space, 3 - change in algae shape from orbicular to discous, 4 - pollution accumulated on algae cells

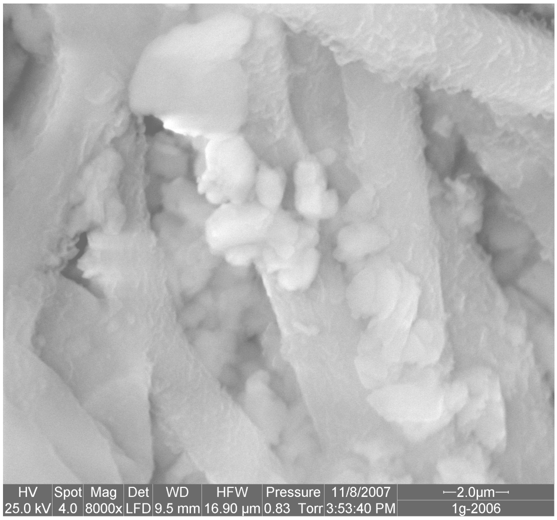

Fig. 23. Mycelium hyphae with pollution on the surface of the cell wall inside the *Hypogymnia physodes* (L.), *Nyl*. thallus, exposed on the crossroad (magnified 40 000x) (M.A. Jozwiak)

Conclusions

The lichens play a very important biogeochemical role in restraining and distributing the nutrient substances and micronutrients but they accumulate also PAHs and heavy metals. They are rated among biologically effective indicators of air pollution. Because they do not have a protective dermal layer and branched system of substance consumption, they must absorb them directly from the air. What is more they have some ecological and physiological requirements what makes them very sensitive to pollutants found in the air. The use of the lichens to show negative environmental changes is nowadays frequently used in particular in the areas under strong anthropogenic pressure on the environment. The monitoring of the environment using the lichens has become widely spread not only for air quality assessment but also as an effective early warning system [45]. The content of SO_2 in the air has significantly decreased since the last decade of the 20th Century because of social and economical changes. On the other hand a pollution by heavy metals and PAHs has increased what is related to the development of means of transportation. It was notified by the European Council and Parliament who in IV Directive UE put an emphasis on the problem of air pollution by As, Cd, Ni, Hg and benzo[a]pyrene as the most toxic PAH compound. The member states were obliged to submit the results of the air quality assessment regarding the level of toxic metals and PAHs until September 30, 2008. Till the end of 2009 the member states must develop and implement corrective measures. The research performed with the use of *Hypogymia physodes* lichen confirmed high concentration of polycyclic aromatic hydrocarbons in the air reaching from 0.56 to 1.22 $mg \cdot kg^{-1}$d.m. The concentration of PAHs in the lichens transplanted in the city of Kielce in 2004-2007 depended on the place of the exposition linked to the emission value and meteorological conditions such as: air temperature, atmospheric precipitation, air relative humidity, wind speed and direction. Obtained concentrations of PAHs in the lichen thalli enable to issue maps of the areas endangered by polycyclic aromatic hydrocarbons in the city what may be suitable for inhabitants as an information about the endangered areas, and for the municipal government on the other hand when developing local land use plans, and in the future for the development of land use plan for the whole commune.

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KUMULACJA WIELOPIERŚCIENIOWYCH WĘGLOWODORÓW AROMATYCZNYCH W PLECHACH *Hypogymnia physodes* (L.)*Nyl.* I ZMIANY W ICH BUDOWIE MORFOLOGICZNEJ

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Abstrakt: Obecnie obok powszechnie znanych zanieczyszczeń gazowych pochodzenia antropogennego, takich jak SO_2, NO_x, CO_2, CO, do groźnych substancji dostających się do powietrza zalicza się wielopierścieniowe węglowodory aromatyczne (WWA). W powietrzu występują w postaci par i aerozoli zalegających na cząstkach pyłu o średnicy 10 µm (PM 10) i 2,5 µm (PM 2,5). W miastach zanieczyszczenie powietrza gazami i pyłem zawieszonym bada się przy użyciu specjalistycznej automatycznej lub półautomatycznej aparatury lub z zastosowaniem procedur analitycznych. Takie tradycyjne podejście, polegające na wykorzystaniu aparatury pomiarowej i analizy chemicznej próbek, nie daje jednak pełnej informacji dotyczącej oddziaływań zanieczyszczeń na organizmy żywe. Dlatego też, w ostatnich latach obserwuje się dynamiczny rozwój technik bioanalitycznych wykorzystujących organizmy żywe jako biowskaźniki. Jednymi z najczęściej wykorzystywanych w bioindykacji organizmów są porosty. Celem badań była ocena zanieczyszczenia powietrza WWA w aglomeracji Kielc z wykorzystaniem *Hypogymnia physodes (L.)Nyl.* Badania przeprowadzono w dwustutysięcznym mieście położonym w południowo-wschodniej Polsce w latach 2004-2007. W mieście, na podstawie poziomu natężenia ruchu kołowego, wytypowano 4 skrzyżowania do badań WWA. Porost *Hypogymnia physodes* przywożono z Puszczy Boreckiej (NE Polska), obszaru, który dla Polski uznawany jest jako obszar wzorcowo czysty. Porosty, na gałązkach o długości 30 cm, transplantowano w wyznaczonych obszarach, po trzy gałązki w każdym punkcie. Przed każdą ekspozycją pobierano próbkę „O", którą do czasu analizy chemicznej przechowywano w zamkniętym pojemniku. Ekspozycja każdorazowo trwała 3 miesiące. W zebranych porostach oznaczano WWA. Analizy dokonywano z zastosowaniem HPLC, kolumna Li Chrospher (TM) 100 RP - 18, detektor UV; λ = 254 nm. Stężenie podano w $mg \cdot kg^{-1}$ suchej masy netto, tj. po odjęciu wartości stężenia WWA oznaczonego w próbce „O". Plechy po ekspozycji badano w mikroskopie elektronowym skaningowym FEI QUANTA 200 z cyfrowym zapisem obrazu. Analiza uzyskanych danych wykazała zróżnicowanie koncentracji zanieczyszczenia na badanych skrzyżowaniach w zależności od natężenia ruchu kołowego i pory roku. Na wartość stężenia WWA w porostach modyfikująco wpływają warunki meteorologiczne. Opad wpływał na obniżenie WWA w powietrzu poprzez wypłukiwanie go wraz z pyłem zawieszonym. Linia regresji dla skrzyżowania I wynosiła $y = 1{,}91759 – 0{,}00674 \cdot x$, przy przedziale ufności p = 0,0308. Najistotniejsza okazała się korelacja między stężeniem WWA a wilgotnością względną powietrza. Zależność ta wskazuje na wzrost stężenia WWA w porostach wraz ze wzrostem wilgotności. Linia regresji dla skrzyżowania I wynosiła $y = –1{,}04196 + 0{,}02897 \cdot x$, przedział ufności p = 0,0505.

Słowa kluczowe: zanieczyszczenia powietrza, bioindykatory, porosty, zmiany morfologiczne