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Assessment of air pollution along express roads and motorways of varied traffic load with the use of bioindicators

Ocena zanieczyszczenia powietrza wzdłuż dróg ekspresowych i autostrad o różnym natężeniu ruchu z wykorzystaniem bioindykatorów

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Streszczenie. Dynamiczny rozwój infrastruktury drogowej w Polsce, wzrost liczby pojazdów o bardzo zróżnicowanym stanie technicznym oraz użytkowanie samochodów pozbawionych układów oczyszczania spalin (filtr DPF) to podstawowe przyczyny zanieczyszczenia powietrza w rejonach przebiegu dróg ekspresowych i autostrad. Wzrost zanieczyszczenia powietrza stwarza konieczność oceny poziomu koncentracji toksyn w obszarach emisji liniowej.

Przeprowadzone badania wykazały możliwość zastosowania w tej ocenie bioindykatorów *Pleurozium schreberi* (Willd.) Mitten oraz *Hypogymnia physodes* (L.). Na podstawie biowskaźników analizowano zmiany w budowie anatomicznej i morfologicznej badanych organizmów, a oceny dokonywano na podstawie zdjęć SEM, uwzględniając stężenie wielopierścieniowych węglowodorów aromatycznych w materiale biologicznym po jego ekspozycji w badanym terenie. Badania objęły również analizę koncentracji WWA w organizmach biowskaźników celem wskazania wpływu wielopierścieniowych węglowodorów aromatycznych na żywe komórki.

Słowa kluczowe: infrastruktura drogowa, *Pleurozium schreberi* (Willd.) Mitten, *Hypogymnia physodes* (L.).

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INTRODUCTION

The Act of 21 March 1985 on public roads (Dz.U. of 2007, No. 19, item 115 as amended) defines motorways and express roads as public roads of limited accessibility, intended only for motor vehicle traffic, equipped with separate one-way roads with split-level junctions. Motorways and express roads in Poland constitute the national road network. They serve the purposes of interregional and international communication. By the end of 2013, there were commissioned 1494.45 km of motorways and 1335.55 km of express roads. According to the Regulation of the Council of Ministers of 20 October 2009, the total network of motorways and express roads in Poland will be about 7480 km (Fig. 1), including about 1990 km of motorways, which will considerably affect air cleanness in the areas where such roads will be exploited. At present, the network of motorways and express roads is being developed quicker due to, among others, large funds from the EU Cohesion Fund. Apart from the unquestionable economic benefits related to this fact, the development of road network causes risks related to air pollution by vehicle traffic. Therefore, it becomes necessary to monitor the condition of the environment, especially the atmospheric air, in the vicinity of motorways and express roads.

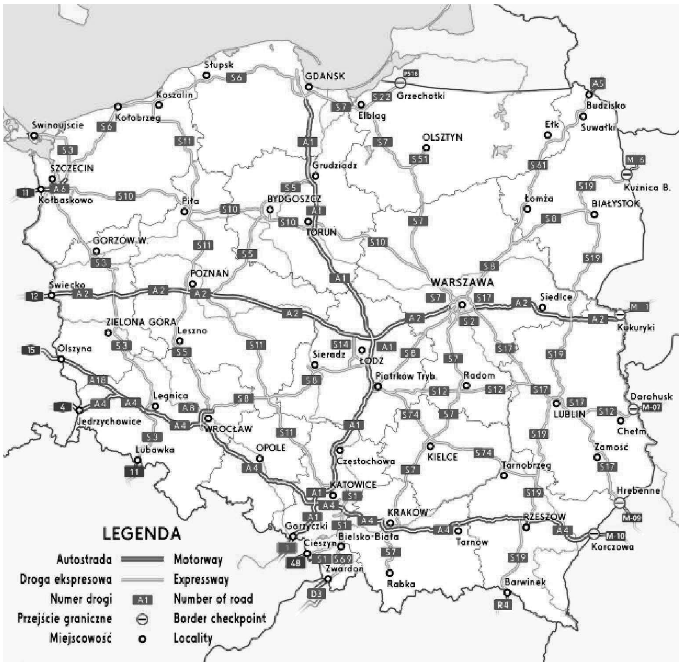

Fig. 1. The outline network of motorways and express roads in Poland

Rys. 1. Przebieg autostrad i tras szybkiego ruchu w Polsce

The level of air pollution can be monitored at little cost through the use of bioindicators along with the modern in-situ testing methods, which consist in the transplantation of bioindicator organisms. The methods of assessing chemical changes in the environment include the observation of the reaction of organisms called bioindicators (Wolterbeek, 2002). Bioindicators are used as biological tests to assess the level of toxins of anthropogenic origin (Dećkowska et al., 2008; Lechnio, Malinowska, 2003). The most popular biotests are mosses and lichens. These organisms absorb chemical substances through ectohydricity, using the organism surface. It enables to determine the dynamics of changes in air pollution (Čeburnis et al., 1999; Fernaandez et al., 2004; Panek, Szczepańska, 2005). Indicators can desorb metals by means of dry deposition or, in the event of high concentration of acidifying gases in the air and precipitation, by means of bioabsorption in the ionised form (Morselli et al., 2003). World research shows that the use of bioindicators in order to asses air condition is very common and brings highly reliable results (Mulgrew et al., 2000; Lechnio et al., 2000; Ashmore et al., 2001; Grodzińska, Szarek-Łukawska, 2001; Couto et al., 2004; Poikolainen et al., 2004; Rühling et al., 2004; Szczepaniak, Bziuk, 2003; Jóźwiak, Jóźwiak, 2009; Jóźwiak, 2012)

APPLIED STUDY METHOD

Bioindication was performed through the use of two indicator organisms, i.e. *Pleurozium schreberi* (Willd.) Mitten and *Hypogymnia physodes* (L.) Nyl. They were exposed along express roads and motorways in South-Central Poland using transplantation method (Photo 1).

Photo 1. Method of exposure *Pleurozium schreberi* (Brid.) Mitt. (Photo by M.A. Jóźwiak)
Fot. 1. Metoda ekspozycji Pleurozium schreberi (Brid.) Mitt. (fot. M.A. Jóźwiak)

The study material was exposed in cases sized 30 cm x 40 cm (*Pleurozium schreberi*) placed 1.5 m above ground level (*Hypogymnia physodes*). The studies were carried out on a six-month basis. The transplant material was subject to microscopic analysis (the microscope Nikon SMZ 1500 with the use the programme NIS-Elements BR and the microscope Nikon A2100). Polycyclic aromatic hydrocarbons (PAHs): from the plant material, there were extracted 50 ml of dichloromethane for 30 minutes. After purifying the extract on stanchions SPE Chromabond SiOH3 ml/500 mg, PAHs were marked using the GC/MS technique with the use of reference curve method (Clarus 680, Clarus600C – PerkinElmer). Object of studies

The study material was collected from the area which was indicated clean (Borki Forest). *Pleurozium schreberi* (Willd.) Mitten is a species from the family of Hylocomiaceae and it is very common in Poland. Bioindication makes use of gametophyte generations that form loose sods: green, green and yellow, and pale green with a red translucent stem. Leaf cells are elongated (Photo 2). Leaf rib is short, double (Photo 3) and yellow.

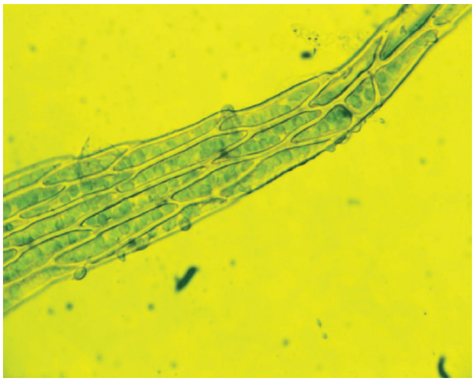

Photo 2. Elongated leaf cells (Photo by M.A. Jóźwiak)

Fot. 2. Wydłużone komórki listka (fot. M.A. Jóźwiak)

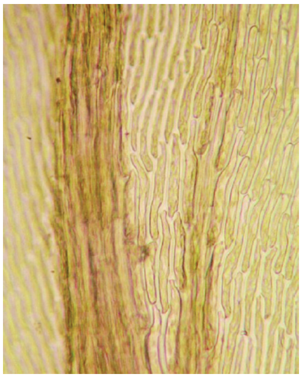

Photo 3. Double leaf rib (Photo by M.A. Jóźwiak)

Fot. 3. Żebro dwuskrzydłowe (fot. M.A. Jóźwiak)

Hypogymnia physodes (L.) is a lichen species belonging to Parmeliaceae family. The habitat of *Hypogymnia physodes* (L.) is mainly the bark of deciduous and coniferous trees. It is common throughout Poland. It forms thalli of leaf, rosette or irregular shapes with a diameter of 2–6 cm (Photo 4). This species is most popular in lichenoindication. It belongs to class 4 of lichen zone scale.

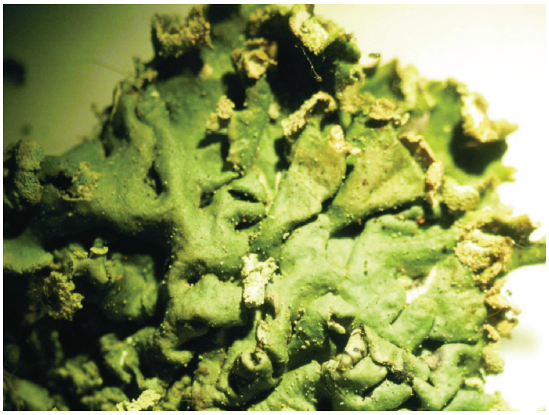

Photo 4. Rosette thallus of *Hypogymnia physodes* (Photo by M.A. Jóźwiak)
Fot. 4. Rosette thallus of Hypogymnia physodes (fot. M.A. Jóźwiak)

AREA OF STUDIES.

The bioindicators were placed in the designated points along the A4 motorway Cracow – Katowice (Poland) and along the S7 express road Kielce – Warsaw (Poland) (Fig. 2). Transplant samples were distributed along each route in five test points, which were defined using GPS. Geographical location of a point according to GPS readings A4 motorway Cracow – Katowice.

A4 motorway Cracow – Katowice
I 500 05' 57'' 190 38' 42''
II 500 O8'63'' 190 26' 62''
III 500 O5' 61'' 190 34'17''
IV 500 04' 76'' 190 45' 83''
V 500 06' 84'' 190 52' 66''
S7 express road Kielce – Warsaw
VI 500 57' 37'' 200 43' 03''
VII 510 31' 50'' 210 05' 59''
VIII 510 31' 50'' 200 56' 29''
IX 510 38' 46'' 200 58' 26''
X 510 34' 17'' 210 0' 02''

Fig. 2. Geographical location of a point according
Ryc. 2. Geograficzna lokalizacja punktów pomiarowych

TEST RESULTS

The concentrations of 16 most representative polycyclic aromatic hydrocarbons are presented in tables 1–6.

Polycyclic aromatic hydrocarbons (PAHs) are compounds that contain more than one condensed aromatic cycle. They usually emerge as a result of incomplete combustion of hydrocarbons. At present, we have the knowledge of over 200 PAHs; 17 of them are marked most often due to their toxicity and adverse influence on human health. Vehicle engines, in particular the high-pressure ones, are a significant source of air pollution due to polycyclic aromatic hydrocarbons. Exhaust gases from diesel engine consist in a mix of hundreds of chemical compounds, which emerge as a result of incomplete combustion of diesel oil and engine oil and the refining additives contained in such oils (Dmuchowski, Orliński, 2003). Harmful compounds are released into the atmosphere in high temperature, in the form of gases and particulate matter. In the gas phase there can be detected uncombusted aliphatic hydrocarbons, nitro-oxidation products, aromatic hydrocarbons, as well as nitrogen, sulphur and carbon oxides. The main ingredient of particulate matter (PM) is elementary carbon (carbon black), on which there are adsorbed both organic and inorganic compounds. PAHs detected in exhaust gases from high-pressure engines may occur in both gase and solid phase. PAHs of low molecular mass, within 157–178 g/mole, occur most frequently in the gas phase. Compounds of medium molecular mass, about 200 g/mole, may occur in both gas and solid phase. In normal conditions, PAHs sublimate easily; therefore, their transition from solid to gas state does not require the transition to liquid state. Compounds of high molecular mass, within 228–278 g/mole, occur mainly in solid state and are adsorbed on the carbon black particles emitted by diesel engines.

Table 1. PAH content in the tissues of the moss *Pleurozium schreberi*, reference sample
Tabela 1. Zawartość WWA w Pleurozium schreberi, próbka odniesienia

WWA	Concentration [μg×g^{-1}×s.m]
Naftalen	0,00
Acenaftylen	0,00
Acenaften	0,00
Fluoren	0,00
Fenantren	0,01
Antracen	0,00
Fluoranten	0,00
Piren	0,02
Benzo(a)antracen	0,00
Chryzen	0,00
Benzo(b)fluoranten	0,05
Benzo(k)fluoranten	0,20
Benzo(a)piren	0,03
Indeno(1,2,3-cd)piren	0,00
Dibenzo(a, h)antracen	0,00
Benzo(g, h, i)perylen	0,00

Table 2. PAH content in the tissues of the moss *Pleurozium schreberi* transplanted along the A4 motorway
Tabela 2. Zawartość WWA w tkankach mchu Pleurozium schreberi transplantowanego przy autostradzie A4

WWA	Concentration [μg×g^{-1}×s.m]
Naftalen	0,00
Acenaftylen	0,18
Acenaften	0,75
Fluoren	0,97
Fenantren	0,32
Antracen	0,26
Fluoranten	0,02
Piren	0,02
Benzo(a)antracen	0,24
Chryzen	0,05
Benzo(b)fluoranten	0,05
Benzo(k)fluoranten	0,57
Benzo(a)piren	0,53
Indeno(1,2,3-cd)piren	0,25
Dibenzo(a, h)antracen	0,00
Benzo(g, h, i)perylen	0,00

Table 3. PAH content in the tissues of the moss *Pleurozium schreberi* transplanted along the S7 express road
Tabela 3. Zawartość WWA w tkankach mchu Pleurozium schreberi transplantowanego przy drodze ekspresowej S7

WWA	Concentration [μg×g^{-1}×s.m]
Naftalen	0,00
Acenaftylen	0,00
Acenaften	0,00
Fluoren	0,00
Fenantren	0,43
Antracen	0,00
Fluoranten	0,01
Piren	0,66
Benzo(a)antracen	0,00
Chryzen	0,00
Benzo(b)fluoranten	0,18
Benzo(k)fluoranten	0,71
Benzo(a)piren	0,61
Indeno(1,2,3-cd)piren	0,29
Dibenzo(a, h)antracen	0,00
Benzo(g, h, i)perylen	0,00

Table 4. PAH content in the tissues of the *Hypogymnia physodes*, reference sample
Tabela 4. Zawartość WWA w Hypogymnia physodes, próbka odniesienia

WWA	Concentration [μg×g^{-1}×s.m]
Naftalen	0,00
Acenaftylen	0,00
Acenaften	0,00
Fluoren	0,00
Fenantren	0,00
Antracen	0,00
Fluoranten	0,00
Piren	0,04
Benzo(a)antracen	0,00
Chryzen	0,00
Benzo(b)fluoranten	0,00
Benzo(k)fluoranten	0,10
Benzo(a)piren	0,025
Indeno(1,2,3-cd)piren	0,00
Dibenzo(a, h)antracen	0,00
Benzo(g, h, i)perylen	0,00

Table 5. PAH content in the tissues of the *Hypogymnia physodes*, A4 motorway
Tab. 5. Zawartość WWA w tkankach mchu Hypogymnia physodes transplantowanego przy autostradzie A4

WWA	Concentration [μg×g^{-1}×s.m]
Naftalen	0,00
Acenaftylen	0,97
Acenaften	1,60
Fluoren	2,19
Fenantren	3,87
Antracen	0,00
Fluoranten	1,64
Piren	2,08
Benzo(a)antracen	0,00
Chryzen	0,00
Benzo(b)fluoranten	3,42
Benzo(k)fluoranten	2,65
Benzo(a)piren	2,34
Indeno(1,2,3-cd)piren	0,00
Dibenzo(a, h)antracen	0,00
Benzo(g, h, i)perylen	0,00
Naftalen	0,00

Table 6. PAH content in the tissues of the *Hypogymnia physodes*, S7 express road
Tabela 6. Zawartość WWA w tkankach mchu Hypogymnia physodes transplantowanego przy drodze ekspresowej S7

WWA	Concentration [μg×g^{-1}×s.m]
Naftalen	0,00
Acenaftylen	1,00
Acenaften	1,50
Fluoren	5,40
Fenantren	6,68
Antracen	1,58
Fluoranten	1,18
Piren	1,33
Benzo(a)antracen	0,00
Chryzen	0,00
Benzo(b)fluoranten	2,81
Benzo(k)fluoranten	2,67
Benzo(a)piren	1,18
Indeno(1,2,3-cd)piren	0,00
Dibenzo(a, h)antracen	0,68
Benzo(g, h, i)perylen	0,00
Naftalen	0,00

Harmful compounds are released into the atmosphere in high temperature, in the form of gases and particulate matter. In the gas phase there can be detected uncombusted aliphatic hydrocarbons, nitro-oxidation products, aromatic hydrocarbons, as well as nitrogen, sulphur and carbon oxides. The main ingredient of particulate matter (PM) is elementary carbon (carbon black), on which there are adsorbed both organic and inorganic compounds. PAHs detected in exhaust gases from high-pressure engines may occur in both gase and solid phase. PAHs of low molecular mass, within 157–178 g/mole, occur most frequently in the gas phase. Compounds of medium molecular mass, about 200 g/mole, may occur in both gas and solid phase. In normal conditions, PAHs sublimate easily; therefore, their transition from solid to gas state does not require the transition to liquid state. Compounds of high molecular mass, within 228–278 g/mole, occur mainly in solid state and are adsorbed on the carbon black particles emitted by diesel engines.

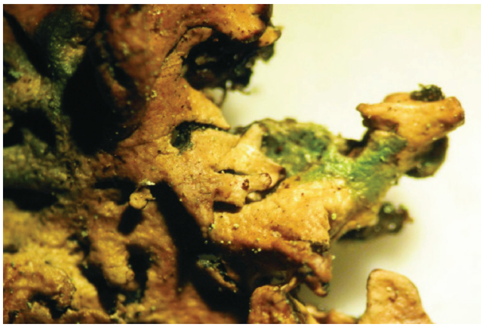

Photo 5. Changes in thallus colour of the transplanted lichens (Photo by M.A. Jóźwiak)

Fot. 5. Kolorystyczne zmiany plechy transplantowanych porostów (fot. M.A. Jóźwiak)

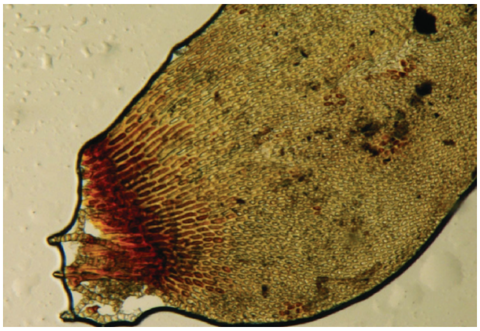

Photo 6. Changes in the tissues of the leaf blade stems of the transplanted mosses (Photo by M. A. Jóźwiak)

Fot. 6. Zmiany w blaszce liściowej transplantowanych mchów (fot. M.A. Jóźwiak)

Morphological changes in lichen thalli and bryophyte leaves that result from environmental stimuli manifested themselves by cell and tissue dysfunctions. As a consequence of these processes, there occur visible macroscopic and microscopic changes. Test results show that the thallus of lichens, as a result of the exposition to traffic pollution, changes its colour due to gradual necrosis of gonidium cells. (Photo 5) These processes lead to breaking rosette fragments due to local thallus necroses. Microscopic analysis of necrotic thalli showed the deformation of photobiont and phycobiont cells. It can be assumed that the changes were caused by stress related to PAH accumulation in lichen cells. In the image of the analysed microscopic changes in bryophyte leaves, extensive colour changes were noticed throughout the middle part of the leaf blade, including the rib and the base of the leaf blade (Photo 6). The scope of discolouration varied but was within the limit from 250 to 400 μm of leaf width, to 600 μm of leaf length. The microscopic analyses did not show any discolouration of leaf rims, which is a result of the guttered-shaped leaf blade – a distinctive feature of this species of moss. Due to the leaf blade gutter, the ionic forms of metals dissolved in precipitation depose at the bottom of leaves, which facilitates their bioaccumulation. The facilitated absorption (ectohydricity) of toxins in these leaf parts also stems from their anatomical structure and the lack of protective epidermis on their top surface.

The result analysis related to PAH content in *Hypogymnia physodes* (Table 1–6) shows that in the vicinity of main roads in Poland, there is a high concentration of aromatic gas compounds such as Acenaphthylene, Acenaphthene, Fluorene and Phenanthrene. Another issue of high concern is the large amount of strongly carcinogenic PAHs in the air. These include Benzo(b)fluoranthene, Benzo(k)fluoranthene and

Benzo(a)pyrene. These compounds adsorb on black coal particles. Therefore, their high concentration is undoubtedly the result of the greater road transport, which is based chiefly on diesel engines. It should also be noted that the majority of cars with high--pressure engines in Poland, due to their age, is not equipped with the modern exhaust gas purifying systems (diesel particulate filter).

The analysis of the results presented in table 1–6 points out the differences in the concentrations of PAHs desorbed in the thalli of the lichens exposed in the vicinity of the A4 and S7 roads. It may stem from the varied traffic load on the tested communication routes. However, it should be noted that PAH contents in bioindicator organism are higher along the S7 road (Tab. 6).

The lower content of PAHs in *Pleurozium schreberi* tissues may result from the manner in which bioindicator is exposed; it is transplanted on the ground level and surrounded by herbacaeous plants, which may constitute a protection against deposition of pollution.

CONCLUSION

Monitoring tests employing bioindication method are used in order to assess the level of pollution on the basis of the reaction of bioindicators. What is especially important is the effect of abiotic factors, including in particular the anthropological toxins, on natural biocenoses (forest environments) and the areas subject to agricultural activity, i.e. agrocenoses. The developing road infrastructure and advancement of motorisation makes it necessary to monitor the condition of the environment along motorways and express roads due to the fact that they are adjacent to the natural forest areas and farmlands.

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