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STATE OF SCOTS PINE NEEDLES' EPICUTICULAR WAXES AND CONTENT OF MICROELEMENTS IN BIOINDICATION

STAN WOSKÓW EPIKUTYKULARNYCH IGIEŁ SOSNY ZWYCZAJNEJ ORAZ ZAWARTOŚĆ MIKROELEMENTÓW W BIOINDYKACJI

Abstract: Studies on the chemical composition and variability of morphological structure of Scots pine (*Pinus sylvestris* L.) allow to assess the health state of trees growing in conditions of exposure to alkaline dust emission. The aim of the studies was the morphological analysis of the epicuticular waxes and the content of microelements in the needles of Scots pine growing in conditions of alkaline pressure, compared to the needles from emission-free areas. The studies were conducted on two-year-old needles collected in the vicinity of cement mills in Sitkowka and Ozarow, located in the Swietokrzyskie Province. The chemical composition analysis of needles indicated higher contents of Cu, Pb and Sr as well as lower content of Mn in the needles from alkalised areas. They were also characterised by higher contents of pH_{KCl} than in the needles from emission-free areas. The images of needles, taken under a Scanning Electron Microscope (SEM), showed definite signs of epicuticular wax erosion in comparison to the needles collected from the control area. According to Turunen's classification, third and fourth degrees of epicuticular wax coverage atrophy were found in two-year-old needles. The analysis of the chemical composition of the needles and the morphology of their surface structure indicated a negative impact of cement dust pollution on Scots pine's assimilation apparatus.

Keywords: alkaline pressure, pine needles, microelements, epicuticular wax structure

Introduction

Scots pine is a species that is highly valued and widely used in bioindicative studies [1, 2]. Its main characteristic as a good bioindicator is, apart from common occurrence,

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a low tolerance to toxic factors [3]. The studies of the variability of anatomical structure of needles and their chemical composition allow to precisely assess the health state of tress growing in conditions of various anthropogenic pressures [4–10]. What is more, the bioindication studies on Scots pine ensure the comparability of results, what distinguishes them from subjective scientific methods including, among others, defoliation [1, 11].

The role of plant epicuticular waxes is not yet known exactly, but a wealth of chemical composition indicates that they have a variety of ecological functions [12, 13]. Chemically, epicuticular waxes are strongly hydrophobic polymers being important mechanical and physiological barriers between the plant and the environment. Epicuticular waxes of coniferous trees are considered to be a good biomarker of air pollution, indirectly indicating the health state of a particular tree [4, 8, 9, 13, 14]. Exposing needles to direct contact with pollution reduces general resistance of trees, affects photosynthesis, respiration and transpiration, as well as causes premature defoliation [15–19]. Describing many changes in structure and chemical composition of epicuticular waxes due to anthropogenic and natural factors is possible thanks to using a Scanning Electron Microscope (SEM). SEM allows to identify damage in epicuticular waxes at an early stage, even before the occurrence of visible symptoms. The most commonly reported changes in the structure of epicuticular waxes include: their underdeveloped crystal structure, coalescence of wax particles, wax erosion, transformation of crystal structured waxes into an amorphous form, as well as changes in chemical composition [8, 9, 13, 20].

The aim of the studies was the morphological analysis of the epicuticular waxes and the content of selected microelements in the needles of Scots pine growing in conditions of constant alkaline pressure, compared to the needles of Scots pine collected from the areas free of emissions.

Object and study methods

The studies were conducted in the years 2008 and 2012 in the forest areas situated in the vicinity of cement mills in Sitkowka and Ozarow, the communes located administratively in the Swietokrzyskie Province (Poland). All study sites were localised at a similar distance of about 0.5 km from the dust emitter. The study sites were in the coniferous forests from the *Dicrano-Pinion* community (degenerative forms) in the fresh coniferous forest habitat with a pine forest stand at the age between 50 and 70 years. For comparative studies, there was selected a site with the *Leucobryo-Pinetum* community located in Wymyslow within the boundaries of the Cisowsko-Orlowinski Landscape Park (Swietokrzyskie Province) which is beyond the reach of industrial emissions.

The study material consisted of two-year-old pine needles collected from each surface of eight randomly selected trees. The samples were dried in a desiccator at 40 ^{o}C for 24 hours, and then crushed in a Fritsch planetary mill and incinerated in an electric oven at 450 ^{o}C. After digesting the samples with HCl-HNO_3, the total contents of selected elements were determined by using ICP-AES method. The state of needles'

surface structure was studied as well. The central parts of five randomly selected needles were sputtered with gold and photographed under a scanning electron microscope JSM-540 with magnification of 2000, 1000 and 500 times [21]. The study results were compared to Turumen's scale of epicuticular wax erosion, which involves six classes:

– 0 (underdeveloped wax structure),
– I (100 % of stomatal wax cover),
– II (71–100 % of stomatal wax cover),
– III (31–70 % of stomatal wax cover),
– IV (0–30 % stomatal wax cover),
– I (0 % of stomatal wax cover).

Results and discussion

The average contents of studied microelements in the needles collected in the study areas indicated considerable variation depending on their locations (Table 1). The pine needles from the alkalised areas were characterised by definitely higher pH_{KCl} values (neutral and slightly acidic pH) in comparison with the needles from the control site (acidic pH). The pH values for the material from the areas surrounding cement mills were also higher than those reported for the areas free of emissions [22, 23]. This indicates a change in the chemical character of the tree stand growing in the areas influenced by the cement mills.

Table 1

Content of microelements and pH_{KCl} values in two-year-old needles of *Pinus sylvestris* (n = 24)

Area		Parameter						
		pH_{KCl}	Ba	Cu	Mn	Pb	Sr	Zn
			[mg/kg d.m.]					
Sitkowka	average		6.3	8.8	71.5	29.5	18.3	61.5
	range	6.1–6.4	6.0–7.1	7.2–10.1	55.0–92.5	20.1–42.5	15.2–22.3	59.5–65.7
	coefficient of variation CV [%]		15.2	41.0	39.0	19.5	11.7	22.1
Ozarow	average		3.5	8.0	125.8	17.8	8.8	46.3
	range	5.5–5.9	3.2–5.9	7.3–8.5	120.5–151.0	8.0–23.5	4.2–8.3	40.6–48.1
	coefficient of variation CV [%]		17.0	35.2	29.5	17.2	20.0	16.5
Control area	average		10.0	5.8	890.8	8.0	3.5	63.1
	range	3.9–4.1	9.0–12.5	4.3–7.0	850.5–990.5	5.1–9.5	3.0–4.6	52.1–65.5
	coefficient of variation CV [%]		12.2	18.0	8.2	17.1	20.2	7.6

The material from the alkalised areas was characterised by higher values of Cu, Pb and Sr as well as lower levels of Mn, Ba and Zn.

The content of Cu was at a similar level in the needles from the areas with confirmed alkaline immission, while the greater dispersion of values was observed for the areas located in Sitkowka. Significantly lower contents of this element were noted for the control area, where its average content was lower by 31 % than that in the needles from the alkalised areas. Copper is a microelement that is essential for proper plant functioning; however, its excess leads to chlorosis. Moreover, if this element is contained in dusts, it has the ability to deposit itself on the surface of leaf blades by binding to the cuticle, and therefore it negatively affects its protective function [24]. The content of heavy metals in pine needles were also being determined by Pomierny [25] who conducted bioindicative studies in the vicinity of Katowice Steelworks. She [25] found that the content of Cu was in the range between 5.4 and 6.6 mg/kg. Nevertheless, these values were lower than those concerning the needles from the alkalised areas in the Swietokrzyskie Province.

The content of most heavy metals in bioindicator's needles is significantly correlated with the concentration of heavy metals in the air [26–29]. That is why, the high content of lead in the needles from the alkalised areas, that was on average three times greater than in the needles from the control area, indicated considerable soil and air pollution by this element [1, 30]. The content of Pb at the level of 43 mg/kg, reported for the material from the vicinity of cement mill in Sitkowka, causes wilting of plants [24]. Hence, the content of this element in the needles collected from the areas with confirmed alkaline immission seems to be the most alarming.

The average content of zinc in aerial parts of plants from the emission-free areas was included in the range between 10 and 70 mg/kg d.m. [24]. Zn, just like Cu, is an essential element in the functioning of organisms and plays an important role in the biosynthesis of enzymes, auxin, and a part of proteins. Zinc deficiency has a destructive impact on a tree stand [1], but its excess (above 100 mg/kg) causes chlorosis [24]. Scots pine is classified as a good Zn accumulator [31]. The needles collected from the control area were characterised by the content of Zn at the level between 52.1 and 65.5, with the average content at 63.1 mg/kg. Similar content of Zn in pine needles (62.5 mg/kg) was observed by Parzych who conducted studies in the area of the Slowinski National Park [32]. The lower content of Zn, even by 26.6 %, was reported for the alkalised areas. Similar value distribution of this element was indicated by Bajorek-Zydron's studies, in which the content of Zn in the needles collected from coal dumps of the mine in Belchatow was at the level of 69,0 mg/kg, but beyond the reach of pollution, the obtained values were relatively lower, *ie* 61.4 mg/kg d.m. and 41.4 mg/kg d.m. [33].

The average content of Mn for the controlled area was at the level of 890.8 mg/kg. Ceburnis and Steines [34], who conducted biomonitoring studies in Lithuania, indicated similar values of this element in pine needles from emission-free areas. The needles collected in the vicinity of cement mills in Sitkowka and Ozarow were characterised by much lower content of Mn, which was included in the range between 55.0 and 151.0 mg/kg. Similar values for Mn in two-year-old needles, exposed to influence of pollution, were noted in Pomierny's measurements [25] in which the needles contained from 73.5 to 132.0 mg/kg of this element. The content of microelements in pine needles was also studied by Miglaszewski, whose studies were conducted in the area of the

Swietokrzyski National Park [35]. According to these results, the content of Mn in two-year-old needles was at the level of 968 mg/kg, and this value was similar to that obtained in the control area located in Wymyslow. The values of other analysed elements, *ie* Pb 3 mg/kg, Cu 4 mg/kg, Ba 4 mg/kg, Sr 5 mg/kg, and Zn 58 mg/kg were at the levels comparable to those observed by Miglaszewski in the control area.

The contents of other analysed elements, *ie* Sr and Ba, were also characterised by considerable variability. However, the content of Ba was lower for the alkalised areas, while the content of Sr was characterised by completely opposite distribution trend. Strontium belongs to the group of elements whose contents in the air are reflected in their contents in plant assimilation apparatuses [34]. Low content of this element in the needles collected from the control area, which was observed at the level between 3.0 and 4.6 mg/kg, coincided with literature data [24]. In the case of Ba, alkaline immission reduces the assimilation of this element by pine needles. The values obtained for the control area, just like for Sr, coincided with literature data [24, 35].

Among the areas remaining under alkaline pressure, the area located in Sitkowka was characterised by higher contents of analysed microelements in the needles (except for Mn). This may be caused by greater pollution emissions from the cement mill than the close proximity to the City of Kielce. High contents of heavy metals in pine needles reduce their durability, which was observed by Lamppu i Hutterm [36]. Mandre et al [37, 38] proved that the needles collected from highly polluted areas are from 26 % to 50 % shorter than the needles collected from control areas.

The high variation of chemical composition of the needles was evidenced by calculated coefficients of variation (CV). The parameter was included in the range of CV = 11.7–41.0 % for the alkalised areas. In the case of the control area, the parameter was relatively lower and was included in the range of CV = 7.6–20.2 %. The statistical analysis showed that the value of pH_{KCl} is significantly correlated by $p \leq 0.05$ with the content of Cu (r = 0.673), Sr (r = 0.793), and Pb (r = 0.602). Significant correlations were also found for the following pairs: Sr – Cu (r = 0.703) and Zn – Ba (r = 0.696).There were no strong and very strong statistical relationships for other analysed heavy metals. Also, Cord and Cord [39], who conducted bioindicative studies, demonstrated significant correlations among selected heavy metals in the areas remaining under alkaline pressure.

It was found, through observing the state of needles' epicuticular waxes, that cement dusts strongly affect their structures, causing coalescence of well-developed wax particles until complete disappearance of structural forms. The wax layer became poorer and gradually eroded. In the case of coniferous trees, the process of stomata filling with a network of wax fibres, under natural conditions, begins in the second year of a needle's life, and then it undergoes gradual erosion that lasts from 4 to 6 years [4]. The SEM images, which were taken under 500 and 1000 times (Fig. 1–4), and present the needles collected from the alkalised areas in comparison to the needles from the control areas, indicated definite erosion of crystal epicuticular waxes. Observation of needles' wax structure degradation within the study sites located in Sitkowka and Ozarow allows to assess the degree of surface erosion. The needles' structure, changed in such a way, may be classified into third and fourth degrees of erosion by Turunen [5],

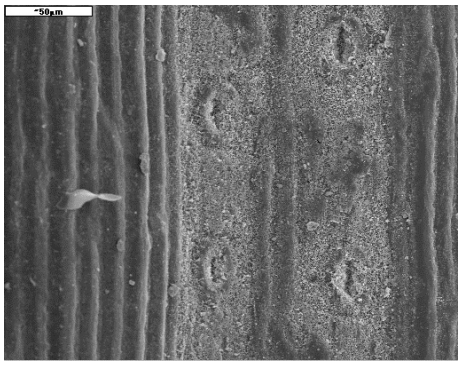

Fig. 1. Natural microstructure of the pine needle's epidermis surface; crystal wax, stomata, control area (SEM image, magnification of 500 times)

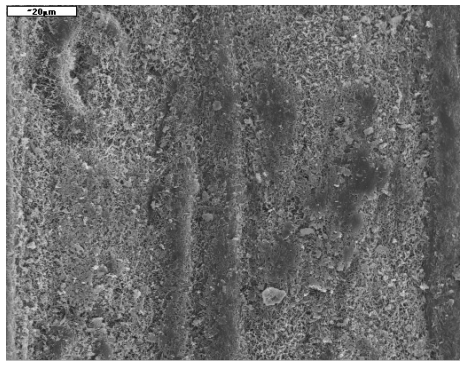

Fig. 2. Natural microstructure of the pine needle's epidermis surface; crystal wax, stomata, control area (SEM image, magnification of 1000 times)

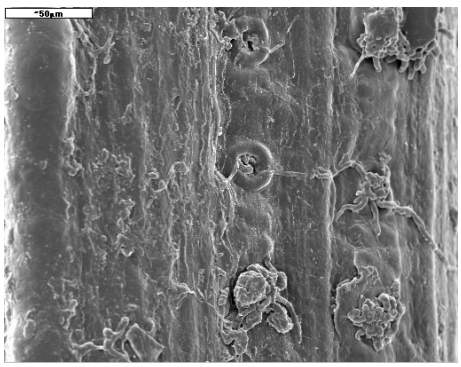

Fig. 3. Changed microstructure of the pine needle's epidermis surface; crystal wax, stomata, study site in Ozarów (SEM image, magnification of 500 times)

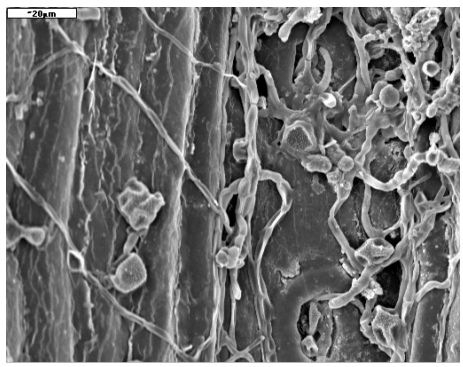

Fig. 4. Changed microstructure of the pine needle's epidermis surface; crystal wax, stomata, study site in Ozarów (SEM image, magnification of 1000 times)

which suggests almost complete decay of wax structures in interstomatal space. The wax layer was discontinued, strongly eroded, locally collapsed, and originally crystal wax became amorphic. Similar observations were the subjects of Staszewski, Bacic et al and Swiercz's studies [9, 17, 40, 42]. Equally interesting were also studies on the epicuticular wax structure conducted by Burkhardt [43] who noticed an amorphous form of epicuticular wax occurring after treatment of its intact form with saline solution. According to the author, the process of wax degradation may be the result and – at the same time – the indicator of a high concentration of aerosols in the air.

Deposition of dust aerosols weakens the health condition of needles, whereas the scale of damage depends on the size of emission and time of exposure [44]. Cement dusts contain particles with a diameter from 20 to 30 µm [40], hence, there is additional risk of bunging the stomata which have a diameter between 8 and 10 µm. Degradation

of needles' wax surface structure affects their average longevity as well. It was observed that Scots pine growing in the alkalised areas sheds elder needles earlier. According to Mäkelä and Huttunena's studies [15], this is a necessity that allows trees to maintain their water balance as elder needles are characterised by higher cuticular than stomatal transpiration. What is more, due to defoliation, the crowns of some pine trees growing within the study sites in the vicinity of cement mills were markedly thinned. The type of thinning the tree crowns may be classified as arch-shaped or bottom-up [45]. Observations made under SEM confirmed that the needles of Scots pine collected from the control area in Wymyslow have a well-preserved network of wax structures (Fig. 1 and 2). Their crowns were dense and had a complete set of needles.

Conclusions

Cement dusts have a strong impact on the chemical state of Scots pine's assimilation apparatuses and on their general state. It was claimed that there are differences in accumulation of elements between the needles collected from the alkalised areas and those from the control areas. Significantly higher contents were noted for Cu, Sr and Pb, whereas the content of Mn was considerably lower. The analysis of the needles' morphological surface properties and various degrees of epicuticular wax preservation, made under SEM, may be useful for indicating degrees of assimilation apparatus deformation through deposition of dust pollutions causing wax layer erosion. It was determined, on the basis of needles' surface morphology observations, that according to Turunen's classification [1992] there are third and fourth degrees of epicuticular wax coverage atrophy. Needles, which lack of natural protection, are easily attacked by filamentous fungi, and severe degradation of wax surface structure involves more frequent defoliation of elder needles.

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STAN WOSKÓW EPIKUTYKULARNYCH IGIEŁ SOSNY ZWYCZAJNEJ ORAZ ZAWARTOŚĆ MIKROELEMENTÓW W BIOINDYKACJI

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Abstrakt: Badania składu chemicznego oraz zmienności struktury morfologicznej igieł sosny zwyczajnej (*Pinus sylestris* L.) pozwalają ocenić stan zdrowotny drzew wzrastających w warunkach narażenia na emisję pyłów alkalicznych. Celem badań była analiza morfologiczna wosków epikutykularnych oraz zawartości mikroelementów w igłach sosny zwyczajnej wzrastającej w warunkach presji alkalicznej, w porównaniu do igieł z terenów wolnych od emisji. Badania prowadzono na igłach dwuletnich, pobranych z okolic cementowni w Sitkówce oraz Ożarowie w województwie świętokrzyskim. Analiza składu chemicznego igieł wykazała wyższą zawartość Cu, Pb i Sr oraz niższą zawartość Mn w igłach z powierzchni alkalizowanych. Cechowały je także wyższe wartości pH_{KCl} niż w igłach z powierzchni wolnych od zanieczyszczenia. Zdjęcia igieł, wykonane pod SEM (elektronowym mikroskopem skaningowym), wskazały na wyraźną erozję wosków epikutykularnych w porównaniu do igieł pobranych z powierzchni kontrolnej. Według klasyfikacji Turunena stwierdzono III i IV stopień zaniku pokrycia wosków epikutykularnych na igłach 2-letnich. Analiza składu chemicznego igieł oraz morfologii ich powierzchni, wskazują na niekorzystny wpływ zanieczyszczenia pyłami cementowniczymi na aparat asymilacyjny sosny.

Słowa kluczowe: presja alkaliczna, igły sosny, mikroelementy, struktura wosków epikutykularnych