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MOSSES AS BIOMONITOR OF AIR POLLUTION WITH ANALYTES ORIGINATING FROM TOBACCO SMOKE

MCHY JAKO BIOMONITOR ZANIECZYSZCZENIA POWIETRZA ANALITAMI POCHODZĄCYMI Z DYMU PAPIEROSOWEGO

Abstract: The aim of the carried out research was the assessment of the possibility to use a popular bioindicator - *Pleurozium schreberi* mosses as a biosensor of the air pollution in living quarters with the analytes originating from tobacco smoke. The moss bag method of active biomonitoring, popular in environmental studies, was applied; the method is based on exposing mosses collected in clean areas in the locations polluted with, for example, heavy metals. However, this experiment involved exposing mosses in living quarters, in which approximately 10 cigarettes were smoked daily (first room - kitchen). For the purpose of comparison, moss samples were also placed in another room (bedroom), which was potentially not polluted. After three months of exposure, the following heavy metals were determined in mosses: Mn, Fe, Ni, Cu, Zn, Cd, Pb and Hg, using the atomic absorption spectrometry method. Additionally, these analytes were also determined in hair samples from the persons smoking in the room and from other smokers; the determined metal concentrations were compared with the results of the studies carried out using hair samples collected from non-smokers. On the basis of carried out research it was confirmed that, among others, the mosses exposed in living quarters accumulate heavy metals, such as Ni, Zn, Pb and Hg, which originate from tobacco smoke. Higher heavy metal concentrations were determined in hair samples from smokers, compared to hair samples from non-smokers.

Keywords: *Pleurozium schreberi* mosses, hairs, heavy metals, cigarette smoke, biomonitoring

Introduction

People have been using stimulants for thousands of years. Tobacco is one of the oldest and most popular stimulants. Tobacco can be snorted, chewed but mainly inhaled, in the form of smoke from burning. Inhalation of the smoke - smoking - considerably increases the influence of the psychoactive substance on an organism. The application of the stimulant via lungs supports its accelerated transfer to the blood circulation system. The lungs contain three hundred million alveoli, with the joint surface of seventy square meters. This area is accessible for the inhaled smoke as well as the chemical compounds

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present in the smoke [1]. Nicotine is the main substance responsible for the psychoactive influence of the smoke. As it is inhaled directly to the lungs and next to bloodstream, it works much faster than any other substance taken in another way, e.g. orally. Smoking causes temporary changes in a smoker's brain. It stimulates and relaxes. This effect is caused by the fact that the substances provided to the organism during smoking are recognised by particular types of cell - the brain nerve cells. Receptors of these cells can bind these substances [2], which changes the way of functioning of nerve cells. The cells become more or less excitable, which changes functioning of the whole brain. This process is perceived as a pleasant experience by a smoker [3].

Human nervous system is based on information transfer through chemical and electric impulses. Neurotransmitters are the chemical substances, which help transfer information and they are indispensable for the proper functioning of the nervous system. Nicotine is a homologue of acetylcholine. Acetylcholine manages the attention mechanisms function. Nicotine directly stimulates the increased dopamine release in the brain. The dopamine release leads to the so-called withdrawal symptoms, which increases the need to remain under the influence of the substance [3].

Tobacco smoke contains, among others, sixty known carcinogenic substances [4]. These include: radioisotopes of radium generated by decay of radon, nitrosamines, benzopyrenes, tar, carbon oxides, hydrogen cyanide, formaldehyde and heavy metals. Their content in tobacco depends on, among others:

- type of tobacco,
- plant development stage,
- physical and chemical characteristics of soil,
- pH of soil - acidic soil enhances better absorption of heavy metals to plants; the pH increase reduces metals absorption by even 50 %,
- chemical form of the present heavy metals,
- used artificial fertilisers, which constitute an additional source of heavy metals available to plants from soil,
- technological processing of tobacco.

Tobacco leaves are most susceptible to heavy metals accumulation. The data from literature confirms accumulation in leaves of such heavy metals as cadmium, lead and zinc [5].

Scientists from University of Washington in Seattle and University of Melbourne analysed the data on smoking from the period 1980-2012. The data was collected in 187 countries. According to the report, approximately 721 million people (over the age of 15 years) smoked in 1980, whereas there were 967 million smokers in 2012. The total number of cigarettes smoked was 4,96 and 6,25 trillion, respectively. We have seen the decrease of the number of smokers in recent years. Approximately 31 % of men and 6.2 % of women smoke. That proportion 30 years ago was, respectively: 41 % and 10.6 % [5].

Mosses as biomonitors of environment pollution with selected analytes

Two methods are used in biomonitoring of atmospheric aerosol pollution with heavy metals: passive biomonitoring, which analyses samples of biota collected from their natural environment and active biomonitoring, which is based on the exposure of biological material, usually in heavy polluted areas, and an analysis of changes, which take place during the exposure. In comparison to the classical monitoring of environment pollution,

the main advantage of biomonitoring studies with the use of mosses is the cheap method of samples collection, which does not require special training and, to a certain extent, allows for easy planning of the research [6-11]. The moss bag method has been widely used in Europe for monitoring of environment pollution with heavy metals, polycyclic aromatic hydrocarbons (PAH) or radionuclides [6]. The technique has been commonly used in assessment of air pollution with trace elements in urban and industrial areas [12]. For example, a study with the use of the moss bag method was carried out in urban areas: in Belgrade, Serbia [13], in Turku, south-western Finland [14], in Chisinau, Republic of Moldova [15] or in Germany (mosses were exposed near the roof of the fermentation chamber in a biogas plant) [16]. All the above mentioned studies confirm good sorption characteristics of mosses and the possibility of using them in biomonitoring of wood and urban areas [17-19]. On the basis of the analysis of own studies' results, it was confirmed that mosses can also be used in assessment of air pollution with heavy metals, originating from, for example, tobacco smoke, in closed spaces.

An average smoker smokes approximately 15 cigarettes per day [20]. A smoker can smoke 246 375 cigarettes in 45 years. Such a large number has a considerable influence on human organism. The analysis of data from literature and the results of own research indicate that smoking influences heavy metals content in human hair. Concentrations of analytes in hair are 50 times higher than in blood or urine. Therefore, they are easier to detect. Hair analysis is a method, which allows to assess the harmful, long-term influence of high concentrations of toxic metals on organisms as well as the degree and speed of their removal [21].

Non-smokers become passive smokers in living quarters, due to the existing communication and ventilation routes.

The aim of the carried out research was the assessment of the possibility to use a popular bioindicator - *Pleurozium schreberi* mosses (the red-stemmed feathermoss) as a biosensor of the air pollution in living quarters, with the analytes originating from tobacco smoke. The research also included a comparison of heavy metals concentrations in hair samples of smokers, with the results of analyses of hair samples collected from non-smokers.

The research methodology

Pleurozium schreberi mosses were collected in the woods located approximately 3 km from the village of Stary Janow, Swietokrzyskie Province, 40 km north of Kielce, brought to the laboratory and cleaned from mechanical impurities. The representative (average) samples of mosses with the mass of 5.00 ± 0.01 g, which included gametophyte with the green part only, were placed in 10 plastic bags and exposed in living quarters (5 bags in the kitchen and 5 in the bedroom) for the period of 3 months. Following the exposure, the moss samples were taken to the laboratory and dried at the temperature of 303 K.

Hair samples were collected and prepared in line with the applicable standards [22, 23]. The material was collected from the occipital region of the skulls of the persons participating in the experiment. Information about the health condition and lifestyle of the persons participating in the study were obtained through a survey filled before sample taking. The maximum hair length was below 4 cm. The collected hair was rinsed in demineralised water, then dried at the temperature below 333 K. Samples were stored in polyethylene bags.

The averaged moss samples with the mass of 0.400 ± 0.001 g d.m. (d.m. - dry mass) and hair with the mass of 0.200 ± 0.001 g d.m. were mineralised in a mixture of nitric acid(V) and hydrochloric acid (HNO_3 65 % : HCl 37 % = 1:3). The process was carried out in a microwave digester Speedwave Four made by Berghof (DE), at temperature of 180 °C. The solutions used in the process were made by MERCK. Heavy metals (Mn, Fe, Ni, Cu, Zn, Cd and Pb) in the mineralised samples were determined by atomic absorption spectrometry method (AAS), using the equipment iCE 3500 made by Thermo Electron Corporation (USA).

Mercury was determined in plant and hair samples (mass of each sample 0.040 ± 0.001 g d.m.) using the mercury analyser AMA 254 made by Altec Ltd.

Quality and quality assurance

Table 1 presents the limits of detection and the limits of quantification of heavy metals for the spectrometer iCE 3500 [24]. The equipment was calibrated with the use of calibration standards from the company ANALYTIKA Ltd. (CZ).

The values of highest concentrations of the models used for calibration (2.0 mg/dm^3 for Cd, 5.0 mg/dm^3 for Cu, Zn, Ni and Pb, 7.5 mg/dm^3 for Mn and 10 mg/dm^3 for Fe) were assumed as the limit of the linear relation of the signal and concentration.

Table 1

Instrument detection limits (*IDL*) and instrument quantitation limits (*IQL*) characteristic for the spectrometer iCE 3500 [mg/dm^3] [24]

Metal	***IDL***	***IQL***
Mn	0.0016	0.020
Fe	0.0043	0.050
Ni	0.0043	0.050
Cu	0.0045	0.033
Zn	0.0033	0.010
Cd	0.0028	0.013
Pb	0.0130	0.070

Table 2

Comparison of measured and certified concentrations in BCR-482 *lichen*

Metal	**BCR-482 *lichen***		**AAS**		***Dev.*** **
	Concentration	**±Uncertainty**	**Average**	**±*SD*** *	
	[mg/kg d.m.]				**[%]**
Mn	33.0	0.5	31.70	0.68	–3.9
Ni	2.47	0.07	2.16	0.32	–13
Cu	7.03	0.19	6.63	0.17	–5.7
Zn	100.6	2.2	95.1	2.3	–5.5
Cd	0.56	0.02	0.53	0.03	–5.3
Pb	40.9	1.4	38.2	1.0	–6.6
Hg	0.00048	0.00002	0.000450	0.000016	–9.8

* - standard deviation; ** - relative difference between the measured and certified concentration $100\% \cdot (c_z - c_c)/c_c$

Instrument detection limits (*IDL*) and quantitation limits (*IQL*) for mercury are, respectively 0.003 ng (0.03 μg Hg/dm^3) and 0.01 ng (0.1 μg Hg/dm^3) in the analysed sample. The equipment was calibrated with the use of calibration standards from the company ANALYTIKA Ltd. (CZ).

Table 2 shows heavy metals concentrations, determined in the certified reference materials as BCR-482 *lichen*, prepared by the *Institute for Reference Materials and Measurements, Belgium*.

Research results and discussion

The graph in Figure 1 presents concentrations of heavy metals, determined in popular cigarette brands (in tobacco and in filter), which were used during the study.

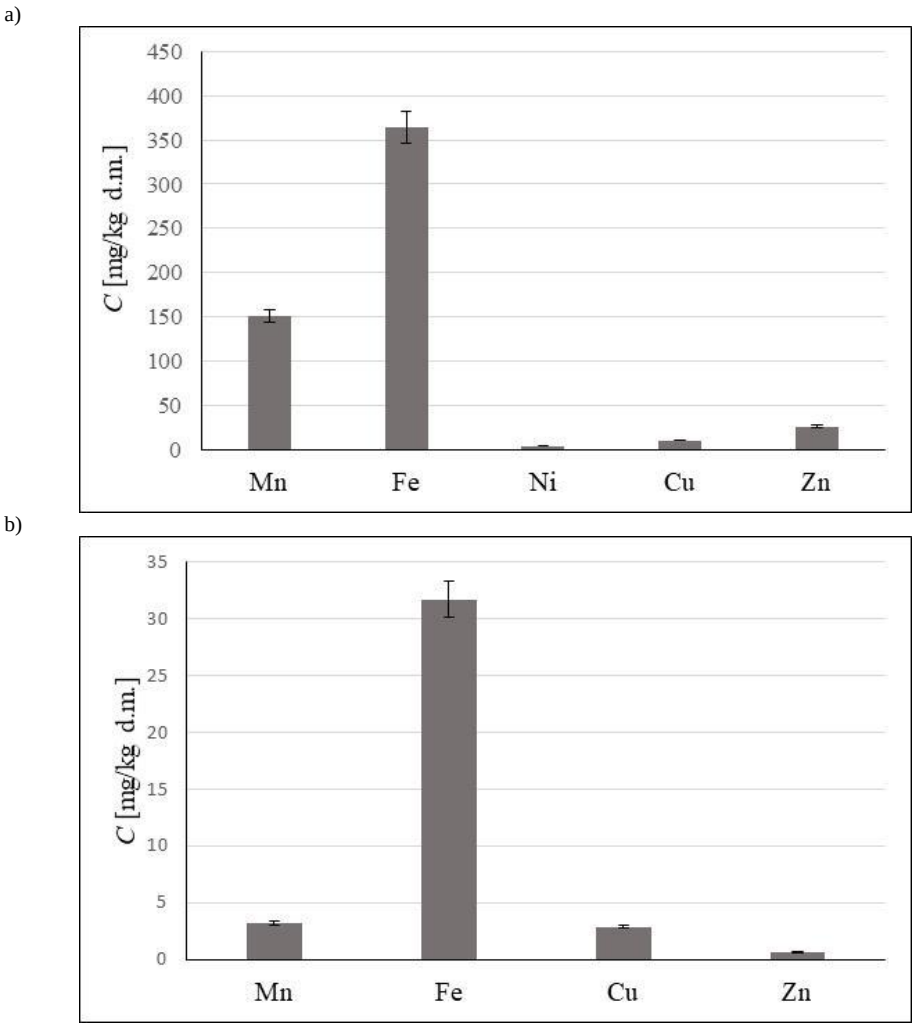

Fig. 1. Concentrations of heavy metals, determined in cigarette samples: a) in tobacco, b) in filter

As confirmed by own research, cigarettes contain heavy metals, among others Mn, Fe, Ni, Cu and Zn. Tobacco is much more contaminated with these analytes than filter. Also

mercury was determined in tobacco (c_{Hg} = 0.0031 mg/kg d.m.). Concentrations of Cd and Pb were below the detection limit of the applied analytical method (c_{Cd} < 0.81 mg/kg d.m., c_{Pb} < 4.38 mg/kg d.m.).

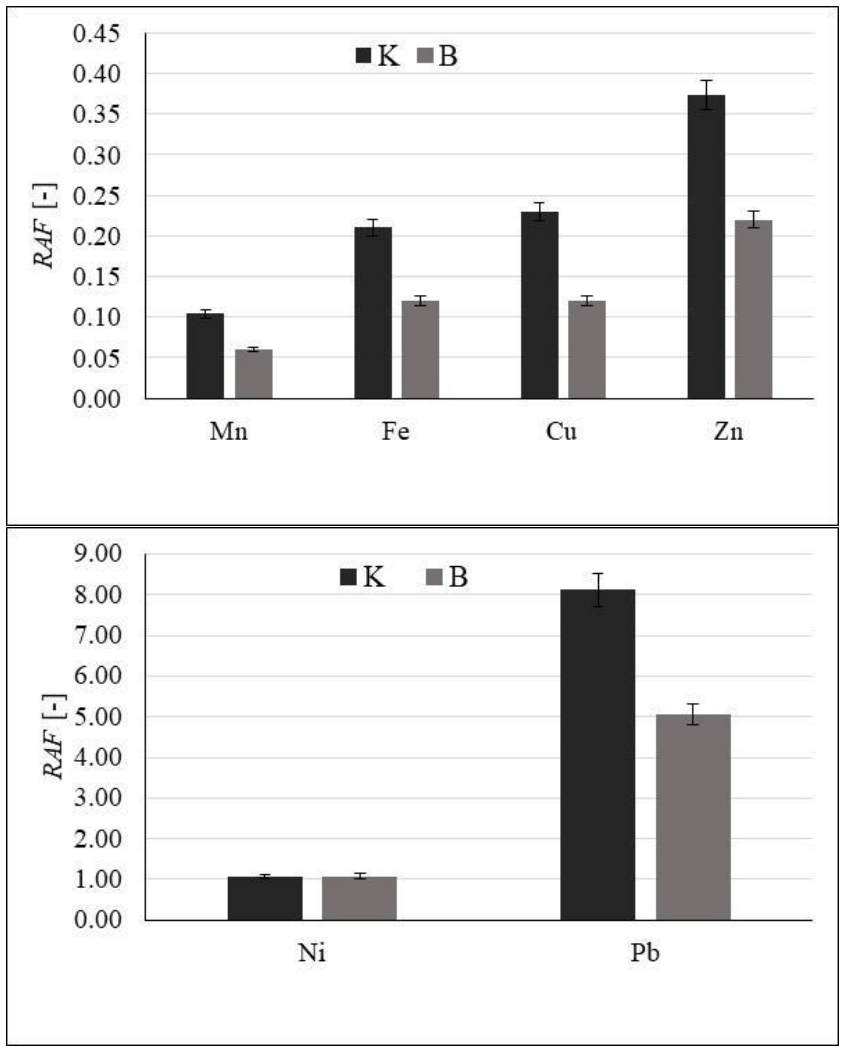

Fig. 2. Heavy metal concentrations increases, determined in the moss samples after three months of exposure in living quarters (K - kitchen, B - bedroom)

The *RAF* - *Relative Accumulation Factor* was used to determine increases of concentrations of the analytes in the exposed moss samples, as defined in [25]:

$$RAF = \frac{C_{i,1} - C_{i,0}}{C_{i,0}}$$

where: $C_{i,1}$ - concentration of an analyte after exposure period [mg/kg d.m.], $C_{i,0}$ - concentration of an analyte before exposure period [mg/kg d.m].

The results of the analysis of the heavy metal concentrations increases, accumulated in the moss samples exposed in living quarters (K - kitchen, B - bedroom) are presented in the graph in Figure 2.

The presented results clearly indicate that the moss samples exposed in the room where cigarettes have been smoked (kitchen) accumulated higher concentrations of heavy metals than in the samples, which were exposed in the bedroom (the smoke-free room). The increase of mercury concentration was also determined in the mosses exposed in the kitchen (RAF_{Hg} = 0.021 mg/kg d.m.). The research demonstrated that the mosses kept in the bedroom (a potentially clean, smoke-free area) also showed increase of the determined elements. This indicates movement of the air polluted with, among others, analytes from tobacco smoke, through communication and ventilation routes.

The analysis included samples of hair of a smoker participating in the experiment, other smokers and non-smoking persons. The distribution of analytes concentrations in hair samples from non-smokers (NS) and smokers (S) is presented in the graph in Figure 3.

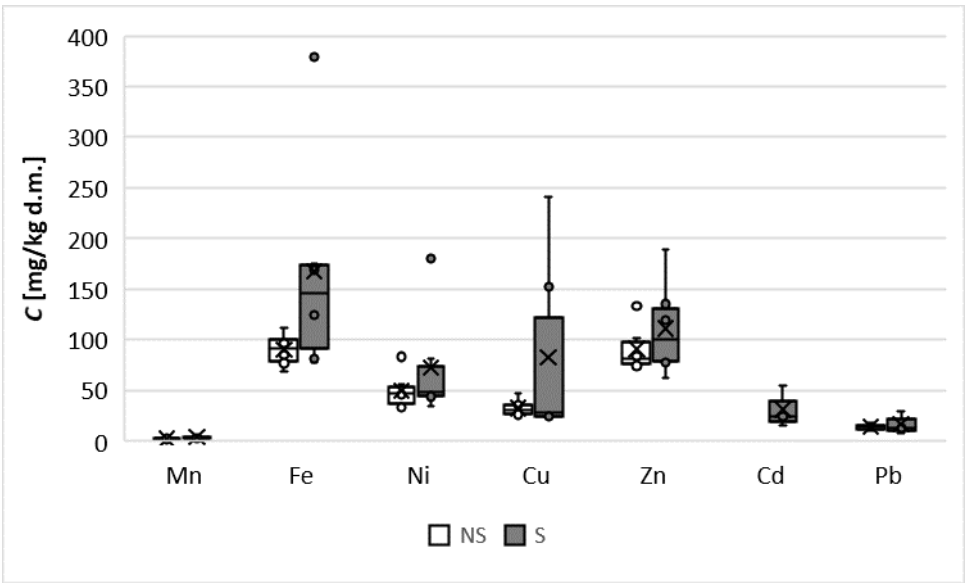

Fig. 3. Comparison of concentrations of the determined heavy metals in hair samples from smokers (S) and non-smokers (NS)

The analysis of the presented results indicates that smoking influences heavy metals content in human hair. In all analysed cases, the average concentrations of heavy metals determined in the group of smokers, are in all analysed cases higher than in non-smokers. The average concentrations are higher from 1.2 times for nickel to even 2.5 times higher for copper. The toxic elements, such as lead and cadmium, are most interesting. The average content of lead is 1.6 times higher in the samples from smokers, in comparison to the concentrations determined in hair from non-smokers. Cadmium concentration in non-smokers was below the detection limits for the applied analytical method, whereas it

was determined on the level of 22.1 mg/kg d.m. in smokers (detection limit for the used AAS instrument for Cd is 13.0 mg/kg d.m). The average concentration of Hg was c_{Hg} = 0.084 mg/kg d.m. in samples from smokers, whereas in those from non-smokers - c_{Hg} = 0.057 mg/kg d.m.

Summary and conclusions

Research centres are very interested in bioindicators of environment pollution because they are simple and not expensive to obtain. On the basis of the carried out research it was confirmed that *Pleurozium schreberi* mosses (the red-stemmed feathermoss) are a good biomonitor of air pollution with analytes from, among others, tobacco smoke, in living quarters.

It was determined that an organism exposed to contact with heavy metals in the form of air pollution (cigarette smoke) accumulates them in hair. The comparison of the obtained results allows to conclude that heavy metals were accumulated in much higher quantities in hair of smokers than non-smokers. This justifies the application of biological material, such as hair, in the health condition checks of humans. Non-smokers become passive smokers in living quarters, due to the existing communication and ventilation routes.

Smoking increases the risk of coronary arteries disease by even 40 %. The risk of myocardial infarction increases by as much as 70 %. Chronic smoking may lead to development of angina pectoris, malignant cancers or increased risk of multiple sclerosis by 40-80 % [26].

Further studies should focus on defining the norms, the excess of which would indicate pathological changes in an organism. The data obtained in this way would support fast and precise diagnosis of a person's health.

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MCHY JAKO BIOMONITOR ZANIECZYSZCZENIA POWIETRZA ANALITAMI POCHODZĄCYMI Z DYMU TYTONIOWEGO

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Abstrakt: Celem przeprowadzonych badań była ocena możliwości wykorzystania popularnego bioindykatora - mchów z gatunku *Pleurozium schreberi* jako bioczujnika zanieczyszczenia powietrza w pomieszczeniach mieszkalnych analitami pochodzącymi z dymu tytoniowego. Zastosowano popularną w badaniach środowiskowych metodę woreczkową biomonitoringu aktywnego (moss bag), polegającą na eksponowaniu mchów pobranych z terenów czystych na obszarach zanieczyszczonych np. metalami ciężkimi. Jednak w tym eksperymencie mchy eksponowano w lokalu mieszkalnym, w którym codziennie wypalanych było około 10 papierosów (pierwsze pomieszczenie - kuchnia). Dla porównania, próbki mchów były również umieszczone w drugim pomieszczeniu (w sypialni) - w pomieszczeniu potencjalnie niezanieczyszczonym. W mchach po trzymiesięcznej ekspozycji oznaczono metale ciężkie: Mn, Fe, Ni, Cu, Zn, Cd, Pb i Hg metodą absorpcyjnej spektrometrii atomowej. Dodatkowo anality te również oznaczono w próbkach włosów osoby palącej w tym pomieszczeniu oraz innych osób palących, a stężenia metali w nich oznaczone porównano z wynikami badań przeprowadzonych z wykorzystaniem próbek włosów pobranych od osób niepalących. Na podstawie przeprowadzonych badań stwierdzono m.in., że mchy eksponowane w pomieszczeniach mieszkalnych akumulują metale ciężkie, m.in. Ni, Zn, Pb i Hg, pochodzące z dymu tytoniowego. Oznaczono większe stężenia metali ciężkich w próbkach włosów palaczy w porównaniu do próbek włosów pobranych od osób niepalących.

Słowa kluczowe: mchy *Pleurozium schreberi*, włosy, metale ciężkie, dym papierosowy, biomonitoring