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Frank Laboratory of Neutron Physics

FINAL REPORT ON THE INTEREST PROGRAMME

*Assessment of the air quality in an industrial
zone using active moss biomonitoring*

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Abstract: Air pollution is one of the major problems of the 21st century, which affects the quality of the environment. Mosses are regarded as one of the main bioindicators of air pollution. Active monitoring is simple to carry out, financially acceptable due to the low cost of material acquisition and it is commonly used in many countries of the world to assess pollution. The technique is successfully applied in urban and industrial areas. The purpose of study was to evaluate the level of air contamination in the area of industrial enterprise in Tula. For study *Pleurozium schreberi*, *Sphagnum fallax* and *Dicranum polysetum* mosses were used. Elements (Mn, Fe, Cu, Zn, Cd and Pb) were determined using atomic absorption spectrometry. The results of the study indicate that the site is contaminated as a result of industrial activities. In the future, long-term biomonitoring studies should be conducted to track changes in pollutant concentrations.

Introduction

Long-term studies using mosses as biomonitors of air quality contribute to the indication of trends and changes in pollution over the years and the identification of sources of emissions of elements accumulated by mosses [1,2]. Although Poland and its neighbouring Central European countries are the most polluted areas in Europe [1], is a recent trend of reduction in some pollutants, but the influence of industry and the increasing share of pollution from urban areas still dominate [2,3]. This is due to the fact that moss chemical composition are specific to land use and moss uptake increase from agricultural to industrial sites [4]. The period of moss uptake is also important, as changes in accumulation are observed from season to season [5], The effect of the moss collection site (e.g., tree canopy) on metal deposition [6] or selecting the most suitable species to assess elemental deposition at a given site [7,8]. The effectiveness of using mosses for biomonitoring in assessing atmospheric deposition of metals is confirmed by long-term studies under the ICP Vegetation program, where changes in the concentrations of heavy metals accumulated by these plants can be recorded over the years [9]. Moss is a good passive sampler for airborne contaminants and can provide valuable information on chemical signature and deposition of metals [10]. Moss biomonitoring can be used to efficiently evaluate regional patterns in atmospheric metal deposition associated with industrial emissions sources [11]. For example, in two metropolitan areas in western Province of Sri Lanka using the moss biomonitoring method selected elements were determined: Zn, Cu, Pb, Ni and Cr, whose origin is traced to petrochemical applications operating in the study area, with mainly Ni and Cr coming from the local petrochemical industry operating around the moss sampling site [12]. Based on metal concentrations in mosses *Polytrichum commune* and *Pleurozium schreberi* the impact of emitters such as: a chlor-alkali plant, a glass smelter, two power plants and a ceramic and porcelain factory was assessed [13]. Neutron activation analysis and atomic absorption spectrometry allowed the determination of more than 30 elements in samples of *P. schreberi* mosses collected in central Russia, in which the main sources of air pollution were identified by factor analysis, which included: industry, oil refinery companies and thermal power plants [14]. Research conducted in a neighboring area (Moscow region) also indicates that the largest anthropogenic impact on air pollution in studied region have industrial activity, thermal power plants and transport [15], and for another study observed a positive change—a reduction in contamination in mosses associated with staying indoors due to the COVID-19 pandemic [16,17].

Despite the many advantages of using mosses in passive biomonitoring, more benefits in terms of the quality of the obtained information on environmental pollution can be obtained by conducting biomonitoring studies under controlled laboratory conditions [18].

The method of active biomonitoring is characterized by such advantages as accurate collection of spatial-temporal data or the simplicity and cost-effectiveness of this method in wide environmental monitoring [19]. Allows survey of any site of interest, is independent of species presence/absence, allows control of some factors, which reduces measurement variability and facilitates data interpretation [20]. In active biomonitoring of mosses, there are a set of factors that need to be

standardized and their variability between each study should be compensated for, such as the location of the exposed samples, the influence of the shape of the biomonitor bag or the method of the bag position itself [21–23]. Nevertheless, this method, as a result of standardization, provides more and more new possibilities and approaches for its use to monitor environmental pollution [24]. Using the moss-bag method with the moss *Sphagnum junghuhnianum*, metal concentrations in mosses were correlated with traffic intensity [25]. In contrast, short-term exposure of mosses of the genus *Sphagnum* allowed to fast screening for the occurrence of methylated volatile arsines or the predominance of particulate As [26]. For the pilot study in Azerbaijan, elevated elemental concentrations obtained as a result of anthropogenic influences were determined at sites that are characterized by cement production, paint production, and oil and gas production [27]. The moss bag technique is also used in urban areas [28,29], where moss exposure successfully allows point identification of pollutants as well as characteristic emitters [29].

The use of moss biomonitoring together with analytical techniques, as the Introduction indicates, provides specific information on environmental pollution. The choice of a given technique (passive, active biomonitoring), determines the obtaining of results of a given quality and, when constructing an experiment, it is necessary to pay attention to what is the main objective of the study of atmospheric aerosol pollution of a given site/area

Project goals

The main purpose of the project is to assess the level of air pollution around one of the industrial enterprises in Tula (RU). So far, only passive biomonitoring studies using mosses have been conducted in the region [30,31]. Therefore, the use of three moss species was undertaken: *Pleurozium schreberi*, *Sphagnum fallax* and *Dicranum polysetum* in the assessment of atmospheric aerosol pollution by selected elements around metallurgical plant in Tula and the effect of the winter period on heavy metal concentrations during 90 days of active biomonitoring exposure.

Scope of work

1. Exposure of moss bags at different sites around the metallurgical plant for three months
2. Preparation of the literature review for the manuscript
3. Description of the analyzed zone
4. Determination of the concentration of the metal in exposed and control moss samples
5. Interpretation of the obtained results

Methods

Exposure of three moss species *Pleurozium schreberi* (*Pl*), *Sphagnum fallax* (*Sp*) and *Dicranum polysetum* (*Dp*) as part of active biomonitoring for three months (November 2021 - February 2022) around Kosogorskii metallurgical plant (Tula, RU).

Moss samples were collected and prepared before exposure following the international guideline ICP Vegetation [32]. Prior to exposure in active biomonitoring, the preparation method affects the initial elemental concentration in mosses, so they were prepared before exposure by earlier study [33]. Mosses were hung at the height of about 2.00 m from the ground and samples were located at 7 sites with 3 bags of each species in every site (9 bags in one site), general 63 samples.

Exposure area

Kosogorskiy metallurgical plant (KMP, Tula) is the largest iron-works plant in Tula region and one of the oldest in Central Russia. The plant is specialized in the production of cast iron foundry, high-carbon ferromanganese, steel castings, pipes, tiles cast iron, spare parts for drilling equipment, building materials, etc. Consequently, the main pollutants in industrial emissions are Fe, Pb, Zn, Cu, and Cd, and sulfur oxide. Thus, in the close vicinity to the plant (400 m from KMP)

the content of Mn and Cd in soil exceed those in the control plot by factors of 16.3 and 92, respectively, and the factors for other metals range from 1.6 (Fe) to 5.7 (Pb) [34].

After three months of exposure, each moss sample, with a mass of 1.000 ± 0.001 g dry mass (d.m.), was mineralized in a mixture of nitric acid and hydrogen peroxide using a microwave oven (Berghof Company, DE) to determine Mn, Fe, Cu, Zn, Cd and Pb. The mineralization process was carried out at the temperature of 180 °C. These metals were determined using an atomic absorption flame spectrometer type iCE 3500 (Thermo Scientific, USA). Concentrations of metals were determined in solution after mineralization filtration and were diluted into volumetric flasks of 25 cm³. Calibration of the spectrometer was performed with standard solutions (ANALYTIKA Ltd., CZ). The values of the highest concentrations of the models used for calibration (2.00 mg/dm³ for Cd, 5.00 mg/dm³ for Cu, Zn and Pb, 7.50 mg/dm³ for Mn and 10.0 mg/dm³ for Fe) were approved as linear limits to signal dependence on concentration. Table 1 presents the instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) of the iCE 3500 spectrometer. Table 2 shows the concentrations of heavy metals in certified reference materials BCR-482 *lichen*, produced at the Institute for Reference Materials and Measurements, Belgium.

Table 1. The instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) for the iCE 3500 (mg/L) spectrometer [35]

Metal	<i>IDL</i>	<i>IQL</i>
Mn	0.0016	0.020
Fe	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* [36]

Metal	BCR-482 <i>lichen</i>		AAS (n = 5)		<i>Dev.**</i> [%]
	Concentration	Measurement uncertainty	Average	± <i>SD</i> * of the concentrations	
		[mg/kg d.m.]			
Mn	33.0	0.50	31.7	0.68	-3.90
Fe	804	160	771	154	-4.10
Cu	7.03	0.19	6.63	0.17	-5.70
Zn	100.6	2.20	95.1	2.30	-5.50
Cd	0.56	0.02	0.53	0.03	-5.30
Pb	40.9	1.40	38.2	1.00	-6.60

* standard deviation.

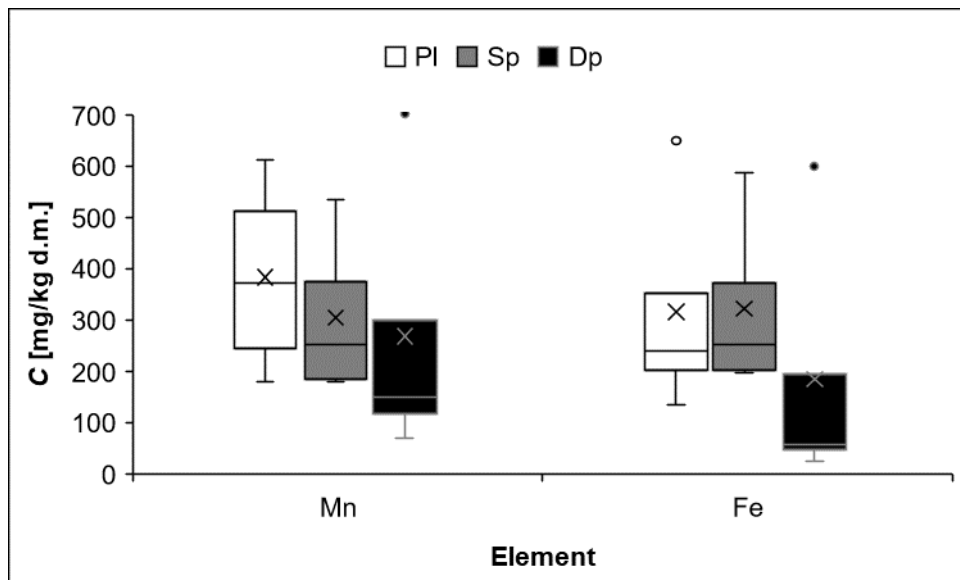
** relative difference between the measured (c_m) and certified (c_c) concentration 100% ($(c_m - c_c)/c_c$).

The *RAF* – Relative Accumulation Factor was used to determine increases of concentrations of the analytes in the exposed mosses samples [37]. The results were interpreted based on the Coefficient of Variation (*CV*), which is frequently used in analysis of biomonitoring research [17,38].

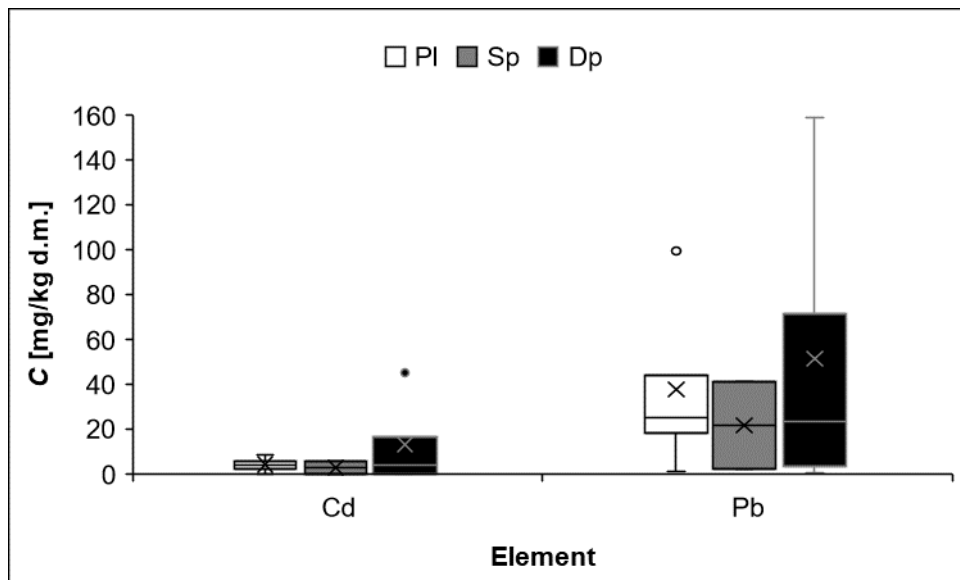
Results

As a first step in the analysis of the results, the distribution of heavy metal concentrations in mosses was evaluated regardless of the exposure site - the results are shown in Fig. 1.

a)



b)



c)

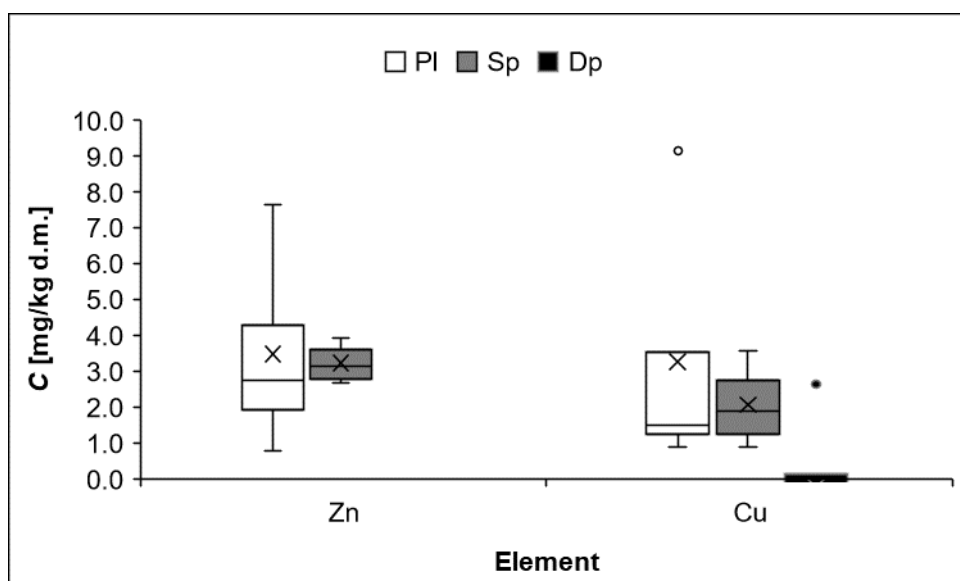


Fig. 1. Concentration distributions for (a) manganese and iron; (b) cadmium and lead; and (c) zinc and copper.

As indicated in Fig. 1. the distribution of elemental concentrations in mosses varied among species and elements. The analysis of the CV coefficient is shown in Table 3.

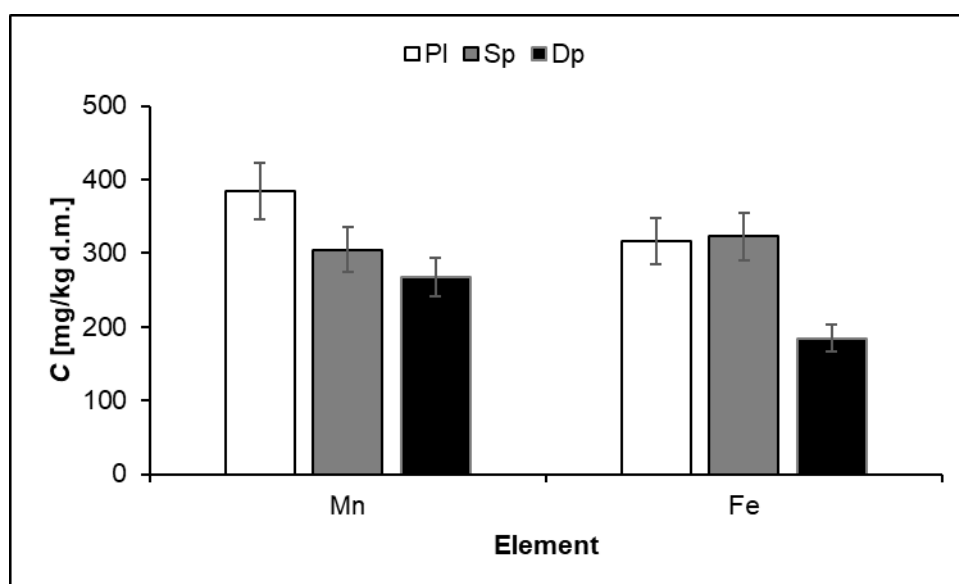
Table 3. Coefficient of variation of elemental concentrations according to moss species [%]

Element/ Species	Mn	Fe	Zn	Cd	Cu	Pb
<i>Pl</i>	51.3	72.0	84.7	85.6	120	114
<i>Sp</i>	54.5	56.6	18.3	114	58.1	103
<i>Dp</i>	109	150	*	165	*	144

* no possibility to determine the CV

The results presented in Table 3 show a rather large scatter and a fair amount of variability in the results across the study area. This indicates that the concentrations change between different moss exposure points. In the next step, the average concentrations of elements accumulated in mosses were analyzed to determine the best biomonitor in relation to selected elements (Fig. 2).

a)



b)

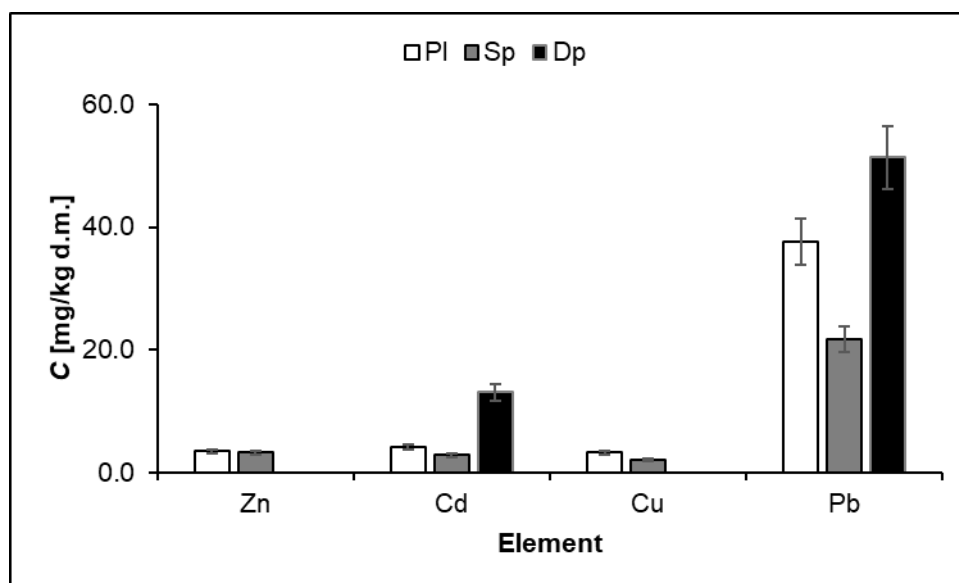


Fig. 2. Average relative concentrations (whiskers are standard deviation) for (a) manganese and iron and (b) zinc, cadmium, copper, and lead.

The results shown in Fig. 2. represent the mean relative concentrations of elements determined in the mosses after exposure. ANOVA analysis indicates that element is statistically significant, $p < 0.001$. Species was not statistically significant. In the next step, the determined *Relative Accumulation Factor (RAF)* values were analyzed as shown in Table 4.

Table 4. *RAF* values for the three moss species for the elements analyzed

Element/ Species	Mn	Fe	Zn	Cd	Cu	Pb
<i>Pl</i>	3.31	0.823	0.072	8.43	0.466	9.58
<i>Sp</i>	4.45	1.65	0.094	6.27	0.684	7.01
<i>Dp</i>	1.48	0.336	*	26.2	*	12.3

* no increments of *RAF*; yellow indicates slight increments: $RAF > 0.50$ and red indicates significant increments in mosses: $RAF > 1.00$

The values shown in Table 4 indicate that, regardless of species, the mosses are primarily significantly enriched in elements after exposure: Mn, Cd and Pb. Additionally, a slight enrichment was recorded for iron for *Pl* species and copper for *Sp* species. Zinc is not an element enriching the exposed mosses.

Discussion

As mentioned in the Introduction, the moss-bag technique finds its application in the assessment of air pollution in urban areas, and one statistical technique that reflects well the changes in elemental concentration is ANOVA, also used by us [39]. For moss transplants *Hylocomium splendens* significant correlations between element concentrations in mosses and bulk deposition (Cd, Ni, Pb and Zn) were found for industrial sites [40]. For our study area, the highest *RAF* values were observed for Cd and Pb of those elements considered (Table 4). As indicated in the "Project goal" so far the Tula site has only been surveyed by passive biomonitoring using different moss species. The site has been contaminated by elements of natural origin (e.g. Zn), from obvious anthropogenic sources: the iron-vanadium industry (i.e. Fe) and other industries and general urban activities (e.g. Mn, Zn, Cu, Pb) [30]. All these elements were determined in our study as part of active moss biomonitoring. In the second publication of the passive technique and metal deposition of the Tula site- the concentrations of some elements have increased over the decade and their labeled concentrations are dangerous to the health and even life of people living in the area [31]. Our 3-month experiment is too short a period of time, performed only during the winter, to come to such conclusions. However, the determination of selected heavy metals in all moss species, indicates air pollution due to active human activities in the area. Biomonitoring studies in similar areas confirm that obtaining high *RAF* values is related to the source of pollution which is heavy industry. In two moss species exposed in Donetsk Region, *RAF* values were 2-3 times lower than those obtained by us for cadmium or lead [41]. The analysis of rare earth elements and the values obtained by the authors, only confirm the air pollution of this region as a result of parks, steelworks, and a power station activities [42].

Due to the location of the measurement site in the immediate vicinity, the area studied is Moscow, about 200 km from Tula. Here, a 10-week exposure of *Sphagnum girgensohnii* made it possible to assess the pollution in this city, where the mosses accumulated the most pollution in open space with traffic, which was also attributed as the source of enrichment of the mosses in particular elements [43]. A 3-month study in the same city (but, in park of the state museum-reserve "Tsaritsyno") with the same indicator species showed low *RAF* values [44]. This proves that moss is a sensitive biomonitor in urban areas, but the land use significantly affects the obtained result of

the concentration of elements accumulated by mosses during exposure. The values obtained in the aforementioned study stand apart from those obtained in the industrial area of Tula that we considered.

Conclusion

The moss-bag technique was first used around one of the industrial enterprises in Tula, RU. The results of the conducted study of 90-day exposure of mosses during the winter period indicate the contamination of the study area with selected heavy metals. The source of pollution is not only active industry, but also low deposition during the heating season. Each of the three moss species used was a good biomonitor of selected elements, which indicates the degree of air pollution in the area. In the future, it would be advisable to continue research on atmospheric aerosol quality using mosses over a long-term period to study seasonal changes and trends and to select the optimum biomonitor of atmospheric aerosol pollution in the area.

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