# NAA FOR SOME METHODOLOGICAL ASPECTS OF BIOMONITORING AIR POLLUTION: BELGRADE CASE STUDY

M. Aničić[1], M.V. Frontasyeva[2], M. Tomašević [1], A. Popovic[3]

[1]*Institute of Physics, 11000 Belgrade, Serbia and Montenegro*
[2]*Joint Institute for Nuclear Research, 141980 Dubna, Russia*
[3]*The Faculty of Chemistry, University of Belgrade, 11000 Belgrade, Serbia and Montenegro*

## Abstract

The level of atmospheric deposition of heavy metals and other elements in Belgrade was evaluated using the moss biomonitoring technique. The concentrations of 36 elements (Na, Mg, Al, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Zn, As, Se, Br, Rb, Sr, Mo, Sb, I, Cs, Ba, La, Ce, Sm, Tb, Dy, Hf, Ta, W, Hg, Th, U) were determined by instrumental neutron activation analysis. The accumulation abilities of two moss species, *Brachythecium sp.* and *Eurhynchium sp.*, were examined as well as two ways of sample preparation prior to analysis.

## Introduction

The moss monitoring technique, first introduced in Scandinavia, has shown to be appropriate for studying atmospheric deposition of heavy metals and other elements as well [1]. Terrestrial mosses have several advantages as biomonitors when compared to higher plants: (1) they lack developed root system, (2) low variability of morphology through the growing season; (3) they lack cuticula; (4) high surface-to volume ratio, and (5) high cation exchange capacity (CEC) [1,2]. It is assumed that moss intakes nutrients directly from the atmosphere.

Bryophytes are especially suitable organisms for monitoring investigations, because procedures of sampling and of chemical analysis are relatively simple and cheap. It is easy to collect these evergreen and perennial plants throughout the year. Most of moss species are widespread; thus, metal concentrations of distant areas can be compared [3].

The present research is focused on the implementation of two moss genera: *Brachythecium sp.* (*B. rutabulum* & *B. salebrosum* ) and *Eurhynchium sp.* (*E. hians* & *E. striatum* ) for assessment of atmospheric deposition of heavy metals and other elements in arid areas similar to that of Belgrade. These moss species, often growing together in turf, are common for the continental area of Balkan including urban areas. The above-mentioned species have already been used for biomonitoring investigations in some European countries [4-6], as alternative to the recommended species *Hylocomium splendens, Pleurosium schreberi,* and *Hypnum cupressiforme* [3,7]. *Brachythecium sp.* and *Eurhynchium sp.*, in [4-6] were used when other adopted species were unavailable. Interspecies calibration showed that they could be used along with *Hylocomium splendens, Pleurosium schreberi,* and *Hypnum cupressiforme.* All mentioned species have similar physiognomy, as they grow in denser mats able to produce a higher rate of bioaccumulation.

The sources and mechanisms other than atmospheric deposition, such as the transfer to moss from local soil dust, throughfall precipitation or remains of other plants, might contribute

[1] Corresponding author: manicic@phy.bg.yu

significantly to the concentration observed [2,8]. Rinsing of moss samples removes plant remains epiphytic organisms, dust, etc. and also reduces local contamination by removing soil mineral particles [9-11]. However, it has been pointed out that moss species have cation exchange capacity (CEC) and washing the moss might significantly distort the results [2,12]. In order to avoid superficial contamination of samples some authors used a jet of air to remove impurity [13]. Surveys on atmospheric depositions, as a rule, are based on total element analysis of unwashed moss samples [1,2].

The main objective of this study was to evaluate the accumulation abilities of *Brachythecium sp.* and *Eurhynchium sp.* as bioindicators of atmospheric heavy metal and other element deposition. A comparison of the unwashed and washed (prior to analysis) moss samples was made and difference in corresponding elemental concentrations was analyzed. The elemental concentrations in moss obtained in 2004 were compared to those obtained in 2000 for the same area of Belgrade after the known NATO military actions.

## Experimental

### *Study area*

The study was carried out in urban area of the city of Belgrade: Kalemegdan Park (H= 116.75 m, $\varphi$ = 44° 49 ' 30" N, $\lambda$ = 20° 27 ' 03" E) and Zemun, ( H= 85 m, $\varphi$ = 44° 51' 19" N, $\lambda$ = 20° 23' 27" E) in the autumn of 2004. Belgrade is the capital of Serbia and Montenegro with population of more than 1.8 million. There are many very old buses and trucks in the streets and it could be a significant major source of pollutants. There are 18 bigger heating plants with a total capacity of 2018 MW, run with natural gas or crude oil and 59 smaller plants run only with crude oil (approximately 193 MW). Fuel used for domestic heating consists mainly of coal or crude oil. The climate of Belgrade is moderately continental, with fairly cold winters and warm summers. The prevailing wind is N-NW, but, also, the characteristic wind is "Košava" (SE-ESE). This wind effectively improves the horizontal dispersion and dilution of pollution in the ground-level atmosphere of the city of Belgrade.

### *Moss sampling*

Collection of moss samples was performed according to proposition of study "Heavy metals in European mosses: 2000/2001 survey" [7]. At both sites, 5 sub-samples for both moss species were collected within a 30 x 30 $m^2$ area, at least 100 m from main roads and 50 m from smaller roads and houses, not directly exposed to throughfall precipitation. Sampling and handling were carried out using plastic gloves and bags.

In the laboratory, the samples were carefully cleaned from impurities, then only green and green-brown moss shots corresponding to two-three last year growth were analyzed.

The samples were sub-divided into two parts. One part was rinsed with double-distilled water for 30 s to remove impurities [10,12]. The rest of the samples were not subjected to washing as recommended in "Heavy metals in European mosses: 2000/2001 survey" [7]. Both parts of the samples were dried for 48 hours at 35°C to constant weight prior analysis.

### *Analysis*

Heavy metals and other element concentrations in the moss samples were determined by the method of instrumental neutron activation analysis (INAA). INAA was performed at the

Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, Russia. Most element concentrations were determined by INAA with detection limits within the range of $10^{-2}$-$10^{3}$ μg/g. For analysis, 5 sub-samples were combined into a well homogenized sample from which three "tablets" were taken for three measurements, both per area and moss species. Approximately 0.3 g of homogenized moss tissue was packed in aluminum cups for long-term irradiation or heat-sealed in polyethylene foil bags for short-term irradiation at the IBR-2 fast pulsed reactor. The irradiation facilities used are described in Table 1. The short irradiation for 2 minutes in Ch2 was used for short-lived radionuclides (Mg, Al, Cl, K, Ca, Ti, V, Mn, I, Dy). The long irradiation for 100 hours in Ch1 was used to determine elements associated with long-lived radionuclides (Na, Sc, Cr, Fe, Co, Ni, Zn, As, Se, Br, Rb, Sr, Mo, Sb, Cs, Ba, La, Ce, Sm, Tb, Hf, Ta, W, Hg, Th, U). Gamma-ray spectra were measured four times using a high-purity Ge detector after decay periods of 5 minutes and 10 minutes following the short irradiation and after 3 days and 20 days following the long irradiation [14].

**Table 1.** Flux parameters of irradiation positions

| Irradiation position | $\Phi_{th} \times 10^{11}$, n· cm$^{-2}$s$^{-1}$ $E$=0-0.55 eV | $\Phi_{th} \times 10^{11}$, n· cm$^{-2}$s$^{-1}$ $E$=0.55-$10^5$ eV | $\Phi_{th} \times 10^{11}$, n· cm$^{-2}$s$^{-1}$ $E$=$10^5$ -25·$10^6$ eV |
|---|---|---|---|
| Ch1 | 0.38 | 0.14 | 7.2 |
| Ch2 | 4.1 | 0.09 | 3.4 |

*Quality control*

To provide quality control (QC), content of elements yielding short- and long-lived isotopes was determined using certified reference materials issued by the International Atomic Energy Agency: lichen (IAEA-336), cabbage (IAEA-359) and standard reference material SRM-1575 (Pine Needles) from the US NIST (National Institute of Standards and Technology). For the short irradiation above-mentioned reference materials were irradiated together with 10 moss samples. For the long irradiation the three reference materials were packed together with 7-9 moss samples in each transport containers.

**Discussion**

In this study, after careful removal of extraneous material from moss samples, a short rinsing procedure of 30 s was applied to a half of the samples to avoid disturbance of CEC [10]. The element concentrations, determined in the moss samples washed in this way, were compared to the moss element concentrations determined in the other half of samples, which were not subjected to washing (Fig. 1). A comparison of the unwashed and washed (prior to analysis) moss samples has not revealed any difference in elemental concentrations. The differences were within the error of used analytical method. The exceptions are a few elements: Se, Ta and Tb (Fig. 1, a)) and Se (Fig. 1, b)) determined in the moss samples of the studied area Zemun, probably due to their low concentrations in the samples (close to detection limit of INAA). Than, it means that gentle cleaning of moss samples prior to analyses was sufficient.

In further data presentations here we have taken into consideration only the results of the unwashed moss concentrations.

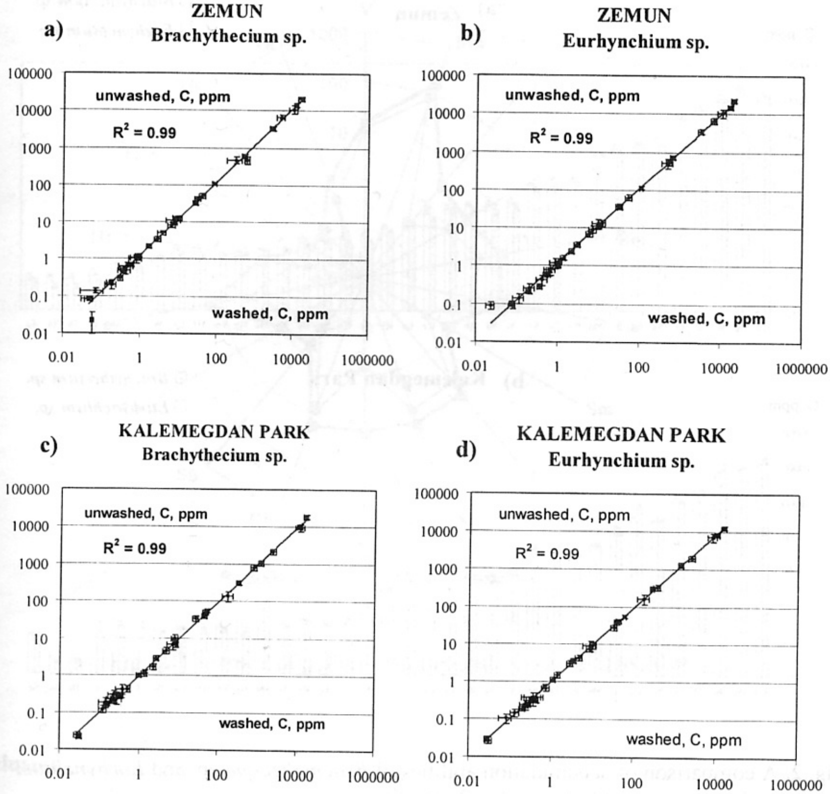
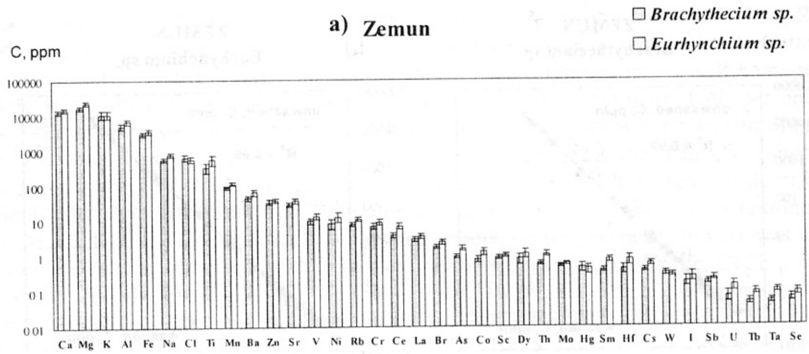



**Fig. 1.** The correlation of moss concentrations between the washed and unwashed samples of *Brachythecium sp.* and *Eurhynchium sp.* at both study areas – Zemun and Kalemegdan Park

The concentration of determined elements for the two most widespread moss species in urban area of Belgrade, *Brachythecium sp.* and *Eurhynchium sp.*, are quite comparable (Fig. 2). For the majority of elements, the obtained element concentrations are within the error estimation (specific for each element - σ). Although, for Ce, As, Co, Th, Sm, Tb, Ta (Fig. 2, a)) and Cl, Co, Mo, U (Fig. 3, b)) a difference between an accumulation abilities of the used species exceed 2 σ. A reasonable explanation for some of these elements may be that the concentrations of some of them were very close to the detection limits (Th, Tb, Ta, U).

The results obtained for *Brachythecium sp.* were used for further estimation of the trend of air pollution in the area of Belgrade.



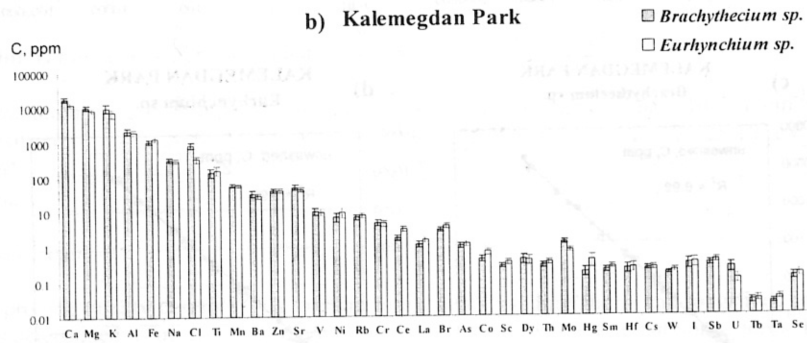




**Fig. 2.** A comparison of accumulation abilities of *Brachythecium sp.* and *Eurhynchium sp.* from Zemun – a) and Kalemegdan Park – b)

The results from this study (the average values for both studied areas – Zemun and Kalemegdan Park) was also compared with the previously obtained results from a location in the area of Belgrade (Avala) for 2000 (in the framework of investigation of northern part of Serbia) [14], (Fig. 3). A decreasing trend of pollution for the elements characteristic of oil combustion, such as: V, Mn, I, As, Br, La, Sm, Cr, Ni, Co, Rb, Sb, Cs, Ce, Tb, Hf, Ta, and Th is evident. Although Avala is much farther from potential sources of pollution than the other two studied places in this investigation (Zemun and Kalemegdan Park), the pollution level was higher at this site. The real explanation of this phenomenon is evident: NATO military actions in Serbia and Montenegro in spring of 1999. Then, the area of Belgrade was disposed to high pollution because of fires subsequent to vast destruction of industrial complexes.

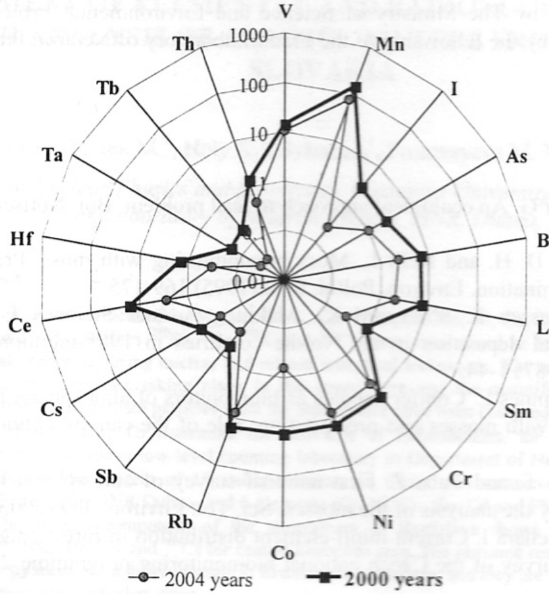



**Fig. 3.** The trend of pollution in Belgrade from 2000 to 2004

## Conclusion

The following conclusions can be drawn from this study:

1. The bryophyte *Brachythecium sp.* and *Eurhynchium sp.* seem to be useful as a bioindicators of elements atmospheric deposition in urban areas of Belgrade where the most frequently used moss bioindicator species *Hylocomium splendens, Pleurosium schreberi* and *Hypnum cupressiforme* are not available. For the majority of the determined elements there are no difference in accumulation abilities of the used moss species. Both studied moss species may be suitable for biomonitoring purposes.
2. A difference between the element concentrations in the washed and unwashed (prior to analysis) moss samples was within the specific analytical error of INAA for majority of the determined elements.
3. A comparison of the results obtained in 2000 and 2004 for the area of Belgrade showed obviously higher concentrations of elements characteristic for oil combustion from fires of devastated petrochemical complexes.

The present study is a part of the project “Air quality studies in urban areas” (project no. 1449) which includes also measurements of heavy metals concentration in suspended particular matters, bulk atmospheric deposition, soil and plant leaves; natural and man-made radionuclides (Be-7, Cs-137, Pb-210) and ground level ozone (Institute of Physics, Belgrade).

This work is supported by The Ministry of Science and Environmental Protection of the Republic of Serbia, and by the fellowship of the Frank Laboratory of Neutron Physics. JINR, Dubna, Russia.

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