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Urban background of air pollution: Evaluation through moss bag biomonitoring of trace elements in Botanical garden

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ABSTRACT

Urban background air pollution is the lowest level of pollution representative for the exposure of general urban population and mainly originates from non-local sources of pollution. Moss bag technique has been predominantly adopted for biomonitoring of trace elements across anthropogenically devastated areas, such as urban and industrial zones. However, the technique has been rarely used for measurement of background air pollution. In this study, element content and concomitant physiological parameters were assessed in the moss *Hypnum cupressiforme* Hedw. after its exposure within the bags in the Botanical garden (Belgrade, Serbia), as the presumable background area. During the summer of 2014, the moss bags were exposed for 60 days in total, and the sample analyses were performed every 15 days. As a control of the measured physiological parameters, a set of the moss bags was kept within the phytotron, under conditions of optimal-like growth. The total content of 21 elements, photosynthetic pigments (chlorophylls and carotenoids), phenolics, and antioxidative capacity were determined in the moss samples. The levels of trace elements were markedly lower within the Botanical garden in comparison to the street ambient, but still significantly higher than in the moss pristine habitat. However, the performed physiological tests indicated that the vulnerability of the moss during exposure time was not caused by the low trace element enrichment, but probably by the harsh meteorological parameters in an urban area. Finally, the Botanical garden could be assumed as control site for measurement of the urban background pollution, and it might be effectively measured by the moss bag technique.

1. Introduction

Over 70% of the human population across Europe lives in urban agglomerations and, to some extent, suffers from influence of air pollution (EEA, 2014). Field measurements highlight a complexity of pollutant emissions, as well as their transport and dispersion across the urban settings (Baldauf et al., 2013). Topography and urbanisation features of the cities cause high spatial variability of air pollutant concentrations.

The total concentration of pollutants comprises those from the explicit local emission sources such as road traffic and chimney stacks; and those called 'background' air pollution that is transported into an area from distant sources by the wind (Hertel and Goodsite, 2009; DEFRA, 2014). Considering the regulatory documents, 'urban background locations' mean places in urban areas where pollutant levels are representative of the exposure level of general urban population (Directive 2008/50/EC). In reality, measurements of urban air pollution are largely available for non-representative sampling sites within the urban area. Air pollution monitoring stations are usually situated at presumably pollution 'hot spots', and rarely in areas with a lower level of air pollution. Thus, data about urban background pollution level are not practically available. Measurements of this kind lack worldwide and these might be only estimated using statistical models (Cohen et al., 2004).

Biomonitoring is a simple method for estimating the pattern of atmospheric pollution in a certain area using living systems, such as mosses or lichens (Markert et al., 2003; Pirintsos and Loppi, 2008; Aničić Urošević et al., 2017). Particularly, active biomonitoring refers to the use of suitable moss or lichen species transplanted from unpolluted area to the sites of interest. This approach enables integrated monitoring of many airborne contaminants simultaneously (i.e., metals and metalloids, trace elements, polycyclic aromatic hydrocarbons – PAHs, radionuclides) (Giordano et al., 2005; Ares et al., 2012; Vingiani et al., 2015; Vuković et al., 2015; Krmar et al., 2016). It is hard

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for any other monitoring approach to enable such a detailed picture of the pollutant variations in space, and at a reasonable cost. The advantages of biomonitor use over regular monitoring are the simplicity of performing and the lack of the need for power supply.

'Moss bag technique' has been developing in last several decades as a method for active biomonitoring of air pollutants (Ares et al., 2012). This method has been particularly useful for conducting a detailed survey in diversely polluted urban microenvironments where native mosses are usually absent because of predominantly paved and landscaped surfaces. Specifically, the moss collected from relatively unpolluted area, with known initial element content, could be exposed in the bag at the site of interest for a certain period acting as an integrated accumulator of the ambient element pollution expressed by net concentrations or relative accumulation factor. However, relocation of moss from their natural habitat for the purpose of active biomonitoring represents a substantial stress for the moss due to changed macro- and microclimate and meteorological conditions. The exposure in the suspended bags is probably a drastic change of moss natural habitat predominantly due to hydric and photo stress (Tretiach et al., 2007). Moreover, in the conditions of air pollution burden, such as in urban area, the physiological parameters of the moss could reflect the heavy metal pollution, especially when ambient pollution is continuous for several weeks or months (Sun et al., 2009).

In this study, active moss biomonitoring was conducted within the Botanical garden with the aim to assess whether the gardens situated in the downtown area could be assumed as a place for the assessment of urban background pollution. In addition, it was investigated whether presumably low trace element load in the *Hypnum cupressiforme* moss bags causes disorder of physiological state of the exposed moss, which implies active or passive element uptake.

2. Materials and methods

2.1. Study area

The study area was Botanical garden "Jevremovac" within Belgrade downtown (Serbia) (44°48′56.9″N; 20°28′23.6″E). The arboretum spreads over the area of 50,000 m^2 of open space and includes over 300 tree species. The Botanical garden is bounded with two streets with very frequent traffic, Boulevard of Despot Stefan and Takovska St., and by the three less car-occupied streets (Fig. 1).

Belgrade has a moderate continental climate. The year-round average temperature is 11.7 °C, the hottest month is July, with an average temperature of 22.1 °C; annual precipitation is about 700 mm. During the study period (July–September 2014), an average temperature was 20.8 °C while the precipitation varied from 8 mm to150 mm with an average value of 48.7 mm (Table 1, Supplementary material).

2.2. Moss sampling and bag preparation

In May of 2014, the moss *H. cupressiforme* Hedw. was collected in the protected area "Vršačke planine', Serbia (44°08′26″N; 21°23′43″E; 230 m a.s.l.) that is recognised as an appropriate pristine area for the moss sampling (Vuković et al., 2015, 2016). The moss was sampled on the rules of the international moss monitoring manual (ICP Vegetation, 2005). In the laboratory, green apical parts of the moss were manually cleaned and separated from the rest of the plant. Subsequently, the selected green parts of the moss were rinsed three times with double-distilled water (approximately 10 L water per 100 g of moss dry weight and 10 min of shaking). The moss was air-dried and gently hand-mixed. Approximately 1.5 g of the moss was packed loosely in 7 × 7 cm nylon net bags with a 2-mm mesh size resulting in a surface area of approximately 30 mg cm^{-2}, which is suggested by Ares et al. (2012) to be the most suitable for maximum uptake (Aničić et al., 2009a; Vuković et al., 2015). The nylon is inert material and does not interfere with the element uptake process while mesh has a homogenising effect on element entrapment acting as a sieve for large airborne particulates (Ares at al., 2012). Moreover, moss material was packed as thin as possible to avoid its overlapping.

To avoid contamination of the moss material, the entire process of moss sampling and bag preparation was conducted wearing polyethylene powder-free gloves. Several moss bags were stored at room temperature in the laboratory conditions for determination of the initial pollutant concentrations in unexposed moss material.

2.3. Experimental design

The study was carried out in the Botanical garden from July to September 2014. The moss bags were exposed to atmospheric deposition for four consecutive exposure periods – 15, 30, 45, and 60 days at two sites – garden boundary and garden inner (Fig. 1). Ten moss bags per study site (four for chemical analysis, and four for physiological tests; two moss bags were extra added for the case of bag loss from any reasons) were hung on plasticized aluminium holders specifically designed for that purpose. The holders were mounted perpendicular to the lampposts at the representative height of 3.5 m (Vuković et al., 2013) in open spaces, out from tree canopies and electric cables. As control samples for the atmospheric deposition of trace elements in open spaces, ten moss bags were sheltered under the roof, close to garden boundary site, and wetted several times per week by spray-distilled water. An aliquot of unexposed moss collected in the same pristine area was used to measure the pre-exposure elemental concentrations (in triplicates).

2.4. Chemical analysis

After each exposure periods, one moss bag was moved per studied site, and further, the moss material were air-dried and homogenised in the laboratory conditions. Each moss sample was analysed in triplicates (n = 3 subsamples × 4 exposure periods × 3 study sites = 36). Approximately 0.3 g of each moss subsample were dissolved with 1 mL of 30% H_2O_2 (Sigma-Aldrich, puriss. p.a.) and 7 mL of 65% HNO_3 (Sigma-Aldrich, puriss. p.a., distilled by the apparatus for acid distillation – BERGHOF, Products + Instruments GmbH, Germany). Further digestion procedure was performed in a microwave digester (ETHOS 1, Advanced Microwave Digestion System, Milestone, Italy) for 45 min at 200°C. The digested samples were diluted with double-distilled water to a total volume of 50 mL.

The concentrations of Al, Ba, Cu, Fe, K, Mg, Mn, Na and Zn were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Thermo Scientific iCAP 6500 Duo, Thermo Scientific, UK). For calibration, a Multi-Element Plasma Standard Solution 4, Specpure (Alfa Aesar GmbH & Co KG, Germany) was used. The concentrations of As, Cd, Ce, Co, Cr, Ni, Pb, Rb, Sb, Sn, Sr and V were determined using inductively coupled plasma-mass spectrometry (ICP-MS, Thermo Scientific iCAP Q, Thermo Scientific, UK). A low-level Elements Calibration Stock, EPA Method Standard (VHG Labs, Manchester) was used for calibration.

Quality control of the element determination was provided through analysis of the analytical blanks and certified reference materials that were analysed once every ten moss samples. As the certified reference materials, the moss *Pleurozium schreberi* M2 and M3 (Steinnes et al., 1997) were used. The recovery of elements from the reference material M2 ranged from 75% to 111% for the majority of the determined elements; however, the recoveries of Ni and Ce were 126% and 56%, respectively. For the reference material M3, the recovery of elements ranged from 80% to 112%, whereas those of Cu and Ce were 63% and 57%, respectively. The data were corrected depending of the element concentration level by the mean recovery value (%) either M2 or M3.