METAL OXIDE NANOPARTICLES BIOMONITORING USING BRYOPHYTES

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Abstract

Hylocomium splendens, species of moss frequently used for biomonitoring purposes, was subjected to metal oxide nanoparticle suspensions (nano-TiO_2, nano-ZnO) in known concentrations in order to assess its applicability in biomonitoring of metal oxide nanoparticle pollution. After weekly subjecting the samples of the moss to the suspensions for up to eight weeks, the samples were digested and the metal content was analyzed by ICP-AES. Both applied suspensions of nanoparticles led to accumulation of the respective metals in the moss thalli - with a clear linear relationship between the amount of metal and the time of the exposition. The significant linearity of the trends and high coefficients of determination assure great accuracy and predictability of the application of *Hylocomium splendens* in future nanoparticle-related biomonitoring surveys.

Keywords: Metal oxides, nanoparticles, pollution, biomonitoring, bryophytes

1. INTRODUCTION

One of the most commonly used nanomaterials are metal oxides, while nanosized zinc oxide (nano-ZnO) and nano titanium dioxide (nano-TiO_2) are the most extensively used in manifold fields and applications [1]. It is, thus, conceivable that their unwanted presence in the environment will continue to grow as well [2]; increasing presence of a compound in the environment, in turn, leads to the heightened need for its detection and monitoring. Biomonitoring - sensu Markert et al. [3] - is a method of using organisms or their parts to quantify the pollutant levels in the environment and it is both affordable and convenient way of environmental monitoring.

Suitable biomonitor species should be generally available, easy to handle and its interaction with the particular pollutant should be known. Good biomonitor, while not being negatively affected by its presence, is able to accumulate the pollutant in its tissues, most of the biomonitors are, therefore, fungi or plants. So far, most of the research conducted dealt with the toxicity of metal oxides nanoparticles to plants [4] but the topics such as the uptake mechanisms, distribution, translocation and accumulation in the plant body have not been fully understood yet, though it was confirmed several times that both uptake and distribution differ from species to species as well as they depend on the particular nanomaterial [5].

Many species of bryophytes - and mosses in particular - are frequently employed in biomonitoring of both air and water pollution [6]. This is facilitated by the fact that they possess all the aforementioned traits of excellent biomonitors. Nevertheless, neither nanoparticle exposition effect on bryophyte physiology nor the bioaccumulation patterns of nanoparticles in their thalli have been explored so far. Hence, the most important questions regarding their possible use as biomonitors of nanoparticle pollution are yet not answered. So far the only study of nanoparticle exposition in bryophytes was performed on a species that is neither used nor suitable as biomonitor: iron nanoparticles were applied to the bryophyte *Physcomitrella patens* in the study of Canivet et al. [7]. In the first attempt of nanoparticle pollution biomonitoring [8], authors analyzed amount of silver in bryophyte species *Brachythecium rutabulum* and *Hypnum cupressiforme* - species occasionally used in biomonitoring surveys - and found significantly more accumulated silver in bryophytes growing close to the nano-Ag production plant than in those growing elsewhere. Nevertheless, the specific relationship between the concentration of the nano-sized pollutant in the environment and in the presumptive biomonitor was not uncovered.

The aim of this study was to find the accumulation rates of nano-ZnO and nano-TiO_2 exposed to the bryophyte species commonly used in biomonitoring - *Hylocomium splendens* and, thus, assess its prospects in nanoparticle-related pollution biomonitoring.

2. MATERIALS AND METHODS

Bryophyte material of the *Hylocomium splendens* species was collected in the area of Beskydy Protected Landscape Area (Czechia), in the altitude of approximately 520 m a.s.l. close to the border with Slovakia; the site was chosen for both its distance of the pollution sources and abundance of the species. *Hylocomium splendens* is one of the species recommended by the European moss biomonitoring manual [9] and proved to be viable biomonitor in the preceding study [10]. Only apical segments (2-4 cm) of the moss gametophytes were collected [11] Boquete et al., 2014, vinyl gloves were used for the manipulation of the material. Collected material was transferred to the laboratory in plastic bags, then washed with distilled water in order to remove adhering matter and dried to constant weight under laboratory conditions while being spread on blotting paper.

Nano-ZnO and nano-TiO_2 suspensions in deionized water were prepared in 1 $g.l^{-1}$ concentrations using ultrasound (30 minutes), the quality of the suspension was assessed by turbidimetry, pH was measured before and after application of the ultrasound to ensure no chemical alteration of the suspensions. Nano-ZnO and nano-TiO_2 particles preparation and characterization were described in details in Mamulová Kutláková et al. [12] and Seidlerová et al. [13].

Homogenized material was divided into samples of roughly 0.5 g of dry weight whilst first two were immediately placed to desiccator later to be analyzed, the rest was placed in plastic Petri dishes. All samples were then subjected to 5 ml of the suspension - either nano-ZnO or nano-TiO_2 - and, on a weekly basis, two samples, one from each set, were collected and placed to desiccator until the time of analysis. Due to less moss material available when assessing nano-TiO_2 exposition, these samples were collected first after two weeks of exposition and then only six more times; samples exposed to nano-ZnO were collected weekly for the total of eight weeks.

After the exposition of all of the material, the samples were washed in distilled water for 30 s to remove the particles adhering to the surface of the thalli; 30 s is the maximum time recommended because prolonged exposition to distilled water may lead to the disruption of the plant cell wall [14], dried to constant weight at 50 °C [15], and pulverized. Both original moss and exposed samples were decomposed thermally in concentrated HNO_3 (p.a.) for the samples exposed to nano-ZnO suspension and H_2SO_4/HNO_3 (p.a.) mixture for those exposed to nano-TiO_2 suspension. Concentrations of Zn and Ti in the samples were determined using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, Spectro Ciros Vision EOP, Germany). Linear models of accumulation were constructed and visualized in R version 3.1.2 [16], as were all the statistical analyses.

3. RESULTS AND DISCUSSION

ICP-AES measurements proved that the amount of the determined metals in the original moss material was trivial (< 0.01 % for both the metals) which supported the assumption that it, indeed, originated from an unpolluted site. For the exposed samples, the amount of the particular metal distinctly followed linear trend depending on time of the exposition, the linearity was found to be statistically significant ($\alpha < 0.05$). The trends are presented in **Figure 1** for the samples exposed to the suspension of nano-ZnO and in **Figure 2** for the samples exposed to the suspension of nano-TiO_2. There was no apparent visual difference between the trends nor were the calculated coefficients of determination substantially different (nano-ZnO: $R^2 = 0.9623$; nano-TiO_2: $R^2 = 0.9503$). The similarity of the trends was confirmed by performing analysis of covariance (ANCOVA); judging from its outcome, the null hypothesis of their similarity cannot be rejected ($p = 0.684$). Not only was the trend similar, the accumulated absolute amounts of the metals were also not dissimilar - paired t-test did not reject the null hypothesis of them being from the same distribution (p = 0.2873).

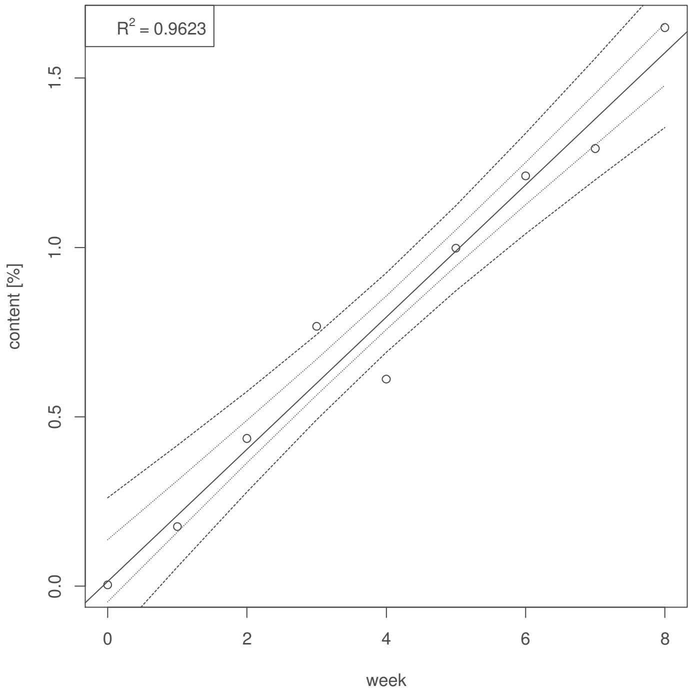

Figure 1 Concentration of zinc in the thalli of *Hylocomium splendens* depending on the time of exposition

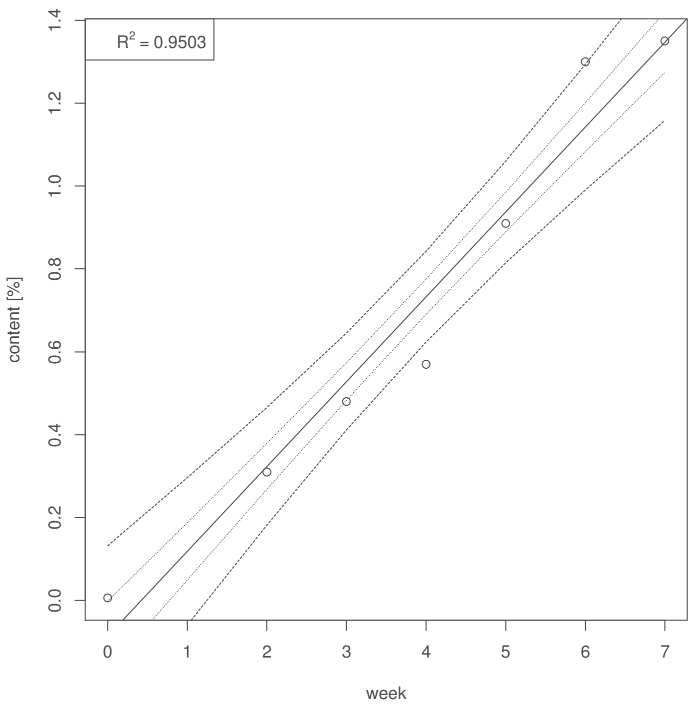

Figure 2 Concentration of titanium in the thalli of *Hylocomium splendens* depending on the time of exposition

It is apparent that both the nanomaterials introduced to the bryophyte samples led to steady and predictable accumulation of it in the bryophyte thalli. Since the samples were washed prior analysis, it was sufficiently assured that the metal determined would be, indeed, only the one penetrating the thalli and accumulated in the intracellular and intercellular space of the moss. *Hylocomium splendens* material showed no apparent preference of accumulation regarding the particular nanomaterial and can be, hence, used in future biomonitoring surveys dealing with - at least - the two metal oxide nanomaterials hereby assessed.

However, two major objections arise. First, since this was a pilot study, the nanomaterial was introduced to the moss thalli in environmentally rather unreasonable concentration and second, the nanomaterial introduced to the moss thalli was suspended in water. Environmentally more plausible concentrations are yet to be tested, nevertheless, this should not be a difficult task to perform. Principal issue is, however, elsewhere: though mosses do absorb their nutrients as well as contaminants from the atmospheric moisture, the design using suspended particles in water is far from ideal in that it does not account for dry deposition which would be much more challenging to simulate.

4. CONCLUSION

The study presents first attempt to assess the possibility of metal oxide nanoparticle pollution biomonitoring by the means of the moss *Hylocomium splendens*. The results indicate that the use of this moss in nano-biomonitoring is both reasonable and recommended since the concentrations of the particular metals in the moss thalli was significantly dependent on the time of exposition of the moss samples to the nanosized metal oxide. The linearity and high coefficients of determination of the obtained accumulation trends allows accurate determination of the presence of the pollutant in the environment from the analysis of the moss thallus. Further study is needed both in terms of environmentally more reasonable amounts of nanomaterial introduced to the presumptive biomonitor and possible simulation of dry deposition together with assessment of its impact on both the bryophyte physiology and accumulation rates.

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