FACTORS AFFECTING DISTRIBUTION OF Zn IN THE MOSS THALLI AFTER NANO-ZnO EXPOSURE

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Abstract

Samples of the moss *Pleurozium schreberi* were subjected to the nano-ZnO suspension under various conditions and the distribution of the accumulated zinc in their thalli was determined. To assess the presence of the accumulated zinc on the surface of the plant, in the extracellular and intracellular space as well as in the residual fraction, sequential elution technique using distilled water, $NiCl_2$ solution, HNO_3 followed by a total decomposition in acid mix was used. Data were then analysed using multiple Analysis of variance in order to identify the most important factors affecting the accumulation and distribution of Zn in the plant. Among all the applied treatments - washing x not washing, irrigation x no irrigation and oven drying x exsiccation at the ambient temperature - washing and irrigation prior to the exposure affected the Zn distribution the most. At the same time, some of the treatments may have been detrimental to the integrity of the moss cells and, hence, alter the observed distribution of Zn in the samples. In terms of total Zn content accumulated in the moss thalli, not washing following by irrigation prior to the exposure is hereby recommended for biomonitoring studies.

Keywords: Moss, ZnO, nanoparticles, accumulation, sequential elution

1. INTRODUCTION

Although some research has been conducted on the toxicity of metal oxides nanoparticles to plants [1], particular uptake mechanisms, distribution, translocation, and accumulation in the plant body are mostly unknown [2]. Bryophytes are species of which many are being routinely used in air or water pollution assessment [3] making them good model organisms, however, the effects of nanoparticle exposure on bryophytes and the nanoparticles accumulation patterns in their thalli are greatly understudied. No signs of stress or toxicity of iron nanoparticles were found when applied to the bryophyte *Physcomitrella patens* though they entered its thallus (and cells) [4,5]. The accumulated nanoparticles were, however, not quantified - only confirmed by qualitative methods. During the first attempt of nanoparticle pollution monitoring, determined amount of silver in bryophyte species *Brachythecium rutabulum* and *Hypnum cupresiforme* was significantly higher in bryophytes near the nano-Ag production plant than in the background [6]. The particular process of accumulation and capacity of the bryophytes to accumulate the nanoparticles are, thus, still unknown. The presented study aims to assess the distribution of Zn in moss thalli after exposure to nano-ZnO (surface, extracellular, intracellular and residual fraction) and to determine the sample preparation factors affecting the accumulation of zinc in these fractions.

2. MATERIALS AND METHODS

The moss samples of *Pleurozium schreberi* (Brid.) Mitt. species were collected using vinyl gloves in the Beskydy Protected Landscape Area (Czechia), far from the sources of pollution. Only 2-4 cm long apical segments of the gametophytes were collected following the published recommendations [7]. *Pleurozium schreberi* was chosen for its established ability to accumulate nanoparticles and since it is frequently applied in biomonitoring studies [8].

After their transport to laboratory, samples were divided to sixteen groups of treatments, four replications each. The treatment of the sample consisted of either washing or not washing, irrigation or no irrigation one week

prior to the exposure, exposure to nano-ZnO or control and, finally, drying in the oven (50 °C) or at the ambient temperature after the exposure and prior to the analysis. Nano-ZnO suspension (c = 0.1 g.l^{-1}) used for exposure was prepared from nano-ZnO (prepared according to the process further described in Mamulová Kutláková et al. [9]) by sonicating the nano-ZnO in de-ionized water for 30 minutes.

The exposure of the moss samples to the suspension took place for the total of five days, two 2.5 ml exposures per week, for the control samples, the same procedure was followed using pure de-ionized water. After the exposure and drying, sequential elution was applied following Pérez-Llamazares et al. [10] in 3 or (more precisely) 4 steps:

I. Surface fraction - samples (ca 0.5 g) were shaken for 30 s in 50 ml distilled water. Leachates were filtrated, filtrate stabilized and analysed, moss material was dried at ambient temperature.
II. Extracellular fraction - samples were shaken in 50 ml of 20 mmol.l^{-1} $NiCl_2$ in two steps (for 45 and 30 min) between which the extraction agent was renewed. Leachates were filtrated, filtrate stabilized and analysed, moss material was dried at ambient temperature.
III. Intracellular fraction - samples were shaken for 30 min in 1 mol.l^{-1} HNO_3. Samples were filtrated and analysed, moss material dried to constant weight.
IV. Residual fraction - ca 0.25 g of each sample was placed to a PTFE container and thermally dissolved in the mixture of chemicals ($HF+HNO_3+H_2O_2$), filtrated and diluted to a common volume.

After drying, all filtrates and digested residual matter were analysed for Zn content by Inductively coupled plasma atomic emission spectroscopy (ICP-AES).

To find which factors affected the Zn content in the individual thallus fractions of *Pleurozium schreberi* and the total amount of Zn in the thallus, the results were statistically assessed using multiple Analysis of variance (ANOVA) in R programme [11]. Since the results were not normally distributed and, hence, parametric tests could not be applied, the results were logarithmically (common logarithm) transformed prior to the analyses.

3. RESULTS AND DISCUSSION

3.1. Total content

The total Zn content and its distribution was affected by the sample treatment as is apparent from **Figure 1** (Un, W - unwashed, washed; Dr, We - not irrigated; irrigated, N, Y - exsiccated at the ambient temperature, 50 °C oven drying) where only the samples exposed to nano-ZnO suspension are plotted for clarity. Washed and wetted samples had the lowest total Zn content. The highest total Zn concentration observed was in the samples unwashed but irrigated prior to the exposure - approximately 25 % more than the lowest observed concentration. Post-exposure drying of the samples had a negative effect on the total Zn amount in most of the cases except for the irrigated moss samples.

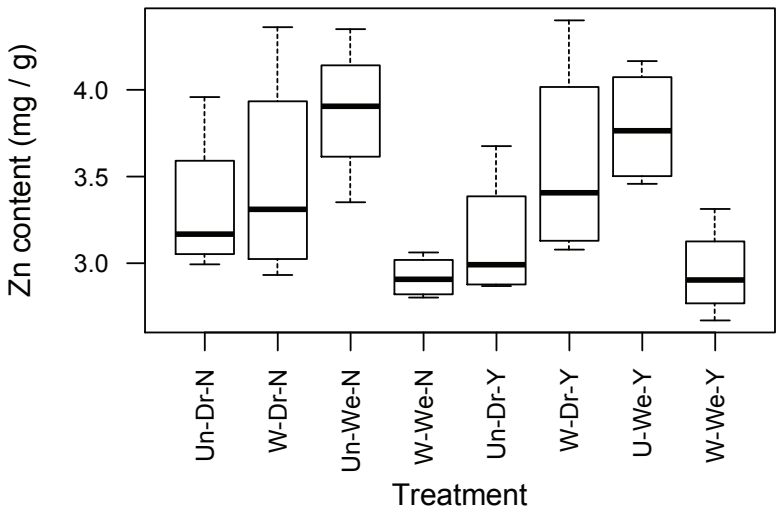

Figure 1 Boxplots of the total Zn content in moss thalli depending on the treatment

3.2. Fractions

The effect of the individual treatments as well as their combinations on the Zn amount in the thallus fractions (and total amount) was assessed using multiple Analysis of variance (ANOVA) the results of which are presented in the **Table 1**. Unsurprisingly, the concentration of the zinc was affected the most by the exposure itself - in all the fractions as well as in the case of the total assessment. In the unexposed samples, the zinc concentration was negligible and, although it proved the pristine status of the collection site, were thus excluded from the figures hereby presented.

Table 1 Effect of the treatments and their combination on Zn content in the moss thalli fractions

	Surface	Extracellular	Intracellular	Residual	Total
NP	***	***	***	***	***
washing	***	-	***	*	-
irrigation	-	***	***	**	***
oven dried	-	-	-	-	-
NP:washing	***	***	***	-	-
NP:irrigation	***	***	***	*	***
washing:irrigation	***	-	**	.	-
NP:oven dried	-	-	-	-	-
washing:oven dried	-	-	-	-	-
irrigation:oven dried	-	-	-	*	-
NP:washing:irrigation	-	***	-	-	*
NP:washing:oven dried	-	-	-	-	-
NP:irrigation:oven dried	-	-	-	-	-
washing:irrigation:oven dried	-	-	-	-	-
NP:washing:irrigation:oven dried	-	-	-	-	-

Significance codes: '***' 0.001; '**' 0.01; '*' 0.05; '.' 0.1; '-' no significance

In the intracellular and surface fractions, the highest Zn content was found in washed unirrigated samples, less in unwashed irrigated, unwashed unirrigated and the least was found in washed irrigated samples. In the samples not subjected to the oven-drying procedure after the exposure, more Zn was found on the surface and in the extracellular spaces of the thallus than in the samples oven-dried (with the exception of the unwashed irrigated samples). In the extracellular space, the concentration of Zn was the highest - approximately 20x higher than on the surface and two times more than in the moss cells. Overall, the samples that were irrigated prior to the exposure tended to have higher Zn content in their cells - phenomenon also, interestingly, observed in the control samples. Highest cellular content of Zn was observed in unwashed, irrigated samples, then in both washed irrigated and unirrigated, and, finally, in the unwashed unirrigated, the Zn cellular content was the lowest. Decrease of cellular Zn content was observed consistently in all the cases when the samples were unirrigated and then subjected to the post-exposure oven-drying, however, this decrease was found not to be significant - this was also true for the observed increase of Zn in all the irrigated samples when subjected to oven-drying. The graphical representation of the example of the relationships

between the Zn content in the thallus fractions (intracellular and extracellular) of the exposed samples depending on the varying sample treatment is presented in the form of a conditional plots (**Figure 2** and **3**).

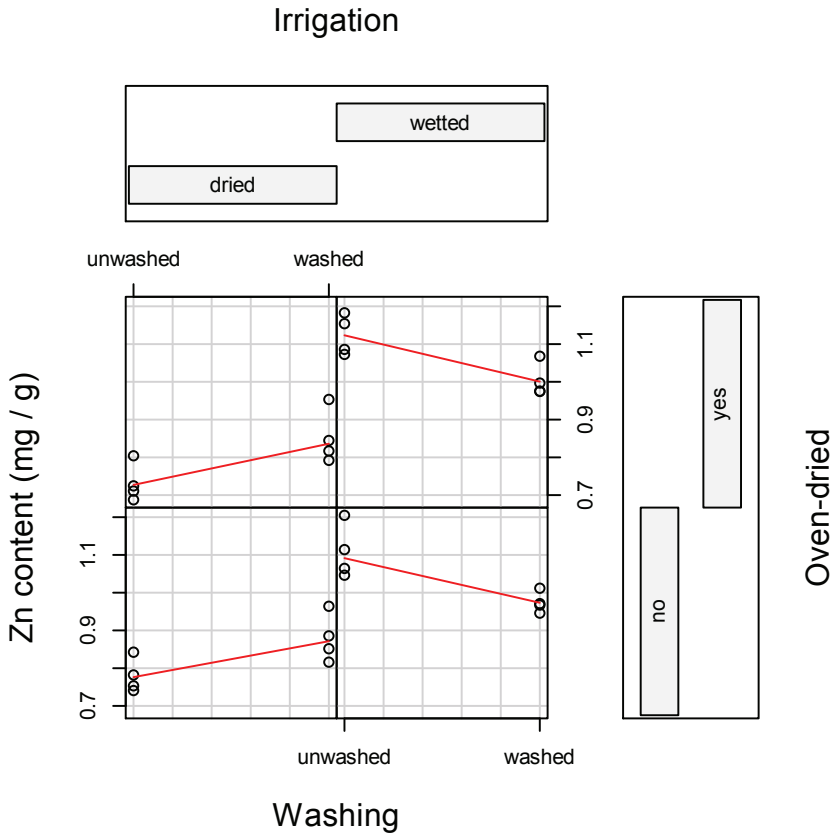

Figure 2 Conditional plot of Zn content in the intracellular fraction of the exposed samples

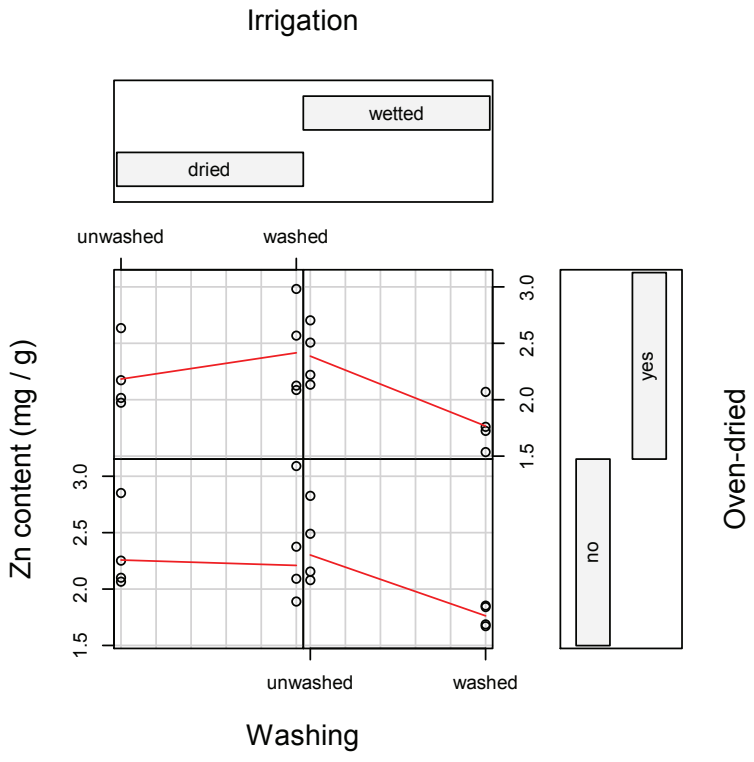

Figure 3 Conditional plot of Zn content in the extracellular fraction of the exposed samples

It has to be noted that the amount of Zn in the particular fractions of the thallus differed among treatments, hence, it is conceivable that the treatments led to disruption of the cell membranes.

4. CONCLUSIONS

Zn from ZnO nanoparticle exposure clearly penetrated both the thalli and the cells of the moss although, using the hereby applied analytical method (ICP-AES), it was impossible to assess whether it remained in the metal oxide nanoparticle form. Both the total and the localized Zn content in moss thalli was altered by the sample treatment prior to the exposure. Nano-ZnO exposure leads to differing Zn content in thalli fractions in *Pleurozium schreberi*. The most important factors affecting the Zn content both in the individual thallus fractions and in the whole thallus were washing and irrigation prior to the exposure.

From the practical, biomonitoring, point of view, the most favourable treatment of the exposed moss samples is the one bringing the highest yield of the monitored element, hence, not to wash and irrigate the moss prior to the exposure seems is to be recommended. However, for decision on the proper treatment when the distribution of the element is of the interest such as when the uptake and translocation of the nanoparticles in the plant is the aim of the study, the question of the preservation of the cell membrane integrity of the used moss plant has yet to be assessed since the obtained results suggest it may be a prominent issue.

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