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Comparative Toxicity of Heavy Metals Cd, Pb, and Zn to Three Acrocarpous Moss Species using Chlorophyll Contents

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Abstract

Mosses have often been used for biomonitoring because of their diversity of habitats, structural simplicity, and rapid multiplication rate. This research aimed to study the tolerance of heavy metal in 3 species of terricolous mosses i.e., *Barbula consanguinea*, *Hyophila apiculata*, and *H. involuta.* The gametophores of mosses were immersed at 4 concentrations of each of the heavy metals Cd, Pb, and Zn as well as in controls with no heavy metal. After 10, 20, and 30 days of exposure, mosses were extracted for chlorophyll-a in ethanol. The extracted chlorophyll-a was analyzed by spectrophotometer at 664 nm. The results revealed that the amount of chlorophyll-a in all species decreased with increasing concentrations of Cd, Pb, and Zn. The degree of metal toxicity for all species was Cd > Pb > Zn. By comparing the ratio of extracted chlorophyll-a in heavy metal-treated mosses to chlorophyll-a extracted in control mosses, the tolerance against 3 heavy metals in 3 mosses was *H. involuta* > *B. consanguinea* > *H. apiculata*. Possibly, *H. involuta* can be used for biomonitoring of heavy metals in contaminated environments in the future.

Keywords: Bryophyte, Pollutants, Cadmium, Zinc, Lead

Introduction

Heavy metals refer to naturally occurring metals having an atomic number (Z) greater than 20 and an elemental density greater than 5 g cm^{-3} [1]. Some of them are metabolically essential for the normal functioning of organisms, but if deficient or excessive, they can cause physiological stress and have adverse impacts [2]. Other heavy metals such as lead (Pb), cadmium (Cd), aluminum (Al), and mercury (Hg) are harmful if present in organisms at all concentrations [2]. Heavy metals are present in the soil as natural components (natural background levels) that are found at various background levels throughout the world due to their various concentrations in the bedrock. For example, Ni, Cr, and Co are abundant in serpentine soils, whereas Zn, Pb, and Cd are high in calamine soils [3]. Over thousands of years, humans have dispersed heavy metals through various activities such as fertilization, mining, automobile combustion, industrial waste disposal, secondary metal production and recycling, and energy production and emissions from power stations [4], causing elevated levels of heavy metal contamination in soil and water [5]. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb, and Ni [3]. Their adverse effects on human health have been known for a long time [4]. Arsenic, cadmium, chromium, lead, and mercury rank among the priority public health threats [6]. Heavy metals are significant pollutants, once introduced into the environment they are difficult to remove and tend to accumulate in plant and animal tissues through the trophic level [7-8].

Bryophytes have been used for monitoring atmospheric heavy metal pollution in northern Europe since 1968 due to their wide distribution [9] and the ability to accumulate heavy metals in large amounts [10], usually greatly surpassing the absorbing capacity of vascular plants [11]. Some bryophytes were used as bioindicators because of their tendency to grow on substrates containing certain heavy metals [12]. Some bryophytes such as liverwort *Lunularia cruciata* [13] and moss *Taxithelium nepalense* [14] are used as important models for investigating heavy metal toxicity because of their relative morphological, anatomical, and genomic simplicity [7].

Bryophytes were the first green plants to colonize the terrestrial environment [15], for this reason, they evolved mechanisms to cope with elevated amounts of heavy metals present on land [16]. The lack of the cuticle layer on the cell walls, the absence of a root system, high ion-exchange properties, and a large surface-to-weight ratio explain their propensity to react faster to heavy metals than most vascular plants [7] absorbing and retaining ions in high concentrations [17]. Little is known about the maximum tolerance levels of bryophytes for individual heavy metals. It appears that the majority of mosses have an innate tendency for accumulating at least some heavy metals such as Fe, Pb, Zn, and Ni, from the substrate and the atmosphere [11]. For this reason, more toxicological studies in strictly controlled environments are needed to investigate to physiological responses at different exposure levels. The chlorophyll content is an often used parameter for assessing the physiological status and biological activity in plants [18]. Although many studies have investigated heavy metal concentrations in mosses, there are a limited number of experimental studies concerning the effects of heavy metals entering the cells, to determine the threshold for toxic effects [19], and few of these apply chlorophyll concentration as a measure of the stress response [7].

The aim of this research was to investigate the patterns of the chlorophyll content in 3 acrocarpous mosses i.e., *Barbula consanguinea*, *Hyophila apiculata*, and *H. involuta*, in response to exposure to 3 different heavy metals, cadmium (Cd), zinc (Zn), and lead (Pb) in solution under laboratory conditions. We focus on these metals because they are frequently found to contaminate soils concomitantly as a result of mining activity [20].

Materials and methods

Moss species, moss collection, and moss acclimation

We chose 3 common species of acrocarpous mosses i.e., *Barbula consanguinea*, *Hyophila involuta*, and *H. apiculata* for this study. *Barbula consanguinea* frequently grows on damp soils and rocks throughout tropical Asia. *Hyophila involuta* grows on humid silt and rocks throughout tropical and sub-tropical Asia and *H. apiculata* grows on soils under the shade of trees throughout Australia, Brazil, Indonesia, Malaysia, and Thailand [21]. Gametophores of these 3 mosses are small to medium-sized, forming short, dense to scattered turfs. The stems are erect, simple to sparsely branch by innovations [22]. All samples were carefully collected from larger moss colonies together with their native substrates (ca. 1 - 2 cm deep from the ground level), with the help of a small knife, and then placed in zipper bags. To avoid ultrastructural changes or cell destruction caused by dehydration, moss samples were brought to the laboratory and transferred carefully to new pots, together with their native soils. Cultures were kept under moist conditions by spraying regularly with tap water, at room temperature (ca. 25 ± 2 °C), with a 12:12 h light: Dark photoperiod for 1 week. Subsequently, gametophores were cleaned with tap water to remove dirt/soil particles, rinsed twice with distilled water to get rid of ions carried by the mosses or tap water [19], placed on a sterile Petri dish filled with 10 mL of distilled water at room temperature (ca. 25 ± 2 °C) to acclimatize, with a 12:12 h light: Dark photoperiod for 3 - 5 days prior to the onset of heavy metal treatments.

Preparation of heavy metal solutions

Cd solutions were prepared by diluting a cadmium standard solution containing $Cd(NO_3)_2$ in HNO_3 0.5 mol/l to 4 concentrations at 0.05, 0.5, 5, and 50 mg/l, respectively. Pb solutions were prepared by diluting the lead standard solution containing $Pb(NO_3)_2$ in HNO_3 0.5 mol/l to 4 concentrations at 0.05, 0.5, 5, and 50 mg/l, respectively. Zn solutions were prepared by diluting the zinc standard solution containing $Zn(NO_3)_2$ in HNO_3 0.5 mol/l to 4 concentrations at 0.1, 1, 10, and 100 mg/l, respectively. The Zn solutions were doubled compared to Cd and Pb because Zn is generally a trace element for plant metabolism and is thereby expected to be toxic at higher concentrations [23]. Next, 10 mL of all Cd, Pb, and Zn solutions were pipetted onto individual sterile Petri dishes in triplicate for each species. As a control, 10 mL of distilled water was pipetted onto separate Petri dishes, also in triplicate. In total, 117 Petri dishes were prepared for the experiments (4 conc.×3 rep×3 species for Cd + 4 conc.×3 rep×3 species for Pb + 4 conc.×3 rep×3 species for Zn + control×3 rep×3 species = 117 Petri dishes).

Heavy metal treatments of gametophore samples

Ten gametophores (10 gametophores×0.5 g wet weight = 5 g) were placed in each Petri dish filled with the heavy metal solution as well as Petri dishes with distilled water (=control) (39 Petri dishes×10 gametophores×3 species = 1,170 gametophores). All Petri dishes of treated gametophores were kept in the culture at ambient temperature with a light intensity of 37.35 ± 8.52 µmol/s/m^2. A random gametophore was picked out from each replicate Petri dish on days 10, 20, and 30, respectively. The gametophore was placed onto tissue paper to remove excess heavy metal solution or excess water. The gametophore was thoroughly rinsed with distilled water, placed onto tissue paper to get rid of excess water, and weighed using a digital weighing scale (Sartorius, model QUINTIX) to obtain 0.5 g of wet weight/gametophore. If the wet weight deviated greatly from 0.5 g, it was replaced by a new gametophore from the same Petri dish.

Pigment extraction and absorbance measurement

To assess the effects of heavy metals on chlorophyll contents in the moss *Barbula consanguinea, Hyophila involuta*, *H. apiculata,* and gametophores were subjected to pigment extraction and absorbance measurement. The untreated and treated gametophores were rinsed with distilled water several times and placed onto tissue paper to blot excess water or heavy metal solution. Each gametophore was placed into a 1.5 mL microcentrifuge tube, with the addition of 1 mL of 99.9 % absolute ethanol (Qrec). Each microcentrifuge tube was tightly closed and gently shaken to allow the gametophore to be thoroughly immersed in the absolute ethanol at 20 °C for 8 h at room temperature and then centrifuged with a Denville Micro 260D Microcentrifuge at 10,000 rpm for 1 min. 250 µl of the supernatant was pipetted into a microplate and analyzed for the absorbance of the extracted pigment with a microplate reader (BMG LabTech, model SPECTRO star Nano) at wavelengths ranging from 400 - 700 nm. Graphs of the relationship between the absorbance and the wavelength (absorption spectra) from the controls were plotted in **Figure 1**, showing that the extracted pigment was likely Chlorophyll-a (with peaks 436, 470, and 664 nm). In the chlorophyll content analysis in this study, the absorbance of the extracted pigment was subsequently measured at a wavelength of 664 nm.

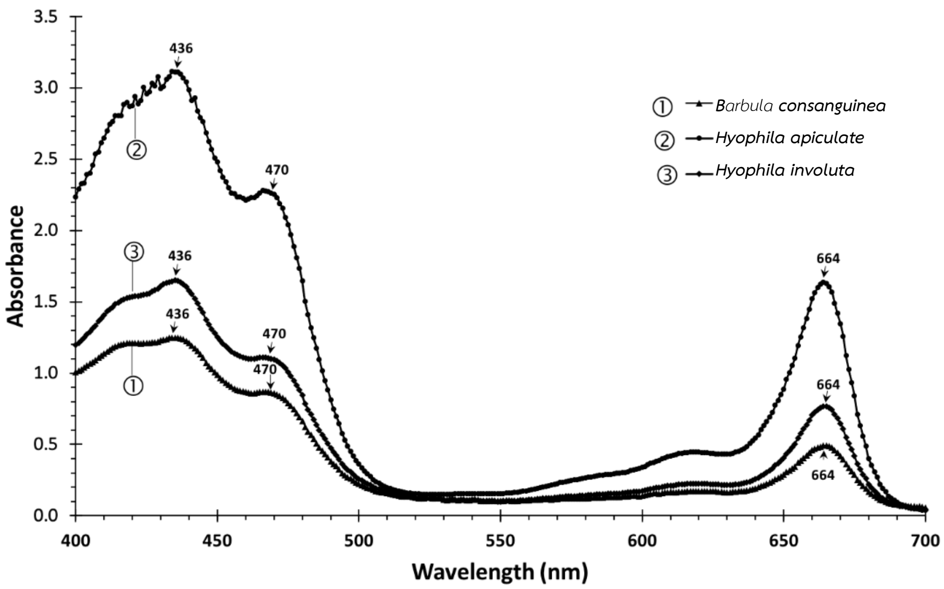

Figure 1 The absorption spectra of ethanol-extracted pigment in the 3 moss species.

Statistical analyses

The absorbance at 664 nm wavelength of pigment (chlorophyll) extracted by ethanol for 8 h from the treated and untreated gametophores was used to determine the relative chlorophyll absorbance ($R_{m/c}$) applying the following mathematical formula:

$$R_{m/c}(\%) = \frac{OD_{664}\text{of extracted chlorophyll in metal-treated mosses}}{OD_{664}\text{of extracted chlorophyll in the control}} \times 100$$

The differences in chlorophyll absorbance ratio were compared between i) gametophores exposed to different concentrations of heavy metals, ii) gametophores exposed to different times of exposure, and iii) gametophores of 3 moss species. The differences in chlorophyll absorbance ratio were compared using one-way analyses of variance (ANOVA). If the ANOVA was significant, Tukey's test was used to calculate the smallest significance between 2 means at a 0.05 significance level. All statistical analyses were run using statistical software, SPSS version 18.0 (SPSS Inc., Chicago, USA) at 95 % confidence.

Results and discussion

The effects of metals on chlorophyll contents

Exposure of 3 mosses i.e., *B. consanguinea*, *H. apiculata*, and *H. involuta* to 3 heavy metals i.e., Cd, Pb, and Zn under laboratory conditions resulted in a decrease in chlorophyll-a contents, with increased in heavy metal concentrations and time of exposure (**Tables 1 - 3**). A decrease in chlorophyll-a contents in moss species after exposure to various concentrations of Cd, Pb, and Zn at different times of exposure confirms that the toxicity of Cd to gametophores is higher than Pb, and Zn at all concentrations for 10, 20, and 30 days of exposure. These observations suggested Cd has the greatest toxic effect and is much stronger than that of Pb and Zn in reducing chlorophyll-a contents in the 3 mosses. Although the treatment with Zn had a 2-fold higher concentration than those of Cd and Pb (**Tables 1 - 3**), the chlorophyll-a contents of Zn-treated gametophores were higher, compared to gametophores treated with Cd, and Pb, particularly for *H. apiculata* and *H. involuta* (**Tables 2** and **3**).

Table 1 Relative chlorophyll absorbance of *B. consanguinea* exposed to different concentrations of heavy metals for 10, 20, and 30 days.

Heavy metal concentration	Relative chlorophyll absorbance at 664 nm (%) at different exposure time (Mean ± SD; n = 3)		
	10 days	20 days	30 days
Cd concentration (mg/l)			
0	100.0 ± 4.7 ^{a,A}	100.0 ± 0.8 ^{a,A}	100.0 ± 13.6 ^{a,A}
0.05	25.9 ± 0.9 ^{b,A}	22.3 ± 1.0 ^{b,B}	22.7 ± 0.5 ^{b,B}
0.5	23.7 ± 0.8 ^{b,A}	19.7 ± 1.0 ^{b,B}	18.1 ± 0.8 ^{b,B}
5	21.9 ± 0.5 ^{b,A}	16.4 ± 1.0 ^{c,B}	15.3 ± 0.8 ^{b,B}
50	19.9 ± 1.6 ^{b,A}	14.2 ± 1.4 ^{c,B}	13.7 ± 0.8 ^{b,B}
Pb concentration (mg/l)			
0	100.0 ± 4.7 ^{a,A}	100.0 ± 0.8 ^{a,A}	100.0 ± 13.6 ^{a,A}
0.05	55.3 ± 4.5 ^{b,A}	48.5 ± 2.7 ^{b,AB}	44.5 ± 1.7 ^{b,B}
0.5	39.9 ± 2.4 ^{c,A}	36.7 ± 0.8 ^{c,AB}	33.0 ± 0.5 ^{bc,B}
5	35.8 ± 1.0 ^{cd,A}	34.1 ± 2.5 ^{c,A}	31.9 ± 1.0 ^{bc,A}
50	29.8 ± 0.7 ^{d,A}	26.6 ± 0.7 ^{d,B}	25.7 ± 1.9 ^{c,B}
Zn concentration (mg/l)			
0	100.0 ± 4.7 ^{a,A}	100.0 ± 0.8 ^{a,A}	100.0 ± 13.6 ^{a,A}
0.1	33.7 ± 0.4 ^{b,A}	32.3 ± 1.5 ^{b,A}	30.5 ± 1.9 ^{b,A}
1	27.5 ± 0.4 ^{bc,A}	26.5 ± 0.5 ^{c,A}	26.6 ± 1.1 ^{b,A}
10	19.7 ± 4.0 ^{c,A}	19.6 ± 3.1 ^{d,A}	19.5 ± 1.3 ^{b,A}
100	19.2 ± 3.3 ^{c,A}	17.3 ± 1.4 ^{d,A}	17.4 ± 1.5 ^{b,A}

^{a,b,c,d}within columns, values with different superscript letters are significantly different ($p < 0.05$) according to Tukey's test.

^{A,B}within rows, values with different superscript letters are significantly different ($p < 0.05$) according to Tukey's test.

Table 2 Relative chlorophyll absorbance of *H. apiculata* exposed to different concentrations of heavy metals for 10, 20 and 30 days.

Heavy metal concentration	Relative Chlorophyll Absorbance at 664 nm (%) at different exposure time (Mean ± SD; n = 3)		
	10 days	20 days	30 days
Cd concentration (mg/l)			
0	100.0 ± 5.7 ^{a,A}	100.0 ± 5.7 ^{a,A}	100.0 ± 15.9 ^{a,A}
0.05	21.7 ± 4.6 ^{b,A}	19.3 ± 1.8 ^{b,A}	18.8 ± 1.7 ^{b,A}
0.5	19.8 ± 1.7 ^{b,A}	7.8 ± 0.2 ^{c,B}	7.2 ± 0.3 ^{b,B}
5	6.7 ± 0.6 ^{c,A}	6.5 ± 0.4 ^{c,A}	5.8 ± 0.5 ^{b,A}
50	6.0 ± 0.2 ^{c,A}	5.4 ± 0.9 ^{c,A}	4.7 ± 0.2 ^{b,A}
Pb concentration (mg/l)			
0	100.0 ± 5.7 ^{a,A}	100.0 ± 5.7 ^{a,A}	100.0 ± 15.9 ^{a,A}
0.05	22.0 ± 3.0 ^{b,A}	18.8 ± 1.4 ^{b,A}	17.1 ± 1.7 ^{b,A}
0.5	15.1 ± 2.0 ^{bc,A}	14.1 ± 0.3 ^{bc,A}	10.7 ± 0.7 ^{b,B}
5	14.3 ± 3.5 ^{bc,A}	12.2 ± 0.7 ^{bc,A}	9.8 ± 0.2 ^{b,A}
50	11.8 ± 2.0 ^{c,A}	7.9 ± 0.8 ^{c,B}	5.7 ± 0.7 ^{b,B}
Zn concentration (mg/l)			
0	100.0 ± 5.7 ^{a,A}	100.0 ± 5.7 ^{a,A}	100.0 ± 15.9 ^{a,A}
0.1	89.0 ± 3.3 ^{a,A}	76.9 ± 5.3 ^{b,A}	69.4 ± 12.4 ^{b,A}
1	60.8 ± 7.3 ^{b,A}	46.1 ± 1.7 ^{c,B}	40.6 ± 4.8 ^{c,B}
10	39.3 ± 5.2 ^{c,A}	35.7 ± 3.0 ^{cd,A}	30.5 ± 1.3 ^{c,A}
100	35.1 ± 9.3 ^{c,A}	27.3 ± 1.7 ^{d,A}	25.0 ± 1.7 ^{c,A}

^{a,b,c,d} within columns, values with different superscript letters are significantly different ($p < 0.05$) according to Tukey's test.
^{A,B} within rows, values with different superscript letters are significantly different ($p < 0.05$) according to Tukey's test.

Table 3 Relative chlorophyll absorbance of *H. involuta* exposed to different concentrations of heavy metals for 10, 20 and 30 days.

Heavy metal concentration	Relative Chlorophyll Absorbance at 664 nm (%) at different exposure time (Mean ± SD; n = 3)		
	10 days	20 days	30 days
Cd concentration (mg/l)			
0	100.0 ± 2.2 ^{a,A}	100.0 ± 19.4 ^{a,A}	100.0 ± 14.6 ^{a,A}
0.05	61.3 ± 5.7 ^{b,A}	46.7 ± 1.6 ^{b,B}	40.2 ± 0.7 ^{b,B}
0.5	50.8 ± 4.9 ^{b,A}	42.2 ± 2.9 ^{bc,B}	35.3 ± 1.2 ^{bc,B}
5	33.5 ± 11.0 ^{c,A}	21.2 ± 1.5 ^{cd,A}	18.8 ± 1.4 ^{cd,A}
50	20.7 ± 3.6 ^{c,A}	17.7 ± 0.7 ^{d,A}	15.2 ± 0.5 ^{d,A}

Heavy metal concentration	Relative Chlorophyll Absorbance at 664 nm (%) at different exposure time (Mean ± SD; n = 3)								
	10 days			20 days			30 days		
Pb concentration (mg/l)									
0	100.0	±	2.2 ^{a,A}	100.0	±	19.4 ^{a,A}	100.0	±	14.6 ^{a,A}
0.05	77.0	±	5.8 ^{b,A}	62.4	±	4.2 ^{b,B}	56.7	±	1.7 ^{b,B}
0.5	51.3	±	3.9 ^{c,A}	44.0	±	0.7 ^{bc,B}	37.0	±	0.9 ^{c,C}
5	44.6	±	3.9 ^{cd,A}	38.6	±	0.3 ^{bc,B}	32.5	±	1.1^{c,C}
50	38.4	±	1.4 ^{d,A}	27.2	±	1.5 ^{c,B}	24.1	±	1.0 ^{c,B}
Zn concentration (mg/l)									
0	100.0	±	2.2 ^{a,A}	100.0	±	19.4 ^{a,A}	100.0	±	14.6 ^{a,A}
0.1	76.1	±	3.2 ^{b,A}	63.6	±	6.8 ^{b,B}	52.0	±	3.6 ^{b,B}
1	61.0	±	8.1 ^{c,A}	52.4	±	3.8 ^{bc,AB}	44.0	±	2.6 ^{bc,B}
10	59.4	±	3.2 ^{c,A}	49.3	±	2.3 ^{bc,B}	42.8	±	1.1 ^{bc,C}
100	40.8	±	5.5 ^{d,A}	32.4	±	0.8^{c,B}	28.5	±	1.4^{c,B}

^{a,b,c,d}within columns, values with different superscript letters are significantly different ($p < 0.05$) according to Tukey's test.
^{A,B,C}within rows, values with different superscript letters are significantly different ($p < 0.05$) according to Tukey's test.

A similar chlorophyll decline induced by heavy metals is also reported in various studies of bryophytes such as in aquatic moss *Fontinalis antipyretica* exposed to Cu [18], mosses *Thuidium delicatulum* (L.) Mitt. and *T. sparsifolium* (Mitt.) Jaeg. and a leafy liverwort *Ptychanthus striatus* (Lehm. & Linderb.) when exposed to Cu, Zn, and Pb [24], moss *Barbula lambarenensis* when immersed in 1,000 and 2,000 ppm of Pb, Cu, Cd, Fe, and V (vanadium) solutions [25], and mosses *Pleurochaete squarrosa* and *Timmiella barbuloides* when exposed to Ni, Pb, and Cr [19]. Detrimental effects of heavy metals on the content of photosynthetic pigments are from i) the inhibition of pigment synthesis [26] and/or ii) oxidative damage to pigments [19]. Excess Cd is responsible for chlorophyll and carotenoid content reduction by inhibiting pigment biosynthesis, leading to a kind of senescence [27], whereas Zn competes with Fe for a site on a particular chlorophyll biosynthetic enzyme in plants [28]. In contrast, Pb has a strong negative effect on chloroplast but most of the Pb is sequestered as phosphate precipitation, which is non-toxic [29]. However, the Pb that is bound to the cell wall is responsible for membrane damage and leakage in bryophytes [30].

Tolerance of heavy metals in bryophytes

In our study, the order of heavy metal tolerance is *H. involuta* > *B. consanguinea* > *H. apiculata* for Cd, *H. involuta* > *B. consanguinea* > *H. apiculata* for Pb, and *H. involuta, H. apiculata* > *B. consanguinea* for Zn (**Tables 1 - 3**), respectively. The toxicity level of metals in plants may differ depending on i) innate properties of heavy metals, by which the most common toxicity order is Hg > Cu > Cd > Ag > Pb > Zn [31], and ii) plant-specific properties [32], where one heavy metal may be more toxic to certain species or group of species than the other [33]. Tolerance to some metals such as Zn can vary widely not only among species but also within species [34]. The samples used in this study were as far as known not exposed to heavy metals prior to the experiment and it would be important to check if these species display selection for increased tolerance in polluted areas. Some heavy metals such as Cd in the environment have no known function in plant metabolism, including bryophytes [23].

The toxicity of metals to bryophyte cells can be expressed in the loss of semipermeable membrane function and therefore plasmolysis [35]. In the treatment of moss *Fontinalis duriaei* with Cd, the cell became plasmolyzed at 100 μg Cd per liter or higher [36]. Petschinger *et al*. [37] determined the metal tolerance of lamina cells to $ZnSO_4$, $ZnCl_2$, and $FeSO_4$ in 3 biomonitoring moss species i.e., *Hypnum* *cupressiforme*, *Pleurozium schreberi*, and *Pseudoscleropodium purum* and 2 metal sensitive species i.e., *Physcomitrium patens* and *Plagiomnium affine* by a viability test involving detection of plasmolysis. They observed that biomonitoring species with long and thin lamina cells and thick cell walls could tolerate better with high levels of metals than species with isodiametric lamina cells and thin cell walls. In our tested mosses, all 3 species are in the same family (i.e., Pottiaceae) where they are similar in terms of lamina cell shape and cell wall thickness. However, the cell shape and cell wall thickness of lamina cells vary depending on the different zone of the leaf. *Hyophila involuta* and *H. apiculata*, with quadrate and thick-walled upper lamina cells and rectangular and thin-walled basal lamina cells, tended to show a relatively higher tolerance to Cd, Pb, Zn concentration than *B. consanguinea*, with quadrate upper lamina cells but elongate-rectangular median and basal cells. Therefore, cell shape as a protoplast-to-wall ratio combined with cell wall thickness could be a good indicator for metal tolerance in bryophytes [37].

Application in biomonitoring programs

Bryophytes are widely used for biomonitoring of atmospheric metal deposition [38], radioisotopes, and multifold chemical pollutants [39] because of their ability to accumulate high concentrations of toxicants without showing physiological impairment [40]. We propose *H. involuta* to be a putative candidate species for biomonitoring of heavy metal contaminated soils in the future. There are 3 reasons why *H. involuta* is beneficial for biomonitoring programs: i) *H. involuta* is a common species thriving on humid soils and rocks throughout tropical and sub-tropical Asia ii) cultivation on soil/ sand substrate in greenhouse conditions is rather easy iii) its reaction norm to concentrations of heavy metals is proportional to the ambient concentrations metal ions over both longer and shorter exposure times. This means that its use in indirect biomonitoring is relatively reliable and cheap in comparison to direct air monitoring which is costly and time consuming. However, we suggest evaluating the capability of heavy metal uptake in *H. involuta* in more detail, including testing whether there is a selection for increased tolerance in polluted areas.

Conclusions

From this study, it can be concluded that the exposure of Cd, Pb, and Zn to mosses *Barbula consanguinea*, *Hyophila involuta*, and *H. apiculata* induced stress and causes chlorophyll-a loss. In comparison among the 3 heavy metals, Cd seems to be the most highly toxic in terms of chlorophyll-a content reduction, followed by Pb and Zn. *Hyophila involuta* is the most tolerant species against heavy metals.

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