Elsevier Editorial System(tm) for Science of the Total Environment

Manuscript Draft

Manuscript Number: STOTEN-D-19-17986R1

Title: Matching times: trying to improve the correlation between heavy metal levels in mosses and bulk deposition

Article Type: Research Paper

Keywords: cadmium; mercury; lead; air pollution; passive and active biomonitoring.

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Abstract: Abstract
The relationship between the concentrations of metals in moss tissues and atmospheric deposition is highly complex, resulting in a general lack of correlations between these two matrices. Here, we tried to improve the significance of the moss-bulk deposition relationship by eliminating the mismatch between the time that the moss tissue selected for analysis is exposed to atmospheric deposition, and the time during which bulk deposition is collected. For this, we analysed the concentrations of Cd, Hg and Pb in new grown tissue of Pseudoscleropodium purum and bulk deposition collected monthly, for one year, in 21 sampling sites (SS) under different degrees of pollution. Additionally, we assessed how different moss tissues, including native moss (green parts and new grown tissues of P. purum) and moss transplants of Sphagnum denticulatum, reflect bulk deposition to find out which moss tissues provide a better estimate of the atmospheric deposition of heavy metals. First of all, our results showed that eliminating the mismatch between native moss exposure time and bulk deposition collection period is not enough to improve their correlation. Environmental variation emerged as the main driver of tissue content variation altering the moss-bulk deposition relationship unpredictably. Secondly, native P. purum represents bulk deposition values better than devitalized transplants by displaying a greater number of significant correlations with bulk deposition. Specifically, green parts of P. purum generally represent better than new grown tissues. Overall, we conclude that neither native mosses nor transplants are good estimators of atmospheric heavy metal deposition rates. However, they are good qualitative indicators of the atmospheric deposition, by allowing us to differentiate SS subject to a wide range of pollution levels. Additionally, green parts of P. purum, and likely of other mosses with similar growth forms, should be used in passive biomonitoring studies to make results from different studies comparable.

Response to Reviewers: Response to Reviewer #1

1. Following the Reviewer #1's concern about the number of significant correlations and the dependency on the different sample size (i.e. samples grouped by SS vs. samples grouped by time) we have included a sentence clarifying this on page 9, lines 217-223:

"Although the lower sample size of the data when grouped by SS (n=12) could also have some effect in the lower number of significant correlations we do not think this would be the major cause of the general lack of correlation (e.g. in Table 2, correlations between NG and BD for Cd in T7 and T8 are significant despite n=13 and n=11 respectively due to the lack of moss growth in many SS during these sampling periods)".

We believe that this is true because: i) we found significant correlations between new grown tissue and bulk deposition rates for the data grouped by sampling period even with low sample sizes (e.g. in Table 2, the Spearman correlation coefficient between NG and BD is significant for Cd in T7 and T8 despite n=13 and n=11 respectively due to the lack of moss growth during these months in some sampling sites), and found no correlations within the same dataset with large sample sizes (e.g. in Table 2, the Spearman correlation coefficient between NG and BD is not significant for Cd in T5 despite n=18). Hence, we believe that if the correlation is strong, small sample sizes will also yield significant correlations; and ii) we took the data grouped by sampling time (n=21) and randomly sampled 12 data points to calculate the significance of the correlation coefficients with smaller sample size. By doing this, we found slight variations in the number of significant correlations between the two sample sizes. These changes consisted some cases on the loss of significance from n=21 to n=12 (as predicted by the reviewer), but in other cases significance was not only maintained, but the P-value decreased.

2. Reviewer #1 wonders why we compare mean concentrations in mosses with concentrations in bulk deposition, however, in this work we did calculate bulk deposition rates as kg*ha-1, although we generally refer to them as bulk deposition for simplicity. We understand this confusion, so we clarified this throughout the manuscript by making the following changes:

Lines 46, 48, 57, 75, 159, 196, 203, 216, 235, 247: add the word "rates".
Lines 80-81: add "...BD rates calculated from samples...".
Line 144: add the sentence "Bulk deposition rates were calculated as mean (n=3) kg·ha-1 at each SS".
Line 193: modify the sentence to "peak BD rates of the different elements do not often match concentration peaks found in moss tissues".

3. The reviewer states that the uncertainty in the NG data (due to the small size of the section studied) is under-communicated and is only discussed for the results of Pb. Thus, he/she is concerned about an over-interpretation of the results of NG. We would like to say that, although it is true that the moss section corresponding to the NG part is pretty small for some sites at specific sampling periods (especially in summer), we only considered moss sections greater than the error made when cutting the moss shoots (as stated on page 5, lines 121-126). Also, we cut these sections from multiple shoots within each sampling site, obtaining enough tissue to perform reliable chemical determinations whose analytical quality were satisfactory (page 6, lines 150-156). Hence, we do trust our results. A priori, we expected a greater number of significant correlations between NG-BD than between GP-BD regardless of how the data were grouped, given that we minimized the mismatch between the time of

exposure of moss tissues and BD collection period. Our results, nonetheless, showed a greater number of significant correlations between GP-BD (compared to NG-BD) for the data grouped by time of exposure. This demonstrates that GP represent BD rates better than NG. However, we do not believe that this is simply due to the small size of the NG section because when the data are grouped by sampling site, the same NG sections represent BD rates better than GP sections. As we exposed on page 10, lines 241-254, we believe that the effect of environmental variation is greater in GP than in NG which could cause the greater mismatch between the concentrations of metals in GP and BD rates under high levels of environmental variation (i.e. when data are grouped by sampling site). GP have been exposed to more environmental events (rain, drought, cold, etc.) that could potentially alter their metal contents directly (e.g. washing, dilution, etc.) or indirectly (e.g. leading to physiological alterations that affect their uptake capacity). The fact that the NG section was small and not present throughout the whole exposure period, emphasizes the lower effect of the environmental variation in it, and the importance of this factor in the final relationship between concentrations of heavy metals in moss and BD rates. Nevertheless, in order to make this message more compelling, we modified the paragraph on page 10, lines 241-254 as follows:

"Secondly, our results show that GP of P. purum represent BD rates better than NG when the effect of environmental variation is minimized (i.e. data grouped by sampling period). This result could be striking given our a priori expectation that minimizing the mismatch between the time of exposure of moss tissues and BD collection period would improve the correlation between NG-BD rate; however, it emphasizes the complexity of the relationship between moss heavy metal contents and BD rates making native mosses (and also moss transplants) unreliable estimators of atmospheric heavy metal deposition rates. Finally, the fact that NG correlates better with BD than GP when environmental variation is high (data grouped by SS) could be due to the greater effect of environmental variation in GP than in NG. Green parts of P. purum have been exposed to more environmental events (rain, drought, cold, etc.) that the NG (younger section that was not present throughout the whole exposure period) which could alter their metal content directly (e.g. washing, dilution, etc.) or indirectly (e.g. leading to physiological alterations that affect their uptake capacity).".

4. Results from the Scheirer Ray Hare test and Dunn's posthoc tests are presented and discussed at the section 3.3. Differences in the magnitude of the concentrations of Cd, Hg and Pb among moss tissue types from line 286. In fact, this entire section attempts to address the different multivariate statistical tests' results.
To make this clearer, since other future readers might have the same concern, we indicate the name of both non-parametric Scheirer Ray Hare test and Dunn's post hoc test at the section 3.3., on page 11, line 288 and 292 (same name as indicated in the lines 168-173).

5, 6 and 7. We have modified the manuscript as the reviewer suggested

Response to Reviewer #3

1. We removed the acronyms in the Abstract as suggested by the reviewer.

2. The reviewer suggests conducting a more complete literature review in the Introduction section, mentioning specifically, the use of moss transplants in Europe. The major goal of this study is to try to optimize the passive biomonitoring method (not the use of transplants) by means of increasing the positive correlations between bulk deposition rates and tissue concentrations in native moss. Regarding this, we checked the bibliography and haven't found any recent research paper addressing the comparison between the concentration of metals in native moss tissues and bulk deposition rates. All the previously published studies in this topic are referred to in the study of Aboal et al. (2010) (cited on page 2, line 41). However, following the reviewer's suggestion, we included some representative references on the native moss in the introduction section. The following citations are included in line (41).

Berg T., Steinnes E., 1997. Use of mosses (Hylocomium splendens and Pleurozium schreberi) as biomonitors of heavy metal deposition: from relative to absolute deposition values. Environmental Pollution, 98(1):61–71.

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3. Following the reviewer's suggestion, we modified the manuscript to better clarify this part. We've expanded the explanation on page 11, lines 277-284:

"These differences are likely caused by variation in moss exposure related factors that affect differently the four matrices. For instance, different exposure times; GP has longer exposure times than NG and T because most of GP fraction corresponds to the fraction of native moss that was present in the study area before the experiment started; while T has overall longer exposure times than the whole NG portion which only represents the portion grown during the last month. Another example could be a different height of exposure to the environment; T are exposed in bags at ~2.5 m from the floor whereas the native moss is collected at ground level"

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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

Cover Letter

Angela Ares

Okinawa Institute of Science and
Technology Graduate University
1919-1 Tancha, Onna, Kunigami District, Okinawa (Japan)

22th of January of 2020

Dear Dr. Paoletti,

Please consider our manuscript, "**Matching times: trying to improve the correlation between heavy metal levels in mosses and bulk deposition"** for publication as a research article in Science of the Total Environment including reviewer's comments.

Terrestrial mosses have been largely used for quantitative estimation of atmospheric deposition of heavy metals, however, increasing amount of evidences are showing that the relation between the concentration in bulk deposition and moss is more complex than initially thought. This study sheds new light on the type of relation between bulk deposition and different type of moss tissues (i.e. native *Pseudoscleropodium purum*, both green parts and monthly grown parts and transplanted *Spahgnum denticulatum*) collected monthly in 21 sampling sites affected by with different pollution levels. The results obtained in this study will improve the applicability of moss technique by contributing to its harmonization.
We believe this manuscript is appropriate for publication in Science of the Total Environment and will be of interest to the journal's diverse readership.

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. Furthermore, we have no conflicts of interest to disclose.

Please address all correspondence concerning this manuscript to me at angela.arespita@oist.jp
Thank you for your consideration of this manuscript.

Sincerely,

Angela Ares

*Title Page

Matching times: trying to improve the correlation between heavy metal levels in mosses and bulk deposition

Boquete, M.T.[1, 2], Ares, A.[3*], Fernández, J.A.[4], Aboal, J.R.[4]

[1] Estación Biológica de Doñana, CSIC, Avenida Américo Vespucio 25, Isla de la Cartuja, 41092 Sevilla, Spain
[2] Department of Integrative Biology, University of South Florida, 4202 E Fowler Ave, Tampa, FL 33620, United States
[3] Marine Biophysics Unit, Okinawa Institute of Science and Technology Graduate University, Kunigami-gun, Okinawa, 904-0495, Japan
[4] CRETUS Institute, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

*A. Ares is the corresponding author

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Matching times: trying to improve the correlation between heavy metal levels in mosses and bulk deposition

Boquete, M.T.[1, 2], Ares, A.[3*], Fernández, J.A.[4], Aboal, J.R.[4]

[1] Estación Biológica de Doñana, CSIC, Avenida Américo Vespucio 25, Isla de la Cartuja, 41092 Sevilla, Spain
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[*]Corresponding author

Abstract

The relationship between the concentrations of metals in moss tissues and atmospheric deposition is highly complex, resulting in a general lack of correlations between these two matrices. Here, we tried to improve the significance of the moss-bulk deposition relationship by eliminating the mismatch between the time that the moss tissue selected for analysis is exposed to atmospheric deposition, and the time during which bulk deposition is collected. For this, we analysed the concentrations of Cd, Hg and Pb in new grown tissue of *Pseudoscleropodium purum* and bulk deposition collected monthly, for one year, in 21 sampling sites (SS) under different degrees of pollution. Additionally, we assessed how different moss tissues, including native moss (green parts and new grown tissues of *P. purum*) and moss transplants of *Sphagnum denticulatum*, reflect bulk deposition to find out which moss tissues provide a better estimate of the atmospheric deposition of heavy metals. First of all, our results showed that eliminating the mismatch between native moss exposure time and bulk deposition collection period is not enough to improve their correlation. Environmental variation emerged as the main driver of tissue content variation altering the moss-bulk deposition relationship unpredictably. Secondly, native *P. purum* represents bulk deposition values better than devitalized transplants by displaying a greater number of significant correlations with bulk deposition. Specifically, green parts of *P. purum* generally represent better than new grown tissues. Overall, we conclude that neither native

mosses nor transplants are good estimators of atmospheric heavy metal deposition rates. However, they are good qualitative indicators of the atmospheric deposition, by allowing us to differentiate SS subject to a wide range of pollution levels. Additionally, green parts of *P. purum,* and likely of other mosses with similar growth forms, should be used in passive biomonitoring studies to make results from different studies comparable.

Keywords: cadmium; mercury; lead; air pollution; passive and active biomonitoring.

1. Introduction

It has been traditionally accepted that terrestrial mosses enable quantitative estimation of atmospheric heavy metal deposition (e.g. Berg et al., 1995; Harmens et al., 2008). However, after a deep review of all the previously published articles about the topic (e.g. Thöni et al., 1996; Berg and Steinnes, 1997; Schintu et al., 2005; Fowler et al., 2006), Aboal et al. (2010) pointed out that this statement was based on studies not always well handled and whose conclusions must be taken with caution. Since then, only two new studies introducing methodological improvements to the experimental design were done to better understand the relationship between heavy metal concentrations in mosses and bulk deposition (BD) rates; these new results showed a general lack of significant correlations between both native (Boquete et al., 2015) and devitalized transplanted mosses (Ares et al., 2015) and BD rates for some of the most commonly studied heavy metals (i.e. Cd, Cu, Hg, Pb, and Zn).

For native mosses, Boquete et al. (2015) suggested that this absence of significant correlations could be related to a mismatch between the time that the moss tissue selected for analysis had been exposed to atmospheric deposition and the time during which BD had been collected. This is due to the fact that at the time at which BD begins to be sampled, there is a pre-existent moss tissue with its own heavy metal concentration. If the growing period of the tissues analysed and BD measuring period do not match, it is very difficult to obtain significant correlations between their concentrations; in fact, these authors showed that the longer the length of the period studied (i.e. above 3 months) the

higher the significance of the relationship between moss and BD rates. On the other hand, the lack of significant correlations for devitalized transplanted mosses seems to be mostly due to environmental conditions (mainly the abundance of rainfall), as the time of exposure of moss and the BD collection period undoubtedly coincide (Ares et al., 2015). Also, it should be noted that devitalization treatment avoids moss growth during transplant exposure and hence weather conditions will rule the heavy metal burden.

Bearing in mind the previous paragraph, it could be assumed that for native mosses it would be possible to improve the significance of the moss-BD relationship if the comparison is made using only the tissue grown during the period in which BD is collected. In fact, Boquete et al. (2015) found that in *Psedoscleropodium purum* the percentage of significant correlations increased with the number of months in the accumulated BD data for Cd and Cu (e.g. from 58% for the monthly BD to 100% for the three-monthly BD for Cu; this pattern was not so clear for Hg, Pb, and Zn). This increase was explained by the fact that at the end of the sampling period, a greater proportion of the tissue analysed would have been exposed to the atmospheric inputs during the period in which BD was accumulated. Nevertheless, so far, no study has directly compared the concentrations of heavy metals in new grown tissue with the BD collected during the same period. Additionally, it still remains unknown how the different moss tissues, including native moss and moss transplants, reflect BD rates, and what the relationship between concentrations of heavy metals in different moss tissues looks like. This knowledge would improve the moss technique by contributing to its harmonization (if different investigators use the same tissues of the same moss species their results could be directly comparable). In light of this, the main objective of this work is to evaluate to what extent the concentrations of heavy metals in new grown tissues of mosses are related to the BD rates calculated from samples collected during the moss growth period, and to find out which moss tissues reflect better the atmospheric deposition of heavy metals.

2. Material and methods

This study was carried out in Galicia (NW Spain), at 21 sampling sites (SS) affected by different levels of pollution. These included areas close to two steelworks (SS1 and SS8-SS11), one wood-works (SS2), two coal fired power plants (SS3-SS7), an aluminium smelter (SS12-SS14), three peri-urban (SS15-SS17) and four rural areas (SS18-SS21). Bulk deposition and native *Pseudoscleropodium purum* (Hedw.) M. Fleisch were sampled monthly, at the same time that moss bags made with devitalized *Sphagnum denticulatum* Brid were exposed, throughout 1 year (January–December, 2010). Part of the data employed in the present work has been previously published in Ares et al. (2015) and Boquete et al. (2015) (see these papers for more details), but all the results presented here are novel.

2.1. Collection of bulk deposition

Three bulk deposition collectors, consisting of a funnel-bottle combination, were placed in each SS. Sampling was carried out in accordance with the recommendations outlined in the Ambient air quality - standard method for determination of arsenic, cadmium, lead and nickel in atmospheric deposition: UNE-EN-standard 15841:2010 established by the European Committee for Standardization (CEN). After each exposure period an aliquot of bulk deposition was acidified with hyperpure HNO_3 (final concentration of 1%) and stored at -20 ºC until analysis.

2.2. Preparation and exposition of S. denticulatum transplants

Moss bags were prepared with the green parts of *S. denticulatum*. The moss was distributed homogeneously inside flat bags (2.5 mg of moss cm^{-2}), with minimal overlapping and compression of the moss. This was then devitalized by drying in an oven at 120 ºC for 24 h. Finally, the bags were vacuum-packed and stored at -20 ºC until use. At each SS and time, one moss bag was hung from a short carbon fibre peg fixed perpendicularly to a pole at a height of ca. 2.5 m over each BD collector (n=3 moss bags per SS and time).

2.3. Collection of native moss

Three samples of native *P. purum* were collected at each SS, as close as possible to the bulk-deposition samplers (the closest sample was obtained at a distance of between 0 and 312 m from the BD sampler) following Fernández et al. (2015) recommendations. Also, growth measurements were done in this species in order to obtain a sample of tissue grown in between sampling times. Growth was measured in terms of length increments of the main stem (Russell, 1984). Thus, as detailed in Boquete et al. (2014), 100 shoots of *P. purum* growing at each site were collected and groups of ten shoots were tied to a thread (at approximately 1 cm from the apex). Each thread was labelled and identified, and each shoot within each thread was assigned a number from 1 to 10 (from one end to the other). Finally, the shoots were placed within the moss carpet at each SS for the duration of the experiment. The length of each moss shoot was measured monthly by taking a photograph of each thread against a background of graph paper (Fig 1A in Boquete et al., 2014) and using ImageJ (https://imagej.nih.gov/ij/). For each SS, monthly growth was then calculated as the median difference in shoot length between one month and the preceding one. Samples of *P. purum* with length increments <1 mm were not cut from the shoots, as this corresponds with the error committed when cutting the segments, as determined by Fernández et al. (2010). Therefore, length increments <1 mm were considered as no growth for the corresponding site and time period.

Moss samples were cleaned prior to analysis by removing plant remains and other foreign material and, taking into consideration the monthly length increments previously estimated, moss shoots were divided in two sections: i) the apical most tissue grown during the last month (specific for each SS), hereafter named as new grown tissue (NG); and, ii) the remaining shoot section corresponding to the green parts (GP), the weighted weight mean average of both parts was calculated. These two sections were weighted (Mettler Toledo XP26), processed, and analysed individually.

2.4. Processing and chemical analysis

135 The concentrations of Cd, Hg, and Pb in bulk deposition samples were determined by
136 inductively coupled plasma mass spectrometry (ICP-MS – VARIAN 820-MS quadrupole
137 spectrometer). Mercury was determined (after filtration of the samples through a 45 μm
138 filter) by ICP-MS (NexION® 300X, Perkin Elmer Inc., Shelton, CT). For control of
139 analytical quality, reference materials (MR1000 and MR10000 for Cd, Cu, Pb, Zn and
140 Environment Canada Total Mercury PT97-Hg07 for Hg) were analysed in parallel to the
141 bulk deposition samples. Percentages of recovery were 108% for Cd, 94% for Pb, and
142 around 103% for Hg. The concentrations of all elements were above the limits of
143 quantification (LOQ), with the exception of Cd (in 9% of the cases) and Hg (in 56% of the
144 cases). Bulk deposition rates were calculated as mean (n=3) $kg \cdot ha^{-1}$ at each SS.
145 Moss samples were homogenized in an ultracentrifuge mill (Restch ZM 200, heavy metal-
146 free) and dried at 40°C. For Cd and Pb determinations, samples were extracted by acid
147 treatment (2% HNO3 v/v) and ultrasound (SONICS Vibra cell VCX 130) according to
148 Barciela et al. (2015), and then, the concentrations of Cd and Pb were determined by
149 graphite furnace atomic absorption spectrometry (Perkin Elmer AAnalyst 600). Mercury
150 was determined using an elemental analyzer (Milestone DMA80). For analytical quality
151 control, duplicate samples were analysed once every nine samples. Two certified reference
152 materials, M2 and M3 (*Pleurozium schreberi*, Steinnes et al., 1997), and analytical blanks (to
153 control for possible contamination) were analysed at the same frequency. The overall
154 percentage error (Cěburnis and Steinnes, 2000) was lower than 9% for all elements. The
155 percentage of recovery ranged between 70% (Hg in M2) and 131% (Pb in M3) and in most
156 cases, was close to 100%.
157 *2.5. Data analysis*
158 The mean concentrations (n=3 replicates, except for Hg in BD) of each element in *P.*
159 *purum* (NG and GP), transplants of *S. denticulatum* (T) and BD rates were grouped both by
160 SS (n = 12 sampling periods) and by sampling time (n = 21 sites). The normality and

161 homocedasticity of the data were checked by means of the Shapiro-Wilks and Barlett tests
162 respectively, revealing that the concentrations of elements in bulk deposition and mosses
163 were not normally distributed even after applying Box-Cox transformations (Box and Cox,
164 1964). Therefore, the non-parametric Spearman's rank correlation test was used to assess
165 the strength of the relationship among the mean concentrations of elements in mosses
166 (different sections of *P. purum* and *S. denticulatum*) and in bulk deposition, in the data
167 grouped both by SS and time.
168 The existence of significant differences in the concentrations of the three elements among
169 the different types of moss tissue (NG, GP, transplants), and across times and sites (Time,
170 SS) as well as their interaction (Tissue:Time, Tissue:SS) was also evaluated in the data
171 grouped by SS and time by means of non-parametric Scheirer Ray Hare test (Dytham,
172 2003) followed by the Dunn's post hoc test for non-parametric multiple pairwise
173 comparisons. All statistical analyses were carried out using R-studio 1.0.153.

174 **3. Results and discussion**

175 *3.1. Correlations between the concentrations of heavy metals in the NG tissue of P. purum and bulk*
176 *deposition*

177 The amount of tissue constituting the NG part of *P. purum* was quite variable across sites,
178 and also changed throughout the duration of the experiment (see Table S.1, Supplementary
179 Material). Total annual growth across sites ranged between 0.87 and 4.8 cm per year, with
180 maximum and minimum growth rates per site ranging between 0.15 - 1.5 cm and 0.00 -
181 0.12 cm per month respectively (all the SS presented no growth - i.e. growth rate = 0.00 cm
182 - in one or more of the summer months, with the exception of SS17). Growth was
183 drastically reduced during summertime, when 62% and 52% of the sites were reported as
184 no growth in July (T7) and August (T8) respectively. These results are in agreement with
185 those found by Boquete et al. (2014) between March 2008-March 2009 in seven sampling
186 sites within the same region (all of them included in the present work: SS3-SS7, SS17 and

187 SS20), showing high spatial and temporal variation and greater growth rates than reported
188 previously for this species in northern regions of Europe (ca. 2.2 cm per year; Kilbertus,
189 1968; Bates, 1987; Leblond et al., 2004).
190 The concentrations of Cd, Hg, and Pb in NG as well as in BD were also highly variable
191 throughout the duration of the experiment (T1-T12) as demonstrated in Figure 1 for 4
192 representative sampling sites (one rural area, two industrial foci, and one urban area). The
193 peak BD rates of the different elements do not often match the concentration peaks found
194 in moss tissues (except for Cd in SS4), suggesting that concentrations of metals in NG and
195 BD do not follow the same patterns of temporal variation. The Spearman's rank
196 correlation coefficients between the NG tissue of *P. purum* and BD rates (Tables 1 and 2
197 for the data grouped by sampling period and site respectively) show that the number of
198 significant correlations varies depending on the elements and how the data are grouped.
199 For the data grouped by time, there are 7 and 3 significant correlations for Cd, and Pb
200 respectively (Table 1). However, the number of significant correlations is much lower for
201 the dataset grouped by SS, with a decrease in the percentage of significant correlations
202 from 58 to 42% for Cd, and 25 to 0% for Pb.
203 The lower number of significant correlations between NG and BD rates in the data
204 grouped by SS is in agreement with the results of Boquete et al. (2015) comparing GP with
205 BD, and Ares et al. (2015) comparing T with BD. These authors suggested that when the
206 data are grouped by SS, the type and characteristics of the atmospheric inputs are
207 homogenized (e.g., gaseous, particulate, particle size, etc. as they originate in the same
208 source) and therefore the variability in the concentrations is mostly due to: i) monthly
209 changes in environmental factors (e.g. temperature, amount of precipitation) that affect
210 pollutant inputs and the mosses uptake capacity (related to their physiological status in case
211 of living mosses); and/or ii) small changes in pollutant emissions. On the contrary, when
212 the data are grouped by exposure period the former variability decreases while the

variability associated with the characteristics of the atmospheric inputs increases. These findings suggest that eliminating the mismatch between native moss exposure time and BD collection period is not enough to improve the correlation between the concentrations of metals in native moss and BD rates, especially under the influence of variable environmental conditions as it happens when grouping the data by SS. Although the lower sample size of the data when grouped by SS (n=12) could also have some effect in the lower number of significant correlations we do not think this would be the major cause of the general lack of correlation (e.g. in Table ,2 correlations between NG and BD for Cd in T7 and T8 are significant despite n=13 and n=11 respectively due to the lack of moss growth in many SS during these sampling periods).

Comparing the number of significant correlations between NG and BD found in this study with those found by Boquete et al. (2015) and Ares et al. (2015) between GP-BD and T-BD respectively using the exact same BD data, sites, and sampling periods, it is noteworthy that: i) for the data grouped by collection period, GP showed a higher number of significant correlations with BD than NG (7 and 3 for Cd and Pb respectively between NG and BD compared to 9 and 11 for Cd and Pb respectively between GP and BD, with lower *p* values in almost all cases), whereas T showed a lower number of significant correlations with BD than NG and GP (only 7 significant correlations between T and BD all of them for Cd); ii) for the data grouped by SS, NG showed a higher number of significant correlations with BD than either GP or T (5, 3 and 0 significant correlations between NG-BD, 2, 2 and 0 between GP-BD, and 0, 0 and 0 between T-BD for Cd, Hg and Pb respectively).

The former results suggest, first of all, that native *P. purum* represents BD rates better than devitalized transplants of *S. denticulatum* regardless of how the data are grouped. Even though the transplantation technique eliminates the variability of the concentrations due to moss growth and metabolism (devitalization), the way in which the moss is exposed (inside

239 a bag hung to a pole at a height of ca. 2.5 m) makes it more susceptible to the variability
240 generated by environmental factors such as rain, wind, etc., which could explain the lower
241 number of correlations with BD compared to native *P. purum*. Secondly, our results show
242 that GP of *P. purum* represent BD rates better than NG when the effect of environmental
243 variation is minimized (i.e. data grouped by sampling period). This result could be striking
244 given our *a priori* expectation that minimizing the mismatch between the time of exposure
245 of moss tissues and BD collection period would improve the correlation between NG-BD
246 rate; however, it emphasizes the complexity of the relationship between moss heavy metal
247 contents and BD rates making native mosses (and also moss transplants) unreliable
248 estimators of atmospheric heavy metal deposition rates. Finally, the fact that NG correlates
249 better with BD than GP when environmental variation is high (data grouped by SS) could
250 be due to the greater effect of environmental variation in GP than in NG. Green parts of
251 *P. purum* have been exposed to more environmental events (rain, drought, cold, etc.) that
252 the NG (younger section that was not present throughout the whole exposure period)
253 which could alter their metal content directly (e.g. washing, dilution, etc.) or indirectly (e.g.
254 leading to physiological alterations that affect their uptake capacity).

255 *3.2. Correlations between the concentrations of heavy metals determined in different moss tissues*

256 The Spearman's rank correlation coefficients between the different moss tissues (NG vs
257 GP, NG vs T, and GP vs T) are presented in Tables 1 and 2 for the data grouped by time
258 and site respectively. First of all, the concentrations of all studied elements in NG and GP
259 were highly significantly correlated for all periods studied (see Table 1), which was expected
260 given that both tissue types originate from the same moss sample. However, again, the
261 number of significant correlations decreased to 14% (Cd) and 24% (Hg and Pb) of the
262 cases for the data grouped by site. The number of significant correlations between native *P.*
263 *purum* and transplanted *S. denticulatum* is greater for GP than for NG for all three elements,
264 despite that the time of exposure to atmospheric heavy metal deposition is more similar

between NG and T than between GP and T. Also, the percentage of significant correlations decreased when data are grouped by sampling site rather than by time (from 42% to 5% for Cd, 42% to 0% for Hg, and 8% to 0% for Pb for the NG-T comparison; and from 58% to 5% for Cd and Hg, and 25% to 0% for Pb for the GP-T comparison).

The lack of significant correlations between the concentrations of the metals studied in the different tissue types could be due to the high levels of temporal variation (T1 to T12) in the concentrations of the three metals in all of them (Figure 1) in combination with the differences among tissues in their capacity to take up heavy metals (discussed in section 3.3.). As pointed out in the previous section for NG and BD, graphs in Figure 1 show large variations in the concentration of Cd, Hg, and Pb in all four matrices (BD, NG, GP, T) with peaks in concentrations not matching neither BD nor tissue types for the most part (maximum and minimum concentrations occur at different times in the different tissues and BD). These differences are likely caused by variation in moss exposure related factors that affect differently the four matrices. For instance, different exposure times; GP has longer exposure times than NG and T because most of GP fraction corresponds to the fraction of native moss that was present in the study area before the experiment started; while T has overall longer exposure times than the whole NG portion which only represents the portion grown during the last month. Another example could be a different height of exposure to the environment; T are exposed in bags at ~2.5 m from the floor whereas the native moss is collected at ground level.

3.3. Differences in the magnitude of the concentrations of Cd, Hg and Pb among moss tissue types

Concentrations of the different elements in the three tissue types (NG, GP and T) are presented for the data grouped by sampling period in Figure 2, and by SS in Figure 3. Results of the non-parametric Scheirer Ray Hare test showed significant interactions Tissue x Time and Tissue x SS for Cd and Hg respectively. The test showed significant differences in the concentrations of all three elements among tissue types in both datasets ($p<0.001$),

among times for Cd ($p<0.05$) and among sites for Hg and Pb ($p<0.001$). When interactions were not significant, Dunn's post hoc tests showed that all three tissues differed significantly in their concentrations of Hg and Pb in the data grouped by time ($p<0.001$ in both cases), and Cd and Pb in the data grouped by site ($p<0.001$ in both cases) with this general pattern: NG > GP > T for Cd, GP > NG > T for Hg, and T > GP > NG for Pb. These differences in the magnitude of the concentrations of the different elements in the three tissue types can be explained by differences in the morpho-physiological characteristics of the tissues, in conjunction with differences in the physico-chemical properties of the metals emitted into the atmosphere, both affecting the final pollutant loads.

First of all, *S. denticulatum* and *P. purum* differ in the concentration and type of organic functional groups capable of binding heavy metals in their cell walls. By comparing devitalized samples of *P. purum* and *Sphagnum sp.*, González and Pokrovsky (2014) found a higher concentration of carboxylic binding sites - responsible for Cd binding - and a lower concentration of sulfhydryl, amine, and polyphenol binding sites - responsible for Hg binding - in *Sphagnum* mosses compared to *P. purum*. This would explain the general lower concentrations of Hg in T than in NG and GP in this study, although not the lower concentrations of Cd found in T. This unexpected result could be due to the effect of the pre-treatment (devitalization by oven-drying at 120ºC) in the chemical composition of transplanted moss (Adamo et al., 2008), which could modify the cation exchange properties of the transplants. Also, *P. purum* is physiologically active throughout the exposure period in this experiment meaning that it actively grows incorporating into its tissues nutrients (and hence heavy metals) available in the environment, whereas *S. denticulatum* is dead and therefore all metal uptake happens through passive processes.

Secondly, the position of the transplants and native mosses with respect to the atmospheric deposition might also affect the capacity of these mosses to take up heavy metals. Native *P.*

317 *purum* is more or less horizontally exposed to deposition, which facilitates ion exchange at
318 the plant-water interface, whereas bags of *S. denticulatum*, exposed in a vertical position,
319 have less time to interact with soluble cations. Finally, *P. purum* and *S. denticulatum* differ
320 greatly in their morphology which may generate important differences in their capacity to
321 capture and retain particles due to variations in gametophyte branching, density, surface
322 area in contact with the atmosphere (surface area/ volume), and degree of compaction of
323 the phyllids (Castello, 2007; Carballeira et al., 2008; Holy et al., 2009). The high capacity of
324 *Sphagnum* species to retain particulate matter (e.g. two thirds of *Sphagnum sp.* biomass
325 correspond to leaves containing hyalocysts which makes them particularly suitable at
326 particle entrapping; Giordano et al., 2005; Bargagli, 2016) would explain its higher
327 concentration of Pb which is mostly found in the insoluble fraction of bulk deposition
328 (Morselli et al., 2003; Rueda-Holgado et al., 2014), and it is found at a greater proportion in
329 dry deposition than other elements (e.g. Cd and Hg) even in unpolluted sites (Connan et
330 al., 2013).
331 On the other hand, the differences in the concentrations of metals between NG and GP
332 are likely due to differences in their exposure times to atmospheric deposition, since GP
333 has overall longer exposure times than NG, combined with putative differences in their
334 physiological activities and the mobility of metals within the moss shoots. This is related to
335 the preferential accumulation of various elements in particular parts corresponding with
336 different ages of moss tissues (Brümelis and Brown, 1997; Boquete et al., 2014). These
337 authors found a tendency for higher concentrations of Hg and Pb in older tissues of *P.*
338 *purum* compared to young ones which agrees with the present results showing higher
339 concentrations of both Hg and Pb in GP compared to NG. These authors pointed out the
340 high covalence index of these elements (Nieboer and Richardson, 1980) to explain their
341 lower mobility within the moss shoots, so that once these metals are taken up by specific

342 tissues they are not easily transported to other parts of the shoots as it happens with other
343 metals.
344 Finally, *P. purum* is a pleurocarpous moss with typical apical elongation. This kind of
345 mosses show almost no, or very tiny branches at the very tip of the shoot (i.e. 1-2
346 apicalmost cm) where elongation is happening, then branching reaches its peak in the
347 young regions after the tip, and finally, many branches, as well as shoot leaves, are lost
348 during tissue ageing (Bates, 1979). In this study, NG always consisted on the unbranched
349 apicalmost part of the shoots (never longer than 1.5 cm, Table 1 supplementary material)
350 whereas the GP consisted on the young region containing completely developed branches
351 which provide GP a greater capacity to retain particulate matter. This would explain the
352 greater concentrations of Pb in the GP of *P. purum*. Finally, despite the greater amount of
353 biomass constituting the GP compared to NG, NG is likely the most physiologically active
354 part of the shoot. Working with a species with similar growth patterns, *Pleurozium schreberi*,
355 Bates (1979) found marked drop in the physiological activity of the tissues beyond the first
356 2 cm of the most apical part of the shoot. Much greater physiological activity in the NG
357 where active elongation of the moss shoot is happening would be responsible for a greater
358 rate of incorporation of heavy metals.

359 **5. Conclusions**

360 Terrestrial mosses have long been used to monitor the atmospheric deposition of heavy
361 metals into terrestrial ecosystems. However, the complex relationship between the
362 concentrations of metals in moss tissues and atmospheric deposition has led to a general
363 lack of significant correlations between these two matrices, preventing the use of moss data
364 to reliably estimate absolute heavy metal deposition rates. In order to contribute to the
365 harmonization of the moss biomonitoring technique, we first tried to improve the
366 significance of the moss-BD relationship, and secondly, we evaluated which moss tissues
367 reflect better the BD levels. Our results demonstrate that: i) environmental factors

368 constitute a pivotal driver of the changes in the contents of heavy metals in mosses, whose
369 variability alters the moss-BD relationship unpredictably; ii) the effect of environmental
370 factors differs for all moss tissue types and for BD causing mismatches in the peaks of
371 concentrations in all four matrices; iii) native *P. purum* represents BD values better than
372 devitalized transplants of *S. denticulatum* regardless of how the data are grouped (by SS or by
373 time); and iv) when environmental variation is reduced, correlations between moss-BD
374 clearly improve for the elements studied, especially for the GP of native *P. purum*. In light
375 of these results we can conclude that neither native mosses nor transplants are good
376 estimators of absolute atmospheric heavy metal deposition rates. However, they allow us to
377 clearly differentiate SS subject to a wide range of deposition levels which makes them very
378 valuable qualitative indicators of atmospheric deposition. Finally, green parts of *P. purum,*
379 and likely of other mosses with similar growth forms, are the best choice for passive
380 biomonitoring of air quality and should be used to make results from different studies
381 comparable.

382 **Acknowledgements**

383 This research was funded by the Spanish Government through MINECO project
384 CTM2011-30305. The authors belong to the Galician Competitive Research Group
385 GRC/GPC2016-002 and to the CRETUS Strategic Partnership (AGRUP2015/02). All of
386 these programmes are co-funded by FEDER (EU). Authors would like to thank the use of
387 RIAIDT-USC analytical facilities.

388

389 **References**

390 Aboal J.R., Fernández J.A., Boquete T., Carballeira A., 2010. It is possible to estimate
391 atmospheric deposition of heavy metals by analysis of terrestrial mosses? Science of the
392 Total Environment, 408: 6291-6297.

393 Adamo, P., Bargagli, R., Giordano, S., Modenesi, P., Monaci, F., Pittao, E., et al., 2008.
394 Natural and pre-treatments induced variability in the chemical composition and
395 morphology of lichens and mosses selected for active monitoring of air-borne elements.
396 Environmental Pollution, 152: 11–19.
397 Ares A., Fernández J.A., Carballeira A., Aboal J.R., 2014. Towards the methodological
398 optimization of the moss bag technique in terms of contaminants concentrations and
399 replicability value. Atmospheric Environment, 94: 496-507.
400 Ares A., Aboal J.R., Carballeira A., Fernández J.A., 2015. Do moss bags containing
401 devitalized *Sphagnum denticulatum* reflect heavy metal concentrations in bulk deposition?
402 Ecological Indicators, 50: 90-98.
403 Barciela M.C., Noya M., López M.D., Aboal J.R., Fernández J.A., Bermejo-Barrera P.,
404 2015. An environmentally-friendly method for As, Cd, Cr, Cu, Ni and Pb determination
405 in terrestrial moss samples by ultrasonic slurry sampling combined with electrothermal
406 atomic absorption spectrometry. Atomic Spectroscopy, 36 (1): 42-48.
407 Bargagli R., 2016. Moss and lichen biomonitoring of atmospheric mercury: A review.
408 Science of the Total Environment, 572: 216-231.
409 Bates J.W. 1979. The relationship between physiological vitality and age in shoot segments
410 of *Pleurozium schreberi*. Journal of Bryology, 10: 339-351
411 Bates, J.W., 1987. Nutrient retention by *Pseudoscleropodium purum* and its relation to growth.
412 Journal of Bryology, 14(3): 565-580.
413 Berg T., Royset O., Steinnes E, 1995. Moss (*Hylocomium splendens*) used as biomonitor of
414 atmospheric trace element deposition: estimation of uptake efficiencies. Atmospheric
415 Environment, 29: 353–360.
416 Berg T., Steinnes E., 1997. Use of mosses (*Hylocomium splendens* and *Pleurozium schreberi*) as
417 biomonitors of heavy metal deposition: from relative to absolute deposition values.
418 Environmental Pollution, 98(1):61–71.

419 Boquete M.T., Aboal J.R., Carballeira A., Fernández J.A., 2014. Effect of age on the heavy
420 metal concentration in segments of *Pseudoscleropodium purum* and the biomonitoring of
421 atmospheric deposition of metals. Atmospheric Environment, 72: 28-34.
422 Boquete M.T., Fernández J.A., Carballeira A., Aboal J.R., 2015. Relationship between trace
423 metal concentrations in the terrestrial moss *Pseudoscleropodium purum* and in bulk
424 deposition. Environmental Pollution, 201: 1-9.
425 Box G.E.P., Cox D.R., 1964. An analysis of transformations (with discussion). Journal of
426 the Royal Statistical Society B, 26: 211–252.
427 Brümelis G., Brown D.H., 1997. Movement of metals to new growing tissue in the moss
428 *Hylocomium splendens* (Hedw.) BSG. Annals of Botany, 79: 679-686.
429 Carballeira C.B., Aboal J.R., Fernández J.A., Carballeira A., 2008. Comparison of the
430 accumulation of elements in two moss species. Atmospheric Environment, 42: 4904-17.
431 Castello M. A., 2007. comparison between two moss species used as transplants for
432 airborne trace element biomonitoring in NE Italy. Environmental Monitoring
433 Assessment, 133: 267–76.
434 Čeburnis D., Steinnes E., 2000. Conifer needles as biomonitors of atmospheric heavy metal
435 deposition: comparison with mosses and precipitation, role of the canopy.
436 Atmospheric Environment, 34: 4265-4271.
437 Connan O., Maro D., Hébert D., Roupsard P., Goujon R., Letellier B., Le Cavelier S., 2013.
438 Wet and dry deposition of particles associated metals (Cd, Pb, Zn, Ni, Hg) in a rural
439 wetland site, Marais Vernier, France. Atmospheric Environment, 67: 394-403.
440 Dytham C., 2003. Choosing and using statistics. Oxford: Blackwell.
441 Fernández J.A., Ares, A., Rey-Asensio, A., Carballeira, A., Aboal, J.R. (2010). Effect of
442 growth on active biomonitoring with moss. Journal of Atmospheric Chemistry, 63(1):
443 1-112010.

444 Fernández J.A., Boquete M.T., Carballeira A., Aboal J.R., 2015. A critical review of
445 protocols for moss biomonitoring of atmospheric deposition: sampling and sample
446 preparation. Science of the Total Environment, 517: 132-150.
447 Fowler D., McDonald A.G., Crossle A., Nemitz E., Leaver D., Cape J.N., et al., 2006. UK
448 heavy metal monitoring network. CEH Project number: C02164.
449 Giordano, S., Adamo, P., Sorbo, S., Vingiani, S., 2005. Atmospheric trace metal pollution
450 in the Naples urban area based on results from moss and lichen bags. Environmental
451 Pollutution, 136: 431-442.
452 Giordano S., Adamo P., Monaci F., Pittao E., Tretiach M., Bargagli R., 2009. Bags with
453 oven-dried moss for the active monitoring of airborne trace elements in urban areas.
454 Environmental Pollution 157: 2798-2805.
455 González A.G., Pokrovsky O.S., 2014. Metal adsorption on mosses: toward a universal
456 adsorption model. Journal of Colloid and Interface Science, 415: 169–178.
457 Harmens H., Norris D.A., Koerber G.R., Buse A., Steinnes E., Rühling Å., 2008. Temporal
458 trends (1990–2000) in the concentration of cadmium, lead and mercury in mosses
459 across Europe. Environmental Pollution, 151: 368–76.
460 Holy M., Leblond S., Pesch R., Schröder W., 2009. Assessing spatial patterns of metal
461 bioaccumulation in French mosses by means of an exposure index. Environmental
462 Science and Pollution Research, 16: 499–507.
463 Kilbertus, G., 1968. Vitesse de decomposition de *Pseudoscleropodium purum* (Hedw.) Fleisch
464 dans la nature. Revue d'Ecologie et de Biologie du Sol, 2: 237e244.
465 Leblond S., Gombert S., Colin J.L., Losno R., Rausch de Traubenberg C., 2004. Biological
466 and temporal variations of trace element concentrations in the moss species *Scleropodium*
467 *purum* (Hedw.) Limpr. Journal of Atmospheric Chemistry, 49: 95-110.

468 Morselli L., Olivieri P., Brusori B., Passarini F., 2003. Soluble and insoluble fractions of
469 heavy metals in wet and dry atmospheric depositions in Bologna, Italy. Environmental
470 Pollution, 124: 457-469.
471 Nieboer, E., Richardson, D.H.S., 1980. The replacement of the nondescript term 'heavy
472 metals' by a biologically and chemically significant classification of metal ions.
473 Environmental Pollution Series B, Chemical and Physical. 1 (1): 3-26.
474 Rueda-Holgado F., Palomo-Marín M.R., Calvo-Blázquez L., Cereceda-Balic F., Pinilla-Gil
475 E., 2014. Fractionation of trace elements in total atmospheric deposition by filtrating-
476 bulk passive sampling. Talanta, 125: 125-130.
477 Russell S., 1984. Growth measurements in bryophytes: a case study. Journal of the Hattori
478 Botanical Laboratory, 56: 147-157.
479 Schintu M., Cogoni A., Durante L., Cantaluppi C., Contu A., 2005. Moss (*Bryum*
480 *radiculosum*) as a bioindicator of trace metal deposition around an industrialised area in
481 Sardinia (Italy). Chemosphere, 60:610-8
482 Steinnes E., Rühling A., Lippo H., Mäkinen A., 1997. Reference materials for large scale
483 metal deposition surveys. Accreditation and Quality Assurance, 2: 243-249.
484 Thöni L., Schnyder N., Krieg F., 1996. Comparison of metal concentrations in three
485 species of mosses and metal freights in bulk precipitations. Fresenius Journal of
486 Analytical Chemistry, 354: 703-8.
487 UNE-EN-standard 15841:2010, 2008. Ambient Air Quality - Standard Method for
488 Determination of Arsenic, Cadmium, Lead and Nickel in Atmospheric Deposition.
489 Established by the European Committee for Standardization (CEN).
490

Table 1. Spearman's correlation coefficients for the mean concentrations (n=3) of Cd, Hg and Pb in bulk deposition and different parts of native and transplanted mosses corresponding to the data grouped by sampling period (n=21). BD: bulk deposition; NG: new grown tissue generated monthly by native *Pseudoscleropodium purum*; GP: green part of *P. purum* shoots; T: transplants of devitalized *Sphagnum denticulatum*; -: coefficients not calculated because there were not enough data available (i.e. degrees of freedom ≤ 0); values in bold: significant correlations; *: $p<0.05$; **: $p<0.001$; ***: $p<0.0001$.

	Cd				Hg				Pb			
	NG-BD	NG-T	NG-GP	GP-T	NG-BD	NG-T	NG-GP	GP-T	NG-BD	NG-T	NG-GP	GP-T
T1	**0.656***	0.355	**0.936*****	0.348	-0.571	0.171	**0.780*****	0.394	**0.535***	-0.034	**0.961*****	-0.079
T2	**0.624***	**0.501***	**0.961*****	**0.529***	0.250	**0.525***	**0.847*****	**0.551***	0.249	0.337	**0.769****	**0.468***
T3	0.499	0.385	**0.864*****	0.158	-	**0.578***	**0.877*****	**0.612***	0.329	0.168	**0.713***	-0.142
T4	**0.806*****	0.297	**0.966*****	0.325	-	0.458	**0.833*****	**0.610***	**0.654***	**0.605***	**0.865*****	**0.466***
T5	0.280	**0.711****	**0.967*****	**0.740****	0.524	**0.523***	**0.802*****	**0.519***	-0.096	-0.249	**0.759****	-0.296
T6	**0.593***	**0.723****	**0.942*****	**0.675****	-	**0.655***	**0.905*****	**0.576***	**0.587***	0.226	**0.655***	0.152
T7	**0.810***	0.429	**1.000*****	**0.755*****	0.000	0.452	**0.786***	**0.435***	0.357	-0.310	**0.976*****	-0.200
T8	**0.709***	0.091	**0.867***	0.148	0.650	0.233	**0.900****	0.327	0.164	0.067	**0.782***	0.166
T9	0.443	-0.325	**0.925*****	-0.274	0.500	0.104	**0.514***	0.291	0.429	-0.179	**0.754***	**-0.455***
T10	**0.721****	**0.546***	**0.889*****	**0.599***	0.042	**0.676***	**0.897*****	**0.634***	0.189	0.133	**0.703***	0.148
T11	-0.179	**0.450***	**0.940*****	**0.442***	-	0.349	**0.968*****	0.265	0.357	-0.326	**0.744****	-0.119
T12	0.500	0.298	**0.933*****	**0.438***	-0.071	0.315	**0.948*****	0.303	0.400	0.207	**0.798*****	-0.019

Table 2. Spearman's correlation coefficients for the mean concentrations (n=3) of Cd, Hg and Pb in bulk deposition and different parts of native and transplanted mosses corresponding to the data grouped by sampling site (n=12). BD: bulk deposition; NG: new grown tissue generated monthly by native *Pseudoscleropodium purum*; GP: green part of *P. purum* shoots; T: transplants of devitalized *Sphagnum denticulatum*; -: coefficients not calculated because there were not enough data available (i.e. degrees of freedom ≤ 0). Values in bold: significant correlations; *: p<0.05; **: p<0.001; ***: p<0.0001.

	Cd				Hg				Pb			
	NG-BD	NG-T	NG-GP	GP-T	NG-BD	NG-T	NG-GP	GP-T	NG-BD	NG-T	NG-GP	GP-T
SS1	**0.738***	0.357	**0.786***	0.091	0.000	-0.086	**0.943***	-0.287	-0.238	0.357	0.071	0.000
SS2	-0.024	-0.248	0.309	0.000	-0.500	-0.067	0.394	0.007	0.350	0.150	0.650	0.280
SS3	0.371	-0.429	-0.095	-0.245	-	0.167	**0.762***	0.119	0.595	-0.048	0.286	-0.259
SS4	0.238	-0.127	0.176	-0.063	**1.000*****	0.042	**0.867***	-0.147	0.150	0.400	**0.933****	0.000
SS5	0.400	0.127	0.000	-0.084	0.500	-0.017	0.250	0.252	-0.436	0.245	-0.009	0.210
SS6	**0.738***	0.030	0.442	0.434	-0.300	-0.139	0.430	-0.007	0.612	0.333	0.612	0.315
SS7	0.450	-0.427	0.600	-0.531	-0.600	0.245	-0.127	-0.182	0.200	0.418	0.491	-0.049
SS8	**-0.609***	0.191	0.155	0.294	0.250	-0.445	-0.327	**0.671***	-0.182	0.218	**0.736***	0.014
SS9	-0.434	0.133	0.294	0.000	0.167	-0.126	0.427	0.035	0.126	-0.014	**0.762***	-0.245
SS10	0.036	-0.143	0.321	-0.315	-	0.700	0.800	0.545	-0.286	-0.179	0.036	-0.308
SS11	0.127	-0.103	0.285	-0.091	0.600	0.152	0.152	0.315	-0.406	0.091	-0.261	0.105
SS12	0.262	-0.248	0.176	**-0.643***	0.700	-0.236	-0.042	0.273	-0.200	-0.115	0.382	-0.259
SS13	0.233	-0.183	0.317	0.112	-	0.167	0.017	0.538	-0.200	0.517	0.317	0.266
SS14	-0.371	-0.371	0.543	0.161	-	0.714	-0.543	0.056	0.600	-0.257	0.543	0.392
SS15	**-0.714***	-0.550	0.183	0.063	-0.500	-0.183	0.500	0.238	-0.033	-0.233	0.617	-0.119
SS16	-0.050	-0.055	**0.758***	-0.329	**1.000*****	-0.345	-0.079	0.490	-0.442	0.467	0.345	-0.063
SS17	0.473	0.210	-0.490	0.154	0.100	-0.084	0.378	-0.266	0.077	-0.056	**0.846****	-0.392
SS18	-0.600	**0.829***	0.086	-0.294	-	0.371	0.600	0.336	0.371	0.257	0.429	0.476
SS19	**0.667***	0.285	0.115	0.091	**1.000*****	-0.612	**0.842***	-0.140	0.139	0.273	**0.697***	0.476
SS20	-0.503	-0.227	**0.800***	-0.196	0.700	-0.145	0.591	0.280	0.418	-0.227	0.418	-0.140
SS21	-0.800	-0.700	0.800	-0.042	-	0.100	**0.900***	0.280	0.400	0.500	0.800	0.028

Figure captions

Fig 1. Mean concentrations (ng g^{-1} dw) of Cd, Hg and Pb (µg g^{-1}) in green parts (GP; light grey bars) and new grow portion (NG; white bars) in native *Pseudoscleropodium purum* and devitalized transplants of *Sphagnum denticulatum* (T; dark grey bars) in relation with bulk deposition load (g ha^{-1}) (solid red line) in 4 sampling sites (SS): SS4 (rural area); SS8, SS12 (industrial areas) and SS17 (urban area) along different periods of exposure

Fig. 2. Concentrations of Cd, Hg (*: ng g^{-1}), and Pb (+: µg g^{-1}) in different parts of native and transplanted mosses for the data grouped by sampling period (n=21). NG: new grown tissue generated monthly by native *Pseudoscleropodium purum* (white series); GP: green part of *P. purum* shoots (light grey); Tr: transplants of devitalized *Sphagnum denticulatum* (dark grey).

Fig. 3. Concentrations of Cd, Hg (*: ng g^{-1}), and Pb (+: µg g^{-1}) in different parts of native and transplanted mosses for the data grouped by sampling site (n=12). NG: new grown tissue generated monthly by native *Pseudoscleropodium purum* (white series); GP: green part of *P. purum* shoots (grey); Tr: transplants of devitalized *Sphagnum denticulatum* (dark grey).

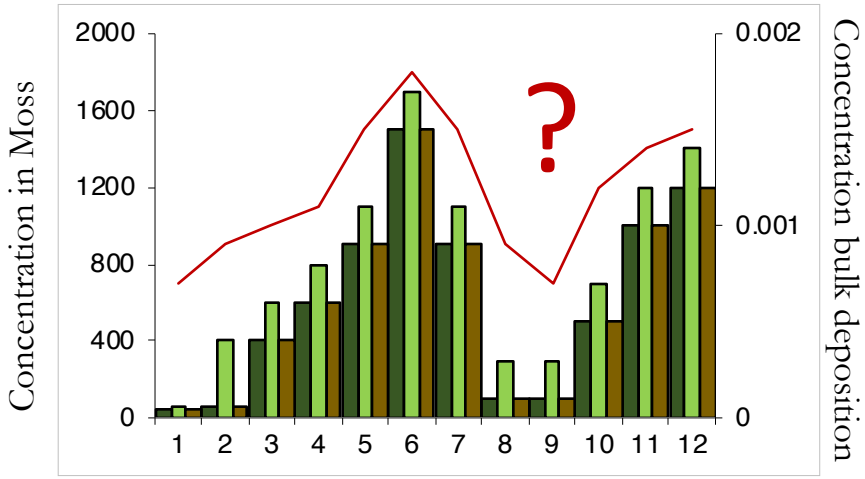
SUPPLEMENTARY MATERIAL

Table S.1: Mean, maximum, minimum monthly growth (cm month^{-1}) and total annual growth (cm) of *Pseudoscleropodium purum* shoots measured as monthly increments in length of the main stem throughout the duration of the study in each sampling site (SS). Only data over 1 mm are showed.

	Mean	Maximum	Minimum	Total
SS1	0.164	0.316	0.106	1.31
SS2	0.401	0.855	0.116	4.01
SS3	0.420	1.110	0.112	3.36
SS4	0.480	1.498	0.148	4.80
SS5	0.221	0.579	0.124	2.44
SS6	0.352	0.737	0.115	3.52
SS7	0.360	0.742	0.132	3.96
SS8	0.220	0.417	0.111	2.20
SS9	0.320	0.752	0.107	3.84
SS10	0.124	0.151	0.101	0.87
SS11	0.245	0.503	0.107	2.45
SS12	0.389	1.101	0.102	3.89
SS13	0.345	0.816	0.114	3.10
SS14	0.261	0.413	0.141	1.57
SS15	0.180	0.351	0.106	1.62
SS16	0.236	0.552	0.105	2.36
SS17	0.307	0.852	0.117	3.68
SS18	0.366	0.601	0.145	2.19
SS19	0.255	0.585	0.103	2.55
SS20	0.377	0.576	0.111	4.14
SS21	0.179	0.346	0.113	0.90

*Graphical Abstract

How is the metal concentration correlation between different moss tissues and bulk deposition?



Bulk deposition

Moss tissues

New grown (NG)

Transplants (T)

Green parts (GP)

Results on correlation:

Moss tissues ≠ Bulk deposition

Cd

Hg

Pb

***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

1.We studied which moss tissues provide a better estimate of the atmospheric deposition of heavy metals

2 Environmental conditions affect the relation between bulk deposition and different moss tissues

3 Native *P.purum* represents bulk deposition better than devitalized *S.denticulatum* transplants

4 Green parts of *P.purum* are the best choice for passive monitoring of air quality

Matching times: trying to improve the correlation between heavy metal levels in mosses and bulk deposition

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Abstract

The relationship between the concentrations of metals in moss tissues and atmospheric deposition is highly complex, resulting in a general lack of correlations between these two matrices. Here, we tried to improve the significance of the moss-bulk deposition (BD) relationship by eliminating the mismatch between the time that the moss tissue selected for analysis is exposed to atmospheric deposition, and the time during which BD is collected. For this, we analysed the concentrations of Cd, Hg and Pb in new grown tissue of *Pseudoscleropodium purum* and BD collected monthly, for one year, in 21 sampling sites (SS) under different degrees of pollution. Additionally, we assessed how different moss tissues, including native moss (green parts and new grown tissues of *P. purum*) and moss transplants of *Sphagnum denticulatum*, reflect BD to find out which moss tissues provide a better estimate of the atmospheric deposition of heavy metals. First of all, our results showed that eliminating the mismatch between native moss exposure time and BD collection period is not enough to improve their correlation. Environmental variation emerged as the main driver of tissue content variation altering the moss-BD relationship unpredictably. Secondly, native *P. purum* represents BD values better than devitalized transplants by displaying a greater number of significant correlations with BD. Specifically, green parts of *P. purum* generally represent better BD than new grown tissues. Overall, we conclude that neither native mosses nor transplants are good estimators of atmospheric heavy metal

deposition rates. However, they are good qualitative indicators of the atmospheric deposition, by allowing us to differentiate SS subject to a wide range of pollution levels. Additionally, green parts of *P. purum,* and likely of other mosses with similar growth forms, should be used in passive biomonitoring studies to make results from different studies comparable.

Keywords: cadmium; mercury; lead; air pollution; passive and active biomonitoring.

1. Introduction

It has been traditionally accepted that terrestrial mosses enable quantitative estimation of atmospheric heavy metal deposition (e.g. Berg et al., 1995; Harmens et al., 2008). However, after a deep review of all the previously published articles about the topic, Aboal et al. (2010) pointed out that this statement was based on studies not always well handled and whose conclusions must be taken with caution. Since then, new studies introducing methodological improvements to the experimental design were done to better understand the relationship between heavy metal concentrations in mosses and bulk deposition (BD); these new results showed a general lack of significant correlations between both native (Boquete et al., 2015) and devitalized transplanted mosses (Ares et al., 2015) and BD for some of the most commonly studied heavy metals (i.e. Cd, Cu, Hg, Pb, and Zn).

For native mosses, Boquete et al. (2015) suggested that this absence of significant correlations could be related to a mismatch between the time that the moss tissue selected for analysis had been exposed to atmospheric deposition and the time during which BD had been collected. This is due to the fact that at the time at which BD begins to be sampled, there is a pre-existent moss tissue with its own heavy metal concentration. If the growing period of the tissues analysed and BD measuring period do not match, it is very difficult to obtain significant correlations between their concentrations; in fact, these authors showed that the longer the length of the period studied (i.e. above 3 months) the higher the significance of the relationship between moss and BD. On the other hand, the

lack of significant correlations for devitalized transplanted mosses seems to be mostly due to environmental conditions (mainly the abundance of rainfall), as the time of exposure of moss and the BD collection period undoubtedly coincide (Ares et al., 2015). Also it should be noted that devitalization treatment avoids moss growth during transplant exposure and hence weather conditions will rule the heavy metal burden.

Bearing in mind the previous paragraph, it could be assumed that for native mosses it would be possible to improve the significance of the moss-BD relationship if the comparison is made using only the tissue grown during the period in which BD is collected. In fact, Boquete et al. (2015) found that in *Psedoscleropodium purum* the percentage of significant correlations increased with the number of months in the accumulated BD data for Cd and Cu (e.g. from 58% for the monthly BD to 100% for the three-monthly BD for Cu; this pattern was not so clear for Hg, Pb, and Zn). This increase was explained by the fact that at the end of the sampling period, a greater proportion of the tissue analysed would have been exposed to the atmospheric inputs during the period in which BD was accumulated. Nevertheless, so far, no study has directly compared the concentrations of heavy metals in new grown tissue with the BD collected during the same period. Additionally, it still remains unknown how the different moss tissues, including native moss and moss transplants, reflect BD, and what the relationship between concentrations of heavy metals in different moss tissues looks like. This knowledge would improve the moss technique by contributing to its harmonization (if different investigators use the same tissues of the same moss species their results could be directly comparable). In light of this, the main objective of this work is to evaluate to what extent the concentrations of heavy metals in new grown tissues of mosses are related to the concentrations of metals in BD collected during the moss growth period, and to find out which moss tissues reflect better the atmospheric deposition of heavy metals.

2. Material and methods

This study was carried out in Galicia (NW Spain), at 21 sampling sites (SS) affected by different levels of pollution. These included areas close to two steelworks (SS1 and SS8-SS11), one wood-works (SS2), two coal fired power plants (SS3-SS7), an aluminium smelter (SS12-SS14), three peri-urban (SS15-SS17) and four rural areas (SS18-SS21). Bulk deposition and native *Pseudoscleropodium purum* (Hedw.) M. Fleisch were sampled monthly, at the same time that moss bags made with devitalized *Sphagnum denticulatum* Brid were exposed, throughout 1 year (January–December, 2010). Part of the data employed in the present work has been previously published in Ares et al. (2015) and Boquete et al. (2015) (see these papers for more details), but all the results presented here are novel.

2.1. Collection of bulk deposition

Three bulk deposition collectors, consisting of a funnel-bottle combination, were placed in each SS. Sampling was carried out in accordance with the recommendations outlined in the Ambient air quality - standard method for determination of arsenic, cadmium, lead and nickel in atmospheric deposition: UNE-EN-standard 15841:2010 established by the European Committee for Standardization (CEN). After each exposure period an aliquot of bulk deposition was acidified with hyperpure HNO_3 (final concentration of 1%) and stored at -20 ºC until analysis.

2.2. Preparation and exposition of S. denticulatum transplants

Moss bags were prepared with the green parts of *S. denticulatum*. The moss was distributed homogeneously inside flat bags (2.5 mg of moss cm^{-2}), with minimal overlapping and compression of the moss. This was then devitalized by drying in an oven at 120 ºC for 24 h. Finally, the bags were vacuum-packed and stored at -20 ºC until use. At each SS and time, one moss bag was hung from a short carbon fibre peg fixed perpendicularly to a pole at a height of ca. 2.5 m over each BD collector (n=3 moss bags per SS and time).

2.3. Collection of native moss

Three samples of native *P. purum* were collected at each SS, as close as possible to the bulk-deposition samplers (the closest sample was obtained at a distance of between 0 and 312 m from the BD sampler) following Fernández et al. (2015) recommendations. Also, growth measurements were done in this species in order to obtain a sample of tissue grown in between sampling times. Growth was measured in terms of length increments of the main stem (Russell, 1984). Thus, as detailed in Boquete et al. (2014), 100 shoots of *P. purum* growing at each site were collected and groups of ten shoots were tied to a thread (at approximately 1 cm from the apex). Each thread was labelled and identified, and each shoot within each thread was assigned a number from 1 to 10 (from one end to the other). Finally, the shoots were placed within the moss carpet at each SS for the duration of the experiment. The length of each moss shoot was measured monthly by taking a photograph of each thread against a background of graph paper (Fig 1A in Boquete et al., 2014) and using ImageJ (https://imagej.nih.gov/ij/). For each SS, monthly growth was then calculated as the median difference in shoot length between one month and the preceding one. Samples of *P. purum* with length increments <1 mm were not cut from the shoots, as this corresponds with the error committed when cutting the segments, as determined by Fernández et al. (2010). Therefore, length increments <1 mm were considered as no growth for the corresponding site and time period.

Moss samples were cleaned prior to analysis by removing plant remains and other foreign material and, taking into consideration the monthly length increments previously estimated, moss shoots were divided in two sections: i) the apical most tissue grown during the last month (specific for each SS), hereafter named as new grown tissue (NG); and, ii) the remaining shoot section corresponding to the green parts, the weighted weight mean average of both parts was calculated (GP). These two sections were weighted (Mettler Toledo XP26), processed, and analysed individually.

2.4. Processing and chemical analysis

134 The concentrations of Cd, Hg, and Pb in bulk deposition samples were determined by
135 inductively coupled plasma mass spectrometry (ICP-MS – VARIAN 820-MS quadrupole
136 spectrometer). Mercury was determined (after filtration of the samples through a 45 μm
137 filter) by ICP-MS (NexION® 300X, Perkin Elmer Inc., Shelton, CT). For control of
138 analytical quality, reference materials (MR1000 and MR10000 for Cd, Cu, Pb, Zn and
139 Environment Canada Total Mercury PT97-Hg07 for Hg) were analysed in parallel to the
140 bulk deposition samples. Percentages of recovery were 108% for Cd, 94% for Pb, and
141 around 103% for Hg. The concentrations of all elements were above the limits of
142 quantification (LOQ), with the exception of Cd (in 9% of the cases) and Hg (in 56% of the
143 cases).
144 Moss samples were homogenized in an ultracentrifuge mill (Restch ZM 200, heavy metal-
145 free) and dried at 40°C. For Cd and Pb determinations, samples were extracted by acid
146 treatment (2% HNO3 v/v) and ultrasound (SONICS Vibra cell VCX 130) according to
147 Barciela et al. (2015), and then, the concentrations of Cd and Pb were determined by
148 graphite furnace atomic absorption spectrometry (Perkin Elmer AAnalyst 600). Mercury
149 was determined using an elemental analyzer (Milestone DMA80). For analytical quality
150 control, duplicate samples were analysed once every nine samples. Two certified reference
151 materials, M2 and M3 (*Pleurozium schreberi*, Steinnes et al., 1997), and analytical blanks (to
152 control for possible contamination) were analysed at the same frequency. The overall
153 percentage error (Cěburnis and Steinnes, 2000) was lower than 9% for all elements. The
154 percentage of recovery ranged between 70% (Hg in M2) and 131% (Pb in M3) and in most
155 cases, was close to 100%.
156 *2.5. Data analysis*
157 The mean concentrations (n=3 replicates, except for Hg in BD) of each element in *P.*
158 *purum* (NG and GP), transplants of *S. denticulatum* (T) and in BD were grouped both by SS
159 (n = 12 sampling periods) and by sampling time (n = 21 sites). The normality and

160 homocedasticity of the data were checked by means of the Shapiro-Wilks and Barlett tests
161 respectively, revealing that the concentrations of elements in bulk deposition and mosses
162 were not normally distributed even after applying Box-Cox transformations (Box and Cox,
163 1964). Therefore, the non-parametric Spearman's rank correlation test was used to assess
164 the strength of the relationship among the mean concentrations of elements in mosses
165 (different sections of *P. purum* and *S. denticulatum*) and in bulk deposition, in the data
166 grouped both by SS and time.
167 The existence of significant differences in the concentrations of the three elements among
168 the different types of moss tissue (NG, GP, transplants), and across times and sites (Time,
169 SS) as well as their interaction (Tissue:Time, Tissue:SS) was also evaluated in the data
170 grouped by SS and time by means of non-parametric Scheirer Ray Hare test (Dytham,
171 2003) followed by Dunn's test for non-parametric multiple pairwise comparisons All
172 statistical analyses were carried out using R-studio 1.0.153.

173 **3. Results and discussion**

174 *3.1. Correlations between the concentrations of heavy metals in the NG tissue of P. purum and bulk*
175 *deposition*

176 The amount of tissue constituting the NG part of *P. purum* was quite variable across sites,
177 and also changed throughout the duration of the experiment (see Table S.1, Supplementary
178 Material). Total annual growth across sites ranged between 0.87 and 4.8 cm per year, with
179 maximum and minimum growth rates per site ranging between 0.15 - 1.5 cm and 0.00 -
180 0.12 cm per month respectively (all the SS presented no growth - i.e. growth rate = 0.00 cm
181 - in one or more of the summer months, with the exception of SS17). Growth was
182 drastically reduced during summertime, when 62% and 52% of the sites were reported as
183 no growth in July (T7) and August (T8) respectively. These results are in agreement with
184 those found by Boquete et al. (2014) between March 2008-March 2009 in seven sampling
185 sites within the same region (all of them included in the present work: SS3-SS7, SS17 and

186 SS20), showing high spatial and temporal variation and greater growth rates than the
187 reported previously for this species in northern regions of Europe (ca. 2.2 cm per year;
188 Kilbertus, 1968; Bates, 1987; Leblond et al., 2004).
189 The concentrations of Cd, Hg, and Pb in NG as well as in BD were also highly variable
190 throughout the duration of the experiment (T1-T12) as demonstrated in Figure 1 for 4
191 representative sampling sites (one rural area, two industrial foci, and one urban area). The
192 peak concentrations of the different elements in BD do not often match the peaks found in
193 moss tissues (except for Cd in SS4), suggesting that concentrations of metals in NG and
194 BD do not follow the same patterns of temporal variation. The Spearman's rank
195 correlation coefficients between the NG tissue of *P. purum* and BD (Tables 1 and 2 for the
196 data grouped by sampling period and site respectively) show that the number of significant
197 correlations varies depending on the elements and how the data are grouped. For the data
198 grouped by time, there are 7 and 3 significant correlations for Cd, and Pb respectively
199 (Table 2). However, the number of significant correlations is much lower for the dataset
200 grouped by SS, with a decrease in the percentage of significant correlations from 58 to 42%
201 for Cd, and 25 to 0% for Pb.
202 The lower number of significant correlations between NG and BD in the data grouped by
203 SS is in agreement with the results of Boquete et al. (2015) comparing GP with BD, and
204 Ares et al. (2015) comparing T with BD. These authors suggested that when the data are
205 grouped by SS, the type and characteristics of the atmospheric inputs are homogenized
206 (e.g., gaseous, particulate, particle size, etc. as they originate in the same source) and
207 therefore the variability in the concentrations is mostly due to: i) monthly changes in
208 environmental factors (e.g. temperature, amount of precipitation) that affect pollutant
209 inputs and the mosses uptake capacity (related to their physiological status in case of living
210 mosses); and/or ii) small changes in pollutant emissions. On the contrary, when the data
211 are grouped by exposure period the former variability decreases while the variability

212 associated with the characteristics of the atmospheric inputs increases. These findings
213 suggest that eliminating the mismatch between native moss exposure time and BD
214 collection period is not enough to improve the correlation between the concentrations of
215 metals in native moss and BD, especially under the influence of variable environmental
216 conditions as it happens when grouping the data by SS.
217 Comparing the number of significant correlations between NG and BD found in this study
218 with those found by Boquete et al. (2015) and Ares et al. (2015) between GP-BD and T-
219 BD respectively using the exact same BD data, sites, and sampling periods, it is noteworthy
220 that: i) for the data grouped by collection period, GP showed a higher number of
221 significant correlations with BD than NG (7 and 3 for Cd and Pb respectively between NG
222 and BD compared to 9 and 11 for Cd and Pb respectively between GP and BD, with lower
223 *p* values in almost all cases), whereas T showed a lower number of significant correlations
224 with BD than NG and GP (only 7 significant correlations between T and BD all of them
225 for Cd); ii) for the data grouped by SS, NG showed a higher number of significant
226 correlations with BD than either GP or T (5, 3 and 0 significant correlations between NG-
227 BD, 2, 2 and 0 between GP-BD, and 0, 0 and 0 between T-BD for Cd, Hg and Pb
228 respectively).
229 The former results suggest, first of all, that native *P. purum* represents BD values better than
230 devitalized transplants of *S. denticulatum* regardless of how the data are grouped. Even
231 though the transplantation technique eliminates the variability of the concentrations due to
232 moss growth and metabolism (devitalization), the way in which the moss is exposed (inside
233 a bag hung to a pole at a height of ca. 2.5 m) makes it more susceptible to the variability
234 generated by environmental factors such as rain, wind, etc., which could explain the lower
235 number of correlations with BD compared to native *P. purum*. Secondly, our results indicate
236 that when environmental variation is high (data grouped by SS) NG correlates better with
237 BD than GP. This could be due to the fact that GP have been exposed to environmental

238 variation for longer than NG, which could cause larger and less predictable changes in the
239 concentrations of heavy metals in this tissue type. However, when the effect of
240 environmental variation is reduced by grouping the data by time, GP provide a better
241 representation of BD than NG, especially for Pb (which is more strongly bound to moss
242 tissues than other elements due to its higher covalent index; Nieboer and Richardson,
243 1980). Although this result could be *a priori* striking, it could be explained by the fact that
244 the NG portion measures, on average for all SS and time periods, only 0.3 cm. These 0.3
245 cm grew throughout the whole exposure period (i.e. 1 month) which means that not even
246 all 0.3 cm (or what corresponded to each site and time period) were exposed to BD during
247 the whole month causing again a mismatch between exposure and BD collection periods.

248 *3.2. Correlations between the concentrations of heavy metals determined in different moss tissues*

249 The Spearman's rank correlation coefficients between the different moss tissues (NG vs
250 GP, NG vs T, and GP vs T) are presented in Tables 1 and 2 for the data grouped by time
251 and site respectively. First of all, the concentrations of all studied elements in NG and GP
252 were highly significantly correlated for all periods studied (see Table 1), which was expected
253 given that both tissue types originate from the same moss sample. However, again, the
254 number of significant correlations decreased to 14% (Cd) and 24% (Hg and Pb) of the
255 cases for the data grouped by site. The number of significant correlations between native *P.*
256 *purum* and transplanted *S. denticulatum* is greater for GP than for NG for all three elements,
257 despite that the time of exposure to atmospheric heavy metal deposition is more similar
258 between NG and T than between GP and T. Also, the percentage of significant
259 correlations decreased when data are grouped by sampling site rather than by time (from
260 42% to 5% for Cd, 42% to 0% for Hg, and 8% to 0% for Pb for the NG-T comparison;
261 and from 58% to 5% for Cd and Hg, and 25% to 0% for Pb for the GP-T comparison).

262 The lack of significant correlations between the concentrations of the metals studied in the
263 different tissue types could be due to the high levels of temporal variation (T1 to T12) in

the concentrations of the three metals in all of them (Figure 1) in combination with the differences among tissues in their capacity to take up heavy metals (discussed in section 3.3.). As pointed out in the previous section for NG and BD, graphs in Figure 1 show large variations in the concentration of Cd, Hg, and Pb in all four matrices (BD, NG, GP, T) with peaks in concentrations not matching neither BD nor tissue types for the most part (maximum and minimum concentrations occur at different times in the different tissues and BD). These differences are likely caused by variation in environmental factors that affect differently the four matrices due to, for example, different exposure times (GP has longer exposure times than NG and T, while T has overall longer exposure times than the whole NG portion), or different degrees of exposure to environmental factors (e.g. T are exposed in bags at ~2.5 m from the floor whereas the native moss is collected on the floor).

3.3. Differences in the magnitude of the concentrations of Cd, Hg and Pb among moss tissue types

Concentrations of the different elements in the three tissue types (NG, GP and T) are presented for the data grouped by sampling period in Figure 2, and by SS in Figure 3. Results of the Scheirer Ray Hare test showed significant interactions Tissue x Time and Tissue x SS for Cd and Hg respectively. The test showed significant differences in the concentrations of all three elements among tissue types in both datasets ($p<0.001$), among times for Cd ($p<0.05$) and among sites for Hg and Pb ($p<0.001$). When interactions were not significant, post hoc tests showed that all three tissues differed significantly in their concentrations of Hg and Pb in the data grouped by time ($p<0.001$ in both cases), and Cd and Pb in the data grouped by site ($p<0.001$ in both cases) with this general pattern: NG > GP > T for Cd, GP > NG > T for Hg, and T > GP > NG for Pb. These differences in the magnitude of the concentrations of the different elements in the three tissue types can be explained by differences in the morpho-physiological characteristics of the tissues, in

289 conjunction with differences in the physico-chemical properties of the metals emitted into
290 the atmosphere, both affecting the final pollutant loads.
291 First of all, *S. denticulatum* and *P. purum* differ in the concentration and type of organic
292 functional groups capable of binding heavy metals in their cell walls. By comparing
293 devitalized samples of *P. purum* and *Sphagnum sp.*, González and Pokrovsky (2014) found a
294 higher concentration of carboxylic binding sites - responsible for Cd binding - and a lower
295 concentration of sulfhydryl, amine, and polyphenol binding sites - responsible for Hg
296 binding - in *Sphagnum* mosses compared to *P. purum*. This would explain the general lower
297 concentrations of Hg in T than in NG and GP in this study, although not the lower
298 concentrations of Cd found in T. This unexpected result could be due to the effect of the
299 pre-treatment (devitalization by oven-drying at 120ºC) in the chemical composition of
300 transplanted moss (Adamo et al., 2008), which could modify the cation exchange properties
301 of the transplants. Also, *P. purum* is physiologically active throughout the exposure period
302 in this experiment meaning that it actively grows incorporating nutrients (and hence heavy
303 metals) available in the environment into its tissues, whereas *S. denticulatum* is dead and
304 therefore all metal uptake happens through passive processes.
305 Secondly, the position of the transplants and native mosses with respect to the atmospheric
306 deposition might also affect the capacity of these mosses to take up heavy metals. Native *P.*
307 *purum* is more or less horizontally exposed to deposition, which facilitates ion exchange at
308 the plant-water interface, whereas bags of *S. denticulatum*, exposed in a vertical position,
309 have less time to interact with soluble cations. Finally, *P. purum* and *S. denticulatum* differ
310 greatly in their morphology which may generate important differences in their capacity to
311 capture and retain particles due to variations in gametophyte branching, density, surface
312 area in contact with the atmosphere (surface area/ volume), and degree of compaction of
313 the phyllids (Castello, 2007; Carballeira et al., 2008; Holy et al., 2009). The high capacity of
314 *Sphagnum* species to retain particulate matter (e.g. two thirds of *Sphagnum sp.* biomass

315 correspond to leaves containing hyalocysts which makes them particularly suitable at
316 particle entrapping; Giordano et al., 2005; Bargagli, 2016) would explain its higher
317 concentration of Pb which is mostly found in the insoluble fraction of bulk deposition
318 (Morselli et al., 2003; Rueda-Holgado et al., 2014), and it has found at a greater proportion
319 in dry deposition than other elements (e.g. Cd and Hg) even in unpolluted sites (Connan et
320 al., 2013).
321 On the other hand, the differences in the concentrations of metals between NG and GP
322 are likely due to differences in their exposure times to atmospheric deposition combined
323 with putative differences in their physiological activities and the mobility of metals within
324 the moss shoots. In a previous study, Boquete et al. (2014) found a tendency for higher
325 concentrations of Hg and Pb in older tissues of *P. purum* compared to young ones which
326 agrees with the present results showing higher concentrations of both Hg and Pb in GP
327 compared to NG. These authors pointed out the high covalence index of these elements
328 (Nieboer and Richardson, 1980) to explain their lower mobility within the moss shoots, so
329 that once these metals are taken up by specific tissues they are not easily transported to
330 other parts of the shoots as it happens with other metals.
331 Finally, *P. purum* is a pleurocarpous moss with typical apical elongation. This kind of
332 mosses show almost no, or very tiny branches at the very tip of the shoot (i.e. 1-2
333 apicalmost cm) where elongation is happening, then branching reaches its peak in the
334 young regions after the tip, and finally, many branches, as well as shoot leaves, are lost
335 during tissue ageing (Bates, 1979). In this study, NG always consisted on the unbranched
336 apicalmost part of the shoots (never longer than 1.5 cm, Table 1 supplementary material)
337 whereas the GP consisted on the young region containing completely developed branches
338 which provide GP a greater capacity to retain particulate matter. This would explain the
339 greater concentrations of Pb in the GP of *P. purum*. Finally, despite the greater amount of
340 biomass constituting the GP compared to NG, NG is likely the most physiologically active

341 part of the shoot. Working with a species with similar growth patterns, *Pleurozium schreberi*,
342 Bates (1979) found marked drop in the physiological activity of the tissues beyond the first
343 2 cm of the most apical part of the shoot. Much greater physiological activity in the NG
344 where active elongation of the moss shoot is happening would be responsible for a greater
345 rate of incorporation of heavy metals.

346 **5. Conclusions**

347 Terrestrial mosses have long been used to monitor the atmospheric deposition of heavy
348 metals into terrestrial ecosystems. However, the complex relationship between the
349 concentrations of metals in moss tissues and atmospheric deposition has led to a general
350 lack of significant correlations between these two matrices, preventing the use of moss data
351 to reliably estimate absolute heavy metal deposition rates. In order to contribute to the
352 harmonization of the moss biomonitoring technique, we first tried to improve the
353 significance of the moss-BD relationship, and secondly, we evaluated which moss tissues
354 reflect better the BD levels. Our results demonstrate that: i) environmental factors
355 constitute a pivotal driver of the changes in the contents of heavy metals in mosses, whose
356 variability alters the moss-BD relationship unpredictably; ii) the effect of environmental
357 factors differs for all moss tissue types and for BD causing mismatches in the peaks of
358 concentrations in all four matrices; iii) native *P. purum* represents BD values better than
359 devitalized transplants of *S. denticulatum* regardless of how the data are grouped (by SS or by
360 time); and iv) when environmental variation is reduced, correlations between moss-BD
361 clearly improve for the elements studied, especially for the GP of native *P. purum*. In light
362 of these results we can conclude that neither native mosses nor transplants are good
363 estimators of absolute atmospheric heavy metal deposition rates. However, they allow us to
364 clearly differentiate SS subject to a wide range of deposition levels which makes them very
365 valuable qualitative indicators of atmospheric deposition. Finally, green parts of *P. purum,*
366 and likely of other mosses with similar growth forms, are the best choice for passive

biomonitoring of air quality and should be used to make results from different studies comparable.

Acknowledgements

This research was funded by the Spanish Government through MINECO project CTM2011-30305. The authors belong to the Galician Competitive Research Group GRC/GPC2016-002 and to the CRETUS Strategic Partnership (AGRUP2015/02). All of these programmes are co-funded by FEDER (EU). Authors would like to thank the use of RIAIDT-USC analytical facilities.

References

Aboal J.R., Fernández J.A., Boquete T., Carballeira A., 2010. It is possible to estimate atmospheric deposition of heavy metals by analysis of terrestrial mosses? Science of the Total Environment, 408: 6291-6297.

Adamo, P., Bargagli, R., Giordano, S., Modenesi, P., Monaci, F., Pittao, E., et al., 2008. Natural and pre-treatments induced variability in the chemical composition and morphology of lichens and mosses selected for active monitoring of air-borne elements. Environmental Pollution, 152: 11–19.

Ares A., Fernández J.A., Carballeira A., Aboal J.R., 2014. Towards the methodological optimization of the moss bag technique in terms of contaminants concentrations and replicability value. Atmospheric Environment, 94: 496-507.

Ares A., Aboal J.R., Carballeira A., Fernández J.A., 2015. Do moss bags containing devitalized *Sphagnum denticulatum* reflect heavy metal concentrations in bulk deposition? Ecological Indicators, 50: 90-98.

Barciela M.C., Noya M., López M.D., Aboal J.R., Fernández J.A., Bermejo-Barrera P., 2015. An environmentally-friendly method for As, Cd, Cr, Cu, Ni and Pb determination

392 in terrestrial moss samples by ultrasonic slurry sampling combined with electrothermal
393 atomic absorption spectrometry. Atomic Spectroscopy, 36 (1): 42-48.
394 Bargagli R., 2016. Moss and lichen biomonitoring of atmospheric mercury: A review.
395 Science of the Total Environment, 572: 216-231.
396 Bates J.W. 1979. The relationship between physiological vitality and age in shoot segments
397 of *Pleurozium schreberi*. Journal of Bryology, 10: 339-351
398 Bates, J.W., 1987. Nutrient retention by *Pseudoscleropodium purum* and its relation to growth.
399 Journal of Bryology, 14(3): 565-580.
400 Berg T., Royset O., Steinnes E, 1995. Moss (*Hylocomium splendens*) used as biomonitor of
401 atmospheric trace element deposition: estimation of uptake efficiencies. Atmospheric
402 Environment, 29: 353–360.
403 Boquete M.T., Aboal J.R., Carballeira A., Fernández J.A., 2014. Effect of age on the heavy
404 metal concentration in segments of *Pseudoscleropodium purum* and the biomonitoring of
405 atmospheric deposition of metals. Atmospheric Environment, 72: 28-34.
406 Boquete M.T., Fernández J.A., Carballeira A., Aboal J.R., 2015. Relationship between trace
407 metal concentrations in the terrestrial moss *Pseudoscleropodium purum* and in bulk
408 deposition. Environmental Pollution, 201: 1-9.
409 Box G.E.P., Cox D.R., 1964. An analysis of transformations (with discussion). Journal of
410 the Royal Statistical Society B, 26: 211–252.
411 Carballeira C.B., Aboal J.R., Fernández J.A., Carballeira A., 2008. Comparison of the
412 accumulation of elements in two moss species. Atmospheric Environment, 42: 4904-
413 17.
414 Castello M. A., 2007. comparison between two moss species used as transplants for
415 airborne trace element biomonitoring in NE Italy. Environmental Monitoring
416 Assessment, 133: 267–76.

417 Čeburnis D., Steinnes E., 2000. Conifer needles as biomonitors of atmospheric heavy metal
418 deposition: comparison with mosses and precipitation, role of the canopy.
419 Atmospheric Environment, 34: 4265-4271.
420 Connan O., Maro D., Hébert D., Roupsard P., Goujon R., Letellier B., Le Cavelier S., 2013.
421 Wet and dry deposition of particles associated metals (Cd, Pb, Zn, Ni, Hg) in a rural
422 wetland site, Marais Vernier, France. Atmospheric Environment, 67: 394-403.
423 Dytham C., 2003. Choosing and using statistics. Oxford: Blackwell.
424 Fernández J.A., Ares, A., Rey-Asensio, A., Carballeira, A., Aboal, J.R. (2010). Effect of
425 growth on active biomonitoring with moss. Journal of Atmospheric Chemistry, 63(1):
426 1-112010.
427 Fernández J.A., Boquete M.T., Carballeira A., Aboal J.R., 2015. A critical review of
428 protocols for moss biomonitoring of atmospheric deposition: sampling and sample
429 preparation. Science of the Total Environment, 517: 132-150.
430 Giordano, S., Adamo, P., Sorbo, S., Vingiani, S., 2005. Atmospheric trace metal pollution
431 in the Naples urban area based on results from moss and lichen bags. Environmental
432 Pollutution, 136: 431-442.
433 Giordano S., Adamo P., Monaci F., Pittao E., Tretiach M., Bargagli R., 2009. Bags with
434 oven-dried moss for the active monitoring of airborne trace elements in urban areas.
435 Environmental Pollution 157: 2798-2805.
436 González A.G., Pokrovsky O.S., 2014. Metal adsorption on mosses: toward a universal
437 adsorption model. Journal of Colloid and Interface Science, 415: 169–178.
438 Harmens H., Norris D.A., Koerber G.R., Buse A., Steinnes E., Rühling Å., 2008. Temporal
439 trends (1990–2000) in the concentration of cadmium, lead and mercury in mosses
440 across Europe. Environmental Pollution, 151: 368–76.

441 Holy M., Leblond S., Pesch R., Schröder W., 2009. Assessing spatial patterns of metal
442 bioaccumulation in French mosses by means of an exposure index. Environmental
443 Science and Pollution Research, 16: 499–507.
444 Kilbertus, G., 1968. Vitesse de decomposition de *Pseudoscleropodium purum* (Hedw.) Fleisch
445 dans la nature. Revue d'Ecologie et de Biologie du Sol, 2: 237e244.
446 Leblond S., Gombert S., Colin J.L., Losno R., Rausch de Traubenberg C., 2004. Biological
447 and temporal variations of trace element concentrations in the moss species *Scleropodium*
448 *purum* (Hedw.) Limpr. Journal of Atmospheric Chemistry, 49: 95-110.
449 Morselli L., Olivieri P., Brusori B., Passarini F., 2003. Soluble and insoluble fractions of
450 heavy metals in wet and dry atmospheric depositions in Bologna, Italy. Environmental
451 Pollution, 124: 457-469.
452 Nieboer, E., Richardson, D.H.S., 1980. The replacement of the nondescript term 'heavy
453 metals' by a biologically and chemically significant classification of metal ions.
454 Environmental Pollution Series B, Chemical and Physical. 1 (1): 3-26.
455 Rueda-Holgado F., Palomo-Marín M.R., Calvo-Blázquez L., Cereceda-Balic F., Pinilla-Gil
456 E., 2014. Fractionation of trace elements in total atmospheric deposition by filtrating-
457 bulk passive sampling. Talanta, 125: 125-130.
458 Russell S., 1984. Growth measurements in bryophytes: a case study. Journal of the Hattori
459 Botanical Laboratory, 56: 147-157.
460 Steinnes E., Rühling A., Lippo H., Mäkinen A., 1997. Reference materials for large scale
461 metal deposition surveys. Accreditation and Quality Assurance, 2: 243-249.
462 UNE-EN-standard 15841:2010, 2008. Ambient Air Quality - Standard Method for
463 Determination of Arsenic, Cadmium, Lead and Nickel in Atmospheric Deposition.
464 Established by the European Committee for Standardization (CEN).
465

Table 1. Spearman's correlation coefficients for the mean concentrations (n=3) of Cd, Hg and Pb in bulk deposition and different parts of native and transplanted mosses corresponding to the data grouped by sampling period (n=21). BD: bulk deposition; NG: new grown tissue generated monthly by native *Pseudoscleropodium purum*; GP: green part of *P. purum* shoots; T: transplants of devitalized *Sphagnum denticulatum*; -: coefficients not calculated because there were not enough data available (i.e. degrees of freedom ≤ 0); values in bold: significant correlations; *: p<0.05; **: p<0.001; ***: p<0.0001.

	Cd				Hg				Pb			
	NG-BD	NG-T	NG-GP	GP-T	NG-BD	NG-T	NG-GP	GP-T	NG-BD	NG-T	NG-GP	GP-T
T1	**0.656***	0.355	**0.936*****	0.348	-0.571	0.171	**0.780*****	0.394	**0.535***	-0.034	**0.961*****	-0.079
T2	**0.624***	**0.501***	**0.961*****	**0.529***	0.250	**0.525***	**0.847*****	**0.551***	0.249	0.337	**0.769****	**0.468***
T3	0.499	0.385	**0.864*****	0.158	-	**0.578***	**0.877*****	**0.612***	0.329	0.168	**0.713***	-0.142
T4	**0.806*****	0.297	**0.966*****	0.325	-	0.458	**0.833*****	**0.610***	**0.654***	**0.605***	**0.865*****	**0.466***
T5	0.280	**0.711****	**0.967*****	**0.740****	0.524	**0.523***	**0.802*****	**0.519***	-0.096	-0.249	**0.759****	-0.296
T6	**0.593***	**0.723****	**0.942*****	**0.675****	-	**0.655***	**0.905*****	**0.576***	**0.587***	0.226	**0.655***	0.152
T7	**0.810***	0.429	**1.000*****	**0.755*****	0.000	0.452	**0.786***	**0.435***	0.357	-0.310	**0.976*****	-0.200
T8	**0.709***	0.091	**0.867***	0.148	0.650	0.233	**0.900****	0.327	0.164	0.067	**0.782***	0.166
T9	0.443	-0.325	**0.925*****	-0.274	0.500	0.104	**0.514***	0.291	0.429	-0.179	**0.754***	**-0.455***
T10	**0.721****	**0.546***	**0.889*****	**0.599***	0.042	**0.676***	**0.897*****	**0.634***	0.189	0.133	**0.703***	0.148
T11	-0.179	**0.450***	**0.940*****	**0.442***	-	0.349	**0.968*****	0.265	0.357	-0.326	**0.744****	-0.119
T12	0.500	0.298	**0.933*****	**0.438***	-0.071	0.315	**0.948*****	0.303	0.400	0.207	**0.798*****	-0.019

Table 2. Spearman's correlation coefficients for the mean concentrations (n=3) of Cd, Hg and Pb in bulk deposition and different parts of native and transplanted mosses corresponding to the data grouped by sampling site (n=12). BD: bulk deposition; NG: new grown tissue generated monthly by native *Pseudoscleropodium purum*; GP: green part of *P. purum* shoots; T: transplants of devitalized *Sphagnum denticulatum*; -: coefficients not calculated because there were not enough data available (i.e. degrees of freedom ≤ 0). Values in bold: significant correlations; *: p<0.05; **: p<0.001; ***: p<0.0001.

	Cd				Hg				Pb			
	NG-BD	NG-T	NG-GP	GP-T	NG-BD	NG-T	NG-GP	GP-T	NG-BD	NG-T	NG-GP	GP-T
SS1	**0.738***	0.357	**0.786***	0.091	0.000	-0.086	**0.943***	-0.287	-0.238	0.357	0.071	0.000
SS2	-0.024	-0.248	0.309	0.000	-0.500	-0.067	0.394	0.007	0.350	0.150	0.650	0.280
SS3	0.371	-0.429	-0.095	-0.245	-	0.167	**0.762***	0.119	0.595	-0.048	0.286	-0.259
SS4	0.238	-0.127	0.176	-0.063	**1.000*****	0.042	**0.867***	-0.147	0.150	0.400	**0.933****	0.000
SS5	0.400	0.127	0.000	-0.084	0.500	-0.017	0.250	0.252	-0.436	0.245	-0.009	0.210
SS6	**0.738***	0.030	0.442	0.434	-0.300	-0.139	0.430	-0.007	0.612	0.333	0.612	0.315
SS7	0.450	-0.427	0.600	-0.531	-0.600	0.245	-0.127	-0.182	0.200	0.418	0.491	-0.049
SS8	**-0.609***	0.191	0.155	0.294	0.250	-0.445	-0.327	**0.671***	-0.182	0.218	**0.736***	0.014
SS9	-0.434	0.133	0.294	0.000	0.167	-0.126	0.427	0.035	0.126	-0.014	**0.762***	-0.245
SS10	0.036	-0.143	0.321	-0.315	-	0.700	0.800	0.545	-0.286	-0.179	0.036	-0.308
SS11	0.127	-0.103	0.285	-0.091	0.600	0.152	0.152	0.315	-0.406	0.091	-0.261	0.105
SS12	0.262	-0.248	0.176	**-0.643***	0.700	-0.236	-0.042	0.273	-0.200	-0.115	0.382	-0.259
SS13	0.233	-0.183	0.317	0.112	-	0.167	0.017	0.538	-0.200	0.517	0.317	0.266
SS14	-0.371	-0.371	0.543	0.161	-	0.714	-0.543	0.056	0.600	-0.257	0.543	0.392
SS15	**-0.714***	-0.550	0.183	0.063	-0.500	-0.183	0.500	0.238	-0.033	-0.233	0.617	-0.119
SS16	-0.050	-0.055	**0.758***	-0.329	**1.000*****	-0.345	-0.079	0.490	-0.442	0.467	0.345	-0.063
SS17	0.473	0.210	-0.490	0.154	0.100	-0.084	0.378	-0.266	0.077	-0.056	**0.846****	-0.392
SS18	-0.600	**0.829***	0.086	-0.294	-	0.371	0.600	0.336	0.371	0.257	0.429	0.476
SS19	**0.667***	0.285	0.115	0.091	**1.000*****	-0.612	**0.842***	-0.140	0.139	0.273	**0.697***	0.476
SS20	-0.503	-0.227	**0.800***	-0.196	0.700	-0.145	0.591	0.280	0.418	-0.227	0.418	-0.140
SS21	-0.800	-0.700	0.800	-0.042	-	0.100	**0.900***	0.280	0.400	0.500	0.800	0.028

Figure captions

Fig 1. Mean concentrations (ng g^{-1} dw) of Cd, Hg and Pb (μg g^{-1}) in green parts (GP; light grey bars) and new grow portion (NG; white bars) in native *Pseudoscleropodium purum* and devitalized transplants of *Sphagnum denticulatum* (T; dark grey bars) in relation with bulk deposition load (g ha^{-1}) (solid red line) in 4 sampling sites (SS): SS4 (rural area); SS8, SS12 (industrial areas) and SS17 (urban area) along different periods of exposure

Fig. 2. Concentrations of Cd, Hg (ng g^{-1}), and Pb (μg g^{-1}) in different parts of native and transplanted mosses for the data grouped by sampling period (n=21). NG: new grown tissue generated monthly by native *Pseudoscleropodium purum* (white series); GP: green part of *P. purum* shoots (light grey); Tr: transplants of devitalized *Sphagnum denticulatum* (dark grey). Main y-axis: concentrations of Cd and Hg in NG, and of Pb in NG and GP; secondary y-axis: concentrations of Cd and Hg in GP and Tr, and of Pb in Tr only.

Fig. 3. Concentrations of Cd, Hg (ng g^{-1}), and Pb (μg g^{-1}) in different parts of native and transplanted mosses for the data grouped by sampling site (n=12). NG: new grown tissue generated monthly by native *Pseudoscleropodium purum* (white series); GP: green part of *P. purum* shoots (grey); Tr: transplants of devitalized *Sphagnum denticulatum* (dark grey). Main y-axis: concentrations in NG; inner secondary y-axis: concentrations in GP; outer secondary y-axis: concentrations in Tr.

SUPPLEMENTARY MATERIAL

Table S.1: Mean, maximum, minimum monthly growth (cm month^{-1}) and total annual growth (cm) of *Pseudoscleropodium purum* shoots measured as monthly increments in length of the main stem throughout the duration of the study in each sampling site (SS). Only data over 1 mm are showed.

	Mean	Maximum	Minimum	Total
SS1	0.164	0.316	0.106	1.31
SS2	0.401	0.855	0.116	4.01
SS3	0.420	1.110	0.112	3.36
SS4	0.480	1.498	0.148	4.80
SS5	0.221	0.579	0.124	2.44
SS6	0.352	0.737	0.115	3.52
SS7	0.360	0.742	0.132	3.96
SS8	0.220	0.417	0.111	2.20
SS9	0.320	0.752	0.107	3.84
SS10	0.124	0.151	0.101	0.87
SS11	0.245	0.503	0.107	2.45
SS12	0.389	1.101	0.102	3.89
SS13	0.345	0.816	0.114	3.10
SS14	0.261	0.413	0.141	1.57
SS15	0.180	0.351	0.106	1.62
SS16	0.236	0.552	0.105	2.36
SS17	0.307	0.852	0.117	3.68
SS18	0.366	0.601	0.145	2.19
SS19	0.255	0.585	0.103	2.55
SS20	0.377	0.576	0.111	4.14
SS21	0.179	0.346	0.113	0.90

Figure

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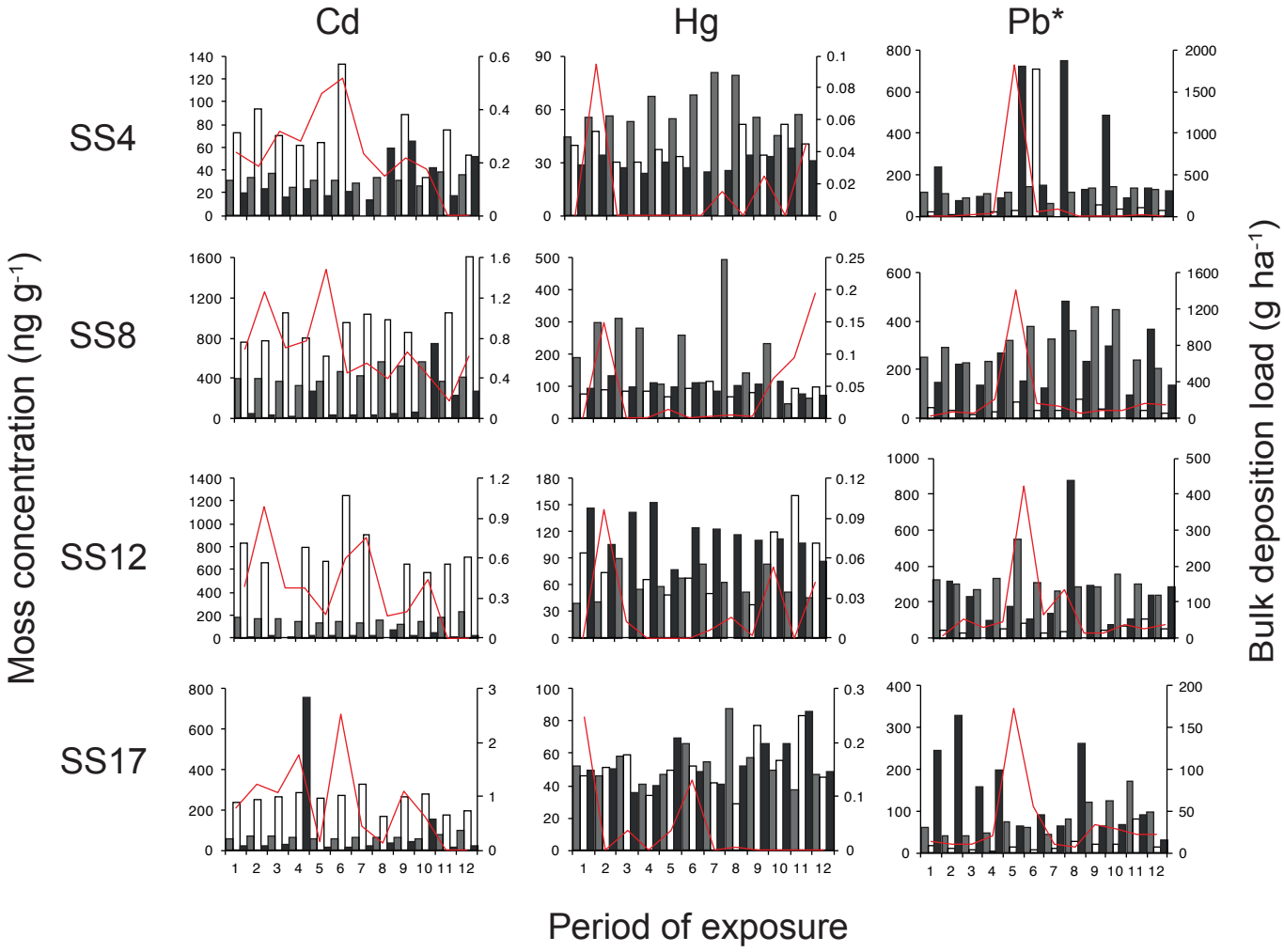

Figure

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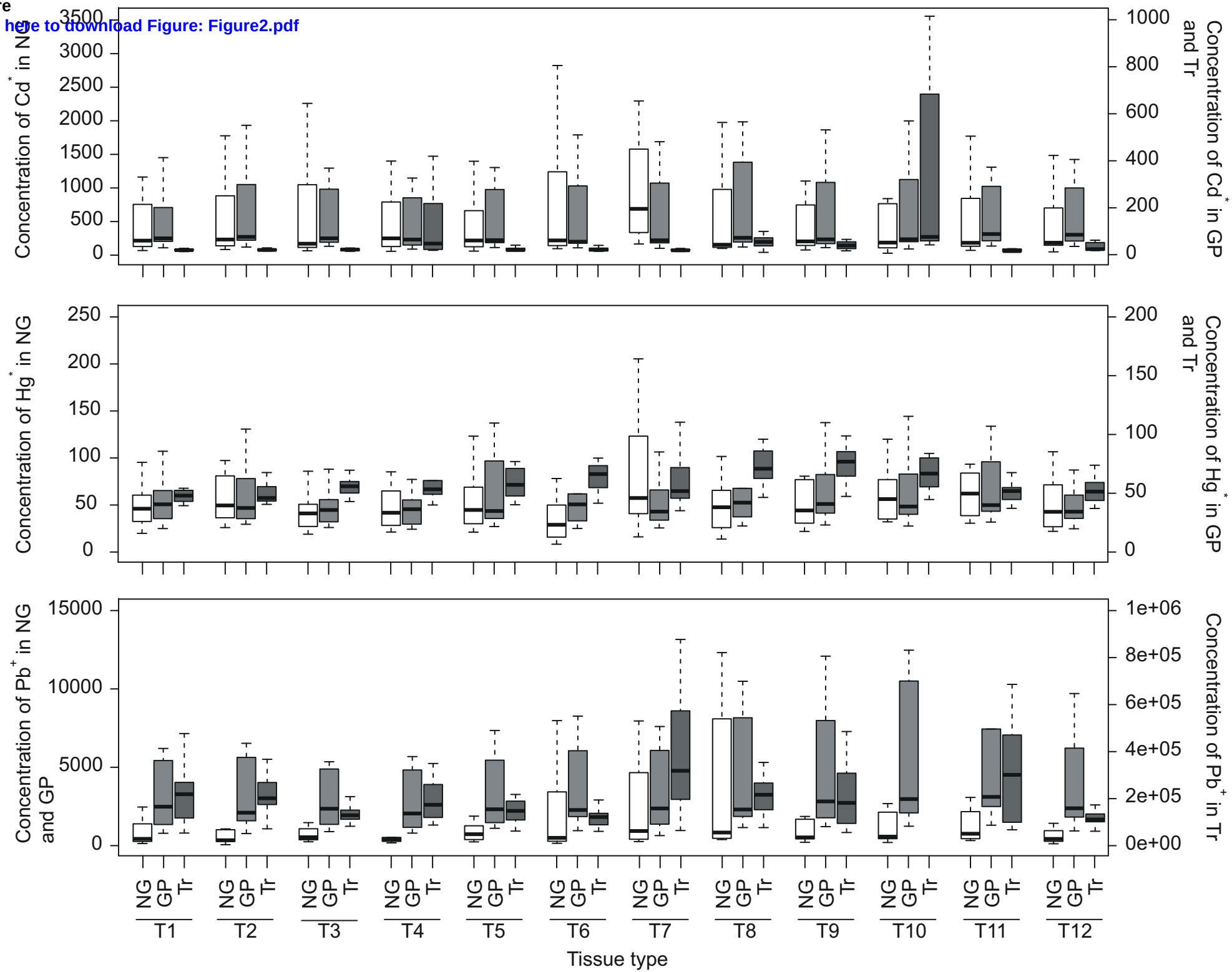

Figure

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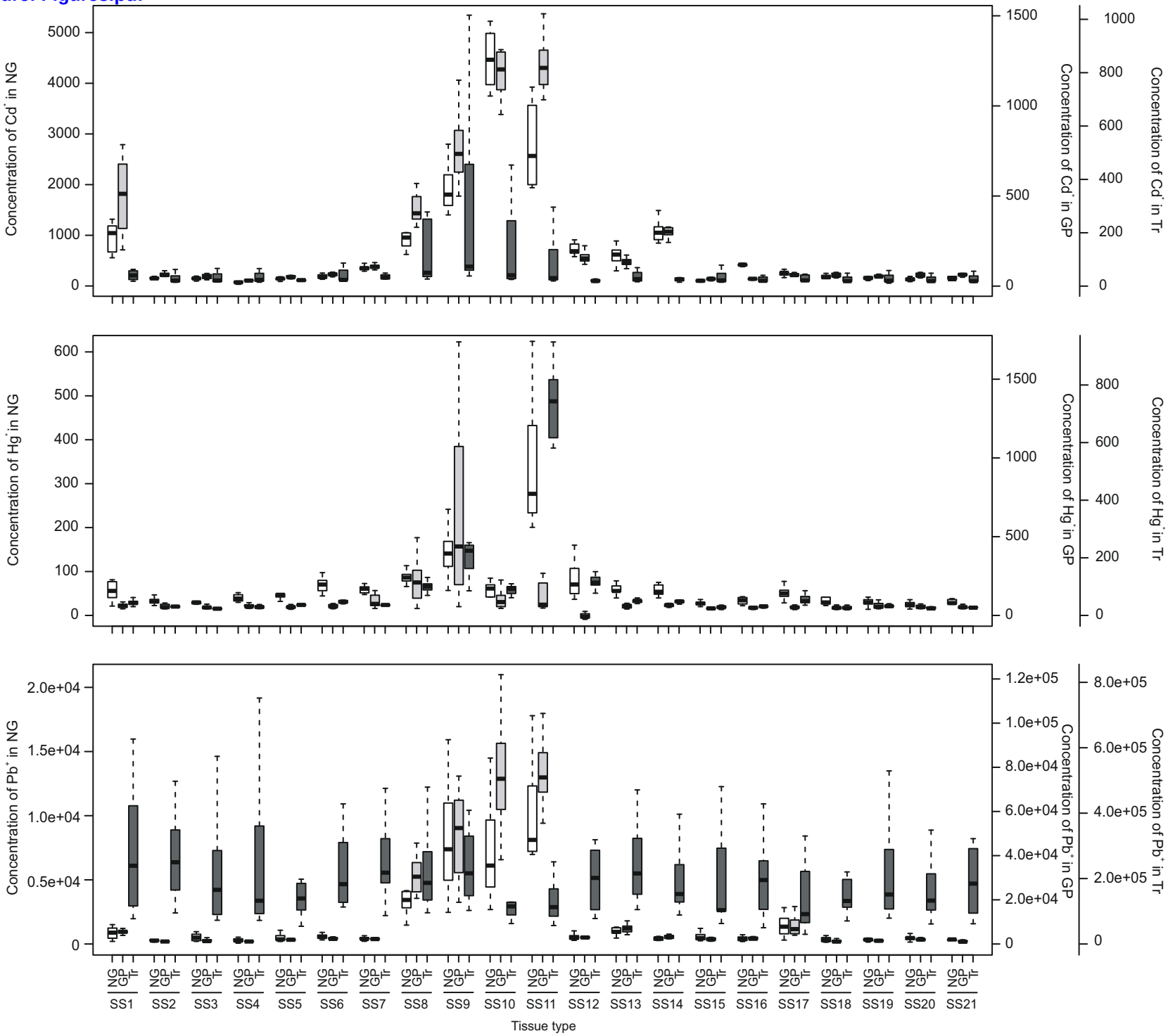

Conflicts of Interest Statement

Manuscript title: "Matching times: trying to improve the correlation between heavy metal levels in mosses and bulk deposition"

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