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ENVIRONMENT RESEARCH

Influence of the storage procedure on the trace element content measured in the aquatic moss *Fontinalis antipyretica* Hedw

Rubén Villares | **María Dolores Vázquez** | **Carlos Real**

Departamento de Bioloxía Funcional, Escola Politécnica Superior de Enxeñaría, Universidade de Santiago de Compostela, Lugo, Spain

Correspondence
Rubén Villares, Departamento de Bioloxía Funcional, Escola Politécnica Superior de Enxeñaría, Universidade de Santiago de Compostela, 27002 Lugo, Spain.
Email: ruben.villares@usc.es

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Abstract

Aquatic bryophytes have been used as pollution biomonitors for decades. Despite this, sample collection and preparation methods have not been standardized, which makes it difficult to compare the results of different studies. Most times, the samples have to be stored before processing, for example, when many of them are collected in a short time, as occurs in extensive pollution studies. Storage must be done in a way that does not change the pollutant concentrations in the samples. We studied whether the concentrations of Al, As, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn in the aquatic moss *Fontinalis antipyretica* were affected by three storage procedures: dry at room temperature, fresh (in refrigerator at 6°C), and frozen at −20°C. In addition, we evaluated whether the subsequent washing of the samples affected the concentrations of these elements differently depending on the storage method. Our results showed that the three methods were, in general, adequate since the concentrations did not change, and we did not observe differences between washed and unwashed samples either. Since the simplest method is refrigeration, we concluded that this is the best of them. However, the concentrations of Hg increased steadily over time in the fresh material, probably because of redistribution after volatilization from the basal parts of the mosses. We believe that the respiration of the plants lowered the concentrations of oxygen inside the hermetically sealed bags containing the samples, thus promoting the reduction of the Hg and its posterior volatilization and redistribution. We did not observe interactions between the storage method and the posterior washing of the samples.

Practitioner Points

- We studied the influence of storage procedure on element content in aquatic mosses.
- The procedures were as follows: dried at room temperature, stored in refrigerator, and frozen.

- The procedures did not change the element contents, except for Hg in refrigerator.
- All the procedures seemed suitable, but refrigeration was the simplest one.

KEYWORDS
biomonitor, bryophytes, metals, river

INTRODUCTION

Aquatic mosses have been used for decades as pollution biomonitors in river ecosystems (e.g., Monaci et al., 2021; Say et al., 1981; Vázquez et al., 2007) because they behave as excellent accumulators of pollutants, such as trace elements (Phillips, 1980; Zechmeister et al., 2003), being *Fontinalis antipyretica* one of the most frequently used species (Debén et al., 2015). However, despite the large number of published works on biomonitoring with river mosses, there is a lack of standardization of the methods (Debén et al., 2015), which makes difficult to compare the results of different studies.

Sample storage is a relevant step in the handling procedure before processing. When many samples are collected in a short time, they cannot be processed immediately, as the preparation for analysis is time-consuming, so they must be stored for some time. There are several alternatives for their storage: drying and storing them at room temperature, freezing, and storing them fresh in a refrigerator (e.g., Fernández et al., 2000; Samecka-Cymermann & Kempers, 1998; Vázquez et al., 2007). However, there is the possibility that the storage method used affects the integrity of the samples and therefore affects the concentration of the trace elements to be studied. For example, although certain species of aquatic bryophytes, such as *F. antipyretica*, can spend some time emerged during the dry season (Cruz de Carvalho et al., 2011), it is known that drying can alter the permeability of cell membranes in mosses, allowing the elements inside the cell to move outside (Brown & Brumelis, 1996; Brown & Buck, 1979). Freezing can also break or alter the cell membranes, favoring the release of intracellular elements (Vázquez et al., 2015). Therefore, keeping the samples humid in a refrigerator seems to be the least aggressive method. However, we did not find studies on the consequences of keeping the moss in these conditions.

Cell alterations are relevant because the next step in many studies is washing the samples (Debén et al., 2015). This is done to remove the matter attached to the exterior of the plant, such as deposits of particulate material or soluble elements present in intercellular spaces. Real et al. (2021) considered this an essential step, since the moss can retain significant amounts of particulate material on its surface (up to 40% of the dry weight of the sample). However, if the storage method weakened the cell walls, cleaning the samples might wash away part of the pollutants accumulated in their interior. Vázquez et al. (2015) found that an increase in washing time can be related to a reduction in the total concentration of more mobile macronutrients in samples of *F. antipyretica*, while the effect on trace metals was minimal. On the other hand, an increase in the concentration of certain elements after washing has also been found in terrestrial mosses, probably because of the solubilization of particles deposited on the surface that ended up incorporated into the moss (Fernández et al., 2010).

Despite the relevance of the storage method, most biomonitoring studies using aquatic mosses do not explain which method did they use. In the review of Debén et al. (2015) on the use of native aquatic bryophytes as biomonitors, they found only 2 out of 73 articles containing information on the storage method used. We know only two published studies addressing the effect of storage on the element concentrations. Wehr et al. (1983) compared samples of two species of aquatic moss dried at room temperature with others stored in a refrigerator (although only overnight). Vázquez et al. (2015), in a study focused on the cellular distribution of several elements, compared their concentrations in different cellular locations in frozen (−20°C for 7 days) and fresh (4°C < 24 h) *Fontinalis squamosa* samples. By contrast, storage effects on trace element concentration have received more interest in biomonitoring with terrestrial mosses (e.g., Aboal et al., 2008; Dołęgowska & Migaszewski, 2020).

Therefore, the aim of this study was to evaluate whether three storage procedures (dried at room temperature, fresh in refrigerator at 6°C and frozen at −20°C) alter the element concentrations in samples of the aquatic moss *F. antipyretica* and whether they interact with the washing procedure applied to the samples.

MATERIAL AND METHODS

Sampling

In July 2016, samples of the aquatic moss *F. antipyretica* Hedw. were collected in three rivers in

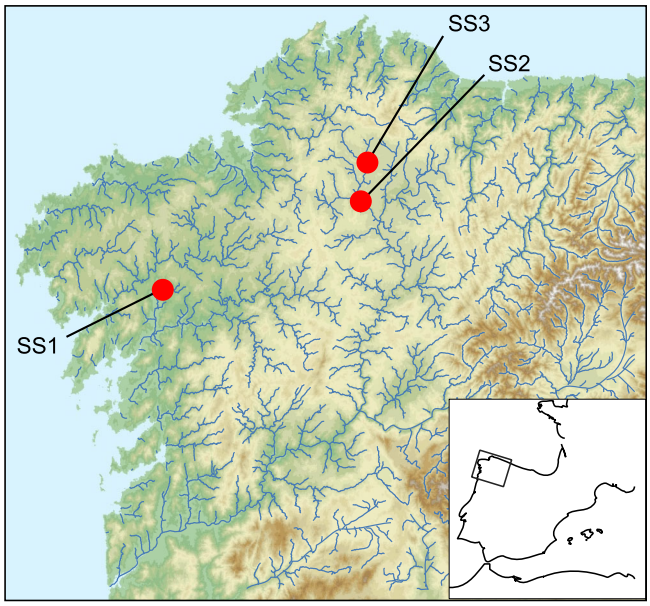

FIGURE 1 Location of sampling sites in the study area.

Galicia (NW of Spain) (Figure 1). The rivers, of different sizes and with different land uses in their basins, were selected to introduce some heterogeneity in the sampling, as would be expected in a biomonitoring study on a regional scale. The study area has a temperate and humid climate. The yearly average temperatures oscillate between 12°C and 13°C, with precipitations above 1000 mm per year, although with a marked summer drought, that causes large seasonal fluctuations in water flow. The sampling points (one in each river) were as follows:

SS1, located on the Tinto River (42° 48′ 56.6″ N, 8° 37′ 37.30″ W [WGS84]; altitude: 77 m a.s.l.), a small river about 5 m wide at the sampling site (SS), surrounded by crops and forested areas.

SS2, Ladra River (43° 9′ 48.30″ N, 7° 43′ 5.80″ W; 395 m a.s.l.), about 20 m wide, in a well-developed riparian forest area, although upriver there are extensive meadows and crops.

SS3, Madalena River (43° 17′ 13.00″ N, 7° 41′ 17.00″ W; 425 m a.s.l.), about 10 m wide, 600 m downstream of the water resource recovery facility of Vilalba (a town of 14,000 inhabitants).

Each sampling point comprised a 100 m long river stretch where the moss grew abundantly, albeit irregularly distributed, forming clumps where there was a solid substrate to attach to, such as rocks or tree roots. Each 100 m stretch was divided into three sections of equal length, and in each section, moss material was collected from 10 to 15 clumps, always submerged at the time of sampling. The samples were washed in situ with water from the river itself to remove any loosely attached sediment, debris, and invertebrates, and they were transported to the laboratory in refrigerated containers.

Storage and washing treatments

Once in the laboratory, the samples collected in each of the three sections of each river were divided into six subsamples (54 subsamples in all: three rivers × three sections × six subsamples), which were subjected to one of the following storage methods:

a. Fresh: samples kept in a refrigerator at 6°C inside closed polyethylene bags for 1 day (processed within 24 h of collection) and 2, 7, and 14 days. The 2 and 7 day data were only available for unwashed samples (see below).
b. Frozen: samples kept at −20°C in closed polyethylene bags for 14 days. Thawing was carried out in a refrigerator at 6°C.
c. Dry: The samples were spread out in plastic trays and kept for 2 days at room temperature for drying. They were then kept for 14 days in plastic containers covered with a sheet of filter paper and at room temperature. Before further processing, the samples were rehydrated with sprayed distilled water (Fernández et al., 2010).

After the corresponding storage time was completed, 14 g (fresh wt.) of apical 3 cm fragments was collected from each subsample. This was enough to get at least 2 g of dry tissue for the chemical analyses. A half of this fresh material was washed; to do this, the segments of moss were placed in 250 mL wide mouth plastic vessels containing 100 mL of distilled water and shaken by hand for 1 min, then the water was removed and another 100 mL of distilled water were added, shaking again for another minute.

The particulate material extracted by washing the samples was stored for further analysis. For this, the washing water was centrifuged (Centronic BL, Selecta, Barcelona, Spain) at 3000 r.p.m. for 3 min, to separate the particulate matter and the water was discarded. The particulate matter was resuspended using <30 mL of distilled water to transfer it to glass vials, where it was left to decant for 2 days, the excess water was retired with a Pasteur pipette, and the remaining material dried for 2 days at 50°C (see more details in Real et al., 2021).

Trace element analysis

The moss samples were dried at 50°C for 48 h and ground in a tangential mill (MM400, Retsch, Haan, Germany), with zirconium oxide grinding containers and balls. Approximately 0.300 g of each subsample were digested with a mixture of 8 mL of HNO3 (69%) and 2 mL of H_2O_2 (33%) in a microwave oven (Ethos1 Plus, Milestone) for 15 min at 190°C and 1000 W. Distilled water (Milli-Q, Millipore) was subsequently added to produce 50 mL of extract. The concentrations of Al, As, Ba, Cd, Cr, Cu, Hg, Fe, Hg, Mn, Ni, Pb, and Zn were determined with Inductively Coupled Plasm-Mass Spectrometry (ICP-MS, mod. 7700x, Agilent). Hg in particulate matter was determined with a Milestone DMA-80 (Direct Mercury Analyzer, Milestone Srl, Sorisole [Italy]) from 20 mg of dried material. To control the quality of the procedure, samples of certified reference material M2 (moss *Pleurozium schreberi*) were analyzed (Steinnes et al., 1997). The measurements were reproducible, showing low standard deviations, comparable with the certified and recommended values.

Statistical analysis

Statistical analysis were performed with R (R Core Team, 2020) and with the IBM SPSS Statistics software, version 24. We did two analysis. The first (we will call it the main experiment for simplicity) was a factorial analysis of variance (ANOVA) to test the influence of SS, Storage Method, Storage Time, and Washing on the element concentrations (a test per element). A second analysis (we will call it the temporal sequence) included only fresh unwashed samples from the three SSs with concentrations measured at 1, 2, 7, and 14 days. In this case, Storage Time was a continuous factor and and SS a qualitative one, so ANCOVA was used to analyze the data.

Data distribution and its influence on the statistical analysis

Initial exploratory analysis of the data for the main experiment revealed that the data were heteroscedastic. Moreover, we found significant linear relationships between the mean and the standard deviation of the groups defined by the factors in the analysis, for all elements except Ba and Mn (Table 1). This showed that the data distribution was gamma. This finding is consistent with the nature of concentration data because the range of the gamma distribution includes positive values only, as the concentrations do, and because this is the distribution to be expected when a solution of some substance is randomly diluted (Ott, 1995), as occurs to the pollutants after being released into a river.

TABLE 1 Determination coefficients and signification levels for the regressions between mean and standard deviations of the data groups resulting from the combination of factors in the main experiment (storage type—three levels, washing—two levels, and sampling site—three levels, i.e., 18 groups).

	R^2	p
Al	0.626	<0.001
Cr	0.368	0.002
Mn	0.152	0.059
Fe	0.301	0.005
Ni	0.493	<0.001
Cu	0.303	0.005
Zn	0.319	0.004
As	0.750	<0.001
Cd	0.442	<0.001
Ba	0.003	0.816
Hg	0.634	<0.001
Pb	0.279	0.008

Note: Each group contained $n = 3$ data.

The analysis was repeated for the data of the temporal sequence. As before, Mn and Ba did not show any relationship between mean and s.d., and Fe and Pb showed a linear but not significant ($p = 0.06$) relationship. In this second analysis, the number of data groups was smaller and the range of the means narrower, which produced a general decrease in the significance of the tests.

Due to the data distribution, it was necessary to use the generalized linear model (GLM) to perform ANOVA and analysis of covariance (ANCOVA) (Chambers & Hastie, 1992; McCullagh & Nelder, 1989). GLM transforms the response variable using a link function. For gamma-distributed data, the reciprocal transformation and the calculations were done on the concentrations^{-1}. We employed *F*-tests to obtain significance values for the factor and interaction effects in GLM-ANOVA (the appropriate test depends on the data distribution) and *t*-tests in GLM-ANCOVA. We summarized the results tabulating the *p*-values produced in the analysis (done with the R function glm).

ANCOVA produces a set of linear models relating the reciprocal of the concentrations to Storage Time, one for each SS. The usual procedure is to start with a full-model, in this case including the factors Storage Time (the slopes of the linear relationships were not equal to zero), SS (the intercepts for the SS were not equal), and their

interaction (the slopes differ between SSs). If some of the factors or interactions are found not significant, then a simplified model can be adjusted that does not include the effects the non-significant elements.

RESULTS

Initial elemental concentrations

Table 2 shows the initial mean elemental concentrations, that is, in mosses processed within the first 24 h after collection. For the unwashed samples, SS1 showed the highest concentrations of Cu, Pb, and Zn, SS3 those of Cr and Hg, and the remaining elements showed maximum values in SS2. For the washed samples, the highest concentrations of Cu, Pb, Zn, and Mn were found in SS1; SS2 showed the highest levels of Ni, As, and Cd, and for the rest of the elements, the highest concentrations were found in SS3. Table S1 of the supporting information shows the means of the concentrations of the determined elements for the groups resulting of the combination of the factors.

Comparison of storage methods after 14 days

The results of the main experiment were summarized in Table 3. None of the interactions was significant, so the effect of each factor on the concentrations could be interpreted independently of the others. The origin of the samples was significant for all elements, which is a consequence of the diverse conditions that the SSs represent.

TABLE 2 Mean concentrations (μg g^{-1}) and standard deviations in washed and unwashed *Fontinalis antipyretica* samples, from the three SSs, processed within 24 h of collection, $n = 3$.

	SS1		SS2		SS3	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Unwashed						
Al	413	49.2	843	262	642	87.3
Cr	0.67	0.15	1.33	0.32	1.53	0.32
Mn	2801	691	3681	1532	1631	335
Fe	1593	317	2746	729	2630	433
Ni	6.21	1.73	14.9	3.19	3.95	0.45
Cu	13.2	2.15	5.02	0.34	6.03	0.71
Zn	279	48.9	81.4	25.9	71.0	10.7
As	0.80	0.17	2.89	0.99	0.79	0.13
Cd	0.46	0.11	0.94	0.21	0.34	0.05
Ba	132	28.7	164	51.5	125	19.5
Pb	4.40	0.74	1.40	0.03	4.08	0.48
Hg	0.046	0.009	0.055	0.010	0.085	0.002
Washed						
Al	376	189	694	262	789	194
Cr	0.52	0.31	1.02	0.17	1.18	0.05
Mn	4274	1484	3440	1153	3476	1377
Fe	1620	457	2260	651	2646	560
Ni	8.14	1.00	14.5	1.93	5.81	1.61
Cu	14.5	3.83	4.81	0.22	7.50	1.29
Zn	379	88.6	77.0	18.8	131	30.1
As	0.93	0.26	2.33	0.65	0.91	0.19
Cd	0.68	0.17	1.04	0.16	0.63	0.22
Ba	175	45.2	150	30.9	201	53.3
Pb	4.30	0.38	1.10	0.07	3.07	0.61
Hg	0.039	0.010	0.050	0.006	0.085	0.006

Abbreviation: SS, sampling site.

The effect of washing was highly significant for Cr and Pb and marginally significant for Cd, Zn, and As. Finally, the conservation method did not produce significant differences in concentrations for most elements. Only Al, Cu, and Hg showed evidence of changes of the concentrations related to this factor.

To determine the conservation methods that altered the concentrations of Al, Cu, and Hg, Dunn–Bonferroni post hoc tests were applied to their data. As there were no differences for these elements between the washed and unwashed samples, the data were pooled to increase the power of the tests. Figure 2 shows the median concentrations for the resultant groups and the significance of the comparisons. Only Al and Hg results are represented because the test did not find significant differences for Cu despite the ANOVA results. There were significantly lower concentrations of Al in fresh and freezed samples but only for SS2. Hg showed increased concentrations in the fresh samples after 14 days in all SSs; the concentrations of the dried moss were higher also but only for SS2.

Temporal evolution of concentrations in the refrigerated samples

The availability of the data for fresh mosses after 1, 2, 7, and 14 days of collection allowed a more detailed study of their temporal changes while they were stored (Figure S1 of the supporting information). As explained in the methods section, we used GLM-ANCOVA to fit linear models to the data, with Storage Time as continuous and SS as categorical independent variables.

We first fitted the full-model (SS, Storage Time and their interaction) to the data of each element and found that the effect of Storage Time was not significant for all elements excepting Hg. The effect of the SS was significant or not depending on the element, but this simply shows the differences in element contents between SSs, as in the main experiment. Note that ANCOVA uses one SS as reference (SS1, actually but could have been any other) and tests for equality of slope and intercept of the resulting linear fit against the other two. It also tests for

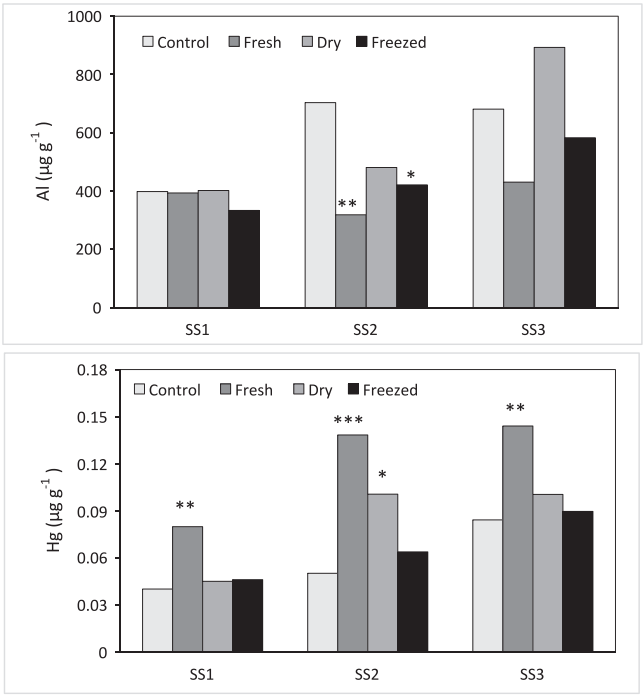

FIGURE 2 Medians of the Al and Hg concentrations in samples of *Fontinalis antipyretica* for the three SS and three conservation methods. The asterisks show the significance of the effect of each conservation method compared with the control for each SS (fresh sample, 1 day), determined using the Dunn–Bonferroni post hoc test for pairwise comparisons (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Note that data from washed and unwashed moss were pooled as there were no significant differences between them (see text); therefore, $n = 6$ for each group.

TABLE 3 Summary of the results of the ANOVA analysis of the main experiment.

Anova term	Al	Cr	Mn	Fe	Ni	Cu	Zn	As	Cd	Ba	Hg	Pb
ConsT	0.044	0.668	0.530	0.115	0.660	0.040	0.454	0.180	0.580	0.357	0.017	0.111
Wash	0.268	<0.001	0.080	0.075	0.372	0.207	0.052	0.029	0.036	0.164	0.089	0.001
SS	<0.001	<0.001	0.006	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.043	<0.001	<0.001
ConsT:Wash	0.582	0.842	0.918	0.366	0.738	0.725	0.955	0.435	0.918	0.801	0.317	0.547
ConsT:SS	0.193	0.599	0.838	0.681	0.901	0.315	0.544	0.367	0.765	0.750	0.635	0.110
Wash:SS	0.707	0.977	0.639	0.770	0.769	0.967	0.240	0.825	0.741	0.929	0.977	0.073
ConsT:Wash:SS	0.986	0.501	0.754	0.970	0.651	0.867	0.650	0.764	0.844	0.733	0.926	0.410

Note: The values are the significance (p-values from F-tests) of the effects: conservation type (ConsT), levels: fresh, dry, freezed; washing (wash), levels: washed, unwashed; sampling site (SS), levels: SS1, SS2, SS3; and of their interactions. The experiment was fully balanced, with $n = 3$ replicates per combination of the levels of the three factors ($N = 3 \times 2 \times 3 \times 3 = 54$ data).

TABLE 4 Summary of the results of the ANCOVA analysis of the temporal sequence.

	Al	As	Ba	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Storage time	0.740	0.600	0.296	0.793	0.536	0.903	0.569	0.001	0.246	0.768	0.900	0.448
Intercept SS2	0.000	0.000	0.038	0.000	0.000	0.000	0.027	0.689	0.045	0.000	0.000	0.000
Intercept SS3	0.035	0.578	0.582	0.166	0.000	0.000	0.089	0.000	0.004	0.025	0.800	0.000
Slope SS2	0.096	0.182	0.023	0.214	0.692	0.551	0.220	0.225	0.015	0.156	0.127	0.050
Slope SS3	0.507	0.970	0.538	0.420	0.644	0.816	0.553	0.166	0.466	0.832	0.088	0.351

Note: The values are the significance (*p*-values from *t*-tests) of the effects: sampling site (SS), levels: SS1, SS2, SS3; storage time, levels: 1, 2, 7, 14 days; and of their interactions. Storage time was considered a continuous factor and SS categorical. The first row contains the *p*-value for the overall effect of the storage time factor. For each SS, a model was fitted to the data and the slopes and intercepts corresponding to SS2 and SS3 were compared with those of SS1. The experiment was fully balanced, with with $n = 3$ replicates per combination of the levels of the two factors ($N = 4 \times 3 \times 3 = 36$ data).

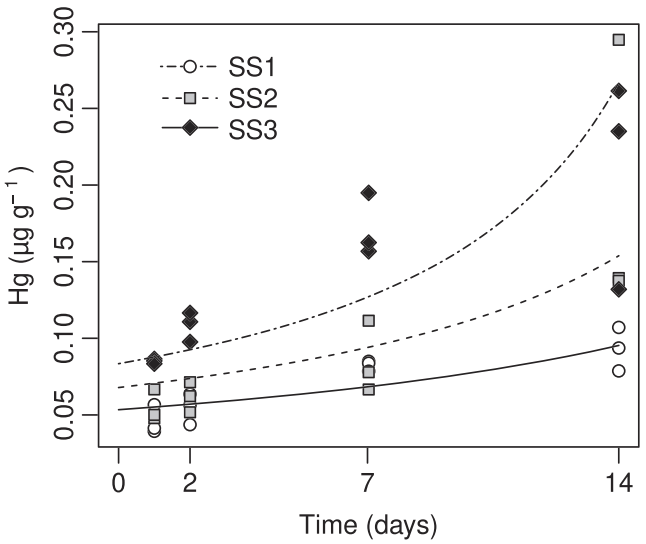

FIGURE 3 Simplified GLM-ANCOVA models adjusted to the Hg data of the temporal sequence, that is, with a common slope. The graph shows the data and model predictions in the back-transformed scale, as the models were fitted to the inverse of the concentrations.

the overall significance of Storage Time. All these tests were summarized in Table 4.

The effect of Storage Time was highly significant for Hg, but the interactions were not. This indicated that the slope was similar for the three SSs, that is, that the process of concentration increase was similar in the three cases. Therefore, we repeated the ANCOVA but without including the interactions to simplify the model. The final model equations (in the transformed scale) were $[\mathrm{Hg}]^{-1} = a_{SS} = 0.5884\, t$ (days), being $a_{SS1} = 18.73\ \mathrm{ppm}^{-1}$, $a_{SS3} = 14.74\ \mathrm{ppm}^{-1}$, and $a_{SS3} = 11.99\ \mathrm{ppm}^{-1}$. However, when they were represented in the original scale (Figure 3), the lines are curved and divergent, and the curvature, and consequently the divergence, increased with the initial contents of the samples.

Particulate material

The increase in Hg contents during storage raised the question of its origin. One possibility was that it was transferred from the particulate matter retained by the moss. If this hypothesis were correct, a reduction in the Hg concentrations of the particulate material along time would be expected. To verify this hypothesis, we analyzed this element in the material extracted by washing. Figure 4 shows the mean concentrations of Hg at 1 day and 14 days in the particulate matter from the three SSs. In all of them, the concentrations increased, so we discarded the particulate matter as the source.

DISCUSSION

The initial mean elemental concentrations were low and within the ranges considered as background or reference in other studies with *F. antipyretica* (Carballeira & López, 1997; Culioli et al., 2009; Vuori & Helisten, 2010). Despite this, there were significant differences between SSs for all elements, as showed by the results of the main experiment and the temporal sequence. Incorporating this variability was one of the objectives of the experimental design.

As a general conclusion, all the methods seem to be adequate for the storage of samples. This factor showed a significant effect only for Cu, Al, and Hg. However, the post hoc tests could not find significant differences for Cu, and for Al, they were found only for SS2, that is, the effect was not consistent across SSs. Therefore, the results were not conclusive for these elements. On the contrary, the concentrations of Hg increased along time for all SSs and in the main experiment and the temporal sequence. For some samples, the increases multiply by 3 or more the initial Hg concentrations. The models adjusted to the temporal sequence data showed that the increase was not lineal but accelerates as time passes and that the effect is

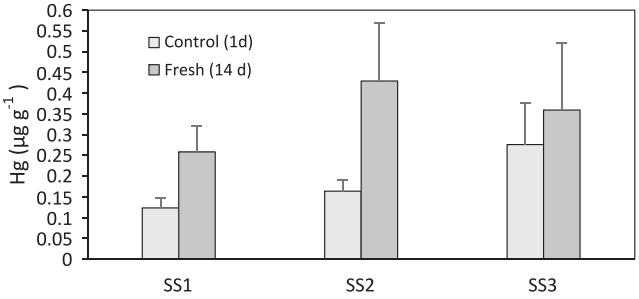

FIGURE 4 Mean and standard deviation (lines) for the concentrations of Hg in the particulate material extracted by washing the fresh moss material (days 1 and 14).

larger for samples with higher initial Hg concentrations. Therefore, we do not recommend storing the samples in confined spaces before Hg analysis.

The models produced by ANCOVA are of limited value from a bioindication perspective. They were a by-product of the statistical technique used, not an objective of this study. They were flawed because the concentration increase cannot proceed indefinitely, as they predict. After some time, the Hg concentration should reached a steady state; that is, it would follow a sigmoidal function of some type. It seems that 14 days only allowed to observe the initial steps of the relocation of Hg.

This behavior of Hg might be probably due to the existence of volatile chemical compounds (Hg^0 and dimethylmercury) that can be produced by bacteria or plants (Ehrlich & Newman, 2008; Kumar et al., 2017). It would be possible that the volatilization in the lower parts of the plant and its subsequent absorption in the apical parts were the cause for the enrichment. We did not measure the concentrations in the old parts, but they certainly made up most of the stored material (as compared to the branch tips which were used for the analysis). Even if only a small part of that Hg was volatilized, it could be enough to increase the concentrations in the apices. These were separated for analysis after the storage period and the samples were kept in hermetic bags, precisely to maintain the samples humid. It is likely that the respiratory activity of the mosses in the dark led to reducing conditions inside the bags that facilitated Hg volatilization. This element volatilizes more easily in soils with low O_2 levels (Obrist et al., 2010). It might be expected the low storage temperature (6°C) would slow the volatilization of Hg. However, according to the equation presented in Pannu et al. (2014) describing its volatilization from a boreal soil, 6% of the total Hg can be lost in 24 h at 6°C as gaseous Hg^0. We discarded the hypothesis of the particulate matter adhered to the moss being a source for this element, as its contents also increased along time, just as the moss tissues did.

Moss stored dry and subsequently rehydrated and washed showed no significant change in element concentrations. Wehr et al. (1983) found that air-dried aquatic mosses had much higher metal concentrations than mosses kept fresh and slightly chilled. They attributed this to the fact that drying can somehow reduce the amount of surface bound element, which was eliminated in a subsequent washing step. They hypothesized that this metal fraction was adsorbed to iron and manganese oxides. It should be noted, however, that these authors analyzed the entire plant, not just the youngest parts where these oxide deposits are less important. This may cause the difference between our results and theirs.

Fernández et al. (2010) observed that both freezing and drying samples of the terrestrial moss *Pseudoscleropodium purum* altered the permeability of the membranes, causing the release of intracellular elements. These authors recommended rehydrating the samples by exposing them to a humidity-saturated atmosphere for 1 week in a refrigerated chamber at 4°C to mitigate this problem. They also observed that the washing of the samples strongly affected the concentrations. However, terrestrial mosses are not subjected to immersion and agitation in water under natural conditions, like the aquatic mosses, so these differences in behavior are not surprising.

CONCLUSIONS

All the storage methods tested seemed suitable for trace element analysis of aquatic moss samples. However, from an operational point of view, refrigeration seems to be the simplest of them. It is as effective as drying and freezing, but it does not require subsequent rehydration, as with drying, nor waiting for thawing. However, the concentrations of Hg can be significantly altered. If this element will be measured, the samples must not be stored in airtight bags to avoid the development of low oxygen concentrations, although in this way there is the possibility of losing some moisture. This should not be a big problem, as the complete drying of the moss did not alter the element concentrations, either.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supporting information of this article.

ORCID

Rubén Villares https://orcid.org/0000-0002-9635-4449

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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