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What dead seaweeds can tell us about metal uptake and their application to control marine pollution

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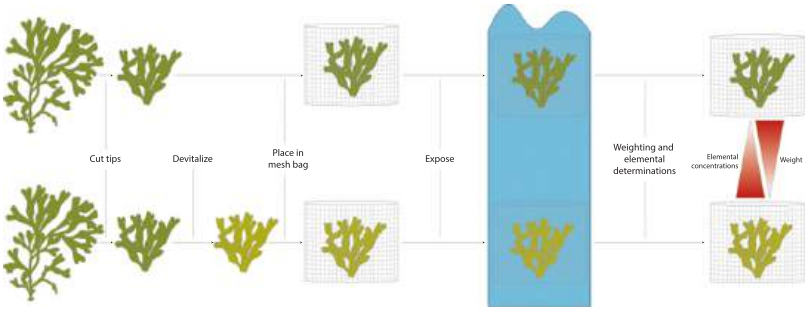
HIGHLIGHTS

- We compared the elemental uptake by fresh and devitalized seaweed transplants.
- Most of the material in devitalized transplants was lost during the exposure period.
- Devitalized samples had higher elemental uptake for most trace elements.
- Physicochemical changes on the seaweeds' surface contribute to the increased uptake.
- Extracellular chemical binding and physical adsorption are relevant uptake pathways.

GRAPHICAL ABSTRACT



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ABSTRACT

The mechanisms of trace element uptake by seaweeds are still unknown, despite being key to understand the impact of pollution in coastal environments. This knowledge gap, in addition to the lack of standardization, have also hindered the use of seaweeds to monitor seawater pollution. To address these shortcomings, we tested the use of devitalization as a pre-exposure treatment for brown seaweed transplants, and we compared devitalized and fresh transplants to gain some insights into the mechanisms of element uptake. We exposed four types of *Fucus vesiculosus* transplants in 6 sites for 4, 8 and 20 days: fresh and devitalized (dried or boiled) algal segments held in mesh bags, and whole algal thalli imitating natural conditions. We then determined he concentrations of 11 trace elements in the algal tissues. The element concentrations were highest in the devitalized transplants, but the material lost consistency and weight throughout the exposure period, limiting their use to short periods. We proposed several factors that may contribute to the different accumulation patterns between treatments, and examined the implications for the uptake mechanisms, revealing that two of the most important are surface adsorption of sediment particles and chemical bounds to extracellular components.

1. Introduction

Pollution is one of the main ways by which human activities damage the environment [6] and is also harmful to human health, being responsible for up to 9 million deaths a year [29]. Many countries and organizations have regulatory agencies and legislation intended to

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control pollution levels and prevent the impact of pollutants on ecosystems and human health. Aquatic environments are particularly sensitive to pollution [23] and contamination is strictly regulated by legislation worldwide, such as the Clean Water Act in the United States [40], the European Water Framework Directive [13] and the Marine Environment Protection Law in China [34]. The regulations apply to coastal waters, which are important for food production, and also to marine ecosystems in general, owing to their importance in primary production and as nurseries [37]. Nonetheless, aquatic environments are severely affected by human activities, as water has been historically used for waste disposal, and persistent pollutants such as potentially toxic elements (PTEs) frequently reach coastal environments [4,42].

The first step in controlling pollution is to obtain reliable information about pollutant levels. The classical approach of measuring the concentration of pollutants directly in seawater has some disadvantages: the analytical determinations can be difficult and costly [28]; there is no temporal representativeness, owing to the rapid rate of replacement of coastal water; and the measurements do not reflect the impact of the pollutants on organisms, which may depend on the chemical species involved. Biomonitoring is an alternative approach that solves these problems by measuring the concentrations of pollutants in organisms exposed to the water rather than directly in the water. This approach is useful for providing information about the environment where the organisms live and about the organisms themselves, which may make it especially valuable for environmental impact assessments. However, this technique also has some disadvantages, such as the lack of comparability of different studies owing to the use of different organisms and methods [15,17].

To standardize the organism used for such studies, the taxonomic group that has attracted the most attention is seaweed. They have been widely used in the past in coastal biomonitoring because they are abundant in most regions throughout the year [17]. Not only this, but measuring the concentration of pollutants in seaweed tissues is also interesting due to their great importance; in coastal ecosystems they play a role as ecosystem engineers [39], and provide food and shelter for many other organisms; and directly for society as we extract countless products from them, including food, biofuels, and substances used in the food and pharmaceutical industries [5,31]. Thus, understanding how seaweed interact with PTEs in the environment is key to predicting how pollution will affect their ecosystem functions, the properties of seaweed-derived products, and to interpreting the results of biomonitoring studies. However, the accumulation mechanisms and factors affecting it are barely understood.

This knowledge gap, in addition to the lack of standardized protocols hinder the application of seaweed for biomonitoring. Some advances have already been made; *F. vesiculosus* has been recommended for the Atlantic region, but the protocol for using this species as a biomonitor is far from being optimized. The active biomonitoring technique, which has proved successful in other cryptogams such as terrestrial mosses [2] and freshwater mosses [10], has been recommended for use with seaweeds [15]. This approach involves culturing or obtaining the plant material from an unpolluted site and transferring it to the test area for exposure. The technique allows the use of pre-exposure treatments, including devitalization. Devitalization consists of killing an organism in a way that preserves the relevant properties for biomonitoring. This method has produced more stable results with other cryptogams [11,2,20], and it has simplified the logistics of the monitoring method, making it possible to preserve the material for longer times. Thus, the present study has two objectives: to compare the loss of material and the ability to accumulate PTEs of live and devitalized transplants of the seaweed *F. vesiculosus*; and to analyze their physicochemical properties and accumulation capabilities to examine the possible uptake mechanisms.

2. Material and methods

2.1. Sampling and pre-exposure treatments

Thalli of the brown algae *Fucus vesiculosus* L. were collected from an unpolluted coastal area (around SS1, Table 1) in NW Spain. Stones of approximate size 30 × 30×20 cm were also collected in the same site. The thalli were rinsed briefly in seawater and transported to the laboratory in polyethylene bags, along with the stones. Some of the thalli samples (25%) were preserved whole in seawater; the other thalli were processed by removing healthy apical segments using glass equipment. The three most apical dichotomies were selected from each thallus, to produce similarly-aged, homogeneous test material [16].

The selected apices were divided into three equal portions (each comprising 25% of the original material collected) and each was subjected to a different treatment: one portion was left untouched; another was oven-dried (at 50 °C for 8 h, 80 °C for 8 h, and 100 °C for 8 h), and the final portion was boiled for 5 min in a 2 L glass beaker heated on a hot plate.

2.2. Preparation and exposure of the transplants

The transplants were prepared by placing the fresh or boiled material (11 g) or the dried material (1.8 g) (the material lost approximately 5/6 of its weight during drying) in 9 × 9 cm flat fiberglass mesh bags (2 mm mesh size). The whole thalli were attached to the stones by gluing the basal part to the stone surface with waterproof silicone sealant. To prevent loss of material but without hampering water flow, the stones with attached seaweeds were covered with 2 cm mesh size polyethylene nets. These transplants imitated natural conditions.

A total of 5 stones each with 15 whole thalli attached were transported to each study site. A total of 45 bag transplants were also exposed in each site, 15 of each type, distributed in 15 strings that were attached to the meshes used to wrap the stones. In addition, 5 transplants of each type were transported to the study site but were not exposed in the water, as a time zero control (T0).

This was done in 6 study sites (SS) in the interior of rias on the NW coast of Spain (Table 1): 5 of the sites were potentially polluted due to their proximity to cities or to current or previous industrial areas, and the other was the same site where the seaweeds were collected.

2.3. Sample collection and processing

In each SS, samples were collected after exposure for 4, 8, and 20

Table 1
List of study sites. The coordinates are in WGS 84 with a web Mercator projection.

Study site	Start date	End date	Coordinates	Pollution source
SS1	31/10/2019	20/12/2019	42.22391, −8.75815	Next to an industrial city of 300,000 inhabitants
SS2	14/11/2019	04/12/2019	42.34626, −8.61331	Next to an old ceramics factory, known to discharge Pb-rich waste into the water
SS3	28/11/2019	18/12/2019	43.49986, −8.17095	Close to an industrial city of 65,000 inhabitants, in a ria with a low water renewal rate
SS4	06/02/2020	26/02/2020	42.94528, −9.17639	Next to an active metallurgic industry
SS5	20/02/2020	11/03/2020	42.40816, −8.6812	Next to a closed chlor-alkali plant, known to discharge Hg-rich waste into the water
SS6	05/03/2020	25/03/2020	42.79022, −8.91764	Not exposed to any known source of metal pollution

days. The length of the exposure period was selected to allow stabilization of element concentrations [41]. At each sampling time, transplants at the end of each string were collected, and the mesh envelopes were opened and 5 thalli removed. Thus, 5 samples of whole fresh seaweed attached to stones and 5 bag transplants subjected to each devitalization treatment were collected each time (i.e. 20 samples each time). The samples were briefly rinsed in the surrounding seawater, stored in polyethylene bags and transported to the laboratory. In addition, on day 20 of the exposure period, a sediment sample (upper 2 mm) was obtained in each SS. In the laboratory, the mesh bags were opened, and the three apical dichotomies were cut from the thalli attached to stones, so that these control samples were comparable to the bag transplants. The samples were then oven dried at 40 °C until constant weight, weighed, and homogenized in a tangential mill with zirconium oxide grinding vessels (Retsch ZM400). The material was stored in hermetically sealed vials in darkness until chemical analysis.

2.4. Chemical analysis

Prior to analysis, the samples were dried again at 40 °C in a forced air oven. The samples (1 g d.w.) were mineralized in Teflon vessels in a microwave oven (Milestone Ethos-1), in 3 successive steps (10 min at 100 °C, 7 min at 150 °C, 25 min at 190 °C), by adding 10 mL of HNO_3 (65%), 2 mL of H_2O_2 (30%) and 2 mL of MilliQ water. The concentrations of Al, V, Mn, Fe, Ni, Cu, Zn, Cd, Ba, and Pb were determined by inductively coupled plasma mass spectrometry (ICP–MS) (Agilent 7700x) by the University Research Support Services Unit (Universidade de Santiago de Compostela). The concentrations of Hg were determined in an elemental analyzer (DMA 80 Milestone) in the Ecology Unit of the same university.

The quality of the samples was ensured by analyzing certified reference materials (CRMs) and analytical replicates every 15 samples, in addition to blanks. The CRMs used were ERM-CD200 (*F. vesiculosus*), BCR-279 (*Ulva lactuca*) and BCR-277R for the sediments. All three materials are certified by the Joint Research Centre of the European Union. The limit of detection (LOD) was calculated as the mean blank value plus 3 times the standard deviation, and the limit of quantification (LOQ) as the mean value plus 10 times the standard deviation.

In order to investigate the causes of the differences in elemental accumulation, stored seaweed samples processed using the same protocol as describe above were analyzed in an FTIR-ATR spectrometer (Agilent Cary 630), in the 400 cm^{-1} to 4000 cm^{-1} region with a resolution of 4 cm^{-1}. A total of 14 samples were analyzed, 2 fresh and 2 dried unexposed (T0) seaweed samples, and 5 fresh and 5 dried samples exposed for 14 days.

2.5. Statistical analysis

All analyses were performed with R software [36]. The normality of the samples was checked using the Shapiro test. The data were not normally distributed either before or after logarithmic transformation. Due to the lack of normality, the differences in the elemental concentrations of different treatments were analyzed by the Kruskal-Wallis test and ad-hoc Dunn tests.

The trends in the elemental concentrations over time were tested by fitting linear models to the untransformed and transformed (logarithmic transformation) data. This was applied to the respective data for each SS and treatment for which material remained after 20 days. The quotient of the sigma values (the sum of the residuals of each model) of the linear and logarithmic model of each data set was calculated, producing a value that indicates whether the trend fitted best to a linear (values <1) or logarithmic (>1) model.

The fraction of the elemental concentrations of the transplants for which the sediment particles attached to the surface are responsible was calculated using Formula 1.

$$y = \frac{[Al]_{Sw}}{[X]_{Sw}} \Big/ \frac{[Al]_{Sed}}{[X]_{Sed}}$$

Formula 1. Calculation of the percentage of the elemental concentrations attributed to the sediment, where 'y' is the portion of the element X attributed to the sediment, $[Al]_{Sw}$ and $[Al]_{Sed}$ are the concentrations of aluminum in seaweed and sediment samples, and $[X]_{Sw}$ and $[X]_{Sed}$ are the concentrations of the studied element.

This was done under the assumption that all the Al in the seaweed samples was derived from the sediment, so that the concentration of any element present in the sediment particles associated with the seaweed samples should thus be proportional to the concentration of Al in the sediment samples. Thus, values < 1 imply that other factors are increasing the concentration of that element in the sample.

The FTIR results were analyzed and visualized using the ChemoSpec package for R [25], including the principal component analysis (PCA). The data were normalized with a probabilistic quotient normalization to remove any possible systematic deviation.

3. Results

The dry weights of the T0 transplants were similar (although weighing the material in different states such as dried or fresh to make the trasnplants led to differences). Thus, for T0, the average dry weight in grams of the apical segments of the whole algae attached to stones, expressed as mean ± sd, was 1.29 ± 0.34; for the fresh transplants, it was 1.53 ± 0.17, for the dried transplants, 1.65 ± 0.20, and for the boiled transplants, 1.05 ± 0.11. However, loss of material during the exposure period led to the final weights being very different (Fig. 1).

The weight of the whole algae attached to stones and the transplants of fresh material did not decrease throughout the experiment; all or most of the devitalized material was lost, in some SS within a week. The low remaining weight of some samples from devitalized transplants made it impossible to perform all chemical analyses, and the concentration of Hg was not determined in these cases.

Regarding the elemental determinations, the results of the quality control were generally satisfactory: the average percentage recovery of the algal CRMs for the certified elements (Cu, Zn, Cd, and Pb) was 84.6% for ERM-CD200 and 86.5% for BCR-279, and in all cases it was higher than 75%. The percentage recovery from sediments was below 40% for all elements except Ni: this was expected as the digestion method used is not designed to extract elements contained in silicates. The global error was below 10% for most elements, except Ni, Zn, Cd, and Pb, for which it was below 14%. For Mn, Fe, Ni, Cu, Zn, Ba, and Hg, the concentrations in all samples were above the LOQ. For some elements, the concentrations in all samples were above the LOD, but a few were below LOQ: 3% for Al, 5% for V, and 0.5% for Cd. Finally, the only element for which the results were not satisfactory was Pb. In this case, the concentrations in 34% of the samples were below LOQ, and in 6% they were under LOD, all non-exposed transplants.

The initial concentrations of elements in the transplants (Figure 1SM) varied slightly depending on the treatment applied; e.g., the boiled transplants consistently had the lowest concentrations of elements like V, Fe, and Ni. Some differences in the concentrations in the T0 transplants in different SS reflected temporal variations in the material collected. However, the differences were not large enough to have a notable impact on the enrichment results, except for Ba in SS4, as the initial high concentrations explain the loss of Ba during the exposure period in the SS.

The net elemental uptake of the transplants, defined as the concentration of each transplant minus the mean concentration of the T0 transplants of the same treatment in the same SS, is shown in Fig. 2 (and Figure 2SM for the remaining elements). Although the final

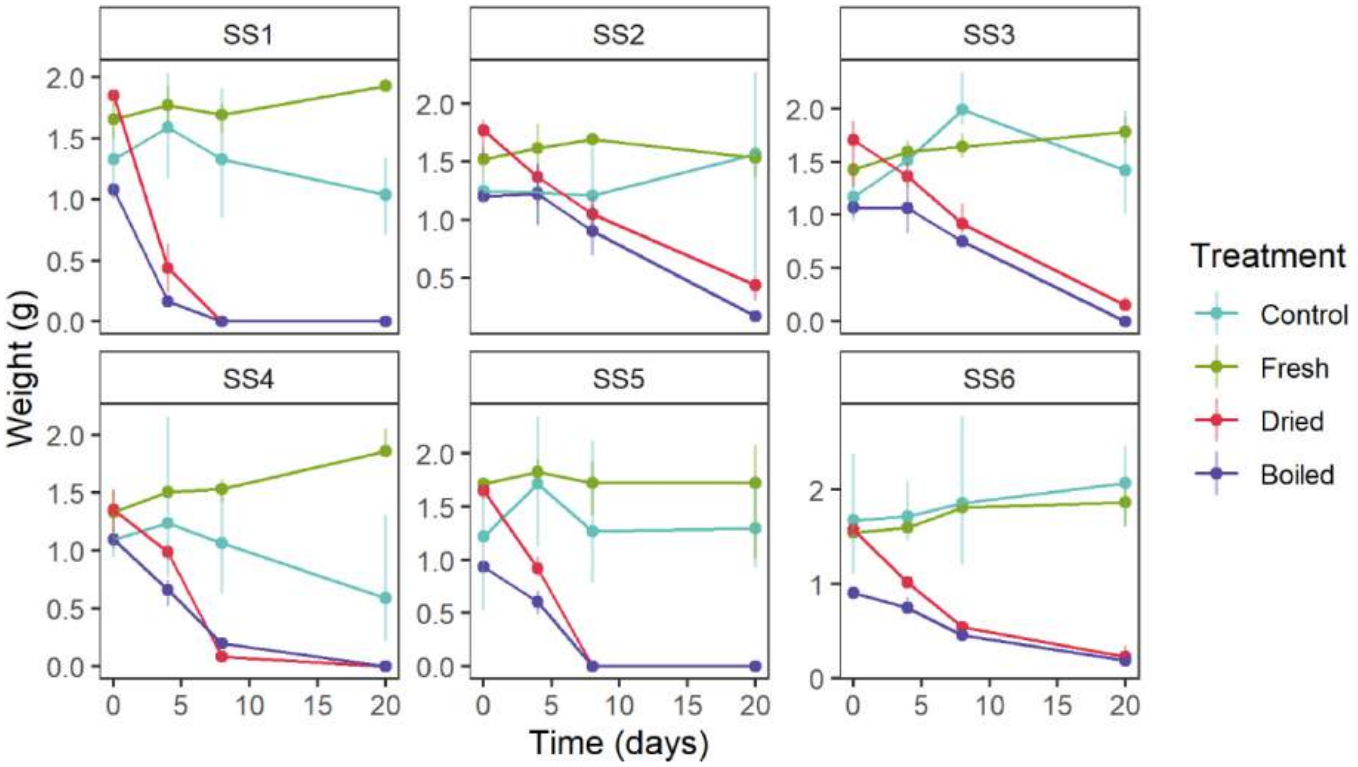

Fig. 1. Weight of the *F. vesiculosus* transplants collected during the exposure period. The points represent the average values, and the vertical bars the maximum and minimum values.

concentrations varied widely depending on the SS, some consistent patterns for each element and treatment were observed. In most cases, the treatments with live seaweed followed the same trend, whereas the devitalized transplants followed a different trend. For Al, V, Fe, Ni, Cu, Hg, and Pb, the concentrations in fresh transplants remained constant or increased slightly during the exposure period, whereas the concentrations in devitalized transplants increased sharply. For Mn, the concentrations in fresh algae remained constant, but the concentrations in devitalized transplants decreased during the 4 first days, before then stabilizing. For the other elements (Zn, Cd, and Ba), the patterns were less clear and varied across SSs.

For most elements and SSs, the concentrations differed significantly depending on the treatment considered. The cases for which the Kruskal-Wallis test revealed differences between treatments are shown in Fig. S3; Dunn test revealed between group differences (indicated by a compact letter display in the same Figure). In most cases, there were no significant differences between the treatments with fresh algae, nor between the devitalization treatments; significant differences were only observed between the live and devitalized transplants.

To evaluate whether the trends in the elemental concentrations during the study period stabilized at a specific value or increased linearly, both linear and logarithmic models were fitted for each element, SS, and treatment for which data for the 20-day exposure was available (Table 1SM). For around half of the treatments, the linear regression provided the best fit and for the other half the logarithmic regression model provided the best fit. The treatments for which the logarithmic model generally yielded the best fits were the devitalized transplants in SS2 and SS3. However, linear models provided the best fits for the devitalized transplants in SS6. Logarithmic models provided the best fits for the concentrations of Mn and Cd in devitalized transplants, owing to the rapid loss of these elements during the first 4 days and subsequent stabilization.

The proportion of the concentration of each element derived from the sediment particles on the surface of the seaweed, which was calculated by comparing the concentrations normalized with Al in the samples and the sediments, is shown in Fig. S4. Although it was assumed that all of the Al in the samples was derived from the sediment, some of the values were higher than 1, indicating that the concentration of those elements in the samples was lower than what the sediment particles would contribute in that case. For most elements, the proportion was similar among sampling points for each element, and higher in devitalized samples than in fresh samples. The element for which the concentrations were highest was Mn, which is consistent with the observed loss of the element during the exposure period. For V and Fe, the ratios were similar among treatments in all SSs except SS4, in which the concentrations of these elements were much higher in the devitalized samples than in the fresh samples ($>$ than 1).

The FTIR spectra of fresh and dried transplants are shown in Fig. 3.

Differences between fresh samples, dried samples, and the respective controls were observed. The complete spectra from 400 to 4000 cm^{-1} and the grouping of the samples by PCA are represented in Figs. S5 and S6.

4. Discussion

Devitalization of *F. vesiculosus* samples led to rapid loss of material during the exposure period. This does not occur in moss, in which the devitalization by boiling and oven-drying with a temperature ramp causes a slight, stable loss of material, even when exposed in rivers [11]. In the present study, almost all the material of devitalized transplants collected after exposure for 20 days exposure was lost, and in some cases there was little to no material remaining after 8 days (Fig. 1). Thus, devitalizing the seaweed is not suitable for transplants intended to be exposed during long periods. For the 4-day exposure, only in a boiled transplant lost all the material, and all dried seaweed transplants had enough material for elemental analysis. Thus, dried transplants could be used for short exposure periods, and would be preferable to boiling as a devitalization treatment as slightly less material was lost and the dried

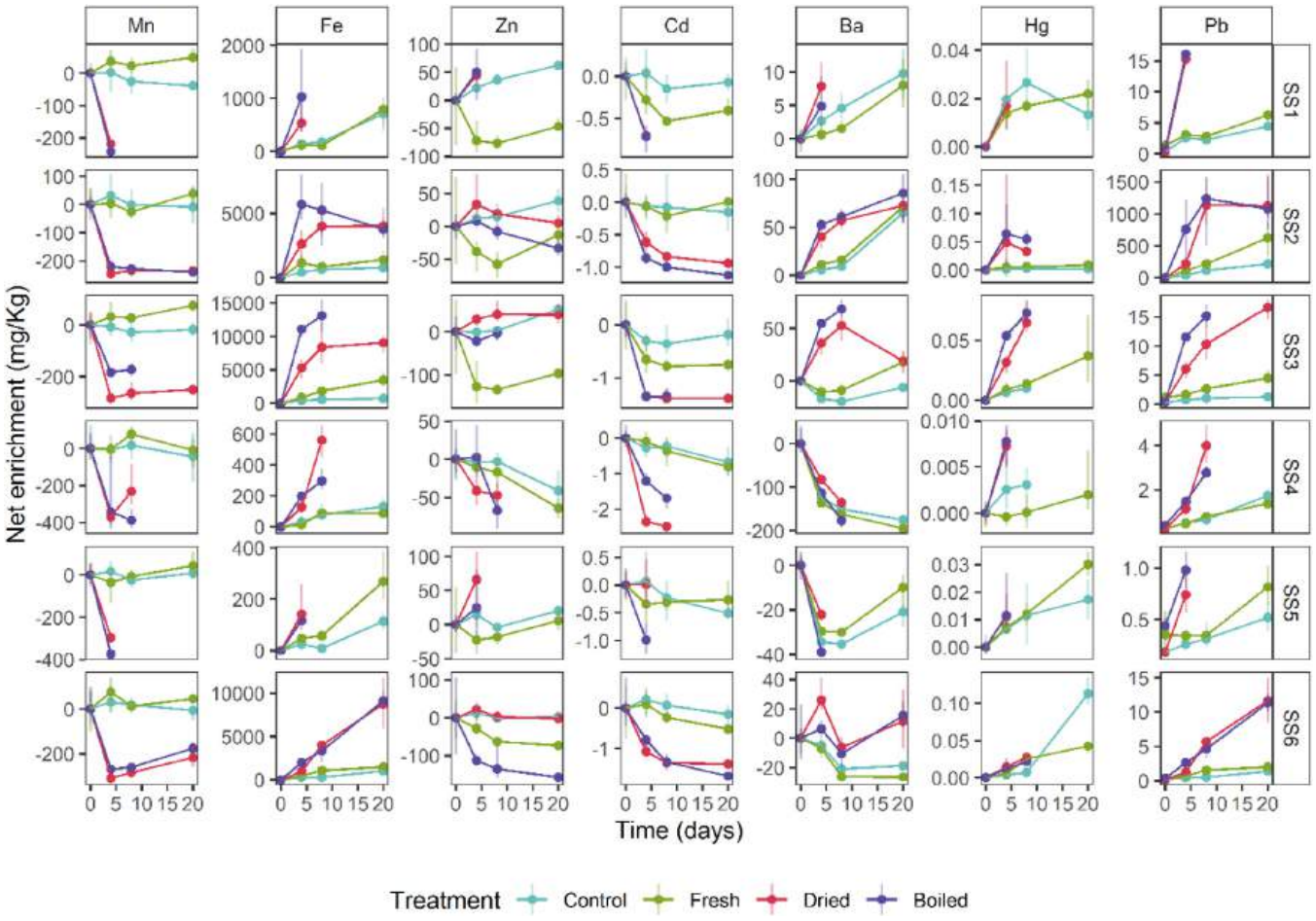

Fig. 2. Net enrichment of the transplants with different treatments throughout the exposure period. The points represent the average of the five experimental replicates, and the vertical bars represent the minimum and maximum concentrations. Al, V, Fe, and Ni followed the same pattern as Cu; the concentrations are shown in Fig. S2.

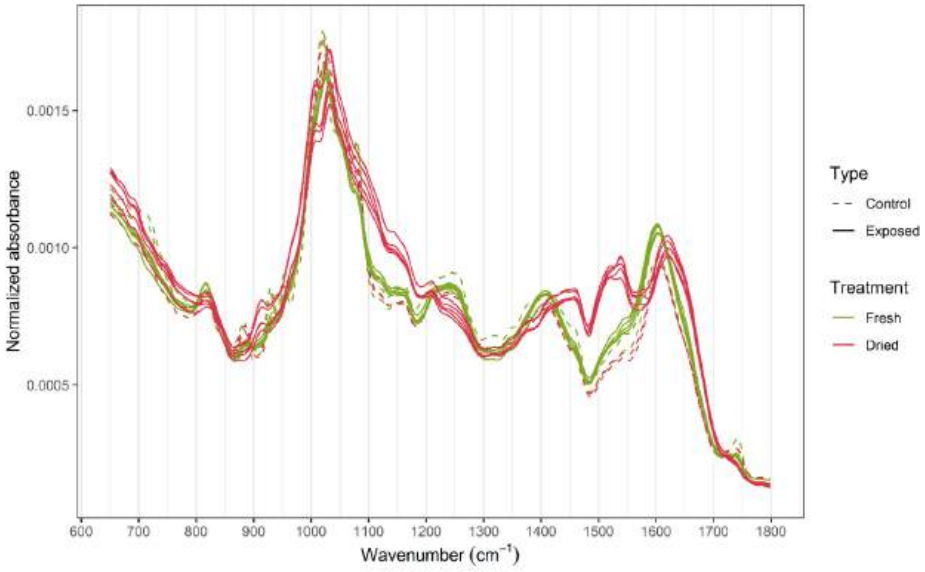

Fig. 3. Normalized absorption spectra of 14 samples in the region from 600 to 1800 cm^{-1}.

material can potentially be preserved for a long time under dry conditions. However, the devitalized seaweeds were much less durable than fresh transplants (either in bags or attached to rocks), the weight of which remained approximately constant. The differences in weight of the fresh seaweeds in bags and on rocks were negligible, and both preserved sufficient material, although the variation in the weight of the

transplants attached to rocks was much greater because they were not weighed before exposure, (the quantity was determined on the basis of the number of thalli). Thus, regarding weight loss, bags with fresh material are as good as the more natural approach of attaching the whole thalli to the rocks.

The other main consideration in selecting the best treatment is the differences in their metal-uptake capacity. The concentrations of most elements were much higher in the devitalized transplants (both methods) than in the live seaweed. Previous studies have shown different effects in metal uptake depending on the organism and element: in terrestrial mosses, *Sphagnum palustre* did not capture more Pb after devitalization [12]; in *Pseudoscleropodium purum* the concentrations of PTEs in live moss ranged from slightly lower to much higher than in oven-dried samples [3]; in *Hypnum cupressiforme* both similar [21] and higher uptake levels [20] have been reported; and the uptake of trace elements in devitalized *Platyhypnidium riparioides* was similar or slightly higher than in live transplants [7]. The only studies that have compared the differences in the accumulation of metals between devitalized and live seaweed are bioremediation studies, in which the biosorption of seaweed biomass (sometimes ground material) has been tested. In these studies, higher levels of uptake by devitalized seaweed have been suggested [32]; however, the only recent study comparing uptake levels showed that live *Ulva lactuca* can remove more Hg from spiked water than devitalized material [27].

The higher element uptake in devitalized transplants than in live transplants supports the hypothesis that the main uptake mechanism of metal accumulation in seaweed is chemical adsorption rather than active internalization [41], as devitalized seaweeds are not capable of the latter. However, the reason why the concentrations of elements in devitalized transplants range from similar to more than one order of magnitude higher than in live transplants requires further explanation. Numerous factors may contribute to these differences: 1) rupture of cell membranes during the devitalization process, which may expose binding sites in the interior of the membranes; 2) heat-induced chemical alteration of the cell walls and membranes, which can increase the affinity for certain elements, as occurs with other pre-exposure treatments [18]; 3) inactivation of the adaptations present in live seaweed to prevent the uptake of toxic elements; the mechanisms involved include the secretion of exudates to capture these elements [38], and possibly the excretion of chelated elements through active transport, both of which would contribute to lowering the concentrations of fresh seaweed; 4) differential loss of material in the devitalized transplants: if all of the components in seaweed do not have the same affinity for each element, the preferential loss of some components over others could cause differences in the accumulation capacity of the different transplants; 5) growth of fresh transplants; in the case of fresh moss transplants, growth has been found to lead to some variation in elemental concentrations due to the differential accumulation of differently aged plant parts [14], in which metal concentrations are different in *F. vesiculosus* [16]; and 6) physical modification of the seaweed surface, allowing pollutant-containing particles to adhere more easily to the surface of the devitalized transplants.

Two results that helped us test these hypotheses were the comparison of Al-normalized concentrations in samples and sediment, and the FTIR spectra of fresh and dried samples. For most elements, Al ratios were higher in devitalized samples, which indicates that a greater percentage of those elements can be accounted for by the contribution from sediments, suggesting that devitalized samples may have more adhered particles than fresh samples. This pattern was observed for almost all of the elements, except V, Fe, and Pb. One possible explanation may be that the sediment is the main contributor to the concentration of these elements in all samples. This is further supported by the fact that SS4 is the only site where there were differences between fresh and devitalized transplants in the Al ratios of Fe and Pb. The origin of these elements is usually geologic, and this SS is affected by a metallurgic industry that may be releasing them in soluble form, so the alteration of their Al ratios may indicate that this is the only case in which the soluble concentrations are high enough to make a significant difference. This result supports the hypothesis that the devitalization process alters the physical properties of the transplants, allowing more sediment particles to adhere to them.

The FTIR spectra of fresh and dried samples showed differences in the IR absorption profiles, and the PCA (Figure 6SM) clustered them in clearly defined groups. The group of samples with the most distinct profiles was the dried transplants after exposure, but differences were also observed in the spectra of exposed fresh samples and the controls, whereas the spectra of the two control groups were much more similar to each other. There are a few significant regions where the different treatments yielded different peaks: the peaks at around 750–950 cm^{-1} have been related to C-O and C-H bonds in uronic and mannuronic residues of alginate polysaccharides [22]; both the controls and exposed fresh samples generated peaks of different intensity at around 820, 880 and 930 cm^{-1}, while the exposed dried samples did not produce peaks at 880 and 930 cm^{-1}, having a peak at 910 cm^{-1} instead. The broad peak from 1220 to 1260 cm^{-1} was common to both controls and fresh samples, but it appeared shifted to 1210 cm^{-1} in exposed dried samples. This peak is related to sulphate ester groups found in fucoidan [8]. In the 1300–1800 cm^{-1} region all except the exposed dried samples also shared the same patterns, with two peaks around 1410 and 1600 cm^{-1}, which have been related to the carboxylate groups present in alginate [22,30]. Exposed dried samples, on the other hand, generated three peaks, with shifts towards higher wavenumbers. The different spectra in these regions imply that the quantities and/or composition of the biomolecules bearing the functional groups to which trace elements attach are also different, and therefore the alterations caused by the devitalization process (at least in the case of oven drying) are partly responsible for the differences in accumulation. However, fresh and dried controls have similar IR profiles, indicating that the heat exposure itself is not sufficient to alter the composition of the seaweed, and most of the changes occur during the exposure period. This suggests that the modifications occur either due to the differential loss of components, or that the devitalization allows chemical alteration to occur during the exposure period, possibly through decomposition.

The fact that these two accumulation mechanisms (adhesion of sediment particles and chemical binding to extracellular components) seem to be able to make a sizable contribution to the accumulation of PTEs suggests that, even in live seaweed, internalization is not a necessary uptake pathway for PTE accumulation. This is key to understand how seaweed interact with pollutants in their environment, for a few reasons: it suggests that most of the PTEs that seaweeds are exposed to are unable to affect the intracellular components, which helps to understand the high tolerance these organisms have to pollution [19]; it also implies that knowing the chemical species of PTEs and whether they are attached to particles is necessary to predict their impact on seaweeds, as the extracellular components are able to capture most species for many PTEs; finally, it also points to the effectiveness of the protection mechanisms seaweeds have against pollutants, since they can control their extracellular composition to avoid internalization [1]. Furthermore, these findings also have profound implications for the interpretation of biomonitoring studies, due to the implication that to extract precise information of the concentrations of seaweeds, it is necessary to know in which cellular compartments the PTEs are.

Another unexpected finding was the variation between elements. Whereas devitalized transplants were able to take up much higher amounts of most elements than live ones, the concentrations of Mn and Cd in them decreased during the exposure period. One possible explanation for the loss of Mn is that, as this element is essential for photosynthesis [24], seaweeds maintain high intracellular concentrations that could be lost due to membrane disruption caused by the devitalization processes. However, this does not explain the decrease in Cd, which is a toxic element with very little biological function, if any. Another possible explanation is the chemical modification of the functional

groups present on the surface of the cells to which PTEs can be bound, such as carboxyl, amino, sulphate and hydroxyl groups [9,26]. The alteration or creation of new functional groups during the devitalization process could affect the affinity of the material for specific elements. However, the uptake patterns of some elements are difficult to explain; for example, it has been suggested that the affinity of chemical elements for different ligands depends on their ionic and covalent index [33,35], but the accumulation of Cu and Cd, which are very similar in this regard, was completely different in the present study.

All of these patterns help us to understand the advantages and limitations of using live and devitalized seaweed transplants. In live transplants, the mechanisms for regulating PTE uptake are the same as in organisms occurring naturally in the ecosystem, and therefore they better reflect the elements affecting them. Live transplants are also more resilient and durable because they can maintain their structure which makes longer exposure periods possible; however, they are still capable of growth, which affects the reliability of the data. Devitalized transplants, on the other hand, have some advantages: 1) elemental concentrations are not affected by growth of the seaweed; 2) the concentrations of most elements are higher, which can be important for the detection of elements that display low concentrations such as Pb; some of the initial concentrations of this element in our experiment were below the LOD, but the post-exposure concentrations of devitalized transplants were much higher; and 3) they are also easier to use from a logistical point of view, as dried seaweed can be kept in the laboratory for longer. It would therefore be possible to collect large amounts of material, dry it, and used it for a long period, thus preventing differences in the initial concentrations such as those we found for Ba.

Based on these properties, both types of transplants may be useful in different scenarios. Fresh transplants would be better for producing results representative of longer periods, and when the objective is to obtain information on the impact of PTEs on the ecosystem. By contrast, devitalized transplants would probably be better for obtaining reliable data on the metal concentrations in seawater in a short time, although further information about the relationship between the concentrations in transplants and water must be obtained to confirm this possibility.

5. Conclusions

- For *F. vesiculosus*, transplants consisting of fresh apices in bags are easier to use and as effective for trace element biomonitoring as transplants of whole thalli (imitating natural conditions).
- Devitalizing the seaweeds affects the consistency and durability of the transplants, leading to complete disintegration of the material in as little as 8 days, making their use for periods longer than a week inviable.
- Uptake of trace elements was much higher in devitalized transplants than in fresh transplants, indicating the possibility of using the former for short exposure periods.
- The physicochemical modifications caused by the devitalization process affect the elemental uptake. After exposure, devitalized transplants contain different polysaccharides and functional groups, which affects their affinity for different elements.
- Devitalized transplants contain higher concentration of elements associated with the sediment, probably due to physical modification of the surface that enables binding of greater numbers of particles.
- PTEs physically or chemically bound to the surface of the seaweeds are very significant for metal accumulation, highlighting the importance of understanding the accumulation mechanisms to understand how they are affected by PTE pollution.

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Environmental Implication

Heavy metals such as the ones studied in this article are some of the pollutants with the greatest impact on coastal environments. This work improves the use of biomonitorization with seaweeds in several aspects: studying the use of devitalization and the application of devitalized transplants; helping to understand the results of biomonitorization studies; and advancing our understanding of the underlying uptake mechanisms. This way, it enables the use of a valuable tool to control pollution in seawater.

CRediT authorship contribution statement

Antón Vázquez-Arias: Investigation, Formal analysis, Writing – original draft. **Jesús R. Aboal**: Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **J. Ángel Fernández**: Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.132216.

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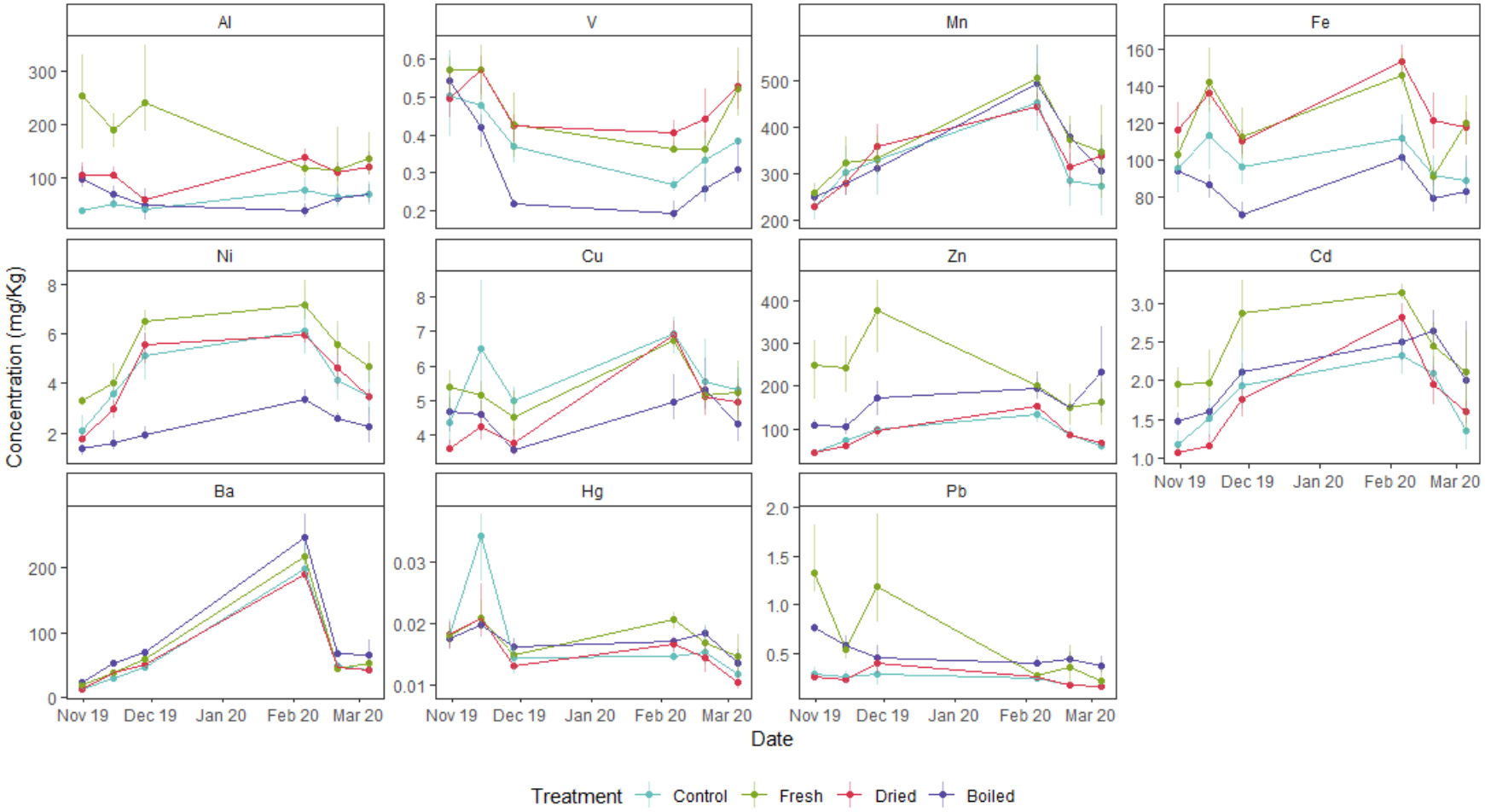

Figure 1SM. Elemental concentrations in pre-exposure T0 transplants collected over time, depending on the treatment. The points represent the average of the replicates and the vertical lines the range from maximum to minimum.

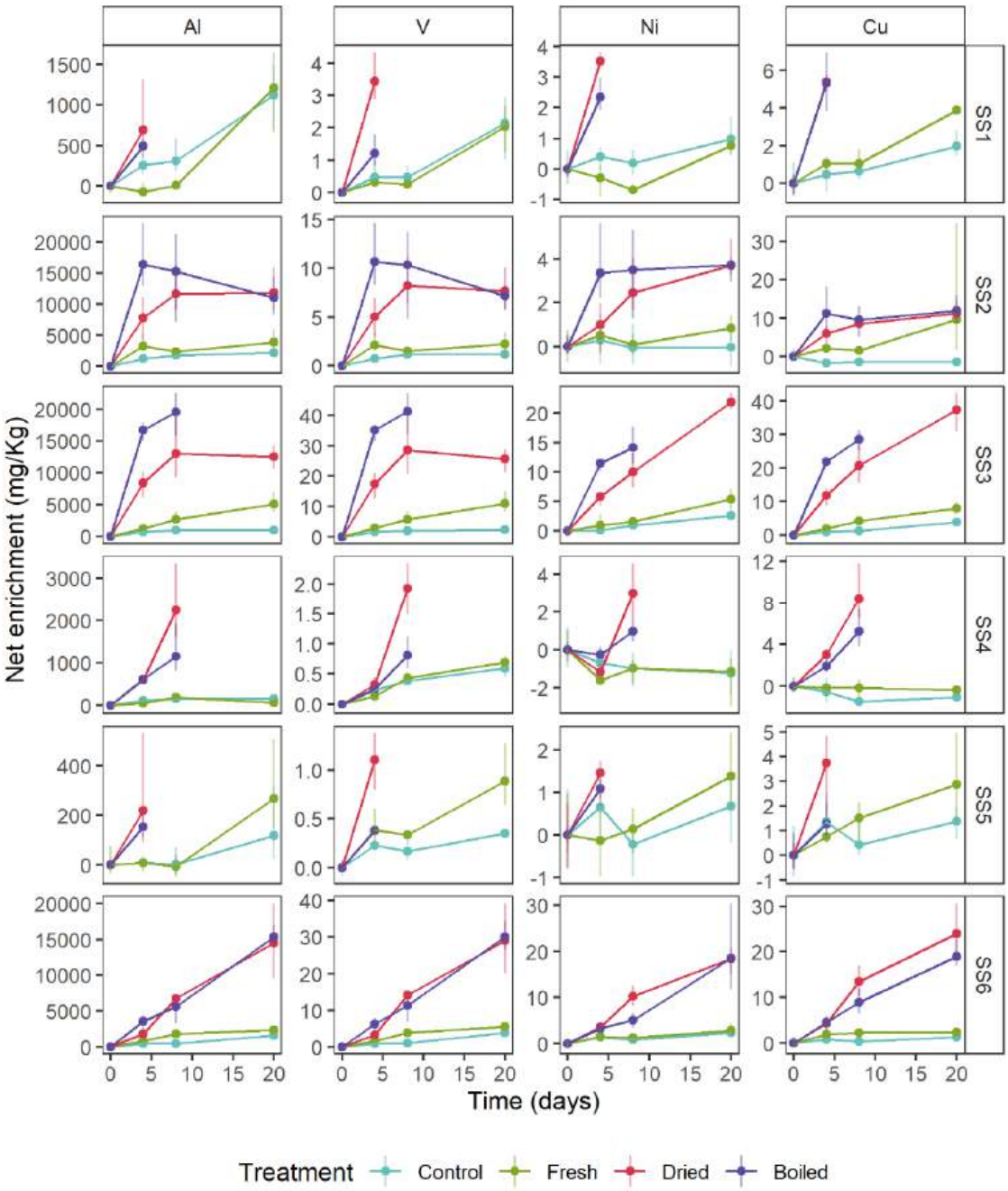

Figure 2SM. Net enrichment of the transplants with different treatments over the exposure period. The points represent the average of the five experimental replicates, and the vertical bars represent the maximum and minimum concentration.

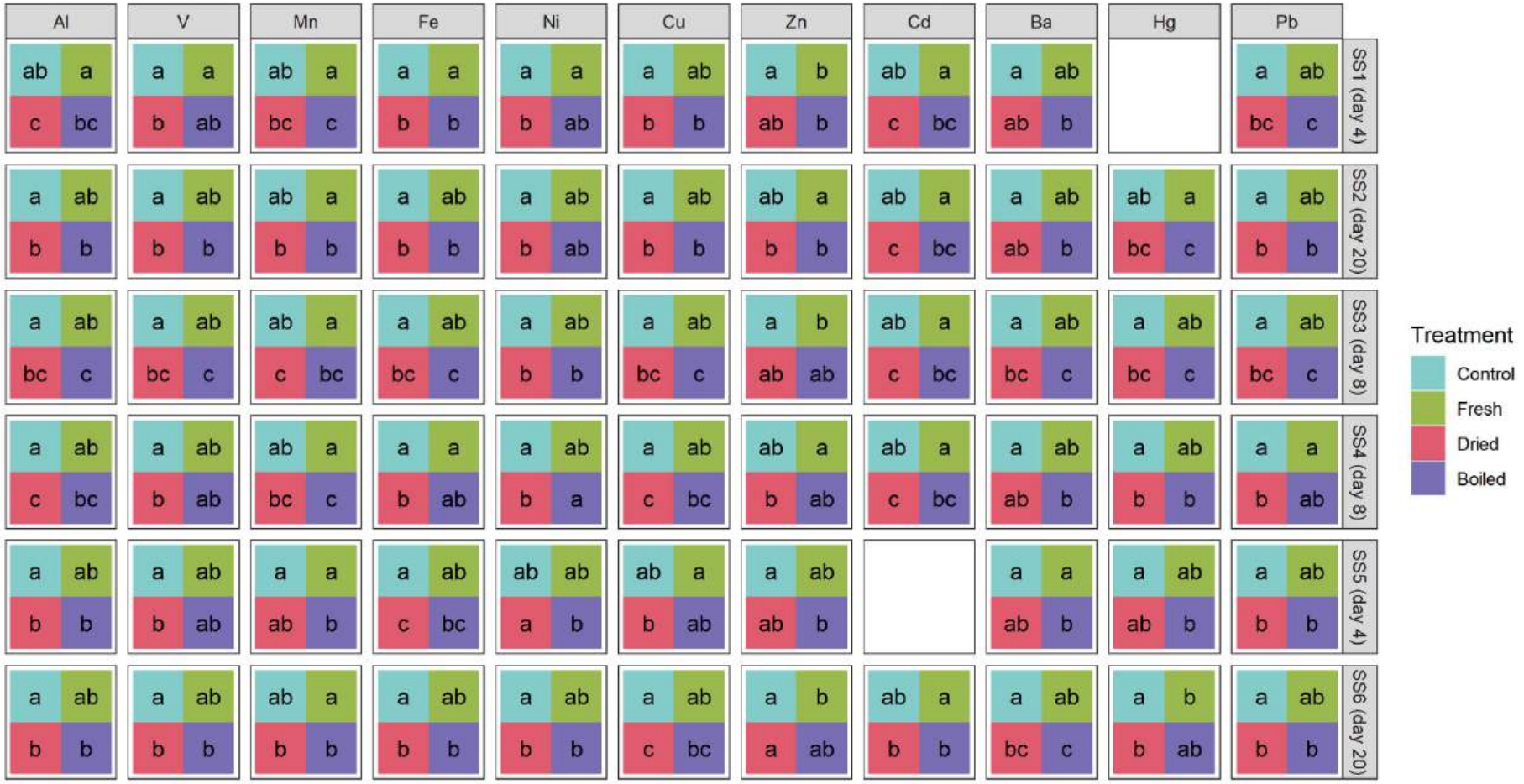

Figure 3SM. Compact letter display showing what treatments had significantly different PTE concentrations. The comparison was performed using the last exposure day for which data of all 4 treatments was available. The corresponding day is shown in the axis y, which applies to all elements but Hg. For Hg, the data used in SS2, SS3 and SS6 was that of day 8, and for SS4 and SS5 that of day 4. The white squares are the cases for which the Kruskal-Wallis results was not significative.

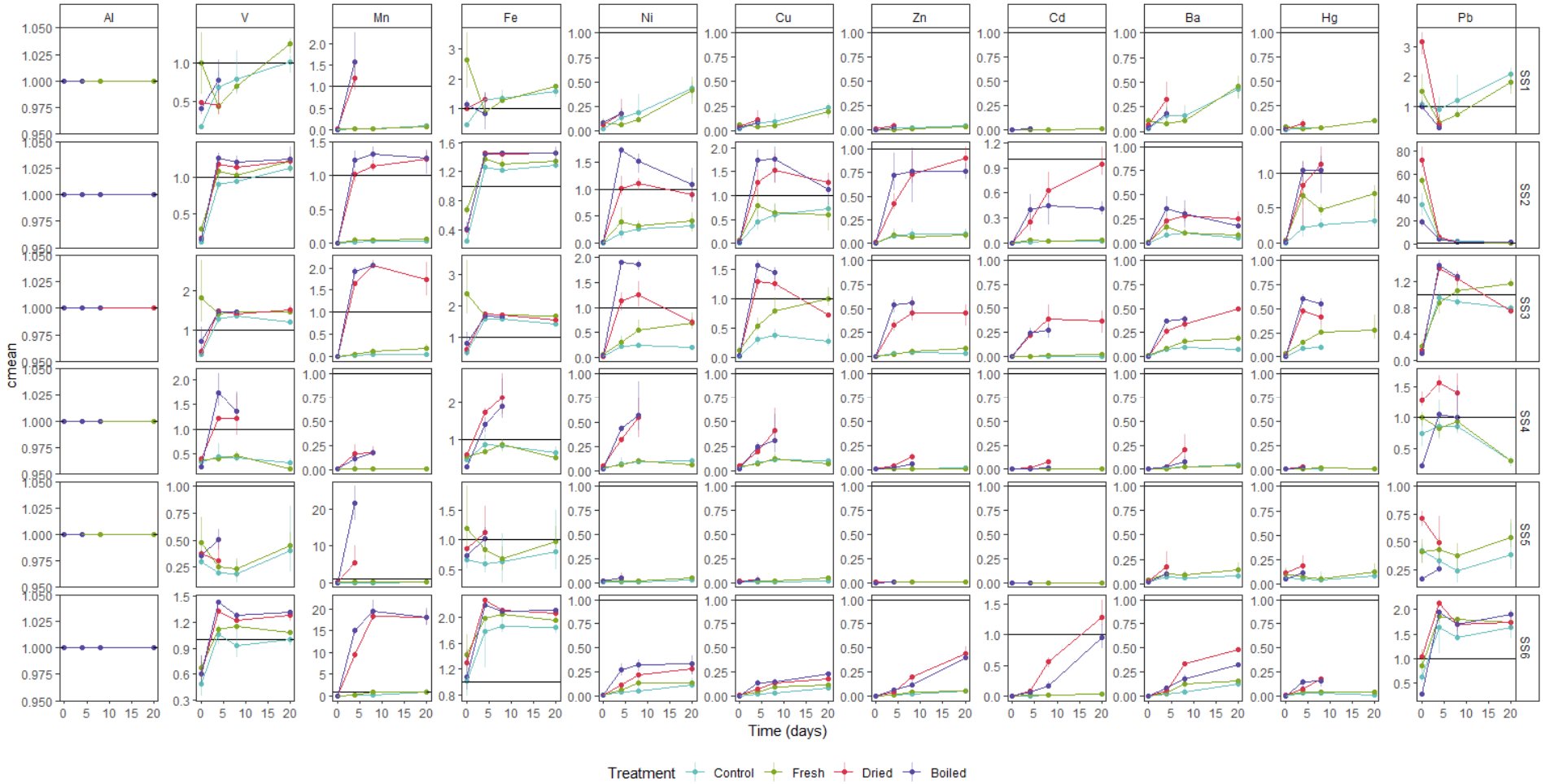

Figure 4SM. Evolution over time of the quotient of the ratio [Al]/[X] in the seaweed samples between the same ratio in the sediment, where X is the element represented.

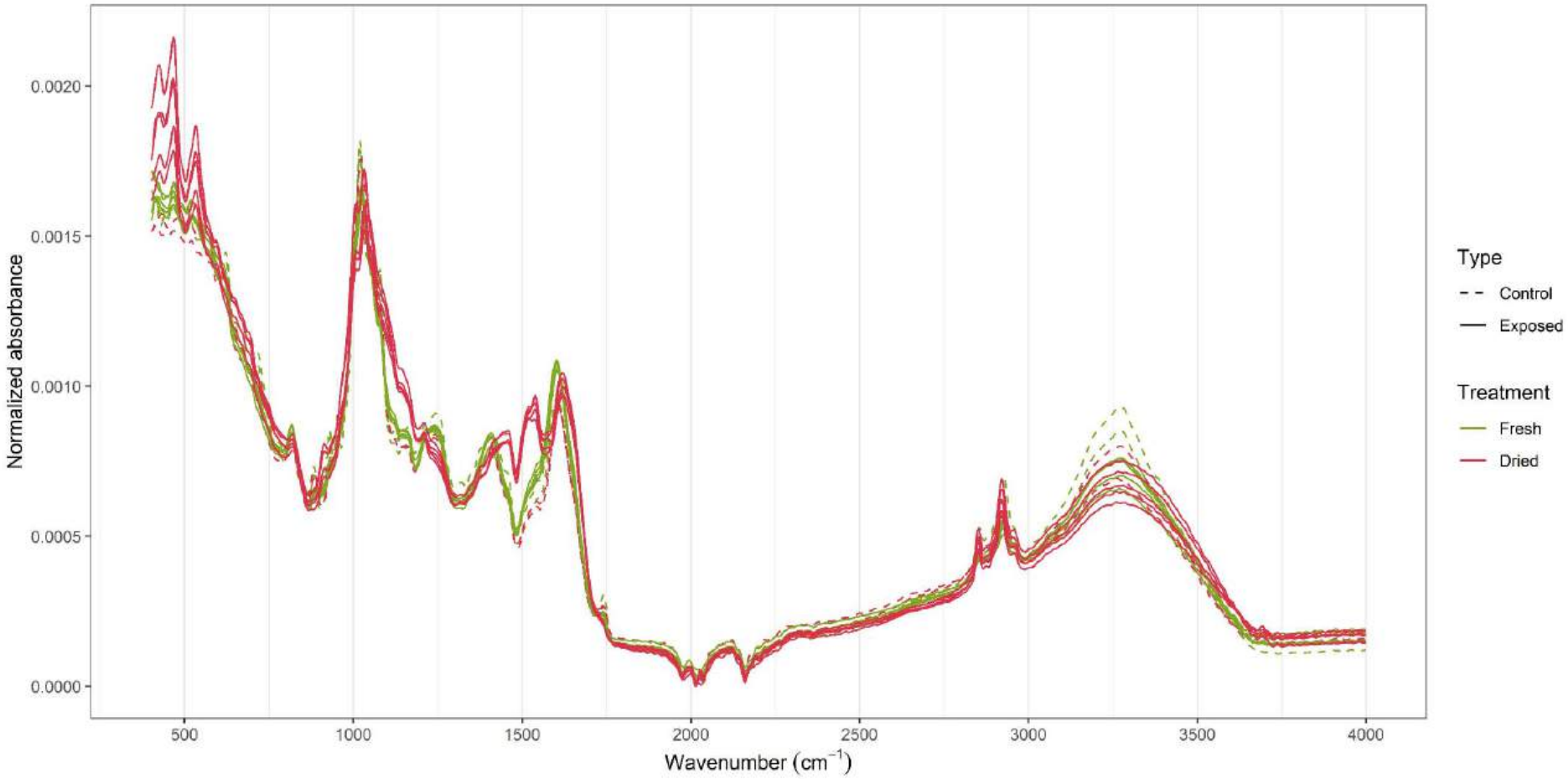

Figure 5SM. Normalized absorption spectra of 14 samples from 400 to 4000 cm^{-1}.

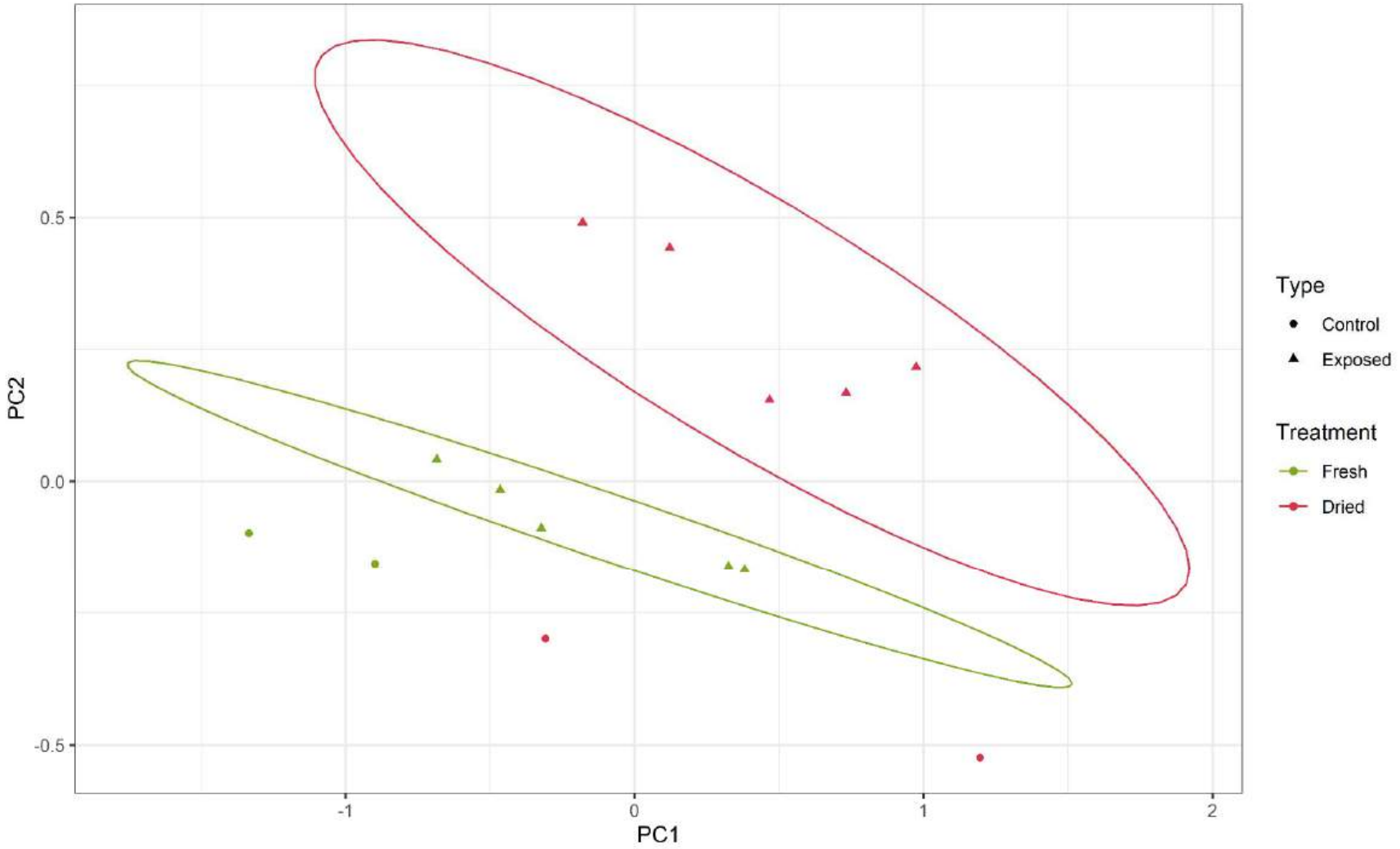

Figure 6SM. Scatter plot of the values of the samples for the first two components of the PCA.

Table 1SM. Quotient of the sigma of the linear model for the concentrations of each element over time divided by the sigma of the corresponding logarithmic model. Values >1 represent better fit for the logarithmic model, and <1 for the linear model. Devitalized transplants are greyed out.

Site	Treatment	Al	V	Mn	Fe	Ni	Cu	Zn	Cd	Ba	Pb	Average
SS1	Control	0,668	0,740	0,976	0,663	0,943	0,864	1,038	1,009	0,819	1,184	0,890
	Fresh	0,646	0,630	1,070	0,625	0,835	0,541	1,126	1,193	0,700	0,643	0,801
SS2	Control	1,392	1,303	0,989	1,356	0,997	1,129	0,890	1,000	0,453	0,731	1,024
	Fresh	1,207	1,228	0,945	1,204	0,981	0,957	1,039	1,004	0,371	0,637	0,957
	Dried	1,520	1,503	1,625	1,521	0,997	1,604	1,003	2,376	1,994	1,146	1,529
	Boiled	1,226	1,233	1,823	1,224	1,308	1,309	0,770	2,239	1,729	1,294	1,415
SS3	Control	1,523	1,572	1,016	1,516	0,782	0,639	0,580	1,058	1,118	1,444	1,125
	Fresh	0,798	0,807	0,948	0,803	0,640	0,739	1,204	1,303	0,763	0,715	0,872
	Dried	1,560	1,518	1,491	1,594	0,452	0,860	1,212	1,705	1,146	1,085	1,262
SS4	Control	1,229	1,169	0,989	0,927	1,043	1,117	0,823	0,958	2,350	0,465	1,107
	Fresh	1,109	0,792	1,004	1,096	1,098	0,996	0,737	0,804	2,955	0,471	1,106
SS5	Control	0,896	1,139	0,999	0,652	0,992	1,040	0,971	0,901	1,257	0,769	0,962
	Fresh	0,765	0,881	0,932	0,584	0,845	0,922	0,974	1,076	1,098	0,712	0,879
SS6	Control	0,732	0,638	0,988	0,721	1,020	0,999	0,999	0,948	1,241	0,663	0,895
	Fresh	1,403	1,224	1,018	1,295	1,034	1,360	1,134	0,915	1,213	1,127	1,172
	Dried	0,673	0,657	1,337	0,636	0,646	0,734	0,971	2,041	1,007	0,618	0,932
	Boiled	0,445	0,431	1,291	0,430	0,707	0,570	1,336	1,520	0,939	0,390	0,806
Average		1,087	1,081	1,121	1,074	0,951	1,019	1,019	1,316	0,867	0,868	