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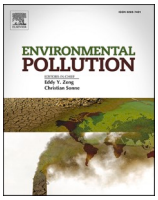

Assessing the effectiveness of seaweed transplants in reflecting seawater pollution levels☆

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ABSTRACT

Seaweed transplants have been widely used to monitor coastal marine pollution, yet their effectiveness in reflecting seawater elemental concentrations remains uncertain. This study investigated the relationship between elemental concentrations in *Fucus vesiculosus* transplants (both fresh and dried) and seawater samples representative of the transplants' exposure period collected using autosamplers. The transplants were deployed across 22 coastal sites in northwest Spain over 14 days. No significant correlations were found between seawater elemental concentrations and those in transplants for any element. Similarly, seawater physicochemical properties (pH, dissolved oxygen, conductivity, and temperature) and the pre-exposure concentrations in transplants had minimal influence on post-exposure levels. Elemental concentrations in native seaweeds at the exposure sites were not correlated with those in transplants (except for Zn in the fresh transplants) and did not reflect seawater concentrations either. These findings highlight that element concentrations in seaweed do not follow a straightforward linear relationship between exposure and tissue concentrations. Instead, they result from a complex interplay of various, yet unknown environmental factors that influence element bioavailability in the water and the physicochemical properties of the seaweed. This complexity calls into question the suitability of seaweed transplants as effective biomonitors for marine pollution.

1. Introduction

Tracking environmental pollutant levels is instrumental to mitigating their potential harm to ecosystems and society. However, there are some challenges associated with measuring pollutant concentrations in environmental media directly: 1) concentrations are often low, necessitating high sensitivity for accurate quantification (Bulska and Ruszczyńska, 2017); 2) concentrations have high spatiotemporal variability, requiring extensive sampling efforts for accurate representation (Elbaz-Poulichet et al., 2006); and 3) similar concentrations can pose different risk levels depending on the pollutants' bioavailability (de Paiva Magalhães et al., 2015). Biomonitoring—measuring pollutant concentrations in organisms to obtain information about the pollution of their environment—helps mitigate these drawbacks, as organisms uptake bioavailable pollutants over time, reaching higher concentrations. This approach has been used with several organisms across different environments. For example, mosses have been used to monitor environmental concentrations of some potentially toxic elements (PTEs) and polycyclic aromatic hydrocarbons (PAHs) (Aboal et al., 2010; Boquete et al., 2015; Foan et al., 2015; Schröder and Pesch, 2010), with varying success depending on the specific pollutant, species, and environmental matrix.

In coastal environments, seaweeds have been regarded as promising biomonitors due to their abundance, year-round availability, sessile nature, and capacity to accumulate high levels of pollutants (Corrias et al., 2020; García-Seoane et al., 2018b). Consequently, they have been used in hundreds of biomonitoring studies using both passive (i.e. using native seaweed, García-Seoane et al. (2018b)), and active (i.e. using transplants, García-Seoane et al. (2018a)) techniques. Among them, brown algae in the genus *Fucus* have been some of the most common species in coastal biomonitoring studies (García-Seoane et al., 2018b).

For biomonitoring to be an effective tool for quantifying

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environmental pollution, it is necessary to understand what environmental parameters are reflected in the biomonitor organism concentrations. Ideally, pollutant concentrations in biomonitors should show a strong positive linear correlation with their environmental levels (Wolterbeek, 2002). For native seaweeds, this relationship has been studied to some extent in the 70s (Foster, 1976; Melhuus et al., 1978), but findings have been inconsistent. More recent studies have also yielded mixed results. While some authors have reported positive correlations between seaweed and seawater concentrations, especially in heavily polluted regions (Bonanno et al., 2020; Chakraborty et al., 2014), most studies have failed to find significant correlations (Akcali and Kucuksezgin, 2011; Chernova and Shulkin, 2019). This lack of correlation may be attributed, to some extent, to a lack of comparability between the sample types, as seaweed concentrations may represent cumulative exposure over a period, while seawater concentrations are snapshots of specific time points. Recent studies with *F. vesiculosus* transplants have demonstrated that their concentrations can take up to several weeks to stabilize for some elements (García-Seoane et al., 2020; Vázquez-Arias et al., 2023b, 2023a). This implies that concentrations in seaweeds take some time to respond to changes in environmental concentrations, so if water concentrations change over time, seaweed and seawater samples collected simultaneously may not be comparable.

Unlike for native seaweeds, the relationship between elemental concentrations in seaweed transplants and their environment has never been addressed, despite the advantage of known exposure times that allow for better comparability. Recent studies supported the use of transplants for biomonitoring (García-Seoane et al., 2018a), and proposed using devitalized material—seaweeds killed without losing biomonitoring properties—as an alternative to fresh material (Vázquez-Arias et al., 2023a). Using devitalized material for biomonitoring has the advantage of preventing unwanted variability induced by growth and metabolism (Fernández et al., 2009), while also making it shelf-stable for easier preservation (Debén et al., 2016). Comparing the elemental concentrations of fresh and devitalized seaweed transplants with those of seawater and native seaweeds could reveal their responsiveness to environmental concentrations and offer insights into the largely unknown accumulation mechanisms and dynamics. For instance, many active biomonitoring studies use pollution indices such as enrichment rates—the difference between pre- and post-exposure concentrations normalized by exposure time—to account for initial variability (Sandu et al., 2012; Sergeeva et al., 2021; Vázquez-Arias et al., 2024b), assuming that transplants integrate pollutants over time rather than reaching a dynamic equilibrium with the medium—an assumption that is yet to be tested.

This study aims to examine the relationship between elemental concentrations in native and transplanted *Fucus vesiculosus* samples and

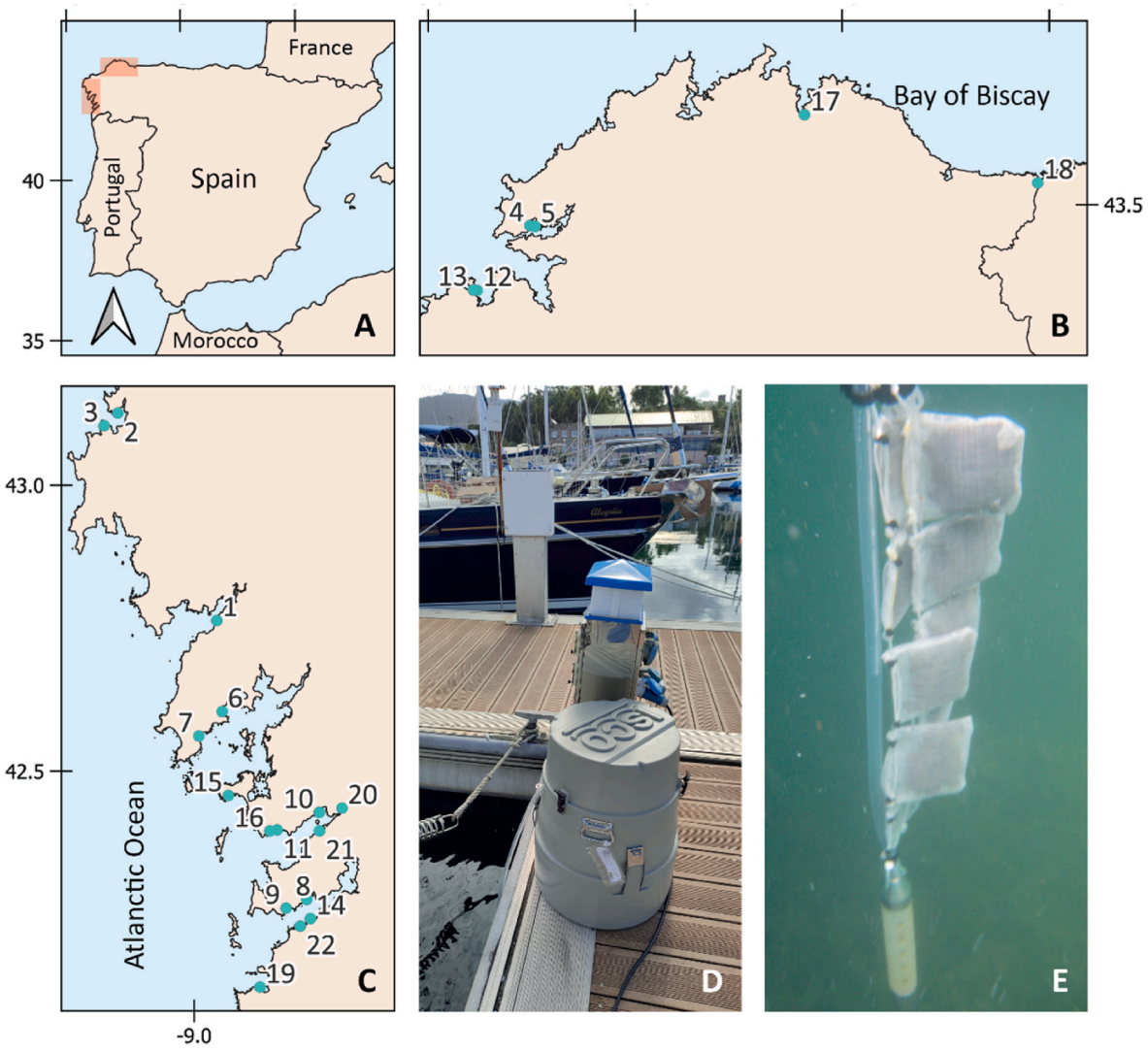

Fig. 1. **A**. Location of the study region in the Iberian Peninsula. **B**. and **C**. Location of the exposure sites. **D**. Body of the autosampler on a floating dock. **E**. Seaweed transplants exposed underwater attached to the hose of the autosampler. Coordinates are in ETRS89.

those in seawater collected continuously over the same exposure period. This will enable testing the following hypotheses: 1) post-exposure PTE concentrations in transplants reflect the total concentration of PTEs in seawater; 2) this relationship is influenced by the physicochemical characteristics of the water; 3) the relationship is also influenced by the metabolic activity of live seaweeds; 4) enrichment rates show stronger correlations with seawater than post-exposure concentrations, assuming that transplants integrate PTEs over time; and 5) transplants reflect seawater concentrations better than native samples due to their matching exposure periods.

2. Material and methods

2.1. Transplant preparation

To compare the concentrations of PTEs in the brown algae *Fucus vesiculosus* L. and in the surrounding water, transplantation experiments were conducted in 22 exposure sites along the coast of Galicia, northwest Spain (Fig. 1), over a ten-month period. Transplant preparation followed the methodology described in Vázquez-Arias et al. (2023a). Thalli of *F. vesiculosus* were collected from a reportedly unpolluted site in northwest Spain (García-Seoane et al., 2019), briefly rinsed in seawater, and transported to the laboratory in polyethylene bags. The thalli were not kept in seawater during transport, as *F. vesiculosus* can tolerate desiccation for hours. There, the material was separated to apply two treatments: one half was preserved in seawater for two days to create fresh transplants, while the other half was processed to produce devitalized transplants. For the devitalized transplants, the three most apical dichotomies were selected from healthy thalli without reproductive structures to ensure homogeneity, as elemental concentrations vary throughout the thallus (García-Seoane et al., 2021). The apices were devitalized by oven-dried at 50 °C for 8 h, 80 °C for 8 h, and 100 °C for 8 h. Once the dry material was ready, fresh transplants were prepared by selecting and cutting the apices of the preserved material in the same manner.

Both fresh and dried transplants were prepared by placing the apices in 9 × 9 cm flat fiberglass mesh bags (2 mm mesh size). Due to the significant weight loss during the drying process (approximately 5/6 of the initial weight), 1.8 g of dry material was used per transplant, compared to 11 g of fresh material.

2.2. Exposure and water collection

To ensure comparability between seaweed and seawater samples, transplants were exposed for two weeks, and composite seawater samples were collected continuously over the same period. This was achieved by the use of autosamplers (3700 Teledyne ISCO, Fig. 1D), that collected 50 mL of water every hour, totaling approximately 16.8 L of water over the 14-day exposure period. The autosamplers were placed in floating docks so that the autosampler's hose tip was continuously submerged despite tidal heigh fluctuations. One autosampler was deployed at each of the 22 exposure sites (Fig. 1), with the hose positioned at a depth of around 4 m, or one to 2 m in exceptionally shallow harbors.

Five transplants of each treatment were attached to the hose tip (Fig. 1E). Additionally, two extra transplants of each treatment were transported to the exposure sites and brought back to the laboratory to obtain the pre-exposure (initial) concentrations of the transplants. A temperature data logger was also attached to the hose tip.

After 14 days, the transplants were collected and placed in plastic bags. At 15 of the 22 exposure sites, native F. vesiculosus populations were present in or around the harbor. At these sites, three native samples were collected and placed in plastic bags. The autosamplers and all seaweed samples were then transported to the laboratory. For each water sample, 50 mL from each bottle were combined to create a ~1.2 L composite sample. The pH, conductivity, and dissolved oxygen (DO) were measured, after which the samples were transferred to polyethylene bottles and frozen until analysis.

The seaweed transplants were removed from the mesh bags, oven-dried at 40 °C until reaching constant weight, weighed, and ground in a tangential mill with zirconium oxide grinding vessels (Retsch ZM400). The homogenized material was stored in hermetically sealed vials in darkness until chemical analysis. Native samples were processed in the same way by selecting the three most apical dichotomies and following the same drying and homogenization procedures.

2.3. Chemical analysis

The seaweed samples were oven-dried again at 40 °C before analysis. One gram of each sample was mineralized in a Teflon vessel in a microwave oven (Milestone Ethos-1) in three steps (10 min at 100 °C, 7 min at 150 °C, 25 min at 190 °C) by adding 10 mL of 65 % HNO_3, 2 mL of 30 % H_2O_2 and 2 mL of MilliQ water. The concentrations of Al, V, Cr, Mn, Fe, Ni, Cu, Zn, Cd, and Pb were determined by inductively coupled plasma mass spectrometry (ICP–MS) (Agilent 7700x) by the University Research Support Services Unit (RIAIDT, Universidade de Santiago de Compostela). Seawater determination by ICP-MS is complicated by the high salt concentrations, which can lead to matrix effects (Søndergaard et al., 2015). To prevent this, seawater samples were diluted by a factor of 5 before being analyzed using the same equipment.

The analytical quality of the seaweed concentrations was validated using analytical blanks, certified reference materials (CRM: ERM-CD200, *F. vesiculosus*, Joint Research Centre of the European Union), and analytical replicates. For seawater samples analytical replicates were used. The average concentrations of the blanks were subtracted from the sample concentrations. Concentration values were above the limit of quantification for all samples but five (4 for Al and 1 for Cd). The average percentage recovery of the CRMs for the certified elements (Cu, Zn, Cd and Pb) were between 90 % and 110 %. The global errors for the seaweed samples, estimated from the analytical replicates, were below 10 % for all elements except Pb, for which it was 15 %. The global errors were much higher in the case of seawater samples, ranging from 8.7 % in Ni to 51 % for Cd. The concentrations of Zn, Cd and Pb in some seawater samples were below the limit of detection, possibly due to the dilution factor. Despite the high analytical variability in seawater, the differences observed between sites were greater than these errors, and thus the results were used for comparison with seaweed concentrations.

2.4. Statistical analysis

All analyses were carried out using R 4.4.1 (R Core Team, 2024). To assess the relationship between elemental concentrations in post-exposure transplants and seawater, enrichment rates and seawater, native seaweeds and seawater, and between the different seaweed sample types (the post-exposure concentrations of fresh and dried transplants and native seaweeds) correlation analyses were performed using the cor_test() function in the rstatix package (Kassambara, 2019). Data was right-skewed, so Spearman correlations were applied.

To evaluate the influence of the physicochemical parameters of seawater on the concentrations of post-exposure transplants and native seaweeds, and the impact of pre-exposure concentrations on the post-exposure concentrations of the transplants, generalized linear models (GLMs) were constructued using the glm() function in the lme4 package (Bates et al., 2015). For transplanted seaweed GLMs, the post-exposure concentrations of each element were used as the response variable, and water concentrations and physicochemical variables (pH, dissolved oxygen, conductivity and average water temperature) as predictor variables. For native samples, the predictor variables were the same, excluding pre-exposure concentrations. As concentration distributions in seaweed were right-skewed, a Gamma distribution with a logarithmic link function was used. Model evaluation was conducted visually using Q-Q residual plots and residuals vs leverage plots. The results were

satisfactory for all models. Data visualization was done using the package ggplot2 (Wickham, 2016).

Bioconcentration factors (BCFs) were calculated as the ratio between elemental concentrations in seaweed and in seawater.

To control the false discovery rate due to the large number of tests performed, p-values were adjusted using the Benjamini and Hochberg (1995) method.

3. Results

The median concentrations and interquartile range of the concentrations of each element in post-exposure seaweed transplants, native seaweeds and seawater can be seen in Table 1SM of the supplementary material. There were large differences in elemental concentrations across different seaweed sample types (i.e. fresh transplants, dried transplants, and native seaweeds). Compared to seawater samples, seaweeds were greatly enriched, displaying BCFs between 493 for V in native samples and 1.9 million for Al in dried samples (Table 2SM). BCFs were highly variable, with the IQR being higher than the median for most elements in all three seaweed sample types.

3.1. Relationship between PTE concentrations in seaweed transplants and seawater

The elemental concentrations in the different seaweed samples plotted against the concentrations of the corresponding seawater samples is shown in Fig. 2 for selected elements, and in Fig. 1SM and 2SM of the supplementary material for the rest. The first two columns of Fig. 2 correspond to seaweed transplants, which had no noticeable correlation to seawater concentrations. This was confirmed by the Spearman correlation coefficients, which were not significant regardless of the treatment (adjusted p-value >0.05).

3.2. Factors potentially influencing the seaweed-seawater relationship

The results of the GLMs used to identify the variables explaining the variability in the concentrations of each element in seaweed samples are shown in Table 1. Seawater concentrations did not have a significant effect on the post-exposure concentrations in transplants for any element.

3.2.1. Initial concentrations

According to our models, the effect of the initial concentrations of the transplants on the post-exposure ones was not significant. The only slight departures from this pattern are Cd and Mn, which had a visually discernible dependence (Fig. 6SM) that was marginally significant in the case of the Mn in the fresh transplants (Table 1).

To study if the enrichment rates, which control for the effect of the initial concentrations, improved the comparability between PTEs concentrations in the transplants and in seawater, we performed correlation tests between the enrichment rates of each element in both types of transplants and in seawater, but none of them were significant (adjusted p-value >0.05).

Pre-exposure concentrations in transplants exhibited seasonal variation, with higher concentrations in winter for half of the elements (Cd, Cu, Mn, Ni, and Zn), and in summer for the other half (Al, Fe, Cr, Pb, and V) (Fig. 5SM) Yet, for most elements, these concentrations were lower than those found in native seaweeds from the exposure sites except for Ni and higher for Mn and Cd (Fig. 3).

3.2.2. Seasonal variation

Post-exposure concentrations also appeared to display seasonal variation for some elements (Fig. 3SM and 4SM). This trend is noticeable for Cd, Ni, Zn, and Mn in the fresh transplants, and for Cd in the dried transplants, all of which showed higher concentrations in winter, consistent with pre-exposure concentrations. In native samples, the seasonal effect was less evident, possibly due to the smaller sample size, which hindered the detection of seasonal trends.

3.2.3. Seawater physicochemical properties

The environmental variables did not have a significant influence on the PTE concentrations of seaweed transplants. The only exception were the concentrations of Cd in the dried transplants, which were positively affected by pH, and negatively by DO and temperature.

Table 1
Parameter estimates for the GLMs modeling elemental concentrations in seaweeds. The response variable for fresh and dried transplants was the post-exposure concentration. Statistically significant values (adjusted p-value <0.05) are in bold and underlined, and marginally significant values (adjusted p-value <0.1) are in bold. Abbreviations: Water conc. = concentration of the element in seawater (μg L^{-1}); Initial conc. = concentration of the element in pre-exposure transplants (μg g^{-1}); DO = dissolved oxygen in seawater (mg L^{-1}); Cond = conductivity in water (mS cm^{-1}); Temp = average water temperature during the exposure period (°C).

	Al	V	Cr	Mn	Fe	Ni	Cu	Zn	Cd	Pb
	Fresh transplants									
Intercept	−1.8	−4.5	−2.6	4.8	1.5	1.6	−0.37	0.69	−0.9	0.15
Water conc.	0.13	0.049	−0.19	0.068	0.042	0.034	0.17	−0.045	−0.28	−2.8
Initial conc.	0.0023	−0.048	−0.66	**0.003**	0.0018	0.099	0.091	0.0089	0.84	0.0089
pH	0.31	0.17	−0.1	−0.013	0.18	−0.013	−0.097	0.076	0.09	−0.46
DO	0.092	0.06	0.1	0.038	0.06	0.059	0.045	0.064	0.054	0.44
Cond	0.11	0.07	0.044	0.013	0.055	0.002	0.031	0.041	0.0015	0.012
Temp	0.0047	0.044	0.11	−0.051	0.032	−0.052	0.061	0.041	−0.054	0.17
	Dried transplants									
Intercept	5.2	1.3	0.3	4	6.8	4.2	−0.078	2.9	−1.2	4.5
Water conc.	0.088	−0.079	0.12	0.18	0.044	0.035	0.15	0.048	0.22	1.4
Initial conc.	−0.0011	−1.4	−2.5	−0.0027	−0.0046	−0.091	0.011	0.0036	0.46	−1.5
pH	0.49	0.11	0.33	0.44	0.36	0.14	0.25	0.38	**0.64**	−0.11
DO	−0.15	−0.082	−0.15	−0.03	−0.14	0.091	−0.036	−0.2	**−0.2**	−0.085
Cond	0.081	0.051	0.064	0.05	0.056	0.0011	0.054	0.037	−0.017	0.025
Temp	−0.16	0.011	−0.064	−0.33	−0.12	−0.2	−0.068	−0.11	**−0.2**	−0.064
	Native seaweeds									
Intercept	4.1	1.3	1.3	6.5	5.5	3.9	2.7	4.3	0.35	2.4
Water conc.	0.1	−0.068	0.13	0.13	0.071	0.035	0.3	−0.029	−0.48	−0.46
pH	0.54	0.22	0.36	0.2	0.56	0.1	−0.11	0.052	−0.0068	−0.15
DO	−0.061	−0.0076	0.034	0.025	−0.1	0.1	−0.055	0.064	0.1	0.21
Cond	0.059	0.02	0.0062	−0.013	0.023	−0.027	0.018	0.029	−0.03	−0.012
Temp	−0.15	−0.079	−0.16	−0.17	−0.15	−0.13	0.01	−0.11	−0.0082	0.037

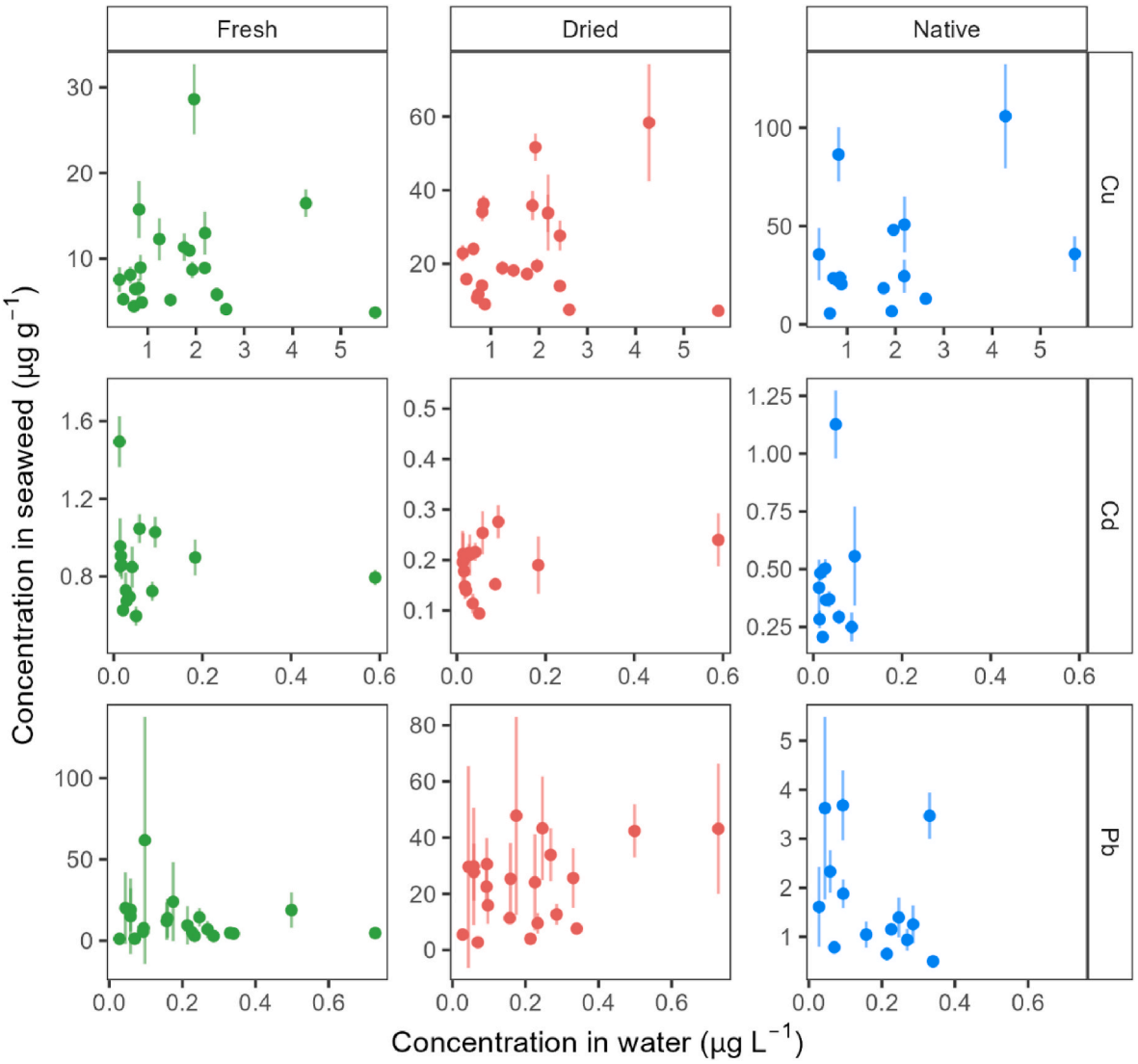

Fig. 2. Scatterplot of the concentrations of Cu, Cd, and Pb in seaweed and seawater. "Fresh" represents the final concentrations in fresh seaweed transplants, "Dried" in dried transplants, and "Native" in native seaweeds. Points represent the mean concentration of samples from each site (5 replicates for transplants and 3 for native samples), and error bars indicate standard deviations.

3.2.4. Seaweed metabolism

The models were similarly ineffective in explaining the PTE concentrations in metabolically active fresh transplants and devitalized dried transplants. The exception was again Cd, which was the only element for which significant contributions from the physicochemical properties of the water were detected.

3.3. Comparison between native and transplanted seaweeds

Results on the comparison of the concentrations between native seaweeds collected from the exposure site and seawater are shown in the last column of Figs. 2 and 1SM and 2SM, and in the last section of Table 1. Their concentrations were notably different from those of the transplants, but mostly in the order of those found in fresh transplants. Similarly to transplanted seaweeds, concentrations in native samples were not correlated to those in seawater.

The GLMs were similar to those constructed for transplants except for the absence of initial concentrations in passive biomonitoring. Seawater concentrations and physicochemical parameters had no significant influence on the PTE concentrations of native transplants.

4. Discussion

4.1. Effect of seawater concentrations on transplant elemental levels

This study is the first to compare elemental concentrations in seaweed transplants with a known exposure period to seawater samples representative of the same period. We detected a wide range of elemental concentrations in seawater (one order of magnitude of variation among sites, which were homogeneously distributed across the range, Table 1SM) which should allow us to detect significant correlations with seaweeds concentrations, if any. However, within our range of concentrations and environmental variables, elemental concentrations in seawater did not explain the concentrations of *F. vesiculosus* transplants, either fresh or dried.

Only a few previous studies have examined the correlation between elemental concentrations in seaweed and seawater, and they have been limited to native seaweeds and discrete seawater samples collected simultaneously. While some of these studies reported finding consistent correlations (Bonanno et al., 2020; Chakraborty et al., 2014; Reis et al., 2016, 2014), most found no relation for most elements and seaweed species (Akcali and Kucuksezgin, 2011; Billah et al., 2017; Boubonari

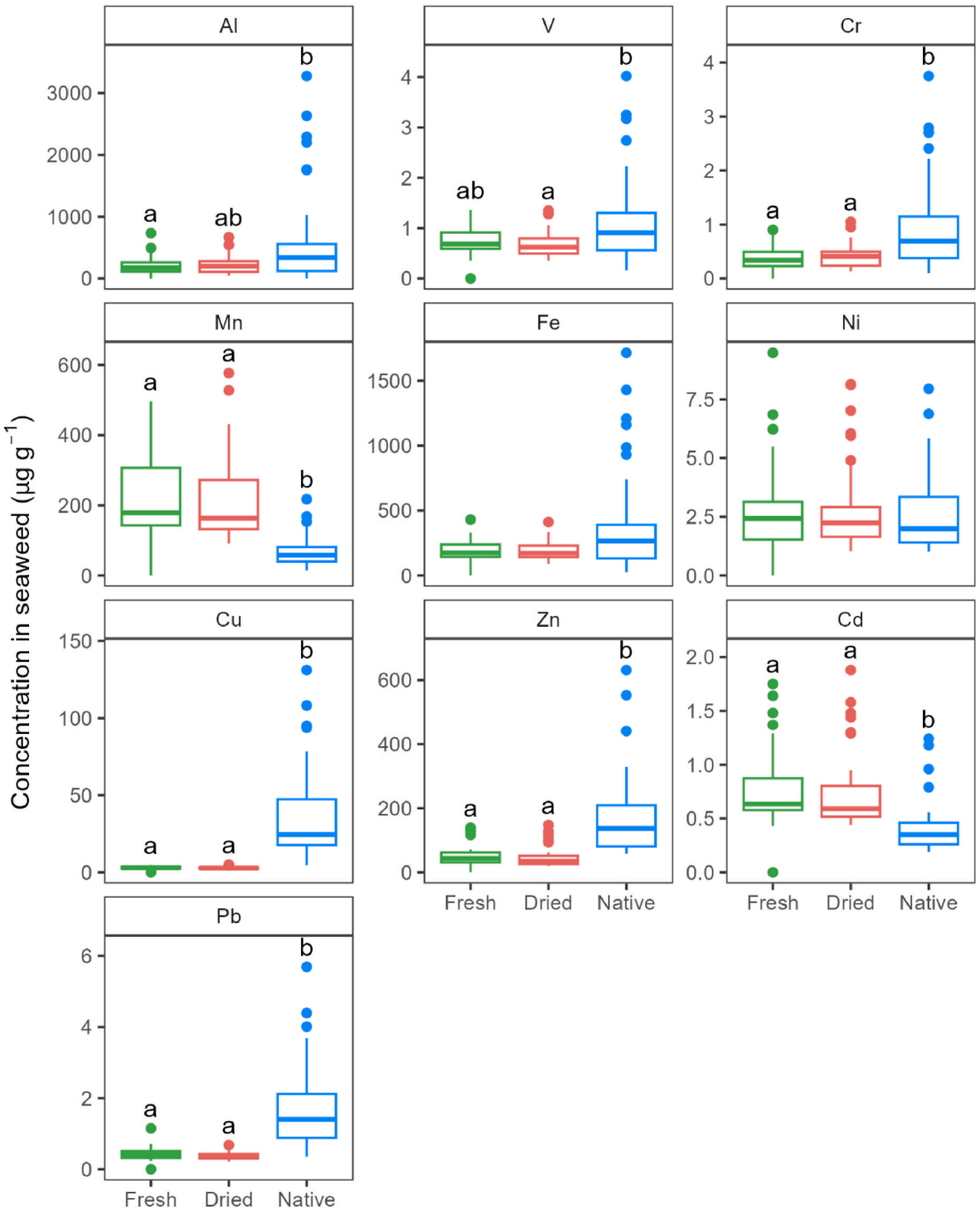

Fig. 3. Boxplots of the pre-exposure elemental concentrations in fresh and dried seaweed transplants made with thalli from a reportedly unpolluted site, and in native seaweeds across the exposure sites. The letters show the groups that were significantly different for the elements in which there were significant differences.

et al., 2008b, 2008a; Chernova and Shulkin, 2019; Malea and Kevrekidis, 2014; Ribeiro et al., 2018). Notably, studies with positive results were often constrained to a rather limited number of sampling sites (Bonanno et al., 2020; Reis et al., 2016), or applied arguably flawed statistical methods (Chakraborty et al., 2014; Reis et al., 2014). Other than the previous studies, positive correlations between seaweed and seawater concentrations were generally rare, and some of the works with the necessary data to study this correlation omitted the comparison (for example Gubelit et al., 2023; Ryabushko et al., 2024), potentially due to, in our opinion, the lack of positive correlations. These findings suggest that seaweed elemental concentrations are not correlated with those of one-time seawater samples.

However, the lack of correlation in those studies is expected due to a major limitation: the mismatch between the seaweed exposure period and the point measurements of seawater. Elemental concentrations in seaweed require time to respond to changes in environmental concentrations as shown in Vázquez-Arias et al. (2023b, supplementary material 4). In that study, Hg, Cr, and Cu reached levels similar to those in native *F. vesiculosus* within 5 days, whereas Zn and Cd required up to 60 days. Another study (Fiévet et al., 2021) quantified the biological

half-life of multiple radioactive isotopes (^{54}Mn, ^{60}Co, ^{65}Z, ^{106}Ru, ^{125}Sb, ^{129}I, ^{137}Cs) in *Fucus serratus* and/or *F. vesiculosus*, with values ranging from 14 to 120 days. Consequently, if concentrations in seawater change over time, discrete water samples may not accurately represent the concentrations in seaweeds exposed over a longer period. Ideally, the exposure period should be similar to the stabilization times to optimize the correlation between seaweed and seawater concentrations, but as this is variable across elements, it is not possible to select a timeframe that is perfectly accurate for all elements. Therefore, our 14-day exposure period might not be ideal for some PTEs, but still offers a more representative sampling compared to single time-point measurements. Yet, despite this improved comparability, neither the correlation analysis nor the GLMs detected any effect of seawater elemental concentrations on those of transplants, contradicting our main hypothesis that *F. vesiculosus* transplants accurately reflect elemental concentrations in seawater.

4.2. Potential influences on the transplant-seawater relationship

Conceivably, the availability of PTEs in the environments must impact their concentrations in seaweeds in some way: for instance, extremely high PTE concentrations have been detected in seaweeds from polluted sites (Vázquez-Arias et al., 2023a). Therefore, there must be other factors explaining the lack of correlation between PTEs concentrations in seaweeds and seawater. Here we studied the effect of the water physicochemical variables, the pre-exposure concentrations of the transplants, and seaweed metabolism.

Seawater physicochemical variables (pH, DO, conductivity and temperature) are known to influence metal bioavailability in marine environments (Hong et al., 2011), but in our study their impact on seaweed metal concentrations was minimal (Table 1). While measuring the physicochemical parameters in the composite sample rather than *in situ* could introduce some noise, if these variables were the primary drivers of the independence between seaweed and seawater concentrations, we would still detect the influence of the physicochemical variables. Therefore, the lack of explanatory power may stem from the complexity of the interaction between water properties and seaweed PTE capture: physicochemical variables can affect bioavailability in non-linear ways, interact with each other, and be element-dependent (Wei et al., 2024). Additionally, physicochemical variables may also be affecting the properties of the surface polysaccharides responsible for PTE capture through processes like the neutralization of acidic groups (Kuzuhara et al., 2018), further complicating their interaction with dissolved elements.

The only element whose concentrations were significantly affected by seawater physicochemical variables was Cd. However, it is possible for this correlation not to be caused by these variables affecting Cd variation. The transplantation experiments were carried out throughout a period of nearly one year. This period was not long enough to test the seasonality of the data statistically, but the post-exposure concentrations of some elements appear to follow seasonal trends (Fig. 4SM and 5SM). Cd concentrations in dried transplants displayed such seasonal variation, which may explain their correlation with seawater parameters such as temperature, which does not affect metal bioavailability, but is seasonal in nature. The seasonal pattern of elemental concentrations in seaweeds has been well documented before (García-Seoane et al., 2021, 2018b), with higher concentrations typically detected during summer, as is the case for Mn, Ni, and Cd concentrations in post-exposure transplants, which are the ones with clear seasonal patterns in this study. These findings suggest that while seasonality may be relevant for explaining elemental accumulation in the transplants, the physicochemical properties of the water are not.

There were large concentration differences among fresh and dried seaweeds, suggesting that the metabolism of the algae and the chemical differences induced by devitalization can affect elemental uptake. However, since seaweed concentrations were not correlated with those of seawater regardless of the treatment, we can conclude that seaweed metabolism is not the primary factor introducing noise in the correlation between seaweed and seawater concentrations.

Another factor that could potentially disrupt this relationship is the variation in the pre-exposure concentrations of the transplants. As the stabilization times of the concentrations of some elements in the transplants might be longer than the exposure period (Fiévet et al., 2021; Vázquez-Arias et al., 2023b), an anchoring effect could affect the post-exposure concentrations. However, this effect was not detected, as post-exposure concentrations were statistically independent from pre-exposure ones, even in cases where they displayed considerable dispersion. The only exceptions were Cd and Mn, where the alignment between pre- and post-exposure concentrations in fresh transplants was notable, albeit statistically insignificant (Fig. 6SM).

These two elements were exceptional in other ways: their concentrations in pre-exposure transplants were higher than those in native seaweeds and post-exposure transplants; they were the only elements with higher concentrations in fresh transplants than in dried ones; and they exhibited some of the clearest seasonal patterns, both pre- and post-exposure. A plausible explanation for this unique behavior is that the high concentrations in the collection site led to the release of Cd and Mn during the exposure period. If the discharge process is slower than the uptake, this would explain why post-exposure concentrations depended on pre-exposure ones only for these elements, as they had not yet stabilized. Furthermore, this effect was observed only in fresh transplants because membrane rupture during drying likely enabled faster release. This would also explain why post-exposure Cd and Mn concentrations in fresh transplants were among the few notably higher in winter than in summer, as this seasonal pattern was evident in pre-exposure transplants and preserved after exposure due to the slow discharge process in fresh transplants.

Other biomonitors, such as mosses, have been proposed to uptake pollutants by integrating them over time in a cumulative process (Harmens et al., 2008). Under that scenario, pre-exposure concentrations would affect post-exposure ones. Although later studies have contradicted this hypothesis (Aboal et al., 2010), many biomonitoring studies still use pollution indices such as enrichment rates to remove the effect of pre-exposure concentrations (e.g. Sergeeva et al., 2021). Contrarily, the independence of pre- and post-exposure concentrations found in this study for most elements supports the hypothesis that the uptake process in seaweeds is not cumulative. Instead, elemental concentrations in seaweeds tend to stabilize over time, reaching a dynamic equilibrium with the environment. In this scenario, including pre-exposure concentrations by using enrichment rates would not remove the effect of initial concentrations, introducing noise instead. The use of enrichment rates in transplants is further complicated by the high variability of the natural concentrations, which follow an intrinsic seasonal pattern already described in the literature (García-Seoane et al., 2021) unavoidable without the use of cultured material.

4.3. Comparisons among native and transplanted seaweeds

As in transplanted seaweeds, the GLMs failed to explain the variability in elemental concentrations in native seaweeds. This is consistent with most previous studies comparing elemental concentrations in seawater and native seaweeds (see section 5.1). Furthermore, concentrations in native seaweeds were not significantly correlated to those of the transplants for most elements. The only exception was Zn, which showed a significant correlation with fresh transplants. The lack of correlation between dried transplants and native seaweeds is expected, as the devitalization process disrupts cell membranes and halts the metabolism. This alteration leads to increase concentration of many elements in devitalized seaweeds, which suggests elemental uptake depends on passive mechanisms such as electrostatic bonds and mineral particle adhesion (Vázquez-Arias et al., 2023a), a conclusion further supported by nanoimaging data (Vázquez-Arias et al., 2024a). However,

the divergence between native seaweeds and fresh transplants is more surprising, as these materials are supposed to be more similar. These discrepancies may be explained by the different exposure periods, phenotypes, and locations (e.g., transplants being permanently submerged in harbors). Regardless of the factors, provided that the use of transplants did not improve the correlations between the concentrations of PTEs in seawater and seaweed, our results did not favor the use of transplants over the collection of native seaweeds, as both proved to be ineffective.

4.4. Implications for biomonitoring

The use of seaweed transplants for monitoring coastal marine pollution has been recommended as a way to improve the temporal interpretation of biomonitoring studies, reduce data variability caused by phenotypic differences, and monitor areas without native seaweeds (García-Seoane et al., 2018a). However, our findings do not support their effectiveness. In this study, elemental concentrations in transplants showed no correlation with those in seawater, even when accounting for the physicochemical properties of seawater. This was also true for native seaweeds, but unlike for transplants, which are exposed under artificial conditions, knowing the natural concentrations in ecologically significant organisms is far more valuable given the potential negative impacts on the ecosystems they inhabit. Seaweed transplants could potentially be used to detect highly polluted places, similarly to what has been proposed for moss transplants (Boquete et al., 2015), but at least in the case of *F. vesiculosus* quantitative biomonitoring of PTEs is not viable.

The lack of the correlation between seaweed and seawater concentrations - a key requirement for an effective biomonitor organism - is likely caused by complex interactions between them. Chemically, physicochemical variables in seawater can have complex, synergistic effects on element bioavailability and on the properties of surface polysaccharides, making these interactions challenging to interpret. Biologically, seaweeds possess distinct uptake and regulatory mechanisms for each element, which significantly affect their concentrations in an element-specific manner. Hence, while seaweed concentrations tend to stabilize over time, eventually reaching equilibrium with their surroundings, the factors driving this equilibrium remain largely unknown. This uncertainty limits the effectiveness of seaweed transplants for biomonitoring purposes, calling into question the validity of numerous past studies that have applied this technique (García-Seoane et al., 2018a).

5. Conclusions

- Under the conditions of our study, *F. vesiculosus* transplants did not reflect the concentrations of chemical elements in seawater.
- Native *F. vesiculosus* performs equally poorly for PTE biomonitoring.
- Seaweeds do not integrate elemental concentrations over time. Rather, they reach a dynamic equilibrium with the environment.
- This equilibrium cannot be reliably predicted based on the elemental concentrations and physicochemical variables of the seawater, likely due to the complex interactions between these variables and the elements in both matrices.
- Seaweed metabolism affects metal accumulation and discharge.
- Uptake depends mostly on passive mechanisms.

CRediT authorship contribution statement

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

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT for assistance with refining language and improving the clarity of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.126456.

Data availability

Data will be made available on request.

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Table 1SM. Median and interquartile range (IQR) of the elemental concentrations in post-exposure fresh transplants (Fresh), post-exposure dried transplants (Dried), native seaweed samples growing in the exposure sites (Native), and seawater samples (Water). Seaweed concentrations are in μg g^{-1}. Seawater concentrations are in μg L^{-1}.

		Al	Cd	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
Fresh	Median	1200	0.842	2.24	7.81	823	209	4.38	8.58	2.73	87.4
	IQR	1100	0.243	2.01	5.87	643	199	2.54	10.7	1.63	63.1
Dried	Median	6970	0.193	10.4	19.1	4030	32.8	10.5	24.7	19.2	80.4
	IQR	9210	0.0685	9	19.9	5080	31.2	8.02	20.4	10.3	45.6
Native	Median	385	0.36	0.693	23.9	255	52.5	1.94	1.39	0.873	136
	IQR	359	0.163	0.887	22.5	223	34	2.07	1.11	0.537	123
Water	Median	4.61	0.0325	0.305	1.61	4.57	1.91	2	0.175	1.77	1.1
	IQR	4.05	0.0477	0.409	1.37	6.64	0.885	1.58	0.176	0.637	1.86

Table 2SM. Median and interquartile range (IQR) of the bioconcentration factors (BCFs) in post-exposure fresh transplants (Fresh), post-exposure dried transplants (Dried) and native seaweed samples growing in the exposure sites (Native). BCFs are in mL g^{-1} (dry weight).

		Al	Cd	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
Fresh	Median	3.4E+05	21838	5224	6044	1.7E+05	1.5E+05	2079	44319	1540	86737
	IQR	3.7E+05	38301	8924	6464	4.8E+05	97531	1848	68651	1122	98387
Dried	Median	1.9E+06	5936	29538	15303	9.2E+05	21900	5138	1.3E+05	11469	52792
	IQR	2.1E+06	6265	28893	14482	1.5E+06	21755	4309	1.8E+05	9209	66966
Native	Median	1.5E+05	12644	2677	23313	79619	36991	1092	8565	493	1.6E+05
	IQR	2.5E+05	13136	3486	18019	2.5E+05	29408	970	30100	481	2.9E+05

Table 3SM. Spearman correlation coefficient (Coef.) and p-value of the Spearman correlation test for correlations between post-exposure fresh transplants (Fresh), post-exposure dried transplants (Dried), native seaweed samples growing in the exposure sites (Native), and seawater samples (Water). P-values have been adjusted with the BH method. Underlined and bolded p-values are significant ($p < 0.05$), and bolded ones are marginally significant ($p < 0.1$).

		Al	V	Cr	Mn	Fe	Ni	Cu	Zn	Cd	Pb
Fresh ~ Dried	Coef.	0.58	0.51	0.52	0.37	0.6	0.57	0.74	0.61	0.65	0.44
	p-value	**0.017**	**0.050**	**0.044**	0.29	**0.012**	**0.022**	**0.0004**	**0.0075**	**0.0037**	0.13
Fresh ~ Native	Coef.	-0.057	-0.25	-0.086	0.42	-0.14	0.29	0.51	0.8	0.21	0.34
	p-value	1	0.53	0.95	0.3	0.9	0.58	0.13	**0.002**	0.67	0.37
Fresh ~ Water	Coef.	0.14	-0.18	-0.12	0.047	0.28	0.027	0.055	0.23	-0.19	-0.22
	p-value	0.91	0.53	0.93	1	0.41	1	1	0.52	0.67	0.42
Dried ~ Native	Coef.	-0.029	-0.29	0.13	0.28	0.075	0.34	0.3	0.53	0.21	0.39
	p-value	1	0.53	0.93	0.57	0.99	0.54	0.45	0.088	0.67	0.3
Dried ~ Water	Coef.	0.3	-0.5	0.17	0.19	0.41	0.037	0.077	0.36	0.25	0.24
	p-value	0.4	0.05	0.91	0.58	0.15	1	1	0.26	0.67	0.42
Native ~ Water	Coef.	0.35	0.23	-0.31	0.26	0.24	-0.15	0.32	-0.16	0.15	-0.42
	p-value	0.4	0.53	0.66	0.57	0.65	0.99	0.45	0.82	0.84	0.3

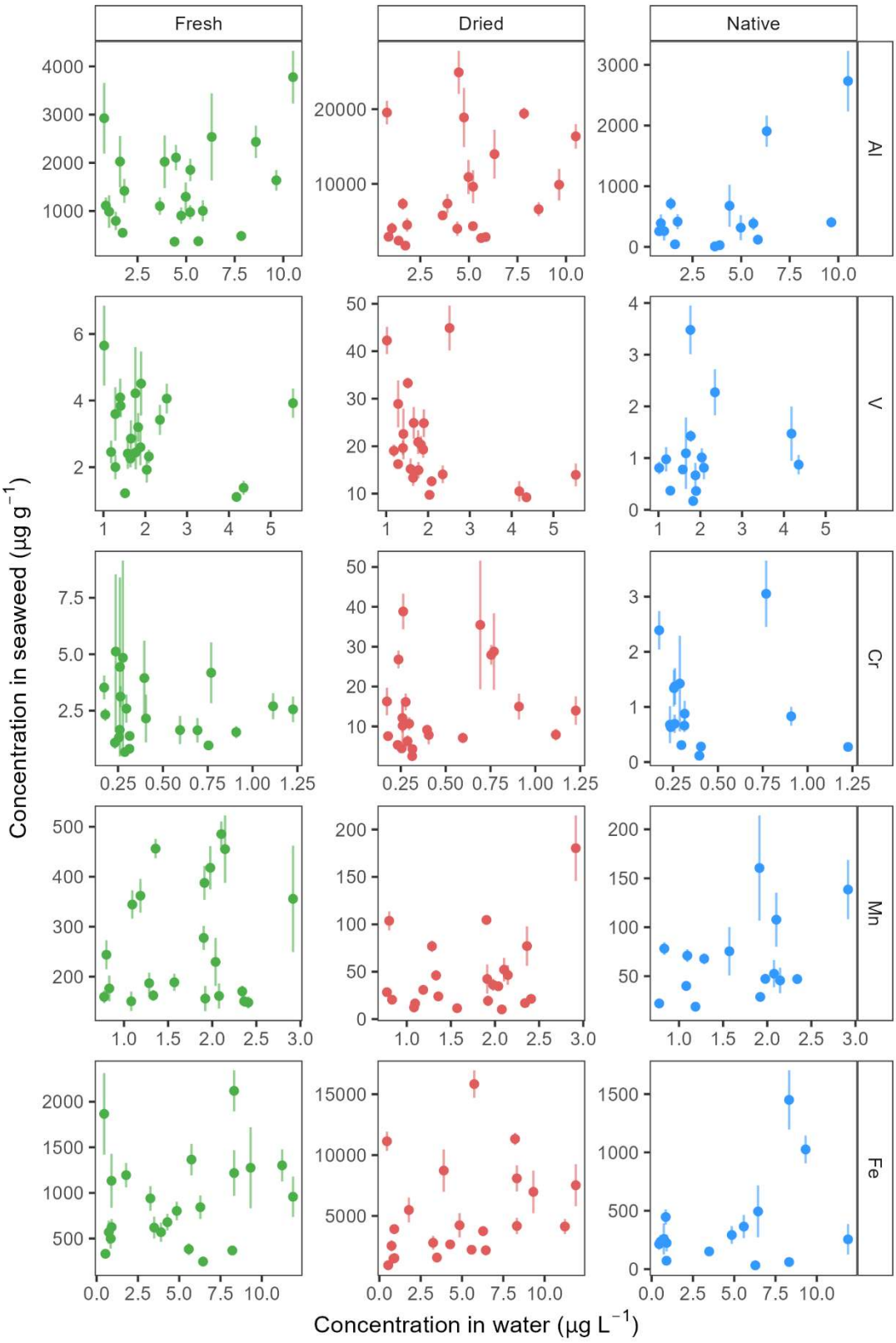

Figure 1SM. Scatterplot of the concentrations of Al, V, Cr, Mn and Fe in seaweed and seawater. "Fresh" represents the post-exposure concentrations in fresh seaweed transplants, "Dried" in dried transplants, and "Native" in native seaweeds. Points represent the mean concentration of samples from each site (5 replicates for transplants and 3 for native samples), and error bars indicate standard deviations.

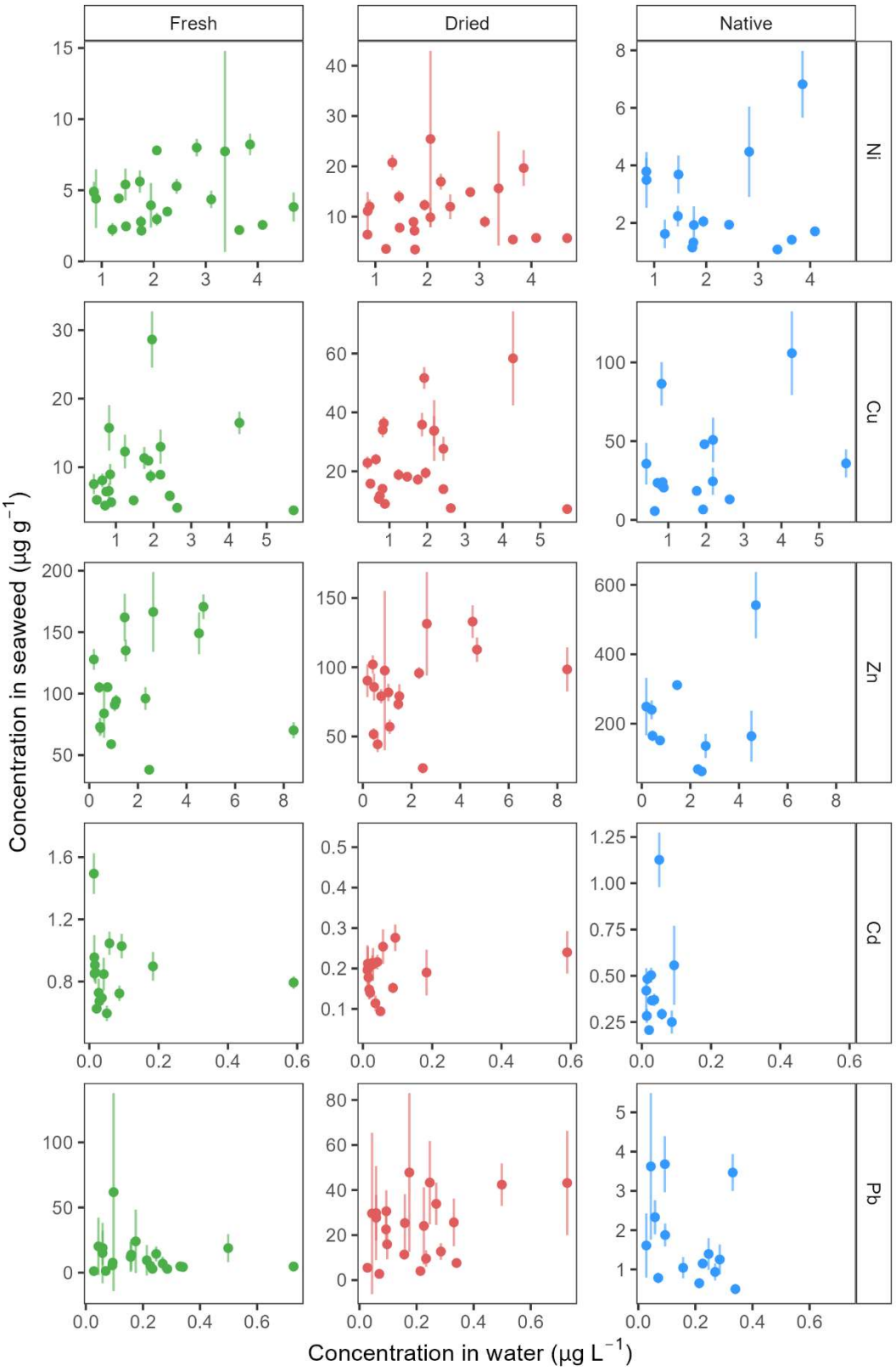

Figure 2SM. Scatterplot of the concentrations of Ni, Cu, Zn, Cd, and Pb in seaweed and seawater. "Fresh" represents the post-exposure concentrations in fresh seaweed transplants, "Dried" in dried transplants, and "Native" in native seaweeds. Points represent the mean concentration of samples from each site (5 replicates for transplants and 3 for native samples), and error bars indicate standard deviations.

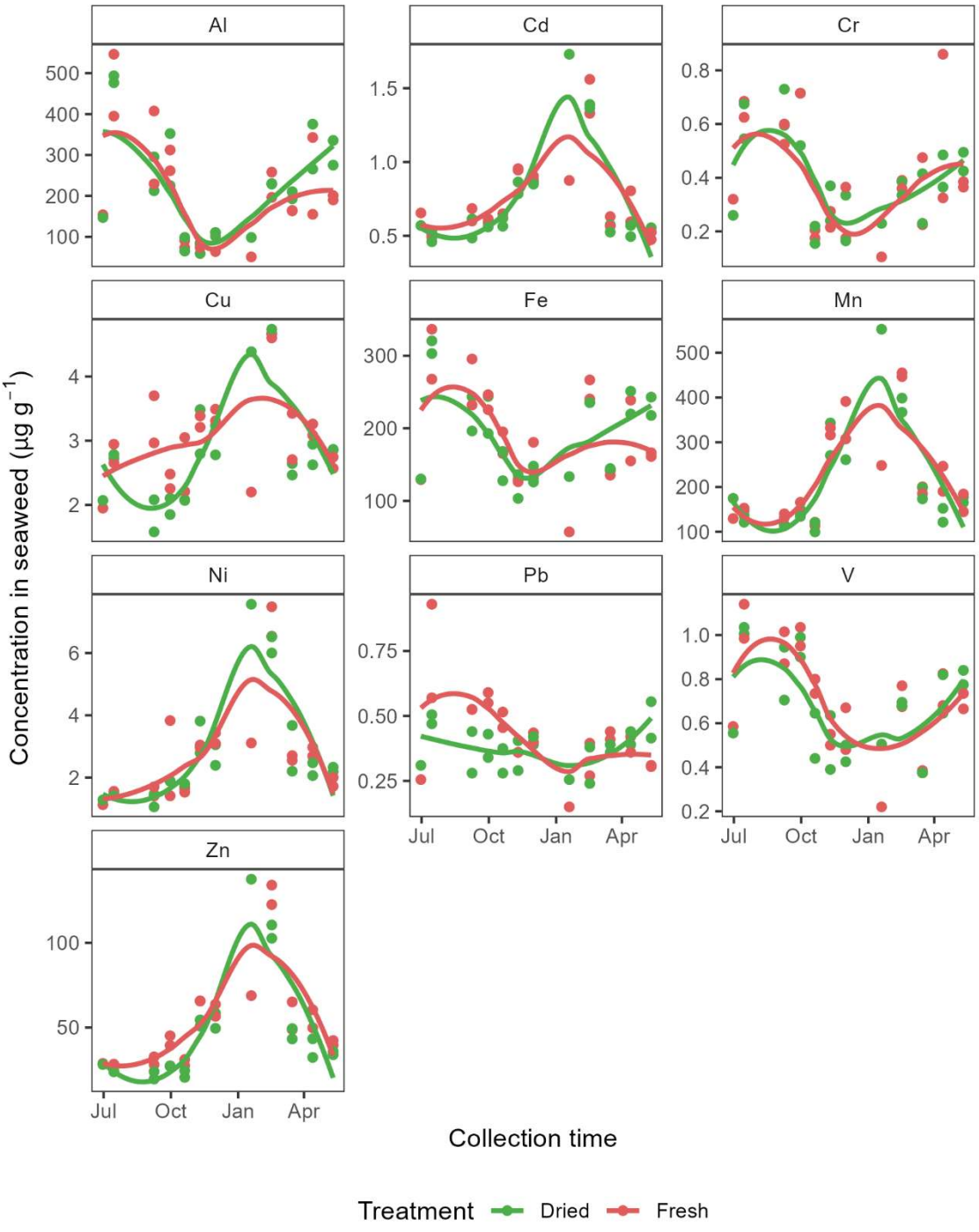

Figure 3SM. Elemental concentrations in pre-exposure fresh and dried seaweed transplants over the time of the experiment. Trend line was generated with a LOESS model.

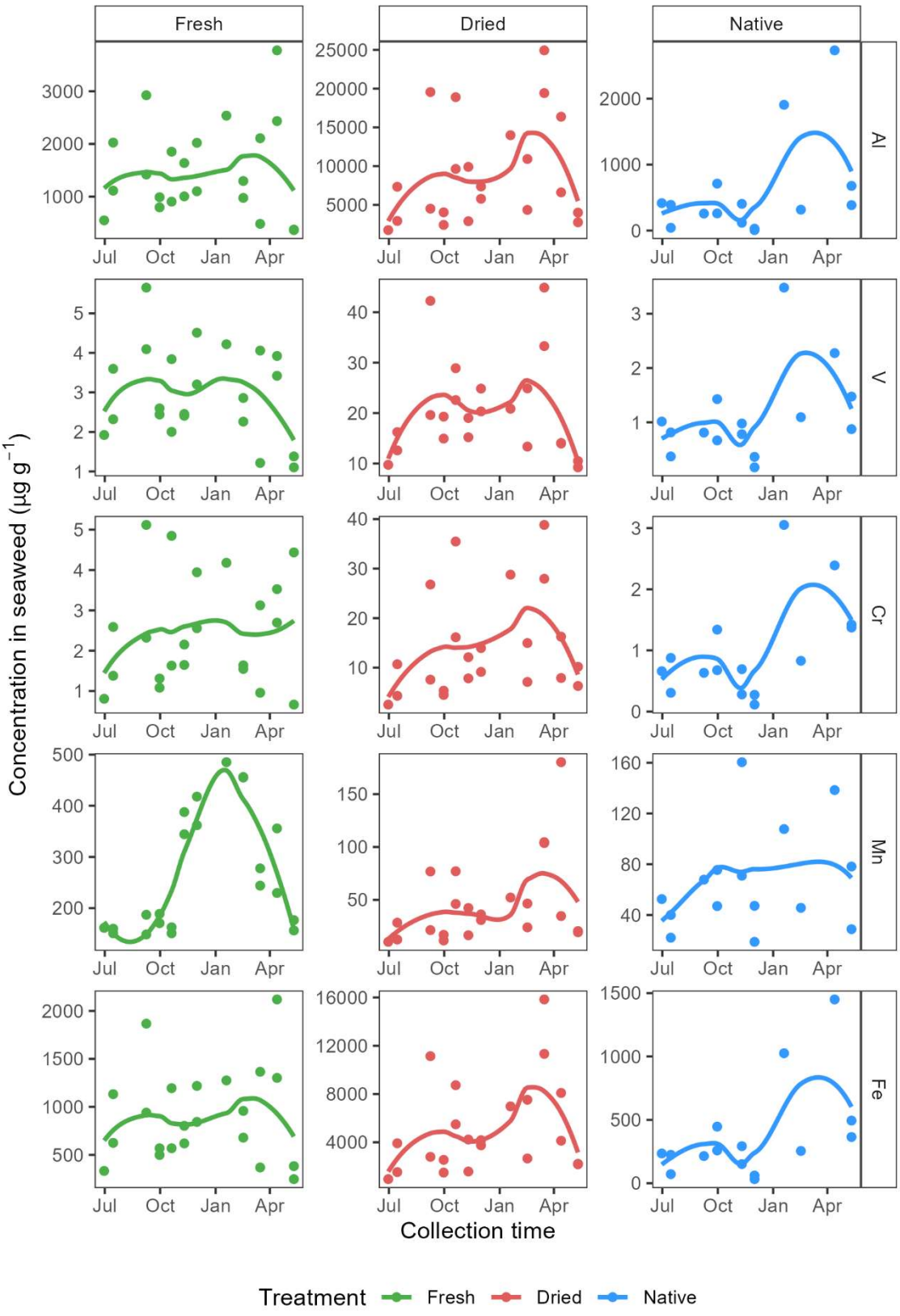

Figure 4SM. Concentrations of Al, V, Cr, Mn, and Fe in post-exposure fresh and dried seaweed transplants and in native seaweeds over the time of the experiment. Trend line was generated with a LOESS model.

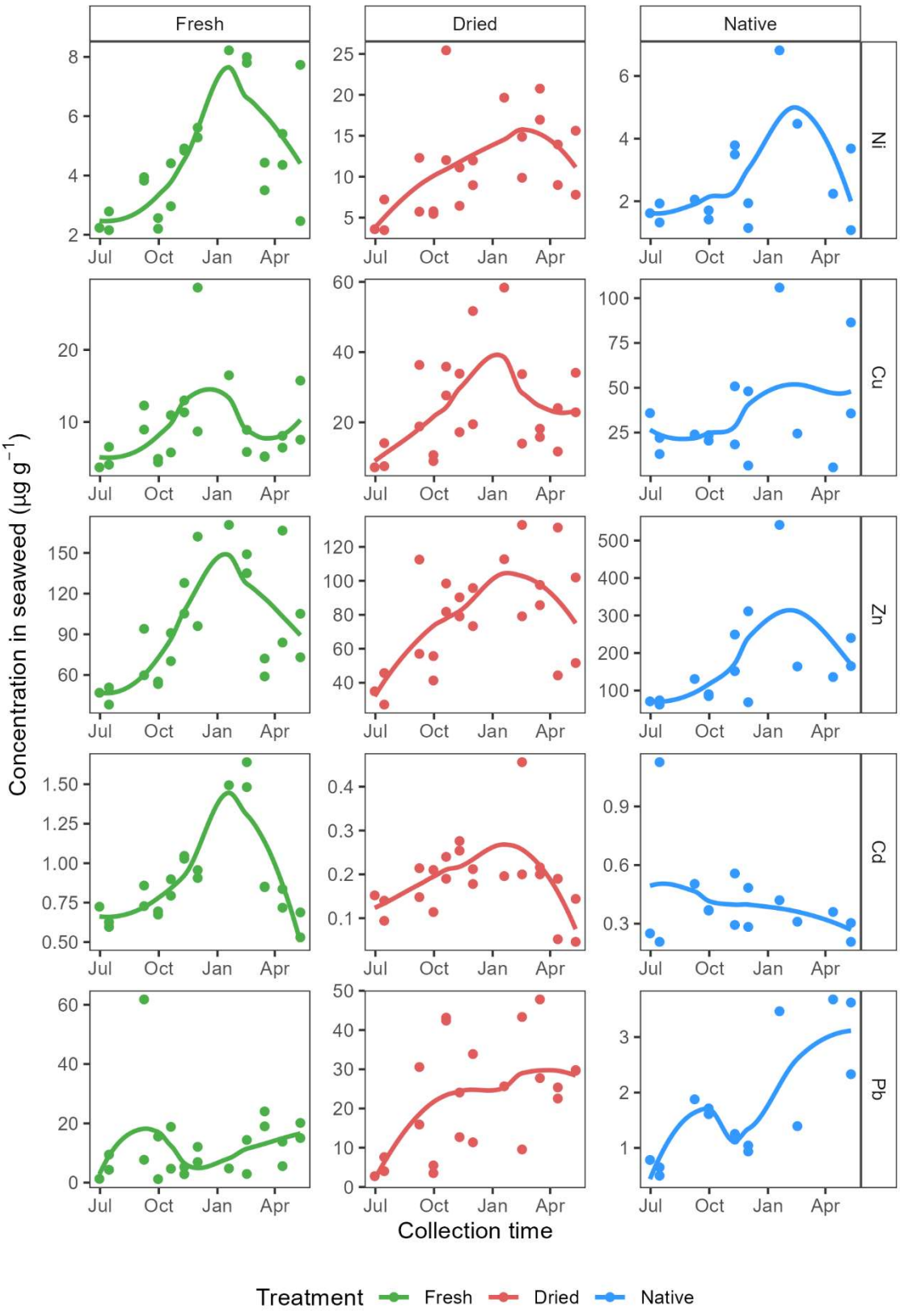

Figure 5SM. Concentrations of Ni, Cu, Zn, Cd, and Pb in post-exposure fresh and dried seaweed transplants and in native seaweeds over the time of the experiment. Trend line was generated with a LOESS model.

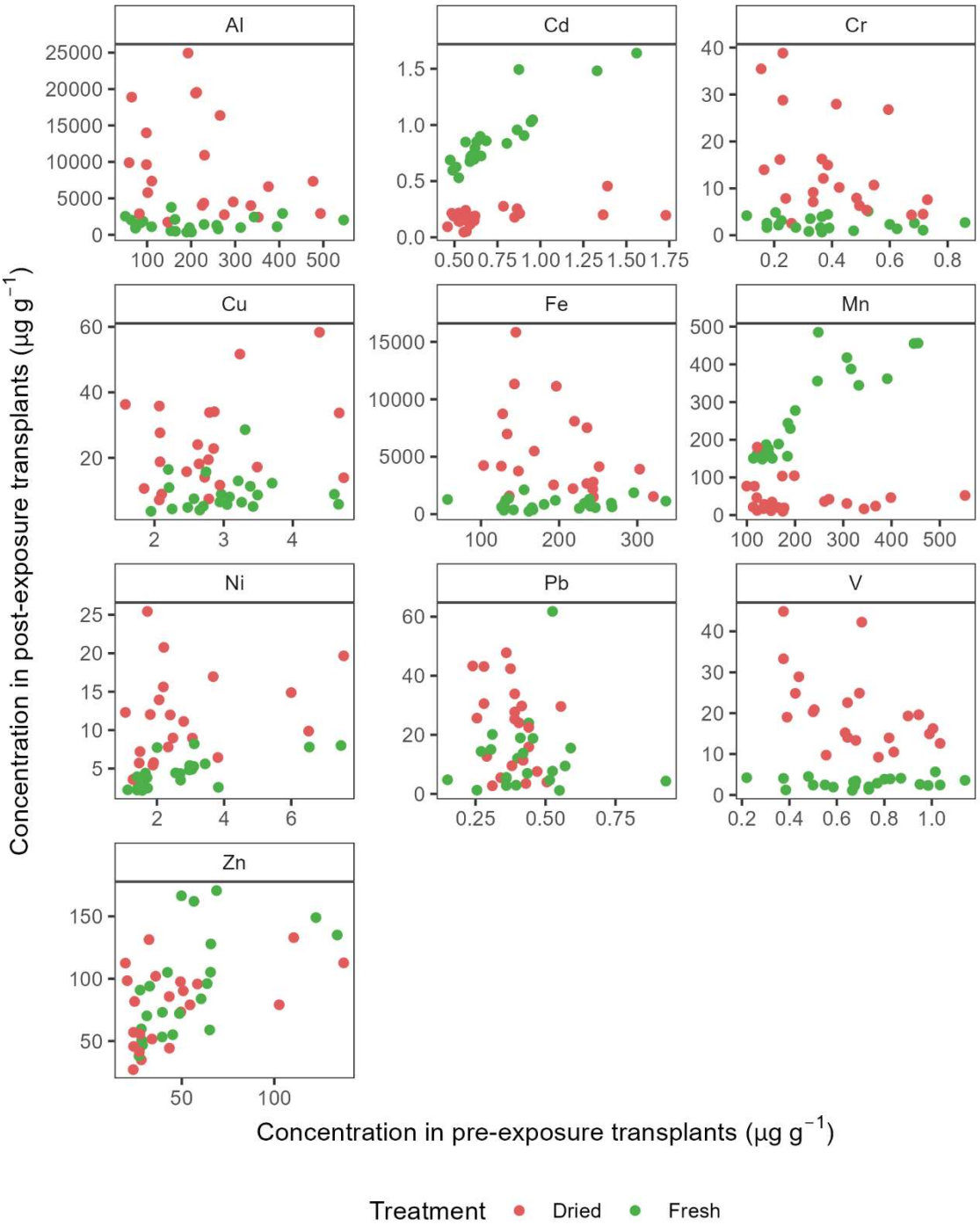

Figure 6SM. Comparison of pre- and post-exposure elemental concentrations in fresh and dried transplants.