ICP-OES Determination of Metal Bioaccumulation in Caulerpa racemosa ramuli from Guánica, Puerto Rico

Paola Díaz
InterAmerican University of Puerto Rico
Gerardo Laureano
InterAmerican University of Puerto Rico
Rosalinda Aybar
InterAmerican University of Puerto Rico
Ramon Rivera
InterAmerican University of Puerto Rico
Angel Núñez
anunez@br.inter.edu

InterAmerican University of Puerto Rico

Short Report

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Abstract

Marine macroalgae have been increasingly utilized in the novel food market due to their richness in essential amino acids, dietary fibers, and bioactive compounds. Among them, *Caulerpa* species, commonly known as sea grapes, have emerged as a valuable commercial resource in Southeast Asia and tropical Australia, where they have been extensively cultivated and studied for their nutritional and biochemical properties. In contrast, little is known about the elemental composition of Caribbean populations. In this study, the ramuli of C*aulerpa racemosa* collected from Guánica, Puerto Rico, were analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES) to determine their elemental composition. Adjacent seawater was analyzed to calculate bioaccumulation factors and evaluate elemental uptake. Results showed a selective absorption profile, with certain trace elements accumulating more than seawater. Cr showed bioaccumulation, indicating *C. racemosa* could be a bioindicator of local metal contamination. The presence of B and Be in tissues also merits further study for ecological and monitoring purposes. Overall, these findings provide new baseline information on the elemental profile of Caribbean *C. racemosa* and support its potential use in marine biomonitoring while highlighting the need for further studies to validate its role in metal remediation and resource recovery.

Introduction

The global demand for sustainable, nutrient-dense food sources has catalyzed the utilization of marine macroalgae within the novel functional food sector (1). Because seaweeds are naturally rich in essential amino acids, dietary fibers, and bioactive compounds, they offer physiological health benefits that extend far beyond basic daily nutrition. Among various marine macroalgae, the green coenocytic macroalgae *Caulerpa* species, commonly known as sea grapes or green caviar, have emerged as highly valuable commercial resources (2). These unique organisms are characterized by a siphonous, single-cell macroscopic structure that lacks internal cross-walls, allowing for highly efficient nutrient transport throughout the entire algal body. Specifically, *C. lentillifera* and *C. racemosa* are the most extensively utilized species for direct consumption and international trade across the Indo-Pacific region (3). These benthic algae have high potential for commercial aquaculture due to their rapid vegetative propagation, which enables substantial biomass yields in relatively short periods (4). However, the functional dietary safety of *Caulerpa* species depends heavily on their specific nutritional and elemental profile, requiring precise analytical quantification (5). Biochemical analyses demonstrate that *C. racemosa* contains appreciable levels of polyunsaturated fatty acids (PUFAs) and bioactive photosynthetic pigments, including chlorophylls and β-carotene, which contribute to its antioxidant capacity (6, 7). Beyond these organic constituents, the mineral-rich ash fraction of *Caulerpa* is substantial, often exceeding 20% of total dry weight (8).

Marine macroalgae naturally absorb and concentrate elemental components directly from their surrounding seawater, enabling them to actively bioaccumulate both essential micronutrients and potentially toxic trace metals within their tissues (4). These algae have been widely used as bioindicators of marine environmental quality and as models for studying elemental bioaccumulation. The elemental composition of *Caulerpa* species has been performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). These methods provide sensitive and accurate quantification of essential minerals while simultaneously detecting potentially hazardous elements, including arsenic, cadmium, and lead, thereby supporting food safety evaluations, environmental monitoring, and regulatory compliance (4, 5).

In Southeast Asia and tropical Australia, *Caulerpa* species have been heavily cultivated, and their biochemical profiles have been extensively studied. In contrast, Caribbean populations remain understudied. Especially with respect to their elemental composition and bioaccumulation profiles. Macroalgal elemental composition is highly dynamic rather than static; it is intimately governed by the physicochemical variables of the local marine environment, including dissolved oxygen, salinity, nutrient availability, and temperature fluctuations (3). More than 20 *Caulerpa* species have been reported from the coastal waters of Puerto Rico (9). Despite this abundance, no studies have comprehensively characterized the elemental bioaccumulation profiles of these native populations. Establishing such baseline data is essential for evaluating their potential as safe novel food sources, assessing environmental quality, and supporting future applications in marine biotechnology. In this study, we employed ICP-OES to characterize the elemental composition of *C. racemosa* collected from Guánica, Puerto Rico. Adjacent seawater was analyzed concurrently to determine bioaccumulation factors and evaluate the species' selective elemental uptake under natural environmental conditions.

Materials and Methods

Study Area and Sample Preparation

Fresh samples of *Caulerpa racemosa* var. Ramuli were collected from the subtidal zone of Tamarindo Beach in Guánica, Puerto Rico, in March 2025. Approximately 1 kg (fresh weight) of algal biomass was manually collected. To establish a baseline for bioaccumulation analysis, adjacent seawater samples were collected in triplicate from the immediate vicinity of the algae. To remove epiphytic organisms and residual marine salts, the algal material was repeatedly washed with distilled water, using a soft brush was gently used to remove debris with minimal damage to the tissue. The samples were air-dried to remove excess surface moisture before morphological dissection. The upright ramuli were carefully separated from the stolons and rhizoids for subsequent elemental analysis. All elemental analyzes were performed using ramulli obtained from a single *Caulerpa racemosa* specimen, with each measurement conducted in triplicate.

Microwave-Assisted Digestion

A total of 1.0g from fresh *C. racemosa* ramuli were weighed and placed in Teflon microwave vessels. 20 mL of 2% nitric acid (HNO_3) was added to each vessel. The samples were digested using a closed-vessel microwave reaction system with a temperature ramp to 200°C, over 15 minutes, and held at that temperature for 15 minutes to ensure complete decomposition. After cooling, the digested material was filtered using filter paper (Whatman No. 41) to remove any particulate.

ICP-OES Analysis

The digested algal samples and filtered seawater samples were analyzed for elemental composition using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The instrument was calibrated using certified multi-element standard solutions following the manufacturers' recommendations. The analyses quantified 61 chemical elements, including major elements, trace elements, heavy metals, and selected rare earth elements in both seawater and *C. racemosa* ramuli. All measurements were performed in triplicate.

Bioconcentration Factor (BCF) Calculation

The Bioconcentration Factor (BCF) was calculated to determine the accumulation efficiency of specific elements from the seawater into the tissues of *C. racemosa*. The BCF was calculated using the following equation:

$$BCF = \frac{Conc.\ Algae}{Conc.\ Seawater}$$

Where Conc. Algae is the concentration of the analyte in the algal tissue, and Conc. Seawater, is the concentration of the analyte in the surrounding seawater (10). Based on the calculated values, elemental accumulation was classified into three categories: low bioconcentration if BCF < 100, moderate bioconcentration if BCF was greater than 100 and less than or equal to 1000, and high bioconcentration if BCF was higher than 1000 (11). Higher BCF values indicate a greater capacity of the algae to accumulate a given element relative to its concentration in the surrounding seawater.

Results

Atomic elements of C. racemosa of ramuli and the surrounding seawater

Elemental profiling detected a total of 61 elements in the adjacent seawater and 11 elements in the *C. racemosa* ramuli biomass. The main elements for each sample type are summarized, with the 15 more abundant in seawater and all elements detected in the algal tissue (Fig. 1). The elementary profile of the ramuli was different from that of the surrounding seawater, indicating selective elemental uptake. Among the detected elements, boron (B), tantalum (Ta), zirconium (Zr), and chromium (Cr) were present at comparatively higher concentrations in the algal tissue, whereas ytterbium (Yb) and manganese (Mn) were detected at the lowest concentrations.

BCF of C. racemosa ramuli

Bioconcentration factors (BCF) were determined to evaluate the ability of *C. racemosa* ramuli to accumulate elements, and these were categorized into high, medium, and low groups (Fig. 2). Rhenium (Re), Zirconium (Zr), and Gadolinium (Gd) showed high accumulation levels (BCF > 1000), with Re reaching the highest overall value, surpassing 10,000. Ytterbium (Yb) and Tantalum (Ta) showed medium accumulation capacity (BCF 100–1000). In contrast, Manganese (Mn), Bismuth (Bi), Lithium (Li), and Chromium (Cr) exhibited low accumulation potential (BCF < 100), with Cr having the lowest BCF among all elements measured.

Heavy Metal Presence in C. racemosa ramuli and the surrounding seawater

Heavy metal concentrations were analyzed across the *C. racemosa* ramuli and the adjacent seawater (Fig. 3). The seawater profile was characterized by the presence of a more diverse heavy metal profile, with Aluminum (Al) present at the highest concentration, followed by Arsenic (As) and Lead (Pb). Nickel (Ni), Cadmium (Cd), and Mercury (Hg) were detected in the seawater samples, but not in the algal tissue. In contrast, the *Caulerpa racemosa* ramuli exhibited a highly selective accumulation pattern. Chromium (Cr) was the only heavy metal detected at an appreciable concentration in the algal tissue (approximately 95), substantially exceeding its corresponding concentration in the surrounding seawater. This pattern suggests preferential accumulation of Cr by *C. racemosa* under the environmental conditions of the study site.

Unique metal presence of C. racemosa ramuli

Boron (B) and beryllium (Be) were detected exclusively in the C. racemosa ramuli and were not detected in the adjacent seawater (Fig. 4). Among these elements, boron was the predominant one, with a concentration of approximately 5,000 μg/kg, whereas beryllium was present at a markedly lower concentration of approximately 450 μg/kg. This represents a tenfold difference in concentration between the two elements.

Discussion and Conclusion

The elemental profiles of *C. racemosa* ramuli, compared to those of the adjacent seawater, suggest its distinct ability to selectively absorb certain elements, rather than reflecting the elemental composition of the surrounding marine environment. Whereas seawater mainly contained elements such as Sulfur (S) and Aluminum (Al), however, only 11 elements were detected in the ramuli, indicating a potential selective accumulation pattern. Among these detected elements, Re, B, Ta, Zr, and Gd, exhibited the highest relative abundances. Important physiological elements such as K, Na, or Ca were not among the 11 elements in the ramuli profile, which might suggest the selective accumulation of trace elements. Rhenium (Re) is exceptionally high, and it overwhelmingly dominates the biomass profile relative to other elements. This significant uptake is not common, so confirmatory analytical methods should be advised. The enrichment of Re, Ta, and Zr, could potentially indicate anthropogenic pollution, as these elements are typically at very low concentrations in natural seawater (12–14), given that these metals are rare in natural seawater. The high bioconcentration factor values (BCF index) observed for of Re, Zr, and Ta support the ability of *C. racemosa* ramuli to accumulate selected trace elements from the surrounding environment (Fig. 2).

Analysis of heavy metals revealed that *C. racemosa* ramuli exhibits a selective accumulation profile. Among the heavy metals analyzed, Cr was the only element showing clear enrichment in the biomass relative the surrounding seawater (Fig. 3). The elevated Cr levels in the ramuli are much higher than the marginal baseline levels found in the marine environment, suggesting environmental exposure to Cr. This is consistent with the reported capacity of *C. racemosa* to accumulate heavy metals (15). Other metals, such as Hg and Ni, were more prevalent in seawater and were not detected in the algae, suggesting limited uptake. The presence of Cr and the absence of Hg in ramuli aligns with studies showing that heavy metal uptake (Hg, As) and distribution vary among ramuli, stolons, and rhizoids (11). In a similar way, B and Be were detected only in the algal tissue (Fig. 4). Boron is an essential micronutrient in marine algae, involved in cell wall polysaccharide cross-linking, and is actively taken up against a concentration gradient across multiple macroalgal lineages (16, 17). Therefore, its enrichment in the ramuli is consistent with physiological uptake and bioconcentration. In contrast, Be has no clear role in algae, and its detection suggests that *C. racemosa* may also accumulate certain non-essential trace elements, supporting further investigation of its potential application as a biomonitoring agent and in marine bioremediation.

In summary, this study highlights the bioaccumulation properties of *C. racemosa* from Guánica, Puerto Rico, by comparing the elemental profiles of its ramuli with that of the surrounding seawater. The ramuli exhibited relatively high bioconcentration factors (BCF) for Re, B and Ta, indicating selective accumulation of these elements. The heavy metal profile revealed selective enrichment together with the accumulation of B and Be, suggesting that C. racemosa has the capacity to accumulate both essential and non-essential trace elements from the marine environment. These findings support the potential of this alga as a biological monitor of trace element contamination and warrant further investigation into its possible applications in marine biotechnology and trace element recovery. Notably, the unusually high concentration of Re should be interpreted cautiously and confirmed using independent ICP-MS analyses.

Limitations

This study was limited to analyzing the ramuli of *C. racemosa* and no other anatomical structures (e.g., stolons, rhizoids) were evaluated for their metal profiles accumulation patterns. In addition, sampling was restricted to a single location and sampling period, which may not fully represent temporal or spatial variability in elemental accumulation. The analysis was limited to calculating BCFs. There were no analyses of metal speciation, bioavailability, recovery efficiency or physiological mechanisms of uptake. Therefore, further studies are needed to validate the observed accumulation patterns, particularly for Re, and to evaluate the ecological and biotechnological significance of these findings.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

There is no conflict of interest.

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Author Contribution

P.D., G.L., and A.N. conceived and designed the study. P.D., R.A., R.E., and G.L performed experiments, collected data, and analyzed and interpreted data. P.D. drafted the manuscript, and G.L. and A.N. finalized the manuscript. A.N. supervised the study and secured funding. All authors contributed to the article and approved the submitted version.

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Data Availability

All data produced by this study are available in the following figshare repository: https://doi.org/10.6084/m9.figshare.32817686.

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Figures

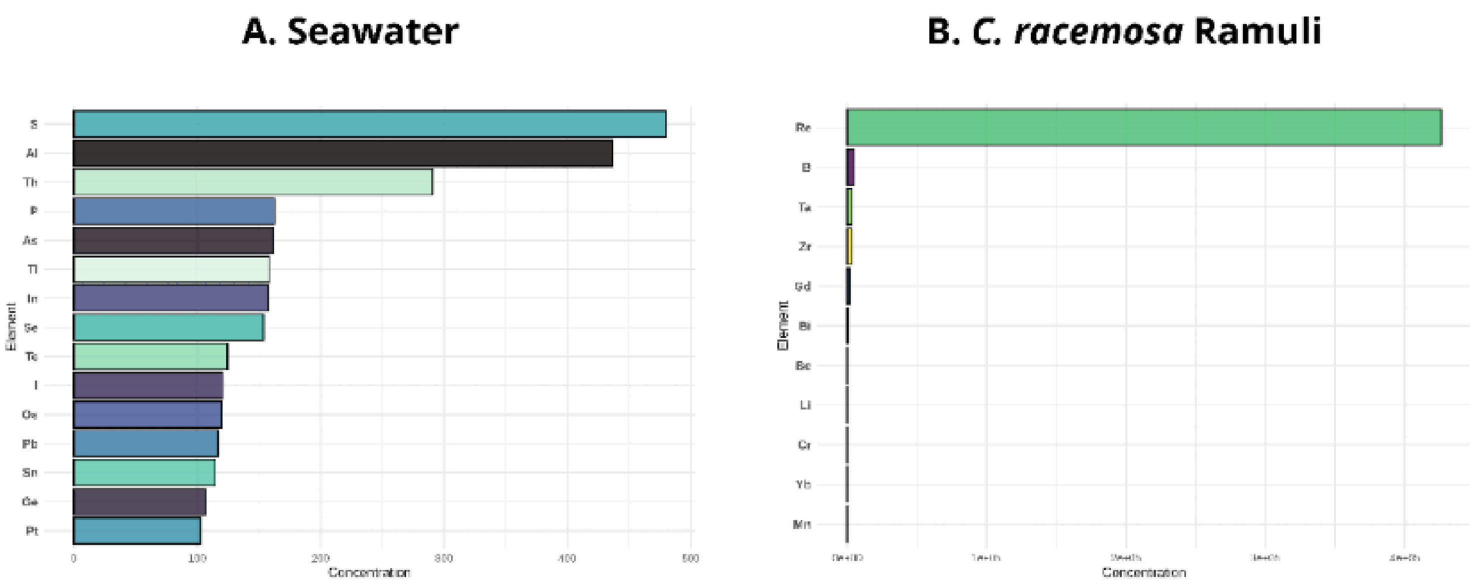

Figure 1

Stacked bar plot showing the distribution of elements in the sample.

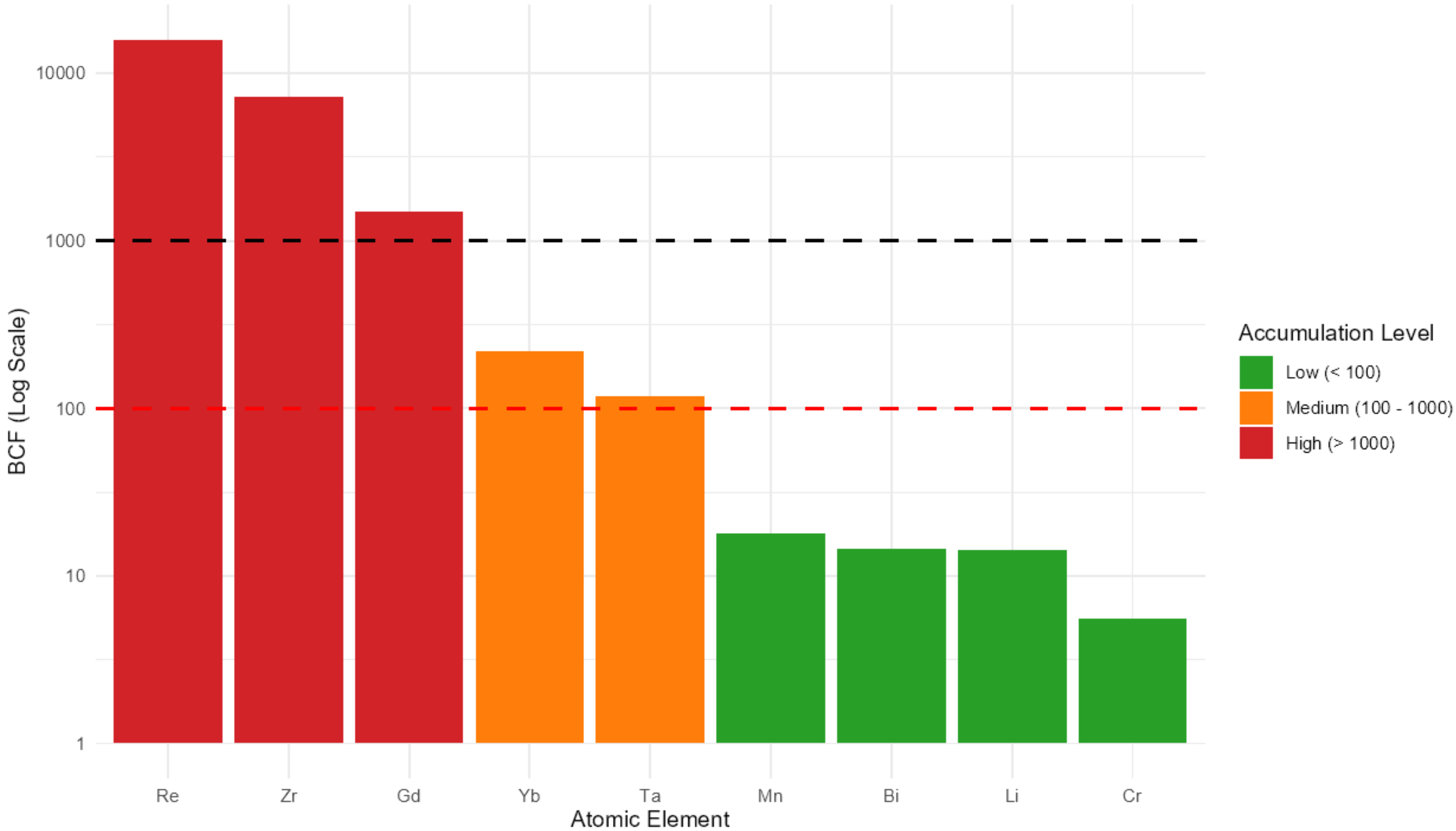

Figure 2

BCF of *C. racemosa* ramuli determined with adjacent seawater. The red line represents the medium BCF threshold, and the black line represents the high BCF threshold.

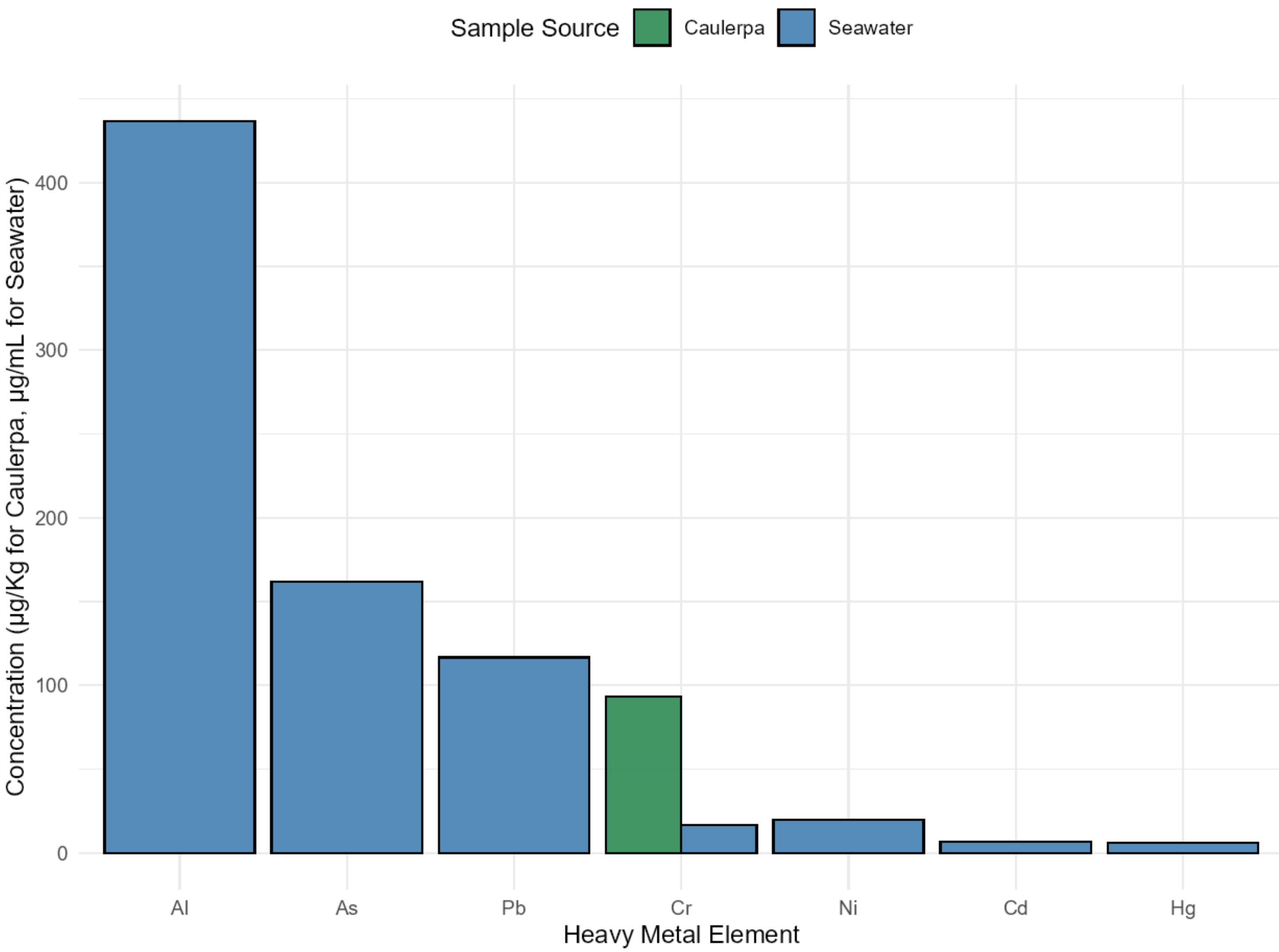

Figure 3

Heavy metal concentration across *C. racemosa* ramuli and adjacent seawater.

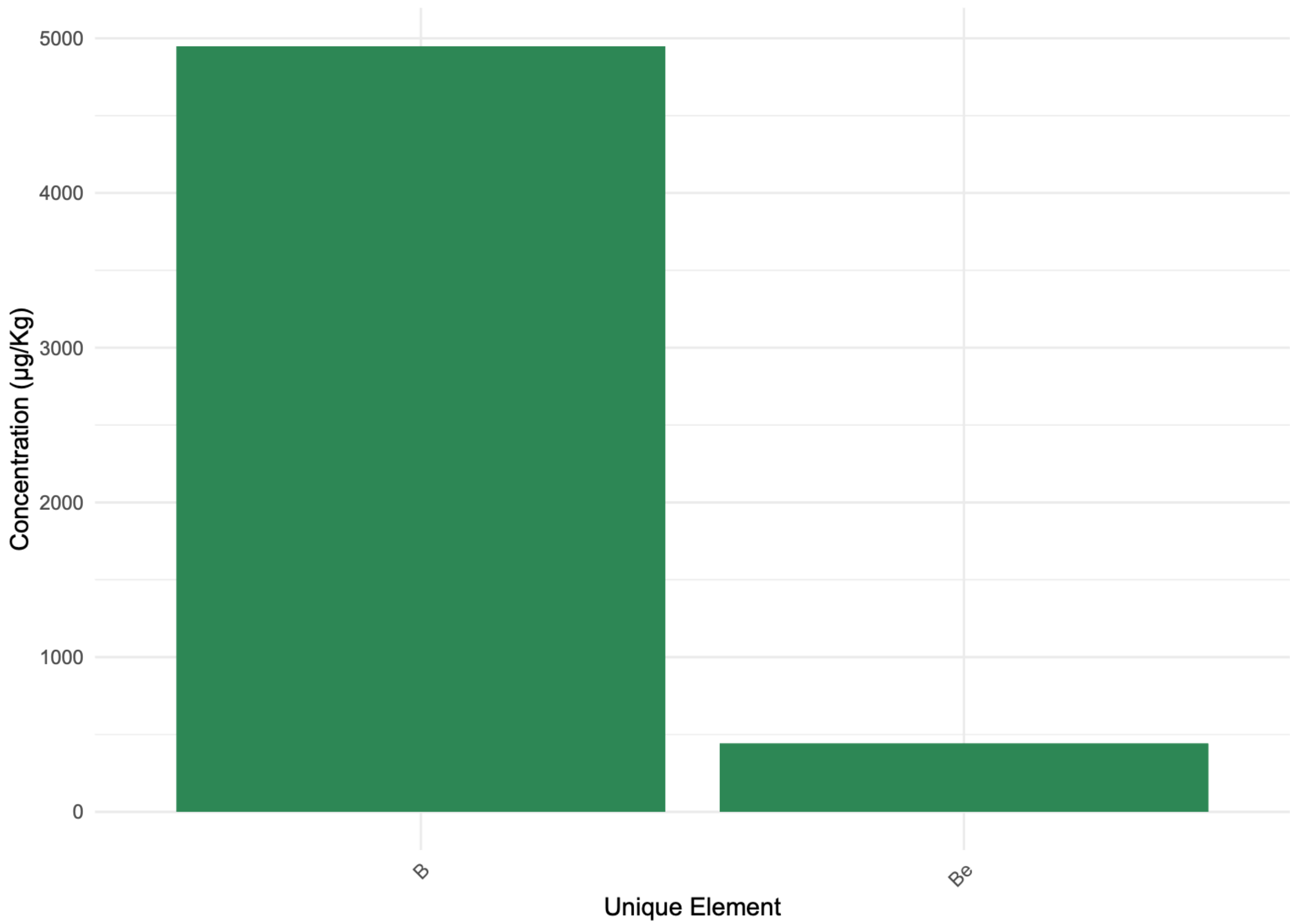

Figure 4

Unique metal presence in *C. racemosa* ramuli in µg/kg.