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Reduced transfer of metals and metalloids from pelagic *Sargassum* spp. accumulated in artificial floating barrier

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Since 2011, holopelagic *Sargassum* have been massively stranding in the coastal environments of the Caribbean Islands inducing damages to coastal ecosystems, public health and the economy. To limit the risks associated with *Sargassum* stranding, floating barriers with nets can be placed in front of sensitive areas, to divert *Sargassum* away from the coast. To evaluate the potential transfer of metallic trace element (MTE) from *Sargassum* to adjacent marine life, seagrasses (*Halophila stipulacea, Thalassia testidinum)* and urchin (*Lytechinus variegatus)* were sampled, both close (0 m) and far (200 m) from barriers installed during 4 years in two bays: Baie Cayol (BC) and Cap Est (CE) in Martinique (FWI). A bay without barriers Baie-Tresor (BT) was also sampled in order to compare the effects of *Sargassum* accumulated in a natural environment versus an environment with floating barriers. The short-term effects of barriers were evaluated by measuring the evolution of MTE after four days, in the algae (*Dictyota* spp.), located close to *Sargassum* accumulations. All sampling was realized during two periods of active (July 2021) and reduced (January 2022) *Sargassum* stranding. The measured concentrations of 19 metal(loid)s trace elements revealed that the proximity of *Sargassum* to the barriers did not increase MTE concentration. The absence of increase in MTE was observed all sites (BT, BC and CE) and during periods of limited and important *Sargassum* stranding. Similarly, translocations of *Dictyota* close to *Sargassum* accumulations did not reveal any increase in MTE concentrations in the algae after 4 days. The present study suggests that the use of barriers to manage *Sargassum* stranding would not constitute an important threat of MTE contamination of marine environments.

Keywords Metals, Metalloids, Dictyota, Seagrass, Mangroves, Arsenic

One of the most diverse genera among brown algae is the genus *Sargassum* including over 350 recognized benthic species[1]. Among this genus, only two species are holopelagic, having their entire life cycle floating in the Atlantic Ocean and reproducing by vegetative fragmentation: *Sargassum fluitans* and *Sargassum natans*[2–5]. Morphological and molecular studies have differentiated three genotypes: *S. fluitans* III and *S. natans* I and VIII[6]. Holopelagic *Sargassum* was first reported during the 15th Century in the Sargasso Sea[7]. Since 2011, *Sargassum* has formed the Great Atlantic *Sargassum* Belt extending from the West African coasts to Brazil, the Caribbean Sea and the Gulf of Mexico. Caribbean islands have to face massive stranding of pelagic *Sargassum* algae (Gower and King, 2011; Széchy et al., 2012) causing several significant issues: (i) ecological damages that threaten endangered species such as turtles[8] and lead to the disappearance of coastal ecosystems[9] (ii) *Sargassum* pose health risk to humans, including respiratory diseases, neurological problems and digestive cardiovascular lesions due to H_2S emitted from decomposed algae[10] (iii) economic issues arise due to the impact on tourism, and obstruction of boat circulation impacting marine trade and fisheries[11]. Pernicious effects can also be associated with MTE due to the ability of *Sargassum* algae to absorb MTE from their environment[12]. In the field, *Sargassum* exhibits high concentrations of metals, especially with arsenic (As) being one of the most consistently abundant elements found across offshore and coastal environments[13–15] throughout the year[16,17]. *Sargassum* has also been

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shown to rapidly accumulate As in experimental conditions[18]. Once they arrive in tropical coastal environments, *Sargassum* quickly releases their metalloid As into mangroves, seagrasses and coral reefs[19]. The metalloid As released by *Sargassum* could be transferred to marine organisms, posing a potential contamination risk for marine life and seafood consumers.

To mitigate the impact of massive accumulations of stranded algae, *Sargassum* can either be harvested from nearshore areas and harbors while they are floating, or once they become stranded[20]. Artificial barriers can also be used to redirect floating algae, preventing their accumulation in sensitive areas. These barriers consist of floating buoys equipped with dragging nets that intercept the path of the floating *Sargassum*. Typically, these devices are installed parallel to the coast, utilizing natural marine currents to guide the algae along these barriers and prevent their massive accumulations.

The aim of the present study is to investigate the potential transfer of metals from *Sargassum* accumulated in artificial barriers to adjacent marine organisms. We analyzed the metal composition of seagrasses (*Halophila stipulacea*, *Thalassia testudinum*) and urchin (*Lytechinus variegatus*) collected both far and close to *Sargassum* accumulated in barriers, in two different bays, as well as in a natural bay without barriers in Martinique (French West Indies). Measurements were conducted during two periods with *Sargassum* stranding activities are active (July 2021), and reduced (January 2022). During both periods, the short-term effects of barriers was assessed by placing the algae *Dictyota* spp. close to accumulated *Sargassum* and measuring changes in their MTE concentrations after four days.

Materials and methods

Study sites

The sampling was conducted on the "windward coast" of Martinique (FWI) which is located on the East side of the Island and is frequently exposed to *Sargassum* strandings (Fig. 1). To assess the characteristics of MTE accumulation attributed to barriers, sampling was carried out in two bays with barriers installed for 4 years: Baie Cayol (BC) and Cap Est (CE). Additionally, a bay without barriers, Baie-Trésor (BT) was sampled. Artificial barrier used in our study allowed small boats to collect *Sargassum* continuously in case of accumulation. According to local and episodic currents, accumulated *Sargassum* can punctually return off shore. Exchange rates of algae have not been quantified and information about resident time of *Sargassum* in barrier is not available.

Sample collection

Sampling took place in July 2021 and June 2022 during periods of respectively significant and reduced stranding. Samples were obtained at different distance from *Sargassum* accumulation (i) in artificial barrier (0 m and 400 m) and (ii) in natural bay (0 m and 400 m). Those distance were chosen arbitrarily in order to be (i) large enough to maximize probabilities to observe different effect of Sargassum accumulation and (ii) small enough to limit bias due to different local environmental conditions.

Phanerogams (*Halophila stipulacea* ($n=30$) and *Thalassia testidinum)* ($n=21$) and urchins (*Lytechin us variegatus*) ($n=27$) were sampled closed to Sargassum accumulations (within 10 m) in barriers (CE and BC) and along the seashore (BT).

A similar sampling approach was implemented at locations far from from *Sargassum* accumulations, more than 200 m away, in each site (Fig. 1). In addition, *Dictyota* spp. algae ($n=37$) were sampled far from the *Sargassum* accumulations (more than 200 m away) and placed in cages attached one meter above the water surface. These cages were positioned close to *Sargassum* accumulations (within 1 m) and far from them (more than 200 m) at each site. After a duration of four days, *Dictyota* spp from each cage were collected for analysis. *Sargassum* accumulated in artificial barrier and natural were not sampled for metal analysis.

Laboratory analysis

Sample preparation

Immediately after collection, the urchins (*L. variegatus*) were dissected and their gonads were collected and extracted. Algae (*Dictyota* spp.) and all parts of the phanerogams (*H. stipulacea* and *T. testidinum*) were gently shaken to remove attached particles and biofilm. All samples were then freeze-dried. Algae and phanerogams were then ground and homogenized using a vibro-grinder with 5 mm zirconium for three min with a frequency of 30 beats per second (Restch© MM200).

Heavy metal analysis

An inductively Coupled Plasma Optical Emission Spectrometer (Spectrometer ICP-OES 700®, Agilent Technologies) was used to analyze a series of 19 elements (Ag, Al, As, Ba, Cd, Co, Ca, Cr, Cu, Fe, Gr, Mn, Mo, Ni, Pb, Se, Sr, V, and Zn) For each sample, a fixed amount of algae (70-80 mg) or fauna (20-30 mg) was placed in a plastic tube containing 1 mL of nitric acid (HN O_3 67%). The powder sample was then mineralized for 3 h at 100 °C using an Environmental EXPRESS HotBlock® − 54. After mineralization, 5 mL of deionized water was added to each sample. The certified reference materials (DOLT-5, TORT-3) were also analyzed following the same process. The metal concentrations in all samples are expressed in $\mu g.g^{-1}$ (ppm) dry weight. A total of 115 samples were analyzed, for each sites CE (33 samples), BC (36 samples) and BT (46 samples).

Data analysis

A Principal Component Analysis (PCA) was performed with RStudio® and R 4.3.1 (R Core Team 2023 [21], using the following packages: FactoMiner (Husson et al., 2020), factoextra (Kassambara and Mundt, 2020), ggplot (Wickham et al., 2020) and corrplot (Wei et al., 2021). The aim was to identify MTE with the most structuring influence among the 19 elements (Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Sc, Se, Sr, Ti, V, Zr and Zn).

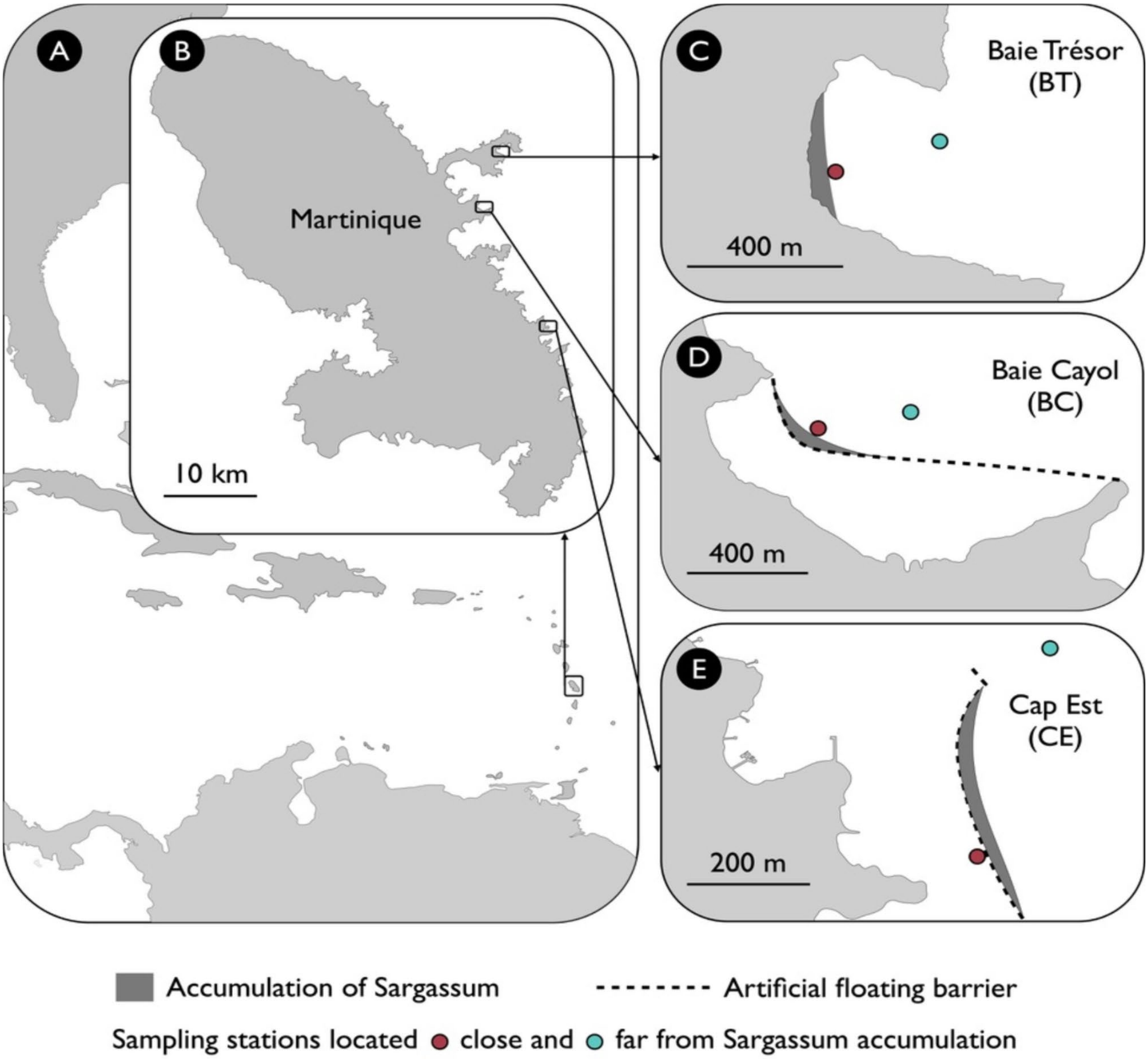

Fig. 1. Location of Martinique in the Caribbean Sea (**A**), location of sampling sites in Martinique (**B**): Baie Trésor (**C**), Baie Cayol (**D**) and Cap Est (**E**).

One-way analysis of variance ANOVA or AOV was used to compare the means of variables in more than two groups of function AOV[21,22].

Results

Principal component analysis

Out of 19 elements analyzed, a total fourteen elements (Al, As, Ca, Cd, Cr, Cu, Fe, Mn, Ni, Pb, S, V, Zn and Zr) were found to be the most abundant and were detected in all samples above the limit of detection (LOD) (Fig. 2). Eight metallic elements (Sr, Sb, Sc, Se, Mo, Na, Mg and K) were below the LOD and therefore were not considered in the analysis.

The first two dimensions of the PCA carried 59.16% (F1) and 16.17% (F2) of the total inertia (Fig. 2). F1 distinctly discriminated the variables Fe (16.20%), Al (15.93%), V (13.18%), Cu (12.59%) and Cr (12.28%) whereas and F2 discriminated As (35.48%), Mn (18.79%) and Ca (13.05%), Zn (13.98%) and S (12.31%).

Together, the first two axes of the PCA discriminated samples of *Dictyota* spp., were characterized by high concentrations in Fe, Al, V, Cu and Cr, and samples of *L. variegatus* were characterized by As, Mn, Zn, Ca and S from *T. testidinum* and *H. stipulacea*. The two phanerogam species showed a large overlap, indicated similar concentrations of As, Zn and S (Fig. 3).

The PCA did not discriminate samples based on the years (2021 and 2022) or the distance from accumulated *Sargassum* found in barriers (CE and CA) and the seashore (BT).

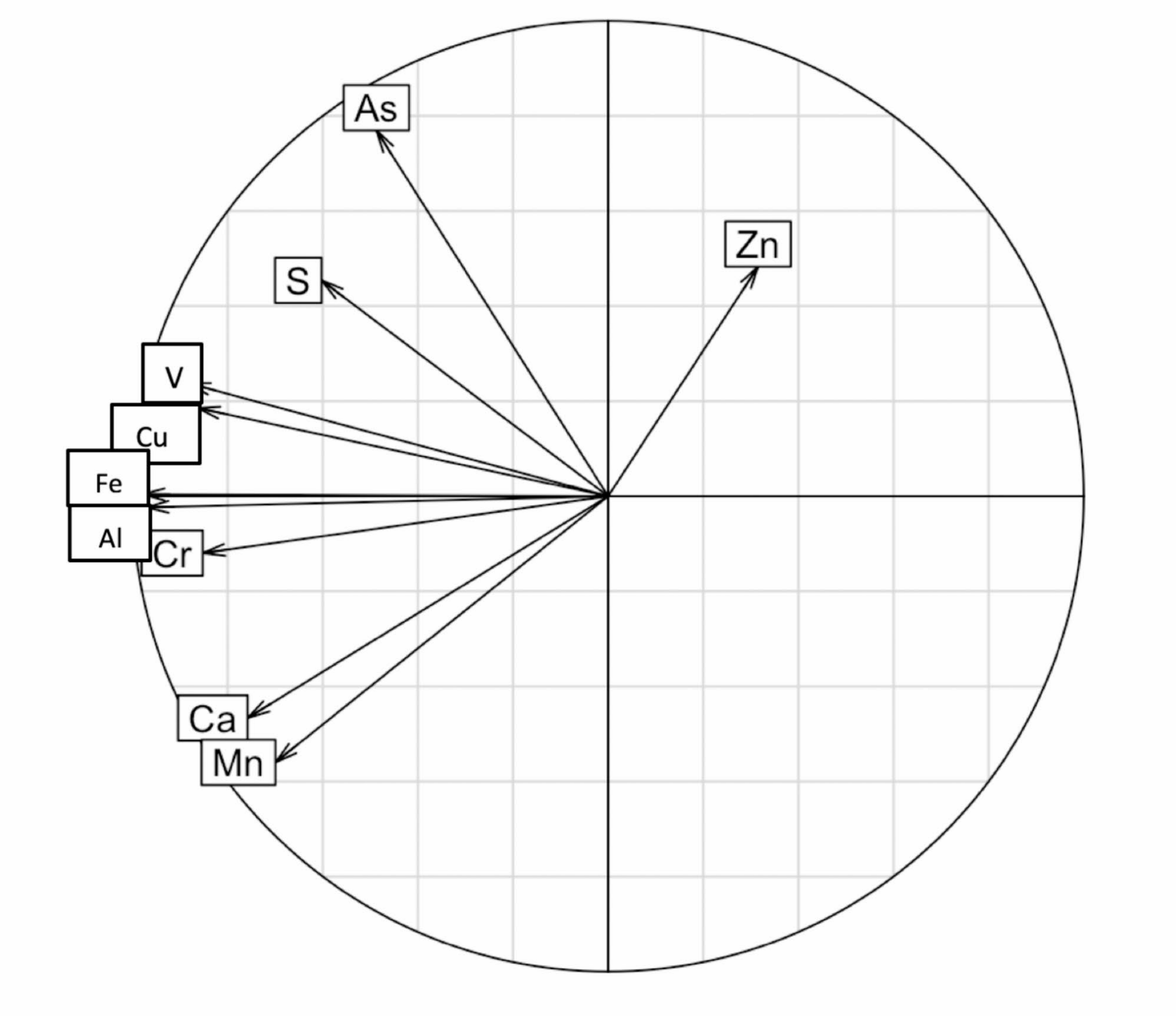

Fig. 2. Circle of correlation of Principal Component Analyses (PCA) showing variables F1 (59.16%) and F2 (16.17%) represents the relationship in organisms (*Dictyota dictyota*; *Thalassia stipulacea*; *Halophila filiforme* and Echninoderm (*Lytechinus variegatus*)) in the three sites (BT, CA and CE) between all the metallic elements (Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Sc, Se, Sr, Ti, V, Zr and Zn).

Concentrations of metals and metalloids

The MTE concentrations were higher in stations CE and BC as compared to BT (Supplementary Table 1), for all samples analyzed (Fig. 4).

Among the four studied organisms, the concentration of the metalloid As were higher in *Dictyota*. spp. presenting arsenic concentration significantly higher than in *T. testidinum*, *H. stipulacea* and *L. variegatus*. (Supplementary Table 2).

The ANOVA tests did not reveal any significant impact of the proximity to accumulated *Sargassum* on the MTE composition of the studied organisms (Fig. 5).

Discussion

The aim of the present study is to evaluate the potential transfer of MTE from *Sargassum* sp. to adjacent marine life. Three species were sampled at varying distances (0 and > 200 m) from the accumulation of *Sargassum* found in two artificial barriers and in a natural bay. These species were chosen as they were the only ones present at all sites.

Out of the 19 metallic trace elements analyzed, three elements (Al, Fe and As) stand out in the PCA analyses, showing higher contributions. The proximity to accumulated *Sargassum* did not lead to an increased concentration of MTE in phanerogams (*T. testidinum and H. stipulacea)* and sea urchin (*L. variegatus)*. Similarly, short-term experiments did not reveal any changes in MTE composition of *Dictyota* spp. when placed near *Sargassum* accumulations during four days.

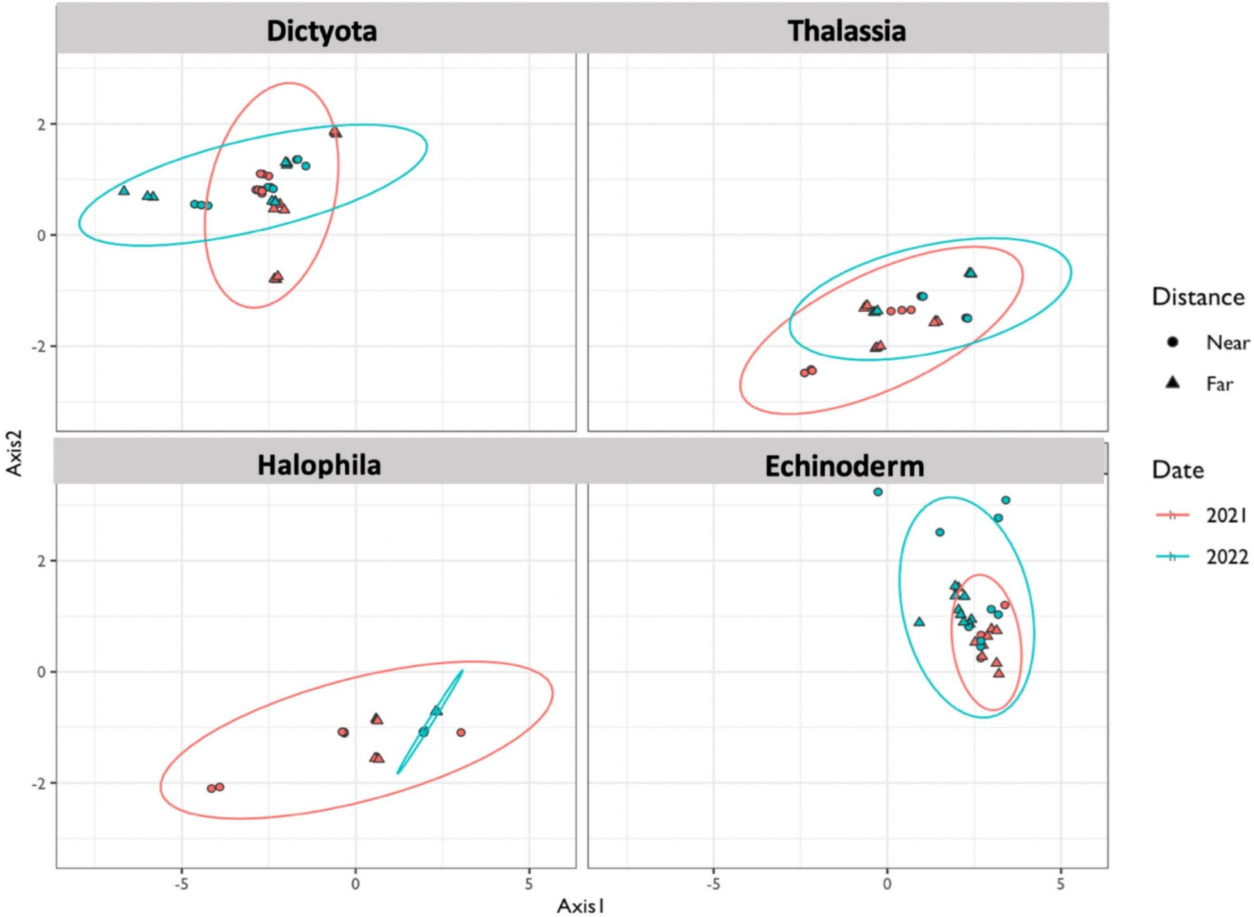

Fig. 3. Principal Component Analysis (PCA) for the four species: *Dictyota dictyota*; *Thalassia stipulacea*; *Halophila filiforme* and Echninoderm (*Lytechinus variegatus)* in the three sites (BT, CA and CE) between all the metallic elements (Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Sc, Se, Sr, Ti, V, Zr and Zn). Color represents different years in 2021 (red ellipse) and 2022 (blue ellipse) and shape represents distance from *Sargassum* accumulation near (circle) and far (triangle).

This lack of effect was observed irrespective of the location of *Sargassum* accumulation, whether it was close to seashore in a natural bay (BT) or in an artificial barrier (CE and CA). Samples with minimal influence from *Sargassum* were collected at a distance exceeding 200 m from the accumulations. These experiments are based on the assumption that the effects on MTE concentrations in other species would be more pronounced with closer the proximity to *Sargassum* accumulations. However, there may be a bias if this spatial scale is not appropriate and if effects occur on a larger scale, impacting control areas as well. During the field sampling, the water appeared brown in color due to leachates near the *Sargassum* accumulations, whereas this turbidity was not observed 10 m away from the *Sargassum*, suggesting a small-scale effect. The eutrophication associated with *Sargassum* accumulation stresses the environment at ecosystem scale and reduces biodiversity in coastal habitats[9]. In the present study, the diversity of benthic species available to evaluate metallic contamination was always higher away from *Sargassum*. Visual observations of water turbidity and diversity of benthic fauna reveals an impact more reduced 200 m away from *Sargassum* accumulation suggesting an adapted sampling scale.

The algae *Dictyota* present the ability to absorb rapidly metals elements like Cr (Chromium) 15 min after exposition (Nandhagopal et al. 2018) and experiments of the present study were conducted during 4 days based on those results. Integration of other metallic compounds can take longer and this potential bias due to experiment duration must be kept in mind.

Spatial transect studies[13,14] as well as temporal surveys[16] both revealed a constant higher concentration of As in pelagic *Sargassum* than in most coastal environment organisms. Once arrived at coast, *Sargassum* rapidly released As[16] and the present study is based on the assumption that proximity with accumulated *Sargassum* increase contamination risks. Sargassum were not sampled during this study as their turn over time is not known in barrier and their metallic concentration would be difficult to link with concentration released in environment.

Brown algae presents high amount of alginate in their cell walls, which gives them a strong affinity with MTEs[12,23,24]. As a result, brown algae are known to be more efficient than other algae and organisms in absorbing and accumulating metals and metalloids like arsenic[25]. This ability leads to a consistent and elevated presence

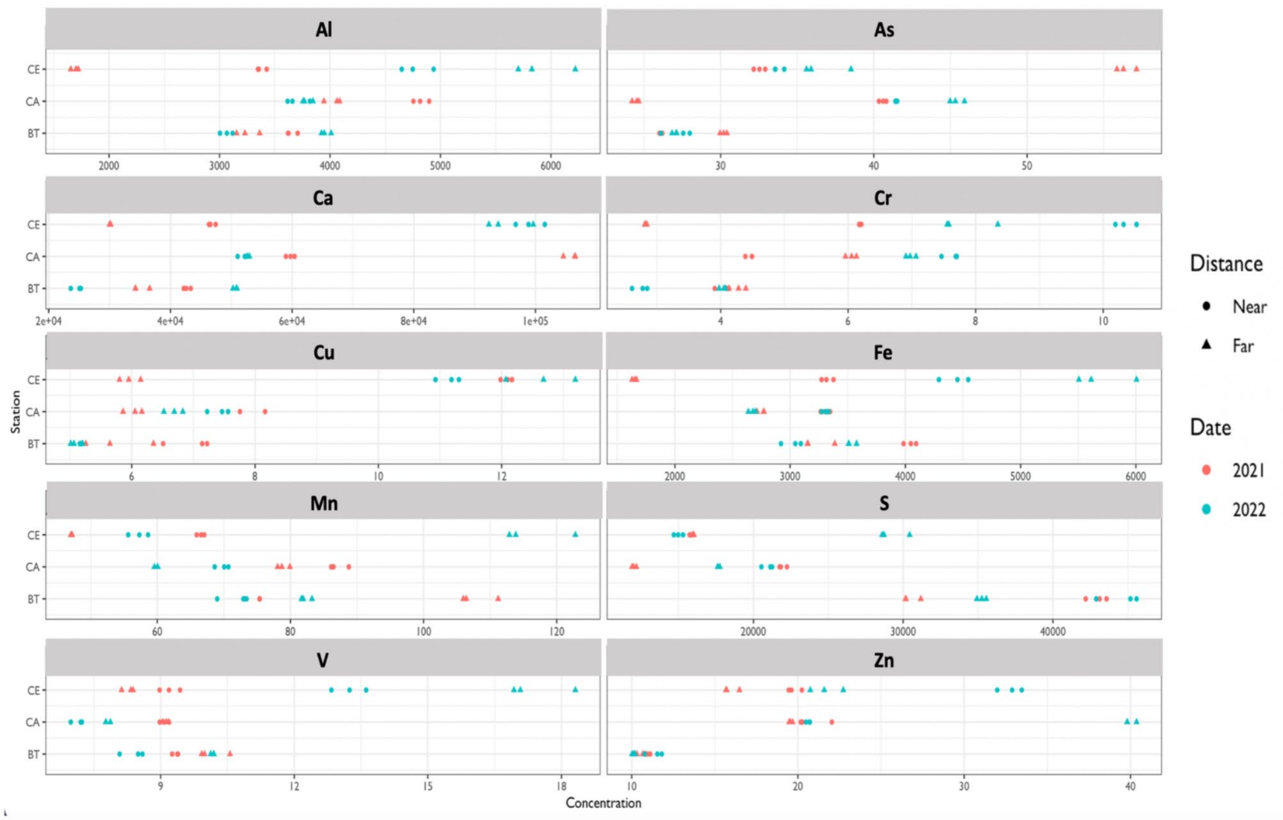

Fig. 4. Concentrations of metallic trace elements (Al, As, Ca, Cr, Cu, Fe, Mn, S, V and Zn) in ppm in: *Dictyota dictyota*; *Thalassia stipulacea*; *Halophila filiforme and Lytchinus variegatus* during 2021 (circle) and 2022 (triangle), near (green) and far (purple) from *Sargassum* accumulation in the three sites (BT, CA and CE).

of As in *Sargassum* over large spatial scales[13,14,26] and over time[16,17]. The genus *Dictyota* has also been found to absorb substantial amounts of metals in the Mediterranean Sea[27] and organochlorine molecules such as chlordecone in French West Indies[28].

Among the metallic trace elements studied, arsenic stands out in the PCA analysis with a higher contribution to the first principal component compared to others elements. Given the consistent presence of As element in *Sargassum*[13–15] this study primarily focuses on the As element. The concentrations of As measured during our campaigns falls within in the range of values regularly measured in macroalgae Chlorophytes (from 0.1 to 23 ppm), Rhodophytes (from 0.1 to 45 ppm) and Phaeophytes (from 1 to 179 ppm)[29].

Due to high level of As found in *Dictyota* spp., this species could serve as a reliable bioindicator with a high sensitivity, enabling the detection of slight changes in MTE concentrations in the environment.

The bioavailability of metals can be influenced by suspended or sedimented organic matter (OM) influencing the metals bioavailability due to its high ability to chelate metal elements[30]. Due to terrestrial runoff and local productions, coastal environments and particularly mangroves present significant amount of OM[31].

OM constitues an ubiquitous sorbent for Arsenic[32–34] with the ability to bind both As(V) and As(III) forming OM-As complexes due to various functionals groups such as sulfhydryl and amine[35,36]. A potential explanation of the absence of As increases in organism located close to *Sargassum* accumulation would be that the high amount of OM in coastal environments reduce As bioavailability.

Once stranded, *Sargassum* are releasing phosphorus in surrounding water[9]. Phosphate ion (PO_4^{3-}) and (AsO_4^{3}) are presenting chemical similarities and As is consequently entering in algal cells using P transportation systems[37]. High amount of P in the environment tend to decrease the amount of As entering in *Sargassum*[38,39]. Arrived in coastal waters *Sargassum* are simultaneously releasing As and P. The amount of As entering in adjacent organisms could be limited through competition with P explaining the reduced effect observed in *Dictyota* spp and phanerogams.

Even if As is entering in brown algae through P transportation systems, brown algae plant have set up a regulation mechanism in order to reduce the toxicity of As. The major part of the arsenate absorbed by the algae is transformed in arsenite As(III)[40–42] and then stocked in the brown algae in the form of nontoxic arsenosugars[29]. Experiments conducted with caged *Sargassum* suggest a rapid release of As[19]. However, the speciation of As released by *Sargassum* is not known and this form could be non-bioavailable explaining the absence of increased As in organisms adjacent to *Sargassum* accumulations.

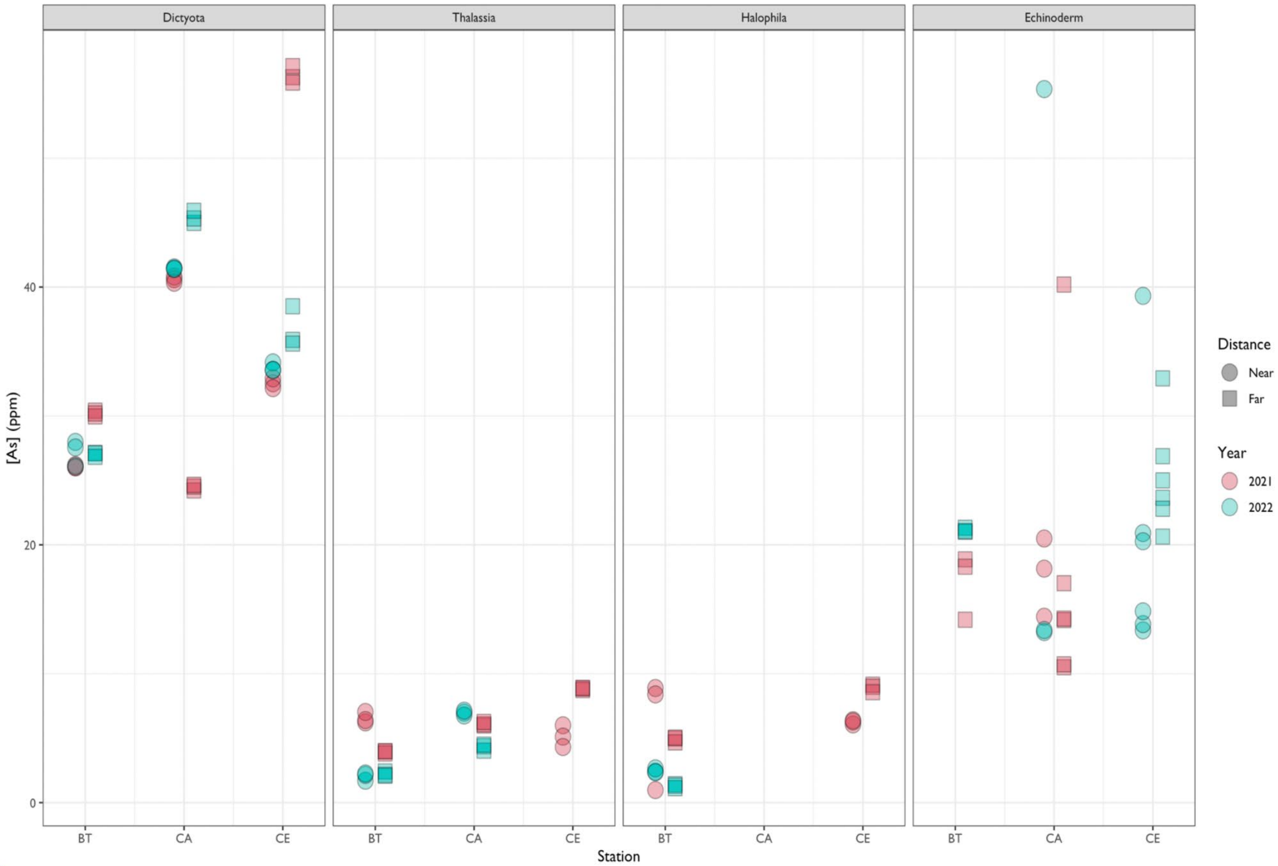

Fig. 5. Concentration (in ppm) of metal concentrations for the As. For the four species: *Dictyota dictyota*; *Thalassia stipulacea*; *Halophila filiforme* and *Lytechinus variegatus*, near (green points) and far (purple points) in the sampling station for the two years (2021 and 2022).

Conclusion

Artificial barriers represent a managing solution to limit the stranding of *Sargassum* and their negative impacts on environment, public health and economy. Sargassum accumulated in barriers can release their heavy metals in coastal environments. To evaluate this risk, two approaches were used in the present study: *i*) observation of metallic compositions of organisms according to distance from accumulated sargassum (during both periods of intense and limited sargassum stranding) and *ii*) temporal evolution of metallic contamination of algae *Dictyota* sp. placed closed to accumulated *Sargassum*. Despite potential bias of this study associated with spatial and temporal scale used, MTE concentrations of organisms were not changed according to their proximity with *Sargassum* accumulations. As a result, the use of barriers to manage *Sargassum* stranding would constitute a limited threat of MTE contamination of marine environment.

Data availability

All the relevant data are provided within the manuscript. The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Author contributions
O-A.C. conceived the research, collected the samples, identified Sargassum species, carried out sample preparation, acquired the data, performed data analyses, conducted the analytical study and interpretation, and wrote the first draft of the paper. P.C. funded the metal analyses and proofread the manuscript B.S-B. performed data analyses, conducted the analytical study and interpretation. P-Y.P. proofread the manuscript, collected the samples and data analyzes.

Declarations

Competing interests
The authors declare no competing interests.

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