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Temporal fluctuation of metallic trace elements concentrations in three morphotypes of floating holopelagic Sargassum from the Caribbean coast (Guadeloupe, French West Indies)

Océanne-Amaya Cipolloni, Patrice Couture, Sébastien Cordonnier,
Pierre-Yves Pascal

► **To cite this version:**

Océanne-Amaya Cipolloni, Patrice Couture, Sébastien Cordonnier, Pierre-Yves Pascal. Temporal fluctuation of metallic trace elements concentrations in three morphotypes of floating holopelagic Sargassum from the Caribbean coast (Guadeloupe, French West Indies). *Marine Pollution Bulletin*, 2024, 201, pp.116229. 10.1016/j.marpolbul.2024.116229 . hal-04578531

HAL Id: hal-04578531

<https://hal.science/hal-04578531>

Submitted on 17 May 2024

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1 **Title: Temporal fluctuation of metallic trace elements**
2 **concentrations in three morphotypes of floating holopelagic**
3 ***Sargassum* from the Caribbean coast (Guadeloupe, French West**
4 **Indies)**

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6
7 **Authors list :** Océanne-Amaya Cipolloni^{1,2*}, Patrice Couture³, Sébastien Cordonnier¹, Pierre-
8 Yves Pascal¹

9
10
11 **Affiliation :**

- 12
13 1. Université des Antilles, Équipe Biologie de la mangrove, Institut de Systématique,
14 Évolution, Biodiversité, ISYEB, UMR 7205, UFR SEN, Pointe-à-Pitre, France
15
16 2. Polytechnic University of Marche & National Biodiversity Future Centre, Dept. of Life
17 and Environmental Sciences, Italy
18
19 3. Centre Eau Terre Environnement, INRS-ETE, Québec, Canada
20
21

22
23 ***Corresponding author:** oceanne.cipolloni@gmail.com
24
25

26 **Abstract**

27 Since 2011, the Caribbean coasts have unprecedented stranding of a pelagic brown macroalgae
28 *Sargassum* inducing damages for coastal ecosystems and economy. This study evaluated the
29 temporal fluctuations of metallic trace elements (MTE) in *Sargassum* freshly arrived on the
30 Caribbean coast. From May 2020 to September 2021, 12 floating samples of three morphotypes
31 (*S. fluitans* III and *S. natans* I and VIII) were regularly collected in the Petit Cul-de-Sac Marin
32 (Guadeloupe, French West Indies). Measured concentrations of 28 metal(loid)s trace elements

33 reveal *i*) an absence of seasonal patterns in MTE concentrations except for metals Fe and Al
34 during 2020 summer *ii*) a regular and high As content during the entire survey *iii*) a similar
35 trend of contamination for each morphotype. The constant and high amount of As implies that
36 stranding management policy and valorization processes of *Sargassum* must consider As
37 contamination and that this vigilance must be constantly along the year.

38 **Keywords (5 words):** Survey, MTE, *Sargassum natans*, *Sargassum fluitans*, Arsenic

39

40 **1 Introduction**

41

42 *Sargassum* (class : Phaeophyceae, order: Fucales) is a group of brown marine algae including
43 more than 350 species (Guiry and Guiry, 2022). Among these genera, only two species are
44 strictly holopelagic: *Sargassum fluitans* and *Sargassum natans* (Dawes and Mathieson, 2008;
45 Parr, 1939; Stoner, 1983) distributed in the Caribbean Sea, at the edge of the Gulf of Mexico
46 and the Azores islands (Lapointe, 1995) including the western Atlantic tropical region and
47 South America from northeastern Brazil (Gower et al., 2013; Guiry et al., 2014; Lapointe,
48 1995).

49 Since 2011, Caribbean coasts are exposed to unprecedented massive stranding of sargassum
50 (Gower and King, 2011) and those floating algae are spotted in places never reported before,
51 such as northeastern Brazil (Széchy et al., 2012). Stranding of sargassum induce ecological
52 damages threatening endangered species such as turtle (Ross and Casazza, 2008; Witherington
53 et al., 2012) and can lead to the disappearance of coastal ecosystems (Gledhiir and Buck, 2012;
54 van Tussenbroek et al., 2017). The decomposition of sargassum accumulated in coastal
55 environments liberates hydrogen sulfide (H_2S) provoking human health issues such as
56 respiratory diseases, neurological problems, digestive and cardiovascular lesions (Resiere et al.,
57 2018). Economy is also affected by the stranding of sargassum with repercussions on tourism

58 and circulation of boats impacting marine trade and fisheries (Langin, 2018; Lapointe et al.,
59 2014).

60 Among those visible impacts, a more pernicious effect could be due to their capacity to absorb
61 metallic trace elements (MTE) from their environment (Volesky and Holan, 1995) notably due
62 to the alginates in their cell walls (Davis et al., 1999; Vieira and Volesky, 2000). *Sargassum*
63 spp. present a MTE profile similar to composition classically found in algae (Devault et al.,
64 2021; Neff, 1997). The metalloid arsenic is usually presenting concentrations above the limits
65 recommended for seaweed utilization in Europe (European Commission, 2019) and agriculture
66 soils in other countries (Rodríguez-Martínez et al., 2020). Experiments approach reveals that
67 As element can be rapidly and highly incorporated by *Sargassum* (Devault et al., 2020; Neff,
68 1997; Penrose and Woolson, 1974). In pelagic environments, total As concentrations of
69 *Sargassum* naturally fluctuate between 80 and 150 ppm (Cipolloni et al., 2022; Dassié et al.,
70 2021; Rodríguez-Martínez et al., 2020). Several studies reveal higher concentrations in
71 *Sargassum* close to the coast than off shore (Fernandez et al., 2007; Rodríguez-Martínez et al.,
72 2020) however this trend is not always observed (Cipolloni et al., 2022; Dassié et al., 2021).

73 Geographical origins of Caribbean *Sargassum* vary along the year (Alleyne et al., 2023) and
74 the discrepancies between studies can be due to different As availability in the environment
75 according to i) different geographical origins and ii) periods of the year (Phillips, 1977). In
76 addition to metal availability in algal environment, their metals absorption is linked with the
77 physiological status of algae, with a higher integration during the growth stage (Dixon, 1996).

78 Total abundance of *Sargassum* and respective abundance of each morphotype are changing
79 along the year suggesting seasonality in growing periods and temporal fluctuations in
80 physiological status of pelagic *Sargassum* at different periods of the year (García-Sánchez et
81 al., 2020).

82 Such temporal variability was previously observed as a survey of stranded *Sargassum* on the
83 Mexican coast revealed higher As concentrations during the rainy season (Magaña-Gallegos et
84 al., 2023; Ortega-Flores et al., 2022). However, such seasonal fluctuations can vary
85 geographically, justifying studies in other locations (Rodríguez-Martínez et al., 2020). The aim
86 of the present study is to evaluate temporal variations of MTE concentrations in three different
87 morphotypes of holopelagic *Sargassum* freshly arrived in coastal waters of Guadeloupe (French
88 West Indies) during a period of 16 months.

89
90

91 **2 Materials and methods**

92

93 **2.1 Study sites**

94

95 The sampling was realized in Guadeloupe (French West Indies) in the Petit Cul-de-Sac Marin
96 (PCSM) (16°13'15N - 61°32'07E) (Fig. 1). Pelagic *Sargassum* are naturally transported by
97 oceanic currents and wind from the Southeast of the Atlantic Ocean toward the North along the
98 Caribbean Islands to the Sargasso Sea and the Gulf of Mexico. *Sargassum* are moving rapidly
99 with a minimum, average and maximum speeds respectively estimated 0.081, 0.32 and 0.46
100 $m \cdot s^{-1}$ (van Sebille et al., 2021). In Guadeloupe *Sargassum* entering in PCSM are rapidly
101 directed to the coast where they strand massively.

102

103

104 **2.2 Land sample collection**

105

106 Algae were sampled at sea every month, between 500m (before their stranding) and less than 5
107 km (Fig. 1). This monthly survey lasted from May 2020 to September 2021. Timing of sampling

108 was chosen *i)* in order to be regularly spaced in time and *ii)* according to *Sargassum* availability
109 and weather conditions.

110 Approximately 4kg of *Sargassum* were manually collected in triplicates from a boat using a
111 landing net with a mesh of 1 x 1 mm. Less than two hours after their collection, morphotypes
112 were separated (*S. fluitans* III, *S. natans* I and *S. natans* VIII), rinsed with fresh water and oven-
113 dried at a temperature of 50°C during 48h.

114

115

116 **2.3 Sample preparation**

117

118 For each sample, 80 g of dried algae were ground and homogenized using a vibro-grinder with
119 10 mm zirconium balls of 10 mm for three minutes with a frequency of 30 beat/s (Retsch® MM
120 400).

121

122 **2.4 Laboratory analysis**

123

124 ***Metal analysis***

125

126 For each sample, 28 elements (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni,
127 P, Pb, S, Sb, Sc, Se, Si, Sr, Ti, V, Zn and Zr) were analyzed using an Inductively Coupled
128 Plasma Optical Emission Spectrometer (Spectrometer ICP-OES 700®, Agilent Technologies).

129 A fixed amount of algal powder (70-80mg) was placed in a plastic tube and 1 mL of nitric acid
130 (HNO₃ 67%) was added. The powdered sample was then mineralized for 4 h at 100°C
131 (Environmental – EXPRESS HotBlock® - 54). After mineralization, 5 mL of deionized water
132 was added to each sample. With the same process, we analyzed the certified reference materials
133 (DOLT-5, TORT-3). All element concentrations in samples of *Sargassum* are expressed in
134 ug.g⁻¹ (ppm) dry weight.

135

2.5 Data analysis

136
137

138 The variance and the homogeneity of metals and metalloid concentration values were verified
139 by the Shapiro's and Level's tests (with significance at the 95 % confidence level). The
140 difference between means concentrations in morphotypes per station and in all stations were
141 tested using the non-parametric test Kruskal-Wallis test.

142 We tested different dependent factors, i.e. years, months and species on the dependent data.

143 We transformed the data, in order to satisfy the criteria of normality and homoscedasticity for
144 parametric tests (Underwood, 1999). Data were tested for homogeneity of variance with the

145 Cochran's test at the 0.05 significance level. Next, we applied a two-ways ANOVA, which
146 were used to evaluate the effect of two groups variable on a response of one variable. The

147 two-ways ANOVA were performed on years, months and species content variables, using the
148 metallic elements as factors (Table 2). Methods of multivariate statistics with Principal

149 Component Analyses (PCA) were executed on RStudio® and RCran, using the following
150 packages: FactoMiner (Husson et al., 2020), factoextra (Kassambara and Mundt, 2020), ggplot

151 (Wickham et al., 2020) and corrplot (Wei et al., 2021) to select the metallic elements with
152 higher influence in data structuration among the 28 elements. Metallic elements below the limit

153 of detection (LOD), for at least one sample, were not considered (B, Ba, Co, K, Mg, Mo, Sb,
154 Sc, Pb, Se, Sr, Ti and Zr).

155

3 Results

156
157

3.1 Metals concentrations

158
159

160 Among the 28 elements analyzed, fourteen elements (Al, As, Ca, Cd, Cu, Fe, K, Mg, Mn, Ni,
161 P, Sc, V and Zn) were the most abundant and were detected in all samples above the limit of

162 detection (LOD). Fourteen metallic elements were below the LOD and were not considered (B,
163 Ba, Co, Cr, Mo, Na, Pb, S, Sb, Se, Si, Sr, Ti and Zr).

164 The metallic elements analyzed (Al, As, Ba, Ca, Cd, Fe, K, Mg, Mn, Na, P, Sc, Si, V, Zn and
165 Zr) varied significantly with the time (year and months) and species ($p < 0.005$, Table 2),
166 demonstrating that metallic traces elements vary according to the time and species.

167 A three-ways ANOVA were performed on Al, As, Fe, Cu and Zn variables, using year, months
168 and species as factors. The relationship between metallic trace elements (Al, As, Fe, Cu and
169 Zn) and the two species (*Sargassum natans* and *fluitans*), year and months were tested with
170 analysis of variance (ANOVA), using parameters of variance to detect possible effects of years
171 months and species. In our study when a significant interaction was revealed between year,
172 months, species and metallic trace elements, an ANOVA three-ways was performed within
173 metallic trace element (Al, As, Fe, Cu and Zn) (only *p-values* are presented in the study).

174 The Al, As, Fe, Cu and Zn of the *Sargassum* varied significantly with year, month and species
175 ($p < 0.05$, Table 2). For the elements As and Cu there was a significant interaction between the
176 factor month and species (Table 2), demonstrating the difference in As and Cu over months
177 depended on species.

178

179

180 **3.2 Principal Component Analysis (PCA)**

181

182 The PCA represented the relationship in *Sargassum* among the metallic elements studied (Al,
183 As, Fe, Sc, Zn, Mn, Ni, V, K, Cu, Ca, Mg and K). The first two dimensions of PCA represents
184 respectively 38.83% (F1) and 13.83% (F2) of the total variance (Fig 2A). F1 distinctly
185 discriminates the variables Fe (15.85%), Al (14.10%), Cu (10.98%), Ca (11.74%) and Zn
186 (8.71%), whereas and F2 discriminates Al (13.24%), Mn (11.41%) and Fe (10.35%) (Fig 2).

187 The PCA clearly discriminates samples from the month of July in 2020, characterized by high
188 concentrations in Al, Fe and Zn (Fig 3). Others months present similar composition in metallic
189 trace element (MTE). MTE concentration fluctuates according to years and months but they
190 followed similar trend in the three morphotypes during all the survey (Fig 4).

191

192 **3.3 Metals and metalloids content**

193

194 The five metallic elements standing out of the PCA analysis (Al, As, Fe, Cu and Zn) were
195 chosen to graphically present their fluctuations over time (Fig 4). Al and Fe presented a
196 significant increase from June to August 2020 (not observed during same months in the
197 following year), while the metalloid As and metals Zn and Cu were constant along the 16
198 months of the temporal monitoring (Fig 3). This trend was similar for each of the three
199 morphotypes.

200

201 **4 Discussion**

202

203 The present study is a survey of the contamination by metallic trace elements (MTE) of each
204 morphotype of holopelagic *Sargassum* (*S. fluitans* III and *S. natans* I and *S. natans* VIII) in
205 coastal Caribbean (Guadeloupe - FWI). This study revealed *i*) an absence of MTE seasonality,
206 that is a seasonal fluctuation, except for the Al and the Fe during 2020, *ii*) a regular and high
207 As content during the entire survey; and *ii*) a similar trend of contamination for each
208 morphotype.

209

210 **Limitation of the study**

211

212 In the present study, each tissue of the macroalgae thalli (leaves, stipes, bladders) were not
213 separately analyzed. Whole *Sargassum* were dried and homogenized through grinding of all
214 tissues together potentially masking specificities of metals composition of each algal tissue

215 (Sadeghi et al., 2014). Our field observations revealed the punctual existence of raft composed
216 principally by *Sargassum* bladders may be implying potential temporal variations of bladders
217 contribution to *Sargassum* biomass. Such variation could represent a bias in our analysis of
218 whole *Sargassum*. However, those fluctuation of bladders abundance could also due to algal
219 mortality as after *Sargassum* death of the *Sargassum* the stipes and fronds tend to sink, leaving
220 behind drifting masses of sole pneumatocysts (Ortega-Flores et al., 2023).

221 The present survey was dependent on the field availability of *Sargassum*. Along the two years,
222 were absent during similar periods (February, March, October and November) highlighting the
223 annual cycle of massive arrival stranding of *Sargassum*. *Sargassum* aggregated in Inter-
224 Tropical convergence Zone (ITCZ), between February and September, and are dissipated
225 between November and January (Franks et al., 2016; Johns et al., 2020; Putman et al., 2018)
226 and similar abundance fluctuations were observed during the present survey in Guadeloupe
227 explaining observed *Sargassum* abundance in Guadeloupe. This unavoidable lack of samples
228 during some periods of the year must be kept in mind in interpreting results.

229

230 **Temporal evolution of MTE**

231 Present temporal monitoring reveals an absence of seasonal patterns in MTE concentrations
232 except higher abundances of Al and Fe during 2020 summer.

233 The desert Sahara is one of the largest hot deserts on earth producing more dust sand than all
234 others deserts cumulated (Prospero et al., 2021), and is one of the main sources of dust on the
235 planet along with the Sahel (D’Almeida, 1985; Schutz et al., 1981). Each year, 400-700 mega
236 tons of dust sand are suspended in the atmosphere (Goudie and Middleton, 2001; Schutz et al.,
237 1981; Swap et al., 1992) and migrates from Africa across the Atlantic Ocean with higher
238 abundances during summer than winter (Gilbert and Sabogal, 2020; Ginoux et al., 2012). This
239 lithometeor phenomena is impacting areas from Florida to Greater and lesser Antilles until

240 French Guiana (Pastore et al., 2021). Dust from Sahara are notably containing N, P and Fe and
241 can increase ocean primary production in low nutrients oceanic regions (Blain et al., 2007;
242 Boyd et al., 2007; de Baar et al., 2005). In the Sargasso sea, similar MTE composition between
243 atmosphere and surface water suggested an important contribution of aeolian sources for Al, Fe
244 and Mn in water (Atkinson and Stefánsson, 1969). Similarly, elements (Al, Ca, K, Mg, Fe, Na,
245 P, S and Zn) from atmospheric deposits collected from 2015 to 2018 in Guadeloupe reveal
246 important fluxes due to Saharan dust with maximum inputs between June and July 2015 until
247 2018 (Xu-Yang et al., 2022). In the present study, higher concentrations of Al and Fe observed
248 in all morphotypes of *Sargassum* from June to August 2020 can potentially be linked with
249 higher inputs of those elements during Saharan dust events. Those peaks coincides with a major
250 Saharan dust flow into the Caribbean region (Xu-Yang et al., 2022).

251 Along the year, *Sargassum* arriving near coasts of lesser Antilles present different origins with
252 algae traveling directly from Africa along 15°N whereas others have a more southern origin
253 and travel along the coast of South America (10°N) (Alleyne et al., 2023). Dust from Sahara is
254 blown to the west depending on the position of the seasonal position on the Intertropical
255 Convergence Zone (ITCZ) (Engelstaedter and Washington, 2007). The ITCZ acts as an
256 effective barrier stopping dust movements into South Atlantic (Huang et al., 2010), and
257 resulting in different biogeochemical provinces of the North and South tropical Atlantic ocean
258 (Schlosser et al., 2014), by changing MTE composition of water during some periods of the
259 year (Álvarez-Salgado et al., 2007, 1999). However, the hypothesis of a large spatial scale
260 origin of *Sargassum* explaining their different MTE composition of *Sargassum* found in
261 Guadeloupe can be reasonably dismissed. One year is necessary for algae to travel from coasts
262 of Africa (15°N) or South America (more Southern than 10°N) (Alleyne et al., 2023). During
263 their long journey to Guadeloupe, *Sargassum* are reproducing vegetatively (Dawes and

264 Mathieson, 2008) and this turnover of biomass should mask or decrease MTE specificities
265 according to *Sargassum* origin.

266 Indeed, algae arriving in summer 2020 in lesser Antilles would come from South America
267 (Alleyne et al., 2023) and should be consequently less influenced by dust with lower Al and Fe
268 composition. In the present study, higher Al and Fe *Sargassum* composition observed during
269 this period suggest that geographical origin of *Sargassum* would have a limited influence.

270 Moreover, a contamination due to coastal proximity remains unlikely as transects from off
271 shore to coast did not revealed modification of element trace metallic composition in *Sargassum*
272 (Cipolloni et al., 2022; Dassié et al., 2021).

273 Arsenic is more abundant in marine waters than fresh waters (Campbell, 1995) and constitute
274 one of the most and widespread metalloids elements (Fattorini et al., 2006; Kevin Henke, 2002)
275 with concentrations in oceanic waters around 1.7 $\mu\text{L/L}$ (Cutter, 1993; Hutchinson and Meema,
276 1987). This total As at the surface water of ocean has several origins *i*) drainage from land *ii*)
277 upwellings from anoxic bottom water of the deep ocean and *iii*) deposit from atmosphere
278 (45,000 metric tons per year) *iv*) geoactivity (volcanos, hydrothermals) (Cutter and Cutter,
279 1995; Waslenchuk, 1978). In the present study, the mean of the metalloid concentration of As
280 was 92.68 ppm in all samples of algae with a maximum value of 118 ppm. Those values are in
281 the range of values previously observed in the *Sargassum* of the Caribbean area with As level
282 fluctuating between 80 and 150 ppm in coastal waters (Dawczynski et al., 2007; García-Sartal,
283 2012; Rodríguez-Martínez et al., 2020), and in the open ocean between 0.1 and 382 ppm (Dassié
284 et al., 2021).

285 The speciation of the As and the chemical form (oxidation and valence state) define the toxicity
286 of the As and it's bioavailability (Dixon, 1996). The total As (tAs) is composed of inorganic

287 As (iAs) and less toxic organic As (oAs) (Andreae and Klumpp, 1979; Howard et al., 1995;
288 Sanders and Windom, 1980) . In the inorganic compounds composed of the arsenite As(III) and
289 arsenate As(V), with the As(III) more toxic than the As(V). Primary producers can present
290 arsenate As(V) tolerance (Knauer et al., 1999). In the brown macroalgae, the As is accumulated
291 in arsenate As(V) form and transformed into less toxic arsenite As(III) (Neff, 1997). The
292 present study reveals a constant and high abundance of the As, in accordance if capacity of
293 marine algae to present As concentration usually between 1 000 and 10 000 times higher than
294 the As concentration in the waters (Borak and Hosgood, 2007).

295

296 **The three genotypes: *S. fluitans* III; *S. natans* (I and VIII)**

297 During the entire survey, evolutions of metal concentrations of algal tissue were significantly
298 similar in the three morphotypes studied. The morphotype *S. fluitans* III present higher
299 concentration of Al, Fe and Cu. Higher concentration in metallic element were previously
300 observed in the two other morphotypes (Ortega-Flores et al., 2022) and in *S. natans* I (Cipolloni
301 et al., 2023). The fixation and absorption of MTE elements is depending of the presence of
302 polysaccharides (alginates and fucoidans) in *Sargassum* algae (Camacho and Hernández-
303 Carmona, 2012; Gobert et al., 2022; Robledo et al., 2021). During the periods of June to
304 September in *S. fluitans* III, some studies showed the abundance of polysaccharides (alginates
305 and fucoidans) and functional groups (uronic acids and sulfate) are higher than during the
306 winter season (Dibner et al., 2022; Ortega-Flores et al., 2022). Such variations in
307 polysaccharide content according to morphotype can explain the specificity of *S. fluitans* III
308 observed the in present study.

309

310 **Management strategies**

311 During the present survey, Al and Fe presented higher concentrations during summer of 2020
312 whereas concentrations of another MTEs remained more stable. As is one of the most
313 commonly toxic metallic metalloid present in the marine environment (Fattorini et al., 2006).
314 In the present study, As concentrations are in the range of abundance previously observed in
315 *Sargassum* from coast (Cipolloni et al., 2023; Rodríguez-Martínez et al., 2020) and off-shore
316 environments (Cipolloni et al., 2022; Dassié et al., 2021). Those concentrations of As remains
317 above the limits accepted by the European norms of marine biology metallic elements (NFU
318 44-051- ISSN 0335-3931) (European Commission, 2019). Our study reveals that independently
319 of month, year and morphotype, *Sargassum* always present high concentrations of the toxic
320 metalloid's arsenic. This characteristic must be considered in the management of *Sargassum*
321 stranding and valorization in order to limit environmental and public health issues.

322 All uses in the agriculture (feed supplement, soil amendments, crop protection, growth
323 substrate), cosmetics, human and cattle food and beverage should be avoided whereas other
324 uses of *Sargassum* with more limited risks regarding As like bioenergy (bioethanol, biodiesel,
325 biogas (biomethane)), bioplastics and constructions materials (Desrochers et al., 2020; Robledo
326 et al., 2021) present more limited risks regarding As and should be favored.

327

328

329 **Author Contributions**

330

331 O-A.C. conceived the research, collected the samples, identified *Sargassum* species, carried out
332 sample preparation, acquired the data, performed data analyses, conducted the analytical study
333 and interpretation, and wrote the first draft of the paper. P.C. funded the analysis research. S.C.
334 collected the samples and carried out some sample preparation. P-Y.P. proofread the
335 manuscript, collected the samples and data analyzes.

336

337

338 **Acknowledgments**

339

340 We thank Brigitta I. Van-Tussenbroek to proofread the manuscript and the correction. We thank
341 the INRS – Institute of National Scientific Research in Québec (Canada) to realize the analyzes
342 of metallic elements. OC was partly supported financially by the institution of Guadeloupe
343 Region.

344

345

346 **Competing interest**

347 The authors declare no competing interest

348

349

350 **Availability of data and materials**

351 All the relevant data are within the paper

352

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615

A)



B

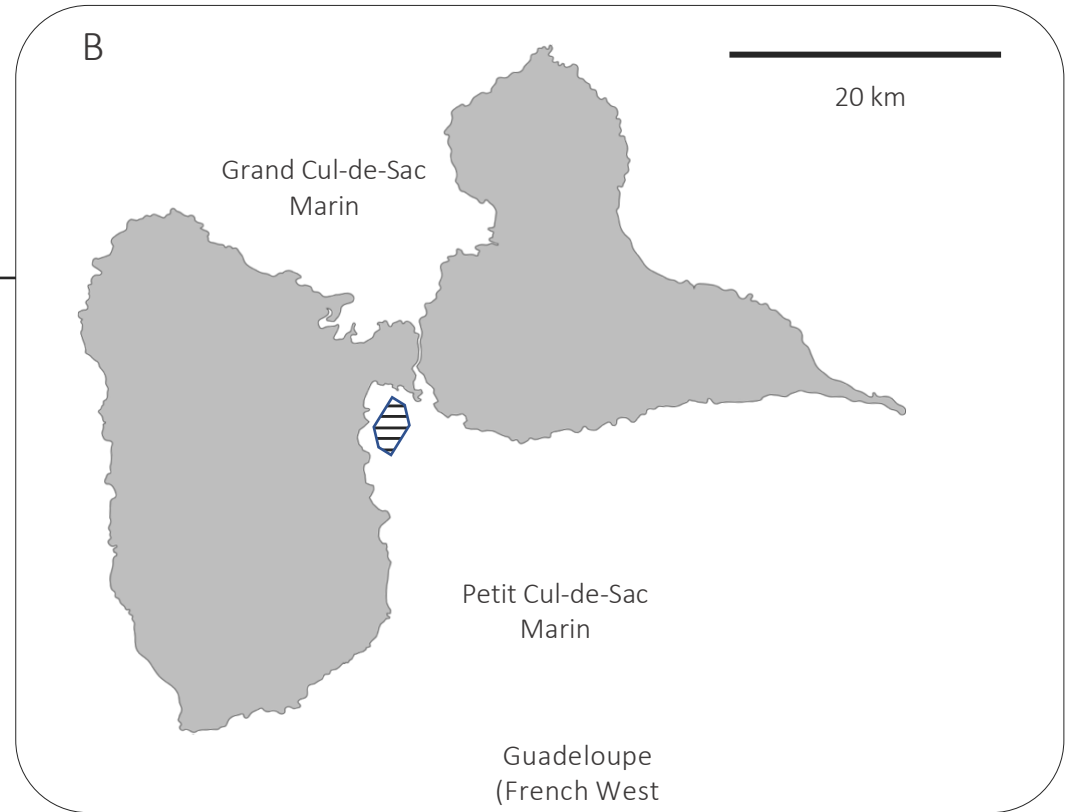


Figure 1 A) Location of Guadeloupe in the Caribbean Island in the Caribbean Sea in the Lesser Antilles **B) Location** of the sampling area with hatch polygon

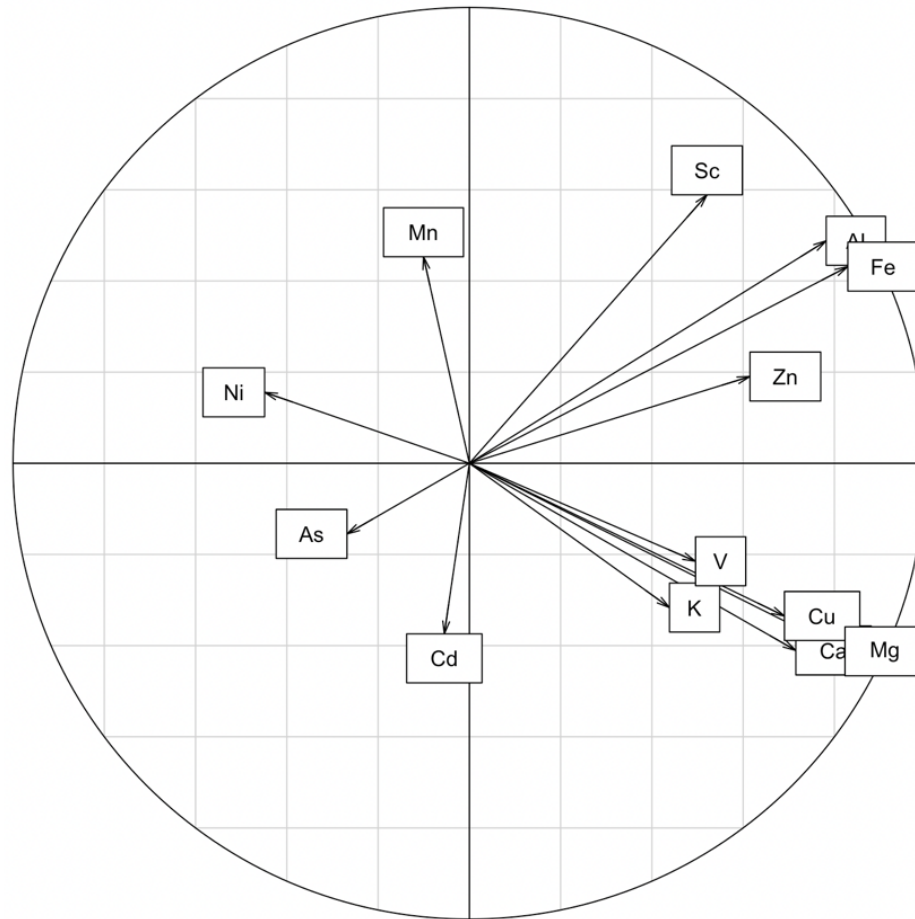


Fig 2. Circle of correlation of Principal Component Analyses (PCA) using observations (3 morphotypes and 7 months) and 13 variables (Al; As; Fe; Sc; Zn; Mn; Ni; V; K; Cu; Ca; Mg; K) with F1 (33.38%) and F2 (13.83%) represents the relationship in *Sargassum* sp. among the metallic elements.

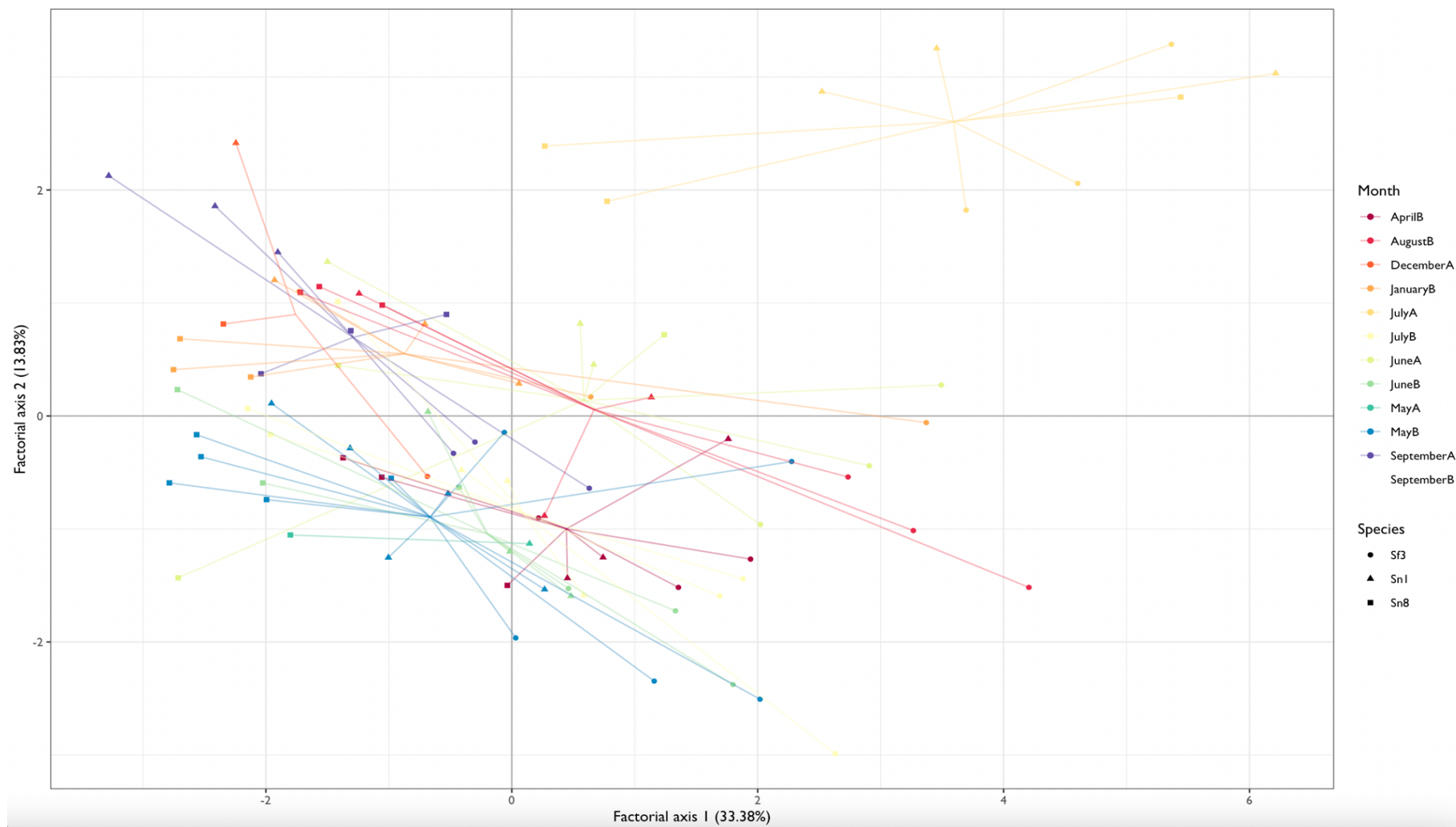


Fig 3. Principal Component Analyses (PCA) using observations (3 morphotypes and 7 months) and 13 variables (Al; As; Fe; Sc; Zn; Mn; Ni; V; K; Cu; Ca; Mg; K) with F1 (33.38%) and F2 (13.83%) presenting observations with different symbol shape (morphotype) and color (months), with the letter A representing 2020 and B representing 2021.

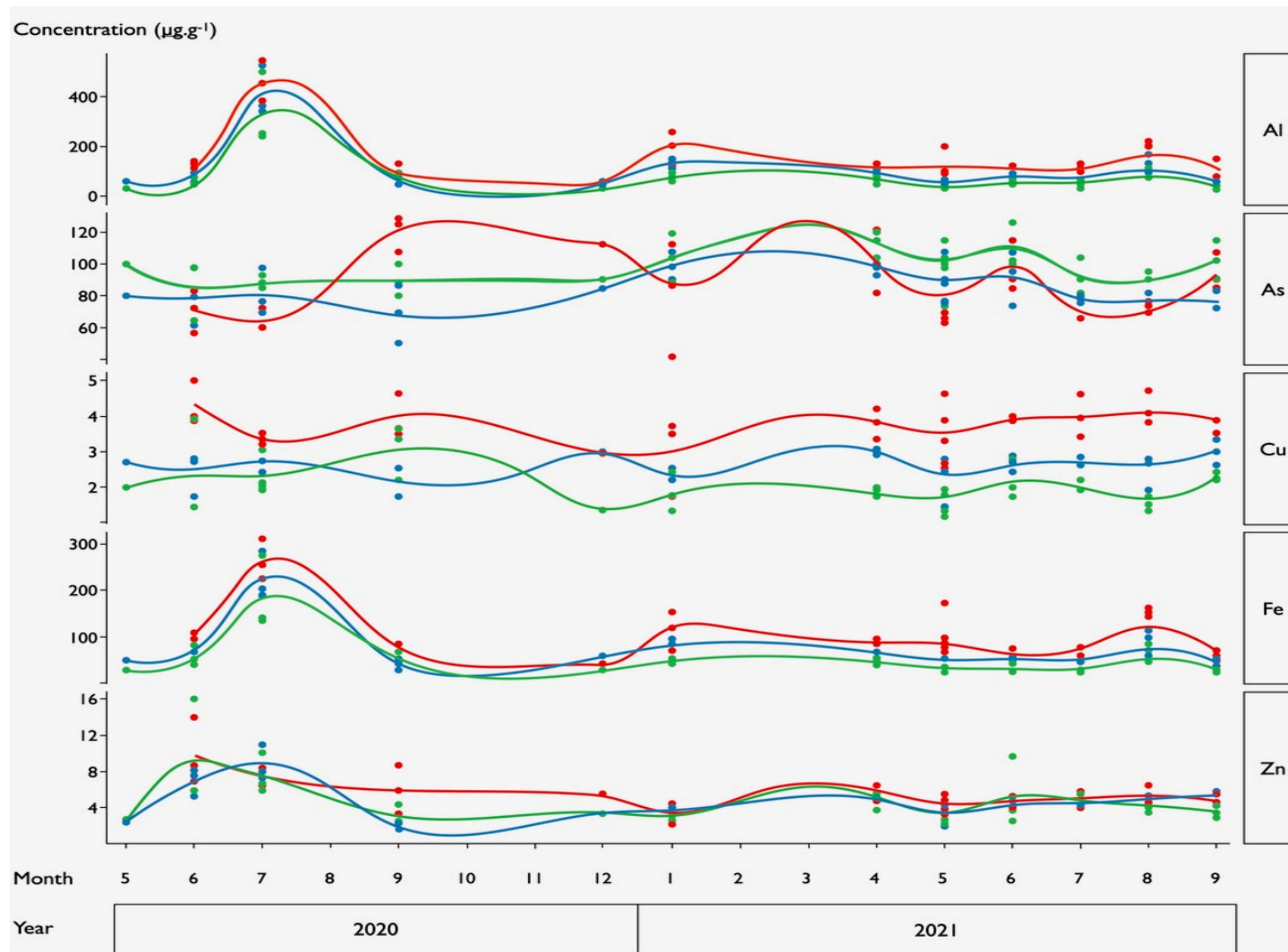


Fig 4 Temporal variability of metal concentrations. Concentrations in Al, As, Cu, Fe and Zn ($\mu\text{g}\cdot\text{g}^{-1}$) in morphotypes *S. fluitans* III (red); *S. natans* I (blue) and *S. natans* VIII (green) during different months in 2020 and 2021.

		<i>Al</i>	<i>As</i>	<i>Fe</i>	<i>Cu</i>	<i>Zn</i>
2020	<i>Sf3</i>	197.96±88.96	88.96±27.34	125.35±73.79	3.63±0.73	6.22±2.90
	<i>Sn1</i>	145.22±134.11	84.60±15.25	93.72±66.83	2.53±0.52	4.94±2.65
	<i>Sn8</i>	109.89±119.50	95.97±14.11	72.23±61.81	2.22±0.78	5.2±3.39
Mean		151.03	89.84	97.10	2.79	5.47
2021	<i>Sf3</i>	119.94±49.85	79.31±14.82	83.87±38.50	3.79±0.60	4.69±0.90
	<i>Sn1</i>	145.22±134.11	145.22±11.43	56.16±20.70	56.16±0.51	4.38±1.04
	<i>Sn8</i>	53.52±26.03	53.52±13.82	38.54 ±16.86	1.86±9.45	4.04±1.74
Mean		106.23	92.68	59.53	20.60	4.37
TORT-3 recovery rate (%SD)		53.22±0.01	77.5 ±0.01	78.24±0.04	77.29±0.04	75.8±0.03
DOLT-5 recovery rate (%SD)		189.17 ±0.01	81.03±0.01	83.05±0.22	81.26±0.01	81.35±0.023

Table 1. Elements concentration (ppm = $\mu\text{g} \cdot \text{g}^{-1}$) of 112 samples of *Sargassum* spp. collected from May 2020 to September 2021 in Guadeloupe (French West Indies) with their respective standard error (standard deviation divided by the squared root of the number of data), and the average values in bold. Recovery rates obtained from the analyses of certified reference material (TORT-3, DOLT-5) are presented for each element.

Variables	Statistical test	Fixed factors						
			<i>Years</i>	<i>Months</i>	<i>Species</i>	<i>Month: Species</i>	<i>Year: Species</i>	<i>Year: Month</i>
Al	Three-ways ANOVA	<i>df</i>	1	7	2	14	2	3
		<i>F</i>	21.8	13.12	9.4	0.62	1.3	58.1
		<i>p</i>	$p < 0.001$	$p < 0.001$	$p < 0.001$	0.83	0.26	$p < 2e-16$
As	Three-ways ANOVA	<i>df</i>	1	7	2	14	2	3
		<i>F</i>	1.2	4.3	10.7	2.5	0.5	2.5
		<i>p</i>	0.33	$p < 0.01$	$p < 0.01$	$p < 0.004$	0.55	0.06
Fe	Three-ways ANOVA	<i>df</i>	1	7	2	14	2	3
		<i>F</i>	52.6	30.6	40.1	1.02	2.01	41.5
		<i>p</i>	$p < 0.001$	$p < 0.001$	$p < 0.001$	0.44	0.1	$p < 1.31e-15$
Cu	Three-ways ANOVA	<i>df</i>	1	7	2	14	2	3
		<i>F</i>	0.182	3.471	73.6	1.2	1.6	0.3
		<i>p</i>	0.67	$p < 0.01$	$p < 0.001$	$p < 0.28$	$p < 0.19$	$p < 0.7$
Zn	Three-ways ANOVA	<i>df</i>	1	7	2	14	2	3
		<i>F</i>	10.3	8.2	2.6	0.5	1.8	6.6
		<i>p</i>	$p < 0.01$	$p < 0.001$	0.07	0.88	0.16	$p < 0.0004$

Table 2. Analyses two-way ANOVA test and the significant result of the p-values ($0,005 < p\text{-value}$) with the significant codes) (***,0.001), (**, 0.01), (*, 0.1).