Title: First assessment of Atlantic open ocean *Sargassum* spp. metal and metalloid concentrations

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Abstract (150 words)

Over the last decade, increasing proliferations of Atlantic *Sargassum* populations have led to massive beaching with disastrous environmental consequences. This study is a preliminary assessment of open ocean Sargassum spp. element concentration to assess their potential contribution on coastal ecosystems. *Sargassum* spp. samples from seven sites, collected along a transect from the center of the Atlantic Ocean to near the coast of Martinique (French West Indies), were analyzed to determine their potential metal and metalloid contamination. Mean element concentrations from the *Sargassum* spp. samples were ranked in the following descending order: As > Fe > Mn > Al > Zn > V > Ni > Cu > Cr > Cd > Hg. Element concentrations are relatively low compared to previous results of beached *Sargassum* spp. except for As that need to be carefully considered before reusing *Sargassum* spp.

Keywords (6max):

Sargassum; metal; metalloid; contamination; Atlantic

I. Introduction

The brown algae *Sargassum* is one of the most diverse marine macro-algae genera with 362 taxonomically accepted species with only two pelagic species: *Sargassum fluitans* and *natans*. These two planktonic species form dense population rafts on the ocean surface, mainly of the Atlantic, and play a crucial role in the wider ecosystem. *Sargassum* spp. are home to a wide range of species (many of which are endemic), provide nurseries and cover habitat for species going from invertebrates to fishes (including commercially important fish species), and endangered turtles (Casazza and Ross, 2008; Witherington et al., 2012).

The presence of free floating *Sargassum* in the Northern Atlantic Ocean is not a new finding; Christopher Columbus reported seeing *Sargassum* spp. as far back as the 15th century. Since 2011, however, proliferation of *Sargassum* spp. populations have been observed in Africa (Oyesiku and Egunyomi, 2014; Addico and Atta deGraft-Johnson, 2016) and in the Wider Caribbean Region (WCR), including locations where they were so far absent or extremely rare (Hu et al., 2016; Sissini et al., 2017). Massive *Sargassum* spp. landings (i.e. arrival of massive amounts of *Sargassum* on beaches) in the Caribbean area, such as the 2011 and the even stronger 2014-2015 events, lead to disastrous consequences with major impacts on public health (Anderson, 2007), fisheries (Solarin et al., 2014), coastal ecosystems (Rodríguez-Martínez et al., 2019), and tourism (Louime, et al., 2017).

Despite their harmful consequences, few studies have been carried out on open ocean pelagic *Sargassum* spp. and especially, to our knowledge, none have been done on their potential role as a carrier of metal pollutants. Brown algae, such as *Sargassum* spp., accumulate heavy metals from the surrounding environment and the concentration in their cell walls may be 20,000 to 40,000 times higher than in the surrounding water (Sudharsan et al., 2012; Sadeghi et al., 2014). The biosorption capability of *Sargassum* spp., or more generally of brown seaweed, have made them flawless biological indicators of heavy metal pollutions (Haug et al., 1974; Butler et al., 1983; Philips, 1990; Khristoforova and Kozhenkova, 2002; Chernova and Sergeeva, 2008; Thangaradjou et al., 2010). Heavy metal concentration in brown algae is proportional to the quantity of bioavailable forms of metals in sea water during the period of algal vegetation (Karthick et al., 2013). Floating *Sargassum* spp. landing in Caribbean coasts are particularly contaminated by As (Devault et al., 2021). *Sargassum* spp. with high levels of metal and metalloid contamination might impact the organisms that feed on them and could also result in the transport of contamination from the open ocean to coastal sites *via* beaching.

Metals and metalloids are naturally occurring elements found throughout the earth's crust. Their oceanic distributions are not homogenous, there is areas of higher metal contents such as region under volcanic activities (Kamenev et al., 2004) and upwelling (Bruland, 1980; Yeats and Campeell, 1983). Anthropogenic activities have however released a large number of heavy metals in the environment leading to large contaminations in coastal and marine environments. Metal pollution can dramatically impact human health, aquatic organisms, and natural ecosystems because of their toxicity, persistence, and bioaccumulation characteristics (DeForest et al., 2007; Karthick et al., 2012). As *Sargassum* spp. continues to be beached in large quantities, we must understand their impacts and how to safely reuse them. Various sargassum valorization exists such as fertilizer for agricultural crops, bioenergy or beauty care products (Chávez et al., 2020). *Sargassum* spp. that reach coastal areas and are finally beached may introduce metal and metalloid contamination; it is consequently crucial to determine their toxicity before their valorization. Depending on their origins and their journey, open ocean free floating *Sargassum* spp. might present different level of metal and metalloid contamination. Therefore, this present study tackles the issue of estimating metal and metalloid concentrations in open ocean pelagic *Sargassum* spp. before their arrival in the Caribbean region. To accomplish these measurements, pelagic *Sargassum* spp. were collected in various locations along a transect from the center of the Atlantic Ocean to near the coast of Martinique (French West Indies). We then make comparisons to previously reported values on metal concentrations on both benthic and planktonic *Sargassum* spp. as well as to open ocean metal concentrations. The ultimate goal of this study is to present a preliminary assessment of open ocean pelagic *Sargassum* spp. metal and metalloid contamination before their arrival in the Caribbean Sea.

II. Material and methods

I.I. Samples collection and preparation

In November 2018, as part of a science participative initiative, a volunteer sailor collected pelagic *Sargassum* spp. samples during a transatlantic sailing cruise. Every time the boat encountered *Sargassum* spp. rafts, a sample of *Sargassum* spp. was collected by hand and the geolocation of the ship position was recorded via Global Positioning System (GPS);

seven sites were sampled following this method (Figure 1 and Table S1). Once collected, *Sargassum* spp. were rinsed with seawater, dried on the ship hull, and stored in plastic bags to avoid potential metal and metalloid contamination from other sources.

In the laboratory, samples were rinsed five times with deionized water and oven-dried at 40°C for 48 hours. There are three main morphotypes of pelagic *Sargassum: Sargassum fluitans* III and *Sargassum natans* I and VIII. Visual identification of the species was established using criteria described in Oyesiku and Egunyomi (2014) and in Fernandez et al. (2017). *Sargassum* spp. in our samples were a mix of the three morphotypes. We did not have enough material, neither enough sites, to be able to discriminate data by morphotype. Our samples are therefore a mixture of the three morphotypes and no distinction in regard of the morphotype will therefore be done and for the remaining of the manuscript, we will only refer as *Sargassum* spp.

I.2. Element analyses

Sargassum spp. samples were ground using an agate mortar into a finely homogenized powder following previously published protocols (e.g. Zou et al., 2015; Kaviarasan et al., 2018; Pan et al., 2018). Powdered samples (100 mg) were weighed and digested with 3 mL of pure nitric acid (67 %). Mineralization was done by heating up the samples at 100 °C for three hours (hot block CAL 3300, Environmental Express,USA), in closed mineralization tubes. After the mineralization, 15 mL of ultra-pure water (Milli-Q,Bedford, MA, USA) was added to each sample. For each site, three aliquots were analyzed except for sites 2 and 4 for which only two aliquots were performed because of the small quantity of *Sargassum* spp. collected (less than 300 mg).

A series of 14 elements were analyzed simultaneously by Inductively Coupled Plasma Optical Emission Spectrometer (700 Series ICP-OES, Agilent): Silver (Ag), Aluminum (Al), Arsenic (As), Cadmium (Cd), Cobalt (Co), Chrome (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), Lead (Pb), Selenium (Se), Vanadium (V), and Zinc (Zn). Certified reference materials DOLT-5 (dogfish (*Squalus acanthias*) liver), TORT-3 (Lobster Hepatopancreas), IAEA-413 (Algae) were analyzed using the same methodology as the *Sargassum* spp. samples; their recovery rates vary between 84.58 and 107.59 % (Table S2). Element concentrations in *Sargassum* spp. samples are expressed in $\mu g\ g^{-1}$ dry weight (dw). For values below the instrument detection limit, theoretical minimum concentration values are calculated (the detection limit of the instrument (in $\mu g.g^{-1}$) multiplied by the volume of the sample (in L) divided by the sample *Sargassum* weight (in g)).

To determine Hg concentrations in *Sargassum* spp. samples, aliquots of ~15 mg of powdered *Sargassum* spp. were analyzed by flameless atomic absorption spectrometry (AMA 254, SYMALAB, France). The validity of the analytical method was verified against a biologic reference material IAEA-407 (fish tissue). *Sargassum* spp. Hg concentration and recovery rates of the reference material are presented in Table S2. There are no Hg values from Site # 2 because there was not enough sample left to perform the analysis.

I.3. Statistical analyses

Differences in mean element concentrations were tested *via* a student's t-test after a Box-Cox transformation (Peltier et al., 1998). The normality of the variance and homogeneity were validated by a Shapiro's and a Levene's test, respectively. For data that yielded neither normal variance nor homogeneity, the difference of the mean significance was determined using a Mann-Whitney u-test. Principal component analyses (PCA) were obtained using the R Cran software (FactoMineR and factoextra packages). The Kaiser criterion was used to select which dimension could be used for interpretation. Pearson product moment correlation coefficients (referred to as correlation) were used to determine the significance of relationships between elements and the three PCA dimensions as well as the relationship between the different *Sargassum* spp. element concentrations. If not specified, the significance was calculated at the 95 % confidence level. For box plot, clusters with the same letter code are not significantly different at the 95% confidence level (t-test or U-test). Horizontal black lines within the boxes mark the median.

III. Results

The element concentrations (and respective standard errors) measured from *Sargassum* spp. collected at the seven sites sampled in this study are presented in Table S2. The three aliquots for Sites 1, 3, 5, 6, and 7 and the two aliquots for sites 2 and 4 yield good reproducibility between replicated with values of relative standard deviation being on average less than 10 % (values not presented here). The quantity of *Sargassum* spp. collected did not permit analysis of more than three aliquots per site but the established reproducibility between them indicates that site-specific *Sargassum* spp. element concentrations are homogeneous and therefore representative of the element concentrations of their *Sargassum* spp. raft source. Out of the 14 elements analyzed, 11 were above detection limits: Al, As, Cd, Cr, Cu, Fe, Mn, Ni, V, Zn, and Hg (Table S2). *Sargassum* spp. Ag, Co, Pb, and Se concentrations are below the detection limits; mean theoretical minimum concentration values and 1σ standard deviation are: 0.0189 ± 0.0021for Ag, 0.0144 ± 0.0016 for Co, 0.0931± 0.0102 for Pb, and 0.1054 ± 0.0116 for Se. Mean elemental concentrations can be sequenced in the following descending order: As > Fe > Mn > Al > Zn > V > Ni > Cu > Cr > Cd > Hg.

To be able to elucidate the role of spatial variability (*i.e.* location) on the concentration of each element, Principal Component Analyses were performed (Figure 2 A et B). The first three dimensions are conserved for interpretation as they passed the Kaiser criterion (the cumulative variance of the first three dimensions explain 90% of the total variance). Correlations between the element concentrations and the three dimensions are presented in Figure 2 C. The dimension 1 explains about 50 % of the total variance (Figure 2.A.). Dimension 1 permits discrimination between Site 1 from Sites 5 and 7. Site 1 is influenced by (and therefore more enriched in) Cr, Fe, As, V, and Al; Sites 5 and 7 are, however, influenced by Cd and Cu. Dimension 2 (Figure 2 A) explains about 22 % of the total variance. Three variables (Mn, Zn, Cu) permit discrimination between Site 6 from Sites 2 and 4; *Sargassum* spp. element concentrations from Site 1 present higher Cu, Mn, and Zn values than Sites 2 and 4. Dimension 3 (Figure 2 B) explains about 15 % of the total variance. The variable Ni, which is only associated with this dimension, permits to oppose Sites 2 and 3 from Site 7 as the site largely affected by Ni.

To visualize the results generated from this study site-specific elemental concentrations, site-specific box plots of *Sargassum* spp. element concentrations were created (Figure 3). For sites for which it was possible to analyze three aliquots (Sites 1, 3, 5, 6, and 7) significant differences between sites are represented by letters. *Sargassum* spp. from the seven sites present different levels of element concentrations and the element repartition is not homogenous between sites. Four different accumulation profiles are observed: i) a significant (at the 95 % significance level; Table S4) decreasing concentration gradient from Site 1 to Site 7 for Al, As, Fe, V, Hg and into a lesser extend Cr, ii) an asymmetric u-shaped profile for Ni with Site 5 presenting the lowest concentration and Site 7 the highest (1.5-times higher than Site 1), each site presents significantly different values, iii) a two sided profile for Cu with low concentrations for Sites 1 to 4 and significantly higher concentrations for Sites 5 to 7, and iv) a random profile for Cd, Mn, and Zn, with Mn and Zn profiles significatively correlated at the 95% significance level (Table S4).

To put these results into perspective, we compared them to a review of previously published *Sargassum* spp. metal(loid) concentrations. First, we compare our open ocean pelagic *Sargassum* spp. element concentrations to concentrations from coastal benthic *Sargassum* spp, coming from a large review of already published data (Table S3 and Figure 3). When looking at the range of variability, our open ocean pelagic *Sargassum* spp. Cd, Cu, Fe, Ni, and Hg values are significantly lower (at the 95 % significance level) while Cr, Mn and Zn values are on the lower range of coastal benthic *Sargassum* spp literature's values. When examining the median element concentrations, the previously published coastal benthic *Sargassum* spp. studies yielded values that are higher than those generated from our open ocean pelagic *Sargassum* spp. samples, ranging from 1.2-times for Zn to 10-times for Fe. Secondly, we looked at three recently published studies that have analyzed coastal planktonic *Sargassum* spp. (Table 1; values not plotted on Figure 3); one from the Dominican Republic's beaches (Fernández et al., 2017), one from the coast of Ghana (Addico and Atta deGraft-Johnson, 2016), and one from the Mexican Caribbean coast (Rodríguez-Martínez et al., 2020). Compared to our open ocean pelagic *Sargassum* spp. element concentrations, planktonic *Sargassum* spp. from the coast of Ghana (Addico and Atta deGraft-Johnson, 2016) yielded concentrations significantly higher (95 % significance level) for Cd, Cu, Fe, Zn, and Hg and significantly lower for As. *Sargassum* spp. from the Dominican Republic (Fernández et al., 2017; Table 1), also yielded concentration values significantly higher (95 % significance level) for Al, Cr, and Hg, in the same range for Cu, Fe, Mn, Ni, V, and Zn and significantly lower for As and Cd. Finally, *Sargassum* spp. from the Mexican Caribbean coast (Rodríguez-Martínez et al., 2020; Table 1) yielded concentration values significantly higher (95 % significance level) for Al, in the same range for As and Cu, and significantly lower for Fe, Mn, V, and Hg.

IV. Discussion

Mean elemental concentrations are sequenced in the following descending order: As > Fe > Mn > Al > Zn > V > Ni > Cu > Cr > Cd > Hg. Levels of essential elements (de Boer et al., 1986; Allan, 1997; Rodrigues Silva et al., 2009; Tamilselvan et al., 2012; Rehder, 2015) like Fe, Mn, Zn, V, and Cu are on average higher than levels of non-essential elements (Rodrigues Silva et al., 2009; Yusuf et al., 2011) like Ni and Cr. The non-essential and mainly toxic elements such as Hg and Cd (Allan, 1997; Tamilselvan et al., 2012; Costa et al., 2017), despite not being highly concentrated, are present in *Sargassum* spp. Both Al and As, in spite of being non-essential elements (Rybak et al., 2017; Al Mamum et al., 2019; Ameri et al., 2020), are present in higher concentrations than most of the essential ones, a distinctive feature already observed in other studies (see Maret, 2016 for a review).

Open ocean pelagic *Sargassum* spp. from this study were collected in November 2018; during that year, the month with the highest coverage (i.e. surface area) of oceanic *Sargassum* spp. near the Mexican Caribbean coastline was recorded in September with 22,900 ha (Chávez et al., 2020). Despite this clear *Sargassum* spp. peak, surface area varied considerably without any clear pattern throughout the rest of the year (Chávez et al., 2020). This variability could explain the relatively low quantity of *Sargassum* spp. rafts encountered and collected during our November cruise compared to the other months.

Results from this study also reveal heterogeneous element repartition in regard to spatial variation. The four distinct accumulation profiles indicate the absence of a clear longitudinal concentration gradient between sites. Element concentrations in *Sargassum* spp. reflect the presence of these elements in ocean water in dissolved forms. The relative concentration of one element versus another as recorded by *Sargassum* spp., however, is not representative of the ratio in seawater. This is because *Sargassum* spp. does not bioaccumulate each element in an identical manner (e.g. Abirhire and Kadiri, 2011; Sadeghi et al., 2014; Chen et al., 2018). Various factors can influence the presence, distribution, and variability of elements in open ocean. Among the natural factors, a potential source of metal and metalloid concentration heterogeneity could be the proximity to upwelling as they are the principal source of surface nutrients and therefore trace metals from the deep ocean (Valdés et al., 2008). Higher concentration of dissolved Zn, Ni, Co and Cd were found in upwelling surface and sub-surface waters (Bruland, 1980; Yeats and Campeell, 1983; Valdés et al., 2008; Kavun and Podgurskaya, 2009; Ahlgren et al. (2014). *Sargassum* sp. from this study do not present specific enrichment in those metal, leading to believe that they might not originated from nor crossed upwelling areas.

The variability of metal and metalloid concentrations in seawater may also be impacted by seasonality and time of day (Philips, 1977). Algae chemical compositions are sensitive to the soluble-trace metal content of their ambient surroundings. However, they do not represent the total metal loads in seawater, as they cannot incorporate metals associated with organic or inorganic particulate matter (Philips, 1977). Moreover, variability in short-term oceanic metal concentrations cannot be responsible for inter-site variability in *Sargassum* spp. since the algae integrate contaminants over their entire life span.

The program GEOTRACES (https://www.geotraces.org/) surveys critical regions of the world's oceans measuring trace elements and their isotopes that are known indicators of important biogeochemical and physical processes. Seawater metal concentrations data from the GEOTRACES dissolved elements database, separated into 5 boxes to represent the position of this present study's sites, are presented in Table 2. The significance of the difference between the different GEOTRACES boxes cannot be established as the number of values per box are less than three. However, a comparison between GEOTRACES and our *Sargassum* spp. data do not reveal a similar pattern. For example, high *Sargassum* spp. values of Fe and Al that discriminate Site 1 from Sites 5, 6, and 7 do not present a similar pattern in GEOTRACES data; boxes representatives of Sites 5, 6, and 7 present similar or higher values of Fe and Al compared to the box representative of Site 1. The absence of a perfect match between *Sargassum* spp. and GEOTRACES metal concentrations is not surprising as GEOTRACES data are from samples taken at a discrete moment in time and space while the *Sargassum* spp. data, as said previously, integrate metal concentrations over their entire life span.

A potential external source of metals and metalloids into the ocean is fluvial inputs *via* river runoff. Various studies have described a decrease in metal concentrations along transects from coastal waters to the open ocean; only a fraction of metals leave the coastal zone and are transported to the open ocean by advection-diffusion processes (Schaule and Patterson, 1981; Symes and Kester, 1985; Landing and Bruland, 1987; Martin and Gordon, 1988). Metals can also enter the ocean by atmospheric inputs; it is well established that the Pb found in the Atlantic Ocean results from the atmospheric input dispersed from the North American continent *via* the westerly winds (Mart et al., 1982). Atmospheric deposition of mercury from continental origin contributes significantly to the variability of surface ocean mixed layer (Zhang et al., 2016). If *Sargassum* spp. element concentrations were influenced by fluvial and/or atmospheric inputs, sites closer to the coast should present higher elemental concentrations. Site 1, the farthest away from the coast, however, yields the highest concentrations in Al, As, Cr, Fe, V, and Hg, which dismantles this hypothesis. This hypothesis is further disproven as all seven sites are relatively far away from any continental inputs, with the closest site (Site 7) located ~200 km away from the nearest coast.

Previous studies have determined that *Sargassum* spp. metal concentrations vary on both spatial and temporal scales (Soerensen et al., 2014; Rodríguez-Martínez et al., 2020). Therefore, the heterogeneity in metal(loid) concentrations observed between this study's seven sites' might be linked to the origin of the *Sargassum* spp. and their journey along different oceanic currents and/or to regions with varied proximities to coastal contaminated areas. In 2018, the year of this study, high densities of *Sargassum* spp. were observed in the

Great Atlantic *Sargassum* belt (Wang et al., 2019). Following the modeling study of Wang et al. (2019), *Sargassum* spp. located in the central Atlantic, like the ones in our study, likely developed locally rather than from seed populations in the Sargasso Sea as proposed by Fernández et al. (2017). It is possible that some of the *Sargassum* spp. may have come from West Africa and bloomed in the central Atlantic, validating the role of the North Equatorial Recirculation Region as a potential source region as proposed by Frank et al. (2016). This hypothesis would suggest the non-influence of previous coastal contamination on the *Sargassum* spp. in our study and would imply relatively low metal(loid) contamination. From their sampling location, *Sargassum* spp. rafts probably traveled along the Loop Current and Gulf Stream to finally enter the North Atlantic Ocean. Some *Sargassum* spp. might have been transported directly into the North Atlantic following the Antilles Current (Wang et al., 2019) while other rafts may have entered the Caribbean Sea (Putman et al., 2019). The Equatorial Atlantic's ocean circulation dynamics play a central role in the transport of *Sargassum* spp. into the Caribbean Sea. Once there, trade-winds are responsible for their beaching by transporting the superficial waters towards the shore, therefore pushing *Sargassum* spp. rafts towards the coast.

Despite the significant differences between site-specific *Sargassum* spp. elemental concentrations, this present study concentrations can still be considered low. As such, these sites are relatively homogenous compared to the large variability observed in the literature review from coastal benthic *Sargassum* spp. (Table S3 and Figure 3). Indeed, most of the coastal benthic *Sargassum* spp. metal concentrations (Cd, Cu, Fe, Ni, and Hg) are significantly higher than values measured in our study. We have to take into consideration that most of the sampling sites from the reviewed coastal benthic *Sargassum* spp. studies are also from highly industrialized coastal areas. As presented above, external inputs that supply trace metal(loid)s to oceanic surface waters, such as river runoff, have a strong effect on coastal waters and therefore on the coastal *Sargassum* spp. metal contaminations.

The comparison between this study's open ocean planktonic *Sargassum* spp. element concentrations to previously published coastal planktonic *Sargassum* spp. metal concentrations show contrasting results (Table 1). Most *Sargassum* spp. metal concentrations (Cd, Cu, Fe, Zn, and Hg) from the coast of Ghana's (Addico and Atta deGraft-Johnson, 2006) present levels significantly higher (up to a factor of one hundred) than values from our study; arsenic concentration are however significantly lower, by a factor of five. According to Addico and Atta deGraft-Johnson (2006), their *Sargassum* spp. samples come from areas associated with intensive mining and industrial activities that might explain the observed high element concentrations. Compared to the results generated in this present study, metal and metalloid concentrations from the Dominican Republic region (Fernández et al., 2017) are in a similar range for Cd, Cu, Mn, Ni, V, and Zn; significantly higher by a factor of 20, 50, and 100 for Cr, Hg, and Al, respectively; and significantly lower by a factor of five for As. The origin of the *Sargassum* spp. samples from the Dominican Republic seem to be more closely linked to sources in the southern latitudes. There they might have proliferated near the mouth of large rivers exposed to coastal contaminations that could explain their high metal and metalloid concentrations (Fernández et al., 2017). The most recent study from Rodríguez-Martínez et al. (2020) present *Sargassum* spp. element concentrations from the Mexican Caribbean coast. Most of their metal and metalloid concentrations are in the same range as presented in our study, even for As. The comparison to these recent studies leads one to conclude that our new assessment of pelagic open ocean *Sargassum* spp. element concentrations are on average in the same range or below already published planktonic *Sargassum* spp. values from non-beached *Sargassum* spp. This validates the hypothesis that pelagic *Sargassum* spp. from this study bloomed in the open ocean and have not yet encountered potential contaminated coastal areas.

Regarding As, it is a metalloid naturally present in the ocean, in concentrations between 15 and 25 nM for hydrogen arsenate (Millero, 2006). Various studies have concluded that brown macro algae rapidly and greatly accumulate dissolved As (e.g. Penrose, 1974; Neff et al., 1997, Devault et al.,2020). Both this present study with samples from the open ocean and studies on the Mexican coast yield similar levels of As concentration, both higher than the ones from the more contaminated coastal areas of Ghana (Addico and Atta deGraft-Johnson, 2006) and Dominican Republic (Fernández et al., 2017). In contaminated areas, heavy metals are highly abundant and could consequently saturate fixation sites of *Sargassum* spp. This competition for binding sites could reduce the number of available sites for As and would explain the low As concentration recorded in *Sargassum* spp. from contaminated areas compared to the open ocean.

A recent study tackling the ecotoxicology effect of nano-plastics on marine organisms analyzed metal contamination in North Atlantic gyre micro-plastics (Baudrimont et al., 2020). Metal contamination in micro-plastics (26.155 $\mu g.g^{-1}$ for Fe; 3.364 $\mu g.g^{-1}$ for Cu; 16.633 $\mu g.g^{-1}$ for Zn; 2.051 $\mu g.g^{-1}$ for Ni; and 0.552 $\mu g.g^{-1}$ for Cd) is within the same range yielded by samples presented in this study's *Sargassum* spp. samples. This could indicate that *Sargassum* spp. can adsorb metals in a manner similar to micro-plastics, or that the metal contamination recorded in *Sargassum* spp. can be due to the presence of nano-plastics attached to them. A more thorough study exploring these mechanisms would be interesting and necessary to validate or refute these hypotheses.

Element concentrations in *Sargassum* spp. presented in this study are well below trace elements limits of the French norm for the enrichment of organic soil product (NFU 44-051-ISSN 0335-3931) for Cd, Cr, Hg, Ni, Pb, Se, Cu, and Zn. Only the concentration of As in *Sargassum* spp. is above the acceptable value of 18 $\mu g.g^{-1}$. This is not surprising as various studies have concluded that brown macro algae rapidly and greatly accumulate dissolved As (e.g. Penrose, 1974; Neff et al., 1997). However, our *Sargassum* spp. samples yielded a mean As concentration (145.62 $\mu g.g^{-1}$) that falls well within the worldwide range of 0.1 - 382 $\mu g.g^{-1}$ for marine algal samples (Neff et al., 1997). That being said, the level of As enrichment might become a concern for the use of *Sargassum* spp. in the industry as it may pose potential health risks. The mean As concentration represents total As in *Sargassum* spp., however, it is already known that only certain forms of As are toxic (e.g. Neff, 1997). Further study would be needed to explore differences in the concentrations of the various forms of As that can be found in *Sargassum* spp. in order to determine the true level of toxicity.

Open ocean *Sargassum* spp., such as the ones analyzed in this study, can therefore be considered as relatively pristine as they did not yield high metal concentrations, aside from the metalloid As. Concentrations from this study can be used as a first assessment for future *Sargassum* spp. contamination studies that endeavor to tackle the issue of estimating coastal contamination. For example, the element concentrations found in the Caribbean beaching *Sargassum* spp. can be studied in the context of our open ocean *Sargassum* spp. samples. While we acknowledge the fact that seven sites are not enough to have a comprehensive understanding of all open ocean *Sargassum* spp. metal and metalloid concentrations, this study still gives a meaningful first assessment. We also recognize the limitation of using a mixture of two or three macroalgae species which can accumulate metal(loids) differently as already pointed out by Milledge et al., (2000). Follow up studies with improved spatial and temporal coverage as well as sample size will be necessary to obtain more detailed calculations as previously developed for algae (Garca-Seoane et al., 2018) and bivalves (Lu et al., 2019), to determine baseline open ocean metal(loid) loads.

V. Conclusions

This study revealed that Atlantic open ocean *Sargassum* spp. do not transport significant metal(loid) loads and could be considered as pristine before reaching the Caribbean Sea, with As as a potential exception that needs to be further explored. These results are reassuring since free floating *Sargassum* spp. provide a habitat for many organisms, including commercially relevant fish that could have been impacted by metal(loid) enrichment. Although the seven sites present statistically significant site-specific differences, the spatial sampling is not sufficient to determine the cause of this variability or to set baseline metal(loid) concentrations representative of all open ocean *Sargassum* spp. populations. Since *Sargassum* spp. present in the western Equatorial Atlantic have a high probability of entering the Caribbean Sea within a year's time (Putman et al., 2019) our studied *Sargassum* spp. rafts might have been transported to the Caribbean region, resulting in massive beaching events. Despite the massive arrival of *Sargassum* spp., the upside is that it is unlikely that these algae would transport any preexisting metal(loids) contamination. These elemental concentrations recorded in the *Sargassum* spp. samples presented in this study can be considered reference values for future work that focuses on *Sargassum* spp. from coastal areas. Additionally, replication studies might be of high interest to further validate our first assessment of open ocean element concentrations and sample sites in closer proximity to the coast may elucidate a potential element enrichment in the *Sargassum* spp. sampled in sites closer to the coast.

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Ethical Approval

This manuscript is an original work and has not been previously published somewhere else nor submitted to more than one journal for simultaneous consideration.

Consent to participate

Not applicable

Consent for publication

Not applicable

Author contribution

EPD carried out sample preparation, analyses and data acquisition; executed the analytical research and interpretation, and served as primary author. PYG carried out statistical analyses. OAC accomplished *Sargassum* species identification. MB obtained the *Sargassum* samples. PYP and MB proofreaded the manuscript. All authors contributed to the article and approved the submitted manuscript version.

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Availability of data and materials

All the data discussed in this study are the one presented in this manuscript's tables.

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