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Inorganic Arsenic in holopelagic Sargassum spp. Stranded in the Mexican Caribbean: Seasonal Variations and Comparison With International Regulations and Guidelines

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Abstract :

Due to the massive proliferation and stranding of holopelagic Sargassum spp. over the last decade, different strategies for the sustainable valorisation of Sargassum biomass have been explored and investigated. One limitation to the development of Sargassum biomass valorisation is related to its high arsenic (As) content. The toxicity of As depends on the chemical forms present and their oxidation or valence state, classified as inorganic and organic compounds, with the inorganic As compounds being much more toxic than the organic ones. The aim of the present study was to determine the inorganic arsenic (iAs) content in holopelagic Sargassum spp. for which almost no information on stranded biomass is available. In this study, we examined the iAs content in the three holopelagic Sargassum morphotypes collected over a seasonal cycle in 2018-2019. The iAs concentrations ranged from 12.7 to 62.9 mg kg-1, representing 14.1% to 81.7% of total arsenic (TotAs). The iAs content was compared between species and seasons and discussed in the context of existing international regulations and guidelines.

Highlights

► Inorganic arsenic (iAs) content was monitored in stranded holopelagic *Sargassum* from the Mexican Caribbean ►The iAs content ranged from 12.7 to 62.9 mg kg^{-1} representing between 14.1% and 81.7% of total arsenic (TotAs) ►The proportion of iAs increased over the period studied ► The highest iAs content was found in the warm-dry season with an annual mean of 41.0 mg kg^{-1} ► The iAs content is well above the levels allowed by international guidelines

Keywords : inorganic arsenic, holopelagic Sargassum, valorisation, regulations

1. Introduction

The influx of massive quantities of holopelagic *Sargassum* spp. has been observed in the Caribbean Sea and the Mexican Caribbean over the last decade, with negative environmental, health and socio-economic impacts (van Tussenbroek et al., 2017; Rodríguez-Martínez et al., 2019). In 2018, the maximum amount of holopelagic *Sargassum* biomass removed along the Mexican Caribbean coast was recorded between March and August, averaging 4.5 x 10^3 m^3 km^{-1} per month (Chávez et al., 2020). The most detrimental consequences occur when the holopelagic *Sargassum* biomass reaches the coast, accumulates and begins to decompose. Stranded, decomposing holopelagic *Sargassum* produces leachates that degrades water quality and threatens to contaminate aquifers with nutrients, salts and metals (Chávez et al., 2020).

To mitigate these impacts, various strategies for sustainable valorisation of *Sargassum* biomass have been explored and investigated (Desrochers et al., 2020; Robledo et al., 2021). For some of these proposed applications, it is crucial to assess the concentration

of toxic chemical elements present in the algae, as *Sargassum* and other brown seaweeds are known to highly uptake some metals and metalloids such as As (Davis et al., 2000). High concentrations of As, especially if As occurs as inorganic species (iAs), can lead to health hazards for humans, animals and the environmental, thus limiting or preventing the use of these algae as fertiliser or compost (Roberts et al., 2015; Veira and López, 2016), for crop and livestock production (Thompson et al., 2020) or for food, textile and pharmaceutical production through alginate use (Barquilha et al., 2019). On the other hand, there are other potential ways to valorise holopelagic *Sargassum* biomass, for which arsenic or metal concentrations do not represent a potential risk, such as biogas and renewable liquid fuel production (Aparicio et al., 2021; Marx et al., 2021), or bioenergy production through anaerobic digestion (Thompson et al., 2019; López-Aguilar et al., 2021). Other potential applications are related to their ability to accumulate certain elements, mainly metals, such as for the production of activated carbon to be used in the context of wastewater treatment (Esmaeili et al., 2015; Francoeur et al., 2021), for bioremediation in coastal ecosystems (López-Miranda et al., 2020; Saldarriaga-Hernández et al., 2020) or as biosorbent materials for the removal of toxic metal ions (Barquilha et al., 2019; Coração et al., 2020) or lanthanides ions (Oliveira et al., 2014).

In order to determine if and how holopelagic *Sargassum* biomass can be used, it is important to evaluate the occurrence of toxic elements and chemical forms in the seaweeds. Among these, the metalloid arsenic (As) is of particular interest, as it has been found in high concentrations in holopelagic *Sargassum* spp collected either off shore in the tropical Atlantic Ocean and in the Sargasso Sea (Cipollini et al., 2022;

Dassié et al., 2022; Gobert al., 2022), near shore some Carribean Islands (e.g. Devault et al., 2022a) or stranded in different beaches of the Caribbean area such as Mexico (Ortega-Flores et al., 2022) or Jamaica (Davis et al., 2021). From a toxicological point of view, the interest in As and its chemical form in algae arises from the fact that the As concentration in marine algae is usually between 1,000 and 10,000 times higher than the As concentration in the waters in which they grow, depending on the algal species (Borak and Hosgood, 2007). However, it is important to define the toxicity of As compounds which depends on their chemical form and their oxidation or valence state. These compounds are classified as inorganic As (iAs) and organic As (oAs). The inorganic pool includes arsenite (As(III)) and arsenate (As(V)) ions and the organic pool includes monomethylarsonic acid (MMA), dimethylarsonic acid (DMA), trimethylarsonic acid (DMA), arsenobetaine (AsB), arsenocoline (AsC) and arsenosugars. It should also be noted that inorganic As compounds are much more toxic than organic ones and that As(III) is more toxic than As(V). Although the organic forms are considered less toxic, they can potentially pose toxicological issues over the mid to long term. In fact, it has been shown that marine bacteria have the capacity to efficiently decompose complex organoarsenicals into their simple organic forms or even into inorganic arsenic (Duncan et al., 2014). For marine brown algae, it has generally been reported that iAs contents range from 2% to 10 % of TotAs with the exception of *Undaria pinnatifida* (Wakame), for which iAs can represent more than 60 % of the TotAs and can exceed 80 mg kg^{-1} (Rose et al., 2007). Recent data obtained by Devault et al. (2022a) indicate that holopelagic *Sargassum* can displayed iAs proportions up to 80%.

There are relatively few studies reporting iAs in holopelagic *Sargassum* biomass (Table 1). A pioneering study by Johnson and Braman (1975), on three samples collected off Florida and on one sample collected in the Sargasso Sea, reported iAs concentrations in the range of 1.9-7.6 and 19.5 mg kg^{-1} ww (wet weight), respectively. More recently, Gobert et al. (2022) found iAs concentrations in samples also collected at sea in the eastern and western tropical Atlantic, generally in the range 11.2-62 mg kg^{-1} dw (dry weight), with the exception of one sample in the Sargasso Sea with a value of 105 mg kg^{-1} dw. For stranded biomass, existing information on iAs concentrations in holopelagic *Sargassum* also appears relatively limited. A technical report (Ocean Harvest Technology, 2016) mentions two iAs values of 27.7 and 68 mg kg^{-1} dw for mixed biomass collected on the beaches of the British Virgin Islands and Martinique, with iAs representing about 70 % of TotAs in each case. On the other hand, the study by Gobert et al. (2022) reported iAs concentrations in the range of 6.2-15.8 mg kg^{-1} dw (iAs:TotAs ratio in the range 16-30 %), for six samples collected on the coast of Martinique. These iAs values were lower than those for biomass collected offshore (i.e. in open ocean or in coastal waters). Devault et al. (2022a) reported iAs values for mixed *Sargassum* biomass collected either on Martinique beaches or near shore. In the same way, they generally found lower iAs values in the stranded samples, especially in the dried biomass, as compared to the floating algae. This comprehensive study by Devault et al. (2022a), which is based on a high number of samples (n=57 for those sampled in Martinique) highlights the high range at which iAs concentrations can occur in holopelagic *Sargassum* (i.e. 3.7-96.3 mg kg^{-1} dw in this case representing 26% to 87% of TotAs).

According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2011), the concentrations of TotAs and iAs in foods such as dried seaweed are extremely variable, ranging from 0.114 to 236 mg kg^{-1} dw for TotAs and from 0.1 to 130 mg kg^{-1} dw for iAs (Almela et al., 2002; Almela et al. 2006), but no guidelines have been established by the Joint FAO/WHO committee for iAs in seaweed for human food or animal feed. On the other hand, a large number of countries have regulations specifying maximum permitted levels of TotAs and iAs in algae, for different uses: food, feed and agricultural soil amendments (Table 2). In the case of iAs, the maximum permitted levels are 2-3 mg kg^{-1} dw or 1-3 mg kg^{-1} dw for feed and food, respectively, depending on the country, and 40 mg kg^{-1} dw for fertilisers and soil improvers in Europe. Before considering any valorisation options for holopelagic *Sargassum* spp., there is an urgent need to better characterize iAs levels in the raw biomass with a more comprehensive view of the factors controlling these levels.

In this study, we report the iAs concentrations for a large number of holopelagic *Sargassum* spp. samples (n=101) collected monthly over an entire seasonal cycle (2018-2019) on the Mexican Caribbean coast. TotAs concentrations in these samples, which were previously reported in Ortega-Flores et al. (2022) ranged from 9.5 to 255 mg kg^{-1} dw and are thus representative to what has been previously and also regularly reported in other studies conducted on holopelagic *Sargassum* spp. either in the Caribbean or in other Atlantic regions. The aim here was to provide much more information on iAs concentrations for stranded biomass, which is virtually absent in the literature. The iAs content was compared between morphotypes and seasons and with

the existing regulations and guidelines to analyse the potential use of holopelagic *Sargassum* spp. biomass that can be harvested along the Mexican Caribbean coast.

2. Materials and methods

2.1 Study site and sampling of algal material

The study site is located in the north section of Quintana Roo state at Puerto Morelos (20°46′07″ N, 86°57′14″W) (Fig 1), where no beach cleaning nor removal of biomass is performed. The Quintana Roo coast is a limestone platform characterized by exposed rocky outcrops alternating with sandy beaches. According to Beddows et al. (2016), the majority of this coastline is a karst geology where freshwater flows through underground river systems and this underground freshwater has very limited impact on the coastal salinity. The tidal regime in the area is microtidal with a typical tidal range of less than 20 cm. The study site, which is a protected sandy beach located in a reef lagoon, is reported as a stable sector to erosion (Vázquez-Delfín et al. 2021). The climate of the region is characterized by three seasons: warm-dry (March-May), rainy (June-October), and winter (November-February, characterized by scattered short showers) (Schmitter-Soto et al., 2002).

Samples of stranded biomass of holopelagic *Sargassum* spp. were similar to those analysed by Ortega-Flores et al. (2022). The samples were collected during monthly visits from June 2018 to May 2019. During each visit, a systematic sampling procedure was established by conducting a 50 m transect running parallel to the shoreline along

the fresh fringe (Ortega-Flores et al. 2022). The specimens collected for this study were thus relatively wet and fresh due to their regular dipping with tide and waves and were not in a state of advanced degradation, which should correspond to stranding times of several hours to a couple of days maximum before sampling. All the algal biomass was collected from ten quadrat plots (0.5 × 0.5 m²) separated by 5 m along the transect. All algae within each plot were collected, placed in appropriate labelled plastic bags and transported in refrigerated boxes to the laboratory for chemical analysis. All holopelagic *Sargassum* specimens collected in each quadrat were classified according to Parr (1939) into the following morphotypes: *S. fluitans* III (hereafter referred to as *S. fluitans*), *S. natans* I, and *S. natans* VIII. In the laboratory, the collected material was visually inspected and any sand deposit was removed by hand, covered with a glove. It is worth noting that no fresh water rinsing or thalli removal was carried out, as the interest of the study was to determine the potential uses of raw sargasso as collected from the sea or beachcast. After an oven-dry at 60 °C for 24 h, the material was then crushed by hand with an agate mortar and stored in containers until chemical analysis.

2.2 Total arsenic data and inorganic arsenic determination

Three replicates for each morphotype per month were selected from the total number of replicates analysed by Ortega-Flores et al. (2022) to obtain reported TotAs and to analyse iAs concentrations (n=101). The determination of TotAs is described in Ortega-Flores et al. (2022). Briefly, TotAs concentrations were measured using an atomic absorption spectrophotometer (XplorAA, GBC Scientific Equipment, Melbourne, Australia), using a hydride generation (HG-AAS). The accuracy of the analytical methods used was validated using a certified standard reference material (IAEA-392, Trace

elements in algae; International Atomic Energy Agency, Vienna, Austria). The recovery was greater than 90 %, and the limit of detection (LoD) was 0.5 mg kg^{-1} for As.

The determination of iAs level in holopelagic *Sargassum* was performed using the protocol described by Gobert et al. (2022), which includes an extraction step and three independent voltammetric measurements of iAs. From the dried and ground samples of holopelagic *Sargassum,* iAs was extracted in water under sonication for 30 min using a sample/ultrapure water mass ratio of 1:100 (typically 0.05 g dw of biomass in 5 mL of ultrapure water). The solution was then centrifuged at 4,000 rpm for 10 min and the supernatant was then separated. Voltammetric analysis was performed using an electrode system (scTRACE Gold, Metrohm) consisting of a gold microwire working electrode, an Ag/AgCl reference and a carbon auxiliary electrode. This system was coupled to a µAutotlab-III potentiostat, controlled by a computer and the data were processed using GPES version 4.9 software. In the measuring cell, 200 µL of biomass extract was added to 11 mL of ultrapure water, 2 mL of electrolyte and 100 µL of a 0.200 mM $KMnO_4$ solution (CHEM-LAB, Belgium). The electrolyte solution consisted of 1 M sulphamic acid (9.71 g, SIGMA-ALDRICH, USA), 0.5 M citric acid (10.51 g, Fluka, Germany) and 0.45 M potassium chloride (3.35 g, SIGMA-ALDRICH, USA) dissolved in 100 mL of ultrapure water using an ultrasonic bath. The electrochemical procedure used consisted of three steps: (1) The cleaning step, consisting of five consecutive cyclic voltammetry scans between -0.2 and 1.0 V, allows the removal of any traces of As or other metals from the electrode surface. (2) The deposition step consists of applying a potential of –1.0 V for 60 s. Under these conditions $H_2(g)$ is generated at the working electrode, allowing As(V) to be reduced to As(III) in the vicinity of the working electrode.

The As(III) formed is then subsequently reduced to As(0), which is adsorbed and accumulated on the gold surface. A second potential of -0.3 V, closest to the As signal, is then applied for 5 s in order to strip from the gold electrode metals other than As, which are also deposited at -1.0 V. (3) The As is then stripped by scanning in square-wave mode (SWV) from -0.3 to 0.4 V. During this step, the As(0) previously adsorbed on the gold surface is oxidised and the current intensity is measured. The parameters of the SWV were: potential step 10 mV, pulse amplitude 20 mV and frequency 100 Hz. The position of the iAs peak is located between 0 and 100 mV and the voltammetric response is obtained from the peak derivative. The concentration of iAs was then obtained after two additions of an As(V) standard solution. This method determines iAs with a detection limit of 0.2 µg L^{-1} and with a precision of ~6 %. A certified reference material of *Sargassum fusiforme* (Hijiki, NMIJ CRM 7405-b) was used to control the accuracy of the method. Each extract was analysed in triplicate and results are expressed as mean ± standard deviation (SD). All data are expressed as mg of iAs per kg algal dry weight (mg kg^{-1} dw).

2.3 Statistical analysis

Normality and homoscedasticity of the data for iAs concentrations were determined by Kolmogorov-Smirnov analysis with Lilliefors and Levene modification, respectively. The data obtained did not meet the assumptions of normality and homoscedasticity, so the data were compared using non-parametric statistics based on the Kruskal-Wallis rank test for differences in iAs content between species and between seasons, followed by a Mann-Whitney post hoc test. Data were statistically analysed using the R program (R Development Core team, 2020).

3. Results

3.1 Inorganic arsenic content in holopelagic *Sargassum*

Inorganic arsenic (iAs) in our samples varied from 12.7 to 62.9 mg kg^{-1} dw with some notable differences according to the morphotypes and the season (Fig. 2). Indeed, the iAs concentrations were statistically different ($p = 0.01$) between *S. fluitans* and S. *natans* VIII on the one hand and *S. natans* I on the other hand (Fig. 2a). *S. fluitans* has the highest annual mean iAs content (37.8 mg kg^{-1} dw) with a range from 20.6 to 71.5 mg kg^{-1} dw, followed by *S. natans* VIII with an annual mean content of 37.7 mg kg^{-1} dw (range 12.6-64.7 mg kg^{-1} dw), and *S. natans* I with an annual mean of 28.0 mg kg^{-1} dw (range 11.9-47.7 mg kg^{-1} dw). Significant differences were observed between the rainy season and the warm-dry season, independent of morphotypes ($p = 0.03$), (Fig. 2b). The highest iAs content was observed in the warm-dry season with a mean of 41.0 mg kg^{-1} dw and a range between 22.0 and 71.5 mg kg^{-1} dw, followed by the winter season (mean 33.8 mg kg^{-1} dw and range 15.5-54.2 mg kg^{-1} dw). The lowest iAs content was observed during the rainy season with a mean of 31.3 mg kg^{-1} dw and a range of 11.9 to 69.3 mg kg^{-1} dw (Fig. 2b).

3.2 Total arsenic and proportion of iAs in holopelagic *Sargassum*

Figure 3a describes the concentrations of iAs and TotAs as a function of time for each sample. *Sargassum natans* VIII and *S. fluitans* morphotypes, with an annual mean TotAs

content of 91.8 and 90.7 mg kg^{-1} dw, respectively, generally showed higher values than *S. natans* I (annual mean TotAs of 68.2 mg kg^{-1} dw). Along the seasonal cycle, the highest TotAs concentrations were observed during the rainy season (with a maximum in August for all morphotypes and values above 200 mg kg^{-1} dw for *S. natans* VIII and *S. fluitans*) and the lowest TotAs concentrations were found over a relatively long period, i.e. from October to May, which includes the winter and the warm-dry seasons. As already mentioned in the previous section, iAs concentrations during the rainy seasons were generally lower than those found later, and especially during the warm-dry period when iAs values were generally found in the range 30-50 mg kg^{-1} dw. These variations of iAs, with a general increase in concentrations over the study period, are therefore opposite to those of TotAs. As a result, the proportion of iAs increased significantly over the study period, with relatively low values during the rainy season (range 14-32 % for the Jun-Sep period), and higher values during the warm-dry season (range 56-80 % for the Mar-May period) (Fig. 3b). Plotting iAs content and iAs proportion as a function of TotAs for each morphotype (Fig. 4) confirms that iAs does not increase proportionally with TotAs content (Fig. 4a). In fact, the iAs proportion remained below 40 % in the samples with TotAs concentrations above 100 mg kg^{-1} dw, whereas iAs contents over 50 % were found in the samples with TotAs concentrations below 50 mg kg^{-1} dw (Fig. 4b).

4. Discussion

4.1 Inorganic arsenic content of holopelagic *Sargassum*

The iAs concentrations and iAs proportions found in our samples were consistent with those previously observed for holopelagic *Sargassum* spp. in different locations (Table 1). However, our values and the ones reported by Devault et al. (2022a) were spread over a wider range, which is probably due to the large number of samples being collected in both studies. With the exception of the study by Johnson and Braman (1975), in which iAs was expressed as wet weight, the iAs concentrations in holopelagic *Sargassum* spp. reported in all studies are generally in the range 10-100 mg kg^{-1} dw. Such a range of iAs concentrations appears to be a common feature of the genus *Sargassum*, including *Sargassum fusiforme* or *S. piluliferum* (Ma et al., 2018). However, low iAs content (< 0.1 mg kg^{-1} dw) and low iAs proportions have also been reported in other species belonging to the order of Fucales (Taylor and Jackson, 2016). Within the brown macroalgae, apart from the genus *Sargassum*, relatively high iAs concentrations were also observed in *Laminaria digitata* (18-62 mg kg^{-1} dw; McSheehy and Szpunar 2000). It is also worth noting that Ender et al. (2019) reported iAs concentrations in this species in the range of 40-80 mg kg^{-1} dw after acidic extraction (1 % nitric acid + 2 % H_2O_2). Apart from *L. digitata,* other Laminariales species have low iAs content with concentrations below 1 mg kg^{-1} dw (Taylor and Jackson, 2016).

In our samples of holopelagic *Sargassum* spp. collected along the seasonal cycle, the iAs concentrations did not vary proportionally with respect to the TotAs content (Fig 4a). Once As is sequestered by the algae, mainly in the inorganic pentavalent arsenate form (AsO_4^{3-}) (Cutter et al. 2001; Wurl et al. 2015), it is likely to be stored in the cell wall which acts as the first barrier to toxic elements. This hypothesis is in line with Ender et al. (2019) who used NanoSIMS elemental imaging to show that most of the arsenic in *L.*

digitata is located in the cell wall, mainly as inorganic arsenic and to a lesser extent as hydrophilic arsenosugars. However, we suggest here that there is probably a limit to the extent to which *Sargassum* spp. can accumulate As in their cell walls, which is related to the number and availability of binding sites on the alginates and/or fucoidans. Once this limit is reached, other storage or release strategies are likely to be activated by the algae. Indeed, different organisms are known to use different ways to resist and detoxify the harmful effects of As, including adsorption on the cell-surface, uptake or storage in the cells (binding to glutathione and/or sequestering in vacuoles or vesicles), oxidation, reduction, methylation, demethylation, and excretion (Ma et al., 2018; Mitra et al., 2017; Zhu et al., 2014). In the case of algae, the cellular uptake, biotransformation and excretion processes of As also differ among species, which can lead to important variations in iAs content. Indeed, Al Mamun et al. (2019), who compared the uptake and metabolism of As in species of algae, reported that the detoxification mechanism of As(V) in the red *Porphyra yezoensis* and the brown *Sargassum horneri* consists of methylation and reduction of As(V), whereas in the brown *S. patens,* the detoxification of As(V) is only associated with reduction. In the case of holopelagic *Sargassum* spp., it should be noted that our measurements of iAs speciation only indicate the occurrence of As(V) and not As(III). As a consequence, no As(V) reduction mechanism seems to operate in these algae.

The analysis of the seasonal variation of iAs demonstrates that iAs concentrations show asynchronous variations compared to those of TotAs (Fig. 3). If the highest values of TotAs were observed during the rainy season, the highest values of iAs were obtained during the warm-dry season. The content of TotAs and iAs can be related to the origin

of the algae, but also to the conditions experienced by these algae during transport and stranding.

According to Johns et al. (2020) study, the North Coast of Quintana Roo can receive sargassum from two main routes: (1) a shorter route through the Great Antilles coming directly from the North West Atlantic including the Sargasso Sea area and a (2) a longer route through the tropical Atlantic and Lesser Antilles. Alleyne et al. (2023) also shows that this latter route through Lesser Antilles can be divided in two sub-origins; one area around 15° N that travels directly E–W across the Atlantic, and another area generally south of 10° N that takes a route close the coast of South America. Higher levels of TotAs in winter, when the north-east trade winds prevail at latitudes below 30°N in the North Atlantic (Herwitz and Muhs, 1995), could correspond to biomass that has grown in the Sargasso Sea and that took a direct route through Greater Antilles. Indeed, the work of Gobert et al. (2022) showed that such biomass, growing under P-limited conditions, is more enriched in As (with values in the range 100-250 mg kg^{-1} dw) compared to biomass collected further south in the tropical Atlantic (with TotAs values usually below 100 mg kg^{-1} dw).

In addition to this control of iAs concentrations linked to the origin of the biomass, it is also important to consider the different conditions encountered by the algae during their transport to the shore and also during their stranding. According to Rodríguez-Martínez et al. (2020), the residence time of Sargasso in near-shore waters (from the Yucatan Current to the shore) is in the order of hours. This authors also indicate that the metal and arsenic composition of the Sargasso is likely controlled by pelagic factors and that it is unlikely that these elements are strongly absorbed in near-shore waters of the

Mexican Caribbean due to absence of major industrial, mining or heavy agricultural activities in the region. However, the experiments by Devault et al. (2022b) suggest that holopelagic *Sargassum* can transudate a high part of their As content in few hours when stressed in seawater. This later could correspond to the step when the algae accumulate near the shore and no longer benefit from conditions to ensure sufficient mixing.

Secondly, the potential exposure of holopelagic *Sargassum* to non-saline water from precipitation can also induce As losses, since iAs is easily extracted with water. Thus, the amount of rainfall during the 2018 rainy season (151.9 mm) in the study area was much higher than that of the 2019 warm-dry period (43.3 mm) (CONAGUA, 2018), which can explain why iAs concentrations, and even more iAs proportions, were lower during the rainy seasons for the three morphotypes. Other parameters that may influence the content in As include temperature, pH or solar radiation (Sari and Tuzen, 2008; Patrón-Prado et al. 2011; Ortega-Flores et al. 2022). Overall, our data indicate that, regardless of the TotAs content, it is essential to determine the iAs content due to intra- and interspecific variation.

4.2 Toxicological guidelines for the proper use of holopelagic *Sargassum* biomass

The iAs content of all samples analysed in our study (range of 12.7 to 62.9 mg kg^{-1} dw) is higher, or even much higher, than the regulatory limit (1, 2 or 3 mg kg^{-1} dw) set by China, Europe, the United States, Australia and New Zealand for the use of algae in animal feed, human food, or any product intended for human consumption (cosmetic, medical) (Table 1). The use of stranded holopelagic *Sargassum* spp. biomass for animal feed or human products would therefore be rejected on the basis of the regulations. In

addition to the above, harvesting of stranded or floating *Sargassum* spp. raises numerous quality concerns: biomass may be potentially degraded, uncertain quality and food safety, lack of traceability, unknown growth history and potential contamination (Devault et al., 2020).

An important aspect to consider is that the main treatments to process seaweed, such as soaking, may have an important impact on the arsenicals present, thereby altering the toxicological risk of the product as sold (Almela et al., 2005). In relation to the good extractability of iAs species in water, it is expected a decrease of iAs concentrations with the rinsing or soaking of biomass. For example, Rose et al. (2007) showed that Hijiki (*Sargassum fusiforme*) soaked for consume displayed much lower iAs concentrations than Hijiki "as sold" (iAs range of 5-23 mg kg^{-1} dw as compared to iAs range of 72-96 mg kg^{-1} dw, respectively). In their study of holopelagic *Sargassum*, Devault et al. (2022a) clearly showed significantly less total As, As(V), Methylarsonic acid and arsenobetain in the dried samples recovered from the beach than in the floating algae suggesting that the loss of the different As species could be related to leaching due to rain or daily dipping by tide.

Regarding the use in feed, there are several studies on the use of *Sargassum* spp. as ingredients in feed formulations (Casas-Valdez et al., 2006; Pham et al., 2006; Marín et al., 2009; Carrillo et al., 2012; Kim et al., 2014). However, the content of TotAs and/or their chemical species in the final product have not been evaluated. According to our results, all three morphotypes of holopelagic *Sargassum* have values that exceed the existing standards for the relevant uses, with the most common value being 40 mg kg^{-1}

dw to 75 mg kg^{-1} dw for TotAs and 1 to 3 mg kg^{-1} dw for iAs in most regulations (Table 1), both for feed and agricultural uses.

The use of holopelagic *Sargassum* with greater acceptance and potential under existing regulations is in the agricultural sector (soil amendment). Stranded seaweed has historically been used in rural coastal areas, fresh or partially dried. This is the most direct and cheapest use and is still practiced locally. Direct application of seaweed requires the establishment of a nutrient management plan, which may take different regulatory forms depending on national legislation. The most controlled aspect of their use is the formation of hydrogen sulphide and the flagged sodium content as a potential contributor to soil salinization. Composting is another approach to using holopelagic *Sargassum*, although the use of "pure" seaweeds is known to be difficult and is usually supplemented with other organic waste. Sembera (2013) and Sembera et al. (2018) studied the use of *S. fluitans* and *S. natans* in co-composting, increasing the amount of *Sargassum* biomass from 2 % to 4 %, and reported that As was more prominent in the unwashed *Sargassum* biomass, but still well within the standards set by the Environmental Protection Agency for safety and for compost quality (Woodbury, 1993). At present, there are companies (Holdex, Idex and Société Martiniquaise des Eaux) that have received permission to incorporate harvested *Sargassum* into their composts. However, the leachate from these composts is an aspect that needs to be studied and monitored.

According to our results, harvesting stranded biomass during the winter season is the best option in terms of TotAs and iAs contents due to their lower proportions. However, it should also be noted that biomass abundance and strandings are usually relatively low

at this time of year, which further limits the potential for valorization of this resource. Another aspect to consider that limits the use of this biomass is its cadmium content. In fact, all the observations made on these stranded biomasses (Ortega-Flores et al., 2022) indicate Cd concentrations close to or even higher than the maximum level of 0.5 or 1 mg kg^{-1} dw allowed for food or feed (Chinese and European regulations) or 1.5 mg kg^{-1} dw allowed for organic fertilizers (European regulation).

5. Conclusion

The iAs content in stranded holopelagic *Sargassum* spp. is reported here for the first time for a full seasonal cycle. This comprehensive data set showed that iAs concentrations varied in the range of 12.7-62.9 mg kg^{-1} dw with *S. natans* I showing a lower iAs content (range 11.9-47.7 mg kg^{-1} dw) than the other two morphotypes. In contrast to TotAs, the iAs content of all morphotypes increased over the study period, resulting in a much higher proportion of iAs in the warm-dry season than in the rainy season. The lower proportions of iAs during the rainy season can be explained by different reasons that can be independent: a different origin of the biomass, a saturation of alginates-fucoidans binding sites in the algal cell walls and/or a release of iAs when biomass is leached by rain. In any case, the high iAs concentrations are such that they limit the potential use/valorisation of this biomass into feed or food products. On the other hand, its use to fertilize agricultural soils could be considered, because the iAs concentrations observed are at levels below or just above the limit of 40 µg g^{-1} for such use. In view of the important damage caused to coastal and reef habitats (e.g. Rodríguez-Martínez et al., 2019), as well as potential alteration of water quality following

the release of iAs (which is outside the scope of this study and not yet addressed by the community), it is undeniable that offshore collection must be the preferred method to collect such biomass. For valorizing a part of this biomass, further research is needed to see how inexpensive methods, such as grinding, drying on sun and/or rinsing with freshwater (on board or at land) following at sea following collection, can eliminate a significant proportion of the iAs and ensure a limited supply of this toxic substance to agricultural soils.

Author Contributions

Paulina Annette Ortega-Flores: Formal analysis, Methodology, Visualization, Writing – original draft. Tristan Gobert: Formal analysis, Methodology, Writing – review. Lia Celina Méndez-Rodríguez: Conceptualization, Investigation, Resources, Writing – review & editing. Elisa Serviere-Zaragoza: Conceptualization, Funding acquisition, Writing – review & editing. Solène Connan, Daniel Robledo, Yolanda Freile-Pelegrín and Juan Antonio de Anda Montañez: Conceptualization, review & editing. Matthieu Waeles: Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

Figure 1. Study area: Puerto Morelos, Quintana Roo, Mexico.

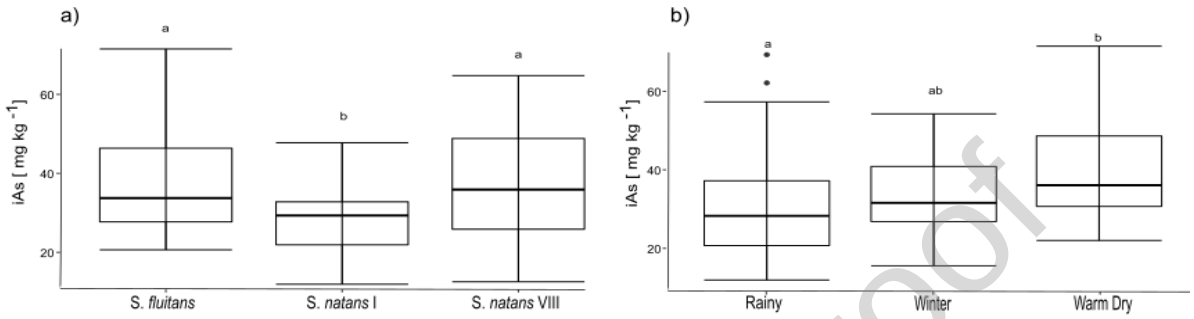

Figure 2. Inorganic arsenic content (iAs) in stranded holopelagic *Sargassum* in Quintana Roo, Mexico during an annual cycle (2018-2019) by a) morphotypes and b) season. Values represent the median, first quartile and third quartile; n = 101. Superscript letters denote statistically significant differences between morphotypes and seasons.

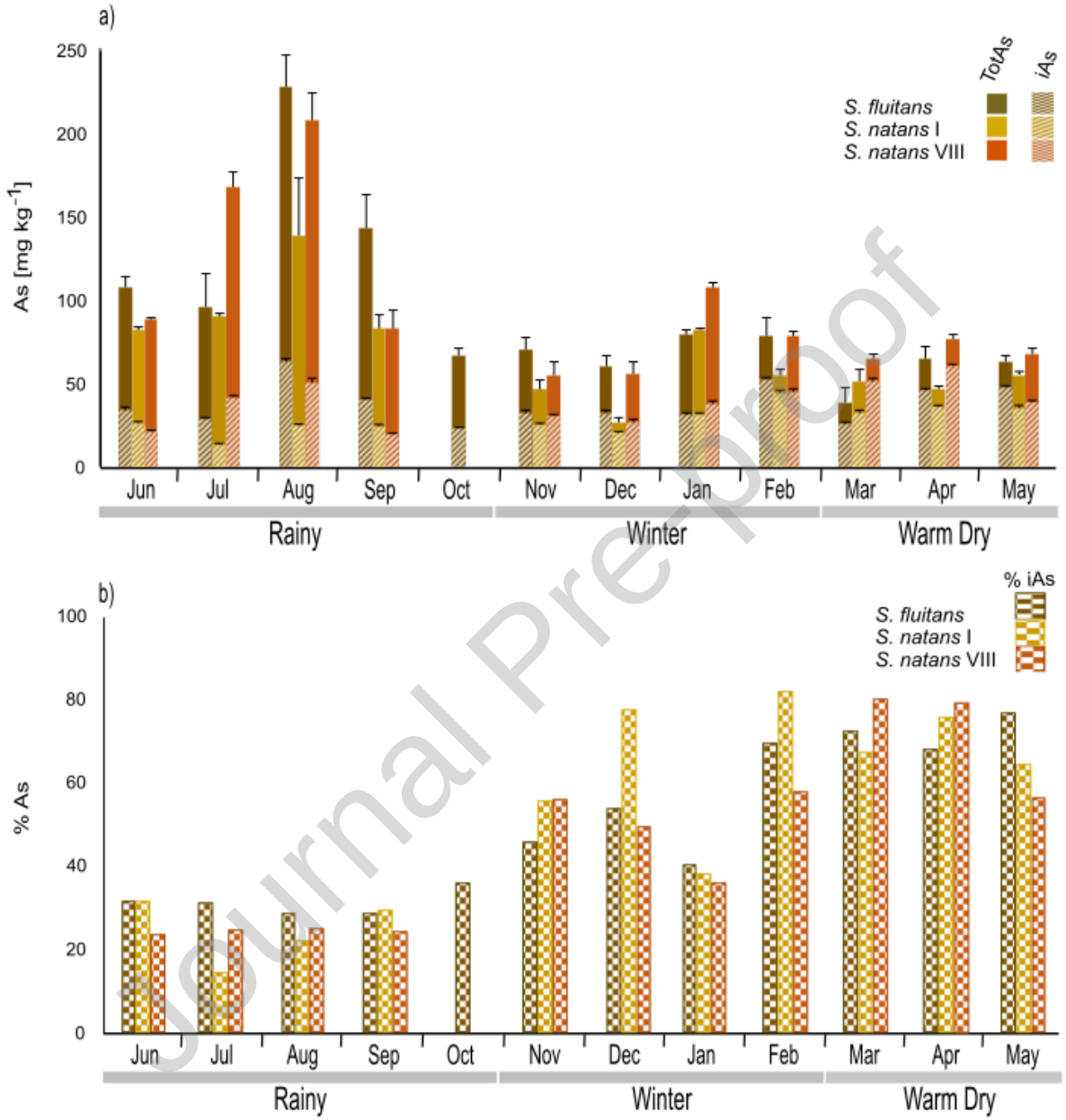

Figure 3. a) Inorganic arsenic (iAs) and total arsenic (TotAs) content (mg kg^{-1} dw, values are mean ± standard error, n=101) and b) iAs proportion (retlivaly to ToAs) in stranded holopelagic *Sargassum* in Quintana Roo, México over an annual cycle (2018-2019). Note that *S. natans* I and *S. natans* VIII morphotypes were not observed in October 2018.

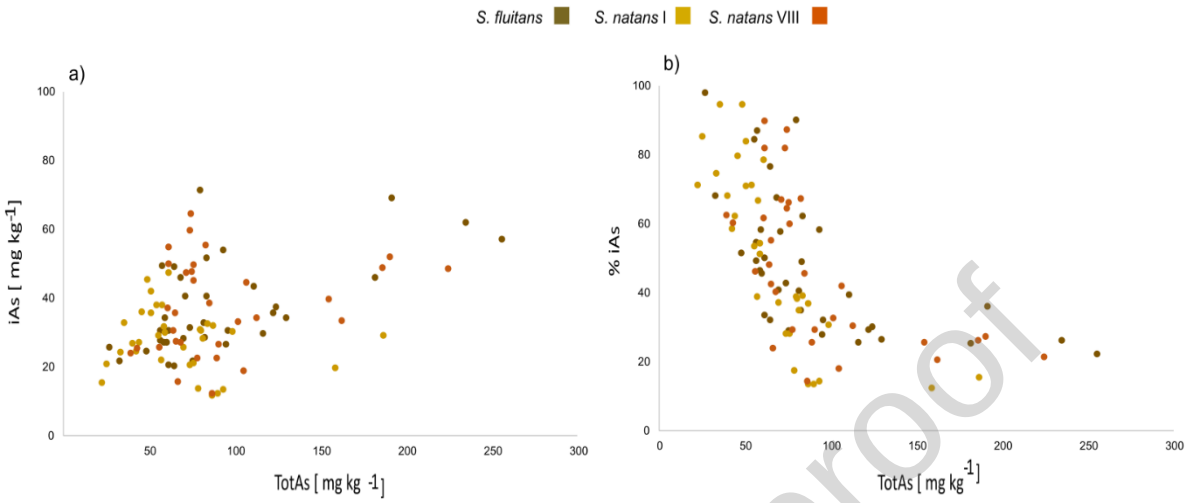

Figure 4. Distribution of data obtained for inorganic arsenic (iAs) content. a) iAs concentrations relative to TotAs content. b) iAs percent present in TotAs content. Data are in mg kg^{-1} dw.

Table 1: iAs concentrations, TotAs concentrations (mg kg^{-1}) and iAs proportions reported for holopelagic *Sargassum* spp. The collection sites and the number of samples for each study (n) is also provided. Concentrations are expressed as dry weight, except from the study by Johnson and Braman (1975) that reported concentrations as wet weight.

Location	Species	n	iAs (mg kg^{-1})	TotAs (mg kg^{-1})	iAs:TotAs (%)	Reference
Offshore						
Sargasso Sea	*S. fluitans*	1	19.5*	19.5*	100%	Johnson and Braman (1975)
South & West offshore Florida	Mixed	3	1.9-7.6*	4.2-8.7*	29-87%	Johnson and Braman (1975)
Tropical Atlantic & Sargasso Sea	*S. fluitans*, *S. natans* I & *S. natans* VIII	35	11.2-105	56-235	16-53%	Gobert et al. (2022)
Near Shore						
Off Caicos Island	*S. fluitans*	1	19.3	58	33%	Nielsen et al. (2021)
Off Martinique Island	Mixed	13	26.6-95.5	47.7-128	45-77%	Devault et al. (2022a)

Stranded						
British Virgin Islands (Beach)	Mixed	1	27.7	45	62%	Ocean Harv. Tech. (2016)
Martinique (Beach)	Mixed	1	68	98	70%	Ocean Harv. Tech. (2016)
Martinique (Beach)	Mixed	44	3.7-96.3	9.5-128	26-87%	Devault et al. (2022a)
Martinique (Beach and Mangrove)	*S. fluitans*, *S. natans* I & *S. natans* VIII	6	6.2-15.8	39.7-51.7	16-30%	Gobert et al. (2022)
Mexican Caribbean (Beach)	*S. fluitans,* *S. natans* I & *S. natans* VIII	34	12.7-62.9	21.7-255	14-82%	This study

* wet weight.

Table 2. Maximum levels of TotAs and iAs (mg kg^{-1} dw) allowed in algae for different applications

Country or agency	**Feed**		**Agriculture**		**Food products/ Human consumption**	
	TotAs	**iAs**	**TotAs**	**iAs**	**TotAs**	**iAs**
Canada[1]	8	-	13 - 75	-	-	-
USA[2]	-	3	41	-	-	3
Mexico[3]	-	-	22 - 75	-	-	-
Costa Rica[4]	-	-	60	-	-	-
Chile[5]	40	-	10- 40	-	-	-
Europe[6]	40	2	30 - 50	40	-	-
France[7]	40	2	18	-	-	3

Australia & New Zealand[8]	-	-	-	-	-	1
China[9]	-	-	-	-	-	1

[1]Canadian Food Inspection Agency (CFIA): RG-8 Regulatory Guidance: Contaminants in Feed and Regulatory requirements for fertilizers and supplements – Index of Trade Memoranda; [2] National Research Council (US NRC): Mineral Tolerance of Animals, Code of Federal Regulations (CFR): 40 CFR 503 and Food Chemicals Codex; [3]Norma Oficial Mexicana (NOM): NOM-147-SEMARNAT-SSA1-2004 and NOM-004-SEMARNAT-2002; [4]Reglamento Técnico Centroamericano (RTCR): 485:2016; [5]Servicio Agrícola y Ganadero (SAG): Resolución n° 7.885; [6]Commission Regulation (EU): n° 2019/1869 and n° 2019/1009; [7]Agence Française De Securite Sanitaire Des Aliments (AFSSA): 2007-SA-0007; [8]Australia New Zealand Food Standards Code: Standard 1.4.1; [9]GB2762-2017 National Food Safety Standard of Pollutants in China.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Inorganic arsenic (iAs) content was monitored in stranded holopelagic *Sargassum* from the Mexican Caribbean
- The iAs content ranged from 12.7 to 62.9 mg kg^{-1} representing between 14.1 % and 81.7 % of total arsenic (TotAs)
- The proportion of iAs increased over the period studied
- The highest iAs content was found in the warm-dry season with an annual mean of 41.0 mg kg^{-1}
- The iAs content is well above the levels allowed by international guidelines