Unusual holopelagic *Sargassum* mass beaching in North West Africa: morphotypes, chemical composition, and potential valorisation

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Highlights

- *Sargassum* mass beaching event in Senegal reported by participatory approach
- Four morphotypes identified: *S. fluitans* III dominant and a putative new morphotype
- Low As, but relatively high Cd and Hg levels in Senegalese *Sargassum vs.* Atlantic Ocean
- High total protein contents and intra-thallus variation of biochemical composition
- High concentrations of rare Pd in *Sargassum* tissue

Abstract

The rapid proliferation of holopelagic *Sargassum* in the tropical Atlantic Ocean presents environmental challenges and economic opportunities. In 2022, Senegal witnessed its first significant holopelagic *Sargassum* beaching event, triggering widespread concern and interest from civil society, industrial sectors and government. This study represents the first analysis of stranded holopelagic *Sargassum*'s morphotypes and chemical composition in North West Africa. We highlight the distinct nature of *Sargassum* stranding in Senegal, dominated by *S. fluitans* III, and describe a novel morphotype. Compared to the tropical Atlantic, Senegalese *Sargassum* displayed lower arsenic concentrations, higher cadmium levels, and increased mercury content. The biochemical analysis revealed high total protein levels in Senegalese samples. Furthermore, variations in biochemical composition within various parts of the *Sargassum* thallus were observed. These findings offer valuable insights into potential applications and limitations. Our study identifies promising uses in Senegal and neighbouring countries, mainly for animal feed and agriculture and in the chemical industry, where notable palladium content is observed and, to a lesser extent, the phenolic compounds and mannitol. Our interdisciplinary approach enhances the global scientific understanding of the *Sargassum* issue and aids in developing sustainable strategies for African coastal regions grappling with climate change and invasive species. With the anticipation of more frequent *Sargassum* beaching events and more generally for seaweed exploitation, we advocate for inter-governmental African organisations to establish standardised norms for their exploitation. We also recommend that the Food and Agriculture Organization/World Health Organization consider incorporating more seaweed in the *Codex Alimentarius*.

Keywords: Invasive species; Participatory approach; Trace metals; Biochemical composition; Exploitation; Senegal

1. Introduction

Under the effect of climate change and eutrophication, the tropical Atlantic Ocean and the Caribbean coasts have been regularly invaded by holopelagic *Sargassum* since 2011 [1]. This periodical phenomenon had severe socio-economic, sanitary, and environmental impacts [2], affecting the African coast [1]. In general, holopelagic *Sargassum* plays an essential role in ocean carbon sequestration [2], supports many marine organisms [3], and has been identified as 'Essential Fish Habitat' [4] [2]. The 'Sargasso Sea' [2], and recently, the 'Gulf of Mexico' and the 'Northern Equatorial Recirculation Region' are source regions of *Sargassum* [5] [2], representing oceanic zones characterized by the most substantial aggregations of this seaweed.

However, driven by environmental alterations affecting the Atlantic circulation and coastal nutrient levels, the system gets imbalanced, and *Sargassum* starts to spread. Consequently, *Sargassum* stranding is now a new norm for countries to adapt.

Besides the recognised massive invasions in the Caribbean and the Gulf of Mexico, *Sargassum* increasingly reaches the African coastline from Morocco to the Gulf of Guinea [2]. Firstly reported from Ghana, West Africa, in 2009, the holopelagic *Sargassum* [6] spread further to Morocco in 2011 [7]. Research suggests that *Sargassum* cycles are closely linked to seasonal changes in sea surface temperature. Nutrient fluxes from the Congo River, Amazon River and Northwest Africa iron-rich dust were raised as a likely cause of massive *Sargassum* blooms in West Africa [7]. Satellite images showed bands of *Sargassum* more or less close to Senegal in 2021 and 2022 [8].

Two major holopelagic *Sargassum* species (*Sargassum fluitans* and *S. natans*) are known from the Atlantic Ocean, of which several distinct morphotypes have been identified [8]. *Sargassum fluitans* III, *S. natans* I, and *S. natans* VIII are the three major morphotypes encountered in the Caribbean [8] [9], without the possibility, for the moment, of elevating them to the rank of three species although their morphology and genetic are different [10]. In addition

to being morphologically distinct, these three morphotypes show distinct ecological, biological, physiological, and chemical traits [8] [11]. The predominance of one morphotype influences the chemical composition of a mixed *Sargassum* content, which has implications for its potential uses [1] [8]. To date, no information is available concerning the identity or chemical composition of morphotypes in Senegal.

Over 20 million tons of drifting *Sargassum* were detected in 2018 within the Atlantic *Sargassum* Belt [12], demonstrating the urgency of finding a solution to handle these substantial drifting biomasses. Clean-up efforts generate costs to national economies of hundreds of millions of dollars [1]. Given its recognised valuable composition (*e.g.* minerals, nutrients, phenolic compounds, proteins, carbohydrates and lipids), there is a growing interest in using the algal biomass for animal feed and human food production, agriculture, energy production (*e.g.* biofuel) and the chemical industry [1] [2]. Before turning the stranded *Sargassum* into business opportunities, it is necessary to know the predominance and composition of the present morphotypes and their level of contamination with pollutants. The World Health Organisation classifies arsenic as one of the ten chemicals of greatest public health concern, and there have been health advisories worldwide concerning arsenic in *Sargassum* [13]. Total arsenic concentrations in marine algae are high and generally range from 10 to 100 mg kg^{-1}, reaching 200 mg kg^{-1} in *Sargassum* [14]. Regarding *Sargassum*, high C:N ratios make them more applicable for biofuel production [15]; a C:N value between 20 and 35 is recommended for biogas production [16]. Using *Sargassum* for biogas production could be difficult, and further work is needed [13].

The present study investigated the morphological and chemical composition of the first massive *Sargassum* stranding at the Senegalese coast to study their preliminary potential value for commercial and societal applications. Various morphotypes of holopelagic *Sargassum* were identified in the beachings, and their chemical content *vs*. nutritional value and toxicity level

were analysed for the first time, and compared with works done in the African, Caribbean and Mexican regions.

2. Materials and Methods

Information was gathered to investigate the massive *Sargassum* stranding using i) a scientific sample collection and ii) an accompanying participatory citizen approach.

2.1 Study area and biomass collection

Senegal is part of the Canary Current Large Marine Ecosystem (CCLME), an area under the influence of currents from the Canary Islands and Guinea [17]. Seven sampling sites (Kayar, Cambérène, Ngor, Somone, Mbour, Joal-Fadiouth in Senegal and Banjul in Gambia) were visited during the stranding events between August 11 2022 and September 17 2022 (Figure 1; Table 1). Ngor site is under the marked influence of untreated urban effluent [18]. The presence of wastewater treatment plants and discharges of treated wastewater characterises the Camberene site. The Somone, Mbour and Joal-Fadiouth sites, situated in the south of Senegal, are marked by a low level of urbanisation [19]. *Sargassum* thalli were collected by hand near the coast at each sampling site, submerged in water up to ~15 cm depth and on the beach. *Sargassum* thalli were collected until they filled ~ three-quarters of a 25-litre icebox. The biomass was transported in empty iceboxes and preserved in a freezer (~ -18 °C) or sun-dried according to the processing method (see below). Twenty-three samples were analysed (Table 1, Figure 1).

2.2 Participatory citizen approach

Building on precedent experiences during former *Ostreopsis* cf. *ovata* and *Noctiluca scintillans* blooms in the area [20] [21], a network of civil society and academic community volunteers was motivated to report the Senegalese 2022 stranding. Additionally to such a participatory approach, a network of fisheries investigators from the National Oceanographic Research Centre (ISRA/CRODT) contributed to this effort. While primarily tasked with monitoring artisanal fishing landings, these investigators willingly gathered information on the presence and rough

estimates of the stranding using digital tablets [22]. These devices facilitated the capture of coastline photographs and close-up images of the *Sargassum*, enabling comprehensive documentation of the events, *e.g.* nuisances, identification of the *Sargassum*, epiphytes and symbiotic organisms (Supplementary S1).

2.3 *Sargassum* and morphotype identification

Sargassum specimens were categorized into morphotypes using established taxonomic keys, employing specific criteria such as vesicle shapes [23] [24], presence of spines on vesicles or stems and measurement of leaf length, width and density [25] [6] [26]. Four distinct morphotypes were delineated following taxonomic keys. *Sargassum natans* I (SnI) was assigned when criteria including elliptical vesicles, the presence of spines on vesicles, and long, narrow blades were met (see Supplementary S2 for illustrations). *Sargassum fluitans* III (SfIII) was identified by elliptical vesicles, the absence of spines on vesicles, and short, wide blades. *Sargassum natans* VIII (SnVIII) was characterized by globular vesicles, long and wide blades, and the absence or rare presence of spines on vesicles; in the latter case, spines were also associated with elliptical vesicle forms.

For a chemical differentiation between the morphotypes, dry samples underwent analysis using a DRX 500 NMR spectrometer equipped with a HR-MAS probe (Proton High-Resolution Magic Angle Spinning Nuclear Magnetic Resonance HR-MAS ^{1}H NMR). The sample consisted of a dry algal stem, which was then placed in a zirconium oxide vial with ~30 µL of deuterated water (D_2O). The assembly was rotor-oriented at the so-called "magic angle" of 54.7° to respect the B0 magnetic field. Good homogenisation was achieved at around 5000 Hz. This approach provides a resolution comparable to liquid solution NMR, facilitating the analysis of the major metabolites within the sample. The outcome was a distinctive chemical fingerprint of the seaweed, allowing for meaningful comparisons and discrimination between different samples. Notably, this technique previously demonstrated its efficacy in distinguishing various

Sargassaceae species, including five within the *Cystoseira* genus [27], two closely related species within the *Turbinaria* genus [28], and diverse populations of European *S. muticum* [29]. Spectra were acquired for each morphotype (SnI, SfIII, SnVIII, and a putative new one (MF) describe in section 3.1) in the current study to corroborate their classification through chemical fingerprinting.

2.4 Analysis of *Sargassum* content

2.4.1 Sample preparation

Except for sun-dried specimens, all samples underwent a brief soak in demineralised water, subsequent drying, and grinding using an MM 200 (Retsch) grinder at 20 Hz for six minutes. Nine samples were oven-dried at 50°C for 48 hours (Table 1), while fourteen samples were freeze-dried using the CHRIST Alpha 1-4 LSC basic freeze dryer with primary desiccation for 69 hours at a set pressure of 1 mbar and secondary desiccation for 2 hours (Supplementary S3). Additionally, two samples were sun-dried to compare to freeze-dried samples for the biochemical composition (Table 1).

2.4.2 Heavy metal analysis

Concentrations of arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), copper (Cu), zinc (Zn) and nickel (Ni) were measured using a Niton XLT900s energy dispersive X-ray fluorescence containing a 50 kV X-ray excitation tube and silver Ag as an excitation source. The amperage was 40 A and a maximum power of 2 W. The resolution was fixed at 178 eV. The samples were measured in the mining Cu/Zn mode. This non-destructive technique measures concentrations independent of the chemical state of an element. The ground sample was transformed into a pellet before analysis. A certified reference material (International Atomic Energy Agency Reference Materials Group, IAEA – 461, Trace Elements in Clam) was used to prepare standard samples and establish the calibration curves. The internal standard method eliminated or assessed

the matrix effect. The results are expressed in parts per million dry weight (ppm DW) after carrying out the analysis in each sample [30] [31].

Concentrations of palladium (Pd), titanium (Ti), lithium (Li), boron (B), barium (Ba), europium (Eu) and aluminium (Al) were measured using an Inductively Coupled Plasma Mass Spectrometry (ICPMS iCAPRQ Thermoscientific). Microwave Digestor-Multiwave GO Plus (Anton Paar) was used for sample digestion using acids (Nitric acid HNO_3 and hydrochloric acid HCl). All elements were measured in total, and if any elements have multiple isotopes, the ICPMS system selects higher mass-intensity isotopes without any interference from any source [32].

2.4.3 Cation analysis

Samples were oven-dried at 60°C for 30 minutes to remove residual moisture. The subsequent mineralization protocol started with weighing 0.5 g of dry sample, which was then carefully placed in a 10 mL tube. Calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), iron (Fe) and manganese (Mn) content analyses were performed with microwave plasma atomic emission spectroscopy (MP AES 4200-Agilent) [33] [34]. Nitric acid (HNO_3) 69% was used as the digesting acid. Next, 3 mL of HNO_3 was added to the tube gradually to avoid foaming, then left overnight (cold digestion). The tubes were placed in a mineraliser (DigiPREP Jr, SCP Science) and preheated at 100°C for two hours. The tubes were then left to cool, and after that, 1 mL hydrogen peroxide (H_2O_2) and 1 mL HNO_3 were added to the tubes and preheated at 100°C for one hour. After mineralisation, the solutions were filtered and adjusted in 100 mL flasks with demineralised water, constituting the solutions to be analysed. After filtration, mineral elements were assayed by MP AES.

2.4.4 Analysis of total phosphorus, total carbon, total nitrogen and ash content

The total phosphorus (P) content analysis was performed with a continuous flow molecular adsorption spectrophotometer SEAL AA3 Analyzer following the same mineralisation and

filtration procedure used for mineral analysis with the MP AES. The analysis uses the Murphey and Riley method [35] (Supplementary S3). The total carbon (C) and total nitrogen (N) content analyses were performed with a CHN Thermo Scientific Flash 2000 Elemental Analyzer. The analysis method is based on the principle of the « Dumas method » [36]. For ash content, samples were burnt in an oven at 500°C for 2 hours after a one-hour stage at 200°C.

2.4.5 Extraction and assay of phenolic compounds and DPPH radical scavenging activity of phenolic extracts

The protocol to extract phenolic compounds was developed and miniaturized from Gager et al. [37]. Briefly, 15 mg of algal powder was extracted in triplicate in 1.5 mL ethanol:water (50:50, v/v) for 15 min in an ultrasound bath, followed by 2 h in the dark at 40°C, under magnetic stirring. Samples were then centrifuged (Eppendorf Centrifuge 5810, Germany) for 5 min, and the supernatant was recovered. The pellet was extracted a second time for 1 h followed by centrifugation. A volume of 3 mL of supernatant was obtained for each sample and evaporated at 40°C using a speedvaccum (DUC-23050-B00, minVac, Royaume-Uni) to obtain the crude extract. Phenolic contents were determined using the Folin-Ciocalteu method, which uses a standard curve of phloroglucinol [38] [39]. The microplates were filled by depositing 20 µL of each of the samples (crude extract or standard), 130 µL of distilled water, 10 µL of Folin-Ciocalteu reagent, and, finally, 40 µL of calcium carbonate. After shaking, the microplates are placed in an oven at 70°C for ten minutes. The reaction was then stopped by setting the microplates on crushed ice. After further stirring, the optical densities (OD) are read at 620 nm using a microplate reader spectrophotometer (S/N 415-0039, Omega, Germany). The standard range is prepared from phloroglucinol of known concentrations and using a stock phloroglucinol solution at 100 mg L^{-1}. All samples were tested in triplicate. The dry crude extract of phenolic compounds was reconstituted to different concentrations to be assayed. Phenolic content is expressed as mg of phenolic compounds per gram of the dry seaweed powder or dry weight (mg g^{-1} DW).

To test the activity of phenolic compounds, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) experiment was used to measure the radical scavenging activity of phenolic compounds. This test was developed by Fukumoto and Mazza (2000) [40], then modified by Molyneux (2004) [41], Turkmen et al. (2007) [42], Le Lann et al. (2008) [28], and Zubia et al. (2009) [39]. All tests were performed in triplicates. The IC50 was then calculated, *i.e.*, the concentration at which 50% of the DPPH free radical is inhibited. Butylhydroxyanisole (BHA), Butylhydroxytoluene (BHT), 6-hydroxy-2,5,7,8-tetramethylchromane-2- carboxylic acid (Trolox), together with Vitamins C and E were used as positive controls. Crude extracts and positive controls were compared and used at three different concentrations (0.1, 0.25 and 0.5 g L^{-1}).

2.4.6 Extraction and assays of mannitol, soluble and insoluble protein

For mannitol, the extraction and quantification procedure followed the protocol established by Chades et al. [43] and Sanchez [44]. All samples and standards were tested in triplicates. The mannitol standard range points were prepared from mannitol dissolved in hydrochloric acid at 0.05 mol L^{-1}. The range was prepared from zero to 1500 nmol mL^{-1}. All samples and standards were tested in triplicates.

For soluble and insoluble proteins, 50 mg of algal powder was placed in an Eppendorf tube, to which a phosphate buffer was added (pH = 8). EDTA was added, followed by 2-mercaptoethanol. After shaking, the samples were placed in an ultrasonic bath to destroy cell-cell bonds. The samples were then stirred again for four hours at room temperature. They were then centrifuged, and the supernatant was used for soluble protein assay. NaOH was added to the residue and shaken for one hour at room temperature. After centrifugation, the supernatant containing insoluble proteins was recovered. Soluble and insoluble proteins were assayed using the BCA (Bicinchoninic acid) method. Two standard ranges were prepared: a first for the determination of soluble proteins with bovine serum albumin (BSA) in distilled water, then a

second for the determination of insoluble proteins with BSA in NaOH at 0.5 mol L^{-1}. All samples and standards were tested in triplicate.

2.5 Statistical tests and comparative analysis

The variables related to content of phenolic compounds, mannitol, soluble and insoluble proteins were tested in triplicate and are presented as mean ± standard deviation. The other variables, tested individually, were reported as measured value. Statistical analyses (normality and homogeneity tests and analysis of variance) were carried out using R Studio (version 4.2.2). Levene's and Shapiro-Wilk's tests were used to check homogeneity and normality. Statistical analyses were performed with a 5% risk. The various grades were compared using a one-factor analysis of variance.

A comparative analysis of heavy metal contents was conducted, explicitly focusing on the comparison with data obtained from 24 other Atlantic regions spanning from East to West Atlantic [1] [6] [45] [13] [46] [47] [48] [49]. For comparison, the measured content or, when relevant, the average value was used. In cases where variability was observed, both maximal and minimal values were considered. When variability was absent, the average value was utilized twice for a more robust and representative comparison. In the comparative analysis, (1) no distinction was made between the different morphotypes; (2) the values below the limit of detection were not considered; (3) no discrimination was done between sea and beach samples (4) nor on the analytical methodology used (Table S1). Data from this study and the ones from the references were displayed on map representations. Data are classified using the method of natural breaks (Jenks) [50] [51]. The Jenks optimisation method, also known as the Jenks natural break classification method, is a data clustering method designed to determine the best arrangement of values into different classes. This method identifies logical breakpoints in a data set by grouping similar values that minimise the differences between data values in the same

class and maximise the differences between classes. This method can be beneficial as it identifies actual classes within the data [52].

The C, N and P mass concentrations were converted into molar concentrations. From that the C:N, C:P, N:P and C:N:P molar ratios were calculated and compared to the Redfield ratio [4] [53] and those in the literature.

3. Results

The participatory approach provided an early warning from Senegalese citizens, who reported the massive arrival of *Sargassum* through visual observations (from air and on sea) on July 25 2022 (Fadel Diedhiou and Yannick Pensard, pers. comm. – Supplementary S1). Massive *Sargassum* grounding was observed in central and south Senegal (from Kayar to Casamance) between July 28 and August 22 and no observation was reported in the northern part of Senegal (Saint-Louis). There was no report of severe human health issues. The participative survey revealed only minor skin irritations reported during recreational swimming in direct contact with *Sargassum*. Additionally, two turtle deaths were documented at the exact location on August 22 2022. However, it was observed that the *Sargassum* invasion might not necessarily be attributed as the direct cause of this mortality.

3.1 Morphotype classification of Senegalese samples

Four distinct morphotypes (SnI, SfIII, SnVIII, and a putative new morphotype (MF)) were identified following taxonomic keys. Some specimens classified as SnVIII showed vesicles similar to *S. granuliferum* [23]. SnVIII can be distinguished from SnI and SfIII based on its larger bladders and blades. The MF is described for the first time, as it was repeatedly observed and did not correspond closely to the taxonomic criteria of SnI, SfIII and SnVIII. Moreover, MF was exclusively identified during collection in Kayar on 17/09/22. This particular morphotype shares identical morphological characteristics with MA, with the notable exception of the presence of spines.

SnI, SfIII and SnVIII were encountered in almost all studied sites. SfIII was the most dominant morphotype. The sample set included SnI, SfIII, SnVIII, and MF. The MF is unrelated to any of the most common morphotypes cited and was of putative species assignment.

3.2 Analysis and determination of morphotypes by HR-MAS ^{1}H NMR

Chemical fingerprinting spectra obtained for each morphotype (Figure 2) showed that all samples presented signals between 3.5 and 4 ppm, confirming the presence of mannitol. Higher signals of mannitol were more characteristic for SnVIII. Signals in the 5.9 and 6.4 ppm zone correspond to the presence of aromatic groups and, therefore, to phenolic compounds. It is difficult to distinguish the morphotypes based on the portion of aromatic groups due to the similarity of the spectra and the non-presence of high peaks in this section of the spectra. Samples assigned to SnI had similar spectra under 3.5 ppm, easily differentiated from the other morphotypes. SfIII might be distinguished from SnVIII by comparison of spectral signals between 1 and 1.5 ppm.

Meanwhile, SfIII might be distinguished from SnI based on comparison of spectral signals under 3.5 ppm. Finally, MF exhibited almost the same morphological characteristics as SnI; one might expect its chemical fingerprint to resemble that of SnI, but this was not the case. The spectra of MF under 1.5 ppm were quite different from those of SnI under 1.5 ppm in their relative peaks. The spectra of MF between 1.0 and 4 ppm were also quite different from those of SnVIII, with the later having higher peaks in this spectral section. The spectrum of MF between 4.5 and 5.0 ppm was clearly different from that of SnI, SfIII and SnVIII. MF had the particularity to present a more marked peak towards 2 ppm than the other morphotypes. Within SfIII, the chemical fingerprinting of one of the samples (sample ID: Mb2) deviates from the chemical fingerprintings of the other samples.

3.3 Elemental variation in holopelagic *Sargassum* across multiple sites

The As concentrations of the mixed morphotypes from Kayar, Camberene, Somone, Mbour and Joal-Fadiouth were 15, 21, 23, 11 and 11 ppm, respectively. Notably, these concentrations exhibited a two-fold difference between minimum and maximum values, with the highest As values observed at the Camberene and Somone sites. In contrast, Pb and Hg showed a relatively more homogeneous spatial distribution across sites, with 1.4 and 1.2-fold differences, respectively. These mixed morphotypes' mean values of Pb and Hg were 1.31 and 0.51 ppm, respectively. Considering all analysed samples (combining values from mixed and single morphotypes), Zn concentrations displayed the highest variability with a five-fold difference, followed by As (three-fold difference), copper (Cu) and cadmium (Cd) with a 1.6-fold difference each (Table 2). The Fe content was higher (almost 2-fold to 4-fold difference) at Joal-Fadiouth (1017 ppm) than at the other sites (552, 464 and 237 ppm for Somone, Kayar and Cambérène, respectively).

3.4 Elementary chemical content, pattern for the mixed morphotypes and single morphotypes of *Sargassum*

3.4.1 Arsenic, cadmium, lead and mercury concentrations

Regarding the heavy metals of the morphotypes from Kayar, the concentrations of As, Cd, Pb, and Hg followed a consistent pattern across SfIII, SnVIII, and SnI, with the order As>Cd>Pb> Hg, except in the case of MF where Cd replaced As (Figure 3). Similarly, the heavy metal concentrations of the mix morphotypes in the areas of Kayar, Camberene, Somone, and Mbour also showed a similar pattern with As>Cd>Pb> Hg, except for Joal-Fadiouth sample, which displayed a Cd concentration higher than the As concentration. Among the morphotypes, concentrations were highest in SnI, followed by SnVIII, SfIII, and MF for all the heavy metals except lead. Concentrations of these elements were generally higher in the mix morphotypes of the Somone site compared to the other sites for all these elements.

3.4.2 Comprehensive elemental content

Concerning all elements analysed in *Sargassum* samples (mixed morphotypes and single morphotype), the most predominant elements followed the order: Ca>K>Mg>Na>Fe> Zn, while the least predominant elements were ranked as Zn>Cu>As>Cd>N>Pb> Hg (Table 3). Ni, Zn, and Cu concentrations were highest in SnI, followed by SnVIII, SfIII, and MF, except for the similar Cu concentrations observed in SnVIII and SfIII (Figure 3). Ca, K, Mg, Na, and Mn concentrations were notably higher in Camberene mixed morphotypes than in other sites, while samples from the Joal-Fadiouth site generally showed lower concentrations for all elements (Figure 4). Additional results on Pd, Ti, Li, B, Ba, Eu, and Al contents (sample ID: Ng) provide a more comprehensive perspective.

3.5 Concentrations of carbon, nitrogen, phosphor, ash

The C:N molar ratios of *Sargassum* from Kayar, Camberene, Somone and Joal-Fadiouth were relatively close, 27, 22, 21 and 24, respectively. The C:P molar ratios of *Sargassum* from Kayar, Camberene, Somone and Joal-Fadiouth were 476, 495, 429 and 628, respectively, also relatively close except for Joal-Fadiouth. The N:P molar ratios of *Sargassum* from Kayar, Camberene, Somone and Joal-Fadiouth were also aligned, measuring 18, 22, 21 and 26, respectively.

Notably, total nitrogen (N) exhibited a limited representation in the principal components analysis (PCA) (Figure S1). The Kaiser-Meyer-Olkin (KMO) accuracy measure was 0.5 for the complete model and each variable. The PCA accounted for 89.9% of the variations (PC1 - 60.6 and PC2 - 29.3%) (Supplementary S4). Preliminary chemometric evaluation of the elements measured through PCA of four mixed morphotypes (ID: Ka, Ca, So, and JF see Table 1) across 17 different parameters revealed positive correlations among the elements Cd, Hg, Ni, P, Pb, Cu, and As, as well as among Mg, Ca, ash, Na, and K. Similarly, positive correlations were observed among Zn, Fe, and C. Conversely, Mn exhibited negative correlations with Pb, Cd, Hg, Ni, P, and Cu, while Fe showed negative correlations with Ash, Na, K, Mg, and Ca (Table

S2; Figure S1). Remarkably, the *Sargassum* from the Joal-Fadiouth site stood apart from the other samples due to its higher loadings of C and Fe (Figure S1).

3.6 Biochemical composition variability in different *Sargassum* morphotypes: phenolic, mannitol, soluble, and insoluble protein analysis

All the morphotypes studied produced phenolic compounds (Table 1), with levels varying from one sample to another, with a maximum factor of six. Despite variations within the same morphotype, no statistically significant difference was observed among the morphotypes ($p = 0.642$). Notably, SnVIII (ID: Ka2) exhibited the highest phenolic content (32.31 ± 0.31 mg g^{-1} DW), followed by SnVIII (ID: Mb1) (21.7 ± 0.52 mg g^{-1} DW) and SnI (ID: Ka1) (20.59 ± 1.05 mg g^{-1} DW). In contrast, SfIII (ID: Ka3) displayed the lowest phenolic content, measuring 5.19 ± 0.49 mg g^{-1} DW. Moreover, while several samples showed no radical scavenging activity, SnVIII (ID: Ka2) demonstrated an interesting radical scavenging activity close to positive controls.

Mannitol contents, expressed in mg g^{-1} of dry matter, were found to be in the same order of magnitude across all Senegalese *Sargassum* samples analysed, ranging from 1.25 ± 0.14 mg g^{-1} DW for SnVIII (ID: Mb1) to 3.27 ± 1.65 mg g^{-1} for SnI (ID: Ka1). The results indicated no significant differences between morphotypes ($p = 0.141$).

Regarding soluble protein contents, all samples analysed displayed varying levels, with no significant differences observed among the morphotypes ($p = 0.501$). Notably, the sample with the highest soluble protein content was SnVIII (ID: Ka2) (251.33 ± 19.12 mg g^{-1} DW), while the sample with the lowest was SnI (ID: Ka5) (78.14 ± 25.28 mg g^{-1} DW). Conversely, the levels of insoluble protein differ significantly between morphotypes ($p = 0.027$), with levels ranging from 91.93 ± 9.31 mg g^{-1} DW for SfIII (ID: Ka3) to 163.94 ± 8.67 mg g^{-1} DW for SnI (ID: Ka1) (Table 1).

3.7 Intra-thallus variability and effect of sun-dried vs freeze-dried methods

Different thallus parts were analysed for the SnVIII morphotype to study the intra-thallus variability of metabolite contents. An observed intra-thallus variation in associated content was observed for each compound analysed. Leaves appeared to exhibit higher phenolic (28.52 ± 4.39 mg g^{-1} DW) and proteins (236.33 ± 3.19 mg g^{-1} soluble DW and 173.21 ± 19.67 mg g^{-1} insoluble DW) contents, while vesicles showed relatively higher levels of mannitol (4.58 ± 1.47 mg g^{-1} DW) (Table 1).

The valuable compounds also varied according to the drying method. The sun-dried samples collected in Gambia (ID: Ga) and Senegal (ID: Ng) exhibited significantly different levels of phenolic compounds (PC) and soluble proteins (SP) compared to the freeze-dried samples (PC: $p = 0.022$; SP: $p = 0.038$). However, the levels of mannitol ($p = 0.582$) were similar to those of the freeze-dried samples.

4. Discussion

4.1 Morphotypes discrimination using HR-MAS ^{1}H NMR

Based on the analysis of spectra reported in the results section, the mannitol signals on the HRMAS spectra may be the part of the spectra that can help distinguish the SnVIII from the other morphotypes. In contrast, the part of the spectra below 3.5 ppm can help to distinguish the SnI from the different morphotypes. However, further research is needed to confirm these observations. For the MF, which is unrelated to any of the most frequently cited morphotypes and whose species assignment is unclear, it could be concluded that MF differs from SnI and SnVIII mainly on the basis of morphological and chemical fingerprinting comparisons (particularly in the apolar area below 3 ppm). However, a difference between SfIII and MF could be suggested mainly based on the peculiarity of MF to present a very distinct peak (doublet) around 2 ppm. Within the SfIII, the chemical fingerprint of one of the samples (sample ID: Mb2) differs from the chemical fingerprints of the other samples. This deviation

458 could be due to the presence of epiphytic films (Supplementary S1, Pictures j, k, l, p and q)
459 which could interfere more or less with the *Sargassum* spectra, depending on their abundance.

460
461 ### 4.2 Concentrations of non-essential elements, heavy metals of Senegalese *Sargassum*:
462 Atlantic comparative analysis
463 #### 4.2.1 Insights into the chemical elements data of Senegalese *Sargassum*

464 When comparing the minimum values of the different studies carried out in the Atlantic area,
465 Senegal consistently recorded the lowest value for As (Figure 5; Table S3). Similarly, when
466 analysing the maximum values, Senegal had the lowest value compared to the other Atlantic
467 stations (Table S4). On the other hand, the concentration of Cd was significantly higher in
468 Senegal, ranging from 3 to 102 times higher than in other regions, except for two sites in Ghana
469 and an area studied in the Middle Atlantic Ocean near to Guadeloupe (French West Indies)
470 (Table S5). This trend persisted when compared with the maximum value, with the same
471 exceptions in Ghana and the Middle Atlantic Ocean near to Guadeloupe (French West Indies)
472 (Table S6). For Hg, the comparative analysis of the minimum and maximum values showed
473 that the concentration in Senegal was more than 40 times higher than in the other countries,
474 with only Ghana recording a value more than two times higher than in Senegal (Tables S7 and
475 S8). The analysis of the minimum Pb values (those of the present study and those of the different
476 studies) showed that the concentration in Senegal exceeded other regions by three to ten times,
477 except for Ghana, where it was 80 times higher than in Senegal, and the Dominican Republic,
478 where it was four times higher (Table S9). Similarly, the maximum Pb value in Senegal
479 exceeded that recorded in other regions by three to twelve times, except in Ghana (208 times
480 higher), the Dominican Republic (seven times higher) and Jamaica Fort Rocky (1.5 times
481 higher) (Table S10).

482 Arsenic concentrations were higher in oceanic than neritic zones (Figure 5A and Figure 5B).
483 This may be due to the antagonistic relationship between As and P [54] [55], as neritic zones are
484 richer in P than oceanic and oligotrophic zones [4], which may imply lower As concentrations

in neritic zones than in oceanic zones. In our present study, working on beached *Sargassum* samples and P-rich waters, thalli presented low contents in As, confirming that Arsenic concentrations in holopelagic *Sargassum* species are controlled by the availability of phosphate, as already demonstrated by Gobert et al. [54].

In rock formations, $CaCO_3$ contains Cd impurities [6]. Significant limestone resources, mainly $CaCO_3$, exist in the Dakar and Thiès regions [56] [57]. Anthropogenic activities, such as the combustion of fossil fuels, poor solid waste and wastewater management, the use of phosphate fertilisers in agricultural activities, and the discharge of untreated industrial wastewater effluents can lead to increased Cd levels in the environment. The fertiliser, pesticide, cement, battery and non-ferrous metal melting industries are known to contribute to Cd pollution [58]. Cd is relatively more soluble in water than many other heavy metals and has a higher mobility [58]. Depending on its origin, phosphate contains high concentrations of heavy metals [59]. In Senegal, the high Cd values may be related to phosphate reserves and phosphoric acid production facilities [60] [61]. We discuss these results below *vs* the choice of how to use *Sargassum* in Senegal.

4.2.2 Comparative elemental analysis of Senegalese *Sargassum*, including rare element observations

Sargassum had more Cu in Senegal than in the Dominican Republic [62] but less than in Ghana [6] and the Mexican Caribbean [45]. Samples from Senegal showed less Mn than those from the Dominican Republic and the Mexican Caribbean and had more Zn than samples from the Dominican Republic and the Mexican Caribbean and less than those from Ghana. Senegalese samples had less Ca, Mg, Na, Ni and more K than those from the Dominican Republic and the Mexican Caribbean. Last, Senegalese samples showed more Fe than those from the Dominican Republic and the Mexican Caribbean but less than those from Ghana.

Some of the elements analysed (palladium (Pd), titanium (Ti), lithium (Li), boron (B), barium (Ba), europium (Eu)) in the single sample (ID: Ng) are rarely reported in the literature for holopelagic *Sargassum*. Senegalese *Sargassum* showed considerably less B (168 ppm) compared to that from the Dominican Republic (102243 - 116294 ppm), while the Ba (13 ppm) and Li (2 ppm) contents of Senegalese *Sargassum* are in the range contents of Dominican Republic (7 - 17 ppm for barium and 0.5 - 3.5 ppm for Li). The Eu content (19) of Senegalese *Sargassum* was considerably higher than that of the Dominican Republic (0.01 - 0.03 ppm). The Al content (16 ppm) from the single sample (ID: Ng) was lower than the median concentration found in the study of Martinez et al. [45]. The Ti concentration found (32 ppm) was lower than the concentration seen in Dominican Republic (37 - 92 ppm) [62] and higher than that from the Mexican Caribbean coast [45], which remained below the limit of detection (LOD: 29 ppm).

Pd is a rare metal whose exceptional properties and rarity are attracting the interest of the world's scientific community [63]. Uses of seaweed extracts (not based on their Pd contents) are reported for benthic *Sargassum* [64] [65] with the use of chemical reagents as a Pd precursor for the synthesis of Pd nanoparticles, as an electrocatalyst [64] and a potential eco-friendly tool in the fight against the protozoan parasite responsible for malaria, *Plasmodium falciparum* [65]. Studies targeting the uses of seaweed based on their content of Pd are not known to our knowledge. Pd concentrations were for the first time determined for twenty-two species of seaweed collected in 1986 from the US Californian coast, but this study excluded holopelagic *Sargassum* [66]. The Pd content of seaweed reported in [66] varied from 0.09 to 0.61 ng g^{-1}, which is far smaller than the value found in Senegal for holopelagic *Sargassum* (2000 ng g^{-1}). Pd content was also reported for benthic *Sargassum* collected in 2016 from different coasts of Sri Lanka [67]. The average content was 30 ± 10 ppm, even higher than the value for

holopelagic *Sargassum* in Senegal (2 ppm). To the best of our knowledge, this present study reports the Pd content in holopelagic *Sargassum* for the first time.

4.2.3 Comparative analysis of heavy metal distribution across Senegalese *Sargassum* morphotypes: contrasting findings with other Atlantic studies

In Senegal, concentrations of As, Cd, Hg, Ni, and Zn followed a pattern with the highest levels in SnI, followed by the SnVIII, SfIII and MF. This order mirrors the findings in Jamaica [1] for As and Ni and aligns with observations in the Turks and Caicos Islands [13], where a higher concentration of As in MA compared to MC and MD was similarly noted. However, the As concentration in the Mexican Caribbean [45] was significantly higher in SnVIII than in SnI. Moreover, SnI showed higher Zn and Cd in Jamaica [1], also confirmed in Senegalese biomasses. In contrast, SfIII had a higher value of Zn for Turks and Caicos Islands. In Jamaica [1] and Turks and Caicos Islands [13], SfIII showed a higher value of Cu; however, in Senegal, SnI had a higher value. In Senegal, the quantity of Pb was higher in SnVIII, followed by SnI, then SfIII. The same observation occurred in the Turks and Caicos Islands [13], where SnVIII also showed a higher value, while in Jamaica [1], SnVIII showed a lower value. The MF in Senegal showed a lower value for each element than the species SnI, SfIII and SnVIII. The findings underscore the complexity of heavy metal distribution in *Sargassum*, emphasizing similarities and regional distinctions compared to broader Atlantic studies.

4.2.4 Comparison of elemental dominance in Senegalese *Sargassum* with global findings: insights into element concentration dependency and environmental variability

In Senegal, beached *Sargassum* showed the highest concentrations of Ca, K, Mg, Na, Fe, and Zn in descending order, while Cu, As, Cd, Ni, Pb, and Hg were found to be the minor predominant elements in the majority of samples, listed in descending order (Table 3). In Turks and Caicos Islands [13] (As>Al>Mn>Cu>Pb>Cr>Cd> Hg), Hg was lower in the relative order *vs* Senegal. They showed that As was more predominant than Cu. Pb was more predominant

than Cd. In Senegal, the results exhibited the opposite. Chen et al. [68] showed on various seaweed samples that elements in seaweed can be sequenced in the following descending order by their mean values Al > Mn > As > Cu > Cr > Ni > Cd > Se > Pb > Hg, where Pb and Hg being the lowest, Cd being more predominant than Pb, which is also the case in Senegal. Chen et al. [68] showed with their ranking that As was more predominant than Cu. In Senegal, the opposite was observed. Compared to other studies [13] [68], the unusual values found in Senegal can be due to the different element concentrations in sampling locations, as the composition of seaweed, including *Sargassum*, depends on their growth environment.

4.2.5 Comparison of carbon, nitrogen and phosphorus ratios in Senegalese *Sargassum* to the Redfield ratio and other Atlantic areas

The P content range for beached *Sargassum* from Senegal (1300 to 1772 ppm) was above those found in the Mexican Caribbean (228 to 401 ppm in [45] and 197 to 472 ppm in [48]). As demonstrated by literature [54] [55], arsenic and phosphorus have an antagonistic relationship, meaning the more *Sargassum* are exposed to a rich phosphorus environment, the more they absorb phosphorus instead of arsenic. The low relative arsenic content in Senegalese *Sargassum* (Tables S3-S4, Figure 4) coupled with the high relative phosphorus in Senegalese *Sargassum* align with the demonstrated relationship and might suggest a P-enrichment in Senegalese waters.

The C:N:P ratios for *Sargassum* were significantly lower in neritic compared to oceanic waters in the western North Atlantic Ocean and Caribbean sea, with no difference between *Sargassum* morphotypes as already demonstrated in this kind of biomass [4]. In Senegal, the molar C:N ratio range (21 to 27) was below the range found in the western North Atlantic Ocean and Caribbean sea [4] for *S. natans* of neritic waters (29 to 35) and in the range for *S. fluitans* of neritic waters (17 to 34). The molar C:P ratio range (429 to 628) in Senegal was above the range found for *Sargassum* of neritic waters (133 to 483) [4]. The molar N:P ratio range (18 to 26)

for mixed morphotypes in Senegal was above the ranges found for *S. fluitans* (7.6 to 12.4) and *S. natans* (8.4 to 14) in neritic Atlantic waters [4]. Compared to the Redfield Ratio C:N:P (106:16:1) and C:N (6.6:1) based on marine phytoplankton, the molar C:N:P ratio mean (507:22:1) and the molar C:N (23:1) ratio mean found in Senegal *Sargassum* suggest like other studies [69] [4], that growth of floating *Sargassum* may be limited by both N and P. The relatively lower production of carbon-rich compounds in phytoplankton compared to *Sargassum* may partly justify the fact that the values of C:N and C:P observed in holopelagic *Sargassum,* including this present study, were considerably higher than Redfield proportions of C:N (6.6:1) and C:P (106:1) [4]. The mean C:N:P ratio found in a global survey of benthic seaweed and seagrasses was 700:35:1, involving a mean C:N of 20, C:P of 700 and N:P of 35. These findings [70] were above the values (C:N:P of 507:22:1, C:N of 23, C:P of 507 and N:P of 22) found in Senegal.

4.2.6 Impacts of location and morphotype composition on carbon and nitrogen ratios in Senegalese *Sargassum* mixtures

Sargassum in oceanic zones (Sargasso sea) has higher C:N ratios than in neritic zones [4]. Neritic zones are associated with a high abundance of fish, whose excretion of ammonium and reactive soluble phosphorus enriches the waters of these zones with nitrogen and phosphorus nutrients. The abundance of fish in neritic zones leads to an increase in the nitrogen content of the *Sargassum* and, consequently, to a decrease in the C:N ratio [4]. The low inter-site variation in nitrogen content and C:N ratios in Senegal may be due to similar fish abundance between the north and south sites.

Inter-site variation in *Sargassum* mixtures' C and N content may be linked to their morphotype composition. The composition of SnI, SfIII and SnVIII differs widely, so the composition of the *Sargassum* mix depends on the ratio of each morphotype within rafts [8]. The study of Alleyne et al. [8] from 2021 to 2022 reported high monthly variation in morphotype

composition of *Sargassum* stranding. In January, the morphotype composition was 67% SnVIII, 25% SfIII and 8% SnI, while in August, it shifted to 14% SnVIII, 67% SfIII and 19% SnI [8].

The inter-site variation in the C content (range: 280835 to 315824 ppm) of the *Sargassum* mixtures may be also explained in relation with the presence of calcified epiphyte films. Calcified epibionts are a source of calcium carbonate, which can lead to carbon dioxide during the CHN analysis combustion reaction, which may increase the measured C content. Observations from this study indicate the presence of films of calcified epibionts, resembling Bryozoans (*Dentitheca bidentata*) and serpulid worm tubes [71], covering the surface of *Sargassum*, particularly on SnVIII (Supplementary S1).

A concentration of 100 and 600 mg kg^{-1} is considered the critical and sufficient iron (Fe) concentration for marine macrophytes [72]. The concentrations of Fe in Senegal were 464, 237, 552 and 1017 mg kg^{-1} DW for Kayar, Cambérène, Somone and Joal-Fadiouth sites, respectively. *Sargassum* from Joal-Fadiouth showed the highest and sufficient Fe content compared with other Senegalese sites. The largest *Sargassum* fragments at Joal-Fadiouth were noted, knowing that Fe can increase the growth rate of *Sargassum* [73]. Preliminary chemometric evaluation of the elements measured by PCA of four *Sargassum* morphotype mixtures showed positive correlations between Fe and C (Table S2, Figure S1). The higher C content of Joal-Fadiouth *Sargassum* may be associated with a higher Fe load. The presence of large holopelagic *Sargassum* fragments at Joal-Fadiouth may be due to the existence of favourable growth conditions in South Senegal. One should note that it was only on this single site that a mixture of holopelagic and benthic *Sargassum* was reported (Supplementary S1).

4.3 Senegalese *Sargassum* mannitol, proteins and phenolic compounds *vs* exploitation

Phenolic compounds are secondary metabolites found, mainly in the form of phlorotannins in Phaeophyceae, and at high levels in Fucales (20-30% DW). These molecules are assumed to function as chemical defences against grazers, pathogens and epiphytes and are involved in photoprotection mechanisms against solar radiation, particularly UV radiation. They have a wide range of biological activities (antimicrobial, antioxidant, antitumoral, antiviral) of high interest for applications in pharmaceutical and cosmetic processes [74]. Phenolic compound levels in Senegal (0.3 - 3% DW) were in line with values found in benthic *Sargassum* species, together with other tropical species, *Turbinaria ornata* (1.12 - 1.58% DW) and *S. pacificum* (formerly *S. mangarevense,* 0.38 - 0.96% DW) [75] (Table 4). Overall, the phenolic levels of Senegalese holopelagic *Sargassum* are higher than the contents of holopelagic *Sargassum* in Jamaica [1] (0.12 - 0.31% DW) and [76] (0.10 -1.05% DW). The range of phenolic compounds of holopelagic *Sargassum* in Turks and Caicos (0.25 - 2.95% DW) [13] was similar to that of Senegalese. The phenolic contents in the brown seaweeds, *Cystoseira tamariscifolia* (10.91% DW) and *Fucus ceranoides* (5.47% DW) [39] were considerably higher than those of Senegalese holopelagic *Sargassum* (Table 4). Phenolic contents of sun-dried samples (0.3 - 0.4% DW) were lower than those of freeze-dried samples (0.5 - 3% DW), suggesting that they either have been degraded by solar radiation or have been released outside the seaweed as a chemical protectant [77]. Moreover, SnI (2% DW) and SnVIII (3% DW) from Kayar produced the most phenolic compounds.

Mannitol is a sugar alcohol produced by photosynthesis. It is universally present in brown algae and can account for 20-30% DW in some brown seaweeds from the genus *Laminaria*. Mannitol exhibits hydrating and antioxidant properties and is used in numerous cosmetic and pharmaceutical applications. Although mannitol produced by chemical synthesis is less expensive than natural mannitol extracted from seaweeds, it is worth using the latter because consumers prefer natural cosmetic products [74]. Overall, the mannitol levels of Senegalese

holopelagic *Sargassum* (0.13 - 0.33% DW) were lower compared to those of brown seaweeds such as *S. vulgare* (3.8 - 11.6% DW), *S. pacificum* (12.2% DW) and *Turbinaria ornata* (5.9% DW) [74]. However, the mannitol levels of Senegalese samples were in line with those of holopelagic *Sargassum* in Jamaica [1] (0.18 - 0.72% DW) (Table 4). Unlike phenolic compounds, mannitol in holopelagic *Sargassum* did not appear to be significantly degraded during sun-drying of the seaweeds (0.19 - 0.25% DW for sun-dried samples and 0.13 - 0.33% DW for freeze-dried samples). All the samples analysed were collected *in situ* near the coast and/or on the beach, with none originating from the open sea. Gloaguen [78] demonstrated that phenolic compound and mannitol levels in holopelagic *Sargassum* collected in West Indies (Guadeloupe and Martinique), varied along a degradation gradient, with higher phenolic compound levels in the open sea decreasing upon beaching and mannitol levels showing the opposite trend, being higher in beached samples.

Total protein contents in Senegalese *Sargassum* varied from 15 to 40% DW. Overall, they were higher than the range found in the Mexican Caribbean, 3 - 11% [48] and Turks and Caicos, 5.2 - 12.7% [79] (Table 4). The Senegalese *Sargassum* were in soluble proteins rather than in insoluble proteins. The protein content of seaweeds varies among different seaweed groups, and it is found to be highest among red seaweeds, then green and brown seaweeds. Generally, brown seaweeds contain protein as low as 3–15% DW compared with green or red seaweeds, having 10–47% DW protein [80]. The highest protein content in Senegalese *Sargassum* (40% DW) was high compared to brown seaweeds, even to the extent that it is close to the reported values in red edible seaweeds *Palmaria palmata* (~35% DW, known as dulse) and *Porphyra* (up to 47% DW, known as nori), two known most famous species with high proteins comparable with high protein pulses such as soybean [80]. The lowest protein content reported in Senegal (15% DW) was close to the range values (11 - 24% DW) found in another edible brown seaweed *Undaria pinnatifida*, known as wakame and as one of the most protein-rich

brown seaweeds [80]. Protein contents found in edible *Sargassum*, *S. fusiforme* (9.1 % DW) [81] and *S. horneri* (5.6 – 12.8 % DW) [82] were smaller than the lowest value reported in Senegal (15 % DW) [14]. A high percentage of protein in seaweeds makes them an interesting source of food and additives [80] and a potential alternative source of proteins for animal feeding [83]. Biomasses of beached *Sargassum* could provide an alternative protein source for livestock feed in West Africa.

It has been shown that the protein content of holopelagic *Sargassum* remains constant with storage time, while lipid and phenolic content decreases with storage time [79]. This last point is of particular importance when considering the valorisation of *Sargassum* in applications focusing on its protein content. A high protein content in *Sargassum* may be correlated with optimal conditions that promote its proliferation to increase. When exposed to an optimal condition to proliferate, *Sargassum* might enter quickly into a growth phase requiring the expression of a high amount of proteins to carry out its metabolic processes [48]. The relationship between the protein content of *Sargassum* and environmental parameters in Senegal should be investigated. In particular, the effect of high nutriment availability in the Senegalese upwelling [84] [85] should be explored.

4.3.1 Comparative analysis of heavy metal concentrations *vs* agricultural and animal feed standards

In agricultural applications, permissible maximum limits for As, Cd, Zn, Cu, and Pb vary depending on the country and governing organisation and no standard norm exits so far in the *Codex Alimentarus*. The reference values considered in this study were based on the maximum limits established by several countries and the European Union, including Austria, Britain, Canada, Germany, Japan, Mexico, and Poland. The maximum recorded arsenic level in Senegal (29 ppm) was below the threshold values set by three countries (Poland 30 ppm, Germany 40

ppm, Austria 50 ppm) and exceeded the recommended limits of four others (Figure S2) [45] [86] [87].

Regarding Cd, the minimum and maximum values observed in Senegal (9 and 15 ppm, respectively) surpassed the threshold established by five countries (Britain 1 ppm, Germany 2 ppm, Poland 3 ppm, Austria 5 ppm, and Canada 8 ppm) but were below that of Mexico (37 ppm) [45] [86] [87]. In the case of Cu and Zn, all Senegalese values fell below the established threshold values [45] [88].

Additionally, for Pb, all Senegalese values were far below the specified threshold levels [45] [88]. Regarding animal feed, all As values in Senegal were below the maximum limit recommended by the European Union for seaweed used in animal feed (40 ppm) [45]. Biomasses of beached *Sargassum* could represent biomass of interest for feeding livestock in Senegal, given their innocuity in term of contaminants and their interesting protein contents.

4.3.2 Composting and anaerobic digestion of Senegalese *Sargassum*

The utilisation of *Sargassum* for composting and anaerobic digestion is contingent on its C:N ratios, where low ratios are advantageous for agricultural applications, while higher ratios are preferable for biofuel production [15]. The United States Composting Council (USCC) recommends a C:N value below 20 for *Sargassum* in compost [89], while a C:N value between 20 and 35 is recommended for biogas production [16]. In the context of composting, the C:N mass ratios for Cambérène (19) and Somone (18) fall within the recommended range, whereas those for Kayar (23) and Joal-Fadiouth (21) slightly deviate from the suggested range. Conversely, for biogas production [16], the ratios show an inverse pattern (Figure S3). Co-digestion of holopelagic *Sargassum* with other locally available feedstock in Senegal, such as cow manure and *Jatropha curcas* cake, might be explored. *Jatropha curcas* cake has a higher C:N ratio (32) and less sodium content (2100 ppm) [90] than Senegalese holopelagic *Sargassum*

(18 – 23 for C:N ratio and 2495 – 6131 ppm for sodium). Even if *Jatropha curcas* cake and cow manure showed less methane potential than the green macroalgae *Ulva lactuca* and *Codium tomentosum* [90], their association with Senegalese holopelagic *Sargassum* for biogas production might provide additional advantages, including salt dilution and C:N ratio increase.

5. Conclusion

The unusual massive beaching reported in Senegal during the summer of 2022 was not observed in 2023, a particularly hot year for the Atlantic Ocean. Four morphotypes were identified, including a morphotype (MF) unrelated to any of the most common morphotypes and of unclear species assignment. The particularity of MF to present a very marked spectral peak towards 2 ppm compared to the other morphotypes might suggest that MF differs from known morphotypes. The absence of particular signals in the spectra of holopolagic *Sargassum* makes their absolute discrimination difficult, while they are different using the morphology. Presently, the profitability of segregating *Sargassum* based on morphotypes for industrial use remains limited, as current valuations focus on the entire biomass, but knowing the predominance of morphotypes can help to predict the average composition. In Senegal, SfIII was the most dominant morphotype. The highest carbon and iron contents of *Sargassum* were reported in the site where we observed the largest fragments of *Sargassum*. We can assume a positive correlation between carbon content, iron content and growth of *Sargassum*.

Further research could explore potential differentiation in composition based on *Sargassum* tissues, emphasising investigating methods of extracting compounds preserved during sun drying, which is more suitable for developing countries and the way of the blue economy. Sun-drying would be suitable for exploiting the mannitol in *Sargassum*. To extract phenolic compounds from Senegalese *Sargassum*, freeze-drying should be preferred, but its cost evaluated. While the potential applications of Senegalese holopelagic *Sargassum* in pharmaceutical and cosmetic industries may not necessitate further exploration (vs mannitol

and phenolic compounds), the substantial presence of palladium remains a noteworthy focus due to its economic importance.

All arsenic values in Senegal *Sargassum* samples were below the maximum limit recommended by the European Union for seaweed used in animal feed, which might be beneficial to investigating their valorisation in the feed sector. Total protein levels in Senegalese *Sargassum* were considerably higher than in other part of the Atlantic Ocean. Senegalese *Sargassum*'s total protein levels were higher than the usual range in brown seaweeds. Biomasses of beached *Sargassum* in Senegal can provide an alternative source of protein for livestock feed even after biomass storage before use. For agriculture applications, all Senegalese values fell below the established threshold values for copper, zinc and lead. However, the recorded levels are above some established limits for arsenic and cadmium. Given that the C:N ratios of some sites fall within the recommended range for composting, the use of Senegalese holopelagic *Sargassum* for agricultural applications might be envisaged.

In North West Africa, we recommend the establishment of standardised thresholds for heavy metals, prioritising cadmium and mercury monitoring. It is concerning that there are no African standards at the continental, regional, or sub-regional levels for the use of seaweed, especially in agriculture and animal feed. With the increasing eutrophication of African waters and the likelihood of more frequent *Sargassum* beaching events, we urge inter-governmental African organisations to develop standardised norms for exploitation. Additionally, we recommend that the Food and Agriculture Organization/World Health Organization consider including more seaweed in the *Codex Alimentarius*.

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Table Captions

Table 1*:* Sample list of holopelagic *Sargassum* collected during the summer of 2022 beaching with date (between 11/08 – 17/09) and location of sampling (between Kayar and Joal-Fadiouth; Sénégal, See Figure 1 for the localization of sites), type of morphotype (M), drying method (Dry) and part of fragment analysed (Part). MM: Mix Morphotypes; SfIII: *Sargassum fluitans* III; SnVIII: *S. natans* VIII; SnI: *S. natans* I; MF: putative new morphotype. E: Entire; V: Vesicles; L: Leaves; S: Stems. Od: Oven dried; Fd: Freeze-dried. As: Arsenic; Cd: Cadmium; Pb: Lead; Hg: Mercury; Cu: Copper; Zn: Zinc; Ni: Nickel; P: total phosphorus; C: total carbon; N: total nitrogen; PC: phenolic compounds; SP: soluble proteins; IP: insoluble proteins. SD Standard deviation. *Other rare elements (Palladium, Titanium, Lithium, Boron, Barium, Europium, and Aluminium) have been analysed for the Ngor sample (Ng). Sampling site: Joal-Fadiouth noted 'JF' and Cambérène 'Camb'.

Table 2: Summary statistics (minimum, first quartile, median, mean, third quartile, maximum and standard deviation) for chemical content (ppm dry weight) and total samples analysed (n) of Senegalese holopelagic Sargassum summer 2022.

Table 3: Rank order of element concentrations (see Table 1) for each *Sargassum* per location in Senegal (Ka: Kayar; Ca: Cambérène; So: Somone, Mb: Mbour; JF: Joal-Fadiouth) and morphotype (MF: putative new morphotype; SfIII: *Sargassum fluitans* III; SnVIII: *S. natans* VIII; SnI: *S. natans* I).

Table 4: Comparative analysis of seaweed composition (arsenic, cadmium, lead, mercury, iron, phosphorus, total protein, phenolic content, mannitol, and ash) with published studies, serving as a basis for comparing the results of the present study. Colours in the table denote instances where the values from the reference study are higher (indicated in brown) or lower (indicated in yellow) than those obtained in the present study. All species noted "*Sargassum* sp" are holopelagic ones. nd: not determined. Source: Bam et al. 2019 [46], Milledge et al. 2019 [13], Cipolloni et al. 2019 [49], Vázquez-Delfín et al. 2018 [47], Davis et al. 2019 [1], Addico and De Graft-Johnson 2016 [6], Tejada et al. 2019 [91], Rodríguez-Martínez et al. 2020 [45], Saldarriaga-Hernandez et al. 2019 [48], Fernández et al. 2017 [62], Oyesiku et al. 2014 [92], Baweja and al. 2016 [80], Nielsen et al. 2020 – 2021 [79], Machado et al. 2022 [76], Zubia et al. 2008 [74], Stiger et al. 2004 [75], Steinberg 1986 [93], Targett et al. 1992 [94], Zubia et al. 2009 [39].

Color should be used for Table 4 in print.

Figure Captions

Figure 1*:* A) Map of Africa with the location of Senegal highlighted in orange. B) Sites observed along the Senegalese coast during the stranding of holopelagic *Sargassum* (summer 2022). Sampling sites of holopelagic *Sargassum* along the Senegalese coast were highlighted with orange points during the sampling of August 11 2022 - September 17 2022. The map was generated using QGIS 3.28 (http://www.qgis.org) with data sourced from GADM maps and data (GADM data (version 4.1) https://gadm.org/download_country.html). GPS coordinates of sampling sites: Grand Côte: two sites Kayar (N 14.91665°; WO17.12398°), Camberene (N 14.76214°; WO17.47393°), Cap-Vert Peninsula (Dakar) one site: Ngor (N 14.75041°; WO17.51113°). Petite Côte (South Senegal) three sites: Somone (N 14.48988°; WO17.08513°), Mbour (N 14.40665°; WO16.96983°), Joal-Fadiouth (N 14.18382°; WO16.86337°). C) Holopelagic *Sargassum* washed ashore on the beach of Mbour, 22 August 2022.

Figure 2: Chemical fingerprints obtained by Proton High-Resolution Magic Angle Spinning Nuclear Magnetic Resonance (HRMAS ^{1}H NMR) to visualise the major metabolites of holopelagic *Sargassum* samples from the Senegalese coast, grouped in four morphotypes (MF: putative new morphotype; SnVIII: *Sargassum natans* VIII; SfIII: *S. fluitans* III; SnI: *S. natans* I). On the left upper corner is the morphotype, and on the right ID identifier of the sample.

Figure 3: Concentrations of elements A) As, Cd, Pb, Hg; and B) Cu, Ni, Zn in Senegalese holopelagic *Sargassum* (summer 2022) according to species (SfIII: *Sargassum fluitans* III,

SnVIII: *S. natans* VIII, SnI: *S. natans* I, MF: putative new morphotype) and sampling sites (Kayar ID Ka, Camberene ID Ca, Somone ID So, Mbour ID Mb and Joal-Fadiouth ID JF).

Figure 4: Concentrations of A) Calcium (Ca), Potassium (K), Magnesium (Mg), Sodium (Na), B) Iron (Fe), Manganese (Mn); C) total nitrogen (N), total phosphorus (P); and D) total carbon, ash in Senegalese holopelagic *Sargassum* during summer 2022, categorized by four sampling sites in Senegal (Kayar ID Ka, Camberene ID Ca, Somone ID So and Joal-Fadiouth ID JF).

Figure 5: Maximum (Max.) and minimum (Min.) content (in ppm) of the four heavy metals As, Cd, Pb and Hg across the tropical Atlantic Ocean in holopelagic *Sargassum*. (A) maximum and (B) minimum arsenic content (data in Table S2 and Table S3); (C) maximum and (D) minimum lead content (data in Table S4 and Table S5); (E) maximum and (F) minimum cadmium content (data in Table S6 and Table S7); (G) maximum and (H) minimum mercury content (data in Table S8 and Table S9). Left-corner rectangles with orange outlines represent the zooms of the juxtaposed study points. [L1]Belize: Central America [46], [L2]Playa Mirador, Tulum [47], [L3]Playa Blanca, Akumal [47], [L4]Playa Xcalacoco, Playa del Carmen [47], [L5]Puerto Morelos [47], [L6]Playa Coral, Cancun [47], [L7]Playa Delfines, Cancun [47], [L8]Mexican Caribbean coast: Contoy island to Xcalak [45], [L9]Quintana Roo, Punta Sur [48], [L10]Quintana Roo, Chen Rio [48], [L11]Quintana Roo, Mezcalitos [48], [L12]Jamaica Fort Rocky [1], [L13]Turks and Caicos Shark Bay South Caicos [13], [L14]Antigua/Barbuda: juncture of Caribbean Sea and Atlantic Ocean [46], [L15]Middle Atlantic Ocean to Guadeloupe (French West Indies) [49], [L16]Barbados: Caribbean [46], [L17,L18,L19,L20,L21,L22]Middle Atlantic Ocean to Guadeloupe (French West Indies) [49], [L23]Senegal [Present study], [L24,L25]Ghana [6]. GPS coordinates are listed in Table S1. Data are classified using the method of natural breaks (Jenks) [50] [51].

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