Research article

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Iodine and fluorine concentrations in seaweeds of the Arabian Gulf identified by morphology and DNA barcodes

Abstract: Even though seaweeds have been recognized as key players in the ocean-to-atmosphere transfer of iodine in other parts of the world, there is a complete lack of knowledge about iodine accumulation in seaweeds of the Arabian Gulf. Similarly, very little is known about fluorine in seaweeds, anywhere in the world. Given that the Arabian Gulf is of particular interest due to being an extreme environment, featuring some of the highest temperatures and salinities observed in any marine water body world-wide, this study endeavoured to conduct a preliminary survey of iodine and fluorine levels in 11 of the most common seaweed species in the region, supported by morphological and molecular (DNA barcode)-based identification. Iodine was determined by inductively-coupled plasma-mass spectrometry, while ion chromatography was employed for analysis of fluoride. Species surveyed included *Iyengaria stellata*, *Padina boergesenii*, *Chondria sp. Dictyota dichotoma*, *Colpomenia sinuosa*, *Feldmannia indica*, *Codium papillatum*, *Sargassum ilicifolium*, *S. ilicifolium var. acaraeocarpum*, *Sargassum asperifolium* and *Sargassum aquifolium*. The findings of *S. ilicifolium* and *S. ilicifolium* var. *acaraeocarpum* reported here are new records both for Kuwait and the Arabian Gulf. *P. boergesenii* and *D. dichotoma* are new records for Kuwait.

Keywords: IC; ICP-MS; iodine; Kuwait.

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1 Introduction

The discovery of iodine (atomic number 53, with an atomic weight of 126.9 Da) is credited to Courtois in 1811, who was trying to manufacture saltpetre (needed to make gunpowder in the context of the Napoleonic wars) from seaweed ash. After the initial discovery, Joseph Gay-Lussac, another French chemist, gave the new element its name iodine (from the Greek word "ιώδης" due to its purple colour; historical details reviewed by Küpper et al. 2011). Iodine levels are much higher in seawater (60 µg l^{-1}, corresponding to 0.47 µM) than in estuaries (about 5 µg l^{-1}, corresponding to 0.04 µM) or rivers (less than 0.2 µg l^{-1}, corresponding to 1.6 nM in some Triassic mountain regions of northern Italy), and, consistent with this, freshwater trout contain 20 µg kg^{-1} of iodine compared to saltwater fishes (herring) with about 500–800 µg kg^{-1} (Venturi et al. 2000). The oceans are the largest reservoir of bioavailable iodine on the planet (Wong 1991) where total iodine levels are typically in the range of 0.5 µM (Truesdale et al. 1995a). From there, the element is

transferred from the oceans to the atmosphere in the form of gas or aerosols and from there, by wind and rain, to land areas (Küpper et al. 2011; Truesdale et al. 1995b). Seaweeds contribute to this through a process called iodovolatilization which was discovered as early as the 1920s (Dangeard 1928; Kylin 1929). It has been proposed that iodide had the ancestral function of an ancient antioxidant in animals and that this resulted in the evolution of the thyroid gland (Venturi and Venturi 1999). Iodine plays a central role in thyroid physiology, being the essential element in thyroid hormones (THS) and the major constituent regulator of thyroid gland function (Thilly et al. 1992, reviewed by Küpper et al. 2011). Iodine from the diet is rapidly and efficiently absorbed (>90%) throughout the gastrointestinal tract (in particular, the duodenum; Yarrington and Pearce 2011). The kelp *Laminaria digitata* accumulates iodine to more than 30,000-times the concentration found in seawater, often representing an average content of 1% of dry weight and much higher levels in young thalli (Küpper et al. 1998). More recently, it was found that, in *Laminaria*, the accumulation of iodide serves the provision of a simple, inorganic antioxidant, which protects the thallus surface against reactive oxygen species (Küpper et al. 2008). If ozone is present, molecular iodine is released, which can undergo further photolytic and oxidative reactions with ozone yielding hygroscopic iodine oxides, resulting in aerosol particle formation, thus impacting coastal climate (Küpper et al. 2008). More recently, it was found that bromide complements iodide as antioxidant in *Laminaria* (Küpper et al. 2013). The world's largest seaweed, the giant kelp *Macrocystis pyrifera*, shares key features of its halogen metabolism with *Laminaria*, even though the overall accumulation of iodine is lower (Tymon et al. 2017). *Macrocystis* forests were found to impact iodine speciation in surrounding seawater (Gonzales et al. 2017). In *Laminaria*, inorganic iodine emission rates exceed organic emissions (i.e. of halocarbons) by several orders of magnitude (Küpper et al. 2008; Palmer et al. 2005). Rates of halocarbon production vary considerably between species (Carpenter et al. 2000) and marine macroalgae are thought to be the dominant source of CH_3I in the coastal ocean (Gschwend et al. 1985; Giese et al. 1999; Itoh 1997). Most studies exploring halocarbon emission by seaweeds were conducted on temperate and some polar seaweed species, while only a few have considered tropical species. Brown seaweeds from temperate regions including Laminariales and Fucales release large amounts of iodinated compounds (Carpenter et al. 2000, reviewed by Küpper et al. 2011; Küpper and Kroneck 2015). Much less is known about seaweeds from tropical regions. In this context, recent studies from Malaysia (Keng et al. 2020; Leedham et al. 2013) have investigated

seaweeds, including *Sargassum binderi*, *Padina australis*, and *Turbinaria conoides*, which were dominant in a tropical coral reef, for their emissions of various volatile halocarbons.

Fluorine (F, atomic number 9, with an atomic weight of 18.9984 Da) is considered an essential element for animals including humans, which is primarily because of its benefits to dental health and its suggested role in maintaining the integrity of bones (Prystupa 2011), while continuing exposure to elevated levels are of concern. According to the World Health Organization (WHO), the recommended upper limit of fluoride in drinking water is between 0.8 and 1.5 mg l^{-1} (WHO 2019). The European Food Safety Authority (EFSA) recommends an intake of 0.05 mg of fluoride per kg of body weight per day for children and adults (EFSA Panel on Dietetic Products, Nutrition and Allergies 2013). Fluoride has been one of the most effective and widespread agents used to prevent dental caries (Petersen 2003). It is important to metabolism, formation and structure of bone and teeth, growth and reproduction and other physiological process in the human body (Liteplo et al. 2002). Fluoride species can be taken up by aquatic organisms directly from the water or, to a lesser extent, via food. Uptake depends on the anthropogenic sources, the local geology and the physicochemical conditions (Camargo 2003; Liteplo et al. 2002). Studies on the distribution of certain halogens in seaweeds show the equivalent concentrations of halogens in seaweeds are in the order I > F > Br > Cl, whereas in seawater the order of concentrations is Cl > Br > F > I (Rao and Indusekhar 1989). Little is known about the biological significance of fluorine in algae. Fluoride possibly increases the growth and metabolic activities of brown seaweeds (Camargo 2003). On the other hand, it was found that aluminofluoride and beryllofluoride, which are structural analogues of vanadate and phosphate, inhibited apo-(halo-)bromoperoxidase (a key enzyme in halogen metabolism of brown and red algae; Küpper and Kroneck 2015) from the North Atlantic brown alga *Ascophyllum nodosum* (Tromp et al. 1991). Haloperoxidases cannot catalyse fluorination reactions because hydrogen peroxide lacks the thermodynamic potential to oxidize fluoride; thus, enzymes catalysing fluorination reactions are not peroxidases (Butler and Sandy 2009). At present, it is not clear whether algae of any phylum contain fluorinating enzymes; in fact, the only fluorinase so far known to science is from the bacterium *Streptomyces cattleya* (O'Hagan et al. 2002). Also, it should be noted that among all marine organisms investigated so far, anywhere in the world, only one marine-derived *Streptomyces xinghaiensis* strain has been reported to produce a fluorinated metabolite, the structurally simple fluoroacetate (Huang et al. 2014; Ma et al. 2016). No fluorinated compounds have been reported from the entire breadth of algal diversity,

including seaweeds. They are mainly producers of chlorinated, brominated and iodinated compounds due to the presence of vanadium haloperoxidases (e.g. Butler and Sandy 2009; Carter-Franklin and Butler 2004; Küpper et al. 2008; Küpper and Kroneck 2015). This may also be due to traditionally much less investigator effort focussing on algae in the natural products community compared to bacteria, fungi, sponges, etc. Considering that algae also harbour abundant and diverse bacterial communities, especially on their surfaces as biofilms, it would be surprising that there are no fluorinated compounds. Young and Langille (1958) found that fluoride content ranges from 3.02 to 18.86, 4.78 to 17.82 and 4.35–20.04 mg kg^{-1} dry weight in green, brown and red algae, respectively. Even though variations in fluoride content are observed in each class of algae, red algae, in general, tend to contain more fluoride compared to brown and green algae. Recently, there has been increasing interest in the potential of dried seaweed biomass for biosorption of fluoride from fluoride-contaminated water. In this context, it was found that dried *Padina* sp. (Phaeophyta) from the Red Sea (Mohamed et al. 2020) and *Gracilaria* sp. (Rhodophyta) from the Bay of Bengal (Babu et al. 2020) could be applied as eco-friendly biosorbents for fluoride.

The Arabian Gulf is a shallow basin that has been in existence, i.e. filled with sea water, for only around 15,000 years. Except at its entrance in the Straits of Hormuz, it is less than 60 m deep and it is located in one of the most arid regions of the world, characterized further by the greatest seasonal temperate range in the world as well as the highest annual sea temperature (Sheppard et al. 2010). The coastal environment of Kuwait (ca. 500 km coastline) can be divided into the Northern Region, Kuwait Bay, and the Southern Region. The Northern Region extends from the northern border of the state to Ras Al-Ardh. This area is influenced by outflows of freshwater from the Shatt Al-Arab waterway. The Southern Region extends from Ras Al-Salmiyah to the southern border of the state. Marine biota of the Gulf are mostly of Indo-Pacific affinity, however cold winds from the Anatolian and Iranian highlands limit the occurrence of more cold-sensitive taxa. For much of its area, the photic depth is 15 m or less. High evaporation (up to 2 m yr^{-1}) results in salinities generally of at least 39, reaching 70 in the Gulf of Salwah off south-western Qatar. The Arabian Gulf is characterized by sandy seabeds and shores on its western side including the Qatar Peninsula with occasional limestone outcrops formed of fossilised reef rock, while genuine rocky shores occur mostly on the eastern (Iranian) shore. Indeed, organisms in the Gulf experience high levels of salinity as well as exceptional levels of temperature stress, which makes the region and its biota interesting models for studying the impacts of climate change (Sheppard et al.

2010). For almost all groups of biota, the Arabian Gulf has to be considered impoverished compared to the neighbouring Indian Ocean, which is due to both harsh environmental conditions and its young age as a sea area, while population densities of several groups of biota are similar to those of other tropical areas (Sheppard et al. 2010). For example, the Arabian Gulf harbours an impoverished subset of reef-building corals of the Indian Ocean (Sheppard 1998). However and despite a fairly general pattern of low diversity for most groups, more in-depth surveys have revealed a higher diversity than expected for some groups such as fish (Krupp et al. 2000; Price et al. 2002) and ocypodid crabs (Al-Khayat and Jones 1996). When the size of the water body was compensated for in a recent study of 2894 species of marine macroalgae from 66 sites in the Indian Ocean region, the Arabian Gulf overall ranked 62nd out of 66 in terms of algal diversity in all phyla/higher divisions, based on species richness, range rarity and average taxonomic distinctness (Price et al. 2006). Soft bottom substrata make up most of the surface of benthic habitats of the Arabian Gulf, yet rocky outcrops and coral reefs are common too and harbour significant biodiversity. Indeed, the largest high-diversity types of benthic environments in the Arabian Gulf are coral reefs and coral-dominated substrata, seagrass meadows and algal beds.

As the hottest and the most saline part of the world's ocean, the Arabian Gulf is a unique environment. Yet, little is known about how organisms in the Arabian Gulf tolerate such extreme conditions and how this tolerance influences biodiversity. In this context, nothing at all is known about the accumulation and metabolism of halogens in seaweeds of the Arabian Gulf. This study was conducted in order to obtain a first insight into whether typical, common seaweeds of the Arabian Gulf, whose identification was underpinned by morphological and DNA barcoding, accumulate any iodine and fluorine at levels comparable to those in colder climates. Inductively coupled plasma-mass spectrometry (ICP-MS) and ion chromatography (IC) were employed as the key technique for this, since it provides excellent selectivity and sensitivity for the measurement of iodine (Oliveira et al. 2010; Pacquette et al. 2012; Picoloto et al. 2015; Schnetger and Muramatsu 1996; Sullivan and Zywicki 2012).

2 Materials and methods

2.1 Algal collections

Sampling locations were selected to represent a variety of geographical and ecological regions along the coastline of Kuwait (Table 1). Seaweed samples were collected during low tide

(maximum tidal range is 3 m) from supratidal to subtidal zones of the intertidal regions of the selected locations. Although a few macroalgae were free-floating, most were found attached to stable substrata such as rocks, dead corals, pebbles, shells and seagrasses. Algal samples were rinsed thoroughly with seawater on-site and placed in plastic bags, transferred to the laboratory and were again washed three times with sea water. Subsequently, the fresh samples were frozen in a freezer for 24 h followed by drying in a freeze dryer (Labconco, USA) at −45 °C for 48 h and kept in the presence of silica gel to prevent re-moisturing.

2.2 Determination of total iodine concentrations in tissue samples

Iodine was extracted from algal tissues by alkaline leaching with tetramethylammonium hydroxide (TMAH; Sigma Aldrich, USA) and the total iodine concentration was subsequently determined by ICP-MS. Replicate field-collected samples of blade, holdfast and stipe of a given species were lyophilized and ground to a fine powder using a mortar and pestle. Approximately 0.1 g of the powdered algal material was incubated with 2 mL of 25% TMAH for approximately 72 h at room temperature. The sample was centrifuged and the supernatant was decanted and diluted to approximately 0.25% TMAH. For the determination of the total element content an Agilent 8800 Triple Quadrupole ICP-MS (Agilent Technologies, UK) equipped with a Scott-type spray chamber and a MicroMist concentric glass nebulizer (Glass Expansion, West Melbourne, Australia) was used. The sample and skimmer cones were composed of Ni. The ICP-MS/MS was operated in oxygen (MS/MS-mode) mode for different elements. Collision/reaction cell (CRC) gas flow rates for oxygen were 30% (~ 0.3 mL min^{-1}). Instrument calibration was performed by employing iodine standards of up to 1000 ng mL^{-1} in dilute TMAH. Duplicate trials were averaged.

2.3 Determination of total fluoride concentrations in tissue samples by ion chromatography (IC)

The dried seaweed (obtained from 4.5 - 11 kg fresh weight) was ground to power with an electric grinder mixture. The powder sample of each species was macerated in water at room temperature. After the filtration, water was evaporated under vacuum using a rotary evaporator down to a final volume of 1 ml (which was adjusted by adding water if appropriate). The obtained crude extracts were then kept in a cold room at 4 °C. After that, 100 μL from each species were diluted to 30,000 μL to fit in the calibration working range. Fluoride concentrations in the seaweed were analysed using a Metrohm IC 882 (Herisau, Switzerland) with conductivity detector, a Metrohm A Supp (150 × 4.0 mm) chromatographic column and metrosep RP guard 3.5. The eluent was 3.2 mM sodium carbonate and 0.1 mM sodium bicarbonate with flow rate of 0.7 m min^{-1}; run time was 7 min with a 20 μL injection volume. Calibration was carried out using 1, 5, 10, and 20 mg L^{-1} fluoride, prepared from 1000 mg L^{-1} fluoride standard stock solution.

2.4 Morphological and molecular identification of seaweed taxa

For each taxon collected, a herbarium voucher specimen was prepared on Bristol paper with a subsample being kept both in silica gel and CTAB for subsequent DNA extraction. Herbarium specimens were deposited in the Kuwait University Herbarium (KTUH).

2.5 DNA extraction

About 20 mg of silica gel-dried or CTAB-stored algal material were ground using a mortar and pestle or QIAGEN Tissue Lyser II (Hilden, Germany) at 30 Hz for 10 min, followed by total DNA extraction using the GenElute™ Plant Genomic DNA Miniprep Kit (Sigma, Aldrich) according to the manufacturer's instructions.

2.6 DNA amplification and sequencing

The extracted DNA was amplified by PCR using 11 primers pairs (Table 2). PCR master mix was prepared using a Taq PCR Kit (Qiagen, Hilden, Germany). Samples were subjected to the PCR programs detailed in Table 3.

PCR products were examined on 0.7% (w/v) agarose gel (Bio-Rad Laboratories, USA) to confirm length and concentration of the PCR products. PCR products were purified using the QIAquick PCR purification kit (QIAGEN, Maryland, USA) and were fluorescently labeled using Big-dye V-3.1 reagent mix (Applied Biosystems/ABI, USA) following manufacturers' protocols. Products were then purified using sodium acetate and ethanol before being sequenced using a 3130 × 1 Genetic Analyzer (Applied Biosystems/ABI, USA). Some were purified and adjusted to the required concentration and sent away for commercial Sanger sequencing (Source Bioscience, UK). Obtained sequences were initially analyzed by comparative methods on established public-domain databases using the BLAST (Basic Local Alignment Search Tools) algorithm (http://www.ncbi.nih.gov) (Altschul et al. 1997). The BioEdit (http://www.mbio.ncsu.edu/ BioEdit/ bioedit.html) program was used for sequence alignment of the forward

Table 1: Locations of sampling sites in Kuwait during the surveys and collections in May and June 2018 and February 2019, respectively.

Date	Location	Coordinates	Offshore seawater surface temperature (°C)
27/05/2018	Salwa Enjefa Beach	29°17′52.4″N 48°05′18.6″E	28.20–28.35
26/05/2018	Bnaider Beach	28°47′01.5″N 48°17′50.6″E	27.75–27.90
27/05/2018, 2/06/2018, 3/06/2018	Abu Al Hasaniya	29°12′19.4″N 48°06′41.5″E	30.15–30.30
2/6/2018	Nuwaiseeb	28°34′35.8″N 48°24′04.0″E	31.20–31.35
6/02/2019	Khiran	28°39′22.8″N 48°23′33.4″E	16.50–16.65
6/02/2019	Lagoon of Khiran resort	28°39′47.2″N 48°22′11.7″E	16.50–16.65

Table 2: Primer pairs and PCR conditions used in this study.

Locus	Marker	Primer	Length of amplified target (bp)	Position	Direction	Sequence 5′-3′	PCR parameters	Reference
Nuclear	nrSSU	NS1F	1100	35	F	GTAGTCATATGCTTGTCTC	4 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 30 s at 62 °C, and 1 min at 72 °C, and finally an elongation step of 5 min at 72 °C.	White et al. 1990
		NS4R		1108	R	CTTCCGTCAATTCCTTTAAG		
	ITS2	ITS KP5	NA		F	ACAACGATGAAGAACGCAG	3 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 30 s at 50 °C, and 1 min at 72 °C, and finally an elongation step of 7 min at 72 °C.	Lane et al. 2006
		ITS KG4			R	CTTTTCCTCCGCTTAGTTATATG		
Chloroplast	*psaA*	*psaA* 870F	890	870	F	GGNGGWYTATGGTTAAGTGA	94 °C for 5 min, 37 cycles of 94 °C for 1 min s, 45 °C for 1 min and 72 °C for 1 min and a final elongation step of 5 min at 72 °C.	Yoon et al. 2002
		*psaA*1760R		1760	R	CCTCTWCCWGGWCCATCRCAWGG		
	psaA	*PsaA*130	840	130	F	AACWACWACTTGGATTTGGAA	94 °C for 5 min, 37 cycles of 94 °C for 1 min, 45 °C for 1 min and 72 °C for 1 minand a final elongation step of 5 min at 72 °C	Yoon et al. 2002
		psaA 970		970	R	GCYTCTARAATYTCTTTCA		
Mitochondrial	COI-3P	COI-789F	589	789	F	TNTAYCARCATTTATTTTGGTT	5 min at 94 °C, followed by 25 cycles of 30 s at 94 °C, 30 s at 46 °C, and 40 s at 72 °C, and finally an elongation step of 5 min at 72 °C	Silberfeld et al. 2010
		COI-1378R		1378	R	TCYGGNATACGNCGNGGATACC		
	COI-5P	GazF2	657	104	F	CCAACCAYAAAGATATWGGTAC	2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 50 °C, and 1 min at 72 °C, and finally an elongation step of 5 min at 72 °C	Saunders 2005, Lane et al. 2007
		GazR2		803	R	GGATGACCAAARAACCAAA		

NA: not applicable.

and reverse sequence. The resulting alignment was corrected manually. The sequence was analyzed again by BLAST for sequence similarities in order to support identification of the seaweed taxa. The sequences were deposited in GenBank (Table 4).

3 Results

Iodine was detectable in all seaweed samples collected from Kuwait, with concentrations ranging from 49.59 µg g^{-1} DW in *Codium* papillatum, 129.04 µg g^{-1} DW in *Chondria* sp. and 925.10 µg g^{-1} DW in *Sargassum asperifolium* (Table 4). Likewise, fluoride levels ranged from 0.20 µg g^{-1} DW in *Sargassum aquifolium* to 72.32 µg g^{-1} DW in *C.* papillatum (Table 3).

Species identification of seaweeds used in this study was based on morphological criteria supported by DNA sequencing and molecular phylogenetics. From a total of 11 seaweed samples, eight sequences were obtained (Tables 3 and 4) which could clearly be recognized as one red, one green, and seven brown algae. Using DNA sequences of different markers the following taxa were identified: *Padina boergesenii* (*psa*A); *Iyengaria stellata* (*psa*A), *Dictyota dichotoma* (nrSSU), *Colpomenia sinuosa* (*psa*A), *S. aquifolium* (COI-3P), *S. ilicifolium* var. *acaraeocarpum* (ITS2), *S. ilicifolium* (COI-5P) and *Chondria* sp. (COI-5P). *Codium papillatum*, *Feldmannia indica* and *S. asperifolium* were identified based on morphological criteria, since no useable sequences could be obtained.

Table 3: Identity of seaweed species, DNA sequences obtained, and iodine and fluorine levels observed in them within the framework of this study.

Species	Phylogenetic affinity	Herbarium code	Date and site collected	Identification based upon /locus sequenced	Iodine concentration (μg g⁻¹ DW = ppm)	(μg g⁻¹ FW)	(mol g⁻¹ DW)	(mol g⁻¹ FW)	(μg g⁻¹ DW = ppm)	(μg g⁻¹ FW)	Fluorine concentration (mol g⁻¹ DW)	(mol g⁻¹ FW)
Chondria sp. C. Agardh	Ceramiales, Florideophyceae, Rhodophyta	BNA260518-1	26/05/2018 Bnaider Beach	COI	129.04	5.80	1.016E-3	4.571E-5	2.84 ± 0.00002	0.13 ± 0.0000009	1.495E-4 ± 1.05272E-09	6.719E-6 ± 4.73E-11
Codium papillatum C.K.Tseng et W.J.Gilbert	Bryopsidales, Ulvophyceae, Chlorophyta	ABUH030618-2	03/06/2018 Abu Al Hasaniya	–	49.59	1.45	3.907E-4	1.141E-5	72.32 ± 0.0051	2.11 ± 0.00015	3.807E-3 ± 2.69E-07	1.111E-4 ± 7.85E-09
Colpomenia sinuosa (Mertens ex Roth) Derbès et Solier	Ectocarpales, Phaeophyceae, Ochrophyta	ANJ270518-1	27/05/2018 Salwa Enjefa Beach	psaA	66.19	1.02	5.216E-4	8.033E-6	7.84 ± 0.000001	0.12 ± 0.0000000	4.127E-4 ± 5.26E-11	6.355E-6 ± 8.11E-13
Dictyota dichotoma (Hudson) J.V. Lamouroux	Dictyotales, Phaeophyceae, Ochrophyta	NUW020618-1	02/06/2018 Nuwaiseeb	nrSSU	85.47	2.76	6.736E-4	2.173E-5	2.07 ± 0.00007	0.07 ± 0.0000023	1.090E-4 ± 3.68E-09	3.515E-6 ± 1.19E-10
Iyengaria stellata (Børgesen) Børgesen	Ectocarpales, Phaeophyceae, Ochrophyta	ABUH060618-1	27/05/2018 Abu Al Hasaniya	psaA	44.55	3.92	3.510E-4	3.094E-5	12.19 ± 0.00005	1.07 ± 0.0000044	6.416E-4 ± 2.63E-09	5.654E-5 ± 2.32E-10
Padina boergesenii Allender et Kraft	Dictyotales, Phaeophyceae, Ochrophyta	ABUH270518-1	27/05/2018 Abu Al Hasaniya	psaA	52.54	2.40	4.141E-4	1.8902E-5	2.6 ± 0.00002	0.12 ± 0.0000009	1.369E-4 ± 1.05E-09	6.246E-6 ± 4.80E-11
Feldmannia indica (Sonder) Womersley et A.Bailey	Ectocarpales, Phaeophyceae, Ochrophyta	ABUH030618-1	03/06/2018 Abu Al Hasaniya	–	229.42	11.87	1.8081E-3	9.357E-5	2.66 ± 0.00001	0.14 ± 0.0000005	1.400E-4 ± 5.26E-10	7.246E-6 ± 2.72E-11
Sargassum ilicifolium var. *acaraeocarpum* Grunow	Fucales, Phaeophyceae, Ochrophyta	ABUH270518-3	26/05/2018 Bnaider Beach	ITS2	177.55	19.95	1.399E-3	1.572E-4	4.63 ± 0.00001	0.52 ± 0.0000011	2.437E-4 ± 5.26E-10	2.738E-5 ± 5.91E-11
Sargassum ilicifolium (Turner) C.Agardh	Fucales, Phaeophyceae, Ochrophyta	KHI060219-1	06/02/2019 Khiran	COI	187.14	31.49	1.475E-3	2.482E-4	0.2 ± 0.000006	0.015 ± 0.0000005	2E-1 ± 3.16E-10	1.515E-2 ± 2.39E-11
Sargassum asperifolium Hering et G.Martens ex J.Agardh	Fucales, Phaeophyceae, Ochrophyta	ABUH270518-4	26/05/2018 Bnaider Beach	–	614.61	30.21	4.843E-3	2.380E-4	0.17 ± 0.000004	0.008 ± 0.0000002	8.948E-6 ± 2.11E-10	4.398E-7 ± 1.03E-11
Sargassum aquifolium (Turner) C.Agardh	Fucales, Phaeophyceae, Ochrophyta	ABUH270518-2	27/05/2018 Abu Al Hasaniya	COI-3P	925.10	123.85	7.290E-3	9.759E-4	17.75 ± 0.00008	2.376 ± 0.0000107	9.343E-4 ± 4.21E-09	1.251E-4 ± `5.63E-10

Table 4: List of seaweeds used in this study with markers used for molecular identification, closest sequence match and accession numbers.

Morphological identification	Locus	Markers	Primer	Closest match	Query cover	Identity	Accession no. of closest match	Genbank accession no.
Feldmannia indica				Identification based on morphology				
Padina boergesenii	Chloroplast	*psaA*	psaA870F psaA1760R	*Padina boergesenii*	100%	100%	JQ364159.1	MT683780
Iyengaria stellata	Chloroplast	*psaA*	PsaA 130F psaA 970R	*Iyengaria stellata*	96%	100%	MN587733.1	MT683777
Dictyota dichotoma	Nuclear	nrSSU	AFP2F AFP1R	*Dictyota dichotoma*	97%	99.8%	AF130702.1	MT657272
Colpomenia sinuosa	Chloroplast	*psaA*	PsaA 130F psaA 970R	*Colpomenia sinuosa*	98%	99.84%	MN587728.1	MT683778
Sargassum aquifolium	Mitochondrium	COI-3P	cox1-789F cox1-1378R	*Sargassum aquifolium*	100%	99.17%	KT266809.1	MT783839
Sargassum asperifolium				Identification based on morphology				
Sargassum ilicifolium var. *acaraeocarpum*	Nuclear	ITS2	ITS KP5 ITS KG4	*Sargassum ilicifolium* var. *acaraeocarpum*	76%	100%	KP720362.1	MT664751
Sargassum ilicifolium	Mitochondrium	COI-5P	cox1-GazF2 cox1-GazR2	*Sargassum ilicifolium*	88%	99.03%	MG893221.1	MT783840
Codium papillatum				Identification based on morphology				
Chondria sp.	Mitochondria	COI-5P	cox1-GazF2 cox1-GazR2	*Chondria* cf. *curdieana*	95.86%	95.5%	MG843862.1	MT683781

4 Discussion

The results presented here constitute the first report of iodine and fluorine concentrations in seaweeds of the Arabian Gulf – and are among the first for warm-temperate and tropical seaweeds. The iodine levels observed in this study are in a range comparable to those reported from the same genera elsewhere (Ar Gall et al. 2004; Küpper et al. 1998; Saenko et al. 1978). Given the established role of iodide in controlling oxidative stress on seaweed surfaces (Küpper et al. 2008) and also given the reduced iodine levels observed in the North Atlantic kelp *L. digitata* during the summer, likely due to high irradiance and high temperatures (Ar Gall et al. 2004), it would be of interest to conduct a seasonal survey of iodine levels in the species which were found to contain the highest iodine levels – in particular *S. aquifolium*, *S. asperifolium* and *S. ilicifolium* var. *acaraeocarpum*. It should be noted that most samples used in the present study were collected during the cold season, when seawater temperatures are 15–20 °C lower than during the summer (Al-Yamani, 2004). Overall, there is a tendency that the brown algae surveyed here have higher iodine levels than the red and especially than the green algae – which is a pattern also reported from another part of the world (Saenko et al. 1978).

Halogens, such as iodine and bromine and their respective organohalides, shape major atmospheric processes mainly through destruction of toxic surface ozone and provision of condensation nuclei for cloud formation (Küpper et al. 2008; Palmer et al. 2005). Seaweed-derived atmospheric iodine emissions also have a high significance for human and animal health by supplying the essential element for thyroid functioning (Küpper et al. 2011). Seaweeds have been identified as major emitters of these elements to the coastal atmosphere in different parts of the world, especially the cold-temperate North Atlantic (Küpper et al. 2011). Most of this knowledge is based on studying the native North Atlantic model seaweed, *L. digitata*, thus constituting a strong sampling bias with regard to species diversity and the geographic extent of this phenomenon. The potential contribution of dominant seaweed species to halogen production along continental coasts in warm-temperate and tropical climates, particularly along the Arabian Gulf, is virtually unknown. Because the Gulf region experiences some of the highest ground-level ozone concentrations in the world (Lelieveld et al. 2009), examining the contribution of region-specific seaweed species to halogen production has direct implications for the understanding of atmospheric/climatic processes and the public health of the region. Gaseous emissions from iodine metabolism in seaweeds have both an atmospheric impact (depleting surface ozone and acting as condensation nuclei for cloud formation) and contribute to the supply of thyroid iodine in the coastal population (Küpper et al. 2011). In this context, a better understanding of the extent to which the region's seaweeds potentially constitute a natural scavenger for harmful ground-level ozone, and contribute to cloud formation and to human thyroid iodine supply, would be desirable.

Also, fluoride was present in similar, significant concentrations comparable to those found for iodine. Given how little is known about fluorine accumulation in seaweeds, this constitutes a particular novelty of the present study in general – not limited to the Arabian Gulf. Interestingly, while *Codium* sp. showed among the lowest iodine levels here, the same sample contained the highest fluorine levels – and vice versa for *Sargassum* species, which were among the strongest accumulators of iodine but among the weakest for fluorine. This deserves further investigation, but at present, one can only speculate about the underlying reasons. Very little is known about the regulation and homoeostasis of halogen accumulation in seaweeds in general. Ar Gall et al. (2004) found iodine levels in the kelp *L. digitata* to be around half of their winter levels during summer, while a competitive inhibition of bromide accumulation by increased, exogenous iodide levels (which may be somewhat reminiscent of the results of the present study) was observed in the filamentous brown alga *Ectocarpus* (Küpper et al. 2018). However, given the very different physico-chemical properties of iodide and fluoride, it seems unlikely that the same transport and storage mechanisms are operative for both. In particular, while iodine and bromine uptake in marine algae is usually driven by V haloperoxidases (Küpper et al. 2011; Küpper et al. 2013; Küpper and Kroneck 2015), this class of enzymes cannot mediate fluoride uptake since hydrogen peroxide cannot oxidize fluoride (Butler and Sandy 2009); thus, another, likely totally different pathway must be at play for fluoride accumulation.

Significantly, the findings of *S. ilicifolium* and *S. ilicifolium* var. *acaraeocarpum* reported here are new records both for Kuwait and the Arabian Gulf, while *P. boergesenii* and *D. dichotoma* are new records for Kuwait. *C. papillatum, P. boergesenii, D. dichotoma, S. asperifolium* and *S. aquifolium* have previously been reported from the Arabian Gulf (John and Al-Thani 2014). Likewise, *I. stellata* and *C. sinuosa* are known specifically from Kuwait (Al-Yamani et al. 2014; Silva et al. 1996). It is worth noting that the DNA sequences reported here are the first for any seaweeds from the entire Arabian Gulf, highlighting also how understudied the region is with regards to molecular algal taxonomy. However, this also highlights a general challenge when working in this region – a robust identification of the taxa investigated in this study will only be possible when a larger dataset is available. Thus, the situation is even more complicated than e.g. in the Mediterranean where a recent review highlighted the need for obtaining DNA barcode sequences for a much larger breadth of taxa (Bartolo et al. 2020).

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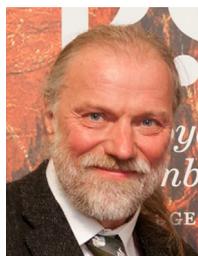
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