

Research article

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Trace element concentrations in seaweeds of the Arabian Gulf identified by morphology and DNA barcodes

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Abstract: Even though seaweeds have been considered a nutrient-rich dietary source of minerals in other parts of the world, there is little knowledge about trace element accumulation in seaweeds of the Arabian Gulf. The Arabian Gulf is of particular interest due to being an extreme environment, as it features some of the highest temperatures and salinities observed in any marine waters in the world. This study determined the minerals contents using inductively-coupled plasma-mass spectrometry (ICP-MS) in 10 of the most common seaweeds of this region (*Iyengaria stellata*, *Padina boergesenii*, *Chondria* sp., *Feldmannia indica*, *Codium papillatum*, *Sargassum aquifolium*, *Ulva chaugulii*, *Ulva tepida* and *Ulva* sp.) supported by morphological and molecular (DNA barcode)-based identification. The finding of *U. chaugulii* reported here is a new record for Kuwait. Most of the seaweeds were rich in essential minerals including Ca, Mg, Na, K, Fe and Zn and their contents were higher than those of other mineral-rich foods. Principal component analysis revealed

species-specific distributions of minerals in seaweeds. *U. tepida* and *I. stellata* were found to be exceptionally rich in most of the macro- and trace elements along with low As and Se, and thus can be utilized for food and feed applications.

Keywords: arsenic; calcium; copper; ICP-MS; iron; Kuwait.

1 Introduction

Seaweeds are used in many coastal countries for human nutrition, in animal feed formulations, and as fertilizer in agriculture, which is partly due to their mineral contents including trace elements (Nabti et al. 2017; Wells et al. 2017). Seaweeds are a rich source of various nutrients including minerals, amino acids, dietary fibres, vitamins, trace elements, and other health-promoting compounds providing benefits beyond basic nutrition (Holdt and Kraan 2011). Seaweeds can be used as a food supplement in order to reach the recommended daily intakes of some macro-minerals and trace elements (Rupérez 2002). Generally, trace elements are important as constituents of bones, teeth, soft tissues, haemoglobin, muscle, blood, and nerve cells, and are vital for overall mental and physical wellbeing (Kuda and Ikemori 2009). Seaweed extracts are characterized by a high concentration of minerals as they concentrate trace elements from surrounding waters, in many cases 10–20 times that of terrestrial plants (Moreda-Pineiro et al. 2011). The mineral content of seaweeds can account for 8–40% of their dry weight. In general, seaweeds contain high concentrations of macronutrients (Ca, Mg, Na, P and K) and trace elements (Br, Co, Cu, Fe, I, Mn, Mo, Se and Zn), which significantly vary among red, brown and green species (Cabrita et al. 2016; Circuncisão et al. 2018). The variations can be attributed to phylogenetic affinity, stage of growth, geographical origin and location, along with seasonal, environmental and physiological variations, submergence and wave exposure (Marinho-Soriano et al. 2006; Van Netten et al. 2000). Moreover, a determining factor for mineral uptake by seaweeds is the content and type of polysaccharides in the cell

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wall structure which may act as chelators (Circuncisão et al. 2018). The accumulation of Mg and Fe seems to be more pronounced in green seaweed species while red and brown seaweeds tend to accumulate higher concentrations of Na, K, Zn, Mn and I (Circuncisão et al. 2018). Brown seaweeds are a good source of magnesium, copper, iron, and iodine as well as other trace elements (Vasuki et al. 2020). Iron is present in seaweeds at higher concentrations than in many common animal and plant food sources (e.g. meats and spinach) due to higher metabolic capacity of seaweeds to directly absorb these elements from the seawater (Smith et al. 2010). Iron was found to be more abundant in brown algae, followed by the red and the green species. Sulphur content was especially high in the red and green seaweeds, compared with the brown seaweeds. The accumulation of iodine in brown algae has been studied extensively (Küpper and Carrano 2019; Küpper et al. 2011); for the Arabian Gulf region, we would like to highlight our recent work which also covers fluorine (Al-Adilah et al. 2020).

Calcium, phosphorus and magnesium, the major minerals in the human body, are abundant elements in macroalgae as well, where they are present in concentrations that surpass those of apples, oranges, carrots, and potatoes (Cardoso et al. 2014). Most seaweeds contain higher concentrations of Na and K than those reported in vegetables. The low Na/K ratios in seaweeds, which are usually below 1:5 (MacArtain et al. 2007), highlight the potential of seaweed supplements for good maintenance of cardiovascular health, since low Na/K ratios are well known to promote lower blood pressure (Perez and Chang 2014). Furthermore, inorganic substances like S, Ca, Mg, Si, Na, K are essential for a wide range of basic physiological functions including the immune system.

On the other hand, seaweeds or macroalgae have been widely used to monitor and to characterize the status of environmental pollution. They play an important role in the nutrient dynamics of coastal systems and reflect changes in water quality efficiently (Wilson 2002). Many studies have been conducted to determine the impacts of heavy metal pollution and bioaccumulation of metals in macroalgae, especially those metals considered as public health threats. Küpper et al. (2002) investigated the formation of heavy metal chlorophylls in brown, red and green algae as a consequence of stress by these metals. Some seaweeds are excellent filtering agents of pollutant trace elements such as arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), and lead (Pb). They remove the toxic materials from the environment and accumulate them in their tissues, which may be 4000–20,000 times more than in the surrounding water (Sudharsan et al. 2012). Accordingly, the accumulation of heavy metals by aquatic biota will affect the food web, which may have implications for human health.

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 fresh water 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 year^{-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 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 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). 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 diversity (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 is known about the accumulation and metabolism of trace metals 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 accumulate trace elements at concentrations comparable to those in colder climates, underpinned by morphological and DNA barcoding-based identification of seaweeds.

Inductively-coupled plasma mass spectrometry (ICP-MS) is an established technique for the analysis of trace metals in

plants (Tokaloğlu 2012) and seaweeds (Soares et al. 2020; Westby 2018) including algal-based food products (Dawczynski et al. 2007). We have previously used the technique for measuring iodine concentrations in Arabian Gulf seaweeds (Al-Adilah et al. 2020). Studies pertaining to the metal bioaccumulation in seaweeds from the Arabian Gulf are lacking and thus an attempt has been made to assess the differences in metal concentrations (Cd, Cu, Mn, Pb and Zn) among algal species collected from Kuwaiti coastal waters, which will also serve as a baseline for future studies in this region.

2 Materials and methods

2.1 Algal collections

Sampling locations were selected to cover a representative variety of geographical and ecological regions along the coastline of Kuwait (Table 1). Fully grown mature seaweed thalli were collected during low tide (the maximum tidal range in Kuwait 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 where

they were again washed three times with seawater. Subsequently, the fresh samples were frozen in a freezer for 24 h followed by drying in a freeze dryer (Labconco, Kansas City, MO, USA) at -45°C for 48 h and kept in the presence of silica gel to prevent accumulation of humidity.

2.2 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 in silica gel for subsequent DNA extraction. Voucher specimens were deposited in the Kuwait University Herbarium (KTUH; Table 1). For three taxa, molecular identification was conducted within the framework of this study – for the others, this has been done and published before (Al-Adilah et al. 2020).

2.3 DNA extraction, amplification and sequencing

About 20 mg of silica gel-dried algal material was 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, USA) according to the manufacturer's instructions. The extracted DNA was amplified by PCR using the primers mentioned in 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 2.

Table 1: Locations of sampling sites in Kuwait during the surveys and collections in May–June 2017 and February 2021.

Species	Phylogenetic affinity	Herbarium code	Date	Location	Coordinates	Offshore seawater surface temperature ($^{\circ}\text{C}$)
<i>Chondria</i> sp. C. Agardh	Ceramiales, Florideophyceae, Rhodophyta	BNA260518-1	26/05/2018	Bnaider Beach	28°47'01.5"N 48°17'50.6"E	27.75–27.90
<i>Iyengaria stellata</i> (Børgesen) Børgesen	Ectocarpales, Phaeophyceae, Ochrophyta	ABUH060618-1	27/05/2018	Abu Al Hasaniya	29°12'19.4"N 48°06'41.5"E	30.15–30.30
<i>Padina boergesenii</i> Allender et Kraft	Dictyotales, Phaeophyceae, Ochrophyta	ABUH270518-1				
<i>Sargassum aquifolium</i> (Turner) C. Agardh	Fucales, Phaeophyceae, Ochrophyta	ABUH270518-2				
<i>Feldmannia indica</i> (Sonder) Womersley et A. Bailey	Ectocarpales, Phaeophyceae, Ochrophyta	ABUH030618-1	3/06/2018			
<i>Codium papillatum</i> C. K. Tseng et W. J. Gilbert	Bryopsidales, Ulvophyceae, Chlorophyta	ABUH030618-2				
<i>Ulva</i> sp.	Ulvales, Ulvaceae, Chlorophyta	DOH010221-1	01/02/2021	Ras Ushairij	29°23'00.7"N 47°49'50.9"E	14.85–15.00
<i>Ulva chaugulii</i> M. G. Kavale et M. A. Kazi	Chlorophyta	DOH010221-2				
<i>Ulva tepida</i> Y. Masakiyo et S. Shimada		DOH010221-5				

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

Locus	Primer	Length(bp)	Position on <i>tufA</i> gene	Direction	Sequence 5'–3'	PCR parameters	References
Chloroplast	tufAF	850–900	35	F	TGAAACAGAAAMAWCGTCATTATGCGCCCTTCNCGAATMGCRAAWCGC	4 min at 94 °C, followed by 38 cycles of 1 min at 94 °C, 30 s at 45 °C, and 1 min at 72 °C, and finally an elongation step of 7 min at 72 °C	Famà et al. (2002)
	tufAR		1108	R			

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). 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.nlm.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 and reverse sequence. The resulting alignment was corrected manually. The sequences were analyzed again by BLAST for sequence similarities in order to support identification of the seaweed taxa. The sequences were deposited in GenBank/NCBI (Table 3).

2.4 Determination of total mineral concentrations in tissue samples

The freeze-dried seaweed samples (in triplicate) were ground with mortar and pestle. Approximately 400 mg of powdered samples were weighed directly into each of the flasks, 5 ml of HNO₃ (37%) and 3 ml of H₂O₂ (30%) added, and the mixture was submitted to microwave-assisted acid digestion (manufacturer of the microwave: Multiwave PRO™, Anton Paar, Graz, Austria) using a temperature-controlled program (Table 4). After cooling, the digested samples were diluted with ultrapure water (Elga DI water system 18.2 Megohm.cm) to 50 ml and the measurements were carried out using ICP-MS where 10 ml of each sample was transferred to a test tube. Prior to starting the analytical measurements, the ICP-MS instrument (Nexion™ 350D, Perkin-Elmer, Waltham MA, USA) was allowed to equilibrate for 30 min. During this warm-up time, the performance check was done using a setup solution (Perkin-Elmer, Waltham MA, USA) containing 1 µg ml⁻¹ of Ba, Ce, Fe, In, Li, Mg, Pb and U. After calibration, the samples were aspirated in the instrument. For some elements, the sample was used without dilution (Se, Mo and As). For others, 100× (Fe, Mo, As, Fe, Zn, Cu, Mn and Ni) or 1000× (Ca, K, Mg, Na and P) dilution. All ICP-MS parameters are shown in Table 5.

2.5 Statistical analysis

All analytical determinations were performed in triplicate ($n = 3$) and the mean values were recorded. The contents of trace elements in different seaweed species were compared by analysis of variance (ANOVA) with values significant at $p < 0.05$. Principal component analysis (PCA) was performed using a web-based software, MetaboAnalyst v 5.0 (<https://www.metaboanalyst.ca>), in order to visualize the distribution of different elements among seaweeds. For this, the elements data matrix was pre-processed by log-transformation and pareto scaling (mean-centered and divided by the square root of standard deviation of each value) prior to PCA.

Table 3: List of the herbarium codes, morphological identification and the closest match of sequences of seaweeds used in this study.

Herbarium	Morphological identification	Locus	Markers	Primer	Closest match	Identity (%)	Accession no. of closest match	GenBank accession no.
DOH010221-1	<i>Ulva</i> sp.	Chloroplasts	<i>tufA</i>	tufAF tufAR	<i>Ulva intestinalis</i>	98.84	KC661439	MW768868
DOH010221-2	<i>Ulva chaugulii</i>	Chloroplasts	<i>tufA</i>	tufAF tufAR	<i>Ulva chaugulii</i>	99.86	MG976863	MW768867
DOH010221-5	<i>Ulva tepida</i>	Chloroplasts	<i>tufA</i>	tufAF tufAR	<i>Ulva tepida</i>	99.31	MG976872	MW768866

Table 4: Microwave-assisted acid digestion program.

Temperature (°C)	Ramp (min)	Time (min)
145	2	10
170	5	5
200	2	15
50	0	10

Table 5: ICP-MS operating conditions.

Operating conditions of ICP-MS (NexION 350D)	
Instrument calibration (range)	0.01–100 ppb
RF power	1600 W
Plasma gas	18 l min ⁻¹
Auxiliary gas	1.2 l min ⁻¹
Nebulizer gas	0.92 l min ⁻¹
Oxygen makeup gas	0.0 l min ⁻¹
Peristaltic pump speed	30 rpm
Stabilization delay	35 s
Replicate/sample	3
Rinse time	35 s
The error/uncertainty	±10%
Limit of detection	0.1 µg l ⁻¹
Nebulizer	MicroMist
Spray chamber	Quartz cyclonic
Torch	High efficiency quartz
Torch injector	1-mm quartz
Cones	Nickel

3 Results

3.1 Identification of seaweeds

Species identification of the seaweeds used in this study was based on morphological criteria supported by DNA sequencing and molecular phylogenetics (Al-Adilah et al. 2020). Using DNA sequences of the *tufA* gene, the additionally collected samples were identified as *Ulva chaugulii*, *Ulva tepida* and *Ulva* sp. (Table 3).

3.2 Mineral contents in seaweeds

Five macroelements, Ca, Mg, Na, K, P and seven trace elements Fe, Mn, Zn, Cu, Ni, As and Se were detected in seaweeds from the Arabian Gulf (Table 6), while molybdenum (Mo) was below the detection limit (0.01 ppb). The contents of different elements significantly differed ($p < 0.05$) between different seaweeds (Table 6).

The distribution of macroelements in seaweeds was in the decreasing order of $Ca > K > Na > Mg > P$ in all species except *Padina boergesenii*, *Codium papillatum*, *U. chaugulii* and *U. tepida*. Mg was the second-most abundant element after Ca in *P. boergesenii* followed by K, P and Na. *C. papillatum*, *U. chaugulii* and *U. tepida* all had strongly varying elemental abundances albeit with Na as the most abundant macroelement (8.0–28.4 mg g⁻¹ DW; Table 6A). The ratio of Na/K varied between 0.11 ± 0.03 (*P. boergesenii*) and 1.3 ± 5.6 (*Ulva* sp.) except in *C. papillatum* that had an exceptionally high Na/K ratio (4.21 ± 0.7). The ranges of concentrations of macroelements in the studied seaweeds were 10.4–110.5 mg g⁻¹ DW for Ca, 3.3–16.0 mg g⁻¹ DW for Mg, 0.4–28.3 mg g⁻¹ DW for Na and 0.5 mg g⁻¹ DW to 3.3 mg g⁻¹ DW for P (Table 6A and B).

Furthermore, Fe was the most abundant trace element in all green, red and brown seaweeds (0.23–3.51 mg g⁻¹ DW). The contents of other trace elements were in the range of 31.01 ± 0.1 mg kg⁻¹ DW to 207.5 ± 1.6 mg kg⁻¹ DW for Mn, 11.41 ± 0.2 mg kg⁻¹ DW to 120.0 ± 2.3 mg kg⁻¹ DW for Zn, 28.1 ± 0.1 mg kg⁻¹ DW to 117.4 ± 0.7 mg kg⁻¹ DW for Cu, 7.1 ± 0.5 mg kg⁻¹ DW to 34.7 ± 0.3 mg kg⁻¹ DW for Ni, 2.6 ± 0.08 mg kg⁻¹ DW to 42.1 ± 0.8 mg kg⁻¹ DW for As and 2.38 ± 0.4 mg kg⁻¹ DW to 11.69 ± 0.1 mg kg⁻¹ DW for Se (Table 6B–D).

3.3 Principal component analysis (PCA)

PCA was performed to reduce the dimensionality of the species/concentrations dataset, in order to identify the seaweed species particularly rich in several minerals at the same time. PCA explained 68.6% of the variation in the

Table 6A: Mineral contents of different seaweeds.

Species	Macroelements					FW: DW ratio
	Ca		Na		K	
	(mg g ⁻¹ DW)	(mol kg ⁻¹ DW)	(mg g ⁻¹ DW)	(mol kg ⁻¹ DW)		
<i>Iyengaria stellata</i>	110.5 ± 1.07 ^a	2.7 ± 0.02 ^a	3.8 ± 0.05 ^h	1.6 × 10 ⁻¹ ± 1.2 × 10 ^{-3g}	1.8 × 10 ⁻¹ ± 9.1 × 10 ^{-4c}	22.0
<i>Feldmannia indica</i>	50.6 ± 0.05 ^b	1.2 ± 0.001 ^d	5.7 ± 0.05 ^f	2.5 × 10 ⁻¹ ± 1.2 × 10 ^{-3e}	2.2 × 10 ⁻¹ ± 1.1 × 10 ^{-3c}	12.9
<i>Padina boergesenii</i>	56.4 ± 0.4 ^c	1.4 ± 0.01 ^g	0.4 ± 0.01 ⁱ	2.1 × 10 ⁻² ± 1.6 × 10 ^{-3h}	1.0 × 10 ⁻¹ ± 5.2 × 10 ^{-3e}	11.4
<i>Sargassum aquifolium</i>	46.8 ± 0.5 ^e	1.1 ± 0.01 ^e	7.1 ± 0.1 ^e	3.0 × 10 ⁻¹ ± 3.7 × 10 ^{-3d}	4.2 × 10 ⁻¹ ± 5.3 × 10 ^{-3b}	33.5
<i>Chondria</i> sp.	55.5 ± 0.2 ^c	1.3 ± 0.01 ^c	5.1 ± 0.1 ^g	2.2 × 10 ⁻¹ ± 1.9 × 10 ^{-3g}	1.8 × 10 ⁻¹ ± 4.7 × 10 ^{-3c}	11.2
<i>Codium papillatum</i>	10.4 ± 0.01 ^h	0.2 ± 0.0003 ^h	26.7 ± 0.1 ^b	1.1 ± 4.2 × 10 ^{-3b}	1.6 × 10 ⁻¹ ± 5.5 × 10 ^{-3cd}	7.3
<i>Ulva</i> sp.	35.1 ± 0.06 ^f	0.8 ± 0.001 ^g	26.0 ± 0.2 ^c	1.1 ± 5.5 × 10 ^{-3a}	5.1 × 10 ⁻¹ ± 9.9 × 10 ^{-4a}	216.8
<i>Ulva chaugulii</i>	22.1 ± 0.3 ^b	0.5 ± 0.008 ^b	28.3 ± 0.03 ^d	1.2 ± 7.9 × 10 ^{-4d}	6.4 × 10 ⁻¹ ± 7.2 × 10 ^{-3c}	219.0
<i>Ulva tepida</i>	68.8 ± 0.5 ^g	1.7 ± 0.01 ^g	7.9 ± 0.1 ^a	3.4 × 10 ⁻¹ ± 3.6 × 10 ^{-3a}	2.0 × 10 ⁻¹ ± 2.9 × 10 ^{-3a}	220.0

Values are given in mean ± SD (n = 3). ^{a-i}Values in row without a common superscript are significantly different at p = 0.05.

Table 6B: Mineral contents of different seaweeds.

Species	Macroelements			Microelements		
	Mg		P	Fe		Na/K
	(mg g ⁻¹ DW)	(mol kg ⁻¹ DW)		(mg g ⁻¹ DW)	(mol kg ⁻¹ DW)	
<i>Iyengaria stellata</i>	3.3 ± 0.1 ^g	1.3 × 10 ⁻¹ ± 4.0 × 10 ^{-3g}	0.9 ± 0.06 ^f	3.0 × 10 ⁻² ± 1.5 × 10 ^{-3f}	2.0 ± 0.2 ^c	3.6 × 10 ⁻² ± 4.9 × 10 ^{-3c}
<i>Padina boergesenii</i>	3.4 ± 0.2 ^d	1.4 × 10 ⁻¹ ± 5.8 × 10 ^{-3d}	1.6 ± 0.03 ^e	5.2 × 10 ⁻² ± 7.5 × 10 ^{-4e}	3.0 ± 0.1 ^c	5.4 × 10 ⁻² ± 2.5 × 10 ^{-3c}
<i>Feldmannia indica</i>	5.3 ± 0.1 ^g	2.2 × 10 ⁻¹ ± 2.5 × 10 ^{-3g}	1.2 ± 0.009 ^c	4.1 × 10 ⁻² ± 2.3 × 10 ^{-4c}	2.0 ± 0.2 ^b	3.7 × 10 ⁻² ± 6.2 × 10 ^{-3b}
<i>Codium papillatum</i>	4.0 ± 0.2 ^f	1.6 × 10 ⁻¹ ± 5.3 × 10 ^{-3e}	0.8 ± 0.005 ^d	2.7 × 10 ⁻² ± 1.3 × 10 ^{-4d}	0.2 ± 0.2 ^e	4.0 × 10 ⁻³ ± 7.0 × 10 ^{-4e}
<i>Sargassum aquifolium</i>	4.7 ± 0.07 ^f	1.9 × 10 ⁻¹ ± 1.7 × 10 ^{-3f}	1.4 ± 0.02 ^f	4.7 × 10 ⁻² ± 7.1 × 10 ^{-4f}	0.4 ± 0.05 ^e	7.5 × 10 ⁻³ ± 1.4 × 10 ^{-3e}
<i>Chondria</i> sp.	3.9 ± 0.02 ^f	1.6 × 10 ⁻¹ ± 6.9 × 10 ^{-3e}	0.5 ± 0.005 ^g	1.8 × 10 ⁻² ± 1.4 × 10 ^{-4g}	1.5 ± 0.1 ^d	2.7 × 10 ⁻² ± 3.0 × 10 ^{-3d}
<i>Ulva</i> sp.	11.5 ± 0.2 ^b	4.7 × 10 ⁻¹ ± 5.2 × 10 ^{-3b}	1.6 ± 0.07 ^c	5.4 × 10 ⁻² ± 1.8 × 10 ^{-3c}	2.2 ± 1.7 ^c	3.9 × 10 ⁻² ± 4.3 × 10 ^{-3c}
<i>Ulva chaugulii</i>	16.0 ± 0.2 ^c	6.6 × 10 ⁻¹ ± 5.8 × 10 ^{-3c}	2.2 ± 0.03 ^a	7.4 × 10 ⁻² ± 9.8 × 10 ^{-4a}	0.5 ± 0.04 ^a	1.0 × 10 ⁻² ± 1.1 × 10 ^{-3a}
<i>Ulva tepida</i>	8.2 ± 0.04 ^a	3.4 × 10 ⁻¹ ± 1.1 × 10 ^{-3a}	3.3 ± 0.03 ^b	1.0 × 10 ⁻¹ ± 9.0 × 10 ^{-4b}	3.5 ± 0.1 ^e	6.2 × 10 ⁻² ± 4.2 × 10 ^{-3e}

Values are given in mean ± SD (n = 3). ^{a-i}Values in row without a common superscript are significantly different at p = 0.05.

Table 6C: Mineral contents of different seaweeds.

Species	Microelements					
	Zn		Se		Mn	
	(mg kg ⁻¹ DW)	(mol kg ⁻¹ DW)	(mg kg ⁻¹ DW)	(mol kg ⁻¹ DW)	(mg kg ⁻¹ DW)	(mol kg ⁻¹ DW)
<i>Iyengaria stellata</i>	15.7 ± 0.1 ^g	2.4 × 10 ⁻⁴ ± 3.8 × 10 ^{-6g}	2.38 ± 0.4 ^e	3.0 × 10 ⁻⁵ ± 1.0 × 10 ^{-5e}	61.9 ± 0.5 ^e	1.1 × 10 ⁻³ ± 1.4 × 10 ^{-5e}
<i>Padina boergeresii</i>	32.3 ± 0.8 ^c	4.9 × 10 ⁻⁴ ± 2.1 × 10 ^{-5c}	2.62 ± 0.2 ^e	3.3 × 10 ⁻⁵ ± 6.0 × 10 ^{-6e}	64.5 ± 0.4 ^b	1.1 × 10 ⁻³ ± 1.2 × 10 ^{-5d}
<i>Feldmannia indica</i>	51.8 ± 0.4 ^f	7.9 × 10 ⁻⁴ ± 1.1 × 10 ^{-5f}	2.68 ± 0.2 ^e	3.3 × 10 ⁻⁵ ± 5.5 × 10 ^{-6e}	115 ± 0.8 ^d	2.1 × 10 ⁻³ ± 2.0 × 10 ^{-5b}
<i>Codium papillatum</i>	37.8 ± 1.2 ^d	5.7 × 10 ⁻⁴ ± 3.0 × 10 ^{-5d}	4.13 ± 0.2 ^b	5.2 × 10 ⁻⁵ ± 6.3 × 10 ^{-6b}	33.0 ± 0.6 ^g	6.0 × 10 ⁻⁴ ± 1.5 × 10 ^{-5g}
<i>Chondria</i> sp.	120.0 ± 2.3 ^a	1.8 × 10 ⁻³ ± 5.7 × 10 ^{-5a}	6.47 ± 0.0.5 ^c	8.1 × 10 ⁻⁵ ± 1.4 × 10 ^{-5c}	72.6 ± 0.6 ^c	1.3 × 10 ⁻³ ± 1.5 × 10 ^{-5c}
<i>Sargassum aquifolium</i>	44.8 ± 0.9 ^e	6.8 × 10 ⁻⁴ ± 2.210 ^{-5e}	7.46 ± 0.1 ^a	9.4 × 10 ⁻⁵ ± 3.3 × 10 ^{-6d}	31.0 ± 0.1 ^g	5.6 × 10 ⁻⁴ ± 4.0 × 10 ^{-6g}
<i>Ulva</i> sp.	49.42 ± 0.8 ^c	7.5 × 10 ⁻⁴ ± 2.1 × 10 ^{-5c}	10.98 ± 0.3 ^a	1.3 × 10 ⁻⁴ ± 7.8 × 10 ^{-6a}	116 ± 1.0 ^f	2.1 × 10 ⁻³ ± 2.6 × 10 ^{-5a}
<i>Ulva chaugulii</i>	11.41 ± 0.2 ^b	1.7 × 10 ⁻⁴ ± 7.×10 ^{-6b}	11.69 ± 0.1 ^c	1.4 × 10 ⁻⁴ ± 4.5 × 10 ^{-6c}	36.0 ± 1.0 ^a	6.5 × 10 ⁻⁴ ± 2.6 × 10 ^{-5f}
<i>Ulva tepida</i>	68.41 ± 1.11 ^h	1.0 × 10 ⁻³ ± 2.7 × 10 ^{-5h}	6.31 ± 0.4 ^a	7.9 × 10 ⁻⁵ ± 9.9 × 10 ^{-6a}	207.5 ± 1.6 ^f	3.7 × 10 ⁻³ ± 4.1 × 10 ^{-5f}

Values are given in mean ± SD (n = 3). ^{a-h}Values in row without a common superscript are significantly different at p = 0.05.

Table 6D: Mineral contents of different seaweeds.

Species	Microelements					
	As		Ni		Cu	
	(mg kg ⁻¹ DW)	(mol kg ⁻¹ DW)	(mg kg ⁻¹ DW)	(mol kg ⁻¹ DW)	(mg kg ⁻¹ DW)	(mol kg ⁻¹ DW)
<i>Iyengaria stellata</i>	3.6 ± 0.2 ^{ef}	4.9 × 10 ⁻⁵ ± 6.3 × 10 ^{-6e}	16.0 ± 0.1 ^e	2.7 × 10 ⁻⁴ ± 4.2 × 10 ^{-6e}	87.9 ± 0.8 ^c	0.001 ± 2.2 × 10 ^{-5c}
<i>Feldmannia indica</i>	9.0 ± 0.09 ^c	0.0001 ± 2.4 × 10 ^{-6c}	20.2 ± 0.2 ^c	3.4 × 10 ⁻⁴ ± 5.6 × 10 ^{-6c}	94.6 ± 0.2 ^b	0.001 ± 5.5 × 10 ^{-6b}
<i>Padina boergeresii</i>	5.5 ± 0.2 ^d	7.3 × 10 ⁻⁵ ± 5.0 × 10 ^{-6d}	17.6 ± 0.4 ^d	3.0 × 10 ⁻⁴ ± 1.0 × 10 ^{-5d}	117.4 ± 0.7 ^a	0.001 ± 1.8 × 10 ^{-5a}
<i>Sargassum aquifolium</i>	42.1 ± 0.8 ^a	0.0005 ± 2.0 × 10 ^{-5a}	8.7 ± 0.1 ^f	1.4 × 10 ⁻⁴ ± 3.9 × 10 ^{-6f}	28.1 ± 0.1 ^f	<DL
<i>Chondria</i> sp.	2.4 ± 0.4 ^g	3.2 × 10 ⁻⁵ ± 1.1 × 10 ^{-5g}	8.1 ± 0.6 ^g	1.3 × 10 ⁻⁴ ± 1.6 × 10 ^{-5g}	<DL	<DL
<i>Codium papillatum</i>	10.7 ± 0.2 ^b	0.0001 ± 6.3 × 10 ^{-6b}	7.1 ± 0.5 ^g	1.2 × 10 ⁻⁴ ± 1.4 × 10 ^{-5fg}	<DL	<DL
<i>Ulva</i> sp.	3.4 ± 0.4 ^{efg}	4.5 × 10 ⁻⁵ ± 1.1 × 10 ^{-5g}	22.5 ± 0.4 ^f	3.8 × 10 ⁻⁴ ± 1.0 × 10 ^{-5b}	35.6 ± 0.2 ^f	5.6 × 10 ⁻⁴ ± 5.9 × 10 ^{-6e}
<i>Ulva chaugulii</i>	2.6 ± 0.08 ^e	3.5 × 10 ⁻⁵ ± 2.2 × 10 ^{-6g}	8.9 ± 0.1 ^a	1.5 × 10 ⁻⁴ ± 3.9 × 10 ^{-6a}	<DL	<DL
<i>Ulva tepida</i>	3.9 ± 0.2 ^g	5.3 × 10 ⁻⁵ ± 5.8 × 10 ^{-6f}	34.7 ± 0.3 ^f	5.9 × 10 ⁻⁴ ± 9.3 × 10 ^{-6f}	45.9 ± 0.2 ^g	7.2 × 10 ⁻⁴ ± 5.5 × 10 ^{-6g}

Values are given in mean ± SD (n = 3). ^{a-h}Values in row without a common superscript are significantly different at p = 0.05. DL, detection limit.

elemental data matrix (PC1 – 44.5% and PC2 – 24.2%). The discriminant variables along PC1 were Na, Se, Cu, Ca, and Fe, while along PC2 they were As, P and Mg. The brown seaweeds *P. boergesenii*, *Iyengaria stellata* and *Feldmannia indica* were positioned together due to their higher contents of Ca and Cu, while *Sargassum aquifolium* was positioned as an outlier due to exceptionally high amounts of As ($42.2 \mu\text{g g}^{-1}$ DW). All three *Ulva* species were positioned separately due to their distinct elemental distributions. *U. tepida* was separated due to its higher concentrations of Fe, Mn and Ni, *Ulva* sp. due to higher concentrations of P and Mg, while *U. chaugulii* was separated due to higher loadings of Na and Se. *Chondria* sp. had the highest content of Zn, while *C. papillatum* had low contents of most of the elements except Na.

4 Discussion

The results presented here constitute the first report of the trace element concentrations in seaweeds of the Arabian Gulf – and are among the first for warm-temperate and tropical seaweeds. The fieldwork of this study coincided with the strongest growth season of seaweeds in the Gulf (spring), ensuring that fully grown mature thalli for each species, the life form contributing the most to benthic primary productivity and standing stock and, thus, of the highest ecological significance, was collected. The concentrations of minerals observed in this study are in a range comparable to those reported from the same genera elsewhere (de la Rocha et al. 2009; Yoganandham et al. 2019). It would be of interest to conduct a seasonal survey of trace elements in the species which were found to contain the highest mineral concentrations – in particular *I. stellata* and *U. tepida*. Members of the genus *Ulva* contained the highest concentrations of several of the trace elements investigated here: *U. tepida* contained the highest Fe, Mn, Ni and P concentrations, while *U. chaugulii* had the highest K, Mg, Na and Se concentrations (Figure 1 and Table 6A–D). Zn concentrations were the same in both species. Further, Ca concentrations were highest in *I. stellata*, while As concentrations were highest in *S. aquifolium* and Cu concentrations in *P. boergesenii*. 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 2014). *S. aquifolium* (collected on 27 May 2018 in Abu Hasaniya), showed a rather high As concentration (Figure 1) compared to the three other brown algal samples investigated here. On the other hand,

comparability is limited, since they belong to different species which may be reflected in different physiological and biochemical adaptations. Similarly high As concentrations have previously been reported for members of Fucales, in particular for the edible brown seaweed *Sargassum fusiforme* (88 mg kg^{-1} ; Holdt and Kraan, 2011). High accumulation of As in seaweeds is a problem for their utilization as food/feed since it is toxic and affects nervous systems (Holdt and Kraan 2011). However, inorganic As taken up by seaweeds can be converted to organic and non-toxic forms such as arsenosugars (Geiszinger et al. 2001; Petursdottir et al. 2016). It is reported that As in *Sargassum* is removed by 89–92% (wet weight) by the cooking process, or effectively removed just by soaking edible brown seaweed and, when fed to mice, a large amount of As was metabolized to dimethylarsenic acid and excreted in urine (Ichikawa et al. 2006, 2010). However, this effect was not observed in vegetables where cooking is only of a very limited value as a means of reducing metal concentrations (arsenic, cadmium, mercury and lead; Perelló et al. 2008) and largely depends on time, temperature and method of cooking. Since this study also covers several heavy metals which can be toxic to seaweeds, animals and humans, several of the results reported here are also relevant in an ecotoxicological context as e.g. a survey of heavy metals in coastal waters of Kuwait (Dawagreh et al. 2019). Also, as the latter paper shows, data about trace metals in sea water are extremely patchy for Kuwait and the Gulf region. Se is incorporated into selenium proteins that have antioxidant and anti-inflammatory effects on the production of active thyroid hormone. Low selenium has been associated with poor immune function and cognitive decline (Mann and Truswell 2017) while excess Se has negative impacts on human health. Se contents observed within the framework of this study were comparable to edible seaweeds ($0.02\text{--}10 \mu\text{g g}^{-1}$ DW; Holdt and Kraan 2011), mustard seeds ($2.08 \mu\text{g g}^{-1}$) and nuts ($19.17 \mu\text{g g}^{-1}$; USDA 2019). Brown seaweeds (except *Laminaria digitata*) generally accumulate high Se ($8\text{--}31.7 \mu\text{g g}^{-1}$ DW; Holdt and Kraan 2011). However, brown seaweeds in our study had much lower Se contents ($2.4\text{--}4.1 \mu\text{g g}^{-1}$ DW; Table 6B).

Seaweeds are known for their high content of macronutrients, such as K, Ca, Na, and Mg, and seaweed extracts are known to maintain an enriched trace element content, valuable for fertilization applications and food supplements (Soares et al. 2020). The low Na/K ratio (0.12–1.3) in all the seaweeds studied here with the exception of *C. papillatum* can be of nutritional importance since a balanced Na/K ratio is important for people especially suffering from

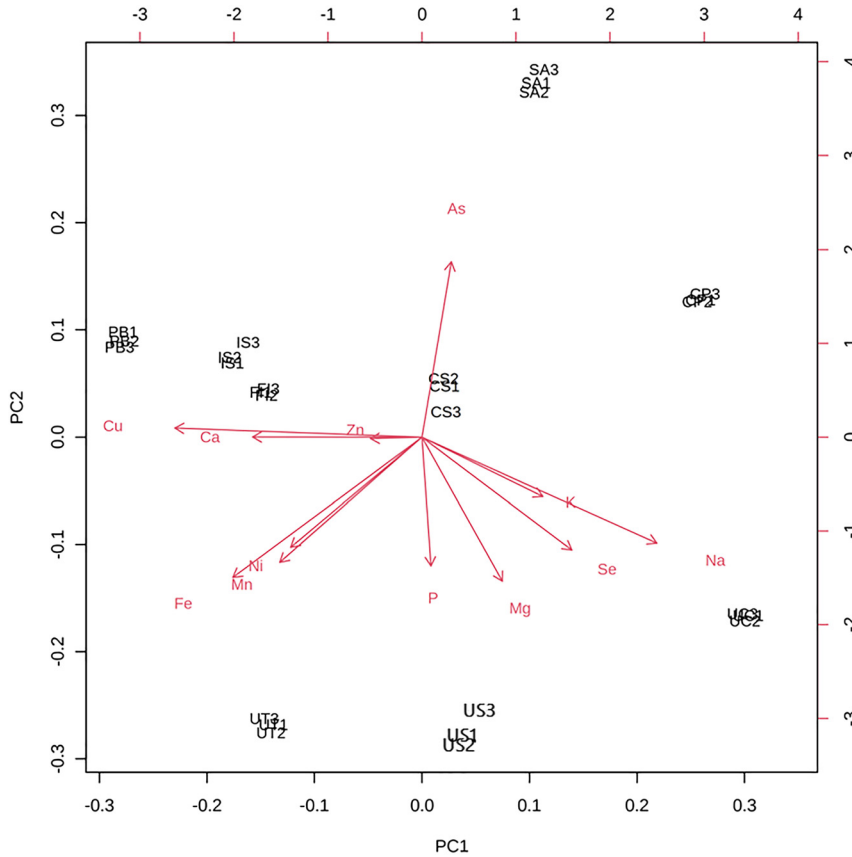


Figure 1: Bi-plot showing distribution of macro- and trace elements in different seaweeds from Arabian Gulf, obtained by principal component analysis with first two principal components (PC1, PC2). Abbreviations: UC, *Ulva chaugulii*; US, *Ulva* sp.; UT, *U. tepida*; CP, *Codium papillatum*; CS, *Chondria* sp.; IS, *Iyengaria stellata*; FI, *Feldmannia indica*; PB, *Padina boergesenii*; SA, *Sargassum aquifolium*.

cardiovascular diseases such as hypertension. Ca, Mg and P are important for bone and teeth health (Muñoz and Díaz 2020) and Ca is also involved in the regulation of heartbeat, nerve transmission, muscle contraction, blood coagulation, and the activation of insulin and the thyroid hormone calcitonin (Mann and Truswell 2017). The contents of Ca, Mg and P in seaweeds from our study are comparable to those of edible seaweeds (Muñoz and Díaz 2020); in fact, Ca content is much higher than those of tofu (21.34 mg g^{-1}) and kale (2.54 mg g^{-1} ; USDA 2019). Fe is an important mineral for human health whose higher demands during growth, high menstrual loss, and pregnancy, often lead to anemia. The high content of Fe ($0.23\text{--}3.51 \text{ mg g}^{-1}$ DW) in the seaweeds could be useful in meeting high Fe demands and is much higher than foods containing high Fe such as dried spearmint (1.23 mg g^{-1}) and whole meat (0.72 mg g^{-1} ; USDA 2019; Muñoz and Díaz 2020). However, Fe can be toxic in excessive amounts (Camaschella 2015) and can lead to tissue damage (Abbaspour et al. 2014). The daily iron requirement amounts to about 25 mg (Munoz and Diaz 2020).

Seaweeds show great variation in nutrient content which are related to species and environmental factors such as water, temperature, salinity, light and nutrients

(Dawes 1998). The usage of mineral supplements in western countries often aims to prevent deficiency of minerals. However, particularly around the Arabian Gulf, nutritional properties of macroalgae are poorly known. This also applies to the understanding of the extent to which the region's seaweeds potentially constitute a natural scavenger for pollution of the seawater. Both aspects constitute a particular novelty of the present study. While *C. papillatum* showed the lowest element concentrations among all nine species investigated here, *Ulva* species and *I. stellata* were among the strongest accumulators.

Seaweeds take up trace elements by both passive and active processes. For instance, Zn uptake is a passive process and is a surface reaction independent of factors influencing metabolism such as temperature, light, pH, or age of the seaweed (Kumar et al. 2011). Cu, Mn, Se, and Ni are transported across the cell membrane into the cytoplasm by a slow active process. Moreover, the uptake process for these elements is dependent on metabolic processes and changes in temperature, light, and life cycle stage of the seaweed (Sanchez-Rodriguez et al. 2001; Kumar et al. 2011).

The finding of *U. chaugulii* reported here constitutes a new record for Kuwait. This species has only recently been described from the west coast of India (Kazi et al. 2016) and was subsequently also reported from the Iranian coast of the Gulf (Pirian et al. 2016). Given the challenges of morphology-based identification in the genus *Ulva*, it has almost certainly been previously been confused with other *Ulva* species and its actual range may be quite large. *C. papillatum*, *P. boergesenii*, *Sargassum asperifolium* and *S. aquifolium* have previously been reported from the Arabian Gulf (Al-Adilah et al. 2020; John and Al-Thani 2014). Likewise, *I. stellata* is known specifically from Kuwait (Al-Adilah et al. 2020; Al-Yamani et al. 2014; Silva et al. 1996). It should be noted that the DNA sequences reported here are among the first for any seaweeds from the entire Arabian Gulf, second only to our recently published study (Al-Adilah et al. 2020), highlighting how understudied the region is also with regards to molecular algal taxonomy. In this regard, the situation in the Gulf 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 number of taxa occurring in the region (Bartolo et al. 2020). However, this also highlights a general challenge when working in this region – a robust, sequence-based identification of the taxa investigated in this study will only be possible when a larger dataset is available.

In conclusion, seaweeds from the Arabian Gulf region are rich in essential minerals including Ca, Mg, Na, K, Fe and Zn. These seaweeds contain higher concentrations of these minerals than other sources of mineral-rich foods and even one gram of dried seaweed can fulfill the dietary requirements. *U. tepida* and *I. stellata* are of nutritional interest for being exceptionally rich in most of the macro- and trace elements while having low As and Se contents compared to other species, which thus, renders them suitable for food and feed applications.

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Frithjof Christian Küpper (left) has held the chair in marine biodiversity at the University of Aberdeen since 2011, after eight years at the Scottish Association for Marine Science (Oban) as a lecturer and reader, studying the biodiversity and biochemistry of marine plants/algae. He conducted graduate studies at Roscoff and Konstanz for a joint French–German PhD. His research found that iodide serves as an inorganic antioxidant in kelp, the first described from a living system, impacting atmospheric and marine chemistry. A certified scientific diver, Frithjof has worked in the Mediterranean, South Atlantic (Ascension, Falkland Islands), Antarctica, the Arctic and the Arabian Gulf.



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Ioanna Kosma holds a B.Sc. in marine sciences and a M.Sc. in “Integrated Coastal Management” from the University of the Aegean (UA), Department of Marine Sciences. Her research background focuses on marine biodiversity and ecology in combination with scientific diving, and she also has experience in marine immunology. She has extensive field work experience, and she has participated in scientific excursions in Cyprus, Kuwait, and Greece. From 2019 she has been working as a research associate at UA (Department of Marine Sciences) and particularly in the Marine Remote Sensing group, participating in various work packages and deliverables.



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Puja Kumari is a Marie Curie (MSCA-IF) postdoctoral research fellow in the School of Biological Sciences, University of Aberdeen. She obtained her PhD in biotechnology through a joint program of MK Bhavnagar University and CSIR-CSMCRI, India. She is the former recipient of ARO (Israel), BIDR-Blaustein (Israel) and JSPS (Japan) postdoctoral fellowships. Her research pursuit included algal stress physiology, lipidomics, metabolomics, chemotaxonomy and algal bioprospection leading to a patent for integrated process for obtaining value-added products from seaweeds without any *in situ* chemical reactions. Currently, she is working on elucidating the role of halogen metabolism in brown algal pathologies.