



Chemical food safety of seaweed: Species, spatial and thallus dependent variation of potentially toxic elements (PTEs) and techniques for their removal

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Abstract

The increasing interest in seaweed as a food resource in Western societies raises concerns about associated potentially toxic elements (PTEs), including arsenic, cadmium, mercury, lead, and iodine. Hence, this study examined variation in PTEs by including samples of four different species (*Ulva lactuca*, *Palmaria palmata*, *Alaria esculenta*, and *Saccharina latissima*) from Ireland, as well as of a single species (*S. latissima*) from three different harvest sites and with different thallus parts analyzed from a single harvest site. Comparing different species from the Irish site, *U. lactuca* showed the overall lowest PTE levels, while *S. latissima* and *A. esculenta* had notably higher levels of arsenic (105 ± 3 and 57.9 ± 5.5 mg kg⁻¹ DW, respectively) and cadmium (0.839 ± 0.046 and 1.03 ± 0.05 mg kg⁻¹ DW, respectively), and *S. latissima* had highest iodine levels (2480 ± 210 mg kg⁻¹ DW). Lead was higher in *P. palmata* and *U. lactuca* but below maximum limits. Further analysis of *S. latissima* from three sites (Ireland, Norway, Sweden) showed site-specific variations in inorganic arsenic (0.192 – 9.69 mg kg⁻¹ DW), lead (0.0751 – 0.269 mg kg⁻¹ DW), and cadmium (0.554 – 1.16 mg kg⁻¹ DW). *Saccharina latissima* from the Swedish site exhibited uneven PTE distribution in tissue of different age. In terms of tolerable intake levels, iodine and cadmium posed most concern for seaweed consumption. After processing, total ash content decreased, leading to reductions in total arsenic (61.1%), inorganic arsenic (92.4%), lead (49.4%), and iodine (72.8%). Cadmium and mercury were difficult to remove. This study offers insights into the chemical food safety of seaweed, both pre- and post-processing, for introduction into expanding European markets.

Keywords Macroalgae · Arsenic · Cadmium · Lead · Mercury · Iodine

Introduction

Global interest in seaweed as a source of food is increasing due to postulated health benefits and sustainability. Although several countries, especially in East- and Southeast Asia, traditionally include macroalgae in their diet, this resource is practically underutilized in other food cultures. In Europe, a growing interest in macroalgae for food consumption is observed, although concern regarding occasional elevated levels of potentially toxic elements (PTEs) occurs and clear

farming- and safety regulations are lacking (Hasselström et al. 2020; Jönsson et al. 2020).

To date, food safety regulations for seaweed in the Western world are scattered. The French Agency for Food, Environmental and Occupational Health & Safety (ANSES 2020) and the Algae Technology & Information Centre (CEVA 2019) have published maximum levels for selected elements in seaweed (see Table 4, below) based on guidelines by the French High Council for Public Health (CSHPF). In addition, the European Food Safety Authority (EFSA) has presented tolerable daily/weekly intake levels (Table S1), and the European Commission has set maximum levels for certain contaminants in foodstuffs (European Commission 2023). These compounds include the four elements arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) which the World Health Organization (WHO) highlighted in the list “The 10 chemicals of public health concern” in 2020 (WHO 2020). In addition, high iodine (I) levels in some seaweed

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species are also considered a human health concern (Banach et al. 2020), and excess intake can lead to thyroid disorders (EFSA 2014). Together they comprise potentially toxic elements (PTEs) in seaweed. Consequently, the levels of these compounds must be investigated for introducing seaweed as a safe food resource in emerging European markets. Therefore, the current study gives a snapshot of the levels of PTEs in different seaweed samples and purposes to relate these to other constituents of the seaweed, intending to increase the current understanding of the safety of consuming seaweed.

One major concern regarding the food safety of seaweeds involves their biosorption capacity. Unlike many land plants, algal thalli take up nutrients directly from the surrounding water and do not utilize root-like functions (Radulovich et al. 2015). The mechanisms of interaction between heavy metals and seaweed are postulated to occur through surface precipitation, ion exchange, and complexation/chelation (He and Chen 2014), as well as surface adsorption, diffusion, and transportation (Sud et al. 2008). The holdfasts resemble roots of land plants, but only act as anchors to avoid drifting with currents and are not primarily involved in nutrition uptake (Pétursdóttir et al. 2019). Hence, seaweeds are susceptible to the quality of the water in which they grow.

Today, heavy metal pollution from anthropogenic sources is a global environmental problem, but the levels in nature vary depending on geographical location (Aziz et al. 2023). Arsenic (total arsenic: tAs) occurs in the environment in inorganic (iAs) and organic (oAs) forms but rarely in its elemental state. Inorganic arsenic exists predominantly as arsenite and arsenate. Arsenosugars comprise the major part of organic arsenic in seaweed, which also contains monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB), and arsenocholine (AsC) (Zhao et al. 2014). Organic forms of arsenic are generally considered less toxic than their inorganic counterparts, although exceptions occur (Hughes 2002). Cadmium compounds are relatively water soluble and are partly released to water environments from boat fuels and paints (Hayat et al. 2019). The anthropogenic source of mercury in oceans comes from atmospheric deposition in the form of Hg(II). One part is reemitted as predominantly Hg(0), and another part is methylated and bioaccumulated (Driscoll et al. 2013). Lead exists in nature in its elemental form and as inorganic and organic compounds. Iodine exists naturally in nature in different oxidative states and in complex organic compounds (Moreda-Pineiro et al. 2011).

In addition to the harvest site, other factors, such as the type of species and age of thallus, also contribute to variances in the overall composition between seaweed samples (Lai et al. 1998; Cherry et al. 2019). Different algae types employ distinct mechanisms for nutrient uptake and synthesize various components comprising the algal body (Roleda and Hurd 2019; Kreissig et al. 2021). Similarly, different

parts of the algae have different metabolic activities and accumulation capacities (Sáez et al. 2012; Pétursdóttir et al. 2019). These variances are best evaluated from kelps, such as sugar kelp (*Saccharina latissima*) predominantly investigated in this study, which grows longitudinally from the meristematic tissue located above the stipe (basal growth) (Nielsen et al. 2014). Hence, distal parts of the kelp comprise older tissue, whereas proximal parts (close to the stipe) are more recently synthesized. Contrasting to the growth pattern in kelps, in *Palmaria palmata* the meristematic cells are located in marginal tissue where vegetative growth occurs and the formation of new fronds from old thalli takes place (apical growth) (Krumhansl et al. 2015; Stévant et al. 2023). *Ulva lactuca* lacks a localized meristem and instead grows uniformly throughout the blade in all directions (diffuse growth) (Krumhansl et al. 2015). Hence, it is more difficult to separate older tissue from younger parts in these species compared to the kelps. Kreissig et al. (2021) demonstrated interspecies variation in element profiles among different seaweeds from Greenland. They further showed that although similar patterns within the same algal families were observed, the element profiles of different thallus parts varied. This variation in composition, particularly regarding PTEs, between species and algal tissue is of interest to investigate further for other Nordic seaweed species, as a basis for developing future standards within the EU.

Recently, various reports within the Nordic countries and the EU have been produced to map the current state of knowledge and provide future perspectives on algal food safety. These reports (FAO and WHO 2022; Hogstad et al. 2023) concluded that more data on PTEs in seaweed is needed to enable proper risk assessments and that safer production and manufacturing methods need to be developed, which motivate the need for the current study. In line with this, previous studies have focused on processing methods for reducing the content of contaminants in seaweed, including blanching (Nielsen et al. 2020; Trigo et al. 2023), soaking/water treatment (Hanaoka et al. 2001; Stévant et al. 2018; Blikra et al. 2021a; Krook et al. 2023), ultrasound (Narukawa et al. 2012; Noriega-Fernández et al. 2021), and pulsed-electric field (Blikra et al. 2022). Hence, post-harvest processing of seaweed has been suggested (Blikra et al. 2021b) as a practice for controlling the PTEs in seaweed products when introduced and expanded on European markets.

While there is a growing interest in seaweed consumption in Europe, regulations are currently scarce, and specific seaweed varieties may contain elevated levels of contaminants, which can impede their safe consumption and commercialization. Hence, this study investigates levels of PTEs in seaweed samples from different 1) species, 2) harvest sites, 3) algae parts, and 4) after processing using six scalable methods for PTE removal. The levels of PTEs are

subsequently correlated with major seaweed compounds, such as polysaccharides. Finally, we believe this study can provide a valuable snapshot of the chemical food safety of seaweed introduced for consumption in expanding European markets.

Material and methods

Seaweed harvested at three sites was used for the different investigations in this study. *Saccharina latissima* (L.) C.E. Lane, C. Mayes, L.D. Druehl, & G.W. Saunders was cultivated by Nordic Sea Farm in the Swedish national park, Koster Sea (N58.863, E11.071). Irish wild seaweed samples of *S. latissima*, *Alaria esculenta* (L.) Greville, *Palmaria palmata* (L.) F. Weber & D. Mohr, and *Ulva lactuca* L. (bladed morphology, currently reannotated as *Ulva fenestrata* Postels & Ruprecht (Roleda and Heesch 2021)) were hand-harvested along the west coast of Ireland by Connemara Organic Seaweed Company within a 24 km radius from Rossaveal, Galway. *Saccharina latissima* from Norway was cultivated and harvested by Seaweed Solutions outside the coast of Trondheim (N63.704, E8.877). Table 1 summarizes the species, harvest site and date, as well as the treatment the algal samples were subjected to in this study. Swedish seaweed was stored frozen until processing and/or analysis, whereas Irish and Norwegian seaweed was stored dried. Irish seaweed was air-dried on racks in the processing

plant in Galway for 20 h at 38 °C and Norwegian *S. latissima* was dried by lyophilisation by the German company Freeze-dry Foods. Swedish *S. latissima* was lyophilized in-house prior to composition analysis.

Investigation of inter-species variation

The algal species *S. latissima* (brown), *A. esculenta* (brown), *P. palmata* (red), and *U. lactuca* (green) harvested from the coast of Connemara, Ireland, were used for the investigation of species dependency in composition analysis. Samples were received dried (air-dried at 38 °C), and further lyophilized to remove potential excess water. Samples were thereafter ground and sieved through 2 mm mesh prior to analysis.

Investigation of spatial variation

To create a snapshot of the geographical impact on the composition of macroalgae, *S. latissima* samples from Sweden (Koster Sea), Ireland (coast of Connemara), and Norway (coast of Trondheim) were analysed. Samples from Norway and Ireland were received dried (freeze-dried and air-dried at 38 °C, respectively), while Swedish seaweed was obtained fresh and immediately frozen after arrival at the lab, and subsequently freeze-dried. All samples were ground and sieved through 2 mm mesh prior to analysis.

Table 1 Overview of the seaweed samples included in the four sub-studies investigating compositional variations between 1) species, 2) harvest sites, 3) thallus parts, and 4) after processing for removal of potentially toxic elements

	Treatment	Species	Harvest site	Growth condition	Harvest date
1. Samples of four species harvested in Ireland	Untreated	<i>S. latissima</i> (SL)	Ireland	Wild	April 2021
	Untreated	<i>A. esculenta</i> (AE)	Ireland	Wild	April 2021
	Untreated	<i>P. palmata</i> (PP)	Ireland	Wild	Sept. 2020
	Untreated	<i>U. lactuca</i> (UL)	Ireland	Wild	April 2021
2. Samples of <i>S. latissima</i> from three harvest sites	Untreated ¹	<i>S. latissima</i> (SL)	Sweden	Cultivated	April 2021
	Untreated	<i>S. latissima</i> (SL)	Norway	Cultivated	May 2019
	Untreated	<i>S. latissima</i> (SL)	Ireland	Wild	April 2021
3. Samples of different thallus parts from Swedish <i>S. latissima</i>	Whole thallus ¹	<i>S. latissima</i> (SL)	Sweden	Cultivated	April 2021
	Blade top	<i>S. latissima</i> (SL)	Sweden	Cultivated	April 2021
	Blade base	<i>S. latissima</i> (SL)	Sweden	Cultivated	April 2021
	Stipe/holdfast	<i>S. latissima</i> (SL)	Sweden	Cultivated	April 2021
4. Samples of processed Swedish <i>S. latissima</i>	Untreated ¹	<i>S. latissima</i> (SL)	Sweden	Cultivated	April 2021
	HPP ²	<i>S. latissima</i> (SL)	Sweden	Cultivated	April 2021
	Soaking, water	<i>S. latissima</i> (SL)	Sweden	Cultivated	April 2021
	Soaking, acid	<i>S. latissima</i> (SL)	Sweden	Cultivated	April 2021
	HAE ³	<i>S. latissima</i> (SL)	Sweden	Cultivated	April 2021
	UAE ⁴	<i>S. latissima</i> (SL)	Sweden	Cultivated	April 2021
	Blanching	<i>S. latissima</i> (SL)	Sweden	Cultivated	April 2021

¹ Same samples ² HPP: High-pressure processing ³ HAE: Heat-assisted extraction ⁴ UAE: Ultrasound-assisted extraction

Variation between different thallus regions

Longitudinal growth of the brown alga *S. latissima* starts from the meristematic tissue just above the stipe (Nielsen et al. 2014). Hence, proximal (basal) regions of the lamina correspond to younger tissue compared to the distal (top) parts. In this study, *S. latissima* cultivated in the Koster Sea on the Swedish west coast was used for comparative analysis of tissues of different age. Algal blades of 80–120 cm were selected and divided into three categories corresponding to 1) blade top samples 2) blade base samples, and 3) stipe/holdfast samples. The mid-section between the blade base and top was discarded. Each of the three thalli sample aliquots was about 150 g wet weight. Samples were lyophilized, ground, and sieved through 2 mm mesh prior to analysis.

Processing for PTE removal

Processing of Swedish *S. latissima* (whole thalli) by six scalable methods was conducted to investigate if potentially toxic elements could be effectively removed (Table 2). The effect of the processing was evaluated by analysis of elements, arsenic speciation, as well as carbohydrate, ash, moisture, and protein content and compared with untreated samples.

High-pressure processing (HPP)

Algal biomass of 100 g was filled in a PA/PE plastic bag and vacuum-packed prior to high-pressure processing (HPP) performed using an Avure AV-10 (Avure Technologies, JBT, USA) according to Jönsson et al. (2023). Samples were subjected to a set pressure of 600 MPa, with actual pressure ranging between 598–605 MPa, for 180 s. The temperature in the vessel increased from 22.1 to 23.6 °C during the run. After HPP, the treated biomass was rinsed with 3x500 mL of distilled water to remove potential extractives. The

Table 2 Specification of processing parameters for each of the six methods employed for the removal of potentially toxic elements from Swedish *S. latissima*

Processing method	Temperature (°C)	Time (min)	pH	Algae/water ratio (% ww/v)
HPP ¹	20	3	-	-
Soaking, Water	30	30	6.5–7.5	20
Soaking, Acid	30	30	3.0	20
UAE ²	30	120	6.5–7.5	20
HAE ³	100	30	6.5–7.5	20
Blanching	80	2	6.5–7.5	-

¹ HPP: High-pressure processing ² HAE: Heat-assisted extraction

³ UAE: Ultrasound-assisted extraction

remaining material was packed into PE-LD zip bags, lyophilized, ground, and sieved through 2 mm mesh, followed by storage at -20 °C until the time of analysis.

Soaking in water

The soaking technique was based on a method by Hanaoka et al. (2001). Samples ($n=3$) of 50 g fresh frozen macroalgae were mixed with 200 mL Milli-Q water in 500 mL bottles. The bottles were directly submerged in an 18 L water bath at 30 °C for 30 min. Thereafter, the water in the bottle was sieved off and the solids were pooled and rinsed with 3x500 mL of distilled water. The seaweed was packed into PE-LD zip bags, lyophilized, ground, and sieved through 2 mm mesh followed by storage at -20 °C until the time of analysis.

Soaking in acid

Soaking in mild acidic conditions was similar to the reduction technique of soaking in water (Hanaoka et al. 2001). Acetic acid of pH~3 was prepared and 200 mL was added to 50 g of fresh frozen seaweed in 500 mL bottles ($n=3$). Samples were processed in an 18 L water bath at 30 °C for 30 min. Successively the acidic solution in the bottle was sieved off and the solids were pooled together and rinsed three times with 500 mL of distilled water. The seaweed was packed into PE-LD zip bags, lyophilized, ground, and sieved through 2 mm mesh followed by storage at -20 °C until the time of analysis.

Ultrasound-assisted extraction (UAE)

The method was based on previous work by Narukawa et al. (2012). Samples ($n=3$) of 50 g fresh frozen seaweed were added to 250 mL bottles together with 200 mL Milli-Q water. The bottles were placed in an ultrasonic bath (Sonorex RK100H, Bandelin, Germany) for 2 h at a temperature of 30 °C. The temperature of the water in the bath increased to a maximum of 48 °C during the processing. Thereafter, the water in the bottles was sieved off and the solids were pooled together and rinsed with 3x500 mL of distilled water. The seaweed was packed into PE-LD zip bags, lyophilized, ground, and sieved through 2 mm mesh followed by storage at -20 °C until the time of analysis.

Heat-assisted extraction (HAE)

Samples ($n=3$) of 50 g fresh frozen seaweed were added to 250 mL bottles together with 200 mL of boiling Milli-Q water. The bottles were submerged in an 18 L water bath at 100 °C for 30 min (Hanaoka et al. 2001; Narukawa et al. 2012). Samples were pooled and washed three times in 500 mL distilled water, followed by packing into PE-LD zip bags, lyophilization, grinding, and sieving through 2 mm

mesh. The samples were stored frozen at $-20\text{ }^{\circ}\text{C}$ until the time of analysis.

Blanching

Samples ($n=3$) of 50 g were immersed in distilled water in an 18 L water bath at $80\text{ }^{\circ}\text{C}$ for 2 min using a sieve. A pressure on top of the sieve made sure all algal samples were submerged in the water. Subsequently, samples were immediately cooled and washed in 1.5 L of $12\text{ }^{\circ}\text{C}$ distilled water. Thereafter, the water was sieved off and the seaweed was packed into PE-LD zip bags, lyophilized, ground, and sieved through 2 mm mesh followed by storage at $-20\text{ }^{\circ}\text{C}$ until the time of analysis.

Composition analysis

Analysis of the different seaweed samples composition was primarily conducted to evaluate variations in PTEs between different samples, to increase the understanding of how these elements distribute among 1) species, 2) harvest sites, 3) thallus parts, and 4) after processing. In addition, several other non-essential and essential elements were analysed, along with total ash content, to examine if there are any correlations to the level of PTEs. Similarly, the two main macronutrients in seaweeds, carbohydrates and protein, were quantified to also evaluate potential relations among the constituents. All chemicals were obtained from Merck/Sigma Aldrich (USA) if otherwise is not stated.

Elemental analysis

The total content of elemental compounds in algal samples ($n=3$, except for the HPP sample $n=2$, due to lack of material) was analysed by an external laboratory, ALS Scandinavia. Their method was developed in-house and accredited by SWEDAC. High-resolution sector field inductively coupled plasma mass spectrometry, HR-ICP-SFMS (Element XR, Thermo Scientific, Germany), was employed to quantify the elements. First, about 1 g sample was pre-treated by heated wet digestion in 10 mL nitric acid (HNO_3) and 0.05 mL hydrofluoric acid (HF) in a graphite heating block at $110\text{ }^{\circ}\text{C}$ for 120 min. The digests were thereafter diluted with Milli-Q water to 30 mL and further diluted with 1.4 M HNO_3 to obtain a final dilution factor of 1000 V/m. Quantification was according to Engström et al. (2004) using the HR-ICP-SFMS configured to add methane to the plasma. For quality assurance, two blanks and two reference standards, tea (GBW 07605, Institute of Geophysical and Geochemical Exploration, China) and wheat flour (NBS 1467a, NIST, USA), were included in the runs. Correction for matrix effects was accomplished by adding $2.5\text{ }\mu\text{L L}^{-1}$ indium as an internal standard to all samples. Analytes detected from

the method included: total arsenic (tAs), silver (Ag), cadmium (Cd), cobalt (Co), chrome (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), lead (Pb), selenium (Se), and zinc (Zn).

The method differed slightly for halogenated compounds, such as iodine (I). Sample preparation was performed by microwave-assisted, closed vessel nitric acid (HNO_3) digestion. The digests were diluted with a mixture of 0.5 % ammonia, 0.01 % Triton X-100, and 0.01 % EDTA prior to analysis by HR-ICP-SFMS (Element XR, Thermo Scientific, Germany). Limit of detection (LOD) and/or limit of quantification (LOQ) is presented in Table S2.

Arsenic speciation

The content of inorganic (iAs) and organic arsenic (oAs) in seaweed samples ($n=3$, except for the HPP sample $n=2$) due to lack of material) was analysed by the Swedish Food Agency. The level of inorganic arsenic and the organo-arsenic compound monomethylarsonic acid (MMA) was analysed by a method SLV-m183-f, based on the Swedish Standard Institute method SS-EN 16802:2014 (SIST 2014). The principle of the analysis was to extract the target compounds from the algal sample (0.2 g) in a solution of 10 mL 65 % 0.1M nitric acid (HNO_3) and 3 %v/v of 30 % hydrogen peroxide (H_2O_2) (diluted to desired content) in a heated ($90\text{ }^{\circ}\text{C}$) water bath. Target compounds were thereafter selectively separated using an anion exchange high-pressure liquid chromatography (HPLC; Agilent 1260, USA) with an analysis colon (Dionex Ionpac AS7, Thermo Fisher Scientific, USA) and pre-colon (Dionex Ionpac AG7, Thermo Fisher Scientific, USA) coupled to an element-specific ICP-MS detector (Agilent 7700x, USA; signal-to-noise ratio of m/z 75). The separation of each sample ran for 10 min at $30\text{ }^{\circ}\text{C}$ using a mobile phase (pH 10.3) of 50 mM ammonium carbonate (Honeywell, USA) and 3 %v/v methanol. For quality assurance, two blanks, a certified reference standard (Rice Flour 1568b, NIST, USA), and two internal standards (Sinlac rice porridge (Nestlé, Switzerland) and algae/2020-07/459 (WFSR, Netherlands)) were included in the runs.

The amount of dimethylarsinic acid (DMA) in the algal samples was analysed using the Swedish Food Agency method, SLV-m274, which is based on previous studies on arsenic speciation (Sloth et al. 2003, 2004). The target compounds were, same as for MMA and iAs, extracted from the algal samples by 65 % 0.1M nitric acid and 3 %v/v of 30 % hydrogen peroxide solution at $90\text{ }^{\circ}\text{C}$ in a water bath. The extract solution was then filtered and analysed by cation HPLC coupled to an ICP-MS detector (signal-to-noise ratio of m/z 75). The separation was conducted in similar conditions as for MMA and iAs, but with a run time of 11 min with a mobile phase of 50 mM pyridine (Alfa Aesar, USA) in 3 %v/v methanol (pH adjusted to 2.7 with formic acid).

For quality assurance, two blanks, and two certified reference standards (Rice Flour SRM 1568b (NIST, USA) and Arsenic Species in Frozen Human Urine SRM 2669 (NIST, USA)) were used.

Moisture content and ash

Water content was determined by gravimetrically measuring the content of bags before and after lyophilization based on the method by Hames et al. (2008). This was combined with analysis of total solids in the remaining powder. Single bags were used.

Total solids and ash content were determined based on a method by Van Wycken and Laurens (2015b). Moisture content was analysed to ensure accurate composition results based on dry weight (DW), and the analysis of ash provides indications of the overall mineral content in the samples. Crucibles were heated in a furnace (Nabertherm Controller B180, Germany) overnight at 575 ± 25 °C to remove potential compostable contaminants and were followingly weighed. Pre-conditioned and pre-weighted crucibles were filled with 100 ± 5 mg of lyophilized ground and sieved (2 mm mesh) algal powder ($n=3$). Total solids were calculated after the samples were heated in an oven (Fratelli Galli Control AG-System, Italy) at 105 °C for 24 h. The same samples were used for the following ash analysis. The crucibles were placed in a furnace and a ramping program was employed. The temperature was ramped from room temperature to 575 °C for 30 min, held at 575 °C for 3 h, and eventually allowed to cool to ambient temperature. Thereafter, the crucibles were once again weighed and the obtained values were used to calculate the ash content of the algal samples.

Total carbohydrates including neutral monosaccharides, mannitol, and uronic acids

Analysis of monosaccharides, mannitol, and uronic acids was based on a method for the determination of total carbohydrates in algal biomass by Van Wycken and Laurens (2015a). Sample ($n=2$) preparation was done by mixing 25 mg algal powder with 250 µL of 72 % (w/w) sulfuric acid (Alfa Aesar, USA) in glass tubes with screw lids. The tubes were successively incubated at 30 °C at 300 rpm for 60 min. Milli-Q water was added to the tubes to dilute the acid to 4 % (w/w). The samples were autoclaved (Systec VE-150, Germany) at 121 °C for 60 min and thereafter centrifuged (Sigma 3-16PK, Germany) at 3000 ×g for 5 min to separate the supernatant from the solids. Finally, the samples were neutralized with 0.1 M Ba(OH)₂·H₂O and filtered into HPLC vials using 0.2 µm syringe filters.

Analysis of neutral monosaccharides and mannitol as well as uronic acids were performed using a High-Performance Anion Exchange Chromatography system equipped with

a Pulsed Amperometric Detector (HPAEC-PAD; Thermo Fisher Scientific, USA) and the analytical column and guard column Dionex CarboPac PA-20 (Thermo Fisher Scientific, USA). Analysis was performed under isocratic conditions, with a flow rate of 0.5 mL min⁻¹ at a temperature of 32 °C. Monosaccharides and mannitol were eluted with elution mixtures A) Milli-Q water (62.5 %) and B) 2 mM sodium hydroxide (37.5 %) for 23 min. For uronic acids the elution mixtures A) Milli-Q water (55 %), B) 1 M sodium acetate in 200 mM sodium hydroxide (15 %), and C) 200 mM sodium hydroxide (30 %), were used for elution for 15 min. Monosaccharide and mannitol standards included: L-fucose, D-glucose, D-xylose, D-galactose, L-arabinose, D-mannose and D-mannitol. Uronic acid standards included: D-mannuronic acid, L-guluronic acid, and D-glucuronic acid.

Protein

Protein content in the algal samples was determined based on the Dumas method for nitrogen analysis by converting nitrogen to protein with a conversion factor of 5 (Angell et al. 2016). Samples of about 25 mg ($n=3$) were weighed into aluminium containers and combusted in a high-temperature (800–1000 °C) chamber in the presence of oxygen using the N/Protein Analyzer Flash EA 1112 Series (Thermo Electron Corp., USA). Following combustion, the formed carbon dioxide, water, and nitrogen were separated in a column and the nitrogen content was measured using a thermal conductivity detector. Aspartic acid of known concentration was used as a reference.

Statistical analysis

Descriptive statistics were applied to all results from composition analysis and presented as means $\pm$ standard deviation. To further assess differences between sample means from composition analysis, one-way ANOVA with Tukey's HSD *post hoc* test was employed at the 95 % confidence interval. Based on the analysis methods employed in the study, it was assumed that the data followed a normal distribution and exhibited homoscedasticity. Pearson's correlation analysis was used to investigate potential relations among sample compounds. Correlation analysis was performed to investigate whether the PTE levels could be related to specific components synthesized by the seaweed, e.g., carbohydrates and protein, and/or other compounds associated with the seaweed, e.g., ash. This was conducted using mean values of analysed compounds for each sub-study separately. Pearson's correlation coefficient (r) of $>|0.7|$ was considered indicative of a strong relationship (Akoglu 2018) at different significance levels ($*p<0.05$, $**p<0.01$, and $***p<0.001$) according to the software. Excel and Jamovi (The-jamovi-project 2022) were used for the calculation of descriptive statistics, and Jamovi was used for ANOVA and Pearson correlations.

Results

This study provides a snapshot of the PTE content (focusing on arseno-compounds, cadmium, mercury, lead and iodine) in different seaweed samples and places these levels in the context of the other compounds present in the seaweed studied to explore the food safety of seaweed for consumption. For this investigation, four groups of samples were analysed: 1) samples of four species harvested in Ireland, 2) samples of *S. latissima* from three harvest sites, 3) samples of different thallus parts from Swedish *S. latissima*, and 4) samples of Swedish *S. latissima* processed for PTE removal. Elemental analysis showed that all samples, regardless of species, harvest site, or part of the thallus, had dominating levels of sodium, iodine, iron, total arsenic, zinc, and manganese.

Composition of different species from a single site

Comparison of four different species from a single site demonstrated that the chemical composition generally differed between species (Fig. 1a, Table 3). *Saccharina*

latissima had significantly ($p<0.05$) higher ash levels than the other species, indicating high mineral content. Surprisingly, the protein content was similar in all species (15.3–17.2 g (100 g)⁻¹ DW). Similar to ash, total carbohydrate content was significantly ($p<0.05$) higher in the two brown seaweeds (*S. latissima* and *A. esculenta*), compared with the red (*P. palmata*) and green (*U. lactuca*) species.

A closer look at the carbohydrates showed that the monosaccharide content mirrored expected polymers from the respective species. *Saccharina latissima* and *A. esculenta* had high levels of guluronic- and mannuronic acids (components of alginate), glucose (component of laminarin), and mannitol. *Palmaria palmata* was rich in galactose (from carrageenan and agar), glucose, and especially xylose (from xylan). *Ulva lactuca*, contained comparatively high levels of arabinose, glucose, and potential components of ulvan (xylose, glucuronic acid, and an unidentified uronic acid expectedly iduronic- or aldobiuronic acid).

Similar to the carbohydrates, the PTE content was shown to differ between species (Fig. 2a). Overall, the two brown kelps had higher levels of arsenic (tAs, iAs, MMA, and

Fig. 1 Variation in composition of main component groups among (a) samples of four species harvested in Ireland, (b) samples of *S. latissima* from three harvest sites, (c) samples of different thallus parts from Swedish *S. latissima*, and (d) samples of processed Swedish *S. latissima*. Concentration of carbohydrates ($n=2$), protein ($n=3$), and ash ($n=3$) are given as mean $\pm$ standard deviation. The unspecified fractions are comprised of components not determined by analysis, e.g., lipids. See Table 3 for mean values and significant differences in the concentrations of each compound between the samples

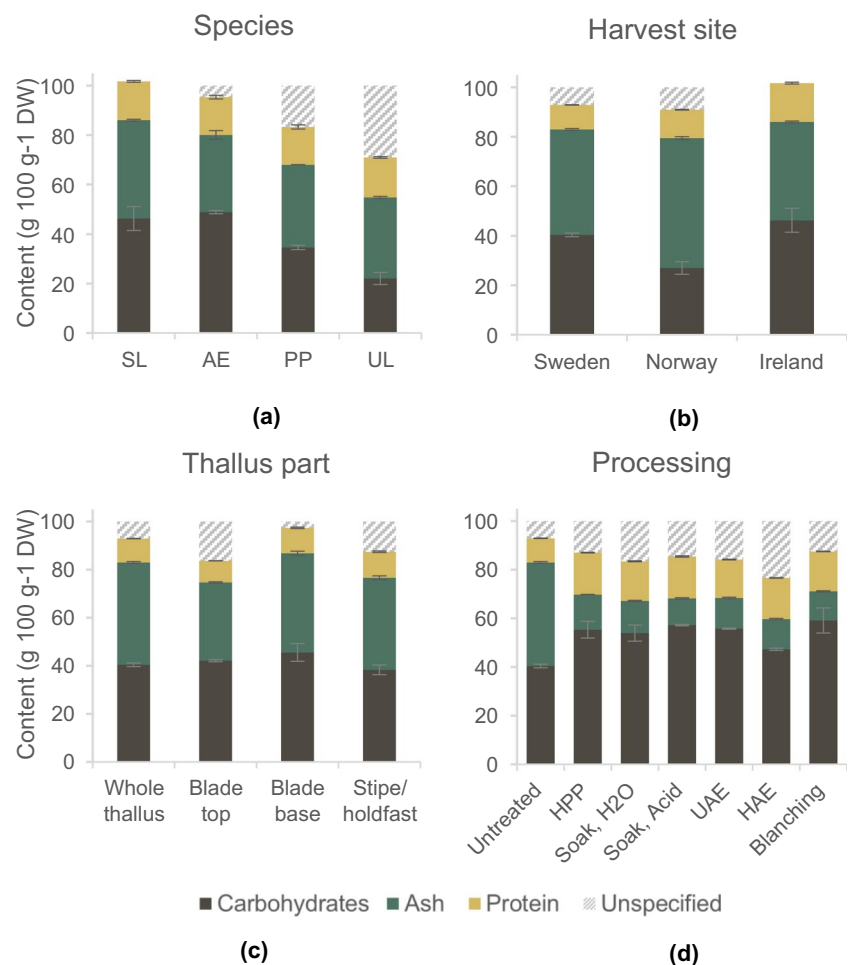


Table 3 Summary of total carbohydrates ($n=2$) including specification of neutral monosaccharides, mannitol, and uronic acids present in algae, as well as a summary of M/G-ratio, ash ($n=3$), and protein ($n=3$)

[g (100 g) ⁻¹ DW]	Neutral Monosaccharides					Mannitol	Uronic Acids			M/G ratio	Total Carbs	Ash	Protein
	Fucose	Arabinose	Galactose	Glucose	Xylose		Mannose	Guluronic Acid	Glucuronic Acid				
Samples of four species harvested in Ireland													
<i>S. latissima</i> ¹	1.22±0.05 ^a	< LOQ	0.76±0.01 ^a	8.14±0.24 ^a	0.34±0.01 ^a	0.51±0.02 ^a	10.0±1.5 ^a	1.21±0.07 ^a	20.5±3.1	2.0	46.3±4.8 ^a	39.8±0.4 ^a	15.7±0.4 ^{ab}
<i>A. esculenta</i>	1.74±0.00 ^{b*}	< LOQ	1.11±0.01 ^b	5.44±0.06 ^b	0.38±0.02 ^a	0.48±0.03 ^{ab}	13.2±0.04 ^b	1.60±0.01 ^a	21.7±0.4	1.6	48.8±0.6 ^a	31.3±1.7 ^b	15.3±0.7 ^a
<i>P. palmata</i>	< LOD	< LOD	4.04±0.13 ^c	3.30±0.03 ^c	26.8±0.76 ^b	< LOD	< LOQ	< LOQ	< LOQ	–	34.6±0.9 ^b	33.3±0.1 ^b	16.2±0.8 ^{ab}
<i>U. lactuca</i> ³	< LOQ	4.78±0.54	0.58±0.02 ^a	5.49±0.66 ^b	2.10±0.24 ^c	0.30±0.08 ^b	< LOQ	5.23±0.52 ^b	< LOQ	–	22.1±2.5 ^c	32.7±0.4 ^b	17.2±0.3 ^b
Samples of <i>S. latissima</i> from three harvest sites													
Sweden ²	1.86±0.10 ^a	< LOQ	0.91±0.02 ^a	6.19±0.25 ^a	< LOQ	< LOQ	7.02±0.20 ^{ab}	0.97±0.01 ^a	13.6±0.4 ^{ab}	1.9	40.4±0.7 ^{ab}	42.6±0.4 ^a	9.9±0.1 ^a
Norway	1.40±0.02 ^b	< LOQ	0.95±0.02 ^a	5.75±0.03 ^a	0.37±0.01 ^a	0.55±0.03 ^a	3.87±1.13 ^a	1.15±0.05 ^{ab}	8.18±1.19 ^a	2.1	27.0±2.5 ^a	52.4±0.6 ^b	11.5±0.2 ^b
Ireland ¹	1.22±0.05 ^b	< LOQ	0.76±0.01 ^b	8.14±0.24 ^b	0.34±0.01 ^a	0.51±0.02 ^a	10.0±1.5 ^b	1.21±0.07 ^b	20.5±3.10 ^b	2.0	46.3±4.8 ^b	39.8±0.4 ^c	15.7±0.4 ^c
Samples of different thallus parts from Swedish <i>S. latissima</i>													
Whole thallus ²	1.86±0.10 ^{ab}	< LOQ	0.91±0.02 ^a	6.19±0.25 ^{ab}	< LOQ	< LOQ	7.02±0.20	0.97±0.01 ^a	13.6±0.41 ^a	1.9	40.4±0.7	42.6±0.4 ^a	9.9±0.1 ^a
Blade top	1.70±0.07 ^a	< LOQ	0.91±0.03 ^a	5.50±0.15 ^a	0.38±0.01 ^a	0.60±0.01 ^a	5.08±0.28	1.13±0.00 ^{ab*}	9.48±0.43 ^a	1.9	42.1±0.4	32.5±0.3 ^b	9.0±0.1 ^b
Blade base	2.03±0.07 ^b	< LOQ	0.83±0.05 ^a	6.69±0.29 ^b	0.44±0.01 ^b	0.57±0.02 ^a	7.18±0.46 ^a	1.39±0.14 ^b	19.1±1.9 ^b	2.7	45.5±3.7	41.3±0.9 ^a	10.6±0.3 ^c
Stipe/holdfast	1.24±0.02 ^c	< LOQ	0.65±0.01 ^b	8.05±0.06 ^c	0.56±0.00 ^c	0.72±0.01 ^b	7.25±0.91	1.28±0.06 ^{ab}	12.0±0.91 ^a	1.7	38.3±2.0	38.3±0.8 ^c	10.9±0.3 ^c
Samples of processed Swedish <i>S. latissima</i>													
Untreated ²	1.86±0.10 ^a	< LOQ	0.91±0.02 ^a	6.19±0.25 ^a	< LOQ	< LOQ	7.02±0.20 ^a	0.97±0.01 ^a	13.6±0.4 ^a	1.9	40.4±0.7 ^{ab}	42.6±0.4 ^a	9.9±0.1 ^a
HPP	2.62±0.08 ^{bc}	< LOQ	1.13±0.04 ^b	8.58±0.07 ^b	0.69±0.01	0.77±0.01	11.5±0.8 ^{ab}	2.49±0.14 ^b	25.9±2.2 ^{bc}	2.3	55.3±3.5 ^{bc}	14.4±0.1 ^b	15.0±0.1 ^b
Soaking, H ₂ O	2.83±0.11 ^b	< LOQ	0.99±0.06 ^{abc}	9.17±0.03 ^{bc}	0.74±0.01	0.82±0.02	12.9±1.5 ^b	2.84±0.22 ^b	23.2±1.3 ^{bc}	1.8	53.9±3.3 ^{bc}	13.2±0.3 ^c	16.3±0.2 ^c
Soaking, Acid	2.62±0.06 ^{bc}	< LOQ	0.99±0.02 ^{abc}	9.44±0.28 ^c	0.72±0.01	0.77±0.12	14.0±0.8 ^b	2.86±0.13 ^b	25.5±0.1 ^{bc}	1.8	57.2±0.3 ^{bc}	11.0±0.3 ^d	17.2±0.3 ^d
UAE	2.84±0.02 ^b	< LOQ	1.01±0.04 ^{abc}	9.36±0.15 ^c	0.74±0.00 [*]	0.84±0.01	12.5±0.7 ^b	2.72±0.07 ^b	25.3±0.4 ^{bc}	2.0	55.8±0.2 ^{bc}	12.6±0.3 ^{cc}	15.8±0.2 ^c
HAE	1.77±0.04 ^a	< LOQ	0.95±0.04 ^{ac}	9.72±0.07 ^c	0.71±0.01	0.75±0.09	9.94±0.01 ^{ab}	2.58±0.05 ^b	20.6±0.3 ^b	2.1	47.3±0.4 ^b	12.4±0.3 ^e	16.9±0.1 ^d
Blanching	2.51±0.09 ^c	< LOQ	1.02±0.01 ^{abc}	9.75±0.22 ^c	0.74±0.02	0.78±0.13	14.3±2.1 ^b	2.88±0.25 ^b	26.7±2.3 ^c	1.9	59.1±5.2 ^c	12.0±0.2 ^e	16.4±0.1 ^c

¹ Same sample. ² Same sample. ³ Unknown uronic acid peaks (0.23±0.0 and 2.72±0.3). * Standard deviation below 0.001. LOD: limit of detection (determined by the instrument). LOQ: limit of quantification (from standard curve). HPP: High-pressure processing. UAE: Ultrasound-assisted extraction. HAE: Heat-assisted extraction.

Results are reported as mean values in g (100 g)⁻¹ algae ± standard deviation and are based on the algal dry weight (DW). Significant (Tukey's HSD, $p < 0.05$) differences within each group of samples are indicated by letters (a-e)

DMA) and cadmium. *S. latissima* had high levels of iodine, while the red and green species were relatively richer in lead. The amount of inorganic arsenic, cadmium, and iodine in *S. latissima*, as well as inorganic arsenic and cadmium in *A. esculenta*, exceeded the maximum levels (Table 4). *Palmaria palmata* and *U. lactuca* met all acceptance criteria presented by ANSES and CEVA. Of the essential minerals, iron appeared at especially high levels in the red and green species (628 and 644 mg kg⁻¹ DW, respectively), making them good sources of iron for a plant-based diet. Further, *P. palmata* was shown to be richer in silver, cobalt, copper, manganese, molybdenum, nickel, and selenium compared to the other species. *Ulva lactuca* had higher levels of chromium and the kelps had higher levels of zinc among the species studied. Interestingly, the levels of elemental compounds appeared linked to the presence of carbohydrates, protein, and ash.

These observed linkages between elements and major compound groups in seaweeds were found from the correlation analysis (Table S3). In the kelps, alginate, and fucose (from fucoidan) correlated positively with arsenic (tAs: $r=0.9$ and $r=0.8$, iAs: $r=0.8$ and $r=0.7$, MMA: $r=0.9$ and $r=0.8$, and DMA: $r=1.0^{**}$ and $r=1.0^{*}$, respectively) and cadmium ($r=1.0^{**}$ respectively $r=1.0^{**}$), whereas iron and lead correlated negatively with alginate and fucose. The M/G-ratio (mannuronic/guluronic acid) of alginate influenced the association of arsenic (tAs, iAs, MMA, DMA) and cadmium to the seaweed, with slightly higher correlation strength for M than G. Finally, sodium correlated negatively with the main monosaccharides of red seaweed, galactose ($r=-1.0^{*}$) and xylose ($r=-1.0^{**}$), indicating that sodium is not associated with red seaweeds by crosslinking to its polysaccharides.

Composition of *Saccharina latissima* from three harvest sites

The growth environment is widely acknowledged to exert a substantial influence on the chemical composition of seaweed. Accordingly, differences in spatial composition for *S. latissima* were demonstrated in this study (Fig. 1b, Table 3, Table S4). Ash was significantly ($p<0.05$) highest in Norwegian samples, followed by Swedish, and Irish, whereas protein content was higher in samples from Ireland, followed by *S. latissima* cultivated in Norway, and Sweden. Total carbohydrate content significantly ($p<0.05$) differed between the higher (Irish) and lower (Norwegian) samples. Alginate levels followed the total carbohydrate pattern, with higher concentrations in Irish samples and lower in Norwegian. Swedish samples were significantly ($p<0.05$) higher in mannitol and fucose (fucoidan) in the comparison between harvest sites.

Elemental analysis (Figure 2b) demonstrated that Irish samples had significantly ($p<0.05$) higher levels of inorganic arsenic than samples from the other harvest sites, which exceeded the maximum level by a factor of 3 (Table 4). Samples from all harvest sites had levels higher than the maximum levels for cadmium and iodine. Highest levels of cobalt, chromium, iron, manganese, nickel, and selenium were observed in Swedish sugar kelp, whereas sodium and zinc were richest in Norwegian samples.

Variation in composition between different thallus parts from *Saccharina latissima*

The growth pattern of *S. latissima* was correlated to the composition of different parts of the blade (Figure 1c, Table 3, Table S5), which was analysed using *S. latissima* of a controlled age cultivated at the Swedish site. Ash content differed significantly ($p<0.05$) between the samples, with highest levels in whole thallus and blade base samples, and lowest in the older blade top samples. The significantly ($p<0.05$) lowest protein level was observed in blade top samples, and highest in blade base and stipe/holdfast. The total carbohydrate content did not differ significantly. However, the blade top had significantly ($p<0.05$) higher mannitol content, whereas the blade base contained significantly ($p<0.05$) higher levels of fucose (from fucoidan) and mannuronic acid, and, hence, alginate with higher M/G-ratio. The stipe/holdfast contained significantly ($p<0.05$) higher levels of glucose (laminarin and cellulose), as well as xylose and mannose (components of fucoidan) compared to the other thallus parts.

From elemental analysis (Figure 2c), it could be concluded that all *S. latissima* parts analysed exceeded the maximum levels for iodine and all samples, except the blade top, exceeded the limit for cadmium. Presence of cadmium correlated with alginate content ($r=0.9$), but at a non-significant level. All other PTEs met the maximum levels. The older tissue in the blade top distinguished itself from the other thallus parts by having significantly higher levels of the following elements, cobalt, chromium, iron, manganese, nickel, selenium, and zinc.

Effect of processing on the composition of *Saccharina latissima*

As part of the investigation into strategies for mitigating PTE levels in seaweed for food, experiments were conducted employing six methods aimed at reducing the content of these elements in Swedish *S. latissima*. Chemical analysis demonstrated that the processing could have a considerable impact on the composition (Figure 1d, Tables 3 and 4, and Figure 2d). Processed samples showed significantly

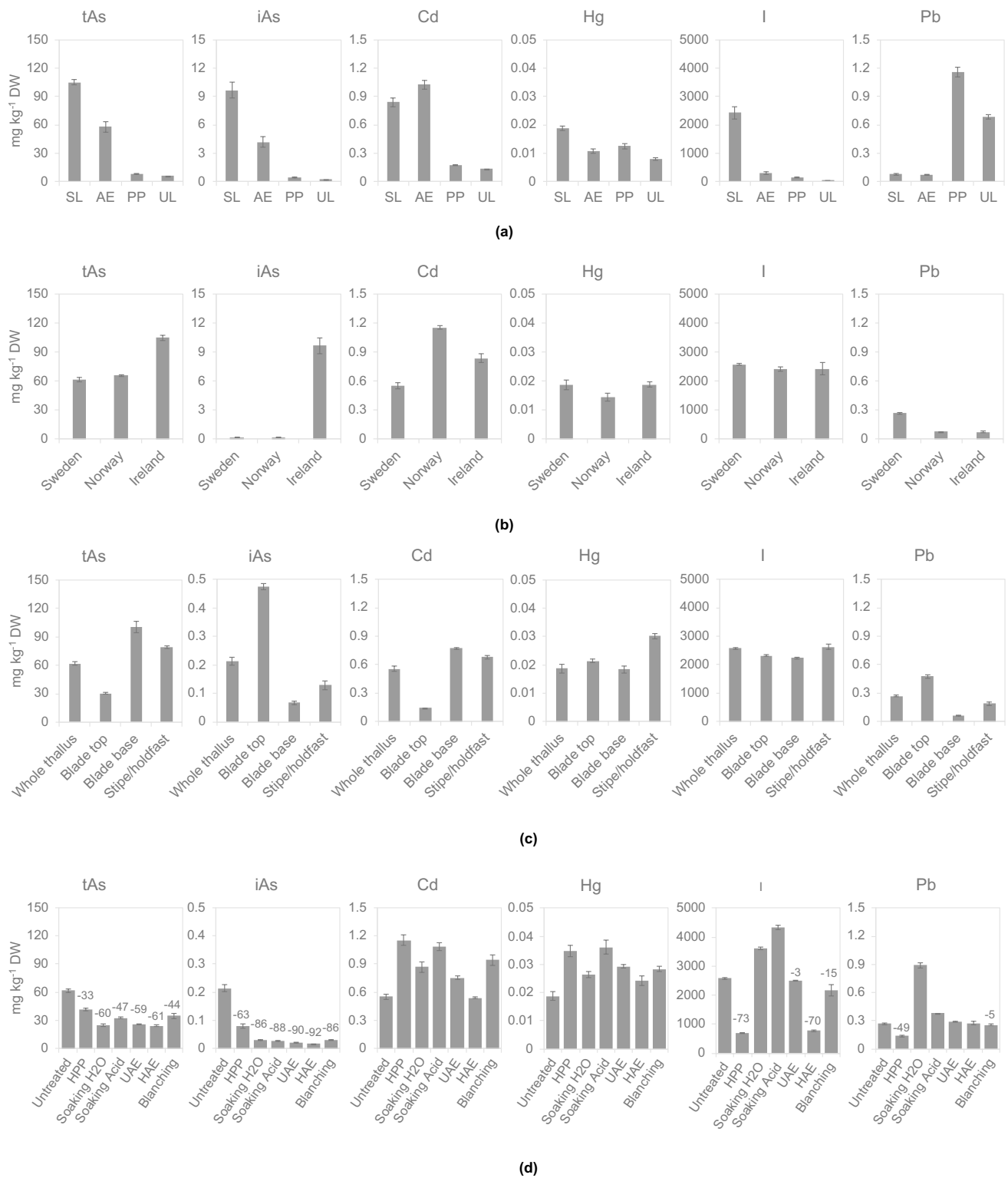


Fig. 2 Composition of PTEs (tAs, iAs, Cd, Hg, Pb, and I) in **(a)** samples of four species harvested in Ireland, **(b)** samples of *S. latissima* from three harvest sites, **(c)** samples of different thallus parts from Swedish *S. latissima*, and **(d)** samples of processed Swedish *S. latissima*

(including relative reduction in %). Concentrations ($n=3$, except for HPP ($n=2$)) are given as mean $\pm$ standard deviation. For mean values and significant differences in the concentrations of each compound between the samples see Table 4

Table 4 Summary of elements and arsenic speciation in all algal samples ($n=3$, except for HPP ($n=2$)) reported as mean values in mg kg^{-1} of algal dry weight (DW) $\pm$ standard deviation. Significant (Tukey's HSD, $p < 0.05$) differences within each group of samples are indicated by letters (a-f)

[mg kg ⁻¹ DW]	Elements										
	Ag	Co	Cr	Cu	Fe	Mn	Mo	Na	Ni	Se	Zn
Samples of four species harvested in Ireland											
<i>S. latissima</i> ¹	0.0190±0.0003 ^a	0.0665±0.0118 ^a	0.174±0.023 ^a	0.759±0.034 ^a	80.4±4.2 ^a	6.04±0.38 ^a	0.242±0.008 ^a	51 600±1 500 ^a	0.257±0.007 ^a	0.0430±0.0131 ^a	36.8±1.3 ^a
<i>A. esculenta</i>	0.0328±0.0010 ^b	0.0466±0.0009 ^a	0.117±0.011 ^a	1.10±0.06 ^a	83.6±5.9 ^a	7.92±0.63 ^a	0.252±0.011 ^a	53 200±1 300 ^a	0.482±0.025 ^b	0.0713±0.0162 ^a	35.4±1.6 ^a
<i>P. palmata</i>	0.208±0.002 ^c	0.476±0.018 ^b	0.776±0.007 ^b	5.07±0.02 ^b	628±170 ^b	228±15 ^b	0.759±0.005 ^b	26 600±340 ^b	7.81±0.073 ^c	0.354±0.023 ^b	27.6±0.5 ^b
<i>U. lactuca</i>	0.0356±0.0017 ^b	0.271±0.010 ^c	1.580±0.330 ^c	2.70±0.30 ^c	644±15 ^b	20.0±1.1 ^a	0.239±0.015 ^a	50 000±2 300 ^a	2.19±0.074 ^d	0.0469±0.0069 ^a	11.8±0.5 ^b
Samples of <i>S. latissima</i> from three harvest sites											
Sweden ²	0.0157±0.0013 ^a	0.138±0.001 ^a	0.764±0.140 ^a	1.17±0.06 ^a	305±4 ^a	14.2±0.7 ^a	0.237±0.016 ^a	55 000±2 500 ^a	0.563±0.081 ^a	0.128±0.035 ^a	26.1±1.5 ^a
Norway	0.00690±0.00043 ^b	0.0822±0.0023 ^b	0.156±0.016 ^b	1.18±0.01 ^a	59.5±5.6 ^b	5.92±0.12 ^b	0.253±0.008 ^b	70 300±1 800 ^b	0.348±0.017 ^b	0.0429±0.0159 ^b	43.5±0.7 ^b
Ireland ¹	0.0190±0.0003 ^c	0.0665±0.0118 ^b	0.174±0.023 ^b	0.759±0.034 ^b	80.4±4.2 ^c	6.04±0.38 ^b	0.242±0.008 ^b	51 600±1 500 ^a	0.257±0.007 ^b	0.0430±0.0131 ^b	36.8±1.3 ^c
Samples of different thallus parts from Swedish <i>S. latissima</i>											
Whole thallus ²	0.0157±0.0013 ^a	0.138±0.001 ^a	0.764±0.140 ^a	1.17±0.06 ^a	305±4 ^a	14.2±0.7 ^a	0.237±0.016 ^{ab}	55 000±2 500 ^a	0.563±0.081 ^a	0.128±0.035 ^a	26.1±1.5 ^a
Blade top	0.0111±0.0007 ^b	0.228±0.012 ^b	0.900±0.052 ^a	1.13±0.04 ^a	536±31 ^b	26.8±0.9 ^b	0.232±0.006 ^a	46 200±450 ^b	0.789±0.019 ^b	0.267±0.049 ^b	36.5±1.1 ^b
Blade base	0.0182±0.0004 ^a	0.0527±0.0043 ^c	0.141±0.008 ^b	0.780±0.022 ^b	77.8±5.0 ^c	6.63±0.04 ^c	0.182±0.008 ^c	56 300±240 ^a	0.195±0.020 ^c	0.0513±0.0221 ^a	13.9±0.2 ^c
Stipe/holdfast	0.0389±0.0021 ^c	0.162±0.013 ^a	0.423±0.034 ^c	1.19±0.03 ^a	218±17 ^d	18.3±0.6 ^d	0.267±0.017 ^b	35 900±1 600 ^c	0.650±0.047 ^a	0.112±0.069 ^a	17.7±0.8 ^d
Samples of processed Swedish <i>S. latissima</i>											
Untreated ²	0.0157±0.0013 ^a	0.138±0.001 ^a	0.764±0.140 ^a	1.17±0.06 ^a	305±4 ^a	14.2±0.7 ^a	0.237±0.016 ^c	55 000±2 500 ^a	0.563±0.081 ^a	0.128±0.035 ^a	26.1±1.5 ^a
HPP	0.0278±0.0005 ^b	0.0595±0.0021 ^b	0.398±0.340 ^{ab}	1.57±0.04 ^b	81.3±1.8 ^b	11.9±0.3 ^b	0.205±0.013 ^{ce}	14 700±460 ^{be}	0.267±0.103 ^b	0.0795±0.0074 ^{ab}	36.1±0.8 ^b
Soaking H ₂ O	0.00520±0.00027 ^c	0.0806±0.0027 ^c	0.304±0.041 ^b	2.66±0.09 ^c	122±9 ^c	15.3±0.7 ^a	0.0448±0.0030 ^a	8 800±350 ^c	0.372±0.007 ^b	0.0578±0.0115 ^{ab}	44.8±1.7 ^c
Soaking Acid	0.00833±0.00041 ^d	0.132±0.005 ^a	0.347±0.025 ^b	2.90±0.08 ^d	170±6 ^d	19.4±0.5 ^c	0.0578±0.0022 ^{ab}	11 000±130 ^{bcd}	0.585±0.013 ^a	0.122±0.042 ^{ab}	70.2±1.0 ^d
UAE	0.00568±0.00013 ^c	0.0921±0.0014 ^d	0.113±0.025 ^b	1.79±0.03 ^e	108±1 ^c	14.2±0.1 ^a	0.0911±0.0031 ^b	11 100±70 ^{bcd}	0.314±0.007 ^b	0.0593±0.0298 ^{ab}	49.8±0.2 ^e
HAE	0.0124±0.0007 ^e	0.0819±0.0018 ^c	0.0939±0.0092 ^b	1.46±0.06 ^b	115±6 ^c	9.58±0.05 ^d	0.258±0.007 ^{cd}	7 700±320 ^{ce}	0.272±0.008 ^b	0.0478±0.0140 ^b	39.5±0.9 ^b
Blanching	0.0122±0.0004 ^e	0.106±0.002 ^e	0.283±0.240 ^b	1.80±0.05 ^e	151±3 ^d	15.6±0.9 ^a	0.202±0.022 ^e	13 200±570 ^b	0.349±0.015 ^b	0.0897±0.0274 ^{ab}	58.6±2.7 ^f
[mg kg ⁻¹ DW]	Arsenic speciation										
	I	As	Hg	Pb	iAs	oAs: MMA		oAs: DMA		% Other oAs compounds	
Maximum levels in seaweeds ¹	2000	-	0.1	5	3	-		-		-	
Samples of four species harvested in Ireland											
<i>S. latissima</i> ³	2 430±210 ^a	105±3 ^a	0.839±0.046 ^a	0.0188±0.0009 ^a	0.0751±0.0067 ^a	9.69±0.80 ^a	0.110±0.006 ^a	1.86±0.03 ^a	88.9		
<i>A. esculenta</i>	303±53 ^b	57.9±5.5 ^b	1.03±0.05 ^b	0.0107±0.0008 ^b	0.0698±0.0082 ^b	4.19±0.54 ^b	0.0676±0.0042 ^b	2.03±0.05 ^b	89.2		
<i>P. palmata</i>	147±14 ^b	7.69±0.45 ^c	0.176±0.006 ^c	0.0124±0.0008 ^b	1.160±0.049 ^b	0.421±0.014 ^c	0.0029±0.0016 ^c	0.514±0.08 ^c	87.8		
<i>U. lactuca</i>	43.7±2.8 ^b	5.73±0.06 ^c	0.134±0.001 ^c	0.00796±0.0053 ^c	0.687±0.023 ^c	0.206±0.003 ^c	0.0027±0.0017 ^c	0.409±0.02 ^c	89.2		
Samples of <i>S. latissima</i> from three harvest sites											
Sweden ⁴	2 580±34	61.7±1.9 ^a	0.554±0.028 ^a	0.0188±0.0015 ^a	0.269±0.001 ^a	0.214±0.013 ^a	0.0780±0.0057 ^a	2.41±0.25 ^a	95.6		
Norway	2 420±77	65.9±0.5 ^a	1.16±0.02 ^b	0.0144±0.0013 ^b	0.0796±0.0009 ^b	0.192±0.007 ^a	0.169±0.004 ^b	1.77±0.14 ^b	96.8		

Table 4 (continued)

Ireland ³	2 430±210	105±3 ^b	0.839±0.046 ^c	0.0188±0.0009 ^a	0.0751±0.0067 ^b	9.69±0.80 ^b	0.110±0.006 ^c	1.86±0.03 ^b	88.9
Samples of different thallus parts from Swedish <i>S. latissima</i>									
Whole thallus ⁴	2 580±34 ^a	61.7±1.9 ^a	0.554±0.028 ^a	0.0188±0.0015 ^a	0.269±0.001 ^a	0.214±0.013 ^a	0.0780±0.0057 ^a	2.41±0.25 ^a	95.6
Blade top	2 320±42 ^b	30.4±0.7 ^b	0.142±0.002 ^b	0.0214±0.0006 ^a	0.477±0.017 ^b	0.475±0.012 ^b	0.0827±0.0060 ^{ab}	2.16±0.08 ^b	91.1
Blade base	2 230±29 ^b	100±6.3 ^c	0.771±0.008 ^c	0.0184±0.0012 ^a	0.0588±0.0054 ^c	0.0675±0.007 ^c	0.0921±0.0021 ^b	2.31±0.22 ^c	97.5
Stipe/holdfast	2 620±89 ^a	79.4±1.4 ^d	0.680±0.021 ^d	0.0302±0.0010 ^b	0.188±0.015 ^d	0.128±0.015 ^d	0.0795±0.0050 ^{ab}	2.02±0.39 ^d	97.2
Samples of processed Swedish <i>S. latissima</i>									
Untreated ⁴	2 580±34 ^a	61.7±1.9 ^a	0.554±0.028 ^a	0.0188±0.0015 ^a	0.269±0.001 ^a	0.214±0.013 ^a	0.0780±0.0057 ^a	2.41±0.25 ^a	95.6
HPP	701±12 ^b	41.3±1.3 ^b	1.15±0.06 ^b	0.0348±0.0020 ^b	0.136±0.006 ^b	0.0798±0.0065 ^b	0.101±0.0001 ^b	1.49±0.07 ^b	96.0
Soaking H ₂ O	3 610±49 ^c	24.9±1.7 ^c	0.869±0.055 ^c	0.0265±0.0010 ^c	0.893±0.028 ^c	0.0298±0.0090 ^c	0.0222±0.0009 ^c	2.15±0.12 ^a	91.1
Soaking Acid	4 320±78 ^d	32.5±1.0 ^d	1.08±0.04 ^b	0.0361±0.0024 ^b	0.379±0.002 ^d	0.0263±0.0013 ^c	0.0207±0.0011 ^c	2.12±0.04 ^a	93.3
UAE	2 510±14 ^a	25.5±0.6 ^c	0.751±0.019 ^d	0.0293±0.0005 ^{cd}	0.290±0.006 ^a	0.0208±0.0014 ^c	0.0239±0.0012 ^c	2.42±0.04 ^a	90.4
HAE	773±26 ^b	24.0±1.0 ^c	0.541±0.009 ^a	0.0243±0.0017 ^{cd}	0.275±0.016 ^a	0.0162±0.0003 ^c	0.0271±0.0014 ^c	3.22±0.07 ^c	86.4
Blanching	2 180±200 ^e	34.7±2.8 ^d	0.941±0.006 ^c	0.0284±0.0010 ^c	0.254±0.008 ^a	0.0303±0.0012 ^c	0.0237±0.0015 ^c	2.96±0.08 ^c	91.3

¹ ANSES (2020) and CEVA (2019). ² European Commission (2023) suggests 3 mg kg⁻¹. ³ Same samples. ⁴ Same samples. HPP: High-pressure processing; UAE: Ultrasound-assisted extraction; HAE: Heat-assisted extraction.

($p<0.05$) lower ash levels compared to untreated material, with a reduction of up to 74.2 %. The reduction in ash correlated positively with arsenic (tAs: $r=0.9^{**}$, iAs: $r=1.0^{***}$), iron ($r=0.9^{**}$), and sodium ($r=1.0^{***}$), which are the main constituents in the mineral fraction according to the current investigation. In contrast to ash, the protein content per algal dry weight increased by 51.5–73.7 % compared to the untreated sample. Hence, processing exerted a concentrating effect. Moreover, when subjecting samples to all the processing methods, the amount of mannitol decreased, whereas all other carbohydrate compounds were on relative terms concentrated in the seaweed. Total carbohydrate content after processing was significantly higher (by 33–46 %) compared to the untreated material, with percentages above 50 % DW for all samples except those from heat-assisted extraction (HAE).

The processing methods applied substantially decreased the levels of some PTEs in *S. latissima*, although a concentrating effect was also observed in some cases (Fig. 2d, Table 4). HPP and HAE decreased iodine to levels significantly below the maximum limit (Table 4). HAE proved to be the only method that did not increase the relative cadmium content, which was significantly ($p<0.05$) concentrated using all other methods. Up to 92.4 % of inorganic arsenic (61.1 % of tAs), 49.4 % of lead, and 72.8 % of iodine could be removed with the different methods. Overall, HAE could reduce the amount of all PTEs to the greatest extent. However, of all PTEs, cadmium and mercury were most difficult to remove.

The correlation analysis (Table S6) indicated some relationship between PTEs, monosaccharides and protein. Protein content appeared to have a negative correlation with total arsenic ($r=-0.9^{**}$) and inorganic arsenic ($r=-1.0^{***}$). The same applied to alginate (tAs: $r=-0.7$ and iAs: $r=-0.8^{*}$) and glucuronic acid, a minor constituent in fucoidan (tAs: $r=-0.9^{**}$ and iAs: $r=-1.0^{***}$). The presence of fucose (in fucoidan) and alginate correlated with cadmium ($r=0.7$ and $r=0.8^{*}$, respectively) and mercury ($r=0.7$ and $r=0.8^{*}$, respectively).

Processing led to a significant reduction in iron and sodium content by all methods, whereas the levels of the other elements after processing were dependent on the method employed.

Discussion

This study confirms that differences in element content and macro-nutritional composition in seaweed samples can depend on species, harvest site, thallus part, and processing method. In several cases, the element content correlated with other analysed components, indicating how these elements interact in the seaweeds.

Biosorption by marine algae is reported to occur through surface precipitation, complexation, or ion exchange, mechanisms predominantly studied in brown seaweed species, e.g. *Sargassum* spp., *Ascophyllum* sp., and *Fucus* spp. (Pan et al. 2000; Davis et al. 2003; He and Chen 2014). Uptake of elemental compounds is postulated to derive from properties of the cell wall polysaccharides where functional groups, such as carboxyl, hydroxyl, sulphate, phosphate, and amine groups, are highly important for metal binding. Ion exchange is an important mechanism for biosorption of heavy metals which replaces light metals (predominantly Ca^{2+} and Mg^{2+} , since monovalent Na^{+} and K^{+} cannot form robust cross-linkages). The affinity of alginate and fucoidan to metal ions depends on stereochemical effects, where larger ions ($\text{Pb}^{2+} > \text{Cu}^{2+} > \text{Cd}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Ca}^{2+} > \text{Mg}^{2+}$) tend to bind better to algal functional groups (Pan et al. 2000; Brownlee et al. 2009). However, in the current study, cadmium bound better to alginate than lead, indicating that electronegativity can play a role.

The largest overall variation in elemental compound content and carbohydrate composition were non-surprisingly found between species (Tables 3 and 4). Lead levels were significantly higher in samples of red and green species but did not exceed the acceptance criteria. This is also seen in the positive correlation between lead, xylose, and galactose. Xylose mainly exists in the form 1,3;1,4- β -D-xylans (mixed-linkage xylans) in the matrix phase in *P. palmata*, while small amounts of 1,4- β -D-xylans have been reported to occur in cell walls (Hsieh and Harris 2019). Galactose is a constituent of agar and/or carrageenan, polysaccharides with sulphated functional groups and hydroxyl constituents (Jönsson et al. 2020).

The two brown seaweeds generally displayed highest content of arsenic, cadmium, and iodine, but with species differences. *Saccharina latissima* proved to have the most concerning iodine levels, regardless of harvest site, in line with previous studies (Roleda et al. 2018; Blikra et al. 2021a). Inorganic arsenic content exceeded the maximum level in the *S. latissima* and *A. esculenta* which were wild harvests from Ireland, whereas the samples of red and green species from the same site were below the maximum level. Samples of cultivated *S. latissima* from Sweden and Norway contained levels below the limits. The reasons for the significant disparity in the levels of inorganic arsenic found in Irish kelps compared to sugar kelp harvested in Sweden and Norway remain uncertain.

Furthermore, alginate (especially mannuronic acid) and fucoidan (with fucose backbone) correlated positively to arsenic and cadmium levels. This can explain the considerably lower cadmium levels in red and green algae, where these polymers are absent (Jönsson et al. 2020). Our data suggest that uronic acids are important in this aspect, but also the structure of the polymer, as glucuronic acids are

present in fucoidan in brown seaweed as well as being prevalent building blocks in the green seaweed polysaccharide ulvan. The total carbohydrate content was, however, significantly ($p < 0.05$) lower in samples from red and green species. In alginate, the proportion of mannuronic- and guluronic acid blocks (M- and G-blocks) is important for uptake of specific ions. In fact, the M/G-ratio determines the strength of alginate gels, where folded G-rich gels are more rigid, and linear M-rich gels are softer. M-blocks appear more selective for Cd^{2+} while G-blocks have a higher affinity for Ca^{2+} (Brownlee et al. 2009). Accordingly, cadmium levels correlated with M/G-ratio in the current study. The correlation between arsenic and alginate may however be influenced by additional factors, as it was found to correlate negatively in samples analysed after processing.

Moreover, differences were observed between *S. latissima* samples from different harvest sites. Overall, the highest level of lead was observed in Swedish samples, cadmium in Norwegian samples, and arsenic in Irish samples. The Norwegian sugar kelp samples comprised remarkably low levels of total carbohydrates which can principally derive from low uronic acid content, and considerably higher ash levels which in turn could depend on high sodium levels (as seen in Table 4). Noteworthy, the Norwegian samples were harvested two years earlier than the samples analysed from the other harvest sites, indicating that the impact of seasonal variation cannot be excluded. Further limitations of the spatial comparison, which can impede the interpretation of the data, involve variation in the post-harvest handling, divergent harvesting months, and differences in sourcing, with Irish seaweed being wild harvested while Swedish and Norwegian samples were cultivated. Moreover, according to Blikra et al. (2021a) the cultivation depth also influences the levels of iodine, but not cadmium and arsenic, in sugar kelp. The observed low carbohydrate levels in combination with high ash levels in Norwegian samples indicated that surface precipitation is the major contributor to overall biosorption as there are fewer cell wall polysaccharides to form complexes and exert ion-exchange with. This was explained from the processing study, where sodium, a main component in the ash fraction, was highly reduced while the carbohydrates were concentrated after treatment.

In addition to species and spatial variations, elemental compounds appeared unevenly distributed in *S. latissima* tissue parts of different ages. The most noticeable difference was observed between the algal top and basal tissue. Mannitol, an important compound for carbon storage, was observed at a high level in the older tissue in the blade top. Moreover, the blade top comprised significantly higher levels of inorganic arsenic and lead but lower cadmium content, whereas the opposite was observed for the basal blade. The observed low cadmium levels in top samples could be attributed to the relatively low levels of mannuronic acid, which

agrees with M-blocks being selective for cadmium ions (Pan et al. 2000). Although the inorganic arsenic content was significantly higher in the top blade samples, the level of total arsenic was lowest in this part of the thallus. Hence, the top blade samples comprised the lowest levels of organic arsenic. Levels of arsenosugars and arsenolipids in different algal parts have previously been studied by Pétursdóttir et al. (2019), who suggested that the distribution of arsenic could depend on different metabolic activities in different parts of the algal thallus. This can relate to the increased levels of carbon storage compounds in the older parts, and possibly to the relatively low levels of organic arsenic in the blade top.

In fact, the arsenic speciation of all samples, regardless of species, harvest site, thallus part, or processing applied, suggested that the main part of the total arsenic consisted of organic arsenic. Of the total arsenic fraction, 86.4–96.0 % comprised unidentified organic arsenic and a relatively small proportion of MMA and DMA. The large share of unspecified organic arsenic compounds are presumably arsenosugars and arsenolipids as previously suggested (Pétursdóttir et al. 2019). This motivates future research for in-depth investigation of the organic arsenic fraction in seaweeds.

As previously mentioned, ANSES and CEVA have provided guidelines for maximum levels in seaweed (Table 4), and EFSA has established maximum tolerable weekly or daily intake levels (TWI or TDI) (EFSA 2006, 2009, 2010, 2011, 2012a, 2012b) for safe consumption of PTE-rich foods. Based on these levels, it can be concluded that iodine and cadmium exert the limiting compounds for the daily intake of seaweeds (Table S1). Consequently, from this data, 13.7 g DW of *U. lactuca*, 4.1 g DW of *P. palmata*, and 2.0 g DW of *A. esculenta* can safely be consumed daily by healthy adults (70 kg). The corresponding number for *S. latissima* is only between 0.2 and 0.3 g DW, and hence, further investigation on PTE removal from *S. latissima* is motivated.

Accordingly, as a final step in this study, the effectiveness of six different processing techniques for Swedish sugar kelp was investigated and demonstrated. Overall, a significant reduction of ash, including arsenic, lead, and iodine (iAs up to 92.4 %, tAs up to 61.1 %, Pb up to 49.4 %, and I up to 72.8 %) was demonstrated. HAE appeared as the most successful method for reduction of PTEs in *S. latissima* reducing the levels of iodine, total arsenic, and inorganic arsenic, whereas cadmium concentration was unaffected. Although HPP significantly reduced four of the six PTEs studied, it tended to drastically concentrate cadmium in the algal material. Since cadmium and iodine levels exceeded the postulated maximum level for the majority of the samples, they must be considered the elements of most concern for seaweed consumption. Only two methods, HPP and HAE, proved capable of reducing the iodine levels below the maximum level, indicating that treatment with high pressure or high temperature is required for its removal. Interestingly, when

comparing soaking in water and soaking in acid, it is evident that cadmium and lead were better reduced in acidic conditions. This indicates that if HAE would be run at acidic conditions, cadmium and lead could potentially be reduced with higher effectiveness.

Total protein and carbohydrate content were concentrated to various extents, which was attributed to the removal of compounds, e.g., ash, which alters the overall composition. It was observed that inorganic arsenic, total arsenic, as well as sodium and iron (considerable levels in the ash fraction), were easily removed by all processing methods. Hence, these compounds were postulated to associate with macroalgae by surface precipitation and possibly also form weak linkages to alginate. On the contrary, cadmium and mercury proved difficult to remove from *S. latissima*. Accordingly, their observed correlation to alginate (especially the M-block), indicated that the interaction could include complexation and/or ion exchange with this polymer. Lead is also expected to associate with macroalgae by ion exchange or complexation (He and Chen 2014), however, no strong correlations were observed in this study. As previously mentioned, the levels of iodine in sugar kelp are most concerning from a food safety perspective. In this study, processing by HPP and HAE increased the daily amount safe to consume from 0.2 g to 0.9 g and 0.8 g, respectively. However, further reduction of iodine would be desirable for introducing seaweed as an ingredient in foods.

Previous studies on PTE reduction from seaweed have mainly focused on arsenic and, more recently, also iodine. Narukawa et al. (2012) removed 85.5 % of total arsenic from hijiki (*Sargassum fusiforme*) by HAE (at 100 °C for 2 h), and 77.4 % by UAE (at ambient temperature for 2h). These are slightly higher removal rates compared to our study with a 60 % reduction of total arsenic for both methods. Hanaoka et al. (2001), similar to our study, demonstrated a removal rate of 42–65 % of total arsenic from hijiki by soaking in water, and showed that while water volume did not affect the reduction, the removal was facilitated by higher water temperature. Other techniques of interest for PTE reduction could include microwave-assisted extraction (MAE) and pulsed electric field (PEF) technology. Removal rates of 77.9 % total arsenic from hijiki by MAE (Narukawa et al. 2012), as well as 42.6 % iodine, 20.7 % mercury, insignificant change of arsenic, cadmium and lead from sugar kelp by PEF (Blikra et al. 2022), was observed in previous studies.

Recently, research has focused on reducing the iodine content since it, together with cadmium, exerts the most concerning elemental constituents from a food safety perspective. Iodine content was reduced by 73 % from *S. latissima* by warm freshwater treatment (45 °C for 120 s) (Krook et al. 2023), up to 93 % by blanching (80 °C for 30 sec, with no significant difference at 45 °C) of *S. latissima* (Nielsen et al. 2020) and similar results (85.9 % reduction

from *S. latissima*) were observed by Trigo et al. (2023). The high reduction rates of iodine from fresh seaweed show that the concerns can easily be overcome by simple scalable methods, however in our study, using thawed sugar kelp, a reduction of merely 15.5 % was observed by blanching (80 °C for 120 s). Some studies have also focused on the more difficultly reduced cadmium. While processing successfully decreased iodine content, the levels of cadmium increased by almost twofold after blanching (Trigo et al. 2023) and by 24 % after boiling (Blikra et al. 2021a). However, Stévant et al. (2018) saw a significant decrease in cadmium content from processing by soaking in hypersaline water (2 M NaCl in water) but not succeeding in dropping the concentrations below acceptance levels. Moreover, Noriega-Fernández et al. (2021) obtained a cadmium reduction of 52 % in *Laminaria hyperborea* by combining UAE (at 50 °C for 5 min) with addition of 1N EDTA, indicating that chelating agents might be necessary for efficient removal. The difficulty of removing cadmium by safe and scalable processes motivates further research on the topic.

Conclusions

This study confirms the importance of analyzing PTEs in seaweeds prior to food consumption as well as the importance of implementing scalable processes for their removal. It creates a snapshot of the variation in PTEs by including samples of four different species (*S. latissima*, *A. esculenta*, *P. palmata* and *U. lactuca*) from one site, as well as of a single species (*S. latissima*) from three different harvest sites, and with different parts analyzed from a single harvest site. The analysis showed that the actual levels of PTEs differed between the species at a single site with comparatively high levels of cadmium and arsenic in the kelps (*S. latissima* and *A. esculenta*), high levels of iodine in *S. latissima*, and with comparatively higher levels of lead in *P. palmata* and *U. lactuca* than in the kelps. The content of especially inorganic arsenic differed in samples of the kelp *S. latissima* from distinct harvest sites, and arsenic and cadmium content varied in different parts of the kelp. When comparing the PTE content in the seaweed samples to tolerable weekly or daily intake levels by EFSA, it was concluded that iodine followed by cadmium exert the most limiting elements for safe seaweed consumption. Processing of *S. latissima* by scalable technologies allowed reduction of the PTEs below maximum levels, with the most efficient technique being heat assisted extraction. However, cadmium was generally difficult to reduce with the selected techniques, motivating further development of processing for its reduction.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

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