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Variations in polyphenol and heavy metal contents of wild-harvested and cultivated seaweed bulk biomass: Health risk assessment and implication for food applications

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ABSTRACT

Seaweeds are increasingly used in European cuisines due to their nutritional value. Many algal constituents, such as polyphenols, are important antioxidants and thus considered beneficial to humans. However, many seaweed species can accumulate heavy metals and exhibit potential health risks upon ingestion. We investigated temporal and spatial variations in polyphenol and heavy metal (As, Cd, Hg, Pb) concentrations of three edible seaweed species. The brown algae *Saccharina latissima* and *Alaria esculenta*, and the red alga *Palmaria palmata* were sourced from natural populations and aquaculture in the NE Atlantic and processed as bulk biomass mimicking industrial scales. The mean polyphenol content was species-specific (*Alaria* > *Saccharina* > *Palmaria*), and highest in winter (for *Alaria* and *Saccharina*) and spring (for *Palmaria*); inter-annual and spatial variations were marginal. Heavy metal concentrations varied between species and depended on collection site, but seasonal variations were minimal. Our data suggest that all three species are good sources of antioxidants, and the heavy metal concentrations are below the upper limits set by the French recommendation and the EU Commission Regulation on contaminants in foodstuffs. A health risk assessment indicated that consumption of these seaweed species poses a low risk for humans with regard to heavy metals. However, an EU-wide regulation on maximal concentration of heavy metals in seaweeds should be established.

1. Introduction

The consumption of seaweeds has been a long tradition in many Asian countries and in some maritime communities across Europe and North America (McHugh, 2003; Mouritsen et al., 2013). Coastal dwellers in, e.g., Indonesia, Malaysia and the Philippines use different species of fresh seaweeds as ingredients in salads and soups. In Ireland and Brittany, seaweeds are commonly used to enrich foods, i.e. to add flavor and to benefit from algal constituents such as natural minerals (Guiry & Blunden, 1991; Holdt & Kraan, 2011). In Norway, consumption and trading of the red alga *Palmaria palmata* has been recorded since the Viking age (Delaney, Frangoudes, & Ii, 2016). Presently, many seaweed species are used as food and supplements due to their nutritional benefits (Holdt & Kraan, 2011). In general, the application of algae as food and nutraceutical ranges from traditional Asian dishes to functional foods in haute cuisine. The increasing interest in seaweeds as nutraceuticals has led to a strong movement to introduce various species into European cuisine (Chapman, Stevant, & Larssen, 2015; Marfaing, 2017; Rioux, Beaulieu, & Turgeon, 2017). Thus, for many maritime communities, seaweeds are not only a valuable source of food for daily consumption but also source of natural products of commercial importance.

Many seaweed species are rich in fibres, minerals, trace elements, proteins, lipids, and certain vitamins (Holdt & Kraan, 2011). Some of these algal constituents possess bioactivities beneficial to humans. For example, algal polysaccharides and polyphenols are of interest not only

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for their antibacterial, antifungal, and antiviral properties but also for their potential to prevent several chronic diseases such as cancer, cardiovascular diseases, obesity, and diabetes (Déléris, Nazih, & Bard, 2016).

Seaweeds thrive in habitats where environmental conditions vary greatly due to the influence of tides. Depending on water level, seaweeds can be subjected to high solar radiation (including UV), pronounced temperature fluctuation and desiccation. A prolonged exposure to environmental stressors may lead to the formation of free radicals and other oxidizing agents in algal specimen. In turn, seaweeds have evolved different strategies to minimize oxidative damage and maintain cellular integrity against these adverse conditions; this ability is often related to the presence of effective antioxidant systems. These systems reduce an accumulation of reactive oxygen species (ROS) and other free radicals and thus prevent an irreversible damage to proteins, amino acids, lipids and DNA. Polyphenols, such as phlorotannins, and sulfated polysaccharides are among the most powerful antioxidant compounds found in different seaweed species (e.g. Balboa, Conde, Moure, Falqué, & Domínguez, 2013; Di et al., 2017; Valentão et al., 2010). Seaweeds rich in polyphenols could serve as a functional ingredient in the human diet, probably preventing chronic diseases. In addition, antioxidant-rich seaweeds may improve food shelf life by reducing a ROS-promoted degradation of oils and fats and improve both nutritional quality and food security, and enhance health-related beneficial properties (Miranda et al., 2016, 2018; Roohinejad et al., 2017).

On the other hand, many algal compounds that are beneficial to humans, also possess one or multiple metal binding sites (Güven, Akyüz, & Yurdun, 1995; Kuyucak & Volesky, 1989; Reddy & Prasad, 1990). For example, the cell wall polysaccharides of brown algae, such as kelps, can have a high affinity to absorb and retain metals from surrounding seawater. In general, algal polysaccharides bind heavy metals to various degrees and their binding affinity is ranked as: alginates (brown algae) > carrageenans (red algae) > agar (red algae) (Güven et al., 1995). In addition, Gekeler, Grill, Winnacker, and Zenk (1988) showed that algal peptides, including those from the brown seaweed *Sargassum muticum*, bind to heavy metal via chelation.

Heavy metals are naturally occurring elements of the earth's crust. They have a high atomic weight and a density at least five times greater than that of water. Human activities in mining and smelting operations, metal producing industries, and in agriculture have drastically altered their geochemical cycles and biochemical balance (Singh, Gautam, Mishra, & Gupta, 2011). The pollution of marine environments by anthropogenic activities (Wang, Xu, Sun, Liu, & Li, 2013) has led concerns about health risks associated with seaweed consumption (Chen, Pan, Huang, & Han, 2018). However, the toxicity of heavy metals depends on multiple factors including chemical speciation and chelation, dose, exposure route, as well as age, gender, and nutritional status of exposed individuals. Arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) are considered highly toxic even at trace levels and rank among the priority metals that are of public health significance (CONTAM Panel, 2010; CONTAM Panel, 2011; EFSA, 2014; EFSA Scientific Committee, 2015; Tchounwou, Yedjou, Patlolla, & Sutton, 2012). They can affect the nervous system and accumulate in human adipose tissue and internal organs, potentially increasing the risk for cancer.

Seaweed consumption across Europe has steadily increased and many raw and processed seaweed products are available for purchase as food or health product. A few studies addressed the potential risk associated with heavy metal contents in edible seaweeds (e.g. Almela, Clemente, Vélez, & Montoro, 2006; Besada, Andrade, Schultze, & González, 2009; Rubio et al., 2017). To date, there is no general agreement on maximum allowable quantities of metals and metalloids in seaweeds. In this regard, there is a call for a national (e.g. Spanish, Norwegian, etc.) or preferably, a pan European regulation on the maximum amount of pollutants in edible seaweeds (Besada et al., 2009). Considering the French recommendation (Afssa, 2007; CSHPF, 1990), the heavy metal concentrations of seaweeds commercialized for human consumption studied by Besada et al. (2009) scored as follows: Hg and Pb are below the limits i.e. a defined threshold concentration, Cd in most samples exceeded the limit, and the total and inorganic arsenic in one species, *Hizikia fusiforme* (= *Sargassum fusiforme*), is very high (total As = 103–147 mg kg^{-1} dry weight; inorganic As = 32–70 mg kg^{-1} dry weight), which would preclude its consumption by humans (Besada et al., 2009). Most studies on economically-important seaweeds primarily addressed As concentration (e.g. Díaz et al., 2012; Ichikawa, Nozawa, Hanaoka, & Kaise, 2010; Khan et al., 2015; Ronan et al., 2017; Rose et al., 2007; Yokoi & Konomi, 2012), even though As may be present in an organic form such as arsenosugars (Taylor et al., 2017), which exhibit a lower toxicity than inorganic As (Almela et al., 2002). In addition, seaweed processing such as washing, soaking and cooking may reduce the total arsenic concentration by as much as 60% (Hanaoka et al., 2001).

In Europe, 95% of marketed algae are intended either for direct (food) or for indirect (phycocolloid, liquid extract as biostimulant) applications. Often, these algae are harvested from natural resources and wild populations (e.g *Ulva* spp., *Palmaria palmata*, *Laminaria digitata*, *Laminaria hyperborea*, and *Ascophyllum nodosum*). To meet an increasing demand for biomass and relieve harvesting pressure on the wild stocks, alternative approaches have been applied and species such as the kelps *Saccharina latissima* and *Alaria esculenta* are grown in aquaculture across various sites in Europe; this cultivation supports an emerging blue bioeconomy (Skjermo et al., 2014).

An increased utilization of seaweeds as nutra- and pharmaceuticals (Déléris et al., 2016; Fleurence, 2016; Kang et al., 2016; Liu et al., 2015; Vonthron-Sénécheau, 2016) at industrial scales has led to a surge in quantifying natural variability of algal compounds. Of particular interests are temporal and spatial variations not only of high-value compounds (e.g. polyphenols) but also of contaminants such as heavy metals. A concomitant assessment of beneficial and harmful algal constituents informs stakeholders and industries about optimal harvesting periods and locations. It further helps to identify the value of seaweed species from various biomass sources (wild or cultivated) and provides information about species selection for industrial applications. This study aimed at quantifying temporal (both seasonal and inter-annual) and spatial (i.e. biogeographic) variations in polyphenols and heavy metal concentrations (As, Cd, Hg, and Pb) of three commercially important and edible seaweed species: the kelps *Saccharina latissima* and *Alaria esculenta* (both Laminariales, Phaeophyceae, Ochrophyta) and the red alga *Palmaria palmata* (Palmariales, Florideophyceae, Rhodophyta). These species were sourced from wild populations and/or from aquaculture in the NE Atlantic of Norway, Iceland and France. Our experimental approach resembled industrial scale processing of seaweed bulk biomass by investigating large quantities of dried and milled (homogenised) algal material. The data presented here provide valuable baseline information on seaweed raw biomass intended for consumption or in applications as nutra- and pharmaceuticals.

2. Materials and methods

2.1. Seaweed biomass collection and processing

The harvest of seaweed biomass considered the following independent variables:

a. Species: The brown seaweed *Alaria esculenta* and *Saccharina latissima*, collectively known as kelps, and the red seaweed *Palmaria palmata*, locally called Dulse or Søl; hereafter, *Alaria*, *Saccharina* and *Palmaria*, respectively.
b. Location: two sites in Norway (Bodø and Trondheim), one site each in Iceland and one site in France.
c. Source: wild-harvest ('wild') and cultivated bulk biomass. Wild biomass was collected in Norway: Trondheimsfjord (Vanvikan), Trondheim (63.551°N, 10.217°E), Skjerstadfjorden, Bodø (67.276

°N, 14.570 °E) and Iceland: Stykkisholmur, Breidafjördur (65.109 °N, 22.772 °W). The two kelps *Alaria* and *Saccharina* were sourced from both wild populations and from aquaculture. Farming sites of kelps are located in Norway: Frøya, Trondheim (63.702°N, 8.872°E) and Morsdalsfjorden, Sund, Gildeskål (67.069 °N, 14.076 °E) and France: CEVA Seafarm, Pleubian (48.847 °N, 3.047 °W). Cultivation were either monoculture (France and Norway) or integrated multi-trophic aquaculture (IMTA) system (Norway only). *Palmaria* was sourced only from wild populations in Norway and Iceland.

d. Season: spring (April and/or May), summer (June and/or August) and autumn (September and/or October).
e. Year: 2015 and 2016.

A standardized protocol for biomass collection, handling and processing was used by the various project partners involved in this study. Collected bulk seaweed biomass included whole algal specimens composed of blades, stipes, holdfasts and sporophylls (specific to *Alaria*). Biomass of entire *Palmaria* specimens was a mixture of male gametophytes and sporophytes; kelp biomass consisted only of sporophytes. Vegetative and reproductive materials were not separated. Large-scale harvesting of cultivated kelps from ropes may lead to partial loss of holdfasts. At least 1 kg of wet biomass per species was harvested: this biomass was comprised of at least 5–10 adult or 50–100 juvenile kelp sporophytes, and at least 300 individuals of *Palmaria*. Following harvest, samples were kept moist, cool and in darkness during transport. In the laboratory, algal biomass was maintained in flowing natural seawater at an ambient temperature (season dependent) until processing. Within 2 h after collection, algal thalli were thoroughly cleaned in a seawater bath by removing visible epibiota and calcareous particles. Subsequently, cleaned biomass was swiftly washed in (sea)water with incrementally decreasing salinities (100%, 50%, 0%), drained of excess water, packed and frozen at −80 °C. Thereafter, samples were freeze-dried and ground to a fine powder (120 μm grain size). The pooled homogenised biomass was analysed in duplicates or triplicates for phlorotannins and heavy metals as outlined below.

2.2. Polyphenolic analysis

The polyphenolic content of algal extracts was determined colorimetrically using the Folin–Ciocalteu reagent according to the method of Ragan and Glombitza (1986). The extraction was performed using 250 mg of ground seaweed biomass in 10 mL of 80/20 (v/v) acetone/water. The mixture was incubated for 1 h in the dark at room temperature. The supernatant was recovered and the pellet was re-extracted a second time under the same conditions. Supernatants from the first and the second extraction were pooled and filtered (0.45 μm). 200 μL of the filtrate were mixed with 1300 μL of dionised water (18.2 MΩ cm) and 100 μL Folin-Ciocalteu reagent (VWR, Germany) followed by the addition of 400 μL of 29% (w/w) Na_2CO_3. After incubation at 45 °C for 30 min in the dark, the absorbance was measured at 760 nm using a UVIKON-XL spectrophotometer (Bio-Tek Instruments, USA); with phloroglucinol as standard reference (99%, Sigma-Aldrich, Steinheim, Germany) for *Saccharina* and *Alaria*, and gallic acid (97.5%, Sigma-Aldrich, Steinheim, Germany) for *Palmaria*. Phloroglucinol is the constitutive secondary metabolite of phlorotannins in brown algae (Ragan & Glombitza, 1986); gallic acid has been identified as constitutive metabolite of phlorotannins in red algae (Souza et al., 2011). Standard curves with concentrations ranging from 0 to 100 μg mL^{-1} of phloroglucinol and gallic acid, respectively, were used for quantification. Phlorotannin contents were expressed as mg phloroglucinol equivalents (PGE) g dw^{-1} for kelps and as mg gallic acid equivalent (GAE) g dw^{-1} for the red seaweed.

2.3. Heavy metal analysis

Total As, Cd, Hg and Pb in dried algal powder from bulk biomass were determined by ICP-MS after mineralization in a closed acid digestion vessel. Briefly, in a 50 mL digestion vessel, 200 mg of freeze-dried sample was mixed with 3 mL of HNO_3 and 1.5 mL of H_2O_2 and digested in a Mars5 microwave oven (CEM, North Carolina, USA), according to method SV-25-02-SN described in Matis Quality manual based on NMKL 186 (2007). The digested sample solution was transferred to 50 mL polypropylene tube, the vessel sparingly rinsed with small amount of deionised water (18.2 MΩ cm) several times. The aliquots were pooled and diluted to 30 mL using deionised water (18.2 MΩ cm). The concentrations of As, Cd, Hg and Pb were determined by ICP-MS (Agilent 7500ce, Waldbronn, Germany). The indium nuclide (^{115}In) was used as an internal standard. Certified reference materials are routinely treated and analysed in the same manner as the samples. For quality assurance, the trace analytical laboratory at the Matís undergoes annual proficiency test with QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) and RIKILT-Institute of Food Safety. Matís is a National Reference Laboratory for heavy metals in food and feed, and takes part in proficiency trainings organized by EU-RL (European Union Reference Laboratory).

2.4. Data analysis

Samples of algal biomass were analysed for polyphenols and heavy metals in duplicates or triplicates, representing technical replicates. The arithmetic mean of these duplicate or triplicate measurements was considered an independent replicate sample (n). Since our analyses were conducted on bulk biomass, which is highly homogenised material comprised of multiple algal specimens, the data were summarized as means and the uncertainty, i.e. the standard errors of means (S.E.).

In this observational study, the explanatory (independent) variables were 'species' (levels: "*Palmaria*", "*Alaria*", "*Saccharina*"), 'year' (levels: "2015", "2016"), 'season' (levels: "spring", "summer", "autumn"), collection 'site' (levels: "Bodø", "Trondheim", "Iceland" for *Palmaria*; "Bodø", "Trondheim", "France" for *Alaria* and *Saccharina*), and biomass 'source' (levels: "wild", "IMTA", "monoculture"; biomass source was investigated only for *Alaria* and *Saccharina*). The response (dependent) variables were concentrations of 'polyphenols', 'As', 'Cd', 'Hg' and ''Pb'. To estimate the biological variance in the response variables for a specific explanatory variable, data were arrayed across all other explanatory variables; for example, the variance of 'polyphenols' for each level of 'year' was obtained by considering the values for 'season', 'site' and, if applicable, 'source'. This approach allowed the application of formal statistical tests to evaluate *effects* of each explanatory variable (Roleda et al., 2018).

The *effect* of 'species' was analysed by non-parametric Kruskal-Wallis H tests (ANOVA assumptions were violated), Mann-Whitney U tests with adjusted P values (Bonferroni procedure) were used for pair-wise comparisons. The *effect* of 'year' was computed by t tests or Welch's t-test if unequal variances were detected. *Effects* of 'season', 'site' and 'source' were analysed by one-way ANOVAs, followed by Tukey's HSD *post hoc* tests for pair-wise comparisons, or, if ANOVA assumptions were violated, by Kruskal-Wallis H tests (followed by Mann-Whitney U tests with adjusted P values) as outlined above for 'species'. For all tests, the significance level α was 0.05. Specifics about each statistical analysis conducted are outlined in the statistical summary tables (Tables 1–4), including test statistics, P values and results of pair-wise comparisons. Statistical analyses were performed using IBM® SPSS® Statistics version 24; data were plotted with SigmaPlot® version 14.

2.5. Biomass quality and health risk assessment

Heavy metal concentrations were compared to those provided by the French recommendation (Afssa, 2007; CSHPF, 1990) to assess the quality of unprocessed seaweed biomass (Table 5). An assessment of potential health risks associated with the consumption of unprocessed

Table 1
Summary of results of statistical analyses. Polyphenols and heavy metal contents in bulk biomass of three edible macroalgal species (*Saccharina latissima*, *Alaria esculenta*, *Palmaria palmata*) were compared by Kruskal-Wallis *H* tests (ANOVA assumptions were violated) followed by Mann-Whitney *U* tests for multiple pair-wise comparisons; the significance level was adjusted according to Bonferroni. Shown are test statistics (χ^2), degrees of freedom (subscript) and *P* values (bold if significant).

		test statistic	*P* value	Direct comparison
Species	Polyphenols	$\chi^2_2 = 46.053$	< **0.001**	*Alaria* > *Saccharina* > *Palmaria*
	As	$\chi^2_2 = 30.474$	< **0.001**	*Saccharina* > *Alaria* > *Palmaria*
	Cd	$\chi^2_2 = 23.673$	< **0.001**	*Alaria* > *Palmaria* = *Saccharina*
	Hg	$\chi^2_2 = 0.221$	0.895	
	Pb	$\chi^2_2 = 7.466$	**0.024**	*Alaria* > *Palmaria* = *Saccharina*

Table 2
Summary of results of statistical analyses. Polyphenols and heavy metal contents in bulk biomass of *Palmaria palmata* were determined between sampling years (2015, 2016), season (spring, summer, autumn), and site (Bodø, Trondheim, France). Statistically significant effects were identified using appropriate tests: independent-sample *t* tests for "year" and "site" (Trondheim was removed from the analysis due to $n = 1$), and 1-way ANOVAs followed by Tukey's *posthoc* tests for "season". If the test assumption of homogeneous variances was violated, appropriate alternative tests were applied: Welch's *t*-test or Kruskal-Wallis *H* tests. Presented are respective test statistics (*t*, *F* or χ^2), degrees of freedom (subscript) and *P* values (bold if significant).

		test statistic	*P* value	Direct comparison
Year	Polyphenols	$t_9 = -0.142$	0.890	
	As	$t_9 = -0.601$	0.563	
	Cd	$t_9 = -0.386$	0.708	
	Hg	$t_9 = 2.133$	0.062	
	Pb	$t_{4.18} = -0.934^{*}$	0.401	
Season	Polyphenols	$F_{2,8} = 14.549$	**0.002**	spring > summer = fall
	As	$\chi^2_2 = 0.167^{**}$	0.920	
	Cd	$F_{2,8} = 0.578$	0.583	
	Hg	$F_{2,8} = 0.405$	0.680	
	Pb	$\chi^2_2 = 4.750^{**}$	0.093	
Site	Polyphenols	$t_8 = 0.186$	0.857	
	As	$t_{5.04} = 3.494^{*}$	**0.017**	Bodø > Iceland
	Cd	$t_{3.11} = -10.558^{*}$	**0.002**	Iceland > Bodø
	Hg	$t_8 = 0.846$	0.422	
	Pb	$t_{3.10} = -0.996^{*}$	0.390	

* Welch's *t*-test.
** Kruskal-Wallis *H* test.

seaweed biomass was conducted according to Chen et al. (2018) by estimating exposure doses (for women and men), the targeted hazard quotient (THQ), and the hazard index.

Exposure doses for each heavy metal were estimated as:

$$Exposure\ dose = \frac{c_i \times d_{dw}}{bw} \quad (1)$$

where c_i is the concentration of the element *i* in dry seaweed biomass (mg (kg dw)$^{-1}$), d_{dw} is the quantity of dry seaweed biomass ingested daily (5.2 g), and bw is the average body weight for Western European women (69.3 kg) and men (86.4 kg), respectively. There are no data regarding seaweed consumption by Western Europeans available; thus, we used average ingestion data from the FAO available for China (5.2 g; Chen et al., 2018). Bodyweight data were obtained from www.worlddata.info (Worlddata.info, 2018).

Here, As was measured as total As, which comprised organic and inorganic As. Adverse effects to human health have been largely associated with inorganic As (Almela et al., 2002). Thus, the proportion (%) of inorganic As (i-As) in total As (tAs) was computed from empirical data (t-As and i-As) presented in Almela et al. (2006) and Díaz et al. (2012): i-As was 4.15% of tAs in *Palmaria* spp. and i-As was 1.72% of t-As in different kelp species (Laminariales).

The THQ_i for each element *i* was determined as:

$$THQ_i = \frac{Exposure\ dose_i}{RfD_i} \quad (2)$$

where the RfD_i is the recommended reference dose for element *i* as provided by the National Center for Environmental Assessment, EPA of the United States (USA EPA, 2007).

The sum of THQ_i for all heavy metals investigated represents the overall hazard index (HI) for the consumption of unprocessed biomass. At HI < 1 the expected health risks are likely to be minimal; at HI > 1 moderate to high health risks may be expected (Chen et al., 2018). This approach of health risk assessment is rather conservative since (1) many Western Europeans likely consume seaweed quantities lower than 5.2 g per day, (2) RfD values are more conservative (Table 6: USA EPA, 2007) than those provided by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) as provisional tolerable weekly intake (PTWI). For example, JECFA's current recommendation on some of the heavy metals' TWIs are as follows: cadmium 2.5 μg (kg week)$^{-1}$ (EFSA CONTAM Panel, 2011), arsenic between 0.3 and 8 μg (kg day)$^{-1}$ (EFSA CONTAM Panel, 2009), methyl mercury 1.3 μg (kg week)$^{-1}$ and total mercury 4 μg (kg week)$^{-1}$ (EFSA CONTAM Panel, 2012), and (3) processing of seaweed biomass likely reduces heavy metal concentrations (e.g. Hanaoka et al., 2001).

3. Result

Mean polyphenol content was species-specific (*Alaria* > *Saccharina* > *Palmaria*; $P < 0.001$; Fig. 1, Table 1). Contents ranged from 14 to 61 mg (g dw)$^{-1}$, 5–15 mg (g dw)$^{-1}$, and 2–6 mg (g dw)$^{-1}$ for *Alaria*, *Saccharina* and *Palmaria*, respectively (Supplementary Table 1).

For each species, polyphenols varied seasonally (Fig. 2), but no intra-annual variation was observed. In *Palmaria*, polyphenols were significantly higher in spring than during summer and autumn (Fig. 2a, Table 2). For the two kelp species, polyphenols were highest in autumn and lowest in spring (Tables 3 and 4). For *Alaria*, polyphenol contents were similarly high in summer and autumn (Fig. 2c); and for *Saccharina*, polyphenol concentrations were similarly low in spring and summer (Fig. 2e). There was no statistically significant difference in polyphenol content detected across the sites investigated (Fig. 2b, d, f).

Mean heavy metal concentration also varied among species. Total As was significantly higher in kelps than in the *Palmaria* (*Saccharina* > *Alaria* > *Palmaria*; Fig. 3a). Both the Cd and Pb concentrations were significantly highest for *Alaria,* and similarly low for *Saccharina* and *Palmaria* (Fig. 3b, d). The Hg concentrations did not statistically differ among species (Fig. 3c). All data, i.e. means and ranges (min-max), are shown in the Supplementary Table 2.

Most heavy metals in different species did not exhibit pronounced seasonality; this is probably associated with the high variability observed (Fig. 4a-l). For example, the Cd content in *Alaria* was slightly, although non-significantly, higher in autumn compared to spring and summer samples ($P = 0.051$; Fig. 4f). Only the Hg content in *Saccharina* varied seasonally peaking during autumn (autumn > summer = spring; Table 4). All data, i.e. means and ranges (min-max),

Table 3
Summary of results of statistical analyses. Polyphenols and heavy metal contents in bulk biomass of *Alaria esculenta* were determined between sampling years (2015, 2016), season (spring, summer, autumn), site (Bodø, Trondheim, France), and source (wild, monoculture, IMTA). Statistically significant effects were identified using appropriate tests: independent-sample *t* tests for "year" and 1-way ANOVAs followed by Tukey's *posthoc* tests for "season", "site" and "source". If the test assumption of homogeneous variances was violated, appropriate alternative tests were applied: Welch's *t*-test or Kruskal-Wallis *H* tests. Presented are respective test statistics (*t*, *F* or χ^2), degrees of freedom (subscript) and *P* values (bold if significant).

		test statistic	*P* value	Direct comparison
Year	Polyphenols	$t_{20} = 0.564$	0.579	
	As	$t_{20} = 1.559$	0.135	
	Cd	$t_{20} = 0.527$	0.604	
	Hg	$t_{9.27} = 4.422^{*}$	**0.002**	2015 > 2016
	Pb	$t_{20} = 0.158$	0.876	
Season	Polyphenols	$F_{2,19} = 4.420$	**0.027**	spring < summer = fall
	As	$F_{2,19} = 3.364$	0.056	
	Cd	$F_{2,19} = 3.507$	0.051	
	Hg	$F_{2,19} = 0.059$	0.943	
	Pb	$\chi^2_2 = 5.523^{**}$	0.063	
Site	Polyphenols	$F_{2,19} = 1.451$	0.259	
	As	$F_{2,19} = 1.123$	0.346	
	Cd	$F_{2,19} = 9.003$	**0.002**	(Bodø^{b} > Trondheim^{a}) = France^{ab}
	Hg	$\chi^2_2 = 1.479^{**}$	0.477	
	Pb	$F_{2,19} = 18.153$	< **0.001**	France > Bodø = Trondheim
Source	Polyphenols	$F_{2,19} = 0.512$	0.607	
	As	$F_{2,19} = 0.683$	0.517	
	Cd	$\chi^2_2 = 4.997^{**}$	0.082	
	Hg	$\chi^2_2 = 2.969^{**}$	0.227	
	Pb	$F_{2,19} = 5.227$	**0.016**	(mono^{b} > IMTA^{a}) = wild^{ab}

* Welch's *t*-test.
** Kruskal-Wallis *H* test.

are shown in the Supplementary Tables 3 and 4

For each species, heavy metal concentrations varied between collection sites (Fig. 5; Tables 2–4). In *Palmaria*, As was significantly higher in Bodø compared to Iceland (Fig. 5a), and Cd contents were significantly higher for Iceland than for Bodø (Fig. 5b). In *Alaria*, Cd ([Bodø > Trondheim] = France; Fig. 5f) and Pb concentrations

Table 4
Summary of results of statistical analyses. Polyphenols and heavy metal contents in bulk biomass of *Saccharina latissima* were determined between sampling years (2015, 2016), season (spring, summer, autumn), site (Bodø, Trondheim, France), and source (wild, monoculture, IMTA). Statistically significant effects were identified using appropriate tests: independent-sample *t* tests for "year" and 1-way ANOVAs followed by Tukey's *posthoc* tests for "season", "site" and "source". If the test assumption of homogeneous variances was violated, appropriate alternative tests were applied: Welch's *t*-test or Kruskal-Wallis *H* tests followed by Mann-Whitney *U* tests with adjusted significance level (Bonferroni). Presented are respective test statistics (*t*, *F* or χ^2), degrees of freedom (subscript) and *P* values (bold if significant).

		test statistic	*P* value	Direct comparison
Year	Polyphenols	$t_{21} = -0.186$	0.854	
	As	$t_{10.79} = 0.765^{*}$	0.461	
	Cd	$t_{21} = -0.764$	0.453	
	Hg	$t_{9.15} = 1.822^{*}$	0.101	
	Pb	$t_{17.75} = -2.207^{*}$	**0.041**	2016 > 2015
Season	Polyphenols	$F_{2,20} = 16.939$	< **0.001**	fall > summer = spring
	As	$F_{2,20} = 1.702$	0.208	
	Cd	$F_{2,20} = 0.375$	0.692	
	Hg	$F_{2,20} = 4.185$	**0.030**	fall > summer = spring
	Pb	$F_{2,20} = 0.402$	0.674	
Site	Polyphenols	$F_{2,20} = 3.134$	0.065	
	As	$\chi^2_2 = 7.639^{**}$	**0.022**	France > Bodø = Trondheim***
	Cd	$F_{2,20} = 14.364$	< **0.001**	Bodø = Trondheim > France
	Hg	$\chi^2_2 = 7.744^{**}$	**0.021**	Bodø = France > Trondheim***
	Pb	$\chi^2_2 = 7.868^{**}$	**0.020**	(France^{b} > Bodø^{a}) = Trondheim^{ab}***
Source	Polyphenols	$F_{2,20} = 1.290$	0.297	
	As	$F_{2,20} = 0.402$	0.674	
	Cd	$F_{2,20} = 1.473$	0.253	
	Hg	$\chi^2_2 = 0.207^{*}$	0.902	
	Pb	$\chi^2_2 = 5.178^{*}$	0.075	

* Welch's *t*-test.
** Kruskal-Wallis *H* test.
*** Mann-Whitney *U* tests.

Table 5
Quality assessment of unprocessed seaweed biomass. Presented are relative quantities of heavy metals (%) according to the French recommendation (Afssa, 2007; CSHPF, 1990). Calculations are based on species-specific heavy metal concentrations; data are shown as means (minimum-maximum).

	CSHPF/Afssa* mg (kg dw)$^{-1}$		Percent of CSHPF/Afssa
i-As†	3	*Palmaria*	12 (10–17)
		Alaria	33 (22–56)
		Saccharina	40 (30–57)
Cd	0.5	*Palmaria*	165 (5–491)
		Alaria	315 (120–524)
		Saccharina	120 (42–198)
Hg	0.1	*Palmaria*	63 (5–314)
		Alaria	58 (4–258)
		Saccharina	33 (1–105)
Pb	5	*Palmaria*	3 (1–13)
		Alaria	5 (1–14)
		Saccharina	4 (1–14)

*Maximum value according to French recommendation.
† i-As was alculated based on the percentage of i-As in t-As presented by Almela et al. (2006) and Díaz et al., 2012 where i-As was 4.15% of t-As in *Palmaria* spp. and i-As was 1.72% of t-As in different kelp species (Laminariales).

(France > Bodø = Trondheim; Fig. 5h) showed significant differences between sites. In *Saccharina*, concentrations of all heavy metals measured exhibited spatial variability (Fig. 5 i–l; Table 4). Samples from France contained highest As, Hg, and Pb, but lowest Cd. By contrast, specimens from Norway possessed highest Cd, but lowest As and Pb; Hg contents in *Saccharina* from Norway were different between the two collection sites (Bodø > Tronheim). Only Hg varied between the two years investigated (2015 > 2016; Table 3). Minor difference in Pb contents were observed in the biomass source of *Alaria* (Mono > IMTA; Table 3).

Based on the French recommendation (Afssa, 2007; CSHPF, 1990), the mean levels of most heavy metals in different species of seaweeds investigated in this study are below (< 100%) the maximum recommended value, except for Cd (> 100%; Table 5). In addition, the maximum level of Hg was above the threshold value, but the average Hg content did not exceed the recommended limit (Table 5).

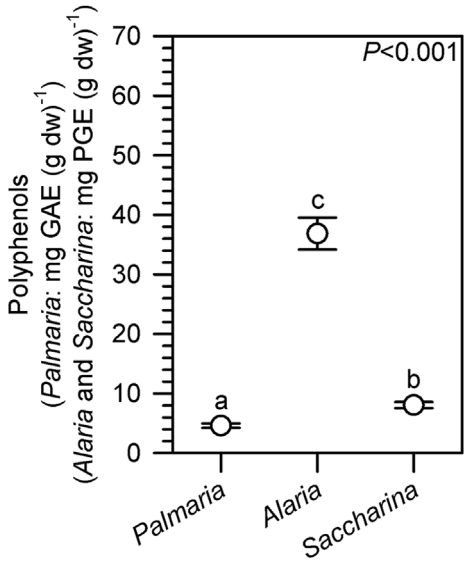

Fig. 1. Variations in polyphenol content in dried bulk biomass of the three edible seaweed species (*Palmaria palmata*, *Alaria esculenta*, *Saccharina latissima*). Data are means ± S.E.; data are given in Table S1 (Supplementary material). Statistical results are summarized in Table 1.

According to the health risk assessment, considering an consumption of 5.2 g dw of seaweed per day (approximately 26 g fresh weight, assuming 80% water content), the exposure dose for a single heavy metal may not exceed 0.118 µg (kg bw day)$^{-1}$ and 0.095 µg (kg bw day)$^{-1}$ on average for women and men, respectively; these highest

Table 6
Estimated exposure of Western Europeans to heavy metals from consumption of unprocessed seaweeds, including a health risk assessment. The targeted hazard quotient (THQ) and the hazard index (HI) are measures of health risk. At HI < 1.0, the expected health risk is minimal. Data are means and maximum (in brackets, to quantify high risk). RfD guidelines are more conservative than those provided by the Joint FAO/WHO Expert Committee as provisional tolerable weekly intake (PTWI) and, thus, were used for this risk assessment.

	RfD*		Women		Men	
			Exposure dose**	THQ	Exposure dose**	THQ
	µg (kg day)$^{-1}$		µg (kg bw day)$^{-1}$		µg (kg bw day)$^{-1}$	
i-As†	0.3	*Palmaria*	0.028 (0.037)	0.09 (0.12)	0.022 (0.030)	0.07 (0.10)
		Alaria	0.073 (0.125)	0.24 (0.42)	0.059 (0.100)	0.20 (0.33)
		Saccharina	0.090 (0.128)	0.30 (0.43)	0.072 (0.103)	0.24 (0.34)
Cd	1	*Palmaria*	0.062 (0.184)	0.06 (0.18)	0.050 (0.148)	0.05 (0.15)
		Alaria	0.118 (0.197)	0.12 (0.20)	0.095 (0.158)	0.09 (0.16)
		Saccharina	0.045 (0.074)	0.04 (0.07)	0.036 (0.060)	0.04 (0.06)
Hg	0.3	*Palmaria*	0.005 (0.024)	0.02 (0.08)	0.004 (0.019)	0.01 (0.06)
		Alaria	0.004 (0.019)	0.01 (0.06)	0.003 (0.016)	0.01 (0.05)
		Saccharina	0.002 (0.008)	0.01 (0.03)	0.002 (0.006)	0.01 (0.02)
Pb	(3.6)§	*Palmaria*	0.012 (0.050)	0.00 (0.01)	0.010 (0.040)	0.00 (0.01)
		Alaria	0.019 (0.053)	0.01 (0.01)	0.015 (0.043)	0.00 (0.01)
		Saccharina	0.014 (0.051)	0.00 (0.01)	0.011 (0.041)	0.00 (0.01)
			$HI_{women\_Palmaria}$	0.17 (0.40)	$HI_{men\_Palmaria}$	0.14 (0.32)
			HI_{women_Alaria}	0.38 (0.69)	HI_{men_Alaria}	0.31 (0.56)
			$HI_{women\_Saccharina}$	0.36 (0.54)	$HI_{men\_Saccharina}$	0.29 (0.43)

*Recommended reference dose (RfD) according to the US EPA (2007), National Center for Environmental Assessment for chronic oral exposure (per kg bodyweight); for Hg, reference dose for chronic inhalation exposure (per kg air).
**Based on daily intake of 5.2 g dw (as determined for China by Chen et al., 2018) and an average body weight of Western Europeans men (86.4 kg) or women (69.3 kg) according to World Data Information (https://www.worlddata.info).
§In 2004, the US EPA considered it inappropriate to develop a reference value for Pb.
†i-As was calculated based on the percentage of i-As in t-As presented by Almela et al. (2006) and Díaz et al., 2012 where i-As was 4.15% of t-As in *Palmaria* spp. and i-As was 1.72% of t-As in different kelp species (Laminariales).

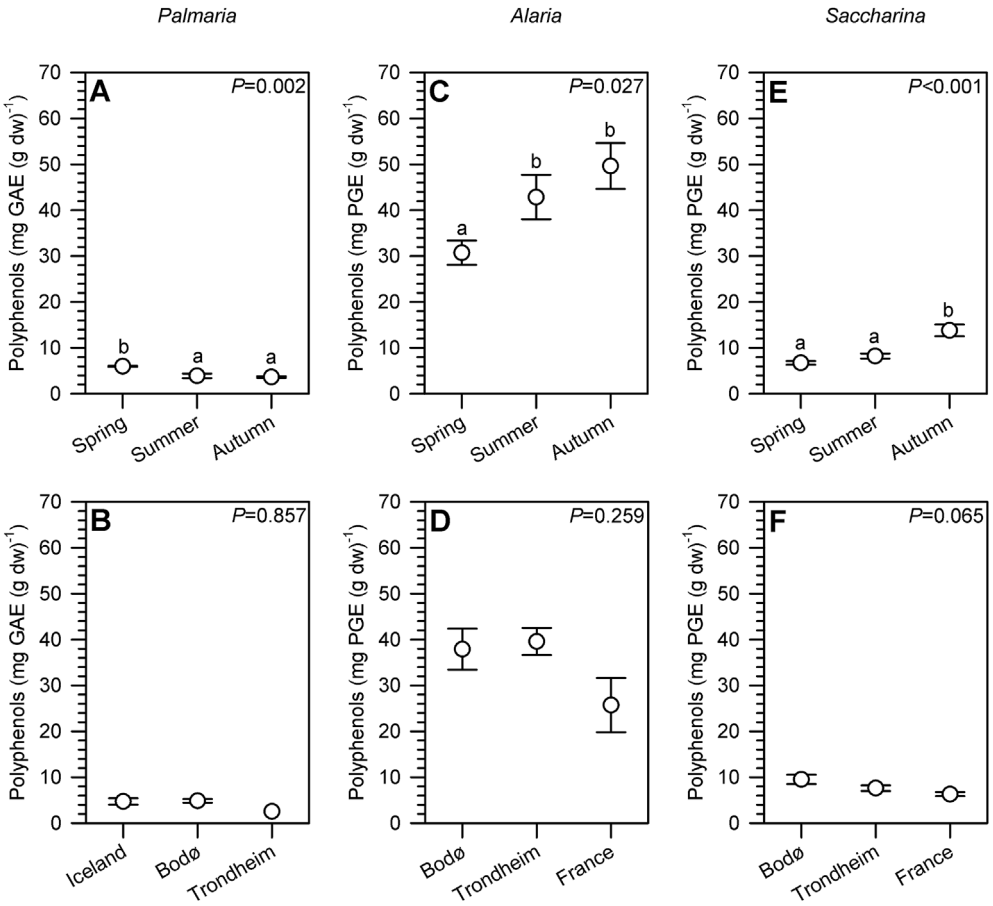

Fig. 2. Variations in polyphenol content in dried bulk biomass of the three edible seaweed species (*Palmaria palmata*, *Alaria esculenta*, *Saccharina latissima*) relative to season, and site. Data are means ± S.E.; data are given in Table S2, S3, and S4 (Supplementary material). Statistical results are summarized in Tables 2–4.

values were determined for Cd in *Alaria* (Table 6). The hazard index (HI) accounts for all heavy metals by considering their sum; HI was smaller than 1.0 for all species, which indicated that heavy metal intoxication from consuming the three edible seaweed species investigated may be minimal (Table 6).

4. Discussion

4.1. Variations in polyphenols

Our study presents the first long-term and large scale study on the polyphenol contents of three commercially important seaweed species in Europe. The antioxidant properties of phenolic compounds found in seaweeds are often associated with phenolic content (Connan, Deslandes, & Gall, 2007). The use of seaweeds in human health and food stability and quality is widely known. Here, variation in polyphenols was largely species-specific and seasonal. No spatial variation was observed within the biogeographic region studied. Previously, species-specific variations have been observed in snapshot measurements (e.g. Ganesan, Kumar, & Bhaskar, 2008; Heffernan, Brunton, FitzGerald, & Smyth, 2015) and numerous seasonal studies on brown seaweeds in specific sites (e.g. Ragan & Jensen, 1978, and references therein; Schiener, Black, Stanley, & Green, 2015).

Among kelps (Laminariales), this study showed that regardless of collection site and season, *Alaria* (3.7 mg (g dw)$^{-1}$) contains higher concentrations of polyphenols than *Saccharina* (0.8 mg (g dw)$^{-1}$). Similarly, Schiener et al. (2015) reported that polyphenol content differs between kelp species from Scotland (converted): *Alaria* (8.7 mg (g dw)$^{-1}$) > *Saccharina* (4.2 mg (g dw)$^{-1}$) > *Laminaria hyperborea* (1.6 mg (g dw)$^{-1}$) = *Laminaria digitata* (1.4 mg (g dw)$^{-1}$). However, concentrations of polyphenols were documented to vary with season in temperate brown seaweeds (Connan, Goulard, Stiger, Deslandes, & Gall, 2004). In this study, lowest polyphenol content among kelps *Alaria* and *Saccharina* were observed during spring (April–May); this is consistent with reports from brown algae from Scotland (Schiener et al., 2015) and Brittany (Connan et al., 2004). On the other hand, seasonal variation in polyphenols appeared to be less pronounced in the kelp *L. digitata* than in fucoids such as *Fucus* spp. and *A. nodosum* (Connan et al., 2004). Regardless of taxonomic group, polyphenol content increased during summer and peaked in early autumn for many fucoids and kelps from Brittany (Connan et al., 2004), Scotland (Schiener et al., 2015) and Norway (Ragan & Jensen, 1978). The seasonal variability observed in polyphenols is probably a response to seasonal changes in abiotic and biotic factors. For example, many polyphenols are important antioxidants and prevent physiological stress arising from exposure to high irradiances, UV and high seawater and air temperatures (Abdala-Diaz, Cabello-Pasini, Perez-Rodriguez, Alvarez, & Figueroa, 2006; Connan et al., 2007; Cruces, Huovinen, & Gomez, 2012; Ragan & Glombitza, 1986; Schoenwaelder, 2002).

Although variability of a multitude of biochemical constituents has been documented for *Palmaria* (e.g., Aguilera, Bischof, Karsten, Hanelt, & Wiencke, 2002; Martinez & Rico, 2002; Rodde, Varum, Larsen, & Myklestad, 2004; Roleda et al., 2018; Schmid, Guihéneuf, & Stengel, 2017), knowledge about seasonality of polyphenols in this red alga is limited. As observed for brown algae, the antioxidative capacity of *Palmaria* increased with increasing subsurface irradiances during the summer in the Arctic (Aguilera et al., 2002), which was associated with an increase in polyphenol as one important antioxidant systems in the species. Here, in contrast to brown algae, the polyphenol content of *Palmaria* was lowest in autumn (no winter measurement) and highest in

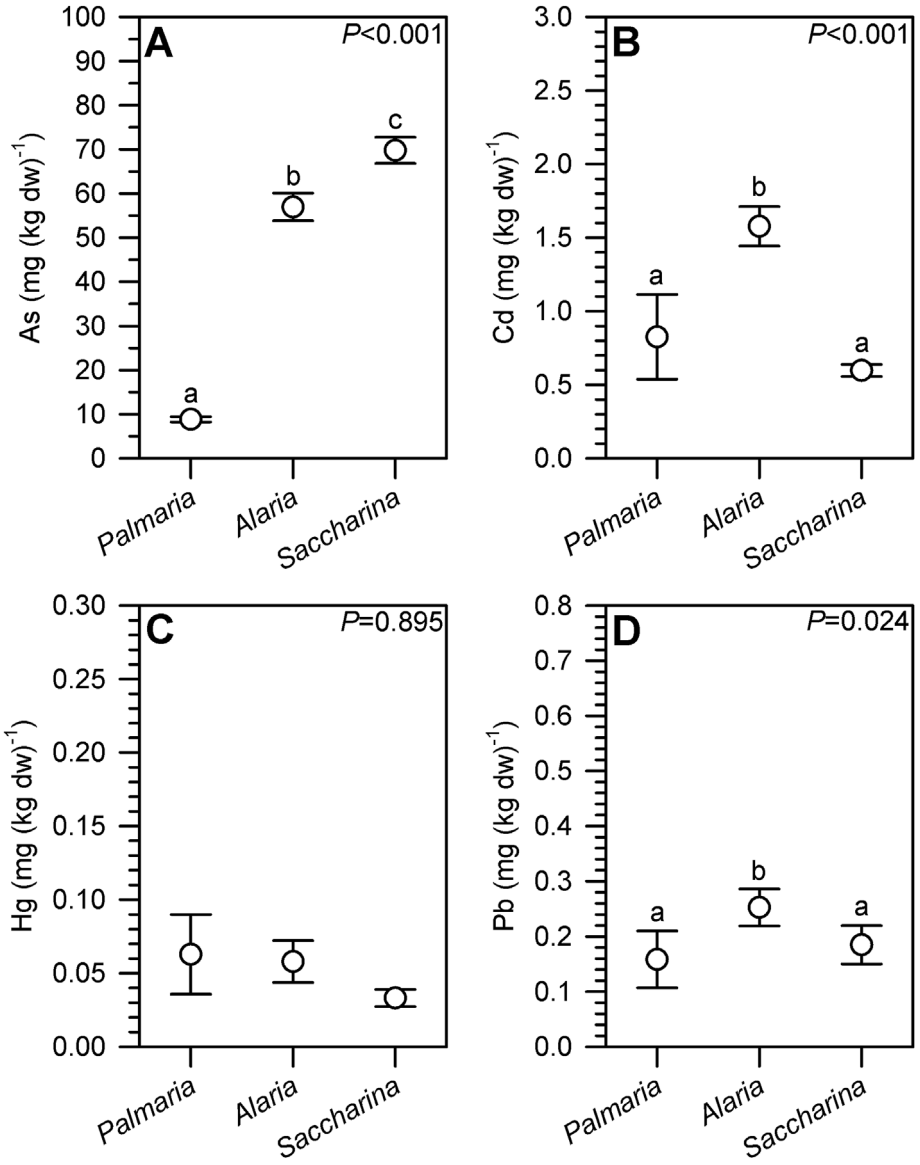

Fig. 3. Variations in heavy metal contents in dried bulk biomass of the three edible seaweed species (*Palmaria palmata*, *Alaria esculenta*, *Saccharina latissima*). Data are means ± S.E.; data are given in Table S1 (Supplementary material). Statistical results are summarized in Table 1.

spring upon exposure to longer daylength and higher irradiances. On the other hand, abiotic factors are particularly stressful for many intertidal red algae during summer; this can lead to oxidative stress, which may contributes to bleaching and low concentrations of polyphenols. Considering that *Palmaria* has approximately 50% and 90% less polyphenols than *Saccharina* and *Alaria*, respectively (Fig. 1). this species may have other antioxidant systems scavenging ROS, e.g. mycosporine-like amino acids (MAAs) (Wada, Sakamoto, & Matsugo, 2015) and enzymatic antioxidants such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX) and peroxiredoxin (PrxR) (Mittler, 2002), to survive environmental stressors in the intertidal zone.

4.2. Variations in heavy metals

The species-specific difference in the accumulation of some heavy metals (e.g. As, Cd, and Pb) is mostly related to their cell wall chemistry. The main chemical groups involved in the biosorption of metallic cation are carboxyl, amino, sulfhydryl, and sulfonate, which are part of the algal cell wall polysaccharides (e.g. alginic acid, sulfated polysaccharides), proteins, and peptidoglycans. The functional groups in algal biomass containing carboxyl have higher affinity with heavy metals than those with sulphate (Güven et al., 1995). The mechanism of mercury adsorption is related to carboxylate groups in the biomass (Carro, Barriada, Herrero, & de Vicente, 2011) while chelation is the main mechanism of the algal biomass to sequester cadmium cation. Lead binding mechanisms include a combination of ion exchange, chelation, and reduction reactions, accompanied by metallic lead precipitation on the cell wall matrix (Raiza, Argaman, & Yannai, 2004). Arsenic uptake depends on adsorption and metabolism-dependent active uptake (Lomax et al., 2011).

Heavy metal concentration in dried commercially available seaweeds intended for human consumption is largely species-specific (Almela et al., 2006; Besada et al., 2009; Khan et al., 2015). The inorganic Arsenic (i-As), which is of major health concern, is generally low in most seaweeds investigated except in brown seaweed species of the family Sargassaceae in the order Fucales (Almela et al., 2006; Khan et al., 2015; Rose et al., 2007): these are *Hizikia fusiformis* (= *Sargassum fusiforme*) and *Sargassum fulvellum* where i-As was found to be 47–80% and 97% of the total Arsenic (t-As), respectively. Other than these

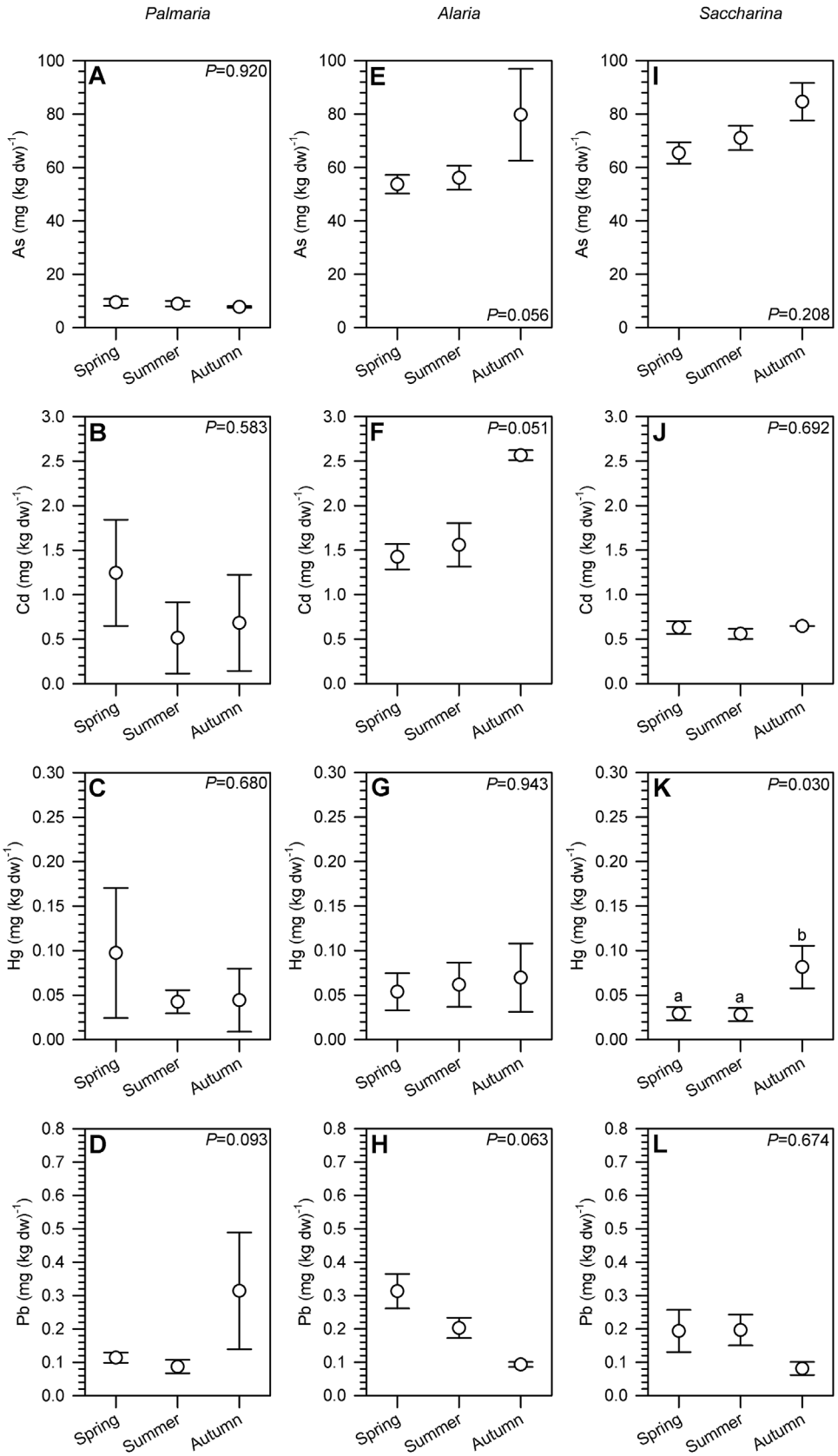

Fig. 4. Seasonal variations in heavy metal contents in dried bulk biomass of the three edible seaweed species (*Palmaria palmata*, *Alaria esculenta*, *Saccharina latissima*). Data are means ± S.E.; data are given in Table S2, S3, and S4 (Supplementary material). Statistical results are summarized in Tables 2–4.

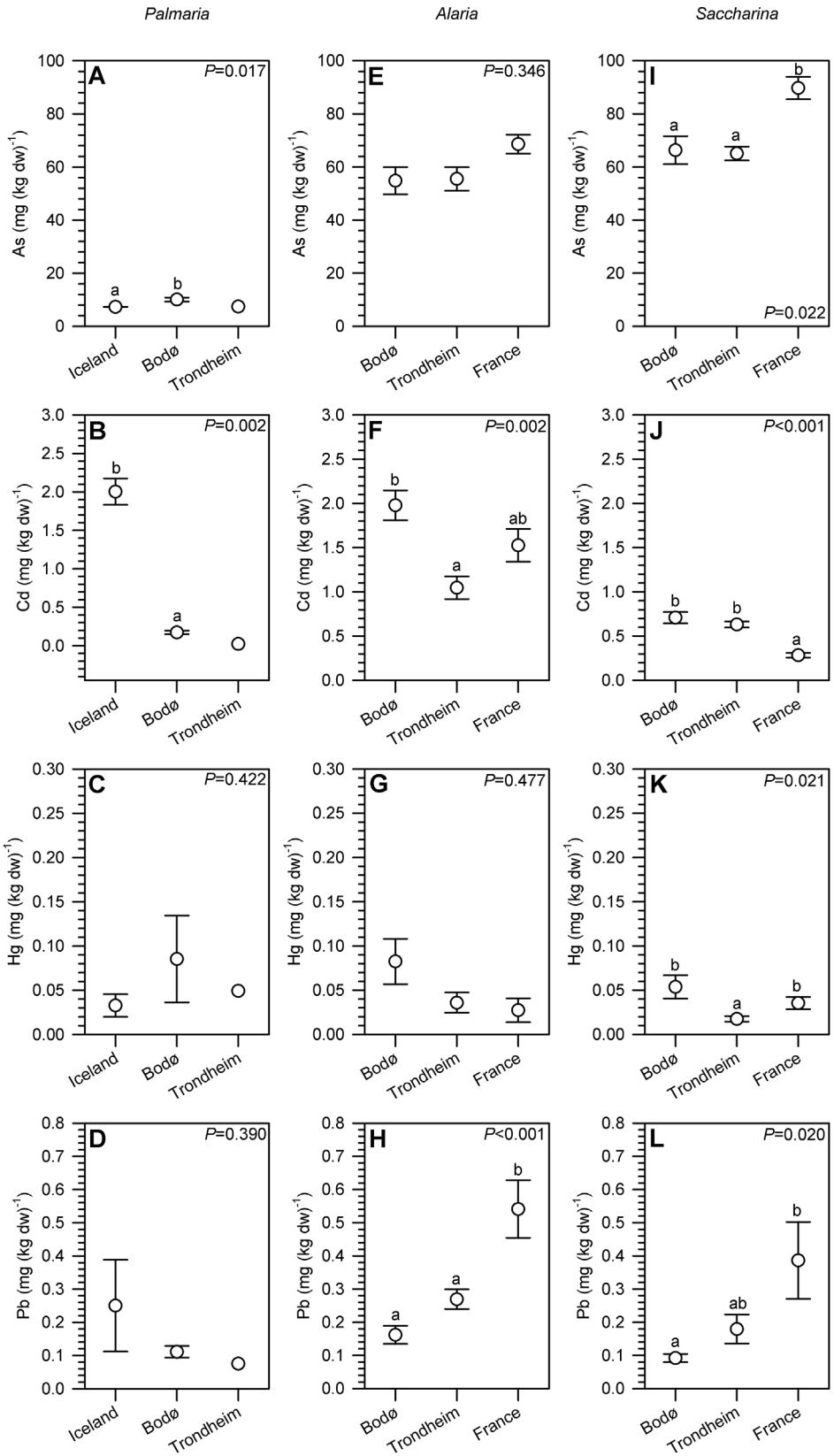

Fig. 5. Spatial variations in heavy metal contents in dried bulk biomass of the three edible seaweed species (*Palmaria palmata*, *Alaria esculenta*, *Saccharina latissima*). Data are means ± S.E.; data are given in Table S2, S3, and S4 (Supplementary material). Statistical results are summarized in Tables 2–4.

extremely high i-As-containing species, green, red and brown seaweeds generally contain on average i-As content of 13, 2.7, 1.6% of the t-As, respectively (computed from data presented by Rose et al., 2007; Almela et al., 2006; Díaz et al., 2012; Khan et al., 2015).

Despite the high As concentration in species of the genus *Sargassum* (=*Hizikia*) (Almela et al., 2006; Khan et al., 2015; Yokoi & Konomi,

2012), washing and soaking before cooking can reduce t-As concentration by 60% (Hanaoka et al., 2001). After digestion, t-As in cooked hijiki (*Sargassum fusiforme*) is mostly excreted and only about 5% of the administered dose is accumulated in mice (Ichikawa et al., 2010).

Different species also exhibit different affinities to heavy metals; for example, affinity towards heavy metal of the brown seaweed *Fucus vesiculosus* is ranked as: Hg > Pb > Cd (Henriques et al., 2017a) while green seaweed *Ulva lactuca* is ranked as: Hg > Cd > Pb (Henriques et al., 2017b). Moreover, heavy metal concentration varied among different kelp species– As: *L. digitata* [96 mg (kg dw)$^{-1}$] > *Alaria* [75 mg (kg dw)$^{-1}$] = *L. hyperborea* [73 mg (kg dw)$^{-1}$] ≥ *Saccharina* [67 mg (kg dw)$^{-1}$]; Pb: *Saccharina* [1.2 mg (kg dw)$^{-1}$] = *Alaria* [1.1 mg (kg dw)$^{-1}$] > *L. digitata* [0.34 mg (kg dw)$^{-1}$] ≥ *L. hyperborea* [0.26 mg (kg dw)$^{-1}$] (Schiener et al., 2015). Our study addressed heavy metal concentrations in biogeographically widely distributed species and we show a different trend in the accumulation of heavy metals: t-As: *Saccharina* [70 mg (kg dw)$^{-1}$] > *Alaria* [57 mg (kg dw)$^{-1}$]; Pb: *Alaria* [0.25 mg (kg dw)$^{-1}$] > *Saccharina* [0.18 mg (kg dw)$^{-1}$]) compared to the study by Schiener et al. (2015), which examined a specific site in Scotland.

The study in Scotland also showed monthly variation in As and Pb among different kelp species, however, the variation is largely random rather than showing a clear seasonal trend (Schiener et al., 2015). We did not observed significant seasonal variation in heavy metal concentration except for higher Hg during autumn in *Saccharina*. Variation in heavy metal concentration was mostly related to location. Interestingly, brown seaweed inhabiting areas, which are contaminated with heavy metals contained higher cell wall polysaccharides, which served as a protective mechanism against toxicity (Andrade et al., 2010).

The mean heavy metal content of *Palmaria* (t-As: 8.8 mg (kg dw)$^{-1}$; Pb: 0.826 mg (kg dw)$^{-1}$; Cd: 0.158 mg (kg dw)$^{-1}$) is in the lower limit range compared to those reported by Almela et al. (2006) in commercial seaweed from Spain and Japan (t-As: 12.6–13.0 mg (kg dw)$^{-1}$; Pb: 1.52 mg (kg dw)$^{-1}$; Cd: 0.147–0.877 mg (kg dw)$^{-1}$). The same is true in terms of the mean heavy metal concentrations of the two kelp species (*Alaria*, t-As: 57 mg (kg dw)$^{-1}$; Pb: 0.253 mg (kg dw)$^{-1}$; Cd: 1.577 mg (kg dw)$^{-1}$ and *Saccharina*, t-As: 70 mg (kg dw)$^{-1}$; Pb: 0.185 mg (kg dw)$^{-1}$; Cd: 0.598 mg (kg dw)$^{-1}$) when compared to other kelp species of the order Laminariales (t-As: 4–116 mg (kg dw)$^{-1}$; Pb: 1.10–2.44 mg (kg dw)$^{-1}$; Cd: 0.074–2.15 mg (kg dw)$^{-1}$) sold as products from Japan, Korea, and Spain (Almela et al., 2006). In this regard, the seaweed biomass harvested in this large-scale study can be considered safe for consumption and as source of molecules for food, feed, and nutraceutical applications.

In the environment, heavy metals originate from various sources, which can be both of natural or anthropogenic origin. Rock weathering and soil formation are the primary natural sources of heavy metals and average natural contents vary between rock types. For example, higher Pb can be measured in granite (17 ppm) compared to and basalt (7 ppm) rock; conversely, higher Cd and Hg is found in basalt (0.21 ppm and 0.09 ppb, respectively) compared to granite (0.13 ppm and 0.03 ppb, respectively), while As is comparable at 2.2 and 2.0 ppm, respectively (Bradl, 2005). Consequently, heavy metal composition and concentrations in water bodies are influenced by rock and sediment type. On the other hand, the principal natural sources of trace metals in the atmosphere are wind-borne, volcanoes, seasalt spray and wild forest fires (Nriagu, 1989). Industrial sources include emissions from transportation, coal combustion, and fugitive particulate emissions (Bradl, 2005). The substances released into the air can be diluted by prevailing air currents, precipitated, or transformed by chemical reactions on their way to the immission location, e.g. the marine environment (Bradl, 2005). The species-specific and site dependent heavy metal concentration observed in Fig. 5 may be related to one or a combination of several factors above. However, we are not able to pinpoint a specific factor that could have contributed to the higher concentration of specific heavy metal in a species as we did not analyze the type of substrate, and the heavy metal concentrations in the seawater, sediment, and atmosphere in each collection site. Moreover, the accumulation of specific heavy metal is also dependent on the adsorption capacity, active-uptake mechanism, and the cell wall chemistry of the seaweed species. For example, a biomonitoring study in the east coast of USA showed that the heavy metal concentrations (e.g. Pb and As) present in the sediment and seawater among different sites are not correlated to the heavy metal concentrations accumulated in different seaweed species e.g. the green *Ulva lactuca* and *Enteromorpha intestinalis* [= *Ulva intestinalis*], and the brown *Fucus vesiculosus* (Chaudhuri, Mitra, Havrilla, Waguespack, & Schwarz, 2007). Moreover, we are tempted to speculate that the higher Cd content in *Palmaria* from Iceland may be related to the volcanism of the oceanic island. However, this hypothesis needs to be validated.

4.3. Regulations

France was the first European country to establish a specific regulation concerning the use of seaweeds for human consumption as non–traditional food substance. In addition, the EU set maximum levels (ML) for different heavy metals in different seafood. ML for Cd is set at 0.05–0.25 mg (kg ww)$^{-1}$ for fish, 1.0 mg (kg ww)$^{-1}$ for different mollusks (including bivalves and cephalopods), and 0.05 mg (kg ww)$^{-1}$ for seaweeds (OJEU L138/75, 2014). ML for Pb is set at 0.30 mg (kg ww)$^{-1}$ for fish and seaweeds, and 1.5 mg (kg ww)$^{-1}$ for different mollusk (OJEU L161/9, 2015) and for Hg for fisheries products in general is set at 0.5–1.0 mg (kg ww)$^{-1}$ (OJEU L364/5, 2006). At present, there is no specified maximum level of As in seafood. The EU recommends member states to monitoring on the presence of As in food during the years 2016, 2017 and 2018 (OJEU L213/9, 2015). The monitoring should include a wide variety of foodstuffs reflecting consumption habits (including fish and seafood) in order to enable an accurate estimation of exposure. The EFSA scientific opinion further recommends that speciation data for different food commodities should be generated to support dietary exposure assessment in order to refine the risk assessment of i-As (OJEU L213/9, 2015). In general, these recommendations apply very conservative ML for seaweeds in comparison to fish and invertebrates. Recently, the EU recommends member states, in collaboration with food and feed business operators, to monitor the presence of As, Cd, Pb, As, Hg and iodine in seaweed, halophytes and products based on seaweed during the years 2018, 2019 and 2020; data will be used to assess dietary exposure (OJEU L78/16, 2018).

4.4. Seaweed safety: health risk assessment

Seaweed consumption is generally considered safe: humans consume large quantities and number of species since centuries. Except for Cd, the mean levels of heavy metals investigated (i.e. t-As, Pb, and Hg) in the three edible seaweed species were below the maximum recommended by CSHPF and Afssa (Table 5). However, health risks in eating heavy-metal contaminated seaweeds can occur when (1) large quantity of seaweeds are consumed at once, and (2) when small quantities of seaweeds are consumed over prolonged periods of time, even if the heavy metal concentration is low or below toxicity levels.

In the absence of European seaweed consumption data, we used a very conservative approach to calculate exposure dose by assuming that Europeans would ingest similar quantities of seaweed as a Chinese consume on average (5.2 g/adult/day), which is higher than that Japanese consume (4 g/adult/day; Zava & Zava, 2011) and lower than average seaweed consumption in South Korean (8.5 g/adult/day; Hwang, Park, Park, Choi, & Kim, 2010). The daily seaweed consumption of Europeans are probably significantly (> 50%) lower than the daily seaweed consumption in China. According to our findings, the t-As, Pb, Cd, and Hg contents of the three edible seaweeds investigated

do not pose a significant health risk to humans (Table 6).

Heavy metals and organic compounds may be accumulated by seaweeds, although our study showed minimal accumulation of heavy metals in seaweeds harvested from non-polluted European waters. High i-As-containing seaweed should be avoided or adequately processed and cooked to eliminate excess i-As. In fact, exposure to heavy metals, e.g. high dietary methylmercury (MeHg), in island and coastal inhabitants comes from eating fish and whale (Dewailly et al., 2008) rather than consuming seaweed.

Surprisingly, the deaths (11–14) and illnesses cases (73) reported from eating seaweeds were not due to heavy metals contamination but due prostaglandin E − 2, a physiologically active lipid, produced by the seaweed species of the genus *Gracilaria* (Cheney, 2016; Hsu, Tsao, Chiou, Hwang, & Hwang, 2007) or the lethal toxins synthesized by epiphytes associated with edible seaweeds species (Cheney, 2016; Haddock & Cruz, 1991; Yotsu-Yamashita et al., 2004). The marine cyanobacterium *Okeania* sp., which synthesizes a lethal toxin polycavernoside can be associated with the tropical edible red seaweeds *Acanthophora specifera* and *Gracilaria edulis* (Navarro et al., 2015; Yotsu-Yamashita et al., 2004). In this regard, consuming seaweeds contaminated with toxic cyanobacteria can expose humans to risks higher than due to heavy metal contamination. This suggest that raw algal material should be pretreated prior to consumption, regardless of species, origin and harvesting time (e.g. Stévant et al., 2018).

5. Conclusion

This is the first long-term investigation into the quality of the three edible seaweed species in the North Atlantic. The distinctive species-specific and seasonal variation in the antioxidant systems between the red and brown seaweeds is likely related to their habitat and exposure to different environmental stressors, and taxonomic (and genetic) differences. Species-specific differences in some heavy metal concentrations can be attributed to the adsorption capacity, uptake mechanism, and the cell wall chemistry. Spatial variation may be associated with heavy metal concentrations at harvesting or cultivation location; this is likely dependent on several natural and anthropogenic factors. Heavy metal concentrations in *Alaria*, *Saccharina* and *Palmaria* were below the French (CSHPF and Afssa) recommendation. A health risk assessment showed that there is low health risk for heavy metals by intake of these seaweed species. However, an EU-wide regulation on maximal concentration of heavy metals in seaweeds should be set-up.

Disclosure

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.foodcont.2018.07.031.

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