Heavy metals and potential dangers in edible seaweed on the market in Italy

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HIGHLIGHTS

- Analysis of 20 metals was performed by ICP-MS.
- Red seaweeds showed higher contents of metals and elements (especially *Porphyra*).
- Seaweeds are a source of essential dietary elements, particularly Mg and Fe.
- Found irregularities on the label of seaweeds about the language and allergens.
- Results obtained from the speciation of arsenic: Phaeophyta had the highest content.
- Brown seaweeds showed remarkably high values of iodine.

Keywords: seaweed, metal, IPC-MS, arsenic, iodine, label

ABSTRACT

The seaweed food has always been important in Asia, but recently increased in the Western diet. *Superfood* known for health benefits and rich in essential elements, can also accumulate high contents of heavy metals and iodine from the environment, becoming a health hazard. In particular for iodine, an appropriate labelling of seaweed is needed to warn the consumer of the potential danger. The aim of the study was to analyse the content of 20 heavy metals in seaweeds, distributed in Italy, by ICP-MS, also determining iodine and arsenic (total and inorganic fraction).

A total of 72 samples of European and Asian seaweed of 8 genera were purchased and the results correlated to the content of heavy metals to the genus, geographical origin and type of sample; 8.33% of the products lacked in the label of the indications of allergens, while 9.72% had irregularities in the label language.

The highest concentration of elements was found in the Rhodophyta. The Aluminum level was the highest in the mixed seaweed (165.39 mg/kg) and for the Cadmium in the Asian seaweed (1.16 mg/kg). The amounts of Iron, Zinc and Magnesium, was highest in the Asian seaweed. The values of Arsenic (total and inorganic contents) were compared with the limits: 2.78% exceeds France and USA limits for inorganic, while higher content of total was found in Phaeophyta, which also showed the highest Iodine content (6770.80 mg/kg) that can be dangerous if not reported correctly in the label.

1. Introduction

Seaweeds have been consumed since ancient times in the Asian countries, but their use has also increased in the European market. In fact, the seaweed production and market are growing strongly: the data relating to the annual world production amounted to 30 million tons per year in 2016 (FAO, 2018).

Their nutritional characteristics and beneficial properties give these *sea vegetables* the qualities of novel food, a functional food legally settled by Regulation (EU) 2015/2283.

In addition, this food has low quantity of calories that can play a role in the body weight control, but also in the prevention of gastrointestinal and cardiovascular diseases and anti-hypercholesterolemic and anti-hypertensive activities (Arulkumar et al., 2019). Studies also showed that a seaweed rich diet is associated with a low incidence of tumors (Cian et al., 2015), showing anti-tumor activities (Arulkumar et al., 2019). In addition, seaweeds can be bioindicators of marine pollution due to the ability to concentrate contaminants (Paz et al., 2019; Shams El-Din et al., 2014).

Seaweeds represented a source of essential trace elements Mg, Cu, Fe, Zn, macronutrients with the role of coenzymes, minerals, antioxidants and vitamins.

But at the same time the consumption of some species of seaweed had been associated with risks, such as toxicity due to high levels of iodine or toxic heavy metals (Pb, Cd, Hg, As, Al) (Bouga et al., 2015) because of the capacity of the seaweed to accumulate them from the environment.

In fact, heavy metals are introduced from anthropogenic sources into the marine environment and they rapidly accumulate in seaweed at high concentrations. In the end, they are introduced in the chain food (Chen et al., 2018).

Iodine is essential for human health, it may be therapeutic in case of nutritional deficiencies, but high values can cause adverse health effects if the consumption is excessive (Banach et al., 2019).

In this scene, a greater transparence in labelling is needed to improve communication to the consumer on the clarity of the species, origin and processing of seaweeds. Attention should be paid to products on the market where edible algae are sold at retail, by checking the labelling and indications given on the iodine content, to detect any anomalies or non-conformities. In addition, another factor that should raise our attention to the safety is the sale in unofficial channels, where seaweeds are sold or cooked. These parallel circuits can represent a risk factor for these functional foods (Prakash et al., 2016).

The present study analysed 72 food products with seaweed for human consumption with the aim to conduct an examination of the labelling and to quantify the content of 20 heavy metals (^{127}I, ^{208}Pb, ^{111}Cd, ^{52}Cr, ^{202}Hg, ^{27}Al, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{66}Zn, ^{78}Se, ^{98}Mo, ^{107}Ag, ^{205}Tl, ^{238}U, ^{123}Sb, ^{51}V, ^{75}As) with the speciation of the metalloid As: total and inorganic arsenic (i-As).

Successively, the concentration of metals analysed by ICP-MS (inductively coupled plasma mass spectrometry) was compared with the origin, the taxonomy (phylum and genera) and the type of sample.

The compliance was compared to Regulation 1169/2011, the main law about the food labelling in the EU, which reports the obligation to indicates the main allergens and to highlights them on the label, including crustaceans, fish and molluscs. Moreover the regulatory references about the law limits on metals were compared, although the scenario is not so well defined.

2 Material and methods

2.1. Sampling

A total of 72 samples were collected (10 were dehydrated) and analysed over a period of 12 months: from December 2017 to December 2018.

The samples were from the groups: brown seaweed (*Himanthalia* *n* = 8, *Saccharina* *n* = 15, *Undaria* *n* = 10, *Ascophyllum* *n* = 1, *Laminaria* *n* = 1), red seaweed (*Porphyra* *n* = 13, *Palmaria* *n* = 9), green seaweed (*Ulva* *n* = 10) and mixed seaweed (*n* = 5).

The samples were purchased in Italy in large-scale retailers, in small ethnic markets and in supermarkets and stored away from light and heat.

The regions of production were different: from China and Korea (Asia) and from France, Spain, Germany and Belarus (Europe). French algae and semi-preserved samples were stored at fridge temperature (5 ± 3°C), while the other products (dried and roasted) were kept at room temperature (20 ± 2°C).

The analysed samples had different types of package: fresh and salted (*n* = 44), fresh (*n* = 1), dried (*n* = 7), roasted (*n* = 11), dried, roasted and salted (*n* = 4) and semi-preserved (*n* = 5).

2.2. Sample treatment and analysis

To 3-5 g of exactly weighted algae, 1-2 mL of distilled water was added in order to moisturize the sample. The samples are then subjected to a wet mineralization process by addition of 10 mL of concentrated nitric acid and heating at 75 ± 10 °C. The sample is taken to the volume of 20 ml with demineralized water and shacked vigorously. Part of the content was poured into 15 ml polystyrene tubes then 1 mL of the obtained solution was diluted to 10 mL with a dilution solution (an aqueous solution of 2% nitric acid with 0.5% hydrochloric acid). For each series of analyses, a reagent blank was mineralized as the samples. In parallel with the evaluations, a reference sample is measured by ICP-MS in order to confirm that the results obtained with the study samples are the expected ones, respecting the measurement uncertainty ranges. The analysis of the mineral components of the sample was performed using an Agilent 7700 inductively coupled plasma mass spectrometer (ICP-MS) equipped with an ASX-500 Series auto sampler. A calibration curve from 0,01 to 100 µg/ml was analyzed for each series of analyses: the correlation coefficient was be equal to or greater than 0.999 for each element subjected to analysis. A mixture of internal standards (each one having a concentration of 1 mg/mL) was infused continuously by a second way of entrance in ICP MS to quantify the samples. The limit of detection (LoD) was set at 0,002 mg/kg and the limit of determination (LoQ) was set at 0,005 mg/kg for all matrices. At the beginning of each measurement cycle, a tuning operation was carried out with a multi-element mixture to check the accuracy of the identification of the m/z ratio values and the accuracy of the instrument.

2.3. Statistical analysis

Shapiro-Wilk test was used to test for normality of distribution of the continuous variables. Continuous data were represented with mean and Standard Deviation (SD) and categorical data were expressed as total numbers and percentages. Samples were grouped for analysis according to the origin (Asia vs Europa), group (brown vs red vs green), genera and type of package of the seaweed.

In the case of non-normal distribution, Mann Whitney U test was used to compare differences between two independent groups whereas the Kruskal–Wallis test was used for comparing two or more independent samples. All data present in table are reported as mean and standard deviation standards, but p-values refer to non-parametric tests. The choice to present average and non-median values is due to the comparability with the results of other studies. All analyses were performed using IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. A p-value <0.05 was defined as statistically significant.

3. Results and discussion

3.1. Content of heavy metals in the analysed seaweed species

The samples were analyzed to determine the metal content and the results are shown in Table 1 as the average concentrations (mg/kg) and standard deviations for each of the 20 metals in the seaweeds according to the origin: Europe and Asia.

Elements in European seaweeds can be sequenced in descending order by mean values: I > Fe > Al > Zn > Mn > As total > V > Cu > i-As > Ni > Pb > Cr > Cd > Mo > U > Co > Se > Ag > Hg > Tl > Sb.

As for the Asian samples, the elements were ranked in this order: I > Fe > Zn > Mn > As total > Al > Cu > i-As > Cd > Cr > V > Ni > Mo > U > Pb > Co > Ag > Se > Sb > Hg > Tl.

As regards toxic metals, the average contents of Al in European seaweed reached the value of 22.95 mg/kg and 9.16 mg/kg in the Asian samples and is greater than the value reported by Rubio et al. (2017). Next, the Cd values reached in the Asian seaweeds were higher (1.16 mg/kg) than the concentration of 0.09 mg/kg in the European samples; this time, value reported by the Asian samples is higher than the value found by Rubio et al. (2017) of 0.44 mg/kg.

Table 1

Mean content ± Standard Deviation (SD) of metals in seaweed samples according to their origin (mg/kg).

Element	**Origin**	
	Europe (*n* = 53)	**Asia** (*n* = 19)
I	106.62 ± 198.78	392.22 ± 1546.56
Pb	0.18 ± 0.29	0.15 ± 0.26
Cd*	0.09 ± 0.24	1.16 ± 1.27
Cr*	0.15 ± 0.13	0.58 ± 2.13
Hg	0.01 ± 0.00	0.01 ± 0.01
Al	22.95 ± 64.33	9.16 ± 9.13
Mn*	4.05 ± 10.07	11.34 ± 12.22
Fe	43.64 ± 77.69	86.87 ± 108.37
Co	0.03 ± 0.05	0.10 ± 0.09
Ni	0.40 ± 0.37	0.33 ± 0.31
Cu*	0.52 ± 0.90	3.89 ± 3.91
Zn*	5.19 ± 8.87	21.35 ± 23.70
Se	0.03 ± 0.04	0.06 ± 0.06
Mo*	0.07 ± 0.08	0.22 ± 0.22
Ag*	0.02 ± 0.02	0.07 ± 0.07
Tl	0.01 ± 0.00	0.01 ± 0.00
U*	0.05 ± 0.05	0.16 ± 0.12

Sb	0.01 ± 0.00	0.01 ± 0.01
V	1.08 ± 2.79	0.51 ± 1.03
Total As*	3.97 ± 4.05	11.22 ± 12.28
Inorganic As	0.51 ± 0.64	1.68 ± 2.21

*$p < 0.05$

The data presented in Table 2 show the average values of the 20 metals in the seaweed according to the taxonomic groups: brown, red and green respectively.

Green seaweeds had the highest content of the Iron macroelements (78.62 mg/kg), while red seaweed showed the highest content of Zn (18.12 mg/kg) and Mn (9.85 mg/kg), with the exception of Iodine which was higher in the brown one. However, predominance of the Mn occurrence in red seaweed was also reported by other authors (Dawczynski et al., 2007; Van Netten et al., 2000). As for the trace elements, the highest content was found mainly in the red seaweeds, with the exception of the metals Cr, Al, Fe, Ni, Ag which were higher in the green and total As in the brown algae. The levels of Hg detected in green seaweed are below the limit of quantification (0.005 mg/kg).

Statistical analysis revealed significant differences between the three taxonomic groups of seaweed for almost all the studied metals, except for Pb, Hg, Mn, Co, Tl, Sb. ($p < 0.05$) These statistical correlations reflect the same reported in literature (Rubio et al., 2017).

Considering the toxic metals, the average Al contents in the samples reached the level of 5.94 mg/kg in the red seaweed, 7.00 mg/kg in the brown and 34.10 mg/kg in the green.

The values reached for Cd are higher in red seaweed (0.93 mg/kg) than literature (Rubio et al., 2017) but aligned for Pb. Moreover,s differences in Cd, Al, As contents between seaweed groups were significant ($p < 0.05$).

Red seaweed showed the tendency of higher concentrations of elements compared to brown and green algae. The metal content in the seaweeds reflects the mineral composition of the taxonomic group (Makkar et al., 2016). The levels of Cd, Cu and Mn are higher in Rhodophyta than those found in brown and green seaweed, a behaviour found also by other authors (Chen et al., 2018). The content of Iodine is higher in the Phaeophyta than other phyla, reaching the value 354.53 mg/kg. As highlighted in literature (Circuncisão et al., 2018), the Phaeophyceae species accumulate particularly high levels of Iodine.

Table 2

Mean metal content ± Standard Deviation (SD) in seaweed samples by taxonomic group: Phaeophyta (brown), Rhodophyta (red), Chlorophyta (green) (mg/kg).

Element	**Phaeophyta**	**Rhodophyta**	**Chlorophyta**
	Brown (*n* = 35)	**Red** (*n* = 22)	**Green** (*n* = 10)
I*	354.53 ± 1140.24	16.28 ± 16.07	10.66 ± 6.74
Pb	0.12 ± 0.25	0.16 ± 0.26	0.16 ± 0.12
Cd*	0.08 ± 0.22	0.93 ± 1.27	0.02 ± 0.01
Cr*	0.10 ± 0.05	0.09 ± 0.09	0.30 ± 0.18
Hg	0.01 ± 0.01	0.01 ± 0.00	-
Al*	7.00 ± 12.38	5.94 ± 5.48	34.10 ± 39.63
Mn	1.99 ± 1.28	9.85 ± 11.95	1.65 ± 0.73
Fe*	17.10 ± 17.58	72.62 ± 105.03	78.62 ± 64.03
Co	0.03 ± 0.03	0.08 ± 0.09	0.03 ± 0.02
Ni*	0.24 ± 0.26	0.42 ± 0.27	0.51 ± 0.33
Cu*	0.28 ± 0.24	3.21 ± 3.92	0.50 ± 0.19
Zn*	4.27 ± 2.82	18.12 ± 23.20	1.58 ± 0.41
Se*	0.02 ± 0.03	0.06 ± 0.06	0.03 ± 0.04
Mo*	0.06 ± 0.05	0.20 ± 0.20	0.03 ± 0.01
Ag*	0.01 ± 0.00	0.05 ± 0.06	0.07 ± 0.00
Tl	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
U*	0.07 ± 0.07	0.11 ± 0.12	0.01 ± 0.01
Sb	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
V*	0.26 ± 027	1.30 ± 1.53	0.65 ± 0.51
Total As*	6.76 ± 8.22	6.24 ± 8.24	0.62 ± 0.16
Inorganic As*	1.08 ± 1.58	0.75 ± 1.16	0.12 ± 0.09

$^{*}p < 0.05$

Table 3 shows the average of the concentrations and standard deviations of the 20 metals in all the genera of algae and the analysis for each taxonomic group showed that *Porphyra* had the highest content of elements, including Cu (4.96 mg/kg), Mo (0.29 mg/kg) and Zn (28.71 mg/kg).

In general, the accumulation of Fe is high in all the types of seaweed: the highest values were found in *Porphyra* (112.29 mg/kg) and mixed algae (196.19 mg/kg). This may be related to the high photosynthesis rates in coastal habitats typical of tropical climates (Chakraborty et al., 2014).

As for Zn, the *Porphyra* genus reported higher values than other studies (Rubio et al., 2017) reaching 28.71 mg/kg, followed by mixed algae (23.45 mg/kg). This element can accumulate prevalently in red (as seen from our data) and in brown macroalgae (Circuncisão et al., 2018).

The wide range of metal concentrations achieved in the eight different genera reflects the importance of biochemical factors that influence the tendencies of each tissue to accumulate metals.

The values of Co are in line with the tendency of the genera reaching maximum values of 0.18 mg/kg (*Ascophyllum*) and minimum values of 0.01 mg/kg (*Saccharina* and *Palmaria*).

Statistical analysis revealed significant differences ($p < 0.05$) between the eight genera of seaweed for almost all the trace elements except for Pb, Tl, Sb.

As for toxic metals (Al, Cd, Pb, As, Hg), the highest levels of Al were found in mixed algae (165.39 mg/kg), followed by the genus *Laminaria* (33.15 mg/kg) and *Ascophyllum* (29.11 mg/kg), while the lowest value was observed in *Himanthalia* (0.71 mg/kg). As also found by Rubio et al., 2017, the accumulation of Al is highly variable in the eight genera of algae. *Porphyra* showed the highest Cd content (1.56 mg/kg), higher than those found by others (Rubio et al., 2017). While for Pb, the maximum content was found in mixed algae (0.56 mg/kg), followed by the *Porphyra* genera (0.17 mg/kg), the *Saccharina* genus (0.17 mg/kg) and finally by *Ulva* (0.16 mg/kg). Indeed, as it is known from literature, the genus *Ulva* has the ability to accumulate the Pb concentration from 500 to 2200 time, demonstrating high ability to remove heavy metals from the surrounding environment (Henriques et al, 2017) and their role as an environmental bioindicator (Shams El-Din et al., 2014).

Table 3

Mean content ± standard deviation of elements in seaweed samples for different genera of seaweeds (mg/kg).

	Genera	I	Ag	Co	Cr	Cu	Fe	Mn	Mo	Ni	Sb
Brown	*Himanthalia* (*n* = 8)	11.55 ± 8.14	0.01 ± 0.00	0.02 ± 0.00	0.10 ± 0.04	0.20 ± 0.08	6.00 ± 0.93	3.25 ± 0.84	0.03 ± 0.00	0.29 ± 0.27	0.01 ± 0.00
	Saccharina (*n* = 15)	303.94 ± 283.05	-	0.01 ± 0.00	0.08 ± 0.03	0.29 ± 0.28	10.65 ± 4.86	1.54 ± 0.99	0.03 ± 0.01	0.16 ± 0.24	0.01 ± 0.00
	Undaria (*n* = 10)	55.56 ± 62.33	-	0.03 ± 0.02	0.12 ± 0.06	0.29 ± 0.28	28.97 ± 23.39	1.29 ± 0.95	0.08 ± 0.04	0.33 ± 0.29	0.01 ± 0.00
	Ascophyllum (*n* = 1)	430.70	0.01	0.18	0.23	0.59	49.42	4.60	0,27	0.22	0.02
	Laminaria (n = 1)	6770.80	-	0.03	0.13	0.43	51.60	3.14	0,12	0.18	0.02
Red	*Porphyra* (*n* = 13)	12.02 ± 9.30	0.08 ± 0.07	0.12 ± 0.10	0.08 ± 0.10	4.96 ± 4.11	112.29 ± 122.82	15.60 ± 12.69	0.29 ± 0.23	0.34 ± 0.28	0.01 ± 0.01
	Palmaria (*n* = 9)	22.43 ± 21.82	0.02 ± 0.00	0.01 ± 0.00	0.11 ± 0.06	0.36 ± 0.07	15.32 ± 8.88	1.54 ± 1.21	0.06 ± 0.03	0.52 ± 0.25	0.01 ± 0.00
Green	*Ulva* (*n* = 10)	10.66 ± 6.74	0.07	0.03 ± 0.02	0.30 ± 0.18	0.50 ± 0.19	78.62 ± 64.03	1.65 ± 0.73	0.03 ± 0.01	0.51 ± 0.33	0.01 ± 0.00
Mixed	(*n* = 5)	45.90 ± 38.40	0.07 ± 0.01	0.15 ± 0.09	2.07 ± 3.93	3.62 ± 2.14	196.19 ± 165.77	25.48 ± 25.85	0.20 ± 0.17	0.91 ± 0.64	0.01 ± 0.00

	Genera	Se	Tl	U	V	Zn	Al	Cd	Hg	Pb	Total As	Inorganic As
Brown	*Himanthalia* (*n* = 8)	0.02 ± 0.03	-	0.11 ± 0.02	0.52 ± 0.16	3.94 ± 0.73	0.71 ± 0.52	0.07 ± 0.01	-	0.06 ± 0.03	4.58 ± 0.52	0.39 ± 0.51
	Saccharina (*n* = 15)	0.02 ± 0.04	-	0.03 ± 0.01	0.13 ± 0.08	3.52 ± 1.50	2.85 ± 1.69	0.04 ± 0.01	0.01 ± 0.00	0.17 ± 0.39	5.18 ± 4.05	0.87 ± 0.88
	Undaria (*n* = 10)	0.02 ± 0.03	-	0.07 ± 0.10	0.09 ± 0.07	4.98 ± 4.54	13.43 ± 18.28	0.16 ± 0.40	0.02	0.10 ± 0.07	6.73 ± 8.09	1.37 ± 1.64
	Ascophyllum (*n* = 1)	0.02	-	0.14	0.70	10.07	29.11	0.03	0.01	0.11	11.05	0.09
	Laminaria (n = 1)	0.03	-	0.22	1.22	5.16	33.15	0.21	0.03	0.11	43.90	7.14
Red	*Porphyra* (*n* = 13)	0.07 ± 0.07	-	0.17 ± 0.12	0.61 ± 1.21	28.71 ± 25.37	7.22 ± 6.05	1.56 ± 1.34	0.01 ± 0.00	0.17 ± 0.30	10.01 ± 9.01	1.27 ± 1.37
	Palmaria (*n* = 9)	0.04 ± 0.05	-	0.01 ± 0.00	2.31 ± 1.43	2.82 ± 1.36	4.23 ± 4.35	0.02 ± 0.01	-	0.14 ± 0.21	0.79 ± 0.35	0.09 ± 0.05
Green	*Ulva* (*n* = 10)	0.03 ± 0.04	-	0.01 ± 0.01	0.65 ± 0.51	1.58 ± 0.41	34.10 ± 39.63	0.02 ± 0.01	-	0.16 ± 0.12	0.62 ± 0.16	0.12 ± 0.09
Mixed	(*n* = 5)	0.07 ± 0.04	0.01 ± 0.01	0.12 ± 0.08	4.56 ± 8.49	23.45 ± 22.82	165.39 ± 184.80	0.60 ± 0.59	0.01 ± 0.00	0.56 ± 0.47	8.76 ± 7.59	0.35 ± 0.40

The group with one sample was excluded from this analysis.

Table 4.

Samples grouped according to the country of origin and comparison between the minimum and maximum values reached for each metal (mg/kg).

Metal	France (*n* = 45)	Belarus (*n* = 4)	Spain (*n* = 3)	Germany (*n* = 1)	Korea (*n* = 11)	China (*n* = 8)
I	2 - 867.1	6.4 – 12.5	58.8 – 61.3	10.80	1.3 – 6770.8	2.4 – 33.7
Pb	0.029 – 1.56	0.036 – 0.061	0.701 – 1.149	0.07	0.007 – 0.27	0.035 – 1.15
Cd	0.006 – 0.09	0.029 – 0.046	0.376 -1.275	0.05	0.042 – 1.31	0.19 – 3.77
Cr	0.037 – 0.57	0.075 – 0.11	0.329 – 0.571	0.09	0.006 – 9.09	0.019 – 0.38
Hg	-	-	0.011 – 0.017	0.01	0.016 – 0.026	0.005 – 0.011
Al	0.007 – 110.91	1.69 – 3.04	5.313 – 331.891	3.18	0.58 – 33.15	1.066 – 20.40
Mn	0.40 – 4.81	2.40 – 3.34	44.01 – 59.874	2.41	0.56 – 15.72	4.39 – 37.39
Fe	4.79 – 195.32	5.22 – 7.45	207.011 – 389.58	13.92	4.73 – 61.60	14.46 – 375.04
Co	0.005 – 0.18	0.008 – 0.014	0.085 – 0.24	0.01	0.005 – 0.067	0.025 – 0.261
Ni	0.027 – 1.21	0.039 – 0.069	0.79 – 1.81	0.04	0.008 – 0.89	0.057 – 0.78
Cu	0.078 – 0.88	0.205 -0.298	3.46 – 4.55	0.27	0.28 – 6.12	2.14 – 10.11
Zn	0.96 – 10.07	1.320 – 3.425	15.19 – 49	4.68	1.57 – 16.44	3.21 – 64.45
Se	0.005 – 0.083	-	0.094 – 0.098	-	0.005 – 0.042	0.01 – 0.20
Mo	0.019 – 0.27	-	0.29 – 0.34	0.01	0.005 – 0.12	0.086 – 0.59
Ag	0.005 – 0.026	-	0.058 – 0.081	-	0.005 – 0.071	0.006 – 0.22
Tl	-	-	0.005 – 0.015	-	-	0.005 – 0.006
U	0.005 – 0.14	0.01 – 0.015	0.049 – 0.22	0.05	0.031 – 0.36	0.006 – 0.36
Sb	0.005 – 0.02	-	0.008 – 0.013	-	0.011 – 0.02	0.006 – 0.026
V	0.012 – 4.80	0.012 – 0.033	1.37 – 19.70	0.15	0.04 – 1.22	0.10 – 4.57
Total As	0.37 – 11.05	0.335 – 0.531	7.28 – 18.36	1.13	0.85 – 43.90	2.03 – 24.03
Inorganic As	0.020 – 2.81	0.05 – 0.09	0.005 – 0.94	-	0.07 – 7.14	0.05 – 3.67

Table 4 compares the average contents based on the countries of origin, demonstrating the wide variability.

It emerges that, as regards macro-elements, France and Spain achieved much higher quantities of Al than other countries, reaching values of 110.91 and 331.89 mg/kg respectively. A similar behaviour is also evident for Fe: both France, Spain and China reported high values 195.32, 389.58 and 375.04 mg/kg respectively. In addition, France and Spain reported high values compared to other countries for the macro-elements Mn, Cu and Zn, reaching higher peaks.

Finally, we report the value reached by Korea of 43.90 mg/kg for the total As. The Regulation of the European Commission (EC) Nº 629/2008 (EC, 2008) and the Regulation of the European Commission (EC) N° 488/2014 (EC, 2014) defined the maximum limit only for Cd on algae (3 mg/kg), based on this the 2.78% of the samples exceed this limit ($n = 2$). The regulation did not define a limit for Al and Pb in algae, but the CEVA (Center d'Etude et de valorization des Algues) in France defined limits for toxic metals and set levels for Cd (0.5 mg/kg dw) and for Pb (5 mg/kg dw) in edible French algae. Comparing our results and considering the 0.5 mg/kg limit for the Cd, 11 samples exceeded it (15.28%), and the origin was mainly from China and Spain, but none of the samples exceeded the limit defined for Pb (5 mg/kg).

Table 5 shows the mean concentration of metals compared to the type of samples as it appears at the opening. The results show the Fe content is higher in the roasted (130.78 mg/kg) and in the dried samples (156.42 mg/kg). About the toxic metal Al, it is prevalent in the dried variety (103.04 mg/kg). But on the other hand, the macro-element Zn is higher in the roasted samples (32.88 mg/kg) followed by the dried ones (20.01 mg/kg). Moreover the data found for Iodine are interesting: depending on the type of the sample, the Iodine content is overall higher in the dried samples (1071.87 mg/kg), fresh (430.70 mg/kg) and fresh and salted (112.60 mg/kg). In fact, the preparation and the cooking methods are factors that influence the final Iodine content in food (Teas et al., 2004). Nitschke and Stengel investigated the influence of the process on the Iodine content also in the *Palmaria* genus and found that washing and dehydration only slightly affected the concentrations of iodine and therefore it is recommended as a conservation technique (Nitschke and Stengel, 2016). Statistical analysis revealed significant differences ($p < 0.05$) between the different preparation of the samples of seaweed for all the metals, except for Tl.

Table 5

Mean concentration of metals compared to the type of sample as it appears at the opening (mg/kg). The group with one sample was excluded from this analysis.

Metal	**Roasted** (*n* = 11)	**Dried** (*n* = 7)	**Dried, roasted and salted** (*n* = 4)	**Fresh** (*n* = 1)	**Fresh salted** (*n* = 44)	**Semi-preserved** (*n* = 5)
I	13.54 ± 9.30	1071.87 ± 2514.86	3.90 ± 2.10	430.70	112.60 ± 210.03	10.02 ± 2.25
Pb	0.19 ± 0.33	0.46 ± 0.42	0.03 ± 0.00	0.11	0.14 ± 0.25	0.05 ± 0.01
Cd	1.73 ± 1.37	0.64 ± 0.59	0.34 ± 0.51	0.03	0.03 ± 0.02	0.04 ± 0.01
Cr	0.10 ± 0.11	1.52 ± 3.35	0.03 ± 0.03	0.23	0.14 ± 0.12	0.08 ± 0.04
Hg	0.01 ± 0.00	0.02 ± 0.01	-	0.01	0.01 ± 0.00	0.01 ± 0.00
Al	8.42 ± 5.86	103.04 ± 152.33	2.09 ± 2.48	29.11	11.81 ± 23.77	2.35 ± 0.72
Mn	18.00 ± 12.33	19.14 ± 23.73	1.77 ± 0.93	4.60	1.65 ± 1.10	2.83 ± 0.43
Fe	130.78 ± 125.14	156.42 ± 151.42	9.20 ± 1.89	49.42	29.62 ± 41.53	8.04 ± 3.39
Co	0.15 ± 0.09	0.11 ± 0.09	0.01 ± 0.00	0.18	0.02 ± 0.01	0.01 ± 0.00
Ni	0.34 ± 0.28	0.78 ± 0.59	0.02 ± 0.01	0.22	0.38 ± 0.30	0.05 ± 0.01
Cu	5.71 ± 4.03	2.93 ± 2.10	0.57 ± 0.05	0.59	0.29 ± 0.17	0.26 ± 0.04
Zn	32.88 ± 25.48	20.01 ± 19.72	3.70 ± 1.07	10.07	3.20 ± 1.64	2.72 ± 1.40
Se	0.10 ± 0.06	0.06 ± 0.04	0.01 ± 0.00	0.02	0.03 ± 0.04	0.01 ± 0.00
Mo	0.33 ± 0.23	0.17 ± 0.14	0.06 ± 0.01	0.27	0.05 ± 0.03	0.01 ± 0.00
Ag	0.09 ± 0.07	0.05 ± 0.03	0.02 ± 0.01	0.01	0.01 ± 0.01	-
Tl	0.01 ± 0.00	0.01 ± 0.03	-	-	0.01 ± 0.00	-
U	0.19 ± 0.12	0.16 ± 0.12	0.06 ± 0.03	0.14	0.04 ± 0.04	0.02 ± 0.02
Sb	0.01 ± 0.01	0.01 ± 0.00	-	0.02	0.01 ± 0.00	-
V	0.71 ± 1.30	3.47 ± 7.19	0.06 ± 0.02	0.70	0.77 ± 1.06	0.05 ± 0.06
Total As	11.55 ± 8.97	17.35 ± 14.88	1.28 ± 0.08	11.05	3.55 ± 3.06	0.59 ± 0.31
Inorganic As	1.18 ± 1.33	2.49 ± 3.14	0.07 ± 0.00	0.09	0.54 ± 0.66	0.07 ± 0.03

3.2. Focus on the content of toxic metals: arsenic (total and inorganic) and iodine

It is important to observe the content of Arsenic, considering the peculiarity of seaweed to be the primary accumulators of As in the marine ecosystem and to play an important role in the food chain (Camurati J. et al., 2019).

The Arsenic values reported refer to the content of total As and inorganic As (i-As). Both compounds are classified by IARC (2009) as carcinogenic to humans, belonging to Group 1 (Camurati J. et al., 2019).

Currently there is no regulatory reference who set a limit (Banach et al., 2019) but considering the maximum limit of 1 mg/kg of i-As defined by Australia New Zealand Food Standard Code (FSANZ), 20.83% of the samples (*n* = 15) exceeded this limit, sometimes reaching very high values up to 7.14 mg/kg.

France and USA authorized a maximum of 3 mg/kg dw of i-As in seaweed based products: in this context 2.78% of the samples (*n* = 2) exceeded the threshold of 3 mg/kg (with the values 5.81 and 7.14 mg/kg).

From the comparison between the total As content and the taxonomy, the total As content is higher in the brown Phaeophyta (6.76 mg/kg), followed by the red Rhodophyta (6.24 mg/kg) and finally the green Chlorophyta (0.62 mg/kg). The highest content of As in brown algae had already been found (Circuncisão et al., 2018), as well as the prevalence of As in Phaeophyta (Van Netten et al., 2000; Dawczynski et al., 2007). This correlation is in line with the literature (Desideri et al., 2017), that reported higher total content of As in brown seaweed, followed by red and green.

As for Iodine, seaweeds are rich in iodine and they can be the cause of the potentially excessive introduction of Iodine, (EFSA, 2006a; SCF, 2002) and attention should be paid (Combet et al., 2014). Exposure to excessive doses of Iodine can lead to the formation of goiter, hypothyroidism, hyperthyroidism and iodism in case of chronic exposure (Bouga et al., 2015). ANSES (Agency for Food, Environmental and Occupational Health & Safety) has acknowledged a concern for risk groups: for those with thyroid dysfunction, pregnant or breastfeeding women (ANSES, 2018; EFSA, 2006, 2014). Legislatively, French recommendations indicate a maximum level of iodine in edible seaweed equivalent to 2000 mg/kg dw (AFSSA, 2009; ANSES, 2018; CEVA, 2014).

As shown in our results, the sample of the genus *Laminaria* (kelp) reached an extremely high value of 6770.80 mg/kg, exceeding the limit defined by the French recommendation by almost 3 times. Also Yeh et al. (2014) described samples with high Iodine mean (2524 mg/kg). Moreover, another study on 12 different algae reported the concentrations of iodine in kelp were above 1500 mg/kg (Teas et al., 2004).

Then we report the Iodine content of the Phaeophyta with the high values of 430.70 mg/kg (*Ascophyllum*) and 303.94 mg/kg (*Saccharina*).

This was also found by other authors who reported that brown algae (especially of the genus *Laminaria* and *Saccharina*) are rich in Iodine (Lüning & Mortensen, 2015; Makkar et al ., 2016).

Another study showed a content of Iodine in *Laminaria* and *Saccharina* that reached values of 230-12000 mg/kg (Holdt and Kraan, 2011).

The authors noticed that factors as season, environment, geography, physiology and their phylum can influence the mineral content of the algae (Holdt & Kraan, 2011; Teas et al., 2004).

Some authors have shown that the Iodine content in *S. latissima* varies significantly between the sampling area, locations and biomass sources (Roleda et al., 2018). In particular, the samples of our research of *S. latissima* species had high Iodine contents. Then we had the results of the genera *Undaria* (55.56 mg/kg), mixed (45.90 mg/kg) and *Palmaria* (22.43 mg/kg).

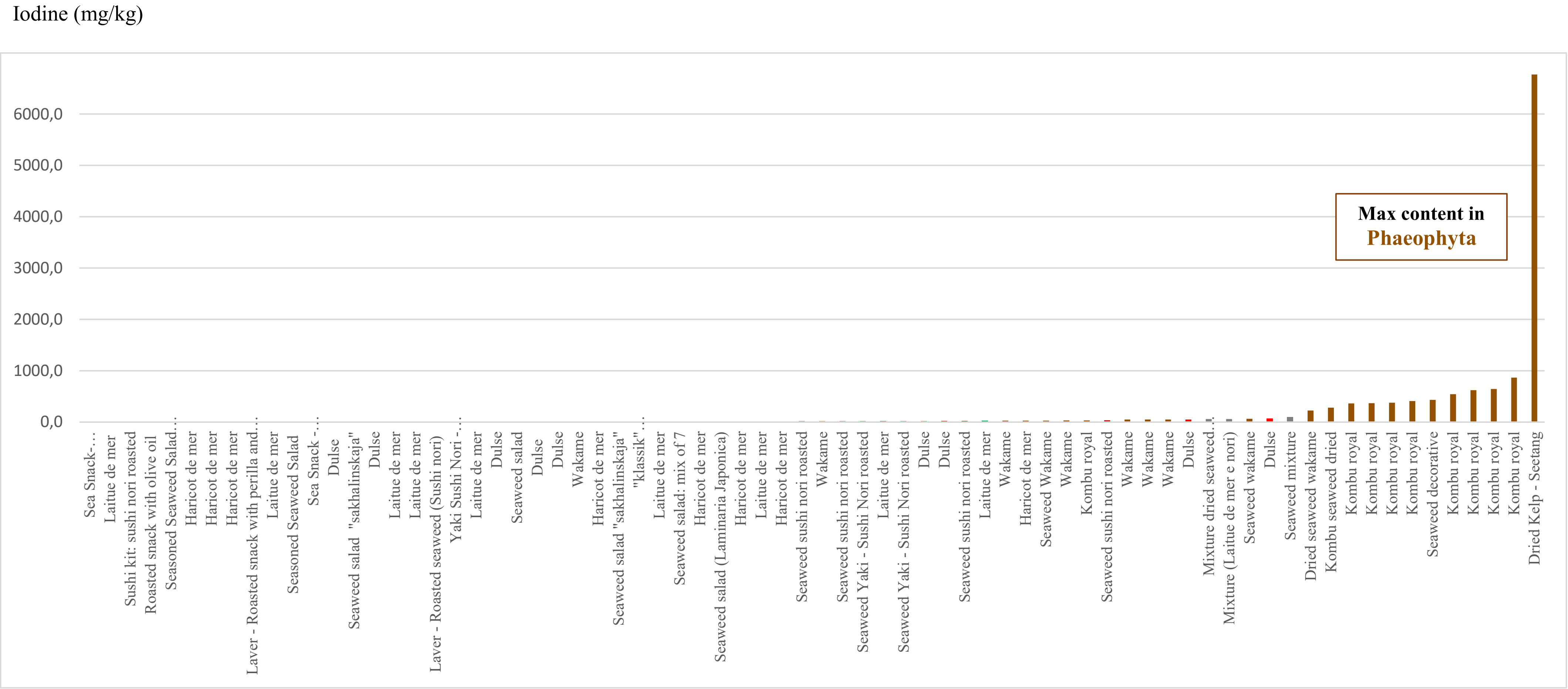
Similarly, to data previously reported (Bouga et al., 2015), we founded that the highest Iodine levels (mg/kg) (Figure 1), belong to the phylum Phaeophyta, followed by Rhodophyta and Chlorophyta.

This is in line with Nitschke & Stengel, who stated that brown algae of the *Laminariales* had Iodine levels of magnitude higher than Rhodophyta and Chlorophyta (Nitschke & Stengel, 2016).

The variability of Iodine observed between species could also be related to differences in Iodine distribution between inorganic and organic forms (Afonso et al., 2018).

Figure 1. Iodine content (mg/kg) of identified seaweed products.

Bar colour indicates the taxonomic group of the seaweeds: 🟩 Chlorophyta (green); 🟥 Rhodophyta (red); 🟫 Phaeophyta (brown)



3.3 Examination of the labels and presence of macro-contaminants

After opening the products, every label was examined with an inspection to verify the correspondence of the species, visible alterations and the possible presence of macro-contaminants. Some contaminants have been found, i.e.: small crustaceans, shells of molluscs and fry, which can represent a potential source of allergens. These allergens are not reported on the label, as required by Annex II of Regulation 1169/2011. In details it was found: a shell of marine gastropod mollusk of the *Gibbula pennanti* species and a crustacean belonging to the order of the Amphipoda, genus *Ericthonius*; a shell of marine gastropod mollusk of the species *Gibbula pennanti*; a crustacean of the order Amphipoda, species *Jassa* cfr *falcata* and a fry of fish of the species *Apletodon* cfr *dentatus*; a Briozoi mass has been found, adhering to the surface of some algae; very small crustaceans Amphipoda, Gammaridea and Isopoda. These potential allergens can be a real danger for consumers, especially for sensitive or allergic people: greater attention should be paid by the producer and the consumer. As for labelling, 8.33% of the products lacked about the indication of allergens in bold. Products from Korea showed various irregularities, mainly related to the language of the label and no indications about allergens. Overall, 9.72% of the products had a non-compliant label language, not following the requirements of Reg. (EU) 1169/2011.

Particular attention must be paid to these products especially the one from non-European origin, because of lack of the mandatory indications relating to allergens and language.

4. Conclusions

The analysis conducted shows the need for a greater attention in the labelling of the seaweed on the market. Most European products are in fact labelled regularly, but irregularities have been found in the language and in lack of allergen indications.

The investigation allowed us to detect the concentrations of heavy metals and correlating them which genus, geographical origin and type of algae, and the results indicate that higher attention must be paid to the necessary correct information to the consumers.

The red seaweeds of the Rhodophyta showed the highest concentration of trace elements and toxic metals, confirming at the same time the importance of seaweed as an essential source of elements introduced with the diet, in particular Fe, Zn and Mn. But the data obtained also demonstrate the importance of monitoring toxic metals in foods where high values of Al, Cd, As and I have been found that can be dangerous for health, suggesting a continuous surveillance in edible seaweeds.

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References

Afonso C., Cardoso C., Ripol A., Varela J., Quental-Ferreira H., Pousão-Ferreira P., Ventura MS., Delgado IM., Coelho I., Castanheira I., Bandarra NM. 2018. Composition and bioaccessibility of elements in green seaweeds from fish pond aquaculture. Food Research International. 105, 271-277. https://doi.org/10.1016/j.foodres.2017.11.015

AFSSA (Agence française de sécurité sanitaire des aliments), 2009. Opinion of the French Food Safety Agency on the recommended maximum inorganic arsenic content of *laminaria* and consumption of these seaweeds in light of their high iodine content. Maisons-Alfort, France

ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail), 2018. Opinion of the French Agency for Food, Envinronmental and Occupational Health & Safety on the risk of excess iodine intake from the consumption of seaweed in foodstuffs. Maisons-Alfort, France

Arulkumar A., Nigariga P., Paramasivam S., Rajaram R., 2019. Metals accumulation in edible marine algae collected from Thondi coast of Palk Bay, Southeastern India. Chemosphere. 221, 856-862. https://doi.org/10.1016/j.chemosphere.2019.01.007

Banach J.L., Hoek-van den Hill E.F., van der Fels-Klerx H.J, 2019. Food safety hazards in the European seaweed chain. Compr. Rev. Food Sci Food Safety. 2020, 1-33. https://doi.org/10.1111/1541-4337.12523

Bouga M., Combet E., 2015. Emergence of Seaweed and Seaweed-Containing Foods in the UK: Focus on Labeling, Iodine Content, Toxicity and Nutrition. Foods. 4, 240-253

Camurati J., Salomone V. 2019. Arsenic in edible macroalgae: an integrated approach. Journal of Toxicology and Environmental Helath, Part. B. https://doi.org/10.1080/10937404.2019.1672364

CEVA (Centre d'Étude et de Valorisation des Algues), 2014. Edible seaweed and French regulation - Synthesis made by CEVA (31/03/2014). Pleubian, France

Chakraborty S., Bhattacharya T., Gurmeet S., Maity JP. 2014. Benthic macroalgae as biological indicators of heavy metal pollution in the marine environments: a biomonitoring approach for pollution assessment. Ecotoxicol. Environ. Saf. 100, 61-68. https://doi.org/10.1016/j.ecoenv.2013.12.003

Chen Q., Pan XD., Huang BF., Han JL, 2018. Distribution of metals and metalloids in dried seaweeds and health risk to population in southeastern China. Scientific Reports, Nature. 8, 3578. https://doi.org/10.1038/s41598-018-21732-z

Cian R.E., Drago S.R., de Medina F.S., Martínez-Augustin O., 2015. Proteins and Carbohydrates from Red Seaweeds: Evidence for Beneficial Effects on Gut Function and Microbiota. Marine Drugs. 13, 5358-5383. Doi: https://doi.org/10.3390/md13085358

Circuncisão A., Catarino MD, Cardoso SM, Silva AMS. 2018. Minerals from Macroalgae Origin: Health Benefits and Risks for Consumers. Marine Drugs. 16, 400

Combet E., Feei Ma Z., Cousin F., Thompson B., Lean MEJ. 2014. Bioavailability and impact on thyroid function of iodine from seaweed in iodine-insufficient women. Br. J. Nutr. 112, 753-761

Dawczynski C., Schäfer U., Leiterer M., Jahreis G. 2007. Nutritional and toxicological importance of macro, trace, and ultra-trace elements in algae food products. J. Agric. Food Chem. 55, 10470-10475

Desideri D., Cantaluppi F., Ceccotto M., Meli MA, Roselli C., Feduzi L. 2016. Essential and toxic elements in seaweeds for human consumption. Journal of Toxicology and Environmental Health, Part A. 79 (3), 112-122. https://doi.org/10.1080/15287394.2015.1113598

EC (European Regulation), 2008. Commission Regulation (EC) No 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Union L173, 6-9

EC (European Regulation), 2011. Commission Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. Off. J. Eur. Union L304, 18-63

EC (European Regulation), 2014. Commission Regulation (EU) No 488/2014 of 12 May 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of cadmium in foodstuffs. Off. J. Eur. Union L138, 75-79

EC (European Regulation), 2015. Commission Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods. Off. J. Eur Union L327, 1-22

EFSA (European Food Safety Authority), 2006. Statement on a request from the Commission related to iodine in seaweed. EFSA J. 4(10), 2. https://doi.org/10.2903/j.efsa.2006.1046

EFSA (European Food Safety Authority), 2014. Dietary exposure to inorganic arsenic in the European population. EFSA J. 12(3), 68. https://doi.org/10.2903/j.efsa.2014.3597

FAO. 2016. The State of World Fisheries and Aquaculture. Contributing to food security and nutrition for all. Food and Agriculture Organization of the United Nations. Rome, 2016.

FSANZ. 2013. Food Standards Australia New Zealand. Survey of inorganic arsenic in seaweed and seaweed-containing products available in Australia. Canberra

Henriques B., Rocha LS., Lopes CB., Figueira P., Duarte AC., Vale C., Pardal MA., Pereira E. 2017. A macroalgae-based biotechnology for water remediation: Simultaneous removal of Cd, Pb and Hg by living *Ulva lactuca.* Journal of Environmental Management. 191, 275-289.

Lüning K., Mortensen L. 2015. European aquaculture of sugar kelp (Saccharina latissima) for food industries: iodine content and epiphytic animals as major problems. Botanica Marina. 58 (6), 449-455. https://doi.org/10.1515/bot-2015-0036

Makkar HPS., Tran G., Heuzé V., Giger-Reverdin S., Lessire M., Lebas F., Ankers P. 2016. Seaweeds for livestock diets: A review. Animal Feed Science and Technology. 212, 1-17. https://doi.org/10.1016/j.anifeedsci.2015.09.018

Nitschke, U., Stengel, D. B. 2016. Quantification of iodine loss in edible Irish seaweeds during processing. Journal of Applied Phycology, 28 (6), 3527–3533. https://doi.org/10.1007/s10811-016-0868-6

Paz S., Rubio C., Frìas I., Gutièrrez A., González-Weller D., Martín V., Revert C., Hardisson A., 2019. Toxic metals (Al, Cd, Pb and Hg) in the most consumed edible seaweeds in Europe. Chemosphere. 218, 879-884. https://doi.org/10.1016/j.chemosphere.2018.11.165

Prakash V., Martin-Belloso O., Keener L., Astley S., Braun S., McMahon H. Lelieveld H (Eds.). 2016. Regulating Safety of Traditional and Ethnic Foods. Academic Press. Waltham, MA. 536 pp.

Roleda MY., Skjermo J., Marfaing H., Jónsdóttir R., Rebours C., Gietl A., Stengel DB., Nitschke U. 2018. Iodine content in bulk biomass of wild-harvested and cultivated edible seaweeds: inherent variations determine species-specific daily allowable consumption. Food Chemistry. 254, 333-339. https://doi.org/10.1016/j.foodchem.2018.02.024

SCF (Scientific Committee on Food), 2002. Opinion of the Scientific Committee on Food on the tolerable upper intake level of iodine. SCF, Brussels, Belgium

Shams El-Din, NG, Mohamedein, LI, El-Moselhy, KhM, 2014. Seaweeds as bioindicator of heavy metals off a hot spot area on the Egyptian Mediterranean Coast during 2008-2010. Environ Monit Assess. 186 (9), 5865-5881. https://doi.org/10.1007/s10661-014-3825-3

Teas J., Pino S., Critchley A., Braverman LE. 2004. Variability of iodine content in common commercially available edible seaweeds. Thyroid. 14 (10), 836-841. https://doi.org/10.1089/thy.2004.14.836

Van Netten C., Hoption Cann SA, Morley DR, van Netten JP. 2000 Elemental and radioactive analysis of commercially available seaweed. Sci. Total Environ. 255, 169-175