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Analysis and Risk Assessment of Seaweed

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Abstract

During the last decade, the interest on the use of seaweed as food or feed, which was before limited to certain European regional subpopulations, has experienced a significant increase in other regions of the EU. In fact, the growing awareness and interest on sustainable and alternative food sources, healthier lifestyles and changes on dietary patterns brought seaweed to the spotlight for the general worldwide cuisine. Due to their high biosorption and accumulation capacity, seaweed can be an important source of increased exposure to persistent and potential harmful elements, such as cadmium (Cd), lead (Pb), mercury (Hg) and inorganic arsenic (iAs), or even some micronutrients, particularly iodine (I), to which an antioxidant role as been described in seaweed. This concentration potential has raised the interest of several Food Authorities regarding the risk of increased exposure to these elements. Moreover, the European Commission requested the collection of monitoring data on their levels aiming to aid the performance of better risk assessments and potentially set maximum levels on the European Legislation. This work aimed to obtain levels of these elements in species of seaweed (*Fucus vesiculosus*, *Fucus serratus*, *Fucus spiralis*, *Fucus evanescens*, *Saccharina latissima*, *Ulva lactuca* and *Cladophora* sp.) cultivated and harvested in Denmark, following European Commission's request. Additionally, a collaboration between Denmark, Ireland, France and the Netherlands was initiated to review and collect all the data available on scientific papers regarding the levels of these contaminants in seaweed worldwide. The final result of this work would be the publication of a review article. This Fellowship also provided on-the-job training on the evaluation of applications of new biocides and participation in the science based advises given to the Danish Food and Veterinary Administration, Danish EPA, the Danish Medical Agency and ECHA.

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1. Introduction

Up to now, mostly used by specific subpopulations in Europe (namely in Iceland, Scotland, Ireland, Wales and France) (Mahadevan, 2015; Tiwari and Troy, 2015), seaweeds or macroalgae have recently experienced and increased interest regarding their use as food and feed. In fact, after being used for centuries as a staple food particularly in Asian countries, seaweeds are expected to become a relevant food and food ingredient in the European market. Seaweeds were brought to the spotlight in the Western world due to their marketing and perception as 'superfood', increased interest in healthier diets and lifestyles as well as on more sustainable food sources and production (Mahadevan, 2015; Mendis and Kim, 2011; FAO, 2018). As a result, a wide variety of seaweed-based or containing products is now more easily available to European consumers, from the traditional sushi to salads, breads pasta, chips and drinks (Bouga and Combet, 2015).

With high nutritional value due to the presence of important macro- and micronutrients including vitamin B12, omega-3 and -6 fatty acids, selenium, iodine and dietary fibre (Aguilera-Morales et al., 2005; Peña-Rodríguez et al., 2011; Gil et al., 2015), seaweeds are also studied as a source of several bioactive compounds with potential health benefits/applications (Holdt and Kraan, 2011; Brown et al., 2014). Seaweeds can also be a source of increased dietary exposure to potential harmful and persistent contaminants (such as inorganic arsenic, lead, cadmium and mercury) as well as some nutrients, such as iodine. In fact, due to the specific characteristics of their cell wall and structure, seaweeds present a high concentration potential for minerals and trace elements present in the surrounding waters. As a result, the levels of these elements are on average several orders of magnitude higher in seaweed than in the water (Jadeja and Batty, 2013; Malea et al., 2015; Bonanno and Orlando-Bonaca, 2018). This concentration potential is behind the extended use of macroalgae in biomonitoring and bioremediation protocols, from where most of the knowledge on the uptake of contaminants by seaweeds has been gathered (Hamdy, 2000; Sheng et al., 2004; Chakraborty et al., 2014; Holan et al., 1993). So far, studies report high intra- and interspecies differences, as well as geographic and seasonal variability in the concentration of different elements in macroalgae (Brito et al., 2012; Ryan et al., 2012; Chakraborty et al., 2014; Malea et al., 2015; Chen et al., 2018).

Iodine is an essential micronutrient for the synthesis of thyroid hormones, which in turn are important for growth, development and metabolism, particularly vital during earlier stages of life (WHO, 2007). Iodine can cause the dysfunction of thyroid gland at high levels of exposure. This is the reason why in 2002, EFSA's Scientific Committee on Food (SCF) suggested a tolerable upper intake level (UL) for adults of 600 µg iodine/day and adjusted this for the remaining age groups based on differences on body surface area (body weight^{0.75}) (European Commission, 2002). Mercury, lead, cadmium, and inorganic arsenic are completely deprived of biological activity in humans and harmful even at trace levels (Bilal et al., 2018), being elements of greater interest for food safety authorities. Inorganic arsenic (IARC, 2012) is classified as carcinogenic for humans while methylmercury (MeHg) (IARC, 1993) and inorganic lead (IARC, 2006) have been classified as possibly carcinogenic for humans, besides being characterised by several other toxic effects in humans, e.g. neurotoxicity and nephrotoxicity.

The toxicological profile and the relative high exposure from other sources of these elements has raised the interest of several Food Authorities concerned with the exposure to excessive levels of these contaminants due to seaweed consumption (FSAI, 2015; Duinker et al., 2016; ANSES, 2018). However, maximum levels for heavy metals and metalloids have been set by the Commission Regulation No 1881/2006 (European Commission, 2006) as amended by Regulation No 629/2008 (European Commission, 2008), in a range of foodstuffs including seafood, seaweeds are not included on the list. Despite being more frequently performed, speciation of arsenic and mercury is still frequently not included despite its importance for the evaluation of the risk associated with consumption and increase consumers' protection. In conclusion, nowadays in Europe, there are no regulation on the maximum levels of these elements in seaweeds as food, besides a maximum limit level of 3.0 mg/kg wet weight for cadmium in 'food supplements consisting exclusively or mainly of dried seaweed or of products derived from seaweed' (European Commission, 2008). Recognising the emergent interest in seaweed and the lack of data on the levels of these contaminants in seaweeds available and or produced in the European market, monitoring data for the most common edible species of seaweeds have been requested by the European Commission to all member states during the period of 2018 to 2020 (European Commission, 2018). The final result of this monitoring action could be the setting of maximum levels for arsenic, lead, cadmium, mercury and iodine for seaweeds as well as providing more data to improve the risk assessments regarding the consumption of this food.

Still, owing to their nutrient density, the invaluable potential of edible seaweeds as a food source should not be neglected. Hence gathering knowledge about contamination patterns and distribution would be of great value to enhance their safe use and health benefits. The main goal of the EU-FORA Fellowship Programme was to monitor the levels of inorganic arsenic, cadmium, lead, mercury and iodine in samples of edible seaweeds cultivated and harvested in Denmark.

2. Description of work programme

2.1. Aims

The main aim of the work programme was the analysis of lead, mercury, inorganic arsenic, cadmium and iodine in samples of seaweed cultivated and harvested in Denmark, following the request on monitoring data by the European Commission (2018). Defining a hypothetical consumption scenario, a risk assessment was performed for the different species of seaweed included in the present study.

2.2. Activities/methods

The work programme included two parts:

- 1) Analysis of iodine mercury, cadmium, lead, and total arsenic using inductively coupled plasma mass spectrometry (ICP-MS). The analysis of inorganic arsenic (in preparation) would be performed using anion-exchange high-performance liquid chromatography (HPLC) coupled to ICP-MS.
 - a) Samples of *Fucus vesiculosus*, *Fucus serratus*, *Fucus spiralis*, *Fucus evanescens*, *Saccharina latissima*, *Ulva lactuca* and *Cladophora* sp. were harvested, freeze dried, pulverised and quantified. The quality of the analytical methods was assured by simultaneous analysis of certified reference materials and adherence to European standard methods (EN15763, EN15111 and EN16802¹). The results are present as $\mu\text{g/g}$ freeze dried weight (fdw);
- 2) Risk assessment regarding the dietary exposure to iodine, cadmium, lead, and mercury due to seaweed consumption was performed considering a single serving size of 5 g of fdw. Species-specific exposure was estimated using the average content for each species as well as the 95th percentile. A similar approach was followed, considering all different species together, as a very rough indicative scenario of what might happen when consumers buy seaweed in store, as frequently the species are not identified and can be picked randomly. The adult population was considered and, when relevant specific high-risk subgroups were discussed.

3. Conclusions

3.1. Occurrence of iodine, cadmium, lead, mercury and total arsenic in Danish seaweed samples

Table 1 summarises the content of iodine, total arsenic, cadmium, mercury and lead analysed for the different species of seaweed. In general, interspecies variability is greater than variability within the same species. Considering all the samples, the levels for the different elements decreased in the order: iodine ranged (17.2–4782 fdw) > total arsenic (3.2–116.7 $\mu\text{g/g}$ fdw) > lead (0.072–9.6 $\mu\text{g/g}$ fdw) > cadmium (0.017–1.97 $\mu\text{g/g}$ fdw) > mercury (0.003–0.042 $\mu\text{g/g}$ fdw).

Despite not useful to perform a risk assessment, the levels of total arsenic were included. This data is important to assess the relative amount of arsenic as inorganic arsenic and better characterise potential inter- and intraspecies variability. However, the content of inorganic arsenic, the species with toxicological relevance to perform a risk assessment, were not available at the time this report was written. Therefore, a risk assessment regarding exposure to inorganic arsenic due to seaweed consumption was not performed on Section 3.2.

¹ The method employed to analyse inorganic arsenic was developed by a group of researchers at the National Food Institute, Technical University of Denmark, in a project under the European Committee for Standardization (CEN). This method has been approved as the European analytical standard [CEN standard (EN16802:2016)] for measuring inorganic arsenic in foodstuffs.

Table 1: Average (\pm SD) and range levels (μ g/g fdw) of iodine, total arsenic, mercury, lead and cadmium

	No. samples	Iodine		Total arsenic*		Mercury		Lead		Cadmium	
		Average (\pm SD)	Range (min–max)								
Phaeophyta (brown algae)											
<i>Saccharina latissima</i>	16	2,302.5 (1,098.18)	333.0–4,782.2	38.324 (8.713)	22.504–54.117	0.016 (0.005)	0.007–0.023	0.257 (0.222)	0.072–0.708	0.682 (0.216)	0.231–0.966
<i>Fucus vesiculosus</i>	27	274.9 (75.87)	137.8–451.2	28.379 (19.690)	10.358–116.677	0.012 (0.007)	0.003–0.042	0.897 (1.730)	0.189–9.601	0.780 (0.372)	0.299–1.969
<i>Fucus spiralis</i>	1	209.52	–	8.940	–	0.019	–	0.956	–	0.464	–
<i>Fucus evanescens</i>	1	394.16	–	14.084	–	0.008	–	0.506	–	0.520	–
<i>Fucus serratus</i>	14	366.46 (197.92)	105.2–961.4	30.269 (9.579)	21.457–56.277	0.009 (0.003)	0.005–0.015	0.465 (0.177)	0.236–0.865	1.044 (0.339)	0.628–1.561
Chlorophyta (green algae)											
<i>Ulva lactuca</i>	2	18.97 (2.52)	17.2–20.8	3.399 (0.293)	3.192–3.606	0.007 (0.002)	0.005–0.008	0.078 (0.019)	0.064–0.092	0.038 (0.030)	0.0168–0.059
<i>Cladophora</i> sp.	1	140.27	–	7.069	–	0.007	–	1.447	–	0.782	–

SD: standard deviation.

*: Data on inorganic arsenic is in preparation.

Table 2: Estimated average and 95th percentile exposure to iodine ($\mu\text{g}/\text{day}$), mercury, lead and cadmium ($\mu\text{g}/\text{kg}$ bw per day) due to the consumption of a single serving size of 5 g fdw of seaweed, considering each species individually and altogether. For species with only one representative sample, the total content was considered for exposure calculation

	Iodine ($\mu\text{g}/\text{day}$)		Mercury ($\mu\text{g}/\text{kg}$ bw per day)		Lead ($\mu\text{g}/\text{kg}$ bw per day)		Cadmium ($\mu\text{g}/\text{kg}$ bw per day)	
	Average	95th percentile	Average	95th percentile	Average	95th percentile	Average	95th percentile
Species-specific exposure								
<i>Fucus vesiculosus</i>	1,374.6	274.9	0.00100	0.0015	0.0747	0.14424	0.0650	0.0928
<i>Fucus serratus</i>	1,832.2	2,523.2	0.00099	0.0010	0.0387	0.05998	0.0870	0.1217
<i>Fucus spiralis</i>	1,047.6	–	0.00161	–	0.0796	–	0.0387	–
<i>Fucus evanescens</i>	1,970.8	–	0.00069	–	0.0421	–	0.0433	–
<i>Saccharina latissima</i>	11,512.3	18,677.2	0.00135	0.0018	0.0214	0.05380	0.0568	0.0772
<i>Ulva lactuca</i>	94.9	86.0	0.00055	0.0004	0.0065	0.00536	0.0031	0.0014
<i>Cladophora</i> sp.	701.3	–	0.00062	–	0.1205	–	0.0652	–
Population of samples								
	4,052.7	13,631.1	0.0010	0.0016	0.0521	0.0786	0.0652	0.1194

bw: body weight.

3.2. Health Risk Assessment

An assessment of potential health risks associated with the consumption of unprocessed seaweed was performed (Table 2). An average consumption of seaweed of 5.2 g/adult per day for Chinese, 4 g/adult per day for Japanese and 8.5 g/adult per day for South Korean has been estimated (Roleda et al., 2019). However, seaweed consumption data for Europeans is absent and, in this report, a single serving size of 5 g (fdw), once a week, was assumed to perform this risk assessment. A body weight (bw) of 60 kg was considered as average body weight for an adult.

Regarding exposure to lead, the previously accepted provisional tolerable weekly intake (PTWI) of 25 µg/kg bw per week was considered, in 2010, by the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) no longer appropriate. The Panel determined a benchmark dose (BMD) lower bound associated with 1% extra risk (BMDL01) for neurodevelopmental effects in children of 12 µg/L in blood, corresponding to 0.50 µg/kg bw per day. A margin of exposure (MOE) of 10 was considered sufficient (exposure ≤ 0.05 µg/kg bw per day) (EFSA CONTAM Panel, 2010). Cereals are the main dietary source of dietary exposure to lead in Europe, with an average dietary exposure in European adults ranging between 0.36 up to 1.24 µg/kg bw per week and 0.80 to 3.10 µg/kg bw in children (EFSA CONTAM Panel, 2010). For high consumers, dietary exposure can reach 2.43 µg/kg bw per week (adults) and 5.51 µg/kg bw per week (children). Therefore, however not being the main dietary source of exposure, increased exposure to lead and its health effects cannot be excluded due to seaweed consumption. This would be of particular relevance for frequent and high consumers and for specific at high risk subgroups, namely children (1–7 years old) and pregnant women due to its critical effects on the neurodevelopment and lower body weight of foetus and children.

Cadmium is primarily toxic to the kidneys and bones and, for non-smokers, diet is also the main source of exposure to this element. Cadmium's TWI was set by EFSA to 2.5 µg/kg bw per week, being reported that, in adults, average exposure to cadmium in Europe is already close or slightly above the TWI. Rice, grains and vegetables are the main food sources of cadmium and, therefore, vegetarians and vegans are at higher risk of exceeding the TWI, as well as children, smokers and people living in highly contaminated areas (EFSA, 2009). Considering the species included in this study, a single serving would contribute with an average intake of 0.003 µg/kg bw to 0.087 µg/kg bw, 1.2–3.5% of the TWI, which is insignificant compared to other sources.

Methylmercury has a defined TWI of 1.3 µg/kg bw per week (neurodevelopmental effects in children), while total mercury has a TWI of 4 µg/kg bw per week (EFSA CONTAM Panel, 2012). In fish and seafood, most of the mercury is considered to be in the form of methylmercury, however, for seaweeds scarce data is available on speciation of this element. However, even when considering the total amount present as methylmercury, in average, the contribution of a single serving of seaweed to total exposure to mercury would be negligible.

On the other hand, intake of iodine due to a single serving of seaweed might easily exceed the UL for iodine (600 µg/day for adults and 200 µg/day for children), as observed for all of the species of brown algae and particularly for *Saccharina latissima* (average > 11,000 µg/day and 95th percentile > 18,000 µg/day). In general, consumption of brown seaweeds rich in iodine, namely *Saccharina latissima*, once a week would not represent a problem for the general healthy population. However, when considering at high risk subgroups: pregnant women (due to the importance of thyroid hormones in fetal development), children and individuals with thyroid dysfunction, a more careful evaluation regarding the species, amount and frequency of seaweed consumed should be done. Species with lower iodine content should be selected and iodine-rich species should be avoided due to lack of knowledge on the long-term exposure effects in fetus and children.

Here, only data on total arsenic was available, which comprises both organic and inorganic arsenic. Inorganic arsenic is the species most well characterised toxicologically and to which health effects have been associated. Therefore, inorganic arsenic is the arsenical form of relevance. Nonetheless, arsenosugars, the most relevant form of organic arsenic in seaweed, undergo extensive metabolism in humans (Leffers et al., 2013 Jul; Van Hulle et al., 2004; Raml et al., 2005, 2009; Wei et al., 2003), and more data on the toxicokinetics and toxicological profile of arsenosugars and their metabolites are warranted.

It is important to caveat that considering the unprocessed seaweed biomass is a conservative approach for exposure assessment, likely leading to overestimation, as the effects of cooking and processing on the final content as well as bioavailability are not taken into account. In fact, it is known that washing and cooking can significantly reduce the levels of several of these elements, namely iodine. Additionally, several other uncertainties exist, particularly regarding the exposure assessment

related with lack of data on iodine content (inter-species and intra-species, seasonal and geographic variability) and consumption data (species, frequency and amount of seaweed consumed) in Europe.

In general, we can conclude that seaweed consumption, by the general population, would be of low health risk for mercury, cadmium, and lead. However, due to their health effects, monitoring the levels of these elements should be performed and potentially, in the future, maximum levels set up for seaweed for food in Europe. For iodine, additionally to setting up maximum levels, the identification of the species on the label of the commercial products could be suggested as a way to decrease the exposure to high levels of iodine. Moreover, this preliminary study highlights the need for more data to be collected so that a more robust risk assessment can be performed. It is considered that the priorities would be the collection of species-specific consumption data and a better characterisation of the content of seaweed on these elements, including speciation data for arsenic.

3.3. Secondary activities

Additional activities taken during this working programme were:

- 1) A literature review of scientific papers and reports published since 1998 on the levels of iodine, cadmium, lead, mercury and inorganic arsenic in seaweeds of interest for human consumption (according to their commercial value, extension of consumption as well as availability of data). Regarding arsenic only studies reporting speciation and levels of inorganic arsenic were included as, so far, this is the species with toxicological relevance. The aim was to gather data for a better characterisation regarding species, season-variability and geographic origin (in preparation for submission);
- 2) Participation on the postgraduate course 'Risk Analysis in Food Safety' including a module in microbiological risk assessment and a second module in chemical risk assessment. Each module included two case studies intended to the elaboration of a risk assessment on a specific microbiological/chemical hazard and elaboration of a report;
- 3) Participation with a poster presentation on the *Open Day* of the National Food Institute (Denmark) as part of the commemorations of its 60th anniversary. The poster was on the work develop by the National Food Institute and DTU on the monitorisation of chemical contaminants in seaweed (particularly iodine), evaluation of efficacy of different processing/preparation methods, risk assessment and elaboration of advises to consumers regarding seaweed consumption.
- 4) On-the-job training on the evaluation of applications and requests, related with biocides products, mainly destined to be used as disinfectant/cleaning agents. Taking part in advice giving to the Danish food and veterinary administration, the Danish Environment Protection Agency and the Danish Medical Agency in a range of different settings.

3.4. Disclaimer

The individual results of the analysis are not included in this report to avoid copyright claims as this research is part of an ongoing research project (being the EU-FORA Fellowship Programme) and the results are intended to be subsequently published in other scientific journals.

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Abbreviations

BMD	benchmark dose
BMDL01	benchmark dose lower bound
bw	body weight
CONTAM	EFSA Panel on Contaminants in the Food Chain
FAO	Food and Agriculture Organization
fdw	freeze dried weight
HPLC	high-performance liquid chromatography
ICP-MS	inductively coupled plasma-mass spectrometry
MOE	margin of exposure
PTWI	provisional tolerable weekly intake
SCF	Scientific Committee on Food
SD	standard deviation
TWI	tolerable weekly intake
UL	tolerable upper intake level
WHO	World Health Organization