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Heavy Metal, Total Arsenic, and Inorganic Arsenic Contents of Algae Food Products

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The total arsenic, inorganic arsenic, lead, cadmium, and mercury contents of 18 algae food products currently on sale in Spain were determined. The suitability of the analytical methodologies for this type of matrix was confirmed by evaluating their analytical characteristics. The concentration ranges found for each contaminant, expressed in milligrams per kilogram of dry weight, were as follows: total arsenic, 2.3−141; inorganic arsenic, 0.15−88; lead, <0.05−1.33; cadmium, 0.03−1.9; and mercury, 0.004−0.04. There is currently no legislation in Spain regarding contaminants in algae food products, but some of the samples analyzed revealed Cd and inorganic As levels higher than those permitted by legislation in other countries. Given the high concentrations of inorganic As found in *Hizikia fusiforme*, a daily consumption of 1.7 g of the product would reach the Provisional Tolerable Weekly Intake recommended by the WHO for an average body weight of 68 kg. A more comprehensive study of the contents and toxicological implications of the inorganic As present in the algae food products currently sold in Spain may be necessary, which might then be the basis for the introduction of specific sales restrictions.

KEYWORDS: Arsenic; inorganic arsenic; lead; cadmium; mercury; edible algae

INTRODUCTION

During recent decades the eating patterns of the Spanish population have undergone marked changes. Innovations in food technology, together with the globalization of markets, have resulted in a significant increase in the number of new foods, both fresh and processed or semiprocessed, available to consumers. Among these products, edible algae have come to form a common part of Spanish diets. A very wide variety of products derived from macroalgae or microalgae can be bought in Spanish stores, with over 30 products of this type already available in 1993 (*1*).

Although macroalgae species are used as a source of hydrocolloids (agar, carrageenans, and alginates), they are mainly used for food, with the great advantage that they need minimum processing after harvest (*2*). No data are available about the consumption of algae in Spain, but this country does seem to be following trends seen in other Western countries, which show an increase in the consumption of these products in recent years (*3*), mainly among groups that could be considered to be extreme consumers, such as people on macrobiotic diets. Consumer interest in these products is due to their nutritional properties, which, although varying from one type of alga to another, can be said to have the following characteristics in general: a high protein content, which in some species, *Porphyra tenera* and *Palmaria palmata*, is almost as high as that of soybean (*4*); an amino acid profile similar to those of leguminosae and eggs (*4*); high levels of dietary fiber, which can be between 33 and 50% of the total product (*5*); and high contents of minerals [calcium, potassium, sodium, iron, iodine, magnesium, and phosphorus (*3*)] and of certain vitamins [A, B_1, B_2, C, and folic acid (*6*)]. Furthermore, medical properties have been described (anti-HIV and antitumor activities and potential contraceptive effects) in relation to numerous edible algae (*7*).

Despite the nutritional properties associated with algae, there is another aspect that should not be forgotten: their capacity to bioaccumulate not only essential elements but also toxic elements such as certain heavy metals and arsenic. This characteristic has encouraged the use of algae as indicators of marine ecosystem pollution (*8*, *9*). The species of alga considered also affects the metal content detected to a considerable extent, although variations have also been demonstrated that are associated with both the origin of the product, which reflects the pollution found in its natural habitat (*7*), and the growth phase and time of year when the samples are gathered (*10*, *11*). As a result, in order to evaluate edible algae and the dietary supplements derived from them, it is necessary to quantify the contaminants (heavy metals and arsenic) they contain as a preliminary step to evaluating their toxicological considerations. Nevertheless, these products do represent a sample type that has been studied very little from the standpoint of chemical

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safety, and their contaminant content is regulated in very different ways by different legislation around the world. In the European Community, Regulation (EC) 466/2001 of the European Commission, dated March 8, 2001, which stipulates the maximum content of certain contaminants in food products (*12*), does not include algae food products; however, some countries, such as France (*5*), do have specific regulation concerning the use of seaweeds for human consumption. There is currently a lack of legislation in this field in Spain.

Most research into macroalgae has focused on the study of minerals rather than on toxic elements. What little information is available about the lead and cadmium contents of such products sold in Spain is restricted to certain specific aspects (*1*, *8*), but none covers mercury content. With respect to arsenic, algae are a primary accumulator in the marine environment and represent an important stage in arsenic metabolism through the food chain (*13*). Information on this contaminant is also sparse and generally refers to levels of total arsenic, which are not useful for evaluating the toxicological risks associated with consumption. The safety of edible algae with respect to this metalloid can be evaluated only after determining the content of inorganic arsenic, a term that includes the most toxic species of arsenic [As(III) + As(V)], based on which the WHO has established a Provisional Tolerable Weekly Intake (15 μg of inorganic arsenic/week/kg of body weight) (*14*).

The quantification of inorganic arsenic in edible algae products is faced with the problem of a lack of specific methodologies. Our laboratory has developed a methodology involving the solubilization of the sample with hydrochloric acid and extraction by means of organic solvents (*15*) that has been applied successfully to a wide range of fish products (*16*). One of the main objectives of the present study was to assess the suitability of this methodology for determining inorganic arsenic in edible algae products. The contents of lead, cadmium, mercury, total arsenic, and inorganic arsenic in a range of samples of algae sold in Spain were therefore determined. The results obtained have been analyzed from legislative and health safety viewpoints.

MATERIALS AND METHODS

Instruments. Determination of total lead and cadmium was performed by graphite furnace atomic absorption spectroscopy (GF-AAS) with a Perkin-Elmer (PE) longitudinal AC Zeeman (Analyst 600) atomic absorption spectrometer, equipped with a transversely heated graphite atomizer and a built-in, fully computer-controlled AS-800 autosampler (Perkin-Elmer Hispania, S.A., Madrid, Spain). Pyrolitic graphite coated tubes with an integrated L'vov platform were used.

The determination of total and inorganic arsenic was performed with an AAS model 3300 (PE) equipped with an autosampler (PE AS-90) and a flow injection system (PE FIAS-400) in order to provide hydride generation in continuous flow mode. The determination of mercury was performed with a flow vapor generation−atomic fluorescence spectrometer (AFS; Millennium Merlin PSA 10.025, PS Analytical).

Other equipment used included a Moulinex Optiquick Duo domestic microwave oven (Moulinex, Valencia, Spain), with a maximum power of 900 W; a PL 5125 sand bath (Raypa, Scharlau, S.L.); a K 1253 muffle furnace equipped with a Eurotherm Controls 902 control program (Heraeus S.A., Madrid, Spain); a KS 125 basic mechanical shaker (IKA Labortechnik, Merck Farma y Química, S.A., Barcelona, Spain); and an Eppendorf 5810 centrifuge (Merck).

Reagents. Deionized water (18 MΩ cm) was used for the preparation of the reagents and standards. All chemicals were of at least pro analysi quality or better. Commercial standard solutions (1000 mg L^{-1}) of As(V), Pb, Cd, and Hg were used (Merck). Calibration standard solutions of As(III) were prepared from a reduced standard solution of As(V).

As a reducing solution for arsenic hydride generation, sodium tetrahydroborate(III) solution (1% m/v) was prepared by dissolving $NaBH_4$ powder in 0.7% m/v NaOH solution and filtering through Whatman No. 42 paper. Fresh $NaBH_4$ solution was prepared daily. As a reducing solution that converts Hg(II) into Hg(0) vapor, 2% m/v $SnCl_2$ was prepared by dissolving $SnCl_2$ powder in 33% v/v HCl (17). The matrix modifier used for determining Cd and Pb was a mixture of $H_2PO_4NH_4$ and $Mg(NO_3)_2$ in 1% HNO_3.

All glassware was treated with 10% v/v HNO_3 for 24 h and then rinsed three times with deionized water before use. The following certified reference materials were employed: *Fucus* sp. (International Atomic Energy Agency, Analytical Quality Control Services, Vienna, Austria); BCR 060 (aquatic plant *Lagarosiphon major*) and BCR-279 (sea lettuce *Ulva lactuca*), both from the Institute for Reference Materials and Measurements (IRMM), Brussels, Belgium.

Sample Collection and Preparation. In Spain, macroalgae are rarely sold fresh; most of them have undergone some form of processing varying from simply drying in the sun to being baked in an oven or flame-dried. These products tend to be sold under a specific name that refers to a certain alga processed in a particular way. In this study, 18 products derived from samples of brown algae (12 samples), red algae (4 samples), and green algae (2 samples), bought in stores in the city of Valencia (Spain), were analyzed. The following derived products were included: wakame and kombu, obtained respectively by drying and cooking the brown algae *Undaria pinnatifida* and *Laminaria japonica*; hijiki (*Hizikia fusiforme*) and arame (*Eisenia bicyclis*), obtained by drying fresh algae; nori and yakinori, obtained respectively by drying and baking the red alga *Porphyra* sp.; dulse, obtained by drying the red alga *Palmaria palmata* (*2*); and AO-nori, obtained by drying the green alga *Ulva pertusa*. The samples are sold in a dried form, and therefore they were not freeze-dried. They were crushed in a mill and stored at 4 °C until analysis.

Determination of Total Arsenic (*16*). The samples (0.25 g) were treated with an ashing aid suspension (20% m/v $MgNO_3$ + 2% m/v MgO) and nitric acid (5 mL of 50% v/v), evaporated to dryness, and mineralized at 450 °C with a gradual increase in temperature. The ash was dissolved in hydrochloric acid (6 mol L^{-1}) and prereduced (5% m/v ascorbic acid + 5% m/v KI). The analytical conditions used for arsenic determination by flow injection−hydride generation−atomic absorption spectrometry (FI-HG-AAS) were the following: loop sample, 0.5 mL; reducing agent, 0.2% (m/v) $NaBH_4$ in 0.05% (m/v) NaOH, 5 mL min^{-1} flow rate; HCl solution 10% (v/v), 10 mL min^{-1} flow rate; carrier gas argon, 100 mL min^{-1} flow rate; wavelength, 193.7 nm; spectral band-pass, 0.7 nm; electrodeless discharge lamp system 2; lamp current setting, 400 mA; cell temperature, 900 °C.

Determination of Inorganic Arsenic (*15*). Water (4.1 mL) and concentrated HCl (18.4 mL) were added to 0.50 g of sample. The mixture was left overnight. The reducing agent was then added (1 mL of 1.5% m/v hydrazine sulfate solution and 2 mL of HBr), and the sample was agitated for 30 s. $CHCl_3$ (10 mL) was then added, and after 3 min of shaking and 5 min of centrifuging (2000 rpm), the chloroform phase was separated. The extraction process was repeated two more times, and the chloroform phases were combined and filtered. The inorganic arsenic in the chloroform phase was back-extracted by shaking for 10 min with 10 mL of 1 mol L^{-1} HCl. The phases were separated by centrifuging at 2000 rpm, and the aqueous phase was then aspirated and poured into a beaker. This stage was repeated once again, and the back-extraction phases obtained were combined. The inorganic arsenic in the back-extraction phase was determined by means of the following procedure: 2.5 mL of ashing aid suspension and 10 mL of concentrated HNO_3 were added to the combined back-extraction phases, dry-ashed, and quantified by FI-HG-AAS in the conditions described previously for the determination of total arsenic.

Determination of Lead and Cadmium. The sample (0.20 g) was placed in a high-pressure poly(tetrafluoroethylene) (PTFE) vessel. Two milliliters of 65% HNO_3 and 1 mL of 35% H_2O_2 were added. The vessel was sealed with a screw cap and placed inside the microwave oven. Samples were irradiated at a 700 W power setting for three cycles of 1 min. After digestion, the vessel was cooled in an ice bath. The solutions were filtered and diluted with water to a final volume of 50 mL. The quantification of Pb and Cd in GFAAS was performed using the standard additions method. The oven program employed is described in **Table 1**.

Table 1. Graphite Oven Operating Conditions for the Determination of Cd and Pb in Algae Products

wavelength (nm)	228.8 (Cd), 283.3 (Pb)
spectral bandwidth (nm)	0.7
background correction	Zeeman
measuring mode	integrated absorption calcd by software
oven tube	transverse heated graphite tubes with integrated L'vov platform
method of calibration	standard additions method
sample vol	10 μL
chemical modifier	0.067 mg of $H_2PO_4NH_4$ and 0.003 mg of $Mg(NO_3)_2$ in 10 μL of HNO_3 1%

oven program

step	temp (°C)	time (s) ramp	time (s) hold	internal Ar (mL min^{-1})
drying	90	10	20	250
drying	120	10	20	250
drying	130	5	40	250
drying	300	5	5	250
pyrolysis	500 (Cd), 850 (Pb)	10	20	250
cooling	20	10	20	250
spraying	1400 (Cd), 1600 (Pb)	0	5	0
cleaning	2450	1	5	250

Determination of Mercury. The sample (0.20 g) was placed in a high-pressure PTFE vessel. Two milliliters of 65% HNO_3 and 1 mL of 35% H_2O_2 were added. The vessel was sealed with the screw cap and placed inside the microwave oven. Samples were irradiated at a 700 W power setting for three cycles of 1 min. After digestion, the vessel was cooled in an ice bath. The solutions, filtered and diluted with water to a final volume of 25 mL, were left for 12 h to eliminate nitrous vapors. The analytical conditions used for mercury determination by continuous flow vapor generation AFS were the following: reducing agent, 2% m/v $SnCl_2$ in 15% v/v HCl, 4.5 mL min^{-1} flow rate; 5% v/v HCl, 9 mL min^{-1} flow rate; carrier gas argon, 0.3 L min^{-1} flow rate; sheath gas argon, 0.3 L min^{-1} flow rate; dryer gas, air, 2.5 L min^{-1} flow rate; specific Hg lamp; fixed 254 nm filter.

RESULTS AND DISCUSSION

Methodologies. The suitability of each of the methodologies used was checked by evaluating their analytical characteristics (limit of detection, precision, and accuracy). *Fucus* sp., *L. major*, and *U. lactuca* were used as reference materials to quantify total As, Pb, Cd, and Hg. In the case of inorganic As there are currently no certified materials, and therefore recovery tests were carried out on the certified reference materials specified and on samples bought in shops to evaluate accuracy. In the case of commercial samples, samples of high and low inorganic arsenic contents were selected for the recovery test. The additions carried out were in accordance with the level of inorganic arsenic present in the samples.

The analytical characteristics of the methodologies used to determine Pb, Cd, Hg, and As are shown in **Table 2**. The results obtained were satisfactory and demonstrate that it is possible to employ these methodologies to quantify the contaminants mentioned in commercial samples of macroalgae. **Table 3** shows the contents of total arsenic and inorganic arsenic, lead, cadmium, and mercury in each of the samples analyzed (mean of three independent analysis). The category to which each of the samples belongs is also indicated, together with its scientific and commercial names.

Total Arsenic. The contents of total arsenic found varied over a wide range of concentrations [2.3−141 mg kg^{-1}, dry weight (dw)]. The highest values were found in products derived from brown algae, with hijiki, from *H. fusiforme*, showing the highest results (115−141 mg kg^{-1} As dw). After hijiki, the samples of *F. vesiculosus* (50 mg kg^{-1} As dw) and kombu (*L. japonica*: 47−53 mg kg^{-1} As dw) show the next highest levels of arsenic content. The remaining products derived from brown algae present lower contents: wakame (*U. pinnatifida*), 32−42 mg kg^{-1} As dw; and arame (*Eisenia bicyclis*), 23.8−30 mg kg^{-1} As dw.

In general, analyses of red algae revealed total arsenic contents of <30 mg kg^{-1} dw. Mention must be made of the differences observed in the total arsenic contents found in the two types of red algae studied. *P. tenera* has arsenic concentrations similar to those found in brown algae (23.7−30 mg kg^{-1} dw), whereas *P. palmata* has far lower values (7.56 mg kg^{-1} dw). The samples of green algae analyzed in this study have the lowest contents of all the algae studied (2.3−5.17 mg kg^{-1} dw).

In general it can be said that, of the products analyzed, those derived from brown algae have higher total arsenic contents than the products derived from red algae, results similar to those of Morita and Shibata (*13*). The total arsenic contents described in the bibliography for edible algae range between 2.0 and 172 mg kg^{-1} dw (*6*−*8*, *13*, *18*−*24*), levels similar to those found in this study.

As mentioned above, Spain does not have any legislation concerning algae food products, so that there is no legal impediment to the sale of the products analyzed. With respect to health considerations, the absence of data concerning the consumption of macroalgae food products in Western countries makes it difficult to calculate intake levels, and most estimates are based on the consumption of the population of Japan, with a daily average consumption of brown algae of 2−3 g and a maximum consumption of 12 g, dw (*25*).

Although total arsenic is not a useful parameter in the study of the toxicological implications derived from the consumption of edible algae, it is nevertheless still used today. Consumption of 3 g of algae per day would give total arsenic intake for each of the samples analyzed ranging from 7 to 423 μg/day. This consumption is similar to data obtained in studies of total diet in which intake was evaluated on the basis of all the food consumed by the population studied (12−345 μg/day) (*26*, *27*), which show that the highest arsenic content is ingested by eating fish and shellfish. When a consumption of 12 g of edible alga/day is considered, intake ranges from 29 to 1763 μg/day—a higher level than that recorded in total diet studies. One may therefore say that even if algae is a product that is not very widely consumed by the general public, regular consumers of edible algae would be considered as a particular group with respect to contaminant arsenic, something that should be studied in more detail.

Inorganic Arsenic. The inorganic arsenic contents found range from 0.15 to 88 mg kg^{-1} dw, levels that are similar to those described by other authors (0.030−62 mg kg^{-1} dw) (*19*, *23*, *28*). A high proportion of the samples analyzed show inorganic arsenic contents of <1 mg kg^{-1} (0.15−0.57 mg kg^{-1} dw). However, products derived from the brown alga *H. fusiforme* have very high contents of inorganic arsenic (83−88 mg kg^{-1} dw), which constitute 60−72% of the total arsenic present in the sample. These results coincide with the data reported in the literature, which reveal that inorganic arsenic represents between 50 and 70% of the total arsenic in *H. fusiforme* (*13*) and between 28 and 63% in ribbon kelp (*24*).

The sale of edible algae is permitted in Spain regardless of its total or inorganic arsenic content. The situation is very different in other countries in the European Union and elsewhere

Table 2. Analytical Characteristics of the Methodologies Used To Determine Total Arsenic, Inorganic Arsenic, Lead, Cadmium, and Mercury in Macroalgae

	As	inorganic As	Pb	Cd	Hg
limit of detection (mg kg^{-1})	0.025	0.014	0.05	0.003	0.003
precision[a] (%)	1	4	3	4	4
accuracy[b]					
Fucus sp.					
found value	42.3–46.2	1.21–1.33	2.09–2.19	0.528–0.550	0.036–0.040
certified value	42.2–46.4	-	1.91–2.47	0.500–0.574	0.032–0.044
Lagarosiphon major					
found value	7.2–7.6	4.50–4.72	61.6–67.2	2.06–2.17	0.314–0.346
certified value	8.00[d]		60.6–67.0	2.1–2.3	0.30–0.38
Ulva lactuca					
found value	2.99–3.17	1.27–1.37	12.6–13.8	0.25–0.29	0.037–0.043
certified value	2.89–3.29		13.12–13.84	0.252–0.296	0.041–0.066[d]
recovery[c]					
Fucus sp. (%)		100			
natural samples (%)		95 (85, 95) 101 (0.25, 0.19)			

[a] Mean RSD obtained from six independent analyses of certified reference material *Fucus* sp. [b] Confidence interval at the 95% confidence level for six independent analyses. [c] Percentage recoveries expressed as mean from three independent analyses. Values in parentheses are the average concentration in the unspiked samples (first value) and concentration of the As(III) added (second value) in mg kg^{-1} of dry weight. [d] Indicative values.

Table 3. Total Arsenic, Inorganic Arsenic, Lead, Cadmium, and Mercury Contents in Macroalgae[a]

type	species	description of product	total As	inorganic As	Pb	Cd	Hg
green	*Enteromorpha* sp.	green nori flakes (dried edible sea algae)	2.3 ± 0.1	0.37 ± 0.07	1.33 ± 0.03	0.03 ± 0.01	20.6 ± 0.4
	Ulva pertusa	AO nori (dried edible sea algae)	5.17 ± 0.05	0.36 ± 0.06	0.93 ± 0.02	0.17 ± 0.01	18 ± 2
red	*Porphyra tenera*	nori (dried edible sea algae)	23.7 ± 0.5	0.57 ± 0.04	0.31 ± 0.06	0.35 ± 0.01	14 ± 2
		nori (dried edible sea algae)	28.3 ± 0.5	0.19 ± 0.02	0.289 ± 0.004	0.18 ± 0.02	4 ± 1
		toasted nori (dried edible sea algae)	30 ± 1	0.314 ± 0.005	0.29 ± 0.02	0.38 ± 0.01	11.3 ± 0.4
	Palmaria palmata	Atlantic dulse (dried tender Japanese sea algae)	7.56 ± 0.02	0.44 ± 0.06	1.1 ± 0.2	0.70 ± 0.03	10.5 ± 0.4
brown	*Eisenia bicyclis*	ise wild arame (dried tender Japanese sea algae)	23.8 ± 0.5	0.17 ± 0.02	0.15 ± 0.08	0.75 ± 0.01	33.6 ± 0.2
		ise wild arame (dried edible sea algae)	29 ± 1	0.185 ± 0.005	0.18 ± 0.01	0.67 ± 0.03	42 ± 3
		arame (dried edible sea algae)	30.0 ± 0.1	0.15 ± 0.06	0.19 ± 0.02	0.74 ± 0.02	38 ± 3
	Undaria pinnatifida	wakame (dried edible sea algae)	32 ± 1	0.15 ± 0.10	<LOD[b]	1.5 ± 0.1	12 ± 1
		wakame (dried edible sea algae)	42 ± 2	0.26 ± 0.03	<LOD	0.13 ± 0.03	23 ± 3
		Japanese wakame (dried tender Japanese sea algae)	34.6 ± 0.3	0.18 ± 0.05	<LOD	1.9 ± 0.1	14 ± 1
	Laminaria japonica	Japanese kombu (dried tender Japanese sea algae)	47 ± 1	0.297 ± 0.001	<LOD	0.15 ± 0.02	30 ± 5
		kombu (dried sea algae)	53 ± 1	0.254 ± 0.005	<LOD	0.30 ± 0.02	37 ± 4
	Fucus vesiculosus	alga fucus	50.0 ± 0.3	0.34 ± 0.04	0.51 ± 0.04	0.55 ± 0.01	36 ± 6
	Hizikia fusiforme	iziki	128 ± 5	88 ± 6	0.63 ± 0.08	1.45 ± 0.14	35 ± 3
		hijiki (dried edible sea alga)	141 ± 6	85 ± 6	0.89 ± 0.15	1.46 ± 0.02	25.9 ± 0.2
		Japanese hijiki (dried tender Japanese sea algae)	115 ± 12	83 ± 5	0.53 ± 0.06	1.0 ± 0.1	30.32 ± 0.03

[a]Three subsamples were analyzed for each type of macroalgae. Values are expressed as mean ± SD. Results for As, Pb, and Cd are expressed in mg kg^{-1} dry weight (dw). Results for Hg are expressed in μg kg^{-1} dw. [b] LOD, limit of detection: Pb = 0.05 mg kg^{-1} dw.

in the world, where arsenic contents are limited on the basis of the inorganic arsenic contents. Thus, regulations in France and the United States has maximum permissible limits of 3 mg kg^{-1} dw inorganic arsenic (*5*), whereas in New Zealand and Australia the limit is as low as 1 mg kg^{-1} dw (*29*). These values were exceeded by the three samples of *H. fusiforme* analyzed, and therefore they could not be sold in the countries mentioned. In this respect, McSheehy and Szpunar (*23*) state that algae from Brittany, China, Iceland, Japan, and Spain were withdrawn from the French market because their inorganic arsenic content was >3 mg kg^{-1} dw. The absence of legislation on this point in Spain means that this country is likely to receive products that are denied entrance into other markets owing to legislation.

With respect to the evaluation of risks, previous studies are rare. A study carried out on kelp supplements (tablets, capsules, and powder) sold in the United Kingdom (*19*) estimated the intake of reducible arsenic by assuming a daily intake based on the manufacturer's recommended maximum dose, which ranged from 4 to 14 g, according to the product. The results showed an intake of <1 μg/day in most of the samples analyzed. However, there was one exception, with a sample of kelp capsules in which a very high content of reducible arsenic was found (50 μg g^{-1}). The authors (*19*) estimated the intake from such kelp samples to be ~700 μg/day, a level 5 times higher than the Tolerable Daily Intake (TDI) recommended by the WHO for a body weight of 68 kg (0.146 mg of inorganic arsenic/day) (*14*). Our study analyzed a wider variety of products, and the results obtained indicate that, in order to evaluate intake levels, the best solution is to consider two groups of algae. With respect to algae with an inorganic arsenic content of <1 mg kg^{-1} dw (84% of the samples analyzed), both the average consumption (3 g/day) and the maximum consumption (12 g/day) give rise to inorganic intake levels of less than the TDI. However, the consumption of samples of *H. fusiforme*, with an inorganic arsenic concentration of >80 mg kg^{-1} dw, would mean intake levels of between 2 (consumption of 3 g/day) and 7 times (consumption of 12 g/day) the TDI.

The effects that continuous high intake of arsenic due to algae may have on consumers' health have not been studied. A toxicological evaluation carried out by Watanabe et al. (*18*) did not find that arsenic produced adverse effects in rats fed with diets containing species of *Hizikia*. However, the metabolic differences between species mean that the conclusions cannot necessarily be extrapolated to humans. Furthermore, it should

be borne in mind that epidemiological and clinical studies indicate that arsenic is a paradoxical human carcinogen that does not easily induce cancer in animal models (*30*). Because no toxicological studies are available as yet, it would seem to be a priority to protect the consumer by carrying out a monitoring program to establish maximum limits of inorganic arsenic content at an international level. The advisability of withdrawing from the market products that systematically have very high contents of the contaminant should not be ruled out.

Lead. The products derived from the brown algae *L. japonica* and *U. pinnatifida* had lead contents below the detection limit of the test method used (0.05 mg kg^{-1} dw). In the other samples the lead content varied between 0.15 and 1.33 mg kg^{-1} dw. The lowest values were found in three samples of arame, made from the brown alga *E. bicyclis* (0.15−0.19 mg kg^{-1} dw), whereas the highest content was found in green nori from the green alga *Enteromorpha* sp. The products derived from *H. fusiforme* varied more (0.53−0.89 mg kg^{-1} dw) than the other brown algae products analyzed. Finally, in the products made from red algae the lead content varied between 0.289 and 1.1 mg kg^{-1} dw.

The differences in lead content are not very pronounced, and it cannot be said that particular types of algae have higher lead contents: green algae, 0.93−1.33 mg kg^{-1} dw; red algae, 0.289−1.1 mg kg^{-1} dw; brown algae, 0.05−0.89 mg kg^{-1} dw. We must remember that the differences recorded for arsenic were as high as 100 mg kg^{-1} dw. The existing data in the bibliography indicate contents that vary from <0.01−163 mg kg^{-1} dw (*1*, *6*−*8*, *21*, *31*, *32*), with levels generally much higher than those found in this study.

The lead content in the samples analyzed in this study did not exceed the content permitted by specific legislation, such as that enacted in France, which limits the concentration of Pb to 5 mg kg^{-1} dw (*5*). With respect to the estimate of intake, assuming a daily consumption of 3 g and the maximum content of the contaminant found in this study (1.3 mg kg^{-1} dw), lead ingestion would be 2% of the TDI established by the WHO (0.24 mg of lead/day for a body weight of 68 kg). If the maximum consumption (12 g/day) is considered, the TDI would also not be exceeded.

Cadmium. The concentrations of cadmium found in this study range between 0.03 and 1.9 mg kg^{-1} dw. The lowest concentration was found in the green algae. Two samples of wakame had the highest concentrations in this study (1.5−1.9 mg kg^{-1} dw), followed by the products made from *H. fusiforme* (1.0−1.46 mg kg^{-1} dw), *E. bicyclis* (0.67−0.75 mg kg^{-1} dw), and the sample of Atlantic dulse (0.70 mg kg^{-1} dw). These values fall within the range found by other authors, 0.020−21 mg kg^{-1} dw (*1*, *6*−*8*, *21*, *32*). As with arsenic levels, the contents were higher in the brown algae tested (0.13−1.9 mg kg^{-1} dw) than in the red algae (0.18−0.70 mg kg^{-1} dw) and green algae (0.03−0.17 mg kg^{-1} dw).

In 10 of the samples analyzed the Cd content was higher than the amounts permitted by specific regulations, such as those enacted in France, which limit the concentration of Cd to 0.5 mg kg^{-1} dw (*5*), and 14 samples exceeded the limit of 0.2 mg kg^{-1} dw imposed in Australia (*33*).

In a worst-case scenario in terms of contaminant intake, that is, a maximum Cd level of 1.9 mg kg^{-1} dw, a consumption of 3 g of this alga would mean an ingestion of 0.006 mg/day of cadmium. This is 8% of the amount established by the WHO (0.068 mg of cadmium/day for a body weight of 68 kg). If consumption of 12 g/day is considered, the intake of Cd would be 34% of the TDI, a very high percentage given that this is the amount received from a single food product. Perhaps it would be advisable to study the toxicological risks related to intake of Cd to which population groups who eat substantial quantities of algae food products are exposed, because, as they are mainly extreme edible algae consumers, most of whom follow macrobiotic diets, they also eat large quantities of vegetables, a food group that also makes a considerable contribution to Cd intake.

Mercury. The algae food products analyzed have mercury levels ranging from 4 to 42 μg kg^{-1} dw. The lowest concentration was found in a sample of nori (*P. tenera*) and the highest in a sample of arame (*E. bicyclis)*. The level in brown algae (12−42 μg kg^{-1} dw) was higher than in red algae (4−14 μg kg^{-1} dw) and green algae (18−20.6 μg kg^{-1} dw).

These contents are lower than those previously described (*7*, *21*) and do not exceed the maximum limit permitted by French regulation (0.1 mg kg^{-1} dw) (*5*). A consumption of 3 or 12 g/day of the alga with the highest mercury content would mean an ingestion 500 and 100 times lower, respectively, than the TDI recommended by the WHO (0.049 mg of mercury/day for a body weight of 68 kg).

Conclusions. A significant number of the macroalgae food products analyzed exceed the contents of cadmium and inorganic arsenic permitted by the few specific laws that have been enacted with respect to these products. The high contents of inorganic arsenic found in *H. fusiforme*, much higher than those of the other edible algae analyzed, demonstrate the need to carry out a more in-depth, tailor-made study of the toxicological implications of inorganic arsenic in algae, which could give rise to specific restrictions on their sale.

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