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Original Article

Trace elements determination in edible seaweeds by an optimized and validated ICP-MS method

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1. Introduction

Marine algae have been consumed traditionally in Asia but only occasionally in other parts of the world (Nisizawa et al., 1987). The increasing popularity of Oriental cuisine and macrobiotic diets in Western countries in recent years has increased the demand for these marine vegetables. From a nutritional point of view, seaweeds are characterized by high concentrations of fibre and minerals (Burtin, 2003; Bocanegra et al., 2003), low fat content and, in some cases, relatively high protein levels (Galland-Irmouli et al., 1999).

Seaweeds may contain a high mineral content, as their cell wall polysaccharides and proteins contain anionic carboxyl, sulphate and phosphate groups that are excellent binding sites for metal retention (Davis et al., 2003). This biochemical cell wall feature makes algal biomass one of the most promising biosorbents available for the removal of toxic heavy metals from industrial wastewaters (Volesky and Holan, 1995; Hardisson et al., 1996;

ABSTRACT

An optimized and validated inductively coupled plasma mass spectrometry (ICP-MS) method was used to analyze trace elements in seaweeds. Different volumes and rates of HNO₃ and H₂O₂, digestion times, and microwave power levels were tested to ascertain the best conditions for sample digestion. Analytical mass and instrumental parameters were selected to assure accurate and precise determination of As, Cd, Co, Cr, Mo, Ni, Pb, Sb, Se, and V by ICP-MS. The method was optimized and validated using biological Certified Reference Materials. In addition, some samples of seaweeds (*Porphyra* and *Laminaria*) from France, Spain, Korea, and Japan were analyzed using the optimized method. *Porphyra* presented higher concentrations of most elements, except for As, than *Laminaria*. Seaweeds from Korea and Japan tended to display the highest concentrations of Pb and Cd. In contrast, Spanish and French samples showed the highest levels of some micro-elements essential to human nutrition.

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Storelli et al., 2001). However, the environmental characteristics of the water in which algae grow (salinity, turbidity, nutrient content, and heavy metal contamination) largely determine the mineral content they can absorb (Lozano et al., 2003; Riekie et al., 2006; Riget et al., 1997; Vasconcelos and Leal, 2001; Marinho-Soriano et al., 2006).

Under similar environmental conditions, mineral composition and content differ greatly between the different families, genera and species of macro-algae. Most of the limited information available on this subject comes from investigations carried out in Asia, as few systematic studies of seaweeds have been performed in Europe or North or South America (Mabeau and Fleurence, 1993; Rizvi and Shameel, 2001; Rupérez, 2002).

Research in this field is of great scientific importance as elements such as As, Cd, Pb, and Sb may be present in seaweeds as a result of environmental contamination while others, including Co, Cr, Mo, Ni, Se, and V are essential for humans. Little information on marine algae is currently available in Food Tables and more data would benefit future nutritional studies.

In order to quantify the mineral concentration in seaweed samples, numerous analytical procedures and techniques have been employed. Almela et al. (2006) used Graphite Furnace Atomic

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Absorption Spectrometry (GF-AAS) to determine As, Cd, and Pb content in seaweeds. This technique is sensitive enough to detect elements present in very small quantities but, like all monoelemental techniques, it is slow and tedious for routine use, particularly when a vast number of elements and samples must be analyzed. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) is a multi-elemental technique, but it is not sensitive enough to detect elements present at the ultra-trace level. Therefore, ICP-OES has been used only for macro-elemental analysis in seaweeds as Fe, Zn, Mg, Mn, and Ca (Munilla et al., 1995; Csikkel-Szolnoki et al., 2000; Domínguez-González et al., 2005).

Inductively Coupled Plasma Mass Spectrometry (ICP-MS), on the other hand, is a very sensitive and precise analytical technique that permits simultaneous determination of a wide range of trace and ultra-trace elements in various biological and pharmaceutical samples, with detection limits in the order of ng·g⁻¹, similar to Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS) (Le Lamer-Déchamps et al., 2003; Huang et al., 2006). However, polyatomic interferences resulting from the combination of matrix ions with Ar may affect this technique. To assure correct results, some of the interferences must be eliminated or controlled by microwave digestion at high temperatures while others have to be corrected using a mathematical equation.

ICP-MS has been validated for analysis of trace metals in plants (Leiterer et al., 1997). However ICP-MS is currently being used to detect metals in seaweeds (van Netten et al., 2000), and algae food products (Dawczynski et al., 2007), but no validation of this method for use in this biological material has yet been published. Thus, the first goal of this study was to optimize and validate the ICP-MS method to determine As, Cd, Pb, Sb, Co, Cr, Mo, Ni, Se, and V in seaweeds. The second goal was to apply the validated method to determine the trace element content of two edible classes of seaweeds (*Porphyra* and *Laminaria*) from two European locations (France and Spain) and two Asian locations (Korea and Japan) in order to enhance the nutritional knowledge of edible seaweeds.

2. Materials and methods

2.1. Instrumentation

A Conterm dry oven from EUROLAB LTD. (Barcelona, Spain) was employed to dry algae samples. Acid digestion of the seaweed samples was performed using a commercial high-pressure laboratory microwave oven (Milestone Ethos 1600 Microwave Labstation, Sorisole, Italy), operating at a frequency of 2450 Hz, with an energy output of 900 W. This microwave digestion system was equipped with ten 100 mL TFM (tetrafluoromethoxy) Teflon closed vessels, and temperature in the interior of the vessels can be monitored with the 300 Automatic Temperature Control Probe. Maximum operating temperature and pressure were 300 °C and 100 bar, respectively.

A VARIAN UltraMass Quadrupole Inductively Coupled Plasma Mass Spectrometer (Varian, Inc. Scientific Instruments Lake Forest, USA) was used for analytical determinations. Operating conditions of the ICP-MS for the analysis of the seaweed samples are shown in Table 1. The instrumental parameters were optimized with a solution containing 10 μ g·L⁻¹ of Be, Ba, Ce, Co, In, Pb, Mg, Tl, and Th (Inorganic Ventures, Lakewood NJ, USA), and were employed for routine laboratory work.

2.2. Trace element determination

As, Cd, Cr, Ni, and V were analyzed in concentrations of $\mu g \cdot g^{-1}$, whereas Co, Mo, Pb, Sb, and Se were analyzed in concentrations of $ng \cdot g^{-1}$. Five measurements were performed from each sample replicate.

Table 1

Operating conditions for ultra-mass ICP Mass Spectrometer.

Parameter	Value
Ins	strument
Spray chamber	Sturmann-Masters
Sample cone	Nickel, 1 mm orifice diameter
Skimmer cone	Nickel, 0.75 mm orifice diameter
Sample update rate	1.0 mL/min
Sample read delay	35 s
Plasm	a conditions
Radiofrequency generator	40 MHz
Incident power	1100 W
Plasma Ar flow rate	16.5 L/min
Nebulizer Ar flow rate	0.87 L/min
Auxiliary Ar flow rate	1.05 L/min
Mass Spec	trometer setting
Scanning mode	Peak Hopping
Resolution	High
Integration time	900 ms
Dwell time	300 ms
Number of readings per replicate	1
Number of replicate	6

2.3. Reagents

All closed vessels, polyethylene flasks and plastic containers were cleaned with HNO_3 (65% w/v, diluted 1/10 with high-purity water) for 24 h and rinsed with high purity water. Subsequently, all material was dried at 50 °C and stored under clean-air conditions. All plastic containers, polyethylene flasks, pipette tips, PFA Teflon digestion vessels (Milestone SRL, Sorisole, Italy) and reagents that came into contact with the samples or standards were checked for contamination. All solvents and reagents were of the highest commercially available purity grade.

De-ionized water with a resistivity of $\geq 18 \text{ M}\Omega \cdot \text{cm}^{-1}$ was obtained by means of a Milli-Q Plus pure water generating system from Millipore (Millipore Molsheim, France) and was employed to prepare all standard and sample solutions. Suprapur grade (Merck, Germany) 65% HNO₃ and 30% w/v H₂O₂ were used for sample dissolution. Monoelemental, high-purity grade 1 g·L⁻¹ stock solutions of As, Cd, Co, Cr, Ge, Mo, Ni, Pb, Re, Rh, Sb, Se, and V were purchased from Merck (Darmstadt, Germany). Plasma torch argon purity was higher than 99.999%.

2.4. Samples

The following Certified Reference Materials (CRMs), which covered the range of the elements studied, were selected: BCR 279 (Sea lettuce, *Ulva lactuca*) for As, Cd, Cr, and Se from Community Bureau of Reference. NCS ZC78006 (Tea) for Co, and Sb from China National Analysis Center for Iron and Steel; NIST 1547 (Peach leaves) for Ni, Pb, Mo, and V from National Institute of Standards & Technology.

Two edible seaweed genera (*Porphyra* and *Laminaria*) of different origins were used. Seaweeds were harvested in geographical areas from Europe and Asia where traditionally they have been used for human consumption. Seaweeds from Japan (Koyo Food Ltd.: Kunga and Mitoku) and Korea (Wesbrag Ltd.: Yang-Tse) were purchased in a local market. Spanish seaweeds were supplied by Algas Atlánticas Algamar S.L. which were harvested in the Spanish northwest coast (Redondela, Galicia) and French samples were harvested in the France northwest coast (Brest, Brittany) by the authors in summer 2004 and they were dried up at low temperatures. Samples from Korea, Japan, and Spain were analyzed just as they were sold. Dried seaweeds were stored in air-tight containers, as they are hygroscopic until undergoing pre-

2.5. Sample preparation and digestion

Approximately 1 g of each dehydrated seaweed sample was rinsed for 3 min with 50 mL ultra-pure water to remove salts, and oven-dried at 55 ± 5 °C for 24 h. Subsequently, seaweed samples and CRMs were homogenized and ground in an agate ball mixer mill to reduce particle size to a maximum of 30 μ m.

In order to optimize digestion, several modalities were assayed: (1) Different concentrations of HNO_3 (from 2.5 mL to 5 mL, in intervals of 0.5 mL) and H_2O_2 (from 0 mL to 2.5 mL, in intervals of 0.5 mL) in 5 mL of acid mixture; (2) Various digestion times (from 5 min to 10 min, in 1 min intervals); and (3) Several microwave oven powers (from 200 W to 650 W, in intervals of 50 W).

The method that used the lowest volume, power, and digestion time was considered the more convenient and finally selected. Two replicate solutions of each sample were prepared by microwave-assisted acid digestion. A set of digestion blanks was also prepared and subjected to the same microwave procedure. A 0.5000 ± 0.0001 g portion of each dry seaweed and CRM was weighed and introduced into a high-pressure closed Teflon decomposition vessel. Then, 5 mL of previously optimized HNO₃ and H₂O₂ mixture was carefully added to each sample and the vessels were gently shaken and sealed. A total of ten sub-samples were evenly spaced on the microwave turntable. Digested samples were transferred to a 50 mL volumetric flask and made up to volume with de-ionized water.

2.6. Test solution and calibration

A multi-elemental standard working solution with the elements to be analyzed at a concentration of $1 \text{ mg} \cdot \text{L}^{-1}$ was prepared by step-wise dilutions of monoelemental stock solutions. This test solution contained 4.8 μ g·L⁻¹ of Rh and 20 μ g·L⁻¹ of Ge and Re that were used as internal standards to correct instrumental drift and possible matrix effects. All the solutions were 1% (V/V) in HNO₃. For calibration, four multi-elemental solutions were prepared with the following concentrations: 0, 3, 10 and 40 ng·mL⁻¹ for As; and 0, 0.8, 3.0 and 10.0 ng·mL⁻¹ for Cd, Co, Cr, Mo, Ni, Pb, Sb, Se, and V.

2.7. Selection of analytical mass

Elements with the highest isotopic abundance were selected as analytical mass, except in the case of Ni and Se (Table 2). These analytical masses are free from isobaric and polyatomic interferences; however, 75As and 51 V would be the only isotopes that high concentrations of chlorine ion could seriously interfere with. Thus, in the present study polyatomic interferences in 75As and 51 V were determined and controlled from chlorine 40Ar35Cl and 35Cl16O, respectively. Solutions with increasing NaCl concentra-

Table 2 Analytical mass selected.

Element	Mass (m/z)			
As	75			
Cd	114			
Со	59			
Cr	52			
Мо	98			
Ni	60			
Pb	206			
Sb	121			
Se	77			
V	51			

tions in high-purity water, covering the 0-3% range, with and without 3 μ g·L⁻¹ of As and V, respectively, were prepared.

2.8. Validation studies

The assays were validated with selected CRMs to assess withinand between-run accuracy and precision. For the within-run assessment, the accuracy and precision of the assays were examined in a single run, with six replicate samples. For the between-run assessment, each CRM was analyzed in six separate runs. Accuracy was defined as the percentage difference between the observed concentration and the expected value (%D). Precision was expressed as the percentage of the variation coefficient (%CV).

Acceptance criteria for accuracy and precision, based on ICH (1996) rules, were as follows: the mean %D and %CV could not exceed $\pm 15\%$; 75% of all samples had to be within $\pm 15\%$ of expected concentrations; no more than one of the six samples within a given concentration could exceed $\pm 15\%$ of their expected values.

Sensitivity of the determination of each chemical element at a specific isobaric mass, indicated in Table 2, was expressed by the slope of the linear regression equation (cps·ng⁻¹·mL). Linearity was assessed by the correlation coefficients of calibration curves and was considered acceptable when $r \ge 0$. 9995. Detection limits were calculated following the IUPAC rules (IUPAC, 1997; Thomsen et al., 2003) on the basis of the 3 σ criterion for ten replicate measurements of blank solutions subjected to the same treatment as the samples.

2.9. Statistical analysis

Two-way ANOVA (genus and country) was performed. When a significant genus–country interaction was found, the country effect was separately tested in *Porphyra* or *Laminaria* by post hoc analysis (Bonferroni test). The effect of genus on each mineral content was tested by unpaired Student's *t*-test. Results were considered significant at p < 0.05. Statistical analysis packages (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Optimization of the sample digestion procedure

The optimized digestion method employed is shown in Table 3. The optimal proportion of HNO_3 and H_2O_2 was 4.5:0.5 (V/V). The proposed digestion method led to a total and simultaneous dissolution of ten samples in only twenty-three min. Other additional advantages of this method include the small amount of reagent used (5 mL), which significantly reduces potential environmental contamination. Moreover, shorter digestion time is required in comparison to traditional methods as those proposed by Rodushkin et al. (1999), Wang et al. (2006), and Farías et al. (2002).

3.2. Selection of analytical masses

The analytical masses selected to perform determinations in the absence of isobaric interferences are indicated in Table 2. The

Table 3Optimized program of sample digestion.

Step	Time (min)	Power (W)
1	2	250
2	2	0
3	6	250
4	5	400
5	2	0
6	6	600

Table 4

Within-run assay accuracy and precision for Certified Reference Materials.

Element	Certified Reference Material	Certified values ($\mu g \cdot g^{-1} DM$)	Within-run assay			
			Found values ($\mu g \cdot g^{-1} DM$)	%D	%CV	
As	BCR 279 Sea lettuce (Ulva lactuca)	3.09 ± 0.20	3.26 ± 0.18	+5.52	5.52	
Cd	BCR 279 Sea lettuce (Ulva lactuca)	0.274 ± 0.022	0.277 ± 0.025	+1.12	9.02	
Со	NCS ZC78006 Tea	(0.20)	0.19 ± 0.02	-5.5	7.89	
Cr	BCR 279 Sea lettuce (Ulva lactuca)	(9.70-11.6)	10.07 ± 0.85	+3.82	8.44	
Ni	NIST1547 Peach leaves	0.69 ± 0.09	0.73 ± 0.10	+5.55	7.28	
Мо	NIST1547 Peach leaves	0.060 ± 0.008	0.057 ± 0.006	-5.00	10.52	
Pb	NIST1547 Peach leaves	0.87 ± 0.03	0.88 ± 0.04	+1.15	9.32	
Sb	NCS ZC78006 Tea	0.037 ± 0.003	$\textbf{0.039} \pm \textbf{0.003}$	+5.40	7.69	
Se	BCR 279 Sea lettuce (Ulva lactuca)	0.593 ± 0.032	$\textbf{0.667} \pm \textbf{0.078}$	+12.50	11.69	
V	NIST1547 Peach leaves	0.37 ± 0.03	$\textbf{0.36} \pm \textbf{0.03}$	-2.70	6.94	

Results are mean ± standard deviation of samples analyzed six times at the same assay. The value in () is for reference only, they are not certified values.

Table 5

Between-run assay accuracy and precision for Certified Reference Materials.

Element	Certified Reference Material	Certified values ($\mu g \cdot g^{-1} DM$)	Between-run assay			
			Found values ($\mu g \cdot g^{-1} DM$)	%D	%CV	
As	BCR 279 Sea lettuce (Ulva lactuca)	3.09 ± 0.20	3.38 ± 0.17	+.38	5.02	
Cd	BCR 279 Sea lettuce (Ulva lactuca)	0.274 ± 0.022	0.279 ± 0.023	+1.82	8.24	
Со	NCS ZC78006 Tea	(0.20)	0.19 ± 0.02	-5.0	8.42	
Cr	BCR 279 Sea lettuce (Ulva lactuca)	(9.70-11.6)	10.10 ± 0.95	+4.12	9.40	
Ni	NIST1547 Peach leaves	7.61 ± 0.48	$\textbf{7.28} \pm \textbf{0.63}$	-4.33	8.65	
Мо	NIST1547 Peach leaves	0.060 ± 0.008	0.055 ± 0.007	-8.33	12.73	
Pb	NIST1547 Peach leaves	1.06 ± 0.10	1.12 ± 0.10	+5.66	8.92	
Sb	NCS ZC78006 Tea	0.037 ± 0.003	0.040 ± 0.002	+8.12	5.0	
Se	BCR 279 Sea lettuce (Ulva lactuca)	0.593 ± 0.032	0.670 ± 0.075	+12.98	11.19	
V	NIST1547 Peach leaves	$\textbf{0.37} \pm \textbf{0.03}$	0.35 ± 0.02	-5.40	6.85	

Results are mean ± standard deviation of samples analyzed in six different assays. The value in () is for reference only, they are not certified values.

potential polyatomic spectral interference of ArCl species on 75As and 51 V affected by chlorine ion was not observed in the present study. The amount of chlorine ion in other types of marine plants should be measured before analyte determination, and only corrected by mathematical equations when this measure affects these analytical masses.

The proposed microwave digestion method avoided potential polyatomic interferences on 52Cr and 59Co isotopic mass by 40Ar12C and 43C16O, respectively. No problems with isobaric interferences arose in the determination of Cd, Pb, Sb, and Mo with the ICP-MS method. These findings coincide with those of Caroli et al. (1999). The 60Ni isotope was chosen for Ni determination, as its use avoids interferences with 23Na35Cl or 58Fe when 58Ni is analyzed.

3.3. Results of validation of methodology

Within and between-run accuracy and precision of the assays are summarized in Tables 4 and 5. The percentage difference between the observed concentration and the expected value (%D) was minimum for Cd and maximum for Se in both the within-run and between-run assays. The % coefficient of variation was minimum for As and maximum for Se and Mo in both types of assays. In all cases, the observed values were within the acceptance criteria outlined above. Good agreement with certified concentrations in CRMs was found in the present study (Tables 4 and 5).

Sensitivities were maximum for V (15574.70 cps ng^{-1} mL) and minimum for Se (95.04 cps ng^{-1} mL). Based on their sensitivity, the elements studied can be ordered according to the following sequence: V > Cr > Ni > Co > Mo > Sb > Cd > As > Pb > Se. The correlation coefficients for the majority of the calibration curves were at least 0.998; thus, displaying good linear relationships throughout the concentration range studied. Consequently, the analytical masses shown in Table 2 were considered optimum for the proposed method. Detection limits are indicated in Table 6. They were comparable with those obtained by other authors in similar samples (Harrington et al., 1999) and appropriate for application in the determination of seaweed mineral content.

3.4. Trace elements in seaweeds

The results of the elemental analysis of seaweed samples are summarized in Tables 7 and 8. With the exception of Co, Ni, and Sb values, mineral content of *Porphyra* (Rhodophyceae) and *Laminaria* (Phaeophyceae) differed significantly (p < 0.05). Struck et al. (1997); Phaneuf et al. (1999); Rupérez (2002) and van Netten et al. (2000) have also reported a wide variation in mineral concentration in different marine algae species, even when differences in environmental factors were minimized (Farías et al., 2002).

The order of element concentrations determined in this study for *Porphyra* was As > Cd > V > Ni > Cr > Pb > Se > Mo > Co > Sb, while that of *Laminaria* was As > V > Cd > Ni > Cr > Pb > Se > Co > Mo > Sb. *Porphyra* contained higher concentrations of most of

Table 6	
Limits of detection of elements.	

Elements	Limits of detection $(\mu g \cdot g^{-1})$
As	$1.33 imes 10^{-3}$
Cd	2.79×10^{-4}
Со	$1.38 imes 10^{-4}$
Cr	4.36×10^{-4}
Мо	$1.54 imes10^{-4}$
Ni	$1.32 imes 10^{-3}$
Pb	$1.74 imes10^{-3}$
Sb	$4.12 imes 10^{-4}$
Se	$2.50 imes 10^{-2}$
V	$5.23 imes10^{-5}$

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Trace elements determined by ICP-MS in *Porphyra* and *Laminaria* from different locations.

Element	Genus	$\text{Mean}\pm\text{SD}$	<i>p</i> < 0.05	95% Confidenc interval
Co (ng·g ⁻¹ DM)	Porphyra Laminaria	$\begin{array}{c} 182\pm58\\ 178\pm96 \end{array}$	NS	155–209 133–223
Mo (ng·g ⁻¹ DM)	Porphyra Laminaria	$\begin{array}{c} 438\pm368\\ 103\pm53 \end{array}$	*	266–610 78–127
Pb (ng·g ^{-1} DM)	Porphyra Laminaria	$\begin{array}{c} 513\pm212\\791\pm519\end{array}$	*	414–612 548–1034
Sb ($ng \cdot g^{-1}$ DM)	Porphyra Laminaria	$\begin{array}{c} 30.5\pm24.9\\ 31.6\pm7.0 \end{array}$	NS	18.8–42.2 28.3–34.8
Se ($ng \cdot g^{-1}$ DM)	Porphyra Laminaria	$\begin{array}{c} 463\pm67\\ 334\pm151 \end{array}$	•	431–494 263–405
As (µg·g ^{−1} DM)	Porphyra Laminaria	$\begin{array}{c} 7.3\pm2.3\\ 43.5\pm10.3\end{array}$	•	6.2-8.3 38.6-48.3
Cd ($\mu g \cdot g^{-1}$ DM)	Porphyra Laminaria	$\begin{array}{c} 2.4\pm1.2\\ 1.0\pm1.1 \end{array}$	•	1.9–2.9 0.48–1.55
$Cr (\mu g \cdot g^{-1} DM)$	Porphyra Laminaria	$\begin{array}{c} 1.0\pm0.2\\ 0.9\pm0.2\end{array}$	•	0.91–1.07 0.77–0.95
Ni (µg∙g ^{−1} DM)	Porphyra Laminaria	$\begin{array}{c} 1.1\pm1.1\\ 1.0\pm0.2 \end{array}$	NS	0.62-1.61 0.86-1.04
$V (\mu g \cdot g^{-1} DM)$	Porphyra Laminaria	$\begin{array}{c} 2.4\pm1.9\\ 1.3\pm1.1 \end{array}$	•	1.5–3.2 0.78–1.83

Values are Mean \pm SD of six samples. NS: Not significant.

 * p < 0.05 significant differences for the same element.

the minerals analyzed than *Laminaria*, while the latter contained much higher levels of As (Table 7). Dawczynski et al. (2007) showed that in the Phaeophyceae mean contents of As were about ten times higher than in Rhodophyceae.

As content in *Laminaria* samples is in the same order than *Laminaria* analyzed by Almela et al. (2002) that was 47–53 μ g·g⁻¹ DM but higher than that analyzed by Dawczynski et al. (2007) (8.42 \pm 2.26 μ g·g⁻¹ DM in food products samples). In fact, the first author pointed out a relation in type of seaweed with As content: Phaeophyceae > Rhodophyceae.

Differences in the biosorption of metals by seaweeds may be explained by differences in the amount and composition of polysaccharides in seaweed cell walls (Burtin, 2003; Davis et al., 2003). The principal components in cell walls of brown alga (e.g. *Laminaria*) are cellulose, fucoidan, alginic acids, and alginates (of which the most important are fucans and laminarans). Consequently, their main active groups are negatively charged functional ones, such as carboxyl and sulphate. Red seaweeds (e.g. *Porphyra*) also contain cellulose but their biosorption properties are characterized by their content in sulphated polysaccharides made of galactans (such as agar and carrageenans) and xylans (Loban and Harrison, 1994; Jimenez-Escrig and Sánchez-Muniz, 2000).

Other factors, including the environmental features of each region (water temperature, pH and salinity), may affect metal accumulation (Struck et al., 1997; Lodeiro et al., 2005; Marinho-Soriano et al., 2006). The relative abundance of a certain element in seawater and its bioavailability may also affect electrostatic adsorption (Sánchez Rodríguez et al., 2001). Moreover, presence of others metals in the seawater can also modulate seaweed metal content. Sánchez Rodríguez et al. (2001) found a linear correlation between some pairs of elements. Other factors that have an important bearing on the concentrations of some metals in seaweeds include the seasonality of the sampling (Hou and Yan, 1998; Vasconcelos and Leal, 2001) and the age of the fronds, which become more retentive over time (Farías et al., 2002).

In the present study, Asian tested seaweeds presented higher levels of Pb and Cd (Pb: 623–1265 ng·g⁻¹ DM; Cd: 1.6–3.1 ng·g⁻¹ DM, data not shown) than their European counterparts (Pb: 317– 403 ng·g⁻¹ DM, Cd: 0.40–1.70 ng·g⁻¹ DM, data not shown), possibly reflecting different levels of environmental pollution. Pollution reports from many parts of the world including China (Almela et al., 2006), India (Rao et al., 2007), Korea (Munilla et al., 1995; Almela et al., 2006), Japan (Munilla et al., 1995; van Netten et al., 2000; Almela et al., 2006), Spain (Almela et al., 2006; Moreda-Piñeiro et al., 2007), Italy (Caliceti et al., 2002), Argentina (Pérez et al., 2007) and Canada (Sharp et al., 1988; Phaneuf et al., 1999; van Netten et al., 2000) offer data regarding Pb levels in seaweeds. Concentrations of this element vary widely between areas studied, but most authors report the highest levels in Japan and Korea.

Table 8

Results of analysis for trace and ultratrace elements from Porphyra and Laminaria from different European and Asian countries.

Element	Genus	France	Spain	Korea	Japan	Genus	Country	Interaction
Co (ng·g ^{-1} DM)	Porphyra	$256\pm31\text{+}^{c}$	$207\pm15\text{+}^{b}$	$142\pm23\text{+}^{a}$	$112\pm15\text{+}^{a}$	NS	***	***
	Laminaria	$\textbf{72.8} \pm \textbf{8.7}^{a}$	99.8 ± 8.8^{b}	253 ± 14^{c}	287 ± 8^{d}			
Mo $(ng \cdot g^{-1} DM)$	Porphyra	$1030\pm40\text{+}^{c}$	$408 \pm 13 \texttt{+}^{b}$	$174\pm48^{\text{a}}$	$140\pm17\text{+}^{a}$	***	•••	***
	Laminaria	$48.4\pm4.4^{\text{a}}$	72.8 ± 3.9^{b}	183 ± 8^{d}	106 ± 10^{c}			
Pb (ng·g ^{-1} DM)	Porphyra	312 ± 22 + ^a	$493 \pm 14 \text{+}^{\text{c}}$	$397 \pm \mathbf{32+^b}$	$848 \pm 53 \textbf{+}^{d}$	***	•••	***
	Laminaria	249 ± 21^{a}	$385 \pm \mathbf{14^b}$	1488 ± 131^{d}	1042 ± 51^{c}			
Sb (ng·g ^{-1} DM)	Porphyra	$23.0 \pm \mathbf{5.7^b}$	$17.0\pm5.4\text{+}^{b}$	$11.0\pm4.4\textbf{+}^{a}$	$71.00 \pm 6.52 + c$	NS	***	***
	Laminaria	$29.0 \pm \mathbf{2.6^b}$	29.2 ± 4.1^{b}	40.8 ± 7.5^{c}	27.20 ± 2.77^a			
Se (ng·g ⁻¹ DM)	Porphyra	$502\pm22\text{+}^{c}$	$536\pm22\text{+}^{c}$	$375\pm24\text{+}^{a}$	$438 \pm 32 \texttt{+}^{b}$	***	***	***
	Laminaria	575 ± 24^{d}	231 ± 13^{b}	201 ± 8^a	328 ± 9^{c}			
As (µg·g ^{−1} DM)	Porphyra	$4.25\pm0.13\textbf{+}^{a}$	$\textbf{8.77} \pm \textbf{1.36+^c}$	$\textbf{6.28} \pm \textbf{0.19+^{b}}$	$9.70 \pm 1.20 \text{+}^{\text{c}}$	***	***	***
	Laminaria	$53.1\pm2.9^{\rm c}$	30.1 ± 7.0^{a}	40.02 ± 3.82^b	50.7 ± 3.8^{c}			
Cd ($\mu g \cdot g^{-1}$ DM)	Porphyra	$\textbf{2.88} \pm \textbf{0.13+}^{b}$	0.46 ± 0.06^a	$3.07\pm0.07\textbf{+}^c$	$3.18\pm0.08\text{+}^{c}$	***	***	***
	Laminaria	0.46 ± 0.08^{b}	0.35 ± 0.10^{b}	0.31 ± 0.07^a	2.95 ± 0.19^{c}			
$Cr (\mu g \cdot g^{-1} DM)$	Porphyra	$0.81\pm0.10\text{+}^{a}$	$1.19\pm0.13\text{+}^{b}$	0.95 ± 0.12^{a}	$1.04\pm0.11\text{+}^{ab}$	***	**	***
	Laminaria	0.98 ± 0.06^{b}	0.65 ± 0.04^a	1.09 ± 0.06^{c}	0.73 ± 0.04^{a}			
Ni (µg·g ^{−1} DM)	Porphyra	$1.22\pm0.20\text{+}^{b}$	$2.76\pm0.16\text{+}^{c}$	$0.27\pm0.07\textbf{+}^{a}$	$0.23\pm0.04\text{+}^{a}$	***	***	***
	Laminaria	0.65 ± 0.04^{a}	1.01 ± 0.074^{b}	1.14 ± 0.04^{c}	1.00 ± 0.07^{b}			
V (µg·g ^{−1} DM)	Porphyra	$5.06\pm0.13\text{+}^{c}$	$3.05\pm0.16\text{+}^{b}$	$0.66\pm0.11\text{+}^{a}$	0.70 ± 0.08^{a}	***	***	***
	Laminaria	0.76 ± 0.03^a	0.67 ± 0.02^a	$\textbf{3.2}\pm0.16^{b}$	0.60 ± 0.07^a			

Values are Mean \pm SD of six samples. '+' Significantly different between alga for the same element and country (p < 0.05). Values for the same alga and element bearing different letters (a, b, c and d) are significantly different (at least p < 0.05). NS = Not significant.

p < 0.01.p < 0.001. Cd levels in the present study were higher in *Porphyra* than in *Laminaria* (Table 7). It agrees with Dawczynski et al. (2007) (*Porphyra* 1.13 \pm 1.06 µg·g⁻¹ DM and *Laminaria* 0.59 \pm 0.21 µg·g⁻¹ DM). On the other hand, results presented in Table 8 indicate that the country of origin represents a significant cause for variation (p < 0.001) in the concentrations of the elements studied. Thus, the highest Cd levels were found in Japanese *Porphyra* samples. Almela et al. (2006) found a wide range of concentrations (0.019–3.09 µg·g⁻¹) for Cd in *Porphyra* from different origins, including Korea and Japan. van Netten et al. (2000), however, reported lower Cd levels in *Porphyra* from Japan (0.27 and 0.83 µg·g⁻¹).

In the present study, Cr values of the two algae genera assayed were significantly different, except in the case of the Korean seaweeds. Cr in studied *Porphyra* is present in a narrow range from 0.81 to 1.19 μ g·g⁻¹ DM and those were similar to reporting by Moreda-Piñeiro et al. (2007) in seaweeds from the Galician Coast (1.31 ± 0.1 μ g·g⁻¹ DM) and Caliceti et al. (2002) in Venice lagoon (0.5–1.0 μ g·g⁻¹ DM), and van Netten et al. (2000) from Japanese samples (<0.5–2.0 μ g·g⁻¹ DM). Values in *Laminaria* agree with those of van Netten et al. (2000) (<0.5–1 μ g·g⁻¹ DM) from Japan and Phaneuf et al. (1999) (1.47 ± 0.94 μ g·g⁻¹ DM) from Canada.

Very high levels of Mo were found in European *Porphyra* samples, especially those from France. Similar Mo content was found by Pérez et al. (2007) in *Porphyra* from Argentina (1.18–1.99 μ g·g⁻¹ DM). Concentrations of elements (Co, Ni, and V) in human food are of great interest, due to their bioactivity and role in human metabolism. The highest levels of Co, Ni, and V in the present study were also found in European *Porphyra* (232 ± 34 ng·g⁻¹ DM, 2.0 ± 0.8 μ g·g⁻¹ DM, 4.1 ± 1.1 μ g·g⁻¹ DM, respectively).

Significant differences in Ni levels between seaweed genera from each location were found (Table 8). Ni levels in *Porphyra* concur with those reported in *Porphyra* columbina (Pérez et al., 2007) (0.74–1.51 μ g·g⁻¹ DM), are slightly higher than those of *Porphyra* vietnamensis (Rao et al., 2007) (0.13 ± 0.01 μ g·g⁻¹ DM) but much lower that those reported in Japanese *Porphyra* tenera (van Netten et al., 2000) (10.17 μ g·g⁻¹ DM). In the current study the highest *Laminaria* Ni levels were found in samples from Korea and there were no differences in samples from Spain and Japan. van Netten et al. (2000) found a wider range (<0.05–1.31 μ g·g⁻¹ DM) in Japanese samples.

Except in Japanese algae, V levels differed significantly between *Porphyra* and *Laminaria*. van Netten et al. (2000) precisely in Japanese samples reported high differences between this seaweed genera (*Porphyra* 1.53–3.96 μ g·g⁻¹ DM and *Laminaria* 0.43–1.17 μ g·g⁻¹ DM). In this work, much higher content of this element for *Porphyra* samples was observed in European locations (4.1 ± 1.1 μ g·g⁻¹ DM, data not shown)) than in Asian locations (0.7 ± 0.1 μ g·g⁻¹ DM, data not shown). Those agree with Farías et al. (2007) in *Porphyra* samples from Argentine coast (3.52–4.54 μ g·g⁻¹ DM) but it does not agree with van Netten et al. (2000).

Present Co data coincide with values reported by Moreda-Piñeiro et al. (2007) from Galicia (Spain) (*Porphyra* $310 \pm 10 \text{ ng} \text{·g}^{-1}$ DM and *Laminaria* $<200 \text{ ng} \cdot \text{g}^{-1}$ DM), but were lower than the concentrations observed by van Netten et al. (2000) from Japan (*Porphyra* 470–566 ng \cdot \text{g}^{-1} DM and *Laminaria* 84–449 ng \cdot \text{g}^{-1} DM) and Pérez et al. (2007) from Argentina (*Porphyra* 380–550 ng \cdot \text{g}^{-1} DM). It has been observed that Co levels were similar in both studied genera

No previously published information is available for Sb concentrations in the two genera of algae tested in the present study. We observed similar Sb levels in *Porphyra* and *Laminaria*, and the highest concentrations were found in the Japanese and Korean samples. Hou and Yan (1998) found 0.121 ng·g⁻¹ Sb in Chinese Pheaeophyceae and 0.141 ng·g⁻¹ Chinese, Rhodophyceae.

Se content of seaweeds in the present study was similar to that reported by van Netten et al. (2000) (*Porphyra* 200 ng·g⁻¹ DM and

Laminaria $<10-570 \text{ ng} \cdot \text{g}^{-1}$ DM). *Porphyra* from Spain and France contained the highest Se levels (Table 8).

4. Conclusions

The optimization and validation of the Inductively Coupled Plasma Mass Spectrometry method permits accurate and precise analysis of As, Cd, Co, Cr, Mo, Ni, Pb, Sb, Se, and V in seaweeds. Applied to seaweeds destined for human consumption, this validated method could help to identify the levels of numerous toxic and bioactive elements in marine algae. In addition to helping complete Food Composition Tables, this method responds to consumer demands for stricter controls by guaranteeing the quality of seaweeds marketed for human consumption.

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