Spectrochimica Acta Part B 131 (2017) 74–78

Contents lists available at ScienceDirect

Spectrochimica Acta Part B

journal homepage: www.elsevier.com/locate/sab

Analytical note

Seasonal determination of trace and ultra-trace content in *Macrocystis pyrifera* from San Jorge Gulf (Patagonia) by Total Reflection X-ray Fluorescence

Vanesa N. Salomone [a,b,*], Marina Riera [a,b,c], Luciana Cerchietti [d], Graciela Custo [d], Claudia Muniain [a,b]

[a] Instituto de Investigación e Ingeniería Ambiental, Universidad Nacional de San Martín, San Martín, Buenos Aires, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[c] Dpto. Biología General, Universidad Nacional de la Patagonia San Juan Bosco, Comodoro Rivadavia, Argentina
[d] Laboratorio de Fluorescencia de Rayos X, Centro Atómico Constituyentes, Comisión Nacional de Energía Atómica, Argentina

ARTICLE INFO

Article history:
Received 13 October 2016
Received in revised form 12 February 2017
Accepted 7 March 2017
Available online 14 March 2017

Keywords:
TXRF
Seaweed
Multi-element analysis
Arsenic

ABSTRACT

Seaweed have a great capacity to accumulate heavy metals in their tissues. The chemical characterization of seaweed is important due to their use in environmental monitoring and human or animal food. The aim of the present study was to evaluate the multi-elemental composition of seaweed from San Jorge Gulf (Patagonia, Argentina) by Total Reflection X-ray Fluorescence (TXRF). The elements As, Br, Cu, Cr, Fe, Mn, Ni, Pb, Rb, Sr, V and Zn were seasonally analyzed and quantified in blades of *Macrocystis pyrifera*. TXRF showed to be a suitable technique for simultaneous multi-element analysis in this kind of samples. The results revealed seasonal variations in the chemical content for some elements; arsenic content was maximum in summer and autumn, iron concentration increased to the winter and zinc concentration was maximum in autumn. The sum of principal micronutrients (Fe + Zn + Mn + Cu) varied between 114 and 171 mg k^{-1} g dw. The total As concentration ranged between 36 and 66 mg kg^{-1}. Lead, nickel and copper were not detected.

1. Introduction

Seaweed are a commercially important, renewable marine resource [1]. The use of macro algae has traditionally focused on the extraction of chemicals components used by pharmaceutical, cosmetics and food industries (production of agar, alginates, carrageenan, etc.). In recent decades, there has been an increase in direct consumption of seaweed in human food (as vegetables and condiments), given their high nutritional value and therapeutic benefits that they provide [2,3]. Interestingly, the demand for algae is also increasing due to its introduction in different areas such as natural fertilization, bioremediation, aquaculture foods and farmer animals, and more recently biofuels and biofilter applications [4,5].

Algae (in particular brown macro algae) are recognized to accumulate metals and metalloids up to levels many times larger than those found in the environment [6,7]. Arsenic occurrence, principally in the form of inorganic arsenic species, is especially important from both an environmental and health standpoint. Farias et al. [8] analyzed macroalgae species from the Antarctic, and they reported that total arsenic concentration was higher in brown than in red macroalgae. Díaz et al. [9] found that the arsenic present in brown algae was primarily as organic arsenic. Studies on heavy metals presence in seaweed used for compost or food reported that toxic elements could represent a risk [10]. Despite their importance, there are few studies on heavy metals in commercially available seaweed [11–15]. Many works have reported the mineral content and chemical composition of brown algae like *Fucus* sp., *Laminaria* sp. and *Undaria pinnatifida* (wakame), among others [11–13]. However, available information on *Macrocystis pyrifera* is limited [9,16–19].

M. pyrifera or giant kelp is a perennial brown macro alga native of Patagonia, Argentina. It is characterized to form dense 'forests', which are the base of many coastal food webs. Primarily, the commercial interest in *M. pyrifera* was related to the production of alginates. Currently, its demand has increased due to the introduction of new uses such as production of bioenergy and animal food, among others [20–22]. In this context, the ability of *M. pyrifera* to remove heavy metals was evaluated [23–25].

There are a variety of techniques and procedures to identify and quantify chemicals such as heavy metals or metalloids, with high sensitivity in algae even in trace amounts. Among them, TXRF analysis is a powerful technique for the elemental analysis of a wide variety of biological and environmental samples [26,27], as summarized in the recently published international Technical Specification ISO/TS

* Corresponding author at: Investigador Asistente-CONICET, Universidad Nacional de San Martín (UNSAM), Instituto de Investigación e Ingeniería Ambiental (3iA), Campus Miguelete, 25 de mayo y Francia, 1650 San Martín, Prov. Buenos Aires, Argentina.
E-mail address: vsalomone@unsam.edu.ar (V.N. Salomone).

http://dx.doi.org/10.1016/j.sab.2017.03.009
0584-8547/

18507:2015 [28]. TXRF presents numerous advantages: it is fast, it uses a small amount of sample, it provides multi-elemental identification in a wide range of concentrations with good precision and accuracy, it does not generate residues and the equipment does not use gases or liquid nitrogen for detection, so the cost of the analysis of samples is low [29]. The last point is especially important when a large number of samples are analyzed. Although a variety of sample types were reported to be analyzed by TXRF, even fresh water algae [30,31], few studies have been performed on marine systems [32] and there are few papers on seaweed [27].

The aim of this research was to evaluate the use of the Total Reflection X-ray Fluorescence spectrometry (TXRF) for seasonal multi-element analysis of a brown alga, *M. pyrifera* from central San Jorge Gulf (Patagonia).

2. Materials and methods

2.1. Equipment

TXRF analysis of samples was performed using a benchtop TXRF equipment (S2 PICOFOX, Bruker, AXS Microanalysis GmbH, Berlin, Germany). The spectrometer consisted of an air-cooled micro focus X-ray Mo tube, a Peltier-cooled, high-resolution XFlash® detector (SDD) and multilayer X-ray optics for beam focusing and monochromatization. The excitation condition was 50 kV and 750 μA. The acquisition time for each spectrum was 300 s. Deconvolution of the spectrum and quantification were performed with Spectra version 7.5.3.0. Software. An MLS-1200 (Milestone, Sorisole, Italy) MW apparatus equipped with ten Teflon vessels was used to acid digestion samples.

2.2. Reagents

Commercially available 1000 mg L^{-1} Cobalt standard solution (Merck) was used for the preparation of the internal standard solution. Analytical reagent nitric acid (Anedra, 65%) was employed after additional purification by sub-boiling distillation in a quartz still. The ultrapure H_2O_2 (Merck) used was analytical reagent grade. The standard arsenic solution (1000 mg L^{-1}) used in validation method was Chemlab. Finally, ultrapure water (18.6 Ω) was required.

2.3. Sampling area

Seasonal sampling was carried out in the subtidal kelp beds in the area of Punta Marques (45°56′ S, 67°32′ W Rada Tilly, San Jorge Gulf, Argentina) during 2014. The four seasons were monitored, summer (February); autumn (April); winter (July) and spring (November). In the South Atlantic, *M. pyrifera* is distributed along the Patagonian coast, from Valdés Peninsula (42°S) to the sub Antarctic Island (60°S); warm summer temperatures may shape the northern limit of its distribution.

2.4. Sample preparation and analytical determinations

Samples consisted in abundant blades of randomly selected individuals of *M. pyrifera*. Each specimen was extracted from the water (but never removed) and some blades were taken, then seaweed were immediately returned to the sea. The blades were picked up from the shallow subtidal area during low tide. After harvest, samples were carefully washed with seawater and then with distilled water to eliminate other attached algae, sediment, organic residues, macrofauna and epibionts. Later, samples were transported to the laboratory at 4 °C, dried in airy atmosphere to constant dry weight and stored in vacuum in polyethylene bags until analysis. Finally, the material was ground to a fine and homogeneous powder and six subsamples were randomly taken. The subsamples were digested using micro wave-assisted acid digestion (MW) in duplicate. In each series, a blank was also prepared and subjected to the same MW procedure as the samples. The total sample number analyzed was 70. Approximately 0.5 g portion of dried algae sample was transferred into Teflon vessels and 3 mL of distilled concentrated HNO_3 and 1 mL of ultrapure H_2O_2 were added. This mixture was subjected to the cycle of MW treatment. The operating conditions for the MW digestion algae are shown in Table 1. Vessels were allowed to cool and a completely clear and homogeneous solution was obtained. Then, the solutions were transferred into a 10 mL flask and brought to final volume with Milli-Q water. One milliliter of each diluted sample was transferred to an eppendorf; then, 10 μL of cobalt solution (100 mg L^{-1}) was added as internal standard and mixed homogeneously. Later, 10 μL of solution was deposited on a reflecting surface and dried using infrared lamp. The quartz surface with the sample was finally placed at equipment for analysis. The elements determined were As, Br, Cu, Cr, Fe, Mn, Ni, Pb, Rb, Sr, V and Zn.

Table 1
MW digestion procedure used to mineralize algae samples.

Sample amount	0.5 g
Reagents	3 mL HNO_3 + 1 mL H_2O_2 (Cycle)
Final volume	10 mL
MW cycle applied power (W)	Time (min)
250	1
0	1
250	5
400	5
650	5
400	5

2.5. Arsenic validation procedure

Arsenic element was selected because of its importance from the environment and health standpoint. Linearity, accuracy, precision and the others parameters were evaluated during validation. Selectivity is not an evaluation parameter of this method. The X-ray fluorescence technique involves a characteristic signal for each element linked to an internal electron transition of the atom. An elemental free response of interferences of other matrix analytes is obtained. The combination of instrumental parameters of measurement guarantees the selectivity and specificity of the method.

A standard certified arsenic solution (1000 mg L^{-1}, Merck) was used for the validation procedure. A calibration curve was performed, and then the linearity range was obtained. For the determination of the other parameters, arsenic solutions of 0.1 mg L^{-1} or 0.02 mg L^{-1} were used.

3. Results and discussion

3.1. Quality control of TXRF analyses

3.1.1. Reference material

The level of accuracy was monitored by adding one sample of vegetable Standard Reference Material, *Ulva lactuca*, (seaweed, BCR 279) or *Citrus* leaves (plant, 1572 NIST) to each digestion series. These materials were digested using the same protocol employed for samples and were measured under the same conditions. The results agree with the values reported for certificated material and are summarized in Tables 2 and 3.

Table 2
Analysis of Standard Reference Material (BCR 279) previous digestion by TXRF. Seven samples were analyzed. Values are expressed as mean ± SD.

Element	Concentration (mg kg^{-1})	
	Certified	Found
As	3.09 ± 0.20	3.24 ± 0.10
Cu	13.14 ± 0.37	11.56 ± 0.25
Pb	13.48 ± 0.36	13.31 ± 0.17
Zn	51.3 ± 1.2	45.6 ± 2.3

Table 3
Analysis of Standard Reference Material *Citrus* leaves (1572 NIST) previous digestion by TXRF. Seven samples were analyzed. Values are expressed as mean ± SD.

Element	Concentration (mg kg^{-1})	
	Certified	Found
As	1.8 ± 0.26	2.2 ± 0.3
Cu	9.5 ± 1	10.3 ± 1.2
Fe	530 ± 59	526 ± 32.7
Mn	82 ± 4	83.3 ± 6.9
Pb	13.5 ± 0.4	13.3 ± 0.2
Zn	107 ± 8.5	106 ± 8

3.1.2. Validation

The analytical validation for arsenic by TXRF was performed. The results are shown in Table 4. This procedure confirmed that the technique is suitable to quantify arsenic in an aqueous medium, showing very low levels of detection and quantification.

3.2. Multi-elemental analysis

The mineral composition of algae varies according to phylum (species), maturity as well as various other factors (e.g. seasonal, inter-annual, wave exposure, environmental, geographical location and physiological variations) [11–13]. The results from San Jorge Gulf are shown in Table 5.

Among the elements analyzed in this work, strontium content was highest. The maximum value was observed in winter (1083 mg kg^{-1} dw) and the minimum in autumn and spring showing similar concentrations (Table 5). High Sr concentrations in brown macro algae are related to the cell wall polysaccharide alginate (which constitutes about 10–40% of brown algae dry weight); in particular, it has been suggested that the main accumulation mechanism for Sr in brown seaweed is an ion exchange between seawater and alginate in the cell wall [33]. The rubidium content showed no seasonal variation; its concentration varied in a small range (20 and 28 mg kg^{-1}). Rubidium level depends on chemical characteristics of the coastal zone rocks and it is rarely studied. Kravtsova et al. [34] evaluated Rb content in brown alga *Cystoseira* and reported values slightly lower than the values found in this study.

Marine algae produce a cocktail of halogenated metabolites with potential commercial value, reflecting the availability of these ions in seawater. Interestingly, bromide is the most frequently used by algae for organohalogen compounds production, although chlorine occurs in higher concentrations than bromine in seawater [35]. Bromine concentration ranged between 50 and 100 mg kg^{-1} dw in summer and winter, respectively. *M. pyrifera* presented appreciable amounts of Fe, Mn and Zn, which are the most important micronutrients for humans. Iron was the most abundant micronutrient except in autumn where the concentration was similar to Zn. The Fe content ranged between 50 and 160 mg kg^{-1} dw for autumn and winter, respectively. Regarding zinc, seasonal variation was less obvious, due to the enormous variability observed among the samples analyzed (see standard deviations). The large individual variation observed can be explained because we did not perform blades analysis considering age or maturity. McKee et al. [36] divided blades of *M. pyrifera* into age categories considering different lengths (blade classes: young, mature and old), and they reported that younger blades had higher alginate content than older blades, confirming intra-plant variation. Metals adsorption capacity of algae is directly related to the presence of the alginate polymer [7]. Based on this, we should have unified the samples for each season (place all blades collected in the same season together) and then perform the analysis. Thus, we would have reduced the observed intra-season variability. Manganese was found in amounts two orders of magnitude lower than Fe, showing no seasonal variation. The sum of principal micronutrients (Fe + Zn + Mn + Cu) was between 114 and 171 mg k^{-1} g dw and was similar to the values reported by Rupérez [12] for brown seaweed (134.1 mg k^{-1} g dw for *Fucus* sp., 101.7 mg k^{-1} g dw for *Laminaria* sp. and 50.6 mg k^{-1} g dw for *Undaria* sp.).

Seaweed exhibit a high affinity for heavy metals, particularly the brown ones. The capacity of algae to accumulate metals is strongly dependent on a variety of factors, namely metal bioavailability in the surrounding water (that vary with temperature, pH, light and nutrient concentration) and the uptake capacity of the algae [37]. Vanadium and chromium are biochemically related to glucose and lipid metabolisms. Vanadium content was low, the maximum concentration was observed in winter (5 mg kg^{-1} dw). These results are consistent with those found in other algae of Patagonia [38]. Chromium was detected only in spring (2 mg kg^{-1} dw). Copper and nickel were below the detection limit (0.16 mg kg^{-1} and 0.9 mg kg^{-1} respectively), which is consistent with that observed by Rupérez [12] and Kolb et al. [39] for brown algae. In a work about *Porphyra columbina* and *Ulva* spp. (red and green macro algae, respectively) performed by Pérez [38] in Patagonia, Cu and Ni content was always below detection limit except in summer.

Toxic elements like lead and arsenic are dangerous for humans even at low levels. Their presence in marine products with commercial potential must be studied. Fortunately, lead content was always below the detection limit (0.1 mg kg^{-1}). However, the total arsenic concentration ranged between 36 and 66 mg kg^{-1} dw. The values found in this

Table 4
Some parameters evaluated during validation process.

Parameter	Result
Linear range[a]	
0.1–100 mg L^{-1}	
Accuracy (bias)[b]	1.9%
Precision (RSD)[c]	5.9%
Level of detection (LD)[d]	1.53 μg L^{-1}
Level of quantification (LQ)[e]	5.09 μg L^{-1}

[a] Calibration curve, $R^2 > 0.996$.
[b] Calculated for a reference arsenic solution 0.100 mg L^{-1} (n = 10). We found an average value 0.102 mg L^{-1} with standard deviation (SD) 0.006 mg L^{-1} and a bias of 1.9%. Accuracy > 95% according to the Student's *t*-test (t = 0.61).
[c] Calculated for a reference arsenic solution 0.100 mg L^{-1} (n = 10). RSD = relative standard deviation.
[d] Calculated for a reference arsenic solution 0.02 mg L^{-1} (n = 10). This value represents, for this work, a LD 0.04 mg kg^{-1} dry weight (dw).
[e] Calculated for a reference arsenic solution 0.02 mg L^{-1} (n = 10). This value represents, for this work, a LQ 0.12 mg kg^{-1} dry weight (dw).

Table 5
Chemical seasonal variation for *M. pyrifera*. Results are expressed in mg kg^{-1} dry weight (dw)[a]. Detection limit: Cu (<0.16 mg kg^{-1}), Ni (<0.9 mg kg^{-1}), Pb (<0.9 mg kg^{-1}) and Cr (<0.8 mg kg^{-1}, except in spring).

	As	Br	Fe	Mn	Rb	Sr	V	Zn
Summer	66 ± 2	50 ± 10	80 ± 10	6 ± 1	23 ± 0.1	840 ± 50	3 ± 1	20 ± 10
Autumn	64 ± 7	60 ± 2	50 ± 20	5 ± 1	*28 ± 1*	680 ± 50	3 ± 1	60 ± 40
Winter	36 ± 1	100 ± 20	160 ± 50	6 ± 1	25 ± 0.3	1083 ± 140	5 ± 1	10 ± 4
Spring	48 ± 4	50 ± 30	90 ± 60	4 ± 1	20 ± 0.3	690 ± 50	<DL	20 ± 10
Detection limit (DL)	0.1	1.20	1.3	0.5	0.8	8.0	1	1.3

The underlined data in the table correspond to the maximum value.
[a] 6 subsamples were analyzed for each season. Values are expressed as mean ± SD.

study were similar to the ones reported by Díaz et al. [9] (68 mg kg^{-1}) for *M. pyrifera* in the Pacific Coast (Punta Arenas, Chile). Seafood usually has significant concentrations of arsenic. High levels of As accumulation in brown seaweeds have been related to high phosphate concentrations, then seaweed take up and bio accumulate arsenate from seawater as a phosphorus analogue [33]. The total amount of As ingested by humans in coast areas depends greatly on the amount of seafood included in their diet (and also, as in non-coastal areas, on the water they consume). Seaweed are a primary accumulator of arsenic in the marine environment, playing an important role in its cycle and in the food chain. Nevertheless, total As content in food has no toxicological value. The organic and inorganic arsenic species differ widely in their toxicity; inorganic forms are in general more toxic than organic ones. Although, the total As concentration is higher in brown algae than in red or green algae; Díaz et al. [9] reported that the percentage of inorganic arsenic with respect to total As was, in general, higher for red and green algae. In particular, for *M. pyrifera* from Punta Arenas (Chile), they found that only 2.5% of the total As was inorganic [9]. Therefore, it is advisable to measure not only the total As concentration but also inorganic arsenic content. There is little legislation on seaweed and harmful metals. France has regulations on the use of seaweed for human consumption and the limit imposed for inorganic arsenic is 3 mg kg^{-1}. The Australian new Zealand Food Standard Code accepts a maximum level of 1 mg kg^{-1} inorganic arsenic in seaweed. In that sense, we consider that further comparative studies in arsenic speciation are needed to verify the real toxicity due to presence of inorganic arsenic in different macroalgae species. In Argentina, there is no specific regulation for algae. Anyway, all the samples of *M. pyrifera* from San Jorge Gulf analyzed exceeded the maximum value of arsenic allowed in solid food products by Argentine Food Code (1 mg kg^{-1}) [40].

Regarding seasonality, our results suggest that element concentrations in *M. pyrifera* are higher in winter. This could be associated with higher water temperature and solar irradiance during the summer, which favor the growth rate and produce a dilution effect, in agreement with Malea et al. [41].

Macrocystis production in Argentina still needs more basic and applied research. The complete nutritional properties of *M. pyrifera* are not well known. TXRF is a suitable technique for multi-elemental content determination in seaweed and such analyses contribute to the knowledge of these algae for a better use and seasonal exploitation.

4. Conclusions

A seasonal study of mineral content of *M. pyrifera* of San Jorge Gulf was performed. The Total Reflection X-ray Fluorescence is an increasingly used multi-element analytical technique and, based on the results presented in this work, an adequate method to determine the elemental composition in previously digested macro algae samples. Although others techniques show very low detection limits for some analytes, those of interest for this work could be quantified with acceptable limits of detection by TXRF, avoiding the use of a more sophisticated and expensive technique. Arsenic content could be quantified with very a low limit of detection as shown in the validation test.

The results of this paper contribute to the recently published Technical Specification ISO/TS 18507:2015, Surface chemical analysis Use of Total Reflection X-ray Fluorescence Spectroscopy in biological and environmental analysis, providing new evidence that confirms the wide variety of biological applications of the technique.

Acknowledgments

The authors are grateful to Dr. Servant for their important collaboration during the TXRF analysis (CNEA), Universidad Nacional de San Martín (UNSAM) for financial support and Municipalidad de Rada Tilly for logistical support during the sampling. VNS and CM are Research Members of the National Research Council of Argentina (CONICET). MR thanks CONICET for doctoral fellowship. The authors declare that they have not conflict of interest and that the research complies with the current laws of the country where it was performed.

References

[1] C. Rebours, E. Marinho-Soriano, J.A. Zertuche-González, L. Hayashi, J.A. Vásquez, P. Kradolfer, G. Soriano, R. Ugarte, M.H. Abreu, I. Bay-Larsen, G. Hovelsrud, R. Rodven, D. Robledo, Seaweeds: an opportunity for wealth and sustainable livelihood for coastal communities, J. Appl. Phycol. 26 (2014) 1939–1951.
[2] S. Mabeau, J. Fleurence, Seaweed in food products: biochemical and nutritional aspects, Trends Food Sci. Technol. 4 (1993) 103–107.
[3] A. Boraso, M. Ciencia, A. Cerezo, Seaweed resources of Argentina, in: A.T. Critchley, M. Ohno, D.B. Largo (Eds.), World Seaweed Resources. An Authoritative Reference System. E-Book.1.0, Eti Information Ltd, 2006.
[4] M.C. Eyras, G.E. Defossé, F. Dellatorre, Seaweed compost as an amendment for horticultural soils in Patagonia, Argentina, Compost. Sci. Util. 16 (2) (2008) 119–124.
[5] N. Wei, J. Quaterman, Y. Jin, Marine macroalgae: an untapped resource for producing fuels and chemicals, Trends Biotechnol. 31 (2) (2013) 70–77.
[6] S. Farias, S. Pérez Arisnabarreta, C. Vodopidez, P. Smichowski, Levels of essential and potentially toxic trace metals in Antarctic macro algae, Spectrochim. Acta B 57 (2002) 2133–2140.
[7] T.A. Davis, B. Volesky, A. Mucci, A review of the biochemistry of heavy metal biosorption by brown algae, Water Res. 37 (2003) 4311–4330.
[8] S. Farías, P. Smichowski, D. Vélez, R. Montoro, A. Curtosi, C. Vodopívez, Total and inorganic arsenic in Antarctic macroalgae, Chemosphere 69 (2007) 1017–1024.
[9] O. Díaz, Y. Tapia, O. Muñoz, R. Montoro, D. Velez, C. Almela, Total and inorganic arsenic concentrations in different species of economically important algae harvested from coastal zones of Chile, Food Chem. Toxicol. 50 (2012) 744–749.
[10] M. Greger, T. Malm, L. Kautsky, Heavy metal transfer from composted macroalgae to crops, Eur. J. Agron. 26 (2007) 257–265.
[11] C. van Netten, S.A. Hoption Cann, D.R. Morley, J.P. van Netten, Elemental and radioactive analysis of commercially available seaweed, Sci. Total Environ. 255 (2000) 169–175.
[12] P. Rupérez, Mineral content of edible marine seaweed, Food Chem. 79 (2002) 23–26.
[13] C. Almela, M.J. Clemente, D. Vélez, R. Montoro, Total arsenic, inorganic arsenic, lead and cadmiun contents edible seaweed sold in Spain, Food Chem. Toxicol. 44 (2006) 1901–1908.
[14] P.V. Subba Rao, V.A. Mantri, K. Ganesan, Mineral composition of edible seaweed *Porphyra vietnamensis*, Food Chem. 102 (2007) 215–218.
[15] V. Besada, J.M. Andrade, F. Schultze, J.J. González, Heavy metals in edible seaweeds commercialised for human consumption, J. Mar. Syst. 75 (2009) 305–313.
[16] R.C. Zimmerman, J.N. Kremer, In situ growth and chemical composition of the giant kelp, *Macrocystis pyrifera*: response to temporal changes in ambient nutrient availability, Mar. Ecol. Prog. Ser. 27 (1986) 277–285.
[17] Y.E. Rodríguez-Montesinos, G. Hernández-Carmona, Seasonal and geographic variations of *Macrocystis pyrifera* chemical composition at the western coast of Baja California, Cienc. Mar. 17 (3) (1991) 91–107.
[18] R. Westermeier, P. Murúa, D.J. Patiño, L. Muñoz, A. Ruíz, D.G. Müller, Variations of chemical composition and energy content in natural and genetically defined cultivars of *Macrocystis* from Chile, J. Appl. Phycol. 24 (2012) 1191–1201.
[19] J. Ortiz, E. Uquiche, P. Robert, N. Romero, V. Quitral, C. Llantén, Functional and nutritional value of the Chilean seaweeds *Codium fragile*, *Gracilaria chilensis* and *Macrocystis pyrifera*, Eur. J. Lipid Sci. Technol. 111 (2009) 320–327.
[20] L.E. Cruz-Suárez, M. Tapia-Salazar, M.G. Nieto-López, C. Guajardo-Barbosa, D. Ricque-Marie, Comparision of *Ulva clathrata* and the kelps *Macrocystis pyrifera* and *Ascophyllum nodosum* as ingredients in shrimp feeds, Aquac. Nutr. 15 (2009) 421–430.
[21] A. Mansilla, M. Ávila, Using *Macrocystis pyrifera* (l.) C. Agardh from southern Chile as a source of applied biological compounds, Rev. Bras. Farmacogn. 21 (2) (2011) 262–267.
[22] D. Briceño-Domínguez, G. Hernández-Carmona, M. Moyo, W. Stirk, J. van Staden, Plant growth promoting activity of seaweed liquid extracts produced from *Macrocystis pyrifera* under different pH and temperature conditions, J. Appl. Phycol. 26 (5) (2014) 2203–2210.
[23] J. Plaza, M. Viera, E. Donati, E. Guibal, Biosorption of mercury by *Macrocystis pyrifera*: influence of zinc, cadmium and nickel, J. Environ. Sci. 23 (11) (2011) 1178–1786.
[24] J. Plaza Cazón, L. Benitez, E. Donati, M. Viera, Biosorption of chromium (III) by two brown algae *Macrocystis pyrifera* and *Undaria pinnatifida*: equilibrium and kinetic study, Eng. Life Sci. 12 (2012) 95–103.
[25] J. Plaza Cazón, C. Bernardelli, M. Viera, E. Donati, E. Guibal, Zinc and cadmium biosorption by untreated and calcium-treated *Macrocystis pyrifera* in a batch system, Bioresour. Technol. 116 (2012) 195–203.
[26] M. West, A.T. Ellis, P.J. Potts, C. Streli, C. Vanhoof, P. Wobrauschek, Atomic spectrometry update – a review of advances in X-ray fluorescence spectrometry and its applications, J. Anal. At. Spectrom. 31 (2016) 1706–1755.
[27] R. Dalipi, E. Marguí, L. Borgese, L.E. Depero, Multi-element analysis of vegetal foodstuff by means of low power total reflection X-ray fluorescence (TXRF) spectrometry, Food Chem. 218 (2017) 348–355.
[28] ISO 18507, Surface Chemical Analysis—Use of Total Reflection X-ray Fluorescence Spectroscopy in Biological and Environmental Analysis, 2015.
[29] L. Borgese, F. Bilo, R. Dalipi, E. Bontempi, L.E. Depero, Total reflection X-ray fluorescence as a tool for food screening, Spectrochim. Acta B 113 (2015) 1–15.
[30] K. Barkacs, A. Varga, K. Gál-Solymos, G. Záray, Direct determination of metal concentrations in freshwater algae by total reflection X-ray fluorescence spectrometry, J. Anal. Atom. Spectrom. 14 (1999) 577–581.

[31] L. Varga, E. Rierpl, A. Tusai, Comparison of slurry preparation and microwave digestion of fresh water algae for multi-element analysis by total reflection X-ray fluorescence spectrometry, J. Anal. Atom. Spectrom. 14 (1999) 881–883.
[32] R.P. Pettersson, A method for trace element determination of marine periphyton communities on discs of float glass (without sample preparation) using total-reflection X-ray fluorescence spectrometry, Spectrochim. Acta B 53 (1998) 101–115.
[33] P. Malea, T. Kevrekidis, Trace element patterns in marine macroalgae, Sci. Total Environ. 494–495 (2014) 144–157.
[34] A. Kravtsova, N.A. Milchakova, M.V. Frontasyeva, Levels, spatial variation and compartmentalization of trace elements in brown algae *Cystoseira* from marine protected areas of Crimea (Black Sea), Mar. Pollut. Bull. 97 (2015) 548–554.
[35] M.T. Cabrita, C. Vale, A.P. Rauter, Halogenated compounds from marine algae, Mar Drugs 8 (2010) 2301–2317.
[36] J.W.A. McKee, L. Kavalieris, D.J. Brasch, M.T. Brown, L.D. Melton, Alginate content and composition of *Macrocystis pyrifera* from New Zealand, J. Appl. Phycol. 4 (1992) 357–369.
[37] I. Sánchez-Rodríguez, M.A. Huerta-Díaz, E. Choumiline, O. Holguín-Quiñones, J.A. Zertuche-González, Elemental concentrations in different species of seaweeds from Loreto Bay, Baja California Sur, México: implications for the geochemical control of metals in algal tissue, Environ. Pollut. 114 (2001) 145–160.
[38] A.A. Pérez, S.S. Farías, A.M. Strobl, S. Camarda, L.B. Pérez, O. Roses, M.A. Fajardo, Studies on the nutritional contribution and seasonal variation of essentials minerals in edible seaweeds, *Porphyra columbina* and *Ulva* spp., from central Patagonia, Argentina, Int. J. Agric. Sci. 2 (6) (2012) 186–197.
[39] N. Kolb, L. Vallorani, N. Milanovic, V. Stocchi, Evaluation of marine algae wakame (*Undaria pinnatifida*) and Kombu (*Laminaria digitata japonica*) as food supplements, Food Technol. Biotechnol. 42 (1) (2004) 57–61.
[40] Código Alimentario Argentino, Capítulo III: Productos Alimenticios. Art. 156 - (Res 1546, 09/17/85), 2012.
[41] P. Malea, A. Chatziapostolou, T. Kevrekidis, Trace element seasonality in marine macroalgae of different functional–form groups, Mar. Environ. Res. 103 (2015) 18–26.