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Full Length Research Paper

Studies on the nutritional contribution and seasonal variation of essentials minerals in edible seaweeds, *Porphyra columbina* and *Ulva* spp., from central Patagonia, Argentina

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Seaweeds are considered a non- traditional food, constituent part of exotic diets or mineral supplement in far from the sea areas. The objective of this work was determining the concentrations of macroelements (Ca, Mg, and P), microelements (Cr, Cu, Fe, Mn, Mo and Zn) and ultratrace (Ni, V) in *Porphyra columbina* and *Ulva* spp. in the San Jorge Gulf (Argentina) during 2002, in order to assess the spatial and seasonal variation of these elements, as well as for determining their nutritional contribution.

These elements were quantified by inductively coupled argon plasma/optical emission spectrometry (ICP-OES) with solid-state detectors. The two algal species under study provide a great amount of essential elements and there is also the possibility of using them to fortify food or as an alternative diet with a very low caloric intake.

KEY WORDS: Mineral composition, macronutrients, micronutrients, seaweeds, trace elements, seasonal variation.

INTRODUCTION

The recent food demand of men and the new trends in the dietary field have caused a turn towards alternative food. Seaweeds have been an essential part of the healthy diet since ancient times. They have been part of the Mediterranean diet since 600 years before Christ. Although approximately 25,000 species of seaweeds are known, only 160 (25 green, 54 brown and 81 red seaweed species) are edible. In countries such as Japan, they constitute up to 25% of the usual diet They are considered a non-traditional food, constituent part of exotic diets or mineral supplement in far-from-the-sea areas (Fajardo et al., 1998). The Japanese eat an average of 1.6 kg of dry seaweeds per capita per year, thus having an estimated intake of 4.38 g in dry weight (dw) per day (Darcy-Vrillon, 1993); and also in all the countries, it should be considered the existence of consumers who eat more than the average in observance with a macrobiotic diet.

Seaweeds from the Argentinean seacoasts constitute economically significant renewable resources. Most of

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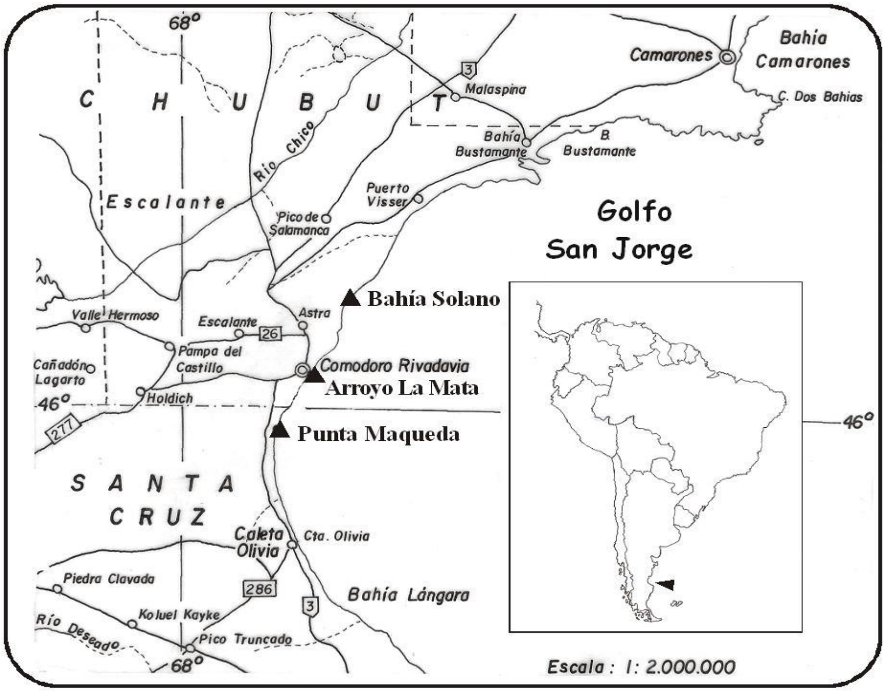

Figure 1: Sampling sites in the San Jorge Gulf (Chubut, Argentina

these algae from the Patagonian coast are exploited to obtain phycocolloids. However, some species are suitable for consumption, such as Porphyra columbina (Montagne, 1842) and Ulva spp. (Fajardo, 1998).

The mineral content of seaweeds is usually high enough (8-40%) and the essential minerals and trace elements needed for human nutrition are part of this content. Edible brown and red seaweeds could be used as a food supplement to help meeting the recommended daily adult intakes of some macrominerals and trace elements (Rupérez, 2002). From a nutritional point of view, seaweeds are characterised by high concentration of fibre and mineral, which have been classified according to nutritional facts as essential and non-essential.

Lead and arsenic concentrations in P. columbina and Ulva spp. do not seem to represent an issue for public health. Nevertheless, all the seaweed samples exceed the values set for cadmium in France, Australia, and New Zealand. An intake of 30 g of seaweed would not exceed cadmium Provisional Tolerable Weekly Intake (Pérez et al., 2011).

The basic aim of this study is to note the concentrations of the macroelements calcium (Ca), phosphorous (P), magnesium (Mg) and of the microelements chromium (Cr), cupper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn) and of ultratrace nickel (Ni) and vanadium (V) in P. columbina and Ulva spp. collected from three beaches on the coasts of San Jorge Gulf, to evaluate the seasonal and the spatial variation of the concentrations of these elements and to determine their nutritional contribution in each sampled area.

MATERIALS AND METHODS

Sampling sites

Species of Porphyra columbina (Montagne, 1842) and Ulva spp. have been collected from the hard subtracts of three sampling stations (Fig. 1) located at the San Jorge Gulf (Argentina):

Bahia Solano (BS) (45°44’ S, 67°25’ W). Located at 30 km to the North of Comodoro Rivadavia. It was not supposed to be influenced by domestic and industrial wastes.

The mouth of the Arroyo La Mata stream (AM) (45°53’ S, 67°32’ W). Area influenced by industrial activity and oil exploitation. This area was assumed to be the most contaminated.Punta Maqueda (PM) (46°00’ S, 67°34’ W). Sampling location placed at 30 km to the South of Comodoro Rivadavia. It was far away from the anthropogenic activities.

Sampling and sample preparation

According to the growth cycles of the two studied species and the technical possibility of collecting

Table 1: Concentrations of Ca, Mg, and P in *Porphyra columbina* (mg/g dw) (n=7)

	Bahía Solano			**Mouth of Arroyo La Mata**			**Punta Maqueda**		
Season	***Ca***	***Mg***	***P***	***Ca***	***Mg***	***P***	***Ca***	***Mg***	***P***
	$\overline{X}$ ± SD (Min-Max) Median (Q_{25}-Q_{75})								
Summer	5.28±1.05 (4.32-6.75) 5.17[a] (4.36-5.95)	5.72±0.80 (4.38-6.64) 5.84[a] (5.46-6.19)	5.20±0.80 (3.79-6.28) 5.39[a] (4.83-5.62)	5.87±1.37 (3.31-7.63) 6.32[a] (5.43-6.50)	6.11±0.75 (4.94-7.28) 6.29[a] (5.69-6.44)	4.07±0.36 (3.43-4.51) 4.06[a] (3.94-4.30)	4.24±1.4 (3.13-5.87) 3.73[a] (3.43-4.80)	5.19±0.77 (4.37-5.9) 5.31[a] (4.84-5.61)	3.34±0.69 (2.46-4.50) 3.18[a] (3.02-3.60)
Autumn	5.54±1.12 (3.46-7.00) 5.46[a] (5.31-6.12)	5.80±0.79 (4.20-6.43) 6.15[a] (5.59-6.32)	6.49±7.04 5.42-7.56 6.48[b] (6.15-6.82)	5.14±0.59 (4.43-6.25) 5.08[a] (4.76-5.34)	5.84±0.53 (5.15-6.84) 5.72[a] (5.60-5.99)	8.05±6.46 7.26-9.22 7.99[b] (7.66-8.28)	4.39±1.15 (3.06-6.10) 4.14[a] (3.5-5.21)	6.04±0.80 (4.99-7.09) 5.80[a] (5.49-6.70)	5.08±1.021 4.02-6.69 4.69[a*] (4.47-5.61)
Winter	3.52±0.87 (2.80-5.00) 2.96[b] (2.87-4.08)	5.39±0.62 (4.76-6.52) 5.03[a] (4.99-5.71)	7.62±1.35 (6.9-10.6) 6.97[b] (6.94-7.44)	3.50±0.72 (2.43-4.27) 3.75[b] (3.13-3.94)	4.77±0.52 (4.14-5.42) 4.66[a] (4.46-5.18)	6.39±0.33 (5.87-6.66) 6.38[a] (6.34-6.66)	2.75±0.47 (2.04-3.31) 2.78[a] (2.48-3.1)	5.29±0.39 (4.75-5.95) 5.26[a] (5.11-5.41)	6.940±0.74 (5.83-7.53) 7.31[b] (6.44-7.46)
Spring	---	---	---	7.62±1.55 (6.52-8.72) 7.62[a] (7.07-8.16)	5.31±3.03 (6.21-7.23) 6.91[b] (6.55-7.06)	6.54±1.53 (4.71-7.33) 7.18[b] (5.96-7.26)	7.10±1.07 (6.19-8.60) 6.82[b] (6.43-7.50)	6.69±0.8 (5.6-7.6) 6.75[b] (6.32-7.11)	6.6±1.2 (5.3-8.1) 6.56[b] (6.23-6.96)

$\overline{X}$: Mean; SD: standard deviation. dw: dry weight. , -- Not sampled
Different superscripts in the same column indicate statistically significant differences: $p<0.05$.
An asterisk (*) in the same line for the same element indicate statistically significant differences: $p<0.05$.

samples, and to the atmospheric conditions, the four seasons of the year were monitored as follows: January (summer), March (autumn), June (winter) and September (spring). Samplings were carried out simultaneously at the three mentioned stations in 2002 and at low tide.

One kilogram of the entire thalli of P. columbina and Ulva spp. was hand-picked in each station taking into account the different algae age, to obtain homogeneous samples, representative of the entire algal population in the area. Table 1

Samples were transported to the laboratory at 4 ºC in plastics bags, scraped and rinsed with distilled water to remove attached seawater, sediment, organics debris, macro fauna and epibiota. From the whole collected sample of each species (1 kg thalli), seven thalli of P. columbina and seven thalli of Ulva spp. were randomly selected and processed individually.

Samples of tissue of whole individuals were dried to constant weight (100 ± 4º C), ground to a fine powder in a porcelain mortar and stored in plastic bags until digestions were performed.

Digestion

Three subsamples (0.100- 0.200 g) of each sample were subjected to acid digestion with 2 or 4 ml using 65% HNO3 (J.T. Baker ACS, USA) in a high pressure poly- tetrafluorethylene (PTFE) vessel. Teflon vessels were sealed with the screw cap and placed inside digestion bombs (Bomb Parr®, Parr Instrument Company, Illinois, USA). Samples were irradiated at

Table 1 (Continuation): Concentrations of Ca, Mg, and P in *Ulva* spp. (mg/g dw) (n=7)

	Bahía Solano			Mouth of Arroyo La Mata			Punta Maqueda		
Season	***Ca***	***Mg***	***P***	***Ca***	***Mg***	***P***	***Ca***	***Mg***	***P***
	$\bar{x}$ ± SD (Min-Max) Median (Q_{25}-Q_{75})								
Summer	10.53±1.67 (8.93-13.16)	31.70±1.76 (29.7-33.8)	3.01±0.63 (2.49-3.71)	7.06±1.29 (5.61-8.84)	32.9±4.1 (27.5-39.9)	1.75±0.31 (1.13-2.04)	13.06±2.46 (10.3-16.6)	27.73±2.90 (22.2-30.9)	1.91±0.19 (1.63-2.12)
	10.09 ^{a} (9.24-11.47)	31.7 ^{a} (30.2-33.1)	2.83 ^{a*} (2.66-3.27)	7.08 ^{a*} (5.97-7.88)	32.8 ^{a} (30.2-35.0)	1.81 ^{a} (1.67-1.93)	12.06 ^{a} (11.67-14.6)	28.88 ^{a} (26.7-29.3)	1.94 ^{a} (1.80-2.04)
Autumn	9.84±1.38 (7.99-11.88)	23.9±1.59 (22.3-26.8)	6.01±9.72 (4.92-7.46)	6.22±1.35 (5.08- 9.10)	20.79±3.31 (18.5-27.8)	3.01±6.17 (2.13-4.07)	8.84±0.65 (8-9.77)	16.94±1.33 (15.76-19.3)	2.81±1.34 (2.59-2.98)
	9.70 ^{a} (9.08-10.61)	23.68 ^{b} (22.98-24.37)	5.90 ^{b*} (5.28-6.597)	5.95 ^{a*} (5.51-6.19)	19.77 ^{b} (18.6-21.2)	2.87 ^{b} (2.77-3.24)	9.00 ^{b*} (8.31-9.26)	16.74 ^{b} (15.96-17.5)	2.82 ^{b} (2.765-2.90)
Winter	9.70±1.47 (6.83-11.77)	30.85±3.61 (23.1-33.7)	4.92±0.55 (3.99-5.87)	15.10±10.0 (5.65-28.99)	28.08±5.03 (22.3-35.0)	3.66±0.44 (3.19-4.53)	10.02±2.29 (7.45-14.00)	29.17±6.29 (23.8-42.8)	3.77±0.51 (3.21-4.68)
	9.87 ^{a} (9.58-10.14)	32.36 ^{a} (30.6-32.8)	4.88 ^{b*} (4.83-5.03)	13.10 ^{b*} (6.18-22.77)	26.56 ^{a} (25.02-31.2)	3.52 ^{b} (3.39-3.78)	9.43 ^{b} (8.66-10.96)	27.46 ^{a} (26.06-28.9)	3.64 ^{b} (3.40-4.01)
Spring	---	---	---	16.88±6.52 (11.9-26.4)	29.61±2.11 (27.9-32.4)	3.90±7.17 (3.06-4.81)	13.20±1.78 (12.6-15.3)	40.76±3.10 (36.8-45.4)	3.14± 3.23 (2.83-3.63)
				14.57 ^{b} (13.6-17.9)	29.02 ^{a} (28.0-30.6)	3.87 ^{b} (3.59-4.18)	13.66 ^{b} (12.63-13.9)	40.65 ^{c*} (39.8-41.1)	3.03 ^{b} (2.92-3.29)

1200 W power in microwave- oven setting for once cycle of 1 minute (Conti et al., 2005).

Analytical determinations

The concentrations of macro and microelements were measured by inductively coupled plasma- optical emission spectrometry (ICP-OES) using a Perkin Elmer Optima 3100 XL spectrometer. Calibration curves were obtained with multi-elemental standards prepared by appropriate serial dilution of commercially available 1000 µg/ mL Ca, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, V, and Zn stock standard solutions (CertiPUR® Merck, Darmstadt, Germany) in 1.5 Mol HNO_3/ L. Samples were diluted to 10 mL with bidistilled water and analysed for metal contents. One sample of CRM and blanks (reagents and digestion blanks) were included in each analytical batch.

Finally, results were expressed in mg/g dry weight (dw), for essential major elements and in µg/ g (dw) for the other elements.

Analysis of certified reference material

The trueness (expressed as bias) of measurements was tested using CRM - BCR 279 (Sea lettuce). One sample of reference material blanks and a replicate of one sample were included in each analytical batch. Results were in agreement with certified values, and average analytical precision was around 5%.

Statistical analysis

Results were expressed as mean ± standard deviation, for descriptive statistic and as median and quartile (Q25, Q75) due to their non-parametrical distribution.The nonparametric Kruskal- Wallis and Mann- Whitney tests were used instead of one way ANOVA because of the large heterogeneity of the variances, with differences being considered at $p< 0.05$. Statistical estimates were done with INSTAT 2.02. $p< 0.05$ was deemed as statistically significant (Conti et al., 2005).

RESULTS AND DISCUSSION

Seasonal variation of macroelement in P. columbina and Ulva spp.

Table 2: Mineral macroelements coverage percentages, for adults (31-50 years old), with an intake of 30 grams of Porphyra columbina and Ulva spp.

	Gender	**Dietary Reference Intakes** mg/d **31 - 50 years old**	**% covered by 30 grams of seaweeds**			
			Porphyra columbina		**Ulva spp.**	
			Annual minimum and maximum	Annual average	Annual minimum and maximum	Annual average
Ca	F and M	RNI [a]: 1000	8-23%	15%	18-44%	31%
Mg	F	RNI [b]: 220	64-94%	79%	231-556%	387%
	M	RNI [b]: 260	54-80%	67%	195-470%	328%
P	F and M	AI [c]: 700	14-34%	24%	8-27%	9%

AI: Acceptable Intake. RNI: Recommended Nutrient Intake. F: Female. M: Male. ([a] Dietary Reference Intakes, 2010; [b]FAO, 2001; [c] Dietary Reference Intakes, 2004).

In P. columbina, Ca showed a significant seasonal variation ($p<0.05$), maintaining the same tendency all year in the three sampling sites (Table 1), with maximum concentrations occurring in spring in AM and in PM. Ca concentrations were statistically similar ($p> 0.05$) among the three sampling sites, all the year.

Mg concentrations also showed a significant seasonal variation ($p< 0.05$) in the sampling sites, with maximum concentration occurring in spring; P did also show a significant seasonal variation ($p< 0.05$) in the three sampling sites (Table 1). Unlike Ca and Mg, maximum concentrations of P were observed during autumn in AM and during winter in PM. P concentrations did not showed significant differences ($p >0.05$) among the three sampling sites in winter, spring or summer, but they did in autumn in PM (Table 1). In BS a summer- to-spring increasing tendency was observed, with the maximum value in winter, although it was impossible to compare spring values because no sampling was performed during that season.

Ca and P concentrations found in this work are of the same order as those reported for some species of Porphyra (hoshinori) in Japan and in the Atlantic coast of Galicia (Spain) (Ródenas de la Rocha et al, 2002). These values are different from those published by Hou and Yan (1998) for the Chinese coasts, with Ca concentrations of 2.4 mg/ g dw and Mg concentrations of 14.5 mg/ g dw, which correspond to half and double of the concentrations found in our area, respectively (Pérez et al., 2007).

In the sampling sites, the bioconcentration pattern of P. columbina decreased as follows: Mg, Ca, and P, the same as that found in the coasts of Pontevedra (Spain) (Ródenas de la Rocha et al., 2002).

In the Ulva spp. thalli collected in BS and PM, Ca concentrations did not show significant variations ($p>0.05$) between summer and winter, but they did when compared to spring concentrations, which

reached the highest levels (Table 1). In AM, this

seaweed showed an

important seasonal variation ($p<0.01$), being autumn the season when Ca concentrations were the lowest and, in agreement with what happens in PM, the highest values were registered in spring. Regarding Ca concentrations from the three sampling sites (Table 1), the highest concentrations occurred in PM and BS during summer and autumn; while in AM, the highest Ca concentrations occurred during winter.

Registering the lowest concentrations, Mg showed seasonal variation ($p<0.05$) in autumn with respect to the other seasons. But for spring, Mg did not show significant differences ($p>0.05$) among the three sampling sites. P is higher in BS than in the other two sampling sites (Table 1), showing a significant seasonal variation with the highest concentrations occurring in autumn ($p<0.05$). A slight seasonal variation ($p<0.05$) occurred in AM and PM, with concentrations going up from summer to spring.

In Ulva spp., Mg and Ca values are similar to those found in species gathered in Greece, China, Spain and the Japanese coasts (Malea and Haritonidis, 2000) and the values of P are higher than those described for the same species in Japan.

The abundance of these metals is similar to that described in the mentioned studies: Mg is more abundant than Ca.

In the three sampling sites, Ulva spp. bioconcentrated a higher amount of Ca and Mg ($p<0.01$) when compared to the annual average concentrations in P.columbina.

Nutritional contribution of the macroelements from P. columbina and Ulva spp. to the diet

Although seaweeds are not traditionally (table 2) used for consumption in our country, including them in thexdiet would represent a great benefit due to their nutritional

Table 3: Concentrations of Cr, Cu, Fe, Mn in *Porphyra columbina* (µg/g dw) (n=7)

	Bahía Solano				**Mouth of Arroyo La Mata**				**Punta Maqueda**			
	Cr	Cu	Fe	Mn	Cr	Cu	Fe	Mn	Cr	Cu	Fe	Mn
	$\bar{x}$ ± SD (Min-Max) Median (Q_{25}-Q_{75})											
S	0.66 ±0.12 (0.52-0.91) 0.61 [a] (0.60-0.68)	5.28±1.09 (3.43-6.64) 5.09 (4.82-6.10)	309±164 (173-666) 275 [a] (225-300)	38.48 ±4.62 (33.16-4.07) 39.2 [a] (34.3-42.1)	0.84 ±0.22 (0.56-1.30) 0.81 [a] (0.76-0.85)	6.82 ±1.22 (4.71-8.46) 6.98 (6.38-7.43)	440±112 (320-660) 430 [a] (370-465)	41.80±4.42 (34.44-8.34) 41.73 [a] (40.26-3.81)	77 ±0.39 (0.53-1.46) 0.76 [a] (0.58-0.64)	1.92 ±1.12 (0.8-3.64) 1.60 (1.10-2.60)	212±64 (155-322) 194 [a] (160-248)	31.74±5.05 (24.32-40.81) 30.8 [a] (30.1-33.1)
A	0.25±0.14 (0.03-0.38) 0.29 [b] (0.18-0.36)	Nd	299±77 (228-467) 279 [a] (268-293)	55.89±10.4 39.64-74.88 54.8 [b] (53.4-57.5)	0.45±0.26 (0.23-0.95) 0.38 [b] (0.24-0.53)	Nd	291±70 (222-391) 258 [b] (242-339)	58.81±14.52 (49.4-74.4) 57.6 [a] (53.2-61.8)	0.48±0.17 (0.20-0.67) 0.56 [b] (0.35-0.60)	Nd	194±32 (155-251) 196 [a] (172-208)	52.4±8.7 (42.6-68.5) 49.4 [a] (48.2-55.0)
W	0.26±0.10 (0.16-0.32) 0.26 [b] (0.24-0.29)	Nd	152±32 (115-195) 141 [b] (129-177)	52.4±15.5 (42.2-86.4) 46.9 [a] (15.5-51.8)	0.11±0.05 (0.10-0.12) 0.10 [c] (0.07-0.11)	Nd	112±34 (91-172) 101 [c] (92-101)	49.6±12.2 (30.8-617) 50.8 [a] (45.8-58.8)	0.24±0.06 (0.14-0.31) 0.24 [c] (0.21-0.28)	Nd	177±32 (123-216) 173 [a] (170-196)	54.5 ±4.6 (47.9-57.6) 54.9 [a] (50.5-57.1)
Sp	---	---	---	---	0.32±0.09 (0.26-0.38) 0.32 [b] (0.28-0.35)	Nd	272±138 (120-392) 306 [b] (120-392)	80.9±21.0 (56.6-93.8) 92.2 [b] (74.39-92.9)	0.14±0.12 (0.02-0.31) 0.11 [d] (0.02-0.22)	Nd	225±82 (159-335) 203 [a] (159-336)	62.6±11.4 (53.7-78.2) 59.2 [a] (54.2-67.5)

$\bar{x}$: Mean; SD: standard deviation. dw: dry weight. Nd: not detected, -- Not sampled. S: Summer; A: Autumn; W: Winter; Sp: Spring
Different superscripts in the same column indicate statistically significant differences: $p<0.05$.
An asterisk (*) in the same line for the same element indicate statistically significant differences: $p<0.05$.

Table 3 (Continuation): Concentrations of Cr, Cu, Fe, Mn in *Ulva* spp. (µg/g dw) (n=7)

	Bahía Solano				Mouth of Arroyo La Mata				Punta Maqueda			
	Cr	**Cu**	**Fe**	**Mn**	**Cr**	**Cu**	**Fe**	**Mn**	**Cr**	**Cu**	**Fe**	**Mn**
	$\bar{x}$ ± SD (Min-Max) Median (Q_{25}-Q_{75})											
S	1.05±0.21 (0.72-1.25) 1.13 [a*] (0.93-1.20)	3.82±2.64 (1.06-7.04) 3.11 [a] (1.83-6.15)	380±206 (258-797) 314 [a] (271-334)	51.40 ±19.0 (18.11-75.56) 51.76 [a] (49.67-59.63)	1.14±0.30 (0.96-1.81) 1.05 [a] (0.96-1.13)	3.77 ±1.85 (1.39-5.88) 3.16 [a] (2.99-5.42)	532±245 (221- 896) 474 [a] (402-681)	15.14±6.52 (9.63-28.06) 13.18 [a] (10.41-7.13)	0.84±0.19 (0.55-1.19) 0.82 [a] (0.76-0.89)	1.69 ±1.40 (0.39-3.86) 1.02 (0.87-2.51)	201±41 (155-265) 201 [a] (169-221)	8.13±1.46 (6.03-9.52 8.91 [a] (6.99-9.22
A	0.91±0.47 (0.57-1.59) 0.74 [b*] (0.58-1.06)	Nd	531±310 (196-905) 431 [b] (265-766)	72.24 ±34.83 (24.62-103.8) 84.1 [b] (44.9-99.8)	0.52±0.19 (0.31-0.86) 0.51 [b] (0.38-0.59)	Nd	495±67 (414-573) 493 [a] (437-555)	29.24±9.81 (23.72-49.63) 26.1 [b] (24.0-30.5)	0.40±0.15 (0.36-0.56) 0.43 [b] (0.36-0.48)	Nd	347±69 (244-417) 350 [b] (305-403)	16.12±2.2 (13.2-19.2 15.8 [b] (14.5-17.8
W	0.36±0.18 (0.12-0.67) 0.34 [c] (0.24-0.47)	Nd	305± 92 (205-461) 285 [a] (232-359)	25.80 ±6.27 (21.25-32.15 22.2 [c] (21.5-28.6)	0.28±0.21 (0.08-0.73) 0.25 [b] (0.17-0.29)	Nd	565± 261 (102-811) 590 [a] (515-754)	22.75±10.56 (11.53-35.39) 25.0 [b] (12.3-29.5)	0.58±0.33 (0.22-1.15 0.52 [b] (0.33-0.77)	Nd	238±109 (132-394) 205 [a] (154-317)	20.24±4.5 (14.4-26.3 20.2 [c] (16.9-23.3
Sp	---	---	---	---	0.76±0.41 (0.35-1.31) 0.70 [c*] (0.53-0.93)	Nd	1121±172 (978-1312) 1072 [b] (1025-1192)	61.0±23.25 (38.67-85.05) 59.3 [c] (48.9-72.2)	0.11±0.04 (0.08-0.16) 0.10 [c] (0.09 – 0.13)	Nd	119±43 (75-177) 118 [a] (83-146)	15.49±5.6 (38.67-85. 15.0 [b] (10.6-19.0

$\bar{x}$: Mean; SD: standard deviation. dw: dry weight. Nd: not detected, -- Not sampled. S: Summer; A: Autumn; W: Winter; Sp: Spring
Different superscripts in the same column indicate statistically significant differences: $p<0.05$.
An asterisk (*) in the same line for the same element indicate statistically significant differences: $p<0.05$.

value. Previous studies determined that the daily consumption of 30 g of dry P. columbina would cover the recommended nutrient intake (RNI) for vitamin C, depending on the season of collection (Fajardo, 1998). Table 2 shows the Dietary Reference Intakes (DRIs 2010) for Ca, Mg (FAO, 2001) and P (DRIs, 2004) and the coverage rates depending on this level.From the nutritional point of view, it is recommended that the ratio Ca/P in the diet be equal or superior to the unit to avoid the decrease of the Ca absorption.

A rich in phosphorous and poor in calcium diet can result in the appearance of secondary, hyperparathyroidism, with the subsequent loss of bone mass, which would cause demineralization (Portela, 2003). This study found an annual average Ca/P ratio of 0.8 in P. columbina, and of 2.9 in Ulva spp.

This ratio in P. columbina agrees with that obtained in previous studies carried out in the PM area (Fajardo, 1988) and with that found by Nisizawa in Japan, who reports a ratio of 0.68. Rather, in Ulva spp., the Ca/P ratio disagrees with that found in Ulva spp. by the same author, who reported a ratio of 12 (Nisizawa, 1987). Despite the great difference, the Ca/P ratio in Ulva spp. remains higher than in P.columbina.

Seasonal variation of Cr in P. columbina and Ulva spp.

There is no significant difference ($p>0.05$) in Cr concentrations among the three sampling sites in summer and in winter (Table 3), but there is in autumn and spring ($p<0.05$), tending to decrease from summer to spring. No significant difference ($p>0.05$) was registered among the three sampling sites in summer and in winter, but it was in spring and in autumn ($p<0.05$). Comparing the results obtained in 2000, it is observed that there was a decrease in the concentration, reason why it would be possible to state that there was a decrease in Cr concentrations over those years.

Seasonal variation of Cu in P. columbina and Ulva spp.

Cu concentrations showed significant differences in the sampling sites ($p< 0.05$), for both species, only during summer. For the other seasons, Cu values are below the method detection limit (Table 3). Cu concentrations in the samples of Porphyra columbina showed a significant difference ($p<0.05$) among the three sampling sites, the highest concentrations occurring in AM and the lowest concentrations, in PM). In Ulva spp., the lowest concentrations occurred in PM, while no significant difference was found between BS and AM ($p<0.05$). P.
columbina has more bio -concentrated Cu than Ulva spp. in BS and in AM.

Seasonal variation of Fe in P. columbina and Ulva spp.

Fe in P. columbina showed seasonal variation ($p<0.05$), with higher concentrations occurring in summer; being AM the sampling site where these seaweeds showed the highest concentration; the lowest values were observed in winter (Table 3). These values match those found for the same species in the Venetian Lagoon (Italy) (Caliceti et al., 2002)

In Ulva spp., the highest Fe concentrations were obtained in AM in spring. These values are comparable to those reported by Caliceti et al. (2002) in Italy and by Phaneuf (1999) in Canada.

In PM and BS, the highest concentrations were observed in autumn, similarly to what Haritonidis and Malea (1999) observed in Greece and Villares, in Spain (Villares et al., 2002), where the highest Fe concentration also occurred in autumn, when biomass is minimal. However, Misheer (2006), in South Africa found the highest values in winter and the lowest during summer and autumn.

Seasonal variation of Mn in Porphyra columbina and Ulva spp.

In P. columbina, Mn concentrations were similar ($p>0.05$) among the three sampling sites with the same tendency all through the year (Table 3). Mn concentrations are of the same order than those described for seaweeds from the coasts of China (Hou and Yan, 1998). In the three sampling sites, Mn showed a significant seasonal variation ($p<0.05$), with maximum concentrations occurring in autumn in BS and in spring in AM.

In a study carried out by Misheer et al. (2006) in Kwazulu-Natal, on the South African coasts, a seasonal variation is described, with maximum concentrations in autumn, alike the samples collected in BS. All year, Mn concentrations in Ulva spp. were lower than those reported by Malea and Haritonidis in the Thermaikos gulf, by Hou in China, by Misheer in the coasts of South Africa (Malea and Haritonidis, 2000; Hou and Yan, 1998; Misheer et al. 2006) and of the same order than those reported by Chaudhuri, (2007) in seaweeds gathered in the in the peninsula of Delmarva (USA).

Seasonal variation of Mo in Porphyra columbina and Ulva spp.

The analysis of Mo concentrations for both species allowed the observation of seasonal variations ($p<0.05$) with the same tendency (Table 4).

Literature contributes very little data on Mo in algae. Nevertheless, similar values to those determined in this work were found in two red algae from Antarctica: 0.80 ± 0.05 and 1.02 ±0.05 µg/g d.w. in Palmaria decipiens

Table 4: Concentrations of Mo, Zn, Ni, and V in *Porphyra columbina* (µg/g dw) (n=7)

	Bahía Solano				**Mouth of Arroyo La Mata**				**Punta Maqueda**			
	Mo	**Zn**	**Ni**	**V**	**Mo**	**Zn**	**Ni**	**V**	**Mo**	**Zn**	**Ni**	**V**
	$\overline{x}$ ± SD (Min-Max) Median (Q_{25}-Q_{75})											
S	1.23±0.13 (1.02-1.39) 1.24 ^{a} (1.17-1.33)	42.9±5.7 (34-49) 44.0 ^{a} (40.0-46.5)	0.74±0.24 (0.51-1.14) 0.65 (0.56-0.89	3.65 ±0.41 (3.24-4.42) 3.60 ^{a} (3.36-3.80)	1.21±0.14 (0.99-1.38) 1.19 ^{a} (1.15-1.32)	70.1±6.8 (58-80) 70.0 ^{a} (68.0-73.5)	1.51±0.3 7 (1.0-2.0) 1.51 (1.2-1.8)	4.54±0.30 (4.14-4.94) 4.61 ^{a} (4.31-4.74)	1.18±0.14 (0.99-1.42) 1.16 ^{a} (1.12-1.24)	21.1±5.2 (15-29) 20.3 ^{a} (17.6-24.2)	0,95±0,38 (0,49-1,4) 0,81 (0,67-1,31)	3,52±0,37 (3,15-4,19) 3,49 ^{a} (3,26-3,66)
A	0.40±0.16 (0.37-0.48) 0.43 ^{b} (0.08-0.598)	33.3±11.9 (15.2-54.3) 34.1 ^{a} (27.4-37.2)	Nd	5.17±0.61 (4.36-6.41) 5.15 ^{b} (5.02-5.14)	0.48±0.07 (0.40-0.60) 0.46 ^{b} (0.42-0.54)	37.1±7.8 (27.86- 48.56) 34.7 ^{b} (31.4-42.8)	Nd	5.87±0.48 (5.24-6.73) 5.89 ^{a} (5.57-6.05)	0.29±0.11 (0.04-0.38) 0.32 ^{b} (0.29-0.35)	10.87±7.7 (1.93-20.82) 9.3 ^{b} (4.4-17.9)	Nd	3,79±1,25 (2,85-6,42) 3,23 ^{a} (3,04-3,98)
W	0.69±0.10 (0.478-0.79) 0.70 ^{c} (0.69-0.75)	58.3±4.2 (51.0-62.9) 60.0 ^{b} (55.9-61.2)	Nd	6.33± 0.40 (5.85-7.12) 6.17 ^{c} (6.13-6.45)	0.73±0.13 (0.56-0.92) 0.70 ^{c} (0.68-0.77)	9.86±4.76 (4.58-17.39) 9.33 ^{c} (7.53-10.52)	Nd	5.51± 0.42 (5.10-6.20) 5.48 ^{a} (5.24-5.54)	0.67±0.13 (0.47-0.86) 0.65 ^{c} (0.60-0.76)	0.07±0.02 (0.04-0.11) 0.07 ^{c} (0.05-0.10)	Nd	6,31± 0,47 (5,81-6,56) 6,22 ^{b} (5,99-6,52)
Sp	---	---	---	---	1.10±0.12 (0.99-1.23) 1.09 ^{a} (1.03-1.16)	43.8±16.5 (32.2-55.6) 43.9 ^{b} (32.2-55.5)	Nd	5.84±1.20 (4.45-6.60) 6.46 ^{a} (5.45-6.53)	1.04±0.09 (1.02-1.15) 1.05 ^{a} (0.99-1.10)	10.1±5.1 (4.3-14.2) 11.7 ^{b} (4.3-14.1)	Nd	6,21±0,43 (5,68-6,74) 6,22 ^{b} (6,05-6,38)

$\overline{x}$: Mean; SD: standard deviation. dw: dry weight. Nd: not detected. -- Not sampled. S: Summer; A: Autumn; W: Winter; Sp: Spring
Different superscripts in the same column indicate statistically significant differences: $p<0.05$.
An asterisk (*) in the same line for the same element indicate statistically significant differences: $p<0.05$.

Table 4 (Continuation): Concentrations of Mo, Zn, Ni, and V in *Ulva* spp. (µg/g dw) (n=7)

	Bahía Solano				Mouth of Arroyo La Mata				Punta Maqueda			
	Mo	**Zn**	**Ni**	**V**	**Mo**	**Zn**	**Ni**	**V**	**Mo**	**Zn**	**Ni**	**V**
	$\bar{X}$ ± SD (Min-Max) Median (Q_{25}-Q_{75})											
S	0.67±0.42 (0.22-1.17) 0.66 [a] (0.31-0.99)	31.30±11.4 (20.4-50.1) 28.8 [a] (22.55-36.63)	4.11±1.34 (2.09-5.45) 3.79 (3.07-4.79)	4.13±3.91 (3.90-4.59) 3.91 [a] (3.90-4.25)	0.61±0.20 (0.43-1.05) 0.57 [a] (0.53-0.59)	17.4±7.1 (11.3-31.6) 16.3 [a] (12.6-18.6)	1.22±0.44 (2.08-8.46) 1.05 (0.95-1.40)	5.57±1.73 (4.27-9.42) 5.01 [a] (4.85-5.30)	0.54±0.06 (0.46-0.60) 0.55 [a] (0.50-0.58)	21.87±2.71 (16.7-24.7) 21.9 [a] (21.05-23.85)	0.99±0.99 (0.74-1.36) 1.05 (0.78-1.23)	4.30±1.40 (3.56-7.47) 3.79 [a] (3.73-3.92)
A	0.24±0.09 (0.06-0.30) 0.27 [b] (0.26-0.29)	21.31±4.08 (16.27-27.39) 21.22 [a] (17.2-22.1)	Nd	7.23 ±1.77 (4.97-9.15) 7.84 [b] (5.78-8.39)	0.32±018 (0.11-0.69) 0.33 [b] (0.23-0.35)	29.7±12.3 (15.4-36.8) 36.70 [b] (9.7-36.7)	Nd	7.84±0.41 (7.41-8.55) 7.75 [b] (7.59-8.01)	0.18±0.08 (0.06-0.30) 0.18 [b*] (0.14-0.21)	14.26±2.98 (10.57-19.67) 14.6 [b] (12.3-15.1)	Nd	4.36±0.69 (3.59-5.13) 4.63 [a] (3.88-4.83)
W	0.32±0.037 (0.28-0.37) 0.31 [b] (0.29-0.35)	27.31±15.23 (10.14-54.4) 22.4 [a] (21.0-31.1)	Nd	8.43 ±0.39 (7.76-9.03) 8.46 [c] (8.29-8.58)	0.26±0.055 (0.23-0.36) 0.25 [b] (0.23-0.29)	4.16±0.65 (3.51-4.82) 4.15 [c] (3.83-4.47)	Nd	8.30±1.19 (5.92-9.75) 8.43 [b] (8.18-8.81)	0.30±0.07 (0.22-0.41) 0.29 [c] (0.24-0.36)	26.32±14.33 (17.8-44.6) 28.9 [a] (20.0-33.5)	Nd	8.47±1.04 (7.47-10.5) 8.08 [b] (7.75-8.89)
Sp	---	---	---	---	0.30±0.08 (0.20-0.41) 0.31 [b] (0.27-0.34)	14.3±4.10 (10.3-18.5) 12.24 [a] (7.9-15.3)	Nd	11.17±2.38 (8.71-14.4) 10.76 [c] (10.1-11.7)	0.31±0.03 (0.28-0.35) 0.29 [c] (0.28-0.34)	1.30±0.23 (1.29-1.35) 1.29 [c] (1.20-1.29)	Nd	9.04±0.82 (8.2-10.29) 9.03 [b] (8.42-9.28)

$\bar{X}$: Mean; SD: standard deviation. dw: dry weight. Nd: not detected. , -- Not sampled. S: Summer; A: Autumn; W: Winter; Sp: Spring
Different superscripts in the same column indicate statistically significant differences: $p<0.05$.
An asterisk (*) in the same line for the same element indicate statistically significant differences: $p<0.05$.

and Georgiella confluens, respectively (Farías et al. 2002). In the Chilean coasts, Vasquez and Guerra (1996) determined higher Mo values than those found in this work in Porphyra columbina and Ulva spp.

Seasonal variation of Zn in P. columbina and Ulva spp.

In AM as well as in PM, the variation of the Zn concentration follows the pattern in which maximum concentrations occur in summer, followed by a decrease in autumn and winter, and a slight increase during spring. The lowest concentrations were observed in PM, all year long (Table 4).

In Ulva spp., a significant seasonal variation is observed all year long and there is not a single tendency or an unique pattern of variation among the three sampled sites ($p<0.05$) (Table 4). The maximum concentrations in AM are observed in autumn; while in PM, they are observed in winter. Haritonidis et al. (1999), in Greece, reported high values of Zn in summer and in autumn and they attribute them to the fact that during these seasons, photosynthesis and respiration are more intense. These factors would reinforce the power of taking in metals of this algal species. This behaviour is similar to that observed in AM. Similar results were reported by Hou and Yang in 1998 (16 µg/g dw), by Villares et al. in 2002 (18 µg/g dw) and by Malea and Haritonidis in 2000 (16.9 µg/g dw). Higher values than those were reported by Caliceti (2002) in Italy with 64 µg/g dw; and lower values were reported by Chaudhuri (2007) in the peninsula of Delmarva, on the eastern coast of USA, which fluctuated between 6 and 12 µg/g dw. Fe, Zn and Cu concentrations decrease in both species as follows: Fe, Zn, Cu. This same pattern is also described by Haritonidis and Malea (1999), Malea and Haritonidis (2000) and Caliceti et al. (2002).

Nutritional contribution of the microelements from Porphyra columbina and Ulva spp. to the diet

In order to study the nutritional contribution of Cr, Cu, Fe, Mn, Mo and Zn when algae as P. columbina and Ulva spp. are added to human consumption, it was calculated the percentage of coverage of the daily requirements for a woman of 31-50 years old who eats 2 spoonfuls (30 g) of dry and grinded seaweed. Table 5 show the percentage of coverage of the microelements under study, considering the daily Recommended Nutrient Intake (RNI), the Acceptable Intake (AI) for each element (DIRs, 2004) and the bioavailability of Fe (10%) and of Zn (15%) (FAO, 2001); as well as the annual minimum and maximum averages.

The bioavailability considered was 10 %. Such availability responds to a diet based on cereals, legumes, roots and tubers, with an inconsiderable amount of meat, fish and vitamin C. These diets are predominant is groups of low socio-economical level in developing countries (FAO, 2001; Portela, 2003).

Regarding the Zn, the bioavailability considered was the lowest (15%) depending on the protein content and the Ca/phytate ratio of the diet (FAO, 2001; Portela, 2003).

The main source of highly available iron for man are the food of animal origin, followed by cereals, vegetables, fruits and their products, which although contribute low or medium bioavailability Fe, they constitute a significant dietary source, due to the amount consumed. In a varied diet, 30-35% of the total consumed iron should come from red or white meat; 20-30% of products containing added iron; and the rest, from vegetables, fruits, tubers and roots (FAO, 2001).

It can be inferred that P. columbina and Ulva spp. are an excellent source of iron, given that the daily intake of two spoonfuls (30 g) of dry and grinded seaweed covers 24% and 42%, respectively, of the Fe daily requirements of a woman of 31-50 years old. It is worth noting that Fe deficiency is one of the most significant nutritional problems of the human race, especially in women.

Seasonal variation of Ni and V in P. columbina and Ulva spp.

For both species, Ni was only detected in summer. In Porphyra columbina, Ni lowest concentrations occurred in BS and PM and the highest ones, in AM (Table 4). A slight seasonal variation was observed in Porphyra columbina for vanadium. There was no significant difference ($p> 0.05$) among the three sampling sites. With respect to the presence of vanadium in the algae, the limited specific references found in the literature were insufficient to draw comparisons. In China, for the same species, Hou et al. (1998) did not detect this element at all, and in Chile, in the Strait of Magellan, Astorga et al. (2008) reported values three times lower (2.60±0.51 mg / g dw) than those determined in this work. Vanadium concentrations are consistent with those found in red algae of Antarctica (0.50 to 38.4 µg/g dw) (Farías et al., 2002).

Maximum Tolerable Intake (MTI) of ultra trace elements contributed by P.columbina and Ulva spp.

In order to assess the possible adverse effects caused by the contributions of Ni and V when these elements enter human food through P. columbina and Ulva spp., the percentage of the MTI that would be achieved with two tablespoons (30 g) of algae ground and dried for subjects greater than 19 years was calculated.

Table 6 shows the percentage of MTI and the minimum and maximum annual average established by DRIs (2001). It follows from the above table that the daily

Table 5: Percentage of coverage of the microelements for an adult (31-50 years old) due to the intake of 30 grams of *Porphyra columbina* and *Ulva* spp.

	Gender	**Dietary Reference Intakes 31 – 50 years old**	**% covered by an intake of 30 grams *of seaweeds***			
			Porphyra columbina		***Ulva* spp.**	
			Annual minimum and maximum	Annual average	Annual minimum and maximum	Annual average
Cr	F	AI[a]: 25 ug/d	13-99%	49%	13-136%	63%
	M	AI[a]: 35 ug/d	8-69%	34%	9-97%	51%
Cu	F and M	RNI[a]: 900 ug/d	6-23%	15%	5-13%	10%
Fe	F	RNI [b]: 29 mg/d*	10-45%	24%	12-110%	42%
	M	RNI [b]: 14 mg/d*	22-92%	50%	25-230%	88%
Mn	F	AI[a]: 1.8 mg/d	51-153%	87%	15-140%	52%
	M	AI[a] : 2.3 mg/d	40-120%	68%	12-110%	41%
Mo	F and M	RNI [a]: 45 ug/d	19-82%	54%	12-67%	24%
Zn	F	RNI [b]: 9.8 mg/d*	0.02-21%	9%	0.4- 11 %	6 %
	M	RNI [b]: 14 mg/d*	0.01-13%	7%	0.3-8%	4%

AI: Acceptable Intake. RNI: Recommended Nutrient Intake. *Considering a bioavailability of 10% for Fe and of 15% for Zn ([a] Dietary Reference Intakes, 2004, [b]FAO, 2001). F: Female. M: Male.

Table 6: Average of Maximum Tolerable Intake for adults older than 19 years old (Ni and V), with 30 g of dried *Porphyra columbina* y *Ulva* spp.

	MTI Adult ≥19 years old	***Porphyra columbina***		***Ulva* spp.**	
		Annual minimum and maximum	**Annual average**	**Annual minimum and maximum**	**Annual average**
Ni	1 mg/d *	1-3 %	2 %	3-11 %	6 %
V	1,8 mg/d *	6-11 %	9 %	4 -13 %	12 %

MTI: Maximum Tolerable Intake. *Dietary Reference Intakes, 2004

intake of 30 g of P.columbina and Ulva spp. does not pose a risk to human health due to the low percentage of IMT reached by the two elements.

CONCLUSIONS

The macroelements in P. columbina and in Ulva spp showed seasonal variation with an increasing tendency in spring. The percentages of coverage of the DRIs in women, considering the annual average content and a consumption of 30 g of dry Porphyra columbina and Ulva spp., were, respectively: calcium, 15% and 31% , magnesium, 54% and 264% phosphorus, 24% and 9%., copper, 15% and 10% , iron, 24% and 42% ,

molybdenum, 54% and 24%, zinc, 9% and 6% , In the samples of P. columbina and Ulva spp., obtained during summer, the concentrations of microelements decrease following this order: Fe, Mn, Zn, Cu in the sampling sites.

The percentages of coverage of the AI in women, considering the annual average content of 30 g of dry Porphyra columbina and Ulva spp. were, respectively:

chromium, 49% and 63% ,manganese, 87% and 52%, The intake of 30 g of Porphyra columbina and Ulva spp. does not pose a risk to human health for the low percentage of MTI reached for the calcium, phosphorous,

magnesium, chromium, cupper, iron, manganese, molybdenum, zinc, nickel and vanadium. Given the nutritional value these ancient food provide, a possibility of using them to fortify food or as an alternative diet with a very low caloric intake emerges.

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