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Nutritive and non-nutritive attributes of washed-up seaweeds from the coast of Ceará, Brazil

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ABSTRACT

This study assesses the nutritive and non-nutritive attributes of washed-up seaweeds from the Brazilian coast. It covers a broad diversity of species (24 red, nine green and four brown) with reasonable levels of proteins (10–14.8%), high ash contents (13–25%), low lipids (below 1%) and high carbohydrate contents (60%). Toxic and/or antinutritional factors were detected, such as low levels of lectins (32 and 64 HU/g of meal for chicken and rabbit trypsin-treated erythrocytes, respectively), tannins (59 mg/100 g), phytic acid (0.45%), high levels of trypsin inhibitors (99.0% inhibition) and -amylase inhibitors (70.5%). The 0/80% fraction showed moderate toxicity to mice (LD_{50} of 63.8 mg kg^{-1}). The presence of heavy metals such as cadmium (0.29 mg/100 g), chromium (0.23 mg/100 g), nickel (0.26 mg/100 g) and vanadium (3.56 mg/100 g) was also detected. Despite moderate toxicity and antinutritional limitations, washed-up seaweeds represent a potential food alternative for humans after appropriate processing and environmental remediation to guarantee food safety.

1. Introduction

The maintenance and improvement of life quality, mainly in developing countries and in poor regions faced with the serious problem of droughts, as in the northeast of Brazil, motivate the search for new substances extractable from renewable natural resources. Seaweeds are one of the richest and promising sources, not yet utilized on a large scale by man. In the Far East and Asian Pacific, people have a long tradition of consuming seaweeds as part of their diet while, in western countries, they are utilized industrially as a source of hydrocolloids, such as agar, carrageen and alginate (Jimenez-Escrig & Sanchez-Muniz, 2000). Over the past few decades, however, the consumption of seaweed products has increased in European countries and today 15–20 edible seaweed species are being commonly marketed for consumption in Europe (Dawczynski, Schubert, & Jahreis, 2007).

The nutritional properties of seaweeds are not as well known as are those of land plants, but several works have shown that they are low in lipids but rich in proteins, non-starch polysaccharides, minerals and vitamins (Darcy-Vrillon, 1993; Dawczynski et al., 2007; Mabeau & Fleurence, 1993). As seaweed polysaccharides cannot be entirely digested by human intestinal enzymes, they are regarded as a new source of dietary fibre and food ingredients (Lahaye, 1991; Mabeau & Fleurence, 1993). The human consumption of seaweed fibre has been proven to be health-promoting, lowering the occurrence of some chronic diseases (diabetes, obesity, heart diseases, cancers), which are associated with the low fibre diets of western countries (Kuda, Goto, Yokoyama, & Fujii, 1998; Kuda, Yokoyama, & Fujii 1997; Southgate, 1990).

Washed-up seaweeds are of different species (red, green and brown), which are taken from their natural habitat and brought to the shore by the action of winds and tides. Since these natural resources are rich in carbohydrates, proteins, vitamins and minerals, it is interesting to assess the possibility of their utilisation as an alternative source of food, replacing, at least partially, the traditional but expensive food sources (MacArtain, Gill, Brooks, Campbell, & Rowland, 2007).

In northeastern Brazil, seaweeds are abundant, easily collected and handled, being mainly utilised as biomass for the treatment of industrial effluents. Nevertheless, it is important to utilise these resources which are usually left on the shore as organic residues.

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It is known that the sustainability of seaweeds and coral natural banks is very important for maintenance of aquatic ecosystems but, at the same time, it is important to exploit them as natural resources. The lack of data concerning exploitation of these seaweed banks (in several regions of Brazil) constitutes a great obstacle to the creation of economical and social development programmes (Iplance, 1995). This work aims to assess the nutritive and non-nutritive attributes of these seaweeds, as well as the degree of contamination by pollutants, in order to verify their viability as food for humans and/or animals.

2. Materials and methods

2.1. Biological and chemical reagents

Soybean trypsin inhibitor (Sigma T9128), α-amylase inhibitor from wheat seed (Sigma A1520), α-amylase from human pancreas (Sigma A9972), trypsin from human pancreas (Sigma–Aldrich T6424), bromelain from pineapple stem (Sigma B5144), papain from papaya latex (Sigma P3250) and subtilisin from *Bacillus licheniformis* (Sigma P5459), were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Pronase, from *Streptomyces griseus* was purchased from Merck (Uppsala, Sweden). Human blood, of types A, B and O, was obtained from Ceará State's Hematology and Hemotherapy Center (Fortaleza, Brazil). Blood (rabbit and chicken) was obtained from animals of colonies maintained at the Federal University of Ceará (Fortaleza, Brazil). Female mice of Swiss strain were obtained from an outbred colony maintained at the Federal University of Ceará (Fortaleza, Brazil). All other chemical reagents used in the experiments were of analytical grade.

2.2. Washed-up seaweeds: collection, species identification and meal preparation

Samples of washed-up seaweeds were collected monthly, over a one year period, at Fleixeira Beach, Trairi, Ceará, Brazil (3° 12′ 0″ south, 39° 22′ 0″ west). The samples were placed in isothermal containers and taken to the laboratory where the epibiota attached to seaweeds were removed. Voucher specimens, for all species, were identified with the help of a stereomicroscope according to Wynne (1998) and deposited at the Algae Herbarium of the Sea Sciences Institute, Federal University of Ceará (Fortaleza, Brazil). The samples were dried and ground in a coffee mill to obtain a fine powder. The meal obtained from the pool of seaweed species (month sample) and from the great pool of all seaweed meals taken together (year sample) were used for chemical and biochemical analyses.

2.3. Proximate composition

The following analyses were carried out: moisture (oven-drying at 60 °C to constant weight), ash (ignition at 550 °C in an electric furnace) and ether extract (Soxhlet apparatus) were determined using standard methods (AOAC, 1990). Nitrogen content was determined using a micro-Kjeldahl method (AOAC, 1990). A conversion factor of 6.25 was used to calculate protein content. Total carbohydrates (including dietary fibre) were determined by difference.

2.4. Antinutritional and toxic factors determination

Tannin content was determined according to the colorimetric method of Folin-Denis described in AOAC (1990). Phytic acid content was determined, following methodology described by Gárcia-Villanova, Gárcia-Villanova, and Ruiz de Lope (1982). For the extraction and determination of trypsin inhibitor activity, fresh or toasted (200 °C/10 min, 20 min and 30 min) samples of seaweeds (10 g) were stirred in 100 ml of distilled water for 1 h at room temperature. This suspension was then filtered through nylon and the filtrate centrifuged at 8000*g*, for 30 min at 4 °C. The precipitate was discarded and 2.5% trichloroacetic acid was added to the supernatant. The solution was left to stand for 30 min and then homogenised and centrifuged at 8000*g* for 30 min at 4 °C. The precipitate was discarded and the new supernatant was utilised for determination of trypsin inhibitors (Hamerstrand, Black, & Glover, 1981). For the extraction and determination of α-amylase inhibitors, a defatted sample of fresh or toasted (200 °C/10 min, 20 min and 30 min) seaweed meal was added to 10 ml of 0.05 M, (pH 7.0) sodium acetate, and left stirring overnight at 20 °C. After that, the extract was filtered through a nylon tissue and centrifuged at 8000*g*, for 30 min, at 4 °C. The supernatant and precipitate were then analysed for -amylase inhibitors according to Bernfeld (1955). The assay of haemagglutinating activity of lectins (native or treated with trypsin, bromelain, papain, subtilisin and pronase, 0.1 mg/10 ml) was done with 2% suspensions of ABO human, rabbit and chicken erythrocytes according to Vasconcelos, Cavada, Moreira, and Oliveira (1991). The assay of acute toxicity was done in female mice with average body weight of 18–20 g by intraperitoneal injection, as described by Vasconcelos, Trentim, Guimarães, and Carlini (1994). Toxic activity was defined as the amount of the fraction 0/80 (g/ kg body weight) capable of causing death in 50% of tested animals.

2.5. Heavy toxic metals

The determination of six elements (cadmium, lead, chromium, nickel, silicon and vanadium) was done by atomic emission spectroscopy (ICP-OES). For each test 200 mg of seaweed meal were treated with 3 ml of concentrated HNO_3 (Merck) and 2 ml of H_2O_2 (30% v/v, Merck). This mixture was heated in a microwave oven (Multiwave, Anton Par) under pressure, with a heating programme set to 20 min and cooling to 15 min. After decomposition, the suspension was diluted to 30 ml with deionized water (Milli-Q) and a calibration curve was prepared, using a multielemental solution for quantitation of heavy metals.

2.6. Statistical analysis

The data of proximate composition were presented as means ± standard deviations of three determinations. Statistical analyses were performed using one way analysis of variance. Multiple comparisons of means' extreme values (lowest and highest) for each component over the year and in the pool of samples were done by ANOVA test. For other analyses, the data were shown as means of three determinations and the standard deviation values were omitted since they were less than 5% of the mean. All computations were done by employing statistical software (StatPlus 2008).

3. Results and discussion

3.1. Samples collection and species identification

There was a broad diversity of washed-up seaweed species (Table 1) over the one year period of sample collection in Fleixeiras Beach. Thirty seven species were identified, these being 24 red (Rhodophyceae), nine green (Chlorophyceae) and four brown (Phaeophyceae). The occurrence of species showed seasonal variation and the average number of seaweed species collected was 18, most of them being red (always present in higher amounts). Some

Table 1
Species of seaweed samples collected from January to December, 2004.

Species of seaweed collected	Months											
	January	February	March	April	May	June	July	August	September	October	November	December
Rhodophyta												
Agardhiellae ramossisima (Harv.) Kylin	x[a]	x	x	x	–[b]	–	–	–	–	–	–	–
Amansia multifida J.V. Lamouroux	x	–	x	x	x	x	x	x	x	x	x	x
Botriocladia occidentalis (Børgesen) Kylin	x	x	x	x	x	x	x	x	x	x	x	x
Bryothamnion seaforthii (Turner) Kütz	x	–	x	–	–	x	x	–	x	x	x	x
Bryothamnion triquetrum (S.G. Gmelin) Howe	–	x	x	x	–	x	x	x	–	x	x	x
Cryptonemia crenulata (J. Agardh) J. Agardh	x	x	x	x	–	–	–	–	x	x	–	–
Digenea simplex (Wulfen) C. agardh	–	x	–	x	x	–	–	–	–	x	–	x
Eucheuma echinocarpus	–	–	–	–	–	–	–	–	–	x	–	x
Galauxaura rugosa (J. ellis & Sol) J.V. Lamour	–	x	–	–	–	–	–	–	–	–	–	–
Gracilaria caudata J. Agardh	–	–	–	x	–	–	x	–	–	x	–	x
Gracilaria cervicornis (Turner) J. Agardh	x	x	x	x	x	x	–	x	x	–	–	x
Gracilaria cornea (Turner) J. Agardh	x	x	x	x	x	x	x	–	x	x	x	x
Gracilaria curtissiae J. Agardh	–	x	–	x	–	x	x	x	–	–	–	–
Gracilaria cearensis (Joly & Pinheiro) in Joly et al	–	–	–		–	–	–	–	–	x	x	x
Gracilaria domingensis Souder ex Kürtzing	–	x	x	x	x	x	x	x	x	x	x	x
Gracilaria ferox (J. Agardh) J. Agardh	x	x	x	–	–	–	–	–	–	x	x	x
Gracilaria birdiae Plastino & Oliveira	x	x	x	x	–	x	x	x	–	x	x	x
Gracilaria occidentalis (Borgesen) M. Bodard	–	–	–	–	–	–	–	–	–	–	–	x
Halymenia sp1	x	x	x	–	x	x	x	x	–	–	x	–
Halymenia bermudensis Collins & Hervey	–	–	–	–	–	–	–	x	x	–	–	–
Halymenia sp.C. Agardh	–	–	–	–	–	–	–	–	x	–	–	–
Hypnea musciformis (Wulfen in Jacquin) Lamouroux	x	x	x	x	x	x	x	x	x	x	x	x
Meristiella echinocarpum (Aresch.) Cheney & Gabrielson		x										
Vidalia obtusiloba C. Agardh	–	x	–	x	x	–	–	x	–	–	–	–
Phaeophyta												
Dictyota mertensii (Martins) Kurtzing	–	–	–	–	–	–	–	–	–	x	x	x
Padina sp.	x	x	x	x	x	x	x	–	x	x	x	–
Sargassum vulgare C. Agardh var. vulgare	x	–	x	x	x	x	x	x	x	x	x	x
Sargassum filipendula C. Agardh	–	–	x	–	x	x	x	–	x	x	x	

[a] Collected.
[b] Not-collected.

species of red seaweeds, such as *Amansia multifida, Botriocladia occidentalis, Hypnea musciformis, Gracilaria cervicornis, G. domingensis*, *G. ferox*, *G. cornea* and *G. birdiae,* showed high occurrence and were collected 9 to 11 times during the year. The green species were always found in small quantities. Among nine species collected, the species *Caulerpa racemosa v. occidentalis* (Forsskål) J. Agardh, *Ulva lactuca* Linnaeus and *Ulva fasciata* Delile were the most frequent. Only four brown species were washed-up in significant amounts, mainly *Sargassum vulgare* and *Padina* sp collected throughout almost the whole year.

3.2. Proximate composition

The proximate composition (Table 2) showed that there were variations in the contents of most components during the year. When statistical analysis was applied to the extreme values of each component over the year, a significant difference ($p < 0.05$) was observed in all components except proteins. Thus, there was seasonality, not only in seaweed species occurrence, but also in the contents of several nutrients. The differences in nutrient contents may be the result of the extraordinary ability of seaweeds to accumulate elements present in water (Carrillo, Castro, Pérez-Gil, Rosales, & Manzano, 1992). Several reports have shown that protein content varies greatly among seaweeds species, depending on the season and environmental growth conditions (Dawczynski et al., 2007). In this work, protein content was determined in a pool of washed-up seaweeds and so variation between species could not be detected during the one year period, except for a slight deviation during the year, from 10.7% in April to 14.8% in December. The pool of washed-up seaweeds, a mixture of brown, red and green seaweeds collected each month during the year, showed 12.2% protein. This represents a reasonable protein level, since it is equivalent to those of some cereal grains normally consumed by humans, such as corn and rice. Moisture content varied from 4.07% to 8.22% whereas ash values were in the range of 13.3% to 25.6% and lipid contents were always below 1% (0.15 to 0.84%). Total carbohydrates, were always about 60% (56.7% to 68.2%). These findings agree with those by Jensen (1993), who showed that nutrient composition of seaweeds varied with species and

Table 2
Proximate composition of meals obtained from washed-up seaweeds collected over a one year period and of the seaweed pool.

Samples	Proximate composition dry basis (%)				
	Moisture	Ash	Lipids	Proteins	Carbohydrates
January	6.77	19.22	0.27	12.7	61.0
February	8.22[a]	17.43	0.25	13.6	60.5
March	6.28	24.84	0.62	11.6	56.7
April	7.23	13.27[a]	0.54	13.0	66.0
May	5.26	16.78	0.15[a]	14.4	63.4
June	5.00	17.87	0.21	14.8[a]	62.1
July	5.54	14.96	0.84[b]	13.2	65.5
August	4.53	16.02	0.30	13.9	65.2
September	4.27	14.81	0.38	12.4	68.2
October	4.07[b]	20.44	0.28	11.6	63.6
November	4.38	25.58[b]	0.68	11.4[a]	68.0
December	4.41	24.25	0.20	10.7	60.4
Pool of samples	7.30[a]	14.63[a]	0.35[c]	12.2 [a]	65.3

Values are means of triplicates. The standard deviation values were omitted since they were less than 5% of mean.
Superscript letters represent extreme values (lowest and highest) for each component during the year and in the pool of samples.
Similar superscript letters in the same column do not differ significantly ($p > 0.05$; ANOVA);
Carbohydrate content was determined by calculating the percentile difference from all the other constituents.

geographical area, water temperature and year season. In addition, protein contents are higher in red and green seaweeds (10% to 30%) whereas the brown ones are a much poorer source of this nutrient (Dawczynski et al., 2007; Mabeau & Fleurence, 1993). The pool of seaweeds showed high ash contents compared to land plants. They varied from 13% (April) to 25% (November), whereas ash values for land plants are in the range 5–10% (USDA, 2001). In fact, Rupérez (2002) has already reported that mineral content in seaweeds is generally high and that the essential minerals and trace elements needed for human nutrition are present in seaweeds. Total carbohydrate content, showed values of around 60% during the year, similar to those reported in the literature (MacArtain et al., 2007). However, it has been shown that seaweeds are very good sources of dietary fibre and, thus, not all of the carbohydrates provide metabolizable energy (MacArtain et al., 2007). It has been shown that the content of total dietary fibre ranges from 33–50 g/100 g d.w. (Lahaye, 1991; Rupérez & Saura-Calixto, 2001). Accordingly, the fibre content of seaweed varieties is higher than those found in most fruits and vegetables and the types and abundance of carbohydrates vary strongly among algae species. The typical algae carbohydrates are not digestible by the human gastrointestinal tract and, therefore, they are dietary fibres. The consumption of this dietary fibre has been related to several health-promoting effects, such as growth and protection of the beneficial intestinal microbiota (Goni, Guidel-Urbano, Bravo, & Saura-Calixto, 2001), reduction of the glycemic response (Goni, Valdivieso, & Garcia-Alonso, 2000), increase in stool volume (Jimenez-Escrig & Sanchez-Muniz, 2000) and reduction of colon cancer risk (Guidel-Urbano & Goni, 2002).

3.3. Antinutrients and toxic factors

Certain substances, with some level of toxicity and/or antinutrient activity, may be present in seafood and seaweeds. Among these are lectins (Rogers & Hori, 1993), which were detected in the total extract of seaweed pool by hemagglutinating assays. Lectins may survive digestion by the gastrointestinal tract of consumers with subsequent binding to membrane glycosyl groups of the cells lining the digestive tract. As a result of this interaction, a series of harmful local and systemic reactions are triggered, placing this class of molecules as antinutritive and/or toxic substances (Vasconcelos & Oliveira, 2004). In this work, no activity was detected against human erythrocytes of the ABO system, either native or treated with bromelain, papain or trypsin. Nevertheless rabbit and chicken erythrocytes showed the highest hemagglutinating activity, 64 and 32 HU/ml, respectively, but this was relatively low when compared to legume seed levels. These erythrocytes showed low activity when treated with subtilisin. Besides, when the extraction was done with acetate buffer, rabbit erythrocytes treated with trypsin showed a hemagglutinating activity of 8 HU/ml. Contrary to our findings, however, Ainouz et al. (1992) have reported (for 27 species of brown, red and green seaweeds from the Ceará Coast) hemagglutinating activity against human erythrocytes of the ABO system treated with trypsin, bromelain and papain. On the other hand, the results shown with rabbit and chicken erythrocytes were similar to ours and showed stronger hemagglutination after treatment with trypsin. The toxic potential of a lectin can be assessed by reactivity with human and animals erythrocyes, treated or not treated with trypsin. According to Grant, More, Mckenzie, Stewart, and Pusztai (1983), lectins strongly agglutinating rabbit, rat, sheep and human (ABO system) erythrocytes (treated or non-enzyme treated) show high oral toxicity to rats, which are usually used as a human model. On the other hand, those lectins that agglutinate only rabbit and rat erythrocytes treated with pronase are essentially non-toxic. The hemagglutinating ativity may be due to lectins, polyphenols, tannins or lipids (present mainly in brown seaweeds), which might interfere in agglutination processes. At the same time, they might dilute the hemagglutinin content due to the large number and quantity of other substances present (Ainouz & Sampaio, 1991).

The 0/80 fraction of the seaweed pool, at concentrations of 30, 50, 75 and 100 mg/kg body weight, was injected intraperitoneously into mice for LC_{50} determination. Symptoms of neurotoxicity were observed and the LC_{50} was 63.8 mg/kg body weight which, according to Hodge and Sterner (1944), is considered moderately toxic.

Tannins, trypsin and alpha-amylase inhibitors and phytic acid are considered antinutritional factors because they might interfere with bioavailability and/or digestibility of some nutrients, such as proteins and trace minerals (Rehman & Shah, 2004). In the present work, trypsin and alpha-amylase inhibitors, tannins and phytic acid were detected in the pool of washed-up seaweeds. The seaweed pool extract, when diluted 1:20, showed 70.5% inhibition of -amylase, which might lead to antinutritional effects (Thompson, 1993). According to Kadam, Smithard, Eyre, and Armstrong (1987), moist heat (boiling, autoclave), reducing agents (sodium sulphite, cysteine), protein fractionation and ethanol extraction improve the quality of proteins from plant seeds due to inactivation and/or elimination of antinutrients. In the present work, dry heat was applied by using a conventional oven at 200 °C for 10, 20 and 30 min to verify the effect upon the levels of trypsin and alpha-amylase inhibitors. After 10 min, alpha-amylase inhibitor was inactivated but no effect was observed on trypsin inhibitors (Table 3). It is known that trypsin inhibitors are heat-resistant when compared to alpha-amylase inhibitors, which show very low heat stability. Thus a heat treatment was used to prevent these effects. The finding that trypsin inhibitors were not inactivated by the heat processing disagrees in part with the data reported by Mubarak (2004), which has shown that application of heat and the use of microwaves are able to reduce or even eliminate antinutrients, mainly protease inhibitors. Trypsin inhibitors are of major concern, since they have been associated with growth inhibition and pancreatic hypertrophy in some experimental animals (Hathcock, 1991). The feeding of purified trypsin inhibitors can potentiate the effects of pancreatic carcinogens (Hathcock, 1991). Protease inhibitors have also been linked to pancreatic cancer in animal

Table 3
Antinutritional and/or toxic factors in the pool of washed-up seaweed samples. Heat stability of trypsin and α-amylase inhibitors.

Samples	Trypsin inhibition (%)	α-amylase inhibition (%)	Tannin (%)	LD_{50} ($(mg.kg^{-1})$[a]	Phytic acid (%)	Hemagglutination (HU/ml)[b]
Pool without heat treatment	99.0	70.5	0.059	63.8	0.45	64
at 200 °C/10 min	98.7	–[c]	nd[d]	nd	nd	nd
at 200 °C/20 min	96.6	–	nd	nd	nd	nd
at 200 °C/30 min	96.9	–	nd	nd	nd	nd

[a] Dose capable of killing 50% of tested mice.
[b] Pool of seaweeds in 0.15 M saline solution + trypsin-treated rabbit erythrocytes. 1HU is defined as the reciprocal of the highest dilution exhibiting hemagglutination.
[c] –: 100% thermal inactivation.
[d] Not detected.

studies, but may also act as anticarcinogenic agents. Animal studies, *in vitro* cell culture work and epidemiological data have shown low cancer mortality rates in human populations with a high intake of protease inhibitors. *In vitro*, protease inhibitors can suppress the malignant transformation of cells induced by different types of carcinogens (Thompson, 1993).

Tannin content was 59.0 mg/100 g, well below that reported by Aguilera-Morales, Casas-Valdez, Carrillo-Domínguez, González-Acosta, and Pérez-Gil (2005) in *Enteromorpha* spp, (62–97.0 mg/100 g sample). Tannins are compounds of intermediate to high molecular weight (up to 30,000 Da), which are highly hydroxylated and can form insoluble complexes with carbohydrates and proteins. In the present work, the method used for quantitating tannins was not specific and could not distinguish low molecular weight phenols (which generally do not adversely affect the nutritional quality) from polyphenols of nutritional concern. The phenolic groups of tannins are bound to enzymes and other proteins by hydrogen bonding to amide groups, and they form insoluble tannin–protein complexes, resistant to digestive enzymes of monogastric animals (Sosulski, 1979). This is the reason why these compounds are considered as antinutrients.

In rice bran, phytic acid content is about 5%, this being one of the highest contents reported for foods. Thus, the values verified in the washed-up seaweeds (0.45%) were quite low but far above those established by Brazilian Legislation (Brasil, 2000), which is below 0.1%. On the other hand, the concentration of 0.45% seen in this work is below those values observed in foods normally consumed by humans, such as common beans (*P. vulgaris* = 1.45%) (Oliveira et al., 2003), refined wheat (2% to 9.6%) (Domínguez, Goméz, & León, 2002), corn (Zea mays = 0.77%), soybean (*G. Max* = 1.5%) and rice bran, with 3.77% (Cheryan, 1980). Nevertheless, the presence of phytic acid in food generally does not imply acute toxicity problems. It depends upon the other ingredients of dietary intake (Patearroyo & Fernández-Quintela, 1995). However, since phytates cannot be absorbed and humans have limited ability to hydrolyse this molecule, adverse effects of this acid upon mineral bioavailability are predicted.

3.4. Toxic metals

Considering that ocean water currents from the surroundings of Pecem Harbour may reach the sites of seaweed collection, the contents of certain elements (cadmium, lead, chromium, nickel, silicon and vanadium) were analysed in order to verify the degree of pollutant contamination in the seaweeds.

The results (Table 4) showed that, among the heavy metals considered to be toxic, cadmium, chromium, nickel and vanadium were detected. Regarding the safe levels of total toxic heavy metals in seaweeds destined for human consumption, the values detected in these seaweeds were high when compared to those established by American legislation (4 mg/100 g dry matter). Vanadium levels (3.56 mg/100 g) *per se* reach almost the maximum established limit. Cadmium levels (0.29 mg/100 g) were greater than those established by Brazilian (0.1 mg/100 g) and French legislation (0.05 mg/100 g). This suggests that the water where seaweed samples were collected is contaminated by pollutants originating presumably from Pecem Harbour (near Fleixeiras). According to Brazilian legislation, the maximum limits of tolerance for heavy metals in food are Cd, 0.1 mg/100 g, lead, 0.8 mg/10 mg), Cu, 0.01 mg/100 g, Cr, 0.1 mg/100 g and Ni, 0.5 mg/100 g). Thus, among the detected toxic minerals in seaweeds, only lead and nickel levels were below the maximum limits. Appropriate measures must be taken to have these sites remediated and to reduce impact on marine biota.

Table 4
Toxic metals and silicon content in the pool of washed-up seaweed samples collected throughout the one year period.

Heavy metals	Content of toxic metals (mg/100 g)
Cadmium (Cd)	0.29
Lead (Pb)	0.36
Chromium (Cr)	0.23
Nickel (Ni)	0.26
Silicium (Si)	989
Vanadium (V)	3.56

Values are means of triplicates. The standard deviation values were omitted since they were less than 5% of mean.

In conclusion, the meal of washed-up seaweeds from Ceará Coast, in Brazil shows antinutrient and/or toxic factors, such as trypsin and α-amylase inhibitors, polyphenol compounds (tannins), lectins, phytic acid and toxic contaminants (heavy metals) which can contribute to the reduction of its nutritional quality. Evidently, these seaweeds are not appropriate for human consumption due to toxic metals contamination and because, even after heat processing, they show trypsin inhibitor activity. In spite of the nutritional and toxic limitations, washed-up seaweeds are potential sources of nutrients and, thus, further studies are necessary to guarantee their safe utilisation for human and animal consumption.

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