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Tracing seaweeds as mineral sources for farm-animals

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Abstract This study characterized the mineral composition of 15 common Portuguese seaweed (green, brown, and red) species. Total measured mineral content ranged from 10.9 g kg^{-1} dry matter (DM) in *Gracilaria vermiculophylla* to 71.0 g kg^{-1} DM in *Codium adhaerens*, calcium being the mineral generally found in higher amounts. Overall, the results suggest that seaweeds have great potential as mineral sources for animal feeding, but a great variability between species was observed regarding their mineral profile. Compared to common animal feed ingredients, the studied seaweeds can be considered as good sources of calcium, magnesium, iron, iodine, copper, manganese, and selenium but are poor sources of phosphorous and zinc. The maximum level of dietary inclusion will be strongly dependent on the mineral profile of the seaweeds. Depending on the seaweed, the upper level of inclusion in poultry and swine diets may reach more than 40 %. The high iodine content of studied seaweeds limits their use in diets for horses, and, to a lesser extent, for ruminants. This work constitutes a paramount contribution regarding the use of seaweeds as mineral sources in animal diets, allowing a more precise choice of the algae species and level of inclusion to be used, thus assuring animal health and strengthening the seaweed industry through this underexploited application field.

Keywords Animal feed · Level of inclusion · Minerals · Seaweeds

Introduction

Seaweeds are a naturally wide available source of biomass, being also easily cultivated, achieving a rapid growth for several species. Compared to land plants, the chemical composition of seaweeds has been poorly investigated. Generally, seaweeds are rich in non-starch polysaccharides, minerals, and vitamins (Urbano and Goñi 2002). Regarding the mineral (ash) content of seaweeds, previous studies refer that this fraction accounts for up to 30–39 % of dry matter (DM) (Rupérez 2002; Burtin 2003; Balboa et al. 2015; Schiener et al. 2015). Due to this high mineral content, some studies have evaluated seaweeds, both naturally collected and enriched seaweeds, as a source of minerals for livestock (Chojnacka 2008; Michalak et al. 2011; Rey-Crespo et al. 2014). It is expected that minerals supplemented to livestock diets in such form can be absorbed with higher efficiency than from inorganic salts (Chojnacka 2008). Indeed, nutritional research indicates that chelated minerals are more efficient than inorganic sources for supplying bioavailable minerals (Evans and Critchley 2014). For example, Rey-Crespo et al. (2014) found an improved mineral status of dairy cattle offered an algae supplement, particularly in iodine and selenium blood and milk levels, suggesting a high bioavailability of seaweed minerals.

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Seaweed composition and growth rate varies according to the species, geographic area, and season of the year (Khairy and El-Shafay 2013), with their elemental content being dependent on several environmental factors such as the concentration of elements in water (Andrade et al. 2004), interactions between elements, salinity, pH, light intensity, and seaweed metabolic requirements (Żbikowski et al. 2006). Seaweed elemental composition would determine their potential use, namely as mineral sources for livestock (El-Said and El-Sikaily 2013; Rey-Crespo et al. 2014).

The present work thus aimed at determining the elemental profile of some green (*Codium adhaerens, Codium vermilara*, and *Ulva* sp.), brown (*Bifurcaria bifurcata*, *Cystoseira usneoides*, *Fucus guiryi*, *Fucus serratus*, *Fucus spiralis*, *Laminaria ochroleuca*, *Pelvetia canaliculata*, *Saccharina latissima*, *Sargassum muticum*, and *Sargassum vulgare*), and red (*Gigartina* sp., and *Gracilaria vermiculophylla*) seaweed species. The studied seaweeds were selected according to their natural presence on the North and Central Portuguese coast and to their ability to be cultivated. To the best of our knowledge, this is the first detailed mineral characterization of the most common seaweeds from the Portuguese coast. This extensive characterization allowed the evaluation of seaweeds as sources of essential, occasionally beneficial, and potentially toxic elements and its impact in their use as feed ingredients. Hence, another aim of this comprehensive mineral characterization was to predict, based on their individual mineral content, the maximum dietary inclusion level of each seaweed in the diets of different animal species. This work would constitute a first step to a full understanding of seaweeds as mineral sources that will certainly impact not only in the development of innovative feeding strategies for animal nutrition but also in the emergence of a new important application field for seaweed industry.

Material and methods

Seaweed samples

A total of 15 seaweed species, including green ($n = 3$), brown ($n = 10$), and red ($n = 2$) algae, with extensive distribution along the North and Central coast of Portugal were studied (Table 1). All seaweeds were naturally harvested with the exception of *Ulva* sp., *Saccharina latissima*, and *Gracilaria vermiculophylla*, which were cultivated in an integrated multitrophic aquaculture (IMTA) system, as described by Domingues et al. (2015). After collection, seaweed samples were rinsed with freshwater to remove epiphytes, detritus, and sand, transported to the laboratory, and dried in a forced air oven at 65 °C until constant weight. The samples were then ground to pass a 1-mm screen and stored in closed containers in a cool and dry place until further analyses.

Analytical methods

Apparatus and reagents

Ultrapure water (>18.2 MΩ cm at 25 °C), obtained with a Sartorius (Germany) Arium pro water purification system, was always used to prepare the solutions and wash the materials.

Only polypropylene labware (pipette tips, volumetric flasks and tubes) was used during the work. Except for the pipette tips (which were used as supplied by the manufacturer), all labware was decontaminated by immersion in HNO_3 10 % (*v*/*v*) solution for at least 24 h and subsequent thorough washing with ultrapure water.

For sample acid digestion, high purity concentrated (≥69 % (*w*/*w*)) HNO_3 (TraceSELECT, Fluka, France) and 30 % (*v*/*v*) H_2O_2 (TraceSELECT Ultra, Germany) were used as received.

Sample solubilization and analytical quality control

A microwave-assisted acid digestion procedure was performed for sample mineralization. For the effect, a Milestone (Sorisole, Italy) MLS 1200 Mega high performance microwave digestion unit equipped with an HPR-1000/10 S rotor was used.

Powdered samples (ca. 500 mg) were directly weighed into the microwave oven PTFE vessels. Then, 5 mL of 69 % (*w*/*w*) HNO_3 and 2 mL of 30 % (*v*/*v*) H_2O_2 were added to each vessel and the mixture was submitted to a microwave heating program (250, 0, 250, 400, and 600 W, for 1, 2, 5, 5, and 5 min, respectively). After cooling, the vessel content was transferred to 25-mL volumetric flasks and the volume was made up with ultrapure water.

For analytical quality control, the certified reference material (CRM) BCR 679 (white cabbage), as supplied by the European Commission—Joint Research Centre, Institute for Reference Materials and Measurements (Geel, Belgium), was used. It was processed as the samples. In the determination of bromine and iodine (and in addition to CRM BCR 679 in the determination of phosphorous), Seronorm Trace Elements Urine (from SERO AS, Billingstad, Norway) was used (after simple sample dilution with ultrapure water).

Ash and elemental analyses

Ground samples were dried at 105 °C for 6 h in order to express their chemical composition in a dry matter (DM) basis. Total ash was determined according to AOAC (1990, ID 942.05).

Sample solutions were analyzed for elemental content by inductively coupled plasma-mass spectrometry (ICP-MS) and flame atomic absorption spectrometry (FAAS) (manganese, iron, calcium, and magnesium). Results were expressed as

Table 1 Species and harvesting area and date of the studied seaweeds

Species	Class	Harvesting area	Harvesting date
Codium adhaerens	Green	Praia do Quebrado, Peniche (39° N, 9° W)	2010
Codium vermilara	Green	Praia do Quebrado, Peniche (39° N, 9° W)	2011
Ulva sp.	Green	Cultivated	2012
Bifurcaria bifurcata	Brown	Praia da Amorosa, Viana do Castelo (41° N, 8° W)	2013
Cystoseira usneoides	Brown	Praia do Quebrado, Peniche (39° N, 9° W)	2010
Fucus guiryi	Brown	Praia da Amorosa, Viana do Castelo (41° N, 8° W)	2013
Fucus serratus	Brown	Praia da Amorosa, Viana do Castelo (41° N, 8° W)	2013
Fucus spiralis	Brown	Praia do Norte, Viana do Castelo (41° N, 8° W)	2013
Laminaria ochroleuca	Brown	Praia da Amorosa, Viana do Castelo (41° N, 8° W)	2013
Pelvetia canaliculata	Brown	Praia do Norte, Viana do Castelo (41° N, 8° W)	2013
Saccharina latissima	Brown	Cultivated	2013
Sargassum muticum	Brown	Praia da Amorosa, Viana do Castelo (41° N, 8° W)	2013
Sargassum vulgare	Brown	Praia do Porto de Areia Sul, Peniche (39° N, 9° W)	2008
Gigartina sp.	Red	Praia da Amorosa, Viana do Castelo (41° N, 8° W)	2013
Gracilaria vermiculophylla	Red	Cultivated	2012

milligram of element per kilogram of plant on a DM basis. FAAS determinations (Ca, Mg, and Fe) were performed using a Perkin Elmer (USA) 3100 flame (air-acetylene) atomic absorption spectrometer. Calibration standards were prepared from 100 mg L^{-1} single-element standard stock solutions (Sigma-Aldrich, USA) of the elements by adequate dilution with HNO_3 0.2 % (*v*/*v*). When required, sample solutions were diluted with ultrapure water to obtain an analytical signal within the linear range of the instrument.

ICP-MS determinations were performed using an iCAP Q (Thermo Fisher Scientific, Germany) instrument, equipped with a MicroMist nebulizer, a Peltier-cooled cyclonic spray chamber, a standard quartz torch, and nickel skimmer and sampling cones. High purity (99.9997 %) argon (BIP, Gasin, Portugal) was used as the nebulizer and plasma gas. Internal standards and tuning solutions were prepared by appropriate dilution of the corresponding AccuTrace Reference Standard (AccuStandard, USA) solutions: ICP-MS-200.8-IS-1 (100 mg L^{-1} of scandium, yttrium, indium, terbium, and bismuth) and ICP-MS-200.8-TUN-1 (100 mg L^{-1} of beryllium, magnesium, cobalt, indium, and lead).

All elements, except bromine, iodine, and phosphorous (see below), were measured in the same analytical conditions. The following isotopes were monitored: ^{7}Li, ^{27}Al, (^{45}Sc), ^{51}V, ^{52}Cr, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{82}Se, ^{85}Rb, (^{89}Y), ^{95}Mo, ^{111}Cd, (^{115}In), (^{129}Tb), ^{202}Hg, ^{208}Pb, (^{209}Bi). Isotopes between parentheses are the internal standards used. Calibration standards were prepared from 100 mg L^{-1} multielement standard solutions: ICP-MS-200.8-CAL1-1 (Isostandards Material, Madrid, Spain), ICP-MS 200.8-CAL2-1 (AccuTrace Reference Standard from AccuStandard, USA), and Plasma CAL Q.C.N.3 (SCP Science, Canada).

Determination of phosphorous

Sample solutions were further diluted with 2 % (*v*/*v*) HNO_3. The elemental isotope ^{31}P was used for quantification and ^{45}Sc as internal standard. Calibration standards were prepared from sodium phosphate dibasic dihydrate (puriss. p.a. grade, Sigma-Aldrich, USA).

Determination of iodine and bromine

The determination of iodine and bromine was performed according to Julshamn et al. (2001). Sample solutions were further diluted (1:45) with ultrapure water and then 1 + 1 with NH_4OH 3 % (*v*/*v*). Elemental isotopes ^{81}Br and ^{127}I were used for quantification and ^{89}Y and ^{115}In as internal standards. Calibration standards were prepared from ULTRAgrade bromate standard solution for ion chromatography (ULTRA Scientific, Italy) and from potassium iodate (Sigma-Aldrich).

Statistical analysis

Simple regression analysis was performed between ash and total identified mineral content. Data on essential and potentially toxic minerals were subjected to factor analyses using the varimax rotation. To evaluate if mineral composition differed between seaweeds, observations were subjected to cluster analyses using the complete linkage method and the squared Euclidean distance measure. Due to the different unities and range, cluster analyses were run with standardized values. All analyses were performed using MINITAB 14 statistical software (Minitab, USA).

Results and discussion

Total identified mineral content

Seaweeds drawn from the water are rich sources of minerals, their content being higher than those reported for the edible land plants (Rupérez 2002). Table 2 presents the ash and total identified mineral content determined in the studied seaweeds. Total mineral content ranged from 10.9 g kg^{-1} DM in *G. vermiculophylla* to 71.0 g kg^{-1} DM in *Codium adhaerens*. Despite these values being lower than the ash content, the total quantified minerals positively correlated with ash content (r=75.9 %; P=0.001). The difference between total ash and identified minerals could depend on their salt content, namely sodium and potassium contents that were not quantified in the present study, but is known to be in large amounts in the seaweeds. For example, Rodríguez-Castañeda et al. (2006) found the highest concentration of sodium (152 g kg^{-1} DM) in *Codium cuneatum* collected in La Paz Bay, Mexico, and El Din and El-Sherif (2012) found up to 9.3 g of potassium per kg DM in seaweeds from Egypt. The studied seaweeds were collected in different years and at different times within the year, thus the variability observed in their composition can be partly explained by their phylogenetic differences, but also by seasonal and geographic conditions (Rodríguez-Castañeda et al. 2006; Riosmena-Rodríguez et al. 2010; Kendel et al. 2013).

Table 2 Ash and total mineral content (g kg^{-1} DM) of the studied seaweeds[a]

	Ash	Total mineral content
Codium adhaerens	727.1	71.0
	(0.00)	(1.42)
Codium vermilara	497.3	24.0
	(0.00)	(1.07)
Ulva sp.	249.7	29.0
	(3.15)	(0.38)
Bifurcaria bifurcata	365.4	17.2
	(0.31)	(3.19)
Cystoseira usneoides	329.2	19.7
	(0.00)	(1.44)
Fucus guiryi	216.7	18.9
	(0.67)	(0.41)
Fucus serratus	235.4	23.8
	(1.03)	(0.29)
Fucus spiralis	276.5	21.6
	(3.04)	(0.01)
Laminaria ochroleuca	266.1	22.7
	(0.84)	(1.19)
Pelvetia canaliculata	245.8	19.9
	(0.64)	(0.13)
Saccharina latissima	171.0	18.9
	(0.74)	(0.71)
Sargassum muticum	222.5	23.2
	(0.44)	(0.64)
Sargassum vulgare	274.3	33.9
	(0.00)	(5.99)
Gigartina sp.	348.4	18.2
	(2.97)	(0.04)
Gracilaria vermiculophylla	278.3	10.9
	(1.34)	(0.40)

[a] Values are expressed as mean (standard deviation, SD) of two replicates.

The present work followed the classification of minerals proposed by Suttle (2010). For the evaluation of seaweeds as mineral sources in animal feeding, a comparison was made between the seaweed's content in individual elements and that of the most often used feed ingredients. To predict the maximum dietary level inclusion of seaweeds, the maximum tolerable level of each mineral for the different animal species as proposed by NRC (2005) was the criterion chosen.

Essential minerals

The first group of minerals targeted by this study comprises a family of chemical elements that are essential to the health and well-being of farm livestock (Suttle 2010): calcium, phosphorous, magnesium, iron, iodine, zinc, copper, manganese, selenium, cobalt, and bromine. In contrast to the other elements, it was only very recently that bromine was established as an essential element, necessary for the basement membrane architecture and tissue development (McCall et al. 2014). The subsequent discussion of essential mineral data will be subdivided in two sections according to their requirement levels: macrominerals (calcium, phosphorous, magnesium) and trace elements (iron, iodine, zinc, copper, manganese, selenium, cobalt, and bromine).

Macrominerals

Table 3 presents the results of the essential macrominerals determined in the 15 seaweeds evaluated. Calcium is the mineral required at higher amounts by animals, the requirements being dependent on animal species, age, and physiological status (Soetan et al. 2010). Seaweeds are one of the most important plant sources of calcium; this representing as high as 70 g kg^{-1} DM (Marsham et al. 2007). In the present study, calcium was the mineral found in higher levels in most seaweed, the highest value being observed in *C. adhaerens*, a green macroalgae, reaching 49.8 g kg^{-1} DM. The lowest content was found in the red seaweeds, *Gigartina* sp. and *G. vermiculophylla* (4.68 and 1.96 g kg^{-1} DM, respectively). When assessing the mineral composition of marine seaweeds from the Egyptian Mediterranean sea coast, El Din and El-Sherif (2012) and El-Said and El-Sikaily (2013) also recorded

Table 3 Essential macrominerals content (g kg^{-1} DM) of the studied seaweeds[a]

	Ca	P	Mg
Codium adhaerens	49.76	0.95	14.93
	(1.311)	(0.056)	(0.271)
Codium vermilara	6.83	1.24	14.61
	(0.285)	(0.029)	(0.369)
Ulva sp.	7.46	1.28	19.54
	(0.250)	(0.073)	(0.087)
Bifurcaria bifurcata	9.08	1.97	5.25
	(1.290)	(0.190)	(0.642)
Cystoseira usneoides	12.60	1.22	4.37
	(0.860)	(0.054)	(0.084)
Fucus guiryi	8.95	1.90	7.02
	(0.108)	(0.089)	(0.049)
Fucus serratus	12.84	2.34	7.24
	(0.001)	(0.079)	(0.031)
Fucus spiralis	10.49	1.56	8.19
	(0.195)	(0.022)	(0.134)
Laminaria ochroleuca	12.55	2.57	6.11
	(0.764)	(0.084)	(0.094)
Pelvetia canaliculata	9.23	1.41	8.12
	(0.036)	(0.024)	(0.059)
Saccharina latissima	9.59	2.26	5.31
	(0.404)	(0.001)	(0.277)
Sargassum muticum	13.02	1.80	7.30
	(0.218)	(0.067)	(0.096)
Sargassum vulgare	27.21	1.06	4.05
	(4.008)	(0.029)	(0.010)
Gigartina sp.	4.68	3.59	8.21
	(0.083)	(0.019)	(0.064)
Gracilaria vermiculophylla	1.96	2.35	4.31
	(0.064)	(0.111)	(0.060)

[a] Values are expressed as means (SD) of two replicates.

the maximum values of calcium in green seaweeds (30.1 and 16.7 g kg^{-1} DM, respectively). Although high dietary amounts of calcium can affect the metabolism of phosphorous, magnesium, and certain trace elements such as zinc (NRC 2001), calcium strong affinity for carboxylic polysaccharides (alginates) may limit its availability in seaweeds (Burtin 2003).

Phosphorus is involved in the metabolism of almost all nutrients through its vital role in both vitamin and enzyme activity (Soetan et al. 2010). The phosphorous content of seaweeds ranged from 0.95 to 3.59 g kg^{-1} DM, in *C. adhaerens* and *Gigartina* sp., respectively, the average value being lower than the most commonly present in feed ingredients (e.g., 2.9–12.0 g kg^{-1} DM in corn grain, wheat grain, rapeseed meal, soybean meal) used in animal nutrition (FEDNA 2010). In seaweeds from tropical environments, a phosphorous content up to 6.25 g kg^{-1} DM was observed (Nascimento et al. 2014). This higher content could be partly explained by the commonly observed saturation of the tropic plants with nutrients, which are sufficient to promote high growth rates and tissue nutrients in suitable concentrations. Higher concentrations of phosphorus might be related to the characteristics of fast growing species, which produce more ATP (Diniz et al. 2012).

The calcium/phosphorous ratio is also important to be considered when feeding animals. Although the absolute ratio depends on the animal species, calcium should always be included in the diets at a higher concentration than phosphorous, which may be difficult to maintain without calcium supplementation in corn-based rations, due to the high concentration of phosphorous and low concentration of calcium in corn grain (FEDNA 2010). With the exception of *G. vermiculophylla*, that presented a calcium/phosphorous ratio of 0.84, all other seaweeds presented higher levels of calcium than phosphorous, thus suggesting seaweeds to be a potential natural source of calcium in animal diets.

Magnesium is a major intracellular cation, being a cofactor of many enzymes, as those involved in cellular respiration, and phosphate transfer reactions (NRC 2005). Magnesium deficiency is more common in ruminants due to their dependency on ruminal magnesium absorption, especially in grazing animals due to the low magnesium content of growing pasture and a relatively high content of antagonists that interfere with their transport across the rumen wall (Martens and Schweigel 2000). The magnesium content of the studied seaweeds was generally higher than that of the most common animal feed ingredients (e.g., 1.0–2.7 g kg^{-1} DM in cereal grains, soybean meal; FEDNA 2010), ranging from 4.05 g kg^{-1} DM in *S. vulgare* to 19.5 g kg^{-1} DM in *Ulva* sp. Conversely, when evaluating seaweeds from Sabah's South China sea, Krishnaiah et al. (2008) found a magnesium content ranging from 5.6 g kg^{-1} DM in *Ulva* sp. to 10.5 g kg^{-1} DM in *S. vulgare*. However, in that study, the magnesium content showed a major seasonal variation (9.25 %).

Trace elements

Seaweeds showed a wide variation on their essential trace element (iron, iodine, zinc, copper, manganese, selenium, cobalt, and bromine) profile (Table 4).

Iron plays an important role in oxygen delivery to the tissues and as a cofactor of several enzymes involved in energy metabolism and thermoregulation (Beard 2001). Livestock dietary requirements of iron range from 50 to 100 mg kg^{-1} DM (NRC 2005). Feeds commonly used for farm animals contain high and variable contents of iron, ranging from 30 to 60 mg kg^{-1} DM in cereal grains, and from 100 to 200 mg kg^{-1} DM in oilseed meals (Suttle 2010). Forages present an iron content quite variable within species and type of soil in which the plants grow (FEDNA 2010; Suttle 2010).

Table 4 Essential trace element levels (mg kg^{-1}) in the studied seaweeds[a]

	Fe	I	Zn	Cu	Mn	Se	Co	Br
Codium adhaerens	3501	475.0	8.00	2.633	45.12	2.658	0.958	1233.3
	(153.4)	(140.93)	(0.288)	(0.366)	(1.809)	(0.160)	(0.088)	(32.19)
Codium vermilara	98	75.4	2.98	0.594	10.31	2.465	0.164	1027.0
	(11.6)	(16.57)	(0.119)	(0.015)	(0.263)	(0.064)	(0.002)	(76.76)
Ulva sp.	139	23.3	16.19	3.356	12.65	1.946	0.252	513.6
	(3.1)	(0.65)	(8.256)	(1.514)	(6.291)	(1.158)	(0.138)	(6.97)
Bifurcaria bifurcata	258	253.8	7.93	0.857	5.82	0.714	0.315	263.0
	(24.6)	(31.24)	(0.263)	(0.164)	(0.075)	(0.352)	(0.004)	(66.43)
Cystoseira usneoides	142	507.2	6.76	1.311	5.99	1.654	0.156	647.7
	(29.1)	(2.87)	(0.654)	(0.171)	(0.580)	(0.210)	(0.012)	(0.76)
Fucus guiryi	132	273.4	45.34	2.090	109.01	0.905	1.485	345.3
	(0.3)	(3.96)	(0.169)	(0.011)	(0.171)	(0.240)	(0.009)	(38.53)
Fucus serratus	310	322.5	52.75	2.685	149.61	1.215	1.964	420.3
	(2.8)	(0.37)	(0.351)	(0.018)	(0.371)	(0.354)	(0.021)	(95.37)
Fucus spiralis	515	232.7	153.62	2.075	62.61	0.807	0.823	335.6
	(9.6)	(12.64)	(4.833)	(0.198)	(1.158)	(0.155)	(0.015)	(2.85)
Laminaria ochroleuca	179	883.5	24.75	1.233	8.62	0.937	0.119	281.4
	(3.0)	(122.56)	(0.528)	(0.152)	(0.160)	(0.175)	(0.006)	(52.86)
Pelvetia canaliculata	202	250.7	66.65	4.523	17.65	1.447	0.523	524.8
	(7.2)	(1.64)	(3.491)	(4.534)	(1.289)	(0.192)	(0.038)	(33.04)
Saccharina latissima	30	957.6	41.55	1.170	3.91	1.300	0.392	552.0
	(0.5)	(44.42)	(3.852)	(0.077)	(0.210)	(0.821)	(0.017)	(126.80)
Sargassum muticum	307	216.0	12.02	2.334	26.72	1.015	0.472	382.2
	(17.7)	(76.79)	(0.431)	(0.314)	(1.404)	(0.141)	(0.035)	(115.99)
Sargassum vulgare	436	583.0	11.74	8.679	24.06	1.447	0.363	490.2
	(101.8)	(127.17)	(0.159)	(0.011)	(0.176)	(0.210)	(0.008)	(38.83)
Gigartina sp.	366	194.1	46.74	2.024	116.22	1.735	0.740	829.3
	(5.9)	(0.20)	(0.123)	(0.075)	(2.036)	(0.602)	(0.020)	(87.04)
Gracilaria vermiculophylla	1049	46.7	32.81	1.998	392.27	1.325	1.534	640.1
	(54.4)	(16.95)	(0.452)	(0.024)	(1.669)	(0.217)	(0.010)	(14.74)

[a] Values are expressed as means (SD) of two replicates.

Regarding seaweeds, El-Said and El-Sikaily (2013) reported the highest iron content in red algal species (789 ±40.0 mg kg^{-1} DM) and the lowest in brown algal species (40.3 ± 4.05 mg kg^{-1} DM). The iron content herein determined also showed a great variation, ranging from 30 mg kg^{-1} DM in *S. latissima* to 3501 mg kg^{-1} DM in *C. adhaerens*, the majority of seaweeds being richer sources of iron than oilseed meals. Although uncommon, if the intake of iron is sufficiently high, signs of toxicosis can occur in most domestic animals. However, the high calcium content of seaweeds can reduce iron toxicity (Prather and Miller 1992) and seaweed toxic potential would depend on the iron bioavailability.

Iodine has a vital function as a constituent of thyroid hormones (Soetan et al. 2010). Iodine dietary requirements were established to be 0.1 mg kg^{-1} DM for horses (NRC 1989), 0.3 to 0.4 mg kg^{-1} DM for growing and laying birds (NRC 1994), 0.14 mg kg^{-1} DM for pig (NRC 1998), and 0.11 to 0.54 mg kg^{-1} DM for sheep and cattle, respectively in summer and winter (ARC 1980). Iodine content of feed ingredients is quite variable, cereals and oilseed meals being poor, animal proteins intermediate ones, and fish meals rich sources (FEDNA 2010). Moreover, the iodine content of plants is highly dependent on the species, climatic and seasonal conditions, and the capacity of the soil to provide iodine (Suttle 2010). In the present study, the cultivated *S. latissima* presented the highest iodine content (958 mg kg^{-1} DM), followed by the naturally collected *L. ochroleuca* (884 mg kg^{-1} DM), which is in agreement with the rankings of iodine content established in previous studies (Burtin 2003; Verhaeghe 2007). This is the reason why brown seaweeds have been traditionally used for treating goiter (Liu et al. 2012). Along with their high iodine content, the weakness of the linkages between polysaccharides and iodine allows its rapid release (Fleurence et al. 1994), making seaweeds a good dietary source of this element.

Zinc is another essential nutrient for animals, functioning largely or entirely in enzyme systems and being involved in protein synthesis, carbohydrate metabolism, and many other biochemical reactions (Miller 1970). The low availability of zinc in vegetable protein sources, and the use of soybean meal in typical diets for swine and chicken contribute to the higher incidence of zinc deficiency in these species. Although parakeratosis can occur in cattle feed diets with low zinc content, it does not seem to be a major nutritional problem in ruminants (Luecke 1984). However, zinc supplementation to dairy cows has been used to improve immune status, thus preventing high somatic cell counts and mastitis rates (Cortinhas et al. 2010). In the present study, the zinc content ranged from 2.98 mg kg^{-1} DM in *C. vermilara* to 154 mg kg^{-1} DM in *F. spiralis*, being generally higher in brown seaweeds. Most seaweeds studied proved to be not a good source of zinc, with contents similar to those of cereal grains normally used in animal feeding. Few seaweed varieties had values higher than those found in oilseed meals, which are also deficient in zinc (e.g., 52–63 mg kg^{-1} DM in soybean meal and rapeseed meal, respectively; FEDNA 2010).

Another important issue is the calcium/zinc ratio, as previous studies suggest the existence of an antagonistic effect between these elements in pigs, chickens, rats, and cattle (Hoekstra et al. 1956; Newland et al. 1958; Thilsing-Hansen and Jorgensen 2001). The calcium/zinc ratio of the analyzed seaweeds was >1 for *B. bifurcata* (1.1), *C. vermilara* (3.8), *C. usneoides* (5.8), *S. vulgare* (15.5), and *C. adhaerens* (41.8), thus requiring some caution in their use to prevent symptoms of zinc deficiency.

Copper is essential for the activity of numerous enzymes, cofactors, and reactive proteins (Suttle 2010). Copper content in typical feed ingredients is insufficient to cover animal requirements, thus it is normally added as a supplement to the diet (Li et al. 2007). In this work, copper content ranged from 0.59 to 8.68 mg kg^{-1} DM in *C. vermilara*, and *S. vulgare*, respectively. Andrade et al. (2006) found a wider range, from 1.6 mg kg^{-1} DM in *Ahnfeltiopsis* sp. to 27.1 mg kg^{-1} DM in *Porphyra* sp. in reference uncontaminated sites, suggesting that macroalgal copper content may be a useful indicator of its biologically available fraction in seawater. Copper poisoning can occur when excessive amounts of copper are ingested, sheep being most affected (Sivertsen and Løvberg 2014). Additionally, factors that alter copper metabolism can influence chronic copper poisoning by enhancing its absorption or body accumulation, namely low levels of molybdenum in the diet. Adequate diets should present a copper/molybdenum ratio between 5:1 and 10:1; a ratio ≤2:1 could result in a severe decrease in copper absorption and copper deficiency (Merck 2010). None of the studied seaweeds had a copper/molybdenum ratio lower than 2:1.

Manganese functions in the body by regulating the activity of certain enzymes, and it is particularly required for normal skeletal development and reproduction (Soetan et al. 2010). Manganese requirements can range from 4 to over 60 mg kg^{-1} of diet DM, being greatly different among animal species and affected by dietary factors that reduce manganese absorption (NRC 1994). Broilers and turkeys have a substantially higher requirement for manganese than do other animals, and to prevent deficiency, manganese is supplemented to the diet, due to its low content in cereal grains (15 to 30 mg kg^{-1} DM; NRC 2005; FEDNA 2010). Concerning ruminant diets, forages vary significantly in manganese content, but it is generally higher than in cereal grains (e.g., 40 mg kg^{-1} DM for dehydrated alfalfa meal; FEDNA 2010). However, the manganese naturally present in forages seems to be poorly utilized by cattle, being usually supplied as a mineral mix added to the complete diet (NRC 2001). *Fucus* species, *Gigartina* sp., and *G. vermiculophylla* had the highest levels of manganese (62.61–392.27 mg kg^{-1} DM), but in order to fulfill the highest requirements (60 mg kg^{-1} diet DM), they would have to be included in the diet at very high rates: 55 % (*F. guiryi*), 40 % (*F. serratus*), 96 % (*F. spiralis*), 52 % (*Gigartina* sp.), and 15 % (*G. vermiculophylla*). A wide range of manganese content was also observed by Smith et al. (2010) in six wild-collected edible New Zealand seaweeds (3.7–192 mg kg^{-1} DM). High levels of calcium, phosphorus, and iron are known to increase manganese requirements in poultry, phosphorus being more important in decreasing manganese absorption (Wedekind et al. 1991; Hansen and Spears 2009). Although the seaweeds studied had higher levels of calcium, the phosphorous content was relatively lower than that found in common animal feed ingredients, suggesting a potential low effect of seaweed ingestion on manganese absorption.

Selenium is an essential trace element important for normal physiology, being a component of glutathione peroxidases, a class of antioxidative enzymes primarily responsible for the reduction of peroxide free radicals and for prostaglandin synthesis, by protecting the oxidative state of lipid intermediates, thus strongly influencing inflammation and immune responses (Hoffmann and Berry 2008; Huang et al. 2012). Selenium requirements are about 0.1 mg kg^{-1} diet DM in uncomplicated situations and 0.3 mg kg^{-1} diet DM when high levels of sulfur or other selenium antagonists are present (Mayland 1994), the intake required to prevent clinical and subclinical signs of deficiency varying with the oxidant stress presented by a given combination of diet and environmental conditions, as well as the level of selenium antagonists (López-Alonso 2012). The selenium content of soil determines its amount in plants (Mora et al. 2008), which can vary as much as 200-fold between the same crops grown in different regions (Yan et al. 2004). However, selenium content is typically low in the commonly used feed ingredients (FEDNA 2010), being normally guaranteed through supplementation with a mineral mix even though the maximal amount added is legally limited to 0.3 mg kg^{-1} DM. Seaweeds are able to accumulate selenium to levels three to four orders of magnitude above its concentration in seawater (Hu et al. 1996) and to transform inorganic selenium into organic species (Yan et al. 2004), which impacts in the bioavailability of this element in the diets that contain seaweeds. In the present study, selenium content ranged from 0.71 mg kg^{-1} DM in *B. bifurcata* to 2.66 mg kg^{-1} DM in *C. adhaerens*. Previous studies reported narrow selenium contents ranging from 0.02 to 0.53 mg kg^{-1} DM (Maher et al. 1992) and 0.05 to 0.51 mg kg^{-1} (Turner et al. 2013). Significantly lower concentrations of selenium have been reported for brown seaweeds relative to green (excluding *Ulva* sp.) and red ones, probably due to the relatively lower concentrations of sulfur-containing amino acids for binding and storage (Maher et al. 1992). From the results we obtained, and in order to satisfy selenium requirements (0.1 mg kg^{-1} diet DM and 0.3 mg kg^{-1} diet DM, NRC 2005), *B. bifurcata* and *C. adhaerens* would have to be included in the diet at a level of 14–42 % and 4–11 %, respectively. However, selenium usually affects organisms in a strictly dosage-dependent manner being essential at low, but toxic at high concentrations. Chronic selenosis is developed when the selenium content in the diet increases up to 3–15 mg kg^{-1} DM (Mayland 1994). According to the results obtained in the present study, selenium toxicosis due to seaweed supplementation would not constitute a problem even if *C. adhaerens* comprised the whole diet.

The only known function of cobalt is to be an essential component of vitamin B$_{12}$ (cobalamin; NRC 2005). Monogastric animals require a dietary source of vitamin B$_{12}$ as mammals lack the ability to synthesize it. In order for ruminal bacteria to synthesize enough vitamin B$_{12}$ to meet the animal requirement, the cobalt content of ruminant diets should be between 0.10 and 0.15 mg kg^{-1} DM (NRC 2005). The cobalt content of the studied seaweeds ranged from 0.12 mg kg^{-1} DM in *L. ochroleuca* to 1.96 mg kg^{-1} DM in *F. serratus*, agreeing with the results from Jayasekera and Rossbach (1996) that found the highest cobalt content in *Fucus* samples from the North Sea. In order to fulfill ruminant's requirement, *F. serratus* would therefore need to be included in the diet at only 7.6 %. The relatively high content of cobalt of some seaweeds would constitute an advantage for their use as most animal feedstuffs have low cobalt levels (<0.0005 mg kg^{-1} DM; NRC 2005).

It was very recently that McCall et al. (2014) established ionic bromide as required for sulfilimine formation within collagen IV, an event critical for basement membrane assembly and tissue development. Bromide is the most likely form of bromine that animals are exposed to through feed and water. Seaweeds are particularly strong concentrators of bromine (Saenko et al. 1978). The studied seaweeds presented bromine levels ranging from 263 mg kg^{-1} DM in *B. bifurcata* to 1233 mg kg^{-1} DM in *C. adhaerens*. Apaydin et al. (2010) found bromine contents ranging from 400 to 1000 mg kg^{-1} in *Ulva lactuca* collected from eight different regions of Istanbul, Turkey. Bromine toxicity has not been a problem in animal nutrition. Hatchling chicks tolerate 5000 mg kg^{-1} DM for 4 weeks without impaired growth or efficiency in feed conversion (NRC 2005). However, maximum tolerable levels are still unavailable for all livestock species.

Similarities among seaweeds according to essential mineral profile

Despite being well known that seaweed composition can greatly vary spatially and temporarily, factor and cluster analysis were applied to identify patterns in our data set that highlight similarities and differences among seaweeds, according to essential mineral composition. The first factor represented 32.0 % of the variation and it was associated with bromine, iron, calcium, selenium, and magnesium, whereas the second factor, responsible for 19.1 % of the variation, was mainly represented by manganese, phosphorous, and zinc (Fig. 1). As shown in Fig. 1a, four clusters can be clearly distinguished. One cluster (C1) includes *F. serratus*, *F. spiralis*, *F. guiryi*, *L. ochroleuca*, *S. latissima*, *B. bifurcata*, *S. muticum*, *P. canaliculata*, *C. usneoides*, and *S. vulgare* for presenting high levels of copper, zinc, and iodine. *Codium adhaerens* constituted cluster 2 (C2), given its high contents of iron, calcium, bromine, and selenium. *Codium vermilara* and *Ulva* sp. formed cluster 3 (C3), having in common a high level of magnesium. Cluster 4 (C4) comprised the red seaweeds, *G. vermiculophylla* and *Gigartina* sp. that presented high contents of phosphorous and manganese. The similarities and differences observed among seaweeds may play an important role on diet formulation regarding the choice and availability of the different seaweeds as a source of individual essential minerals.

Occasionally beneficial mineral elements

According to Suttle (2010), other trace elements may be occasionally beneficial (e.g., molybdenum, chromium, vanadium, nickel, lithium, rubidium). They are needed in very low concentrations (<1 mg kg^{-1} DM). Table 5 presents some occasionally beneficial trace elements found in the studied seaweeds (molybdenum, chromium, vanadium, nickel, lithium, and rubidium).

Animal molybdenum requirements are extremely low, but as it is a component of aldehyde oxidase, sulfite oxidase, and xanthine oxidase, it is probably essential for all higher animals (Hille et al. 2011). In the present study, the molybdenum content varied from 0.141 mg kg^{-1} DM in *B. bifurcata* to

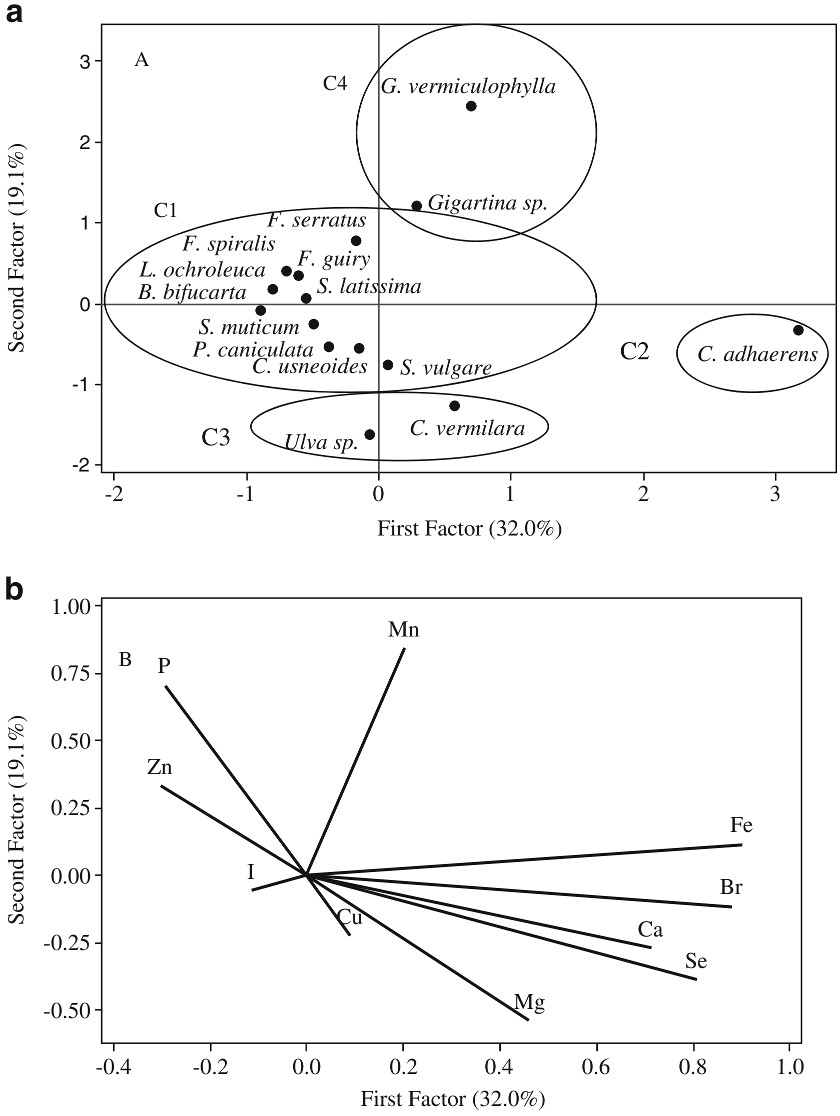

Fig. 1 Projection of seaweed samples (**a**) (variables) and loadings (**b**) by essential mineral content into the plane composed by the first and second factor containing 51.1 % of the total variance

0.537 mg kg^{-1} DM in *G. vermiculophylla*, which is similar to the commonly detected concentrations in cereal grains and straws (0.2 to 0.5 mg kg^{-1} DM, NRC 2005).

In the recent past, it was assumed that practical diets for domestic animals provided sufficient chromium to meet animal requirements. However, there is growing evidence that dietary supplementation with organic chromium may affect animal metabolism and production performance (Amata 2013). NRC (2005) established a maximum tolerable level for chromium (III) at 3000 mg kg^{-1} DM as chromium (III) oxide, and for more soluble forms, a maximum tolerable level of 500 mg kg^{-1} DM for poultry and 100 mg kg^{-1} DM for mammalian species. The studied seaweeds had a chromium content ranging from 0.387 mg kg^{-1} DM in *C. vermilara* to 7.12 mg kg^{-1} DM in *C. adhaerens*, which is below the maximum tolerable level. Devi et al. (2009) also found a great variation in seaweed chromium content, ranging from 3.8 to 41.6 mg kg^{-1} DM in the red alga *Acanthophora spicifera* and the brown alga *Sargassum wightii*, respectively.

A defined biochemical function of vanadium is still unknown in higher animals. The levels of vanadium found in common feedstuffs can be considered adequate, since vanadium deficiencies are hardly seen (NRC 2005). Conversely, vanadium is an essential element of several enzymes in algae (Fries 1982), being reported at levels from 0.3 to 10 mg kg^{-1} DM in seaweeds (Yamamoto et al. 1970). Similarly, in the present study, vanadium content varied between 0.649 and 8.35 mg kg^{-1} DM, respectively in *C. adhaerens* and *L. ochroleuca*. Even if the latter seaweed constitutes the unique ingredient of the diet, it would not reach the level pointed to cause a decrease in animal feed intake (e.g., 10 mg vanadium kg^{-1} body weight of sheep; NRC 2005).

Nickel is essential for some lower forms of life, being a component of ureases in algae (NRC 2005). Nickel is

Table 5 Occasionally beneficial trace element levels (mg kg^{-1}) in the studied seaweeds[a]

	Mo	Cr	V	Ni	Li	Rb
Codium adhaerens	0.287 (0.035)	7.119 (0.279)	8.349 (0.158)	4.260 (0.908)	5.628 (0.017)	12.415 (0.201)
Codium vermilara	0.121 (0.004)	0.387 (0.042)	6.346 (0.529)	1.729 (0.084)	2.413 (0.110)	2.176 (0.036)
Ulva sp.	0.216 (0.095)	1.838 (1.190)	4.446 (2.616)	6.400 (3.415)	0.641 (0.347)	12.835 (7.064)
Bifurcaria bifurcata	0.141 (0.008)	1.109 (0.137)	1.114 (0.349)	0.884 (0.119)	0.815 (0.003)	33.801 (2.092)
Cystoseira usneoides	0.232 (0.057)	0.584 (0.097)	2.953 (0.421)	0.792 (0.066)	0.510 (0.082)	24.852 (0.851)
Fucus guiryi	0.259 (0.043)	0.533 (0.022)	1.128 (0.075)	6.312 (0.021)	0.669 (0.028)	7.907 (0.069)
Fucus serratus	0.285 (0.001)	0.969 (0.065)	1.629 (0.232)	4.658 (0.169)	1.131 (0.026)	8.673 (0.231)
Fucus spiralis	0.237 (0.005)	1.170 (0.056)	1.669 (0.150)	3.953 (0.074)	1.757 (0.073)	10.939 (0.066)
Laminaria ochroleuca	0.197 (0.010)	1.080 (0.082)	0.649 (0.033)	0.971 (0.057)	0.773 (0.009)	21.353 (0.034)
Pelvetia canaliculata	0.243 (0.008)	0.654 (0.059)	1.278 (0.019)	2.083 (0.172)	0.905 (0.075)	8.868 (0.145)
Saccharina latissima	0.235 (0.091)	1.723 (0.026)	1.337 (0.067)	1.377 (0.054)	0.360 (0.025)	12.217 (0.667)
Sargassum muticum	0.312 (0.025)	0.696 (0.101)	1.590 (0.065)	1.991 (0.160)	0.768 (0.044)	13.478 (0.121)
Sargassum vulgare	0.384 (0.005)	1.655 (0.213)	2.637 (0.227)	2.492 (0.039)	0.835 (0.137)	6.362 (0.029)
Gigartina sp.	0.288 (0.007)	0.530 (0.023)	3.806 (0.320)	2.618 (0.014)	1.143 (0.009)	23.231 (0.906)
Gracilaria vermiculophylla	0.537 (0.042)	0.534 (0.020)	3.809 (0.184)	1.481 (0.021)	0.767 (0.059)	18.953 (0.830)

[a] Values are expressed as means (SD) of two replicates.

considered a nonessential nutrient for higher animals, due to its undefined specific biochemical function. Nickel content of seaweeds studied ranged from 0.792 mg kg^{-1} DM in *C. usneoides* to 6.400 mg kg^{-1} DM in *Ulva* sp. A wide range was also reported earlier by Murugaiyan and Narasimman (2013; 8.76 to 232.14 μg mL^{-1}, respectively, in *Chaetomorpha crassa* and *Caulerpa racemosa*). The observed values were much lower than the maximal dietary level able to induce signs of nickel toxicity in chicks, cows, rabbits, and pigs (100 mg nickel kg^{-1} DM supplemented as a water-soluble salt; NRC 2005).

Despite being used as a feed aversion substance for grazing animals, lithium may have some beneficial effects on animals as lithium deficiency has been shown to depress reproductive performance (NRC 2005). In the present study, lithium content ranged from 0.360 mg kg^{-1} DM in *S. latissima* to 5.628 mg kg^{-1} DM in *C. adhaerens*, which is much lower than the maximum tolerable lithium level for domestic animals that is responsible for food aversion and toxicity (25 mg kg^{-1} DM; NRC 2005).

Rubidium has been suggested to be beneficial, or possibly essential, for higher animals, affecting phosphorus, calcium, and magnesium metabolism (NRC 2005). The rubidium content varied from 2.176 mg kg^{-1} DM in *C. vermilara* to 33.881 mg kg^{-1} DM in *B. bifurcata*. This wide range observed could be due not only to the species but also to the individual growth rate of seaweeds, as it is known that the concentration of rubidium decreases as the specific growth rate increases (Rice and Lapointe 1981).

Potentially toxic mineral elements

The group of potentially toxic trace elements includes different metals such as cadmium, mercury, lead, and aluminum and the metalloid arsenic. The content of these potentially toxic elements in the seaweeds studied in this work is presented in Table 6.

Regarding arsenic, some studies suggest a beneficial function of arsenic at very low amounts (e.g., less than 0.035 mg kg^{-1} DM for goats, Anke 1986). In the present study, the arsenic content ranged from 9.44 mg kg^{-1} DM in *C. adhaerens* to 82.46 mg kg^{-1} DM in *C. usneoides*. A wide range of arsenic content was also reported by Chancho et al. (2010; from 1.4 to 117 mg kg^{-1} DM) and Stoeppler (2004; from 1 to 180 mg kg^{-1} DM). The toxicity of arsenic strongly depends on its chemical form (Tchounwou et al. 2012). The majority of arsenic found in algae is virtually nontoxic (organic form); sheep and cattle being even able to develop a taste for arsenic (Clarke and Clarke 1975).

Animals tolerate an acute exposure to 25 mg kg^{-1} DM of cadmium in the diet for a few days (NRC 2005). The cadmium content ranged from 0.065 mg kg^{-1} DM in *G. vermiculophylla* to 1.649 mg kg^{-1} DM in *S. Latissima*, suggesting a low toxicological risk associated to seaweed consumption. Low cadmium content (average value of 0.5 mg kg^{-1} DM) was also observed in commercial edible seaweeds in Korea (Hwang et al. 2010), and in *Gracilaria verrucosa* collected in Thermaikos Gulf, Greece, during the summer (less than 1 mg kg^{-1} DM; Malea and Haritonidis 1999).

In the present study, mercury content ranged from 0.024 mg kg^{-1} DM in *Gigartina* sp. to 0.161 mg kg^{-1} DM in *F. spiralis*, a wider range than that reported in Korea (0.01 to 0.05 mg kg^{-1} DM, Hwang et al. 2010), but lower than the maximum tolerable level for domestic animals (around 1 mg kg^{-1} body weight; NRC 2005).

Lead content ranged from 0.133 mg kg^{-1} DM in *B. bifurcata* to 3.246 mg kg^{-1} DM in *C. adhaerens*. The maximum tolerable level of lead is 250 mg kg^{-1} DM for ruminant animals and ranges from 0.5 to 1 mg kg^{-1} DM for chickens and quails (NRC 2005). Thus, for the last species, the seaweeds presenting high lead levels (e.g., *C. adhaerens, Gigartina* sp., *G. vermiculophylla*) should be used with caution to prevent health problems.

No conclusive evidence exists if aluminum has any essential function in animals (NRC 2005). The aluminum content of the studied seaweeds showed a significant variation, from 11.009 mg kg^{-1} in *S. latissima* to 2803.773 mg kg^{-1} DM in *C. adhaerens*. However, toxicity of orally administered aluminum is not a significant problem in livestock as long as gut and kidney functions are normal. Aluminum toxicity in healthy animals firstly reflects the adverse effect of dietary aluminum on phosphorus utilization (NRC 2005).

Similarities between seaweeds according to potentially toxic mineral profile

Factor and cluster analysis were applied to identify similarities and differences between seaweed samples according to potentially toxic element content. The first factor accounted for 36.0 % of the variation, and it was associated with arsenic, cadmium, and mercury, whereas the second factor, responsible for 23.0 % of the variation, was represented by aluminum and lead (Fig. 2). As shown in Fig. 2a, four clusters can be clearly distinguished. One cluster (C1) included *B. bifurcata*, *L. ochroleuca*, *P. canaliculata*, *S. muticum*, and *C. usneoides* for having high levels of arsenic. *Codium adhaerens* constituted cluster 2 (C2), given its high contents of lead and aluminum. *Codium vermilara*, *Ulva* sp., *Gigartina* sp., *G. vermiculophylla*, and *S. vulgare* formed cluster 3 (C3),

Table 6 Potentially toxic trace element levels (mg kg^{-1}) in the studied seaweeds[a]

	As	Cd	Hg	Pb	Al
Codium adhaerens	9.443	0.117	0.043	3.246	2803.773
	(0.186)	(0.006)	(0.002)	(0.089)	(152.480)
Codium vermilara	18.007	0.089	0.071	0.615	108.212
	(0.422)	(0.022)	(0.080)	(0.019)	(17.360)
Ulva sp.	10.836	0.647	0.067	0.887	121.652
	(5.829)	(0.362)	(0.064)	(0.707)	(76.327)
Bifurcaria bifurcata	58.349	0.148	0.027	0.133	227.645
	(3.056)	(0.014)	(0.010)	(0.024)	(12.256)
Cystoseira usneoides	82.464	0.454	0.037	0.206	138.424
	(2.572)	(0.027)	(0.002)	(0.051)	(57.157)
Fucus guiryi	59.266	1.215	0.038	0.216	122.312
	(0.430)	(0.046)	(0.014)	(0.012)	(12.484)
Fucus serratus	42.428	1.418	0.085	0.501	286.410
	(0.570)	(0.036)	(0.095)	(0.051)	(0.430)
Fucus spiralis	38.616	0.351	0.161	0.844	571.466
	(1.549)	(0.007)	(0.041)	(0.092)	(77.687)
Laminaria ochroleuca	54.138	0.300	0.026	0.134	127.046
	(0.167)	(0.011)	(0.010)	(0.028)	(3.153)
Pelvetia canaliculata	49.426	0.148	0.046	0.373	172.003
	(1.918)	(0.011)	(0.012)	(0.039)	(19.002)
Saccharina latissima	67.074	1.649	0.117	0.196	11.009
	(4.858)	(0.180)	(0.091)	(0.036)	(1.611)
Sargassum muticum	54.360	0.475	0.031	0.432	360.916
	(0.596)	(0.011)	(0.009)	(0.033)	(24.840)
Sargassum vulgare	30.055	0.420	0.032	0.824	579.616
	(0.884)	(0.019)	(0.009)	(0.011)	(89.333)
Gigartina sp.	22.177	0.294	0.024	1.495	310.406
	(0.343)	(0.009)	(0.012)	(0.020)	(29.465)
Gracilaria vermiculophylla	17.579	0.065	0.029	1.120	196.170
	(0.373)	(0.001)	(0.008)	(0.030)	(29.248)

[a] Values are expressed as means (SD) of two replicates.

having in common relatively high levels of lead. Cluster 4 (C4) comprised *F. serratus*, *F. spiralis*, *F. guiryi*, and *S. latissima*, having high contents of cadmium and mercury. The clustering observed provides key information for categorizing seaweeds regarding their harmful potential for animal performance and health.

Maximum dietary level of inclusion of seaweeds

Seaweeds can be used in animal feeding as feed ingredients or as a supplement with prebiotic effects. Indeed, the usual high cost of seaweeds precludes their dietary inclusion at high rates. Conversely, at low levels of inclusion (<2 % of DM intake), seaweeds can improve animal health and productivity by means not conveniently explained by conventional feed analysis, thus exerting a potent prebiotic activity, namely through its wide variety of complex carbohydrates, phlorotannins, and antioxidants (Evans and Critchley 2014). Regarding mineral nutrition, animals have specific requirements for some minerals, but the ingestion of excessive amounts can cause toxicity. Mineral toxicity generally leads to decreased animal performance, anorexia, weight loss, and diarrhea (NRC 2005). Considering the mineral composition of the seaweed species studied here, we calculated the maximum level of its inclusion in diets of poultry, swine, horse, cattle, and sheep, based on two complementary aspects: (i) the maximum tolerable level of mineral element according to NRC (2005) and (ii) the individual element responsible for the maximum limit of inclusion. The calculated values are compiled in Table 7.

For poultry, we may consider the upper level of dietary inclusion of seaweeds as high (>50 %). The only limitation for seaweed inclusion in poultry diets would be *C. adhaerens*

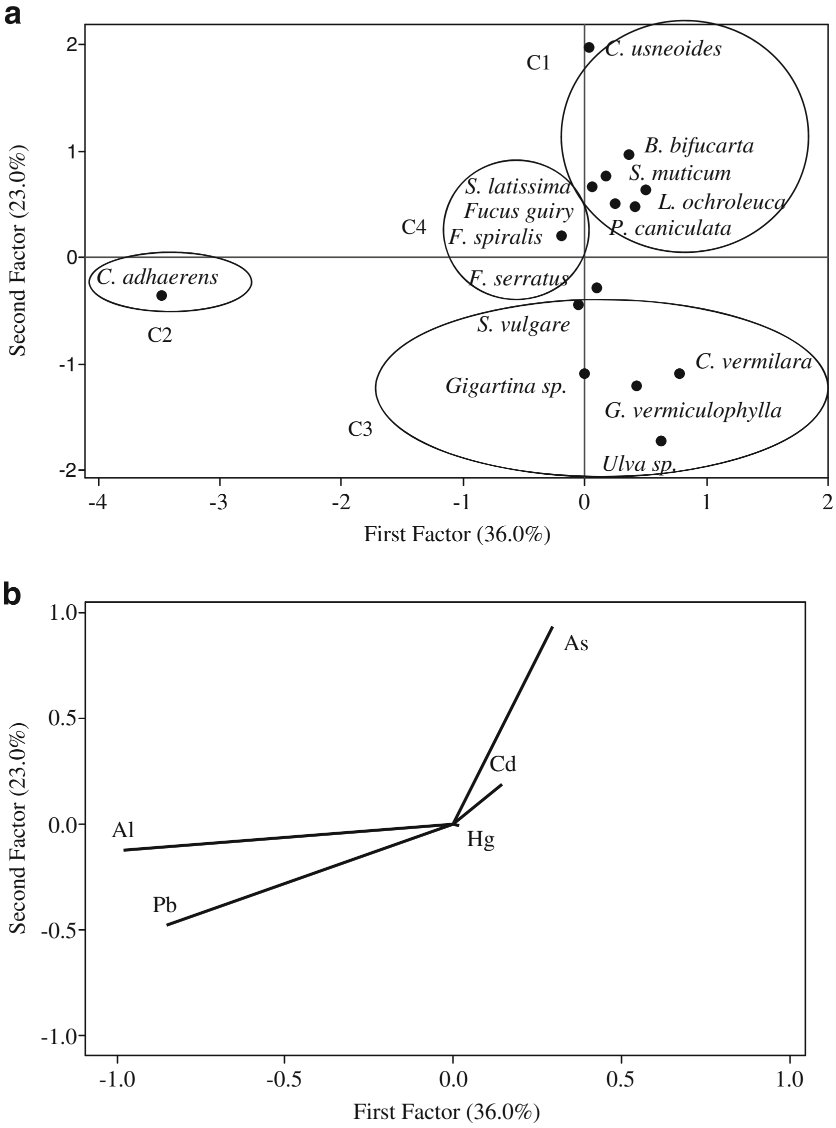

Fig. 2 Projection of seaweed samples (**a**) (variables) and loadings (**b**) by possibly toxic mineral content into the plane composed by the first and second factor containing 59.0 % of the total variance

that should be constraint to 14 % of the diet DM due to its high content of iron, as the maximum tolerable level for this species was set at 500 mg kg^{-1} DM (NRC 2005).

A wide range of seaweed dietary inclusion levels was found for swine, ranging from only 3.6 % for *C. adhaerens* to 43.9 % for *B. bifurcata*, the minerals responsible for inclusion level limit being aluminum, magnesium, and bromine.

Regarding the use of seaweeds in diets for horses, the dietary inclusion level is strongly limited by the iodine content of seaweeds, with the only exception of *B. bifurcata* for which arsenic limits its inclusion to a 51 % level. For horses, the maximum tolerable dietary concentration of iodine is equivalent to an intake of 50 mg of iodine per day for a horse consuming 10 kg of DM daily (NRC 1989).

For ruminant animals (cattle and sheep), the dietary inclusion of seaweeds is mainly limited by iodine content, followed by bromine, and ultimately by magnesium. Inclusion levels ranged from 5.2 % for *S. latissima* to 31.2 % for *G. vermiculophylla*. It should also be noted that acute copper poisoning from seaweed feeding to sheep do not constitute a risk as all studied seaweeds presented copper contents below the toxic level (20–100 mg kg^{-1} DM; NRC 2005) even if the seaweed comprised the whole animal diet.

These results suggest that the level of minerals as magnesium, bromine, iron, iodine, arsenic, and aluminum should be carefully considered when recommending seaweeds for regular animal consumption. Additionally, the salt content of seaweeds (not herein measured) can seriously limit their dietary inclusion as an excessive salt intake can result in loose feces and related problems. Indeed, the study of Smith et al. (2000) demonstrates that for every 0.25 % increase in dietary salt content, an additional 9 g of water is excreted per gram of feces.

Studies evaluating in vivo the dietary inclusion of seaweeds as feed ingredients refer lower inclusion levels in order to prevent negative effects on animal performance. For example, El-Deek and Brikka (2009) pointed a dietary inclusion

Table 7 Maximum level of inclusion (MLI; g seaweed 100 g^{-1} diet DM) of the studied seaweeds in diets of poultry, swine, horse, cattle, and sheep based on the maximum tolerable level of minerals element according to NRC (2005) and mineral element responsible for the maximum inclusion

Seaweed	Poultry		Swine		Horse		Cattle/sheep	
	MLI	Element	MLI	Element	MLI	Element	MLI	Element
Codium adhaerens	14.3	Fe	3.6	Al	1.1	I	10.5	I
Codium vermilara	34.2 (Growing birds)	Mg	16.4	Mg	6.6	I	19.5	Br
	51.3 (Laying hens)	Mg						
Ulva sp.	25.6	Mg	12.3	Mg	21.5	I	30.7	Mg
Bifurcaria bifurcata	51.4	As	43.9	Al	51.4	As	19.7	I
Cystoseira usneoides	36.4	As	30.9	Br	1.0	I	9.9	I
Fucus guiryi	50.6	As	34.2	Mg	1.8	I	18.3	I
Fucus serratus	69.1 G birds)	Mg	33.1	Mg	1.6	I	15.5	I
	70.7 (Laying hens)	As						
Fucus spiralis	61.1 (Growing birds)	Mg	17.5	Al	2.1	I	21.4	I
	77.7 (Laying hens)	As						
Laminaria ochroleuca	34.0	I	39.3	Mg	0.6	I	5.7	I
Pelvetia canaliculata	60.7	As	29.6	Mg	2.0	I	19.9	I
Saccharina latissima	31.3	I	36.2	Br	0.5	I	5.2	I
Sargassum muticum	55.2	As	27.7	Al	2.3	I	23.1	I
Sargassum vulgare	51.5	I	17.3	Al	0.9	I	8.6	I
Gigartina sp.	60.9 (Growing birds)	Mg	24.1	Br	2.6	I	24.1	Br
	91.4 (Laying hens)	Mg						
Gracilaria vermiculophylla	47.7	Fe	31.2	Br	10.7	I	31.2	Br

level of seaweeds from 12 to 15 % in duck starter and grower diets with no adverse effects on growth performance or carcass quality, and Carillo et al. (2012) refer an inclusion of 10 % in laying hen rations in order to increase egg n-3 fatty acids without negatively affecting the albumen height and yolk color. Conversely, some studies have indicated that including as little as 10 % seaweed in a broiler diet reduced growth performance (Ventura et al. 1994). With ruminant animals, higher levels of inclusion have been reported (e.g., 20 % DM basis; Machado et al. 2015), though very small amounts (0.5 % DM basis) have been shown to have beneficial effects in decreasing rumen methane production without adversely affecting rumen fermentation (Kinley and Fredeen 2015). If seaweeds are included in the animal diets for their prebiotic effects, thus at low inclusion rates, the mineral contribution of the seaweeds is much less significant in practical ration balancing.

Conclusions

This is the first detailed characterization of mineral profile of common Portuguese seaweeds, thus contributing to their evaluation as sources of essential, occasionally essential, and potentially toxic elements. Overall, seaweeds presented high levels of macro and trace minerals, the individual content significantly varying among seaweeds. Factor and cluster analysis highlighted the similarities and differences observed between seaweeds regarding their mineral profile which would allow a more precise choice of seaweeds as mineral sources when formulating diets for different animal species and different physiological status.

The level of dietary inclusion of each particular seaweed is strongly dependent on the levels of potentially toxic elements as well as on the levels of essential elements, which when ingested in high amounts can lead to toxicity. The results herein reported suggest that the level of magnesium, bromine, iron, iodine, arsenic, and aluminum should be considered when recommending the studied seaweeds for regular animal consumption. For poultry, the upper level of dietary inclusion of seaweeds is reasonably high (>50 %), with the exception of the iron-rich C. *adhaerens*. A wide range of seaweed dietary inclusion level was found for swine, ranging from only 3.6 % for *C. adhaerens* to 43.9 % for *B. bifurcata*, due to high contents of aluminum, magnesium, and bromine. For horses, the dietary use of seaweeds is strongly limited by their iodine content, exception made for *B. bifurcata*. Similarly, for ruminant animals (cattle and sheep), iodine is the most limiting mineral for the dietary inclusion of seaweeds, followed by bromine and magnesium, yet inclusion levels ranged from 5.2 to 31.2 %. Despite the contribution of this work to a clearer understanding of seaweeds as mineral sources in animal diets, it will be further exploited with the evaluation of seasonal variation in seaweed composition and with living animals.

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