The Elemental Composition of Green Seaweed (*Ulva rigida*) Collected from Çanakkale, Turkey

Nermin Berik[1] , Ekrem Cem Çankırılıgil[2*]

ABSTRACT

Seaweeds or marine algae are rich in terms of minerals. They are used as food source due to the quality of their biological content in many countries. In this study, the elemental composition of green seaweed (*Ulva rigida*) was determined seasonally. Sampling was carried out seasonally from Turkey's Çanakkale strait and the collected algae were analyzed both wet and dried according to the Nordic Committee on Food Analysis (method 186). According to the results; calcium, potassium, magnesium, sodium were found as macro elements and boron, barium, chromium, copper, iron, manganese, zinc as micro elements and determined within the legal limits stated by food codex. However, neither cobalt nor nickel as trace metals were detected in all groups. Moreover, lead and cadmium (which are considered hazardous) were also not detected. While Mg was found to be the highest macro element in all seasons in wet algae, the highest macro mineral varied in dried algae is Ca in spring, K in summer and Mg in both autumn and winter. Fe was found to be the highest micro mineral followed by B, Zn, Mn and Cu both by season and by dried or wet ones (P<0.05).

Keywords: Green seaweed, *Ulva rigida*, trace elements, Çanakkale strait, nutrient

ORCID IDs of the authors:
E.C.Ç.: 0000-0001-5898-4469;
N.B.: 0000-0003-3015-8688

[1]Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Department of Fisheries and Processing Technology, Çanakkale, Turkey
[2]Central Fisheries Research Institute, Trabzon, Turkey

Correspondence:
Ekrem Cem Çankırılıgil
E-mail:
ekremcem19@gmail.com

INTRODUCTION

It is well known that the usage of macroalgae dates back many, many years. Native South Americans used to collect macroalgae in order to build food supplies and illness remedies (12000 years ago) (Ak, 2015; Dillehay et al., 2008). The utilization of algae takes part within the "Materia Medica" which belongs to Shen-Nung in B.C. 2700. However, algae products obtained by scientific methodologies are seen in last century (Sukatar, 2002). The use of Algae is particularly widespread in Southern Asia for nutrition, medicine and cosmetic industries, and fertilizer in agriculture (Atay, 1984; Kodalak, 2008). Sea lettuce is used as an additive for food and provender and is consumed in China, Japan, Korea, Indonesia, Malesia, France, United States, Canada and Scotland, freshly in salads and dried in soups, dishes and sauces

(Mchugh, 2003). Also, they are considered as biologically substantial nourishments (Fleurence, 1999; Rohani-Ghadikolaei, Abdulalian, & Ng, 2012; Sánchez-Machado, López-Cervantes, López-Hernández, & Paseiro-Losada, 2004) and it is significant following fish in aquatic products (Dawczynski, Schubert, & Jahreis, 2007; Fleurence, 1999; Galland-Irmouli et al., 1999; Ortiz et al., 2006).

The Marine Strategy Framework Directive (MSFD) of the European Commission aims to deliver a good environmental status (GES) for the European marine environment by 2020. The eighth and ninth descriptor groups of this directive concern contaminants of fish and other seafood (Law et al., 2010; Swartenbroux et al., 2010). According to this directive, the toxic element compositions of the most commonly consumed fish and seafood species must be moni-

tored in Mediterranean Sea. Therefore, the elemental composition of *Ulva rigida* is important in the usage of a food source. With that feature, *Ulva rigida* can also give an idea about the elemental pollution in the environment (Wan et al., 2017). Moreover, it can be used as an indicator species for lead (Haritonidis & Malea, 1999; Malea & Haritonidis, 2000) and aluminum (Favero, Cattalini, Bertaggia, & Albergoni, 1996). In this study, the elemental composition of *Ulva rigida* were evaluated considering MSFD instructions to highlight *Ulva rigida* as a valuable food source and provide information for further studies in the future.

MATERIAL AND METHODS

Material

In this research, green seaweed (*Ulva rigida*) was used. *Ulva rigida,* also known as sea lettuce, is a cosmopolite species and distributed along the Turkish coasts especially in shallow and rocky bottoms which are rich in nutrients such as nitrogen and phosphorus (Cirik, 2001; Cirik & Cirik, 1999). This algae's tallus size varied from 1-2 cm up to 30 cm and clings to the substrate with a short stem (Irkin, 2009). The green seaweed is shown in Figure 1.

Figure 1. Green seaweed (*Ulva rigida*) collected from Çanakkale, Turkey.
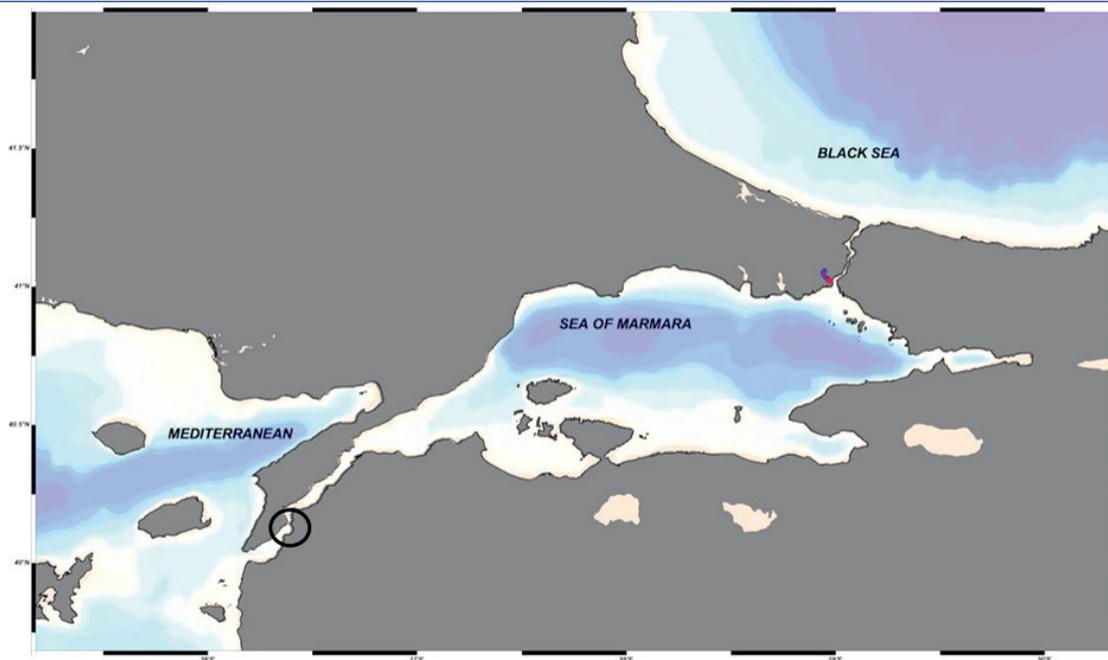
Figure 2. Sampling area in Çanakkale, Turkey. The map was created with Ocean Data View (ODV) Software (40°06'08.6"N, 26°23'13.7"E).

Sampling

Green seaweed samples weighing approximately 2 kg were collected from Kepez station in the Çanakkale strait in the Northern Aegean Sea, Turkey. Sampling was carried out seasonally/quarterly between February/2011 and February/2012. The sampling station of green seaweed (*Ulva rigida*) is shown in Figure 2 and the map used was created with Ocean Data View (ODV) software (Schlitzer, 2019). Samples were stored in 5-liter bottles of sea water taken from the sampling station and transferred to the laboratories in the Faculty of Marine Sciences and Technology in Çanakkale Onsekiz Mart University. Subsequently, the samples were cleaned of any unwanted substances that seaweed carries such as sand particles, epiphyte plants and other aquatic organisms. The sand removal process was carried out by scrubbing with a soft brush in salty water with 0.3% salinity. After this step, the green seaweed samples were separated into two groups. The element analysis was performed on the first group immediately. The second group was dried at 40 °C for 48 hours in a drying oven (Shin Saeng/ SDON-302 model). Finally, the dried samples were then analyzed.

Element Analysis

The element analyses were carried out according to the Nordic Committee on Food Analysis (method 18) (NMKL, 2007) with three parallel and three replicates. These algae are consumed both wet and dried and since some elements can be lost during the drying process, the analyses were carried out with both wet and dried samples. The VH6 Lab Single Element Atomic Absorbsion CRM standards were used in the analyses. Furthermore, the

regulations recommended by the MSFD were applied to avoid secondary contamination in the sample preparations. In analysis, 0.5 mg of the samples were weighed using politetrafloroetilen plates (for microwave use) and they were digested with a 10 milliliter concentrated nitric acid (HNO_3) via Speedwave Berghof model microwave at 160 °C in gradually increasing heat conditions. After the samples were completely burned and had become clear mixtures, they were filtered and diluted with distilled water. In the burning process sequence, the mixtures were analyzed with the ICP-AES (Varian Liberty AX Sequential ICP-AES) in Çanakkale Onsekiz Mart University's Main Laboratory. The elements aluminum (Al), boron (B), barium (Ba), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), lead (Pb) and zinc (Zn) were analyzed. Finally, the data was analyzed and expressed as mg/g for macro elements and µg/g for micro elements on a wet matter basis.

RESULTS AND DISCUSSION

According to the results, four macro elements (calcium, potassium, magnesium, sodium), and seven micro elements (aluminum, boron, barium, chromium, copper, iron, manganese and zinc), were detected. The macro elements were expressed as mg/g and micro elements as µg/g. While magnesium was detected as the most abundant macro element in wet samples in autumn, it was detected as least abundant in summer (P<0.05). The statistical differences were determined between elements seasonally (P<0.05). The elemental composition of dried the seaweeds were

Table 1. Elemental composition of wet green seaweed (*Ulva rigida*)

Elements	Seasons			
	Winter	Spring	Summer	Autumn
Macro elements (mg/g)				
Ca	0.69 ± 0.00[c]	3.23 ± 0.30[a]	0.46 ± 0.02[c]	1.11 ± 0.07[b]
K	5.66 ± 0.03[c]	6.09 ± 0.03[b]	4.68 ± 0.12[d]	7.18 ± 0.11[a]
Mg	5.97 ± 0.06[c]	8.41 ± 0.03[b]	5.69 ± 0.08[d]	8.92 ± 0.09[a]
Na	5.19 ± 0.04[ab]	5.74 ± 0.24[a]	4.44 ± 0.46[b]	4.62 ± 0.40[b]
Micro elements (µg/g)				
Al	89.00 ± 2.27[b]	60.00 ± 0.72[c]	44.93 ± 0.46[d]	110.80 ± 0.20[a]
B	17.67 ± 0.61[c]	15.53 ± 0.31[d]	52.27 ± 0.46[a]	33.53 ± 0.61[b]
Ba	0.53 ± 0.06[b]	0.80 ± 0.00[a]	0.67 ± 0.12[ab]	0.80 ± 0.00[a]
Co	< 0.035	< 0.035	< 0.035	< 0.035
Cr	< 0.010	0.20 ± 0.00[c]	0.60 ± 0.00[a]	0.20 ± 0.00[b]
Cu	3.27 ± 0.76[a]	3.00 ± 0.72[ab]	2.00 ± 0.69[ab]	1.33 ± 0.64[b]
Fe	227.87 ± 3.41[a]	188.20 ± 6.54[b]	124.60 ± 0.87[c]	225.07 ± 1.27[a]
Mn	3.00 ± 0.00[c]	3.07 ± 0.12[c]	4.60 ± 0.00[b]	5.40 ± 0.53[a]
Ni	< 0.035	< 0.035	< 0.035	< 0.035
Zn	13.20 ± 0.20[a]	8.47 ± 0.12[b]	2.00 ± 0.00[d]	4.40 ± 0.00[c]
Toxic elements (µg/g)				
Cd	< 0.035	< 0.035	< 0.035	< 0.035
Pb	< 0.035	< 0.035	< 0.035	< 0.035

*Values are expressed as mean ±SD (n = 3), mean values in row with different superscripts were significantly different (P <0.05).

found to be similar to the wet ones. However, a relative increase was detected in both macro and trace element concentrations of dried samples due to the extraction of water from the samples during the drying process. The macro element composition of *Ulva rigida* is shown in Table 1.

Macro and micro elements accumulate in macroalgae with greater concentrations than the surrounding waters (Bonanno & Orlando-Bonaca, 2018). Iron was found as the most abundant trace element in both wet samples (with the amount of 227.87 µg/g in winter), and dried samples (with the amount of 574.53 µg/g in summer). A noticeable amount of Fe, Al and B were also found in the samples. The Mediterranean Sea is rich in terms of dissolved iron and aluminum (Chou & Wollast, 1997; Guieu et al., 2002) and these elements are usually accumulated in *Ulva rigida* (Favero, Cattalini, Bertaggia, & Albergoni, 1996; Malea & Haritonidis, 2000). Boron, which is found in high concentrations in sea water (Demey et al., 2014; Foster, Strandmann Pogge von, & Rae, 2010), is a mineral that has recently generated benefits for human health (Meacham, Karakas, Wallace, & Altun, 2010; Nielsen, 1997; Nielsen & Meacham, 2016) and it was found in all samples of our study. Also; zinc, manganese, copper and a small amount of chromium and barium were detected in *Ulva rigida*. Only chromium wasn't detected in winter. Chromium, which was detected as 0.20 µg/g in wet samples in fall, wasn't found in dried samples due to the dehumidification.

Two trace elements, cobalt and nickel were not detected in any samples during the whole year. Mediterranean waters are not rich in terms of nickel and cobalt (Achterberg & Van Den Berg, 1997; Swanner et al., 2014). However, according to other research using similar methods, these two elements were detected, albeit in small quantities) in other marine organisms caught from the Mediterranean Sea (Berik, Çankırılıgil, & Gül, 2017; Joksimovic, Tomic, Stankovic, Jovic, & Stankovic, 2011; Mutlu, Türkmen, Türkmen, Tepe, & Ateş, 2012; Türkmen, Türkmen, Tepe, & Çekiç, 2010). Consequently, we might suggest that the cobalt and nickel accumulate less in *Ulva rigida* compared to other elements. According to our analyses, cadmium and lead - among the most dangerous toxic elements - were not detected in green seaweed. Several studies showed that, *Ulva rigida* accumulates these toxic metals, especially lead, in significant amounts. (Haritonidis & Malea, 1999; Saeed & Moustafa, 2013; Ustunada, Erdugan, Yilmaz, Akgul, & Aysel, 2011). This research also showed that, the sampling station of *Ulva rigida* was more reliable in terms of lead and cadmium pollution having regard to these low values. The micro element composition of *Ulva rigida* is shown in Table 2.

As regards the elemental studies on sea lettuces in the Çanakkale Province, these are generally focused on iron (Fe) cadmium (Cd) lead (Pb) zinc (Zn) and copper (Cu) (Ozden & Tuncer, 2015; Ustunada, Erdugan, Yilmaz, Akgul, & Aysel, 2011). As for the present study, we can only compare the results with the previous studies in the Çanakkale Province, in terms of Cu, Fe and Zn. Ustunada, Erdugan, Yilmaz, Akgul, & Aysel (2011) studied the seasonal alterations in the content of Cd, Zn, Pb and Cu in *Ulva*

Table 2. Elemental composition of dried green seaweed (*Ulva rigida*)

Elements	Seasons			
	Winter	Spring	Summer	Autumn
Macro elements (mg/g)				
Ca	2.11 ± 0.07[b]	12.73 ± 0.08[a]	1.56 ± 0.02[c]	2.23 ± 0.03[b]
K	7.01 ± 0.01[c]	7.19 ± 0.02[b]	6.52 ± 0.08[d]	8.17 ± 0.01[a]
Mg	10.55 ± 0.06[a]	8.91 ± 0.04[c]	6.13 ± 0.08[d]	10.22 ± 0.03[b]
Na	6.27 ± 0.27[a]	6.31 ± 0.13[a]	5.83 ± 0.41[ab]	5.21 ± 0.41[b]
Micro elements (µg/g)				
Al	158.00 ± 3.80[c]	211.87 ± 2.66[a]	171.73 ± 6.01[b]	224.60 ± 6.10[a]
B	36.87 ± 0.42[c]	34.73 ± 0.12[d]	59.40 ± 0.69[a]	45.93 ± 1.21[b]
Ba	0.60 ± 0.00[d]	1.27 ± 0.12[b]	0.87 ± 0.12[c]	1.67 ± 0.12[a]
Co	< 0.035	< 0.035	< 0.035	< 0.035
Cr	< 0.010.	0.20 ± 0.00[a]	0.07 ± 0.12[ab]	< 0.010
Cu	6.33 ± 0.64[a]	4.13 ± 0.64[b]	1.00 ± 0.53[c]	1.93 ± 0.46[c]
Fe	293.93 ± 4.12[d]	461.47 ± 8.03[b]	574.53 ± 5.60[a]	399.87 ± 7.91[c]
Mn	5.47 ± 1.50[b]	10.07 ± 0.12[b]	10.27 ± 0.12[a]	6.93 ± 0.12[b]
Ni	< 0.035	< 0.035	< 0.035	< 0.035
Zn	20.13 ± 0.12[a]	17.60 ± 0.20[b]	13.80 ±0.00[d]	14.40 ± 0.20[c]
Toxic elements (µg/g)				
Cd	< 0.035	< 0.035	< 0.035	< 0.035
Pb	< 0.035	< 0.035	< 0.035	< 0.035

*Values are expressed as mean ±SD (n = 3), mean values in row with different superscripts were significantly different (P <0.05).

rigida samples, in six different locations of the Dardanelles (Çanakkale), and did not detect any of the elements in levels greater than 1 μg/g within any of the dry algae samples collected. However, Ozden and Tuncer (2015) did not detect the elements of Pb and Cu in levels above 1 μg/g in the dry samples of *Ulva rigida* collected seasonally from five different locations in the Dardanelles. They also reported that the seasonal average amounts of Zn and Fe were between 1.01-6.5 μg/g and 2.25-10.87 μg/g, respectively. The results of the element quantities in the present study were observed to be higher compared to the abovementioned studies, for example, Cu was between 1.00 and 6.33 μg/g; Fe, 294-575 μg/g; and Zn, 13.80-20.13 μg/g. It is generally considered that the differences in the results between this study and other studies stems from the sampling years and a dissimilarity in sampling locations. The Dardanelles, being a specific current system can contain different proportions of minerals during various years and stations due to taking nutrition particularly from the water bodies originating from the Black Sea. Furthermore, the magnitude of the algae is a distinctive feature in terms of mineral composition. Older algae have better ability of absorbing minerals from sea water.

According to legislation, the acceptable daily limits of copper is 0.5 mg/kg, and manganese is between 0.4-1.0 mg/kg of bodyweight (Codex Alimentarius, 2007; 2011). There are no legal limits for aluminum and barium in meat products but the EFSA (2013) stated that aluminum exposure should not exceed 1 mg/kg weekly for humans and the Codex Alimentarius Committee (1996) stated that the acceptable barium limit is 1 mg/l in drinking water. Nickel and chromium were found very low - just slightly higher than the detection limits. Additionally, certain beneficial elements, such as boron and zinc, should be present in foods in the amounts of 0.5-0.4 mg/kg and 15 mg/kg of body weight, respectively (Codex Alimentarius, 2007; Jopp, 2012; WHO, 2013).

CONCLUSION

In this research, the elemental composition of green seaweed (*Ulva rigida*) and level of food safety was evaluated. In sum, *Ulva rigida* is rich in terms of certain beneficial micro elements such as iron, boron and zinc – and within acceptable values. Furthermore, the elements lead and cadmium (considered as hazardous), were not detected. With these features and the supporting studies related to nutrient content for this species, *Ulva rigida* we can suggest that the seafood sector is a convenient and secure food source if it is collected from not-polluted and safe areas.

Conflict of Interest: Authors state that there is no conflict of interests.

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