

Macro and Trace Element Levels of Green Algae *Codium fragile* (Suringar) Hariot 1889 From Dardanelles (Çanakkale/Turkey)

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ABSTRACT

Heavy metal pollution is one of the important pollution causes for water resources in the world. Algae can be used as an indicator species for the accumulation of heavy metals. Therefore, macro (Na, K, Mg, P, Ca, B) and trace element (Co, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn)

values were analyzed by ICP-OES in the species in our study.

In this study, we aimed to determine the element concentrations of *Codium fragile* (Suringar) Hariot 1889 macroalgae collected from Dardanelles (Çanakkale-Turkey) coastal area (40°14'27.03"N 26°32'29.74"E during summer season in 2018. Elemental concentrations of *C. fragile* were determined as following order: Na>Ca>Mg>K>Fe>P>Mn>Pb>B>Zn>Cu>Ni>Cr>Co=Mo=Cd respectively in our study. We also found the Cd, Co and Mo elements below the limit values. In *C. fragile*, macro elements values were higher than the trace elements values. Fe is the most common trace element after macro elements in all algae samples. Fe trace element is followed by Mn and Pb elements.

We think that high metal concentrations in the Dardanelles affect the water quality negatively and may pose a threat to the living creatures living there, and we believe that serious measures should be taken to reduce continuous input in the Dardanelles, which has high shipping and tourism potential.

Key Words: Algae, *Codium fragile*, Dardanelles, Elemental composition, Heavy metal.

Yeşil Alg *Codium fragile* (Suringar) Hariot 1889 (Çanakkale/Türkiye)'in Makro ve İz Element Düzeyleri

ÖZ

Ağır metal kirliliği, dünyadaki su kaynaklarının önemli kirlilik nedenlerinden biridir. Algler ağır metaller birikimi bakımından indikatör tür olarak kullanılabilir. Bundan dolayı çalışmadaki türümüzde makro (Na, K, Mg, P, Ca, B) ve eser elementlerin (Co, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn) değerleri ICP-OES ile analiz edilmiştir.

Çalışmamızda, Çanakkale (Çanakkale-Türkiye) kıyı alanından (40 ° 14'27.03 "K 26 ° 32'29.74" D) 2018 yılı yaz sezonunda toplanan *Codium fragile* (Suringar) Hariot 1889 (Cholorophyta) makroalg örneklerinin elementel konsantrasyonunu belirlemeyi amaçladık. *Codium fragile* elementel konsantrasyonları sırasıyla aşağıdaki gibi belirlenmiştir: Na> Ca> Mg > K> Fe> P> Mn> Pb> B> Zn> Cu> Ni> Cr> Co = Mo = Cd. Ayrıca Cd, Co ve Mo elementlerini sınır değerlerin altında bulduk. *C. fragile* alginde makro elementler eser elementlerden daha fazla

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miktarlarda bulunmuştur. Fe, tüm alg örneklerindeki makro elementlerden sonra en yaygın bulunan eser element olarak belirlenmiştir. Fe iz elementini Mn ve Pb elementleri takip etmiştir.

Çanakkale Boğazı'ndaki yüksek metal konsantrasyonlarının su kalitesini olumsuz etkilediğini ve orada yaşayan canlılar için tehdit oluşturabileceğini düşünüyor, denizcilik ve turizm potansiyeli yüksek Çanakkale Boğazı'nda sürekli girdiyi azaltmak için ciddi önlemler alınması gerektiğine inanıyoruz.

Anahtar Kelimeler: Alg, Ağır metal, *Codium fragile*, Çanakkale, Element kompozisyonu.

1. Introduction

In the face of a significant increase in industrialization and urbanization in coastal areas, marine environments are exposed to human factors, especially in terms of pollution (Tanyolaç, 2009). Marine pollution from untreated wastewater and industrial waste dumping is one of the most common and most damaging forms of pollution to the entire coastal marine ecosystem of the planet (Espinosa et al., 2007). Heavy metal pollution is one of the most important causes of pollution in lakes and seas around the world. Traditional solution technologies take a long time and are quite expensive. Therefore, it is necessary to use low-cost and environmentally friendly technology to rate contaminated soil and water resources, especially in developing countries. (Abdallah and Abdallah, 2008). Macroalgal species are generally preferred as bioindicator organisms to measure heavy metal levels in both the seas and the freshwaters worldwide. The study was conducted to determine the accumulation of heavy metals and trace elements in water and macroalgae of Dardanelles (Çanakkale/Turkey).

Fuel oil, sewage, wastewater, and other waste from ships substances cause pollution in the seas. Heavy metal pollution in the gulf and inland seas is more important and effective than the pollution in offshore. For this reason, the Dardanelles, which is an important transition point of the polluted waters coming from both the Black Sea and the Marmara Sea and reaching the Mediterranean via upper currents, is under the influence of both lower and upper currents. However, heavy metal pollution carried from the Dardanelles to the Mediterranean by the upper stream is more than the pollution transported from the bottom to the Black Sea. It is stated that the increase in heavy metals and trace elements in the Dardanelles is caused by wastewater that disrupts the optimum stability of the aquatic environment (Üstünada et al., 2011).

Turkey has made many efforts to determine the heavy metal pollution in the sea. Some of these studies are; Sukatar and İlkme (1984), Kuyucak and Valesky (1986), Güven et al., (1992), Batkı et al., (1999), Çetingül et al., (2000), Kut et al., (2000), Topçuoğlu et al. ., (2001, 2002, 2004, 2010), Nuhoglu et al., (2002), Erakın (2005), Özden (2006), Süren et al., (2007), Balkıs et al., (2007), Tuzen et

al., (2009), Boran and Altınok (2010). There is not enough work carried out in this direction in the Dardanelles. Except for recent work by Üstünada (2011) on the absorption of macro and trace elements by the marine alga, no detailed study of the trace elements in the green algae, nor any attempt to correlate the trace elements in seawater with those in the algae, appears to have been carried out. For this reason, the following is a brief resume of the work that has been done in this field.

Seaweeds are used as metal samples and biomonitors of contamination. (Al-Homaidan, 2007, Kamala-Kannan *et al.*, 2008). Macrophytes are preferred in pollution studies due to their ability to accumulate metals dissolved in water or particulate form, and they reflect the pollution in the environment more accurately because they are perennial algae. For this purpose, species belonging to *Ulva Linnaeus*, *Codium Stackhouse*, and *Cystoseira Agardh* taxa are generally used (Mohamed and Khaled 2005; Fytianos et al., 1999). Macroalgal species are generally preferred as indicator organisms to measure heavy metal levels in both the seas and the fresh water in the world. Green algae can attract and store many heavy metal ions in the water because of the high negativity on their surface. This is a factor that makes the use of algae important in determining and removing water pollution. (Alp *et al.*, 2012). Recently, many researchers have focused their work on algae heavy metal absorption under both experimental and natural conditions.

Species belonging to the genus *Codium* are potential species that can be used in biological monitoring for trace metals in the Dardanelles because these species have important features such as being sought for biological monitoring, being easily identified and collected, being in coastal waters throughout the year, being able to accumulate metals much more than their concentrations in water, and show the distribution in all coastal areas.

The systematic classification of the *C. fragile* type used in our study is as follows.

Classification:

Empire	: Eukaryota
Kingdom	: Plantae
Subkingdom	: Viridiplantae
Infrakingdom	: Chlorophyta
Phylum	: Chlorophyta
Subphylum	: Chlorophytina
Class	: Ulvophyceae
Order	: Bryopsidales
Family	: Codiaceae
Genus	: <i>Codium</i>
Species	: <i>Codium fragile</i> (Suringar) Hariot 1889

(https://www.algaebase.org/search/species/detail/?species_id=3638&sk=0&from=results)

Codium sp. is a marine algae and it is stated that there are approximately 50 species in the world (Hoffman et al., 2011; Taylan and Hoşsucu, 2008). The genus of *Codium* currently contains about 125 species widely distributed in the world seas, except for polar regions, and is found mostly in temperate and subtropical regions (Topçuoğlu et al., 2010). We used the *C. fragile* (Suringar) Hariot 1889) in our study (Figure 1).

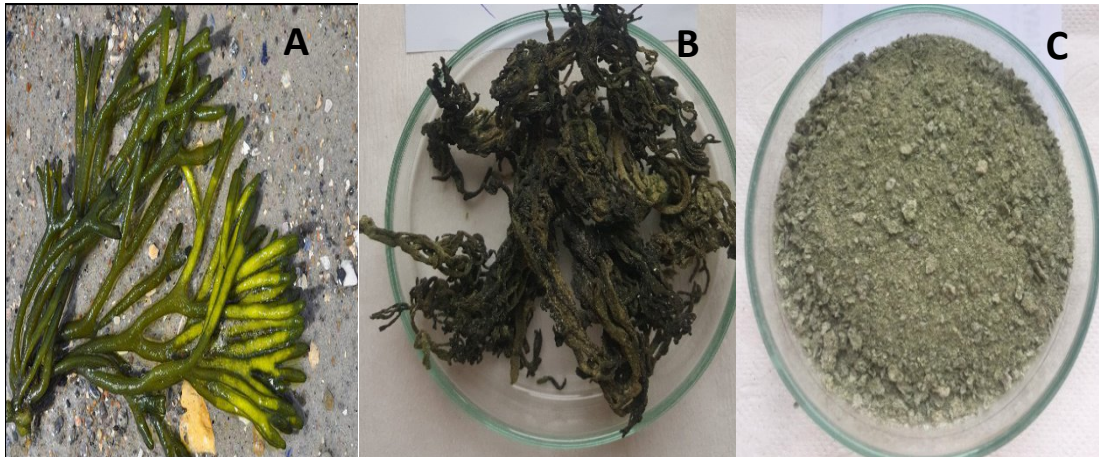


Figure 1. A. *Codium fragile*(Suringar) Hariot 1889 in natural habitat. B. Dry form of *C. fragile* C. Powder of *C. fragile*

C. fragile is a dark green alga that ranges from 10 to 40 cm and consists of branching cylindrical sections. It grows on the blurred slopes of tubular fingers hanging from rocks at a depth of 1-2 m (Mavili, 2011). *C. fragile* is consumed by humans and used by the aquaculture industry as food for invertebrates.

We aimed in this study to determine the availability of macroscopic green algae from the Dardanelles for absorption applications. For this purpose, we investigated both macro element values and trace element values and evaluated the results by comparing algae and water samples.

About the properties of the macro elements, calcium can be found at very different concentrations in the water. Calcium and magnesium are the most important ions that give water hardness. Potassium is the seventh of the elements found most abundantly in the earth's crust (Fowler, 1979). However, the amount of potassium in natural waters is low. Sodium is the most commonly found element in the earth's crust. Seawater has % 2.6-2.7 sodium chloride. Phosphorus is found in organic and inorganic forms in natural waters and is an essential element for plant and animal growth (Dadolahi-Sohrab et al., 2011).

Cobalt is one of the trace elements necessary for life and because of its deficiency, activation of certain enzymes can stop. Chromate compounds can be found in waters only with contamination. Copper available in water is related to the pH of water, carbonate concentration, and other anions. While the amount of iron in nature is quite a lot in nature, it is found in small quantities in natural waters. Manganese is dissolved in soil because of climatic events and gets into the water. The lack of molybdenum limits the primer production and algae growth is warned if it is added to water. Most nickel salts are water-soluble and they are found in surface waters at level 5-20 µg/ L. The average amount of lead at lakes and rivers on earth is about 1-10 micrograms. Selenium can get into aquatic environments through industrial waters. Zinc is one of the important elements of life (Tüzen et al., 2009).

2. Materials and Methods

2.1. Sampling procedures

Algal samples were collected from Yapıldakaltı coast of in Çanakkale City (Çanakkale-Turkey), at the end of the summer in 2018, the place of the intensive population of algae. Among the various

organisms, algal samples were obtained via scuba diving. The collected samples were placed in sterile glass bottles and transferred to the laboratory in the cold chain. Following the removal of the necrotic materials by using distilled water, the dried algae were pulverized and stored at room temperature. The satellite image of the area where the samples were collected was given in Figure 2. Coordinates of the station are N 40°14'27.03" E 26°32'29.74".

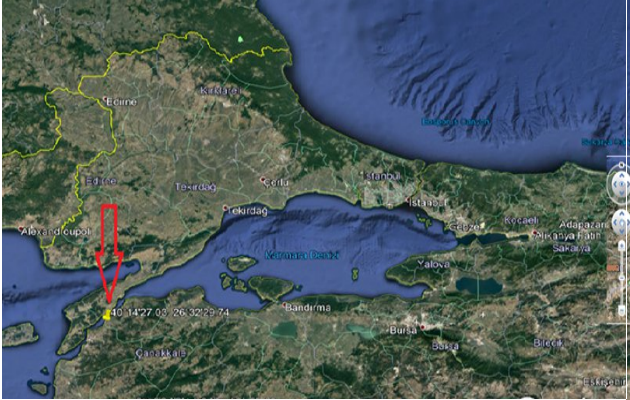


Figure 2. Satellite image of algae collected from Dardanelles

2.2. Analytical Methods

Before analysis, samples were placed in durable glass bottles and dried at 105 °C for 24 hours in a drying oven. 0.2 g of the dried samples were taken and 5 mL nitric acid (65 % HNO₃ mL⁻¹) and 2 mL of purified water were added and kept at room temperature for 24 hours. Then, samples were dissolved in microwave. The samples removed from the microwave were cooled and filtered through a 100 mm blue band filter paper, then transferred to falcon tubes and 25 mL volume was completed with deionized water (Rao, 1986). The samples were analyzed by ICP-OES (Varian Vista).

The wavelengths measured of the elements are respectively B 249,772, Ca 317,933 nm, K 766,491 nm, Mg 279,553 nm, Na 589,592 nm, P 213,618 nm, Co 238,892, Mo 202,032 nm, Cd 214,439 nm, Cr

267,716 nm, Cu 327,395 nm, Fe 238,204 nm, Mn 257,610 nm, Ni 231,604 nm, Pb 220,353 nm and Zn 213,857. Analyzing conditions of ICP-AES is as follows: instrument: ICP-AES (Varian-Vista), RF Power: 0.7-1.5 kW (1.2-1.3 kW for Axial), plasma gas flow rate (Ar): 10.5-15 dm³/min. (radial) 15 (axial) , auxiliary gas flow rate (Ar): 1.5, viewing height: 5-12 mm, copy and reading time: 1-5 s (max. 60 s) , copy time: 3 s (max. 100 s).

2.3. Statistical Analysis

All statistical analyses were performed by using the SPSS15 software package. The Pearson Correlation Matrix was applied to evaluate the relationship between each of the metal parameters within the *C. fragile*.

3. Results

In this study, the concentrations of the elements were determined that accumulate in *C. fragile* collected from Çanakkale, macro, and trace elements of algal species and aquatic environment were compared. The mean and standard deviation values of macro and trace element contents of algae and water samples are given in Table 1.

Table 1. Mineral and trace element concentrations obtained from algae and water samples

n=3	<i>Codium fragile</i> (mg/kg ⁻¹) Mean		
	Mineral Elements	Trace Elements	
Ca	84826.5	Cd	BDL*
K	4707.4	Co	BDL*
Mg	10604.7	Cr	4.18
Na	93182.9	Cu	7.68
P	932.26	Fe	2611
BDL*: Below detection limit		Mn	1.84
		Mo	BDL*
		Ni	5.58
		Pb	46.08
		Zn	16.75

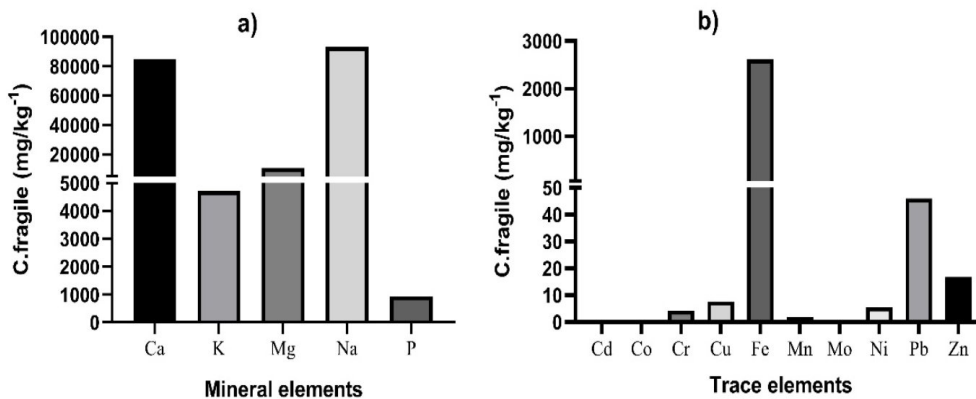


Figure 3. Levels of mineral (A) and trace elements (B) (mg/kg⁻¹) in *C. fragile*.

According to Table 1, when the accumulation amounts of the elements in the algae samples are taken into consideration, it is determined that the first orders are taken by the macro elements. Cobalt, molybdenum, and cadmium are below the limit values in the measurements. Iron was the most abundant trace element after macro elements in all samples. Also, lead, manganese were followed by

iron and chromium is the least trace element that can be detected in all of the samples. In our study, it was determined that the most trace element accumulation was iron in algal species. According to some other studies, it is seen that the most accumulation in the algal species is in iron (Foster,1976; Akçalı and Küçüksezgin, 2011; Alp et al.,2011).

Table 2. The relation between the metal parameters of the samples

	Ca	K	Mg	B	Na	P	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ca	1.000												
K	-0.500	1.000											
Mg	0.866	-0.328	1.000										
B	-0.938	0.770	-0.638	1.000									
Na	0.756	-0.945	0.327	-0.936	1.000								
P	0.971	-0.277	0.961	-0.826	0.577	1.000							
Cr	-0.861	-0.010	-0.999	0.630	-0.318	-0.958	1.000						
Cu	0.863	0.006	0.999**	-0.633	0.322	0.959	-0.997	1.000					
Fe	0.862	0.009	0.998**	-0.631	0.319	0.958	-0.998	0.999**	1.000				
Mn	0.948	-0.750	0.661	-0.999	0.925	0.843	-0.653	0.657	0.655	1.000			
Ni	0.868	-0.864	0.503	-0.987	0.981	0.723	-0.495	0.498	0.496	0.981	1.000		
Pb	0.826	-0.901	0.434	-0.971	0.993	0.667	-0.425	0.429	0.426	0.963	0.997*	1.000	
Zn	0.799	-0.921	0.391	-0.958	0.998*	0.631	-0.381	0.385	0.383	0.949	0.992	0.999*	1.000

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

The relation between the metal parameters of the samples taken from the *C. fragile* collected from Dardanelles was determined by the Pearson Correlation Analysis and was given in Table 2. The relations were determined between the metals in the *C. Fragile* ($p < 0.05$). Pb metal showed a positive correlation with Ni and Zn metals while Zn metal showed a positive correlation with Na metal. Cu and Fe metals had positive correlation with Mg metal while Cu metal showed a positive correlation with Fe metal ($p < 0.01$)

As a result of our study, sodium, which has the highest value in sodium brittle, was measured concerning the macro elements.

About trace elements, iron has the highest value in *C. fragile* was measured. Elemental concentrations of *C.fragile* were determined as following order:

Na>Ca>Mg>K>Fe>P>Mn>Pb>B>Zn>Cu>Ni>Cr>Co=Mo=Cd respectively.

In general, all macro and trace elements except for cobalt and selenium are found in algal samples more than the values found in water.

4. Conclusions

According to these results, it can be said that algae have a high absorption ability. According to PCA analysis, macro and trace elements showed a

positive and negative correlation with each other in *C.fragile*.

In 1940, Öy determined the physiological importance of iron, copper, manganese, and boron and discussed these elements in several species of algae. Iron varied between 120-1330 mg/kg dry matter, while *Ascophyllum nodosum* contained 1.1-1.4 mg/kg copper, *Laminaria* 4 mg/kg, *Fucus serratus* 5.8-17.4 and *F. vesiculosus* 3.4-8.4 mg/kg manganese was present in the Fucaceae to the extent of 100-130 mg/kg and boron 100 mg/kg. (Öy,1940).

In other studies, the Pb value was between 0.02-2.5 µg /g⁻¹ in the Aegean Sea for brown alga *Cystoseira* species (Sawidis et al., 2001), and 3.13-12.50 µg / g-1 in Iskenderun Bay. (Olgunoğlu and Polat 2007) and in the Black Sea, 3.7 µg / g⁻¹ (Topcuoğlu et al 2004), it was observed that the Pb values measured in this study (46.08 mg / kg) were quite high.

It was concluded that the highest concentration of *Codium fragile* was measured as Fe and some studies (Malea and Haritonidis, 1999; Conti and Cecchetti,2003) in which the order in heavy metal concentrations (Cd<Cr<Cu<Zn<Pb<Fe) are found to be parallel. Malea and Haritonidis (1999) determined this order as Cd <Cu <Pb <Zn <Fe, and Conti and Cecchetti (2003) stated it as Cd <Cr <Cu <Pb <Zn.

Atıcı et al., (2008), determined that accumulation of heavy metals (Cd, Pb, Hg, Cr) in water and plankton of Sarıyar Dam Lake (SDR) was seasonally studied from April 2000 to December 2004 and they

recorded high concentrations of all heavy metal in the plankton. Hg was lowest and Pb highest; but the concentration of each metal varied seasonally in their study, but we obtained that *Codium fragile* were determined as following order: Na>Ca>Mg>K>Fe>P>Mn>Pb>B>Zn>Cu>Ni>Cr>Co=Mo=Cd respectively in macroalgae in this study (Atıcı et al.,2008).

High levels of heavy metals were found in *C. fragile* algae. These high concentrations are caused by anthropogenic discharges of pollutants into the coastal areas of the Dardanelles. There are many industrial facilities such as large, medium, and small scale iron and steel facilities, fertilizer factories, liquid gas, and coal transportation piers, beverage and fruit juice factories along the coastline of the Dardanelles; their wastes, as well as the pesticides used in this region where agriculture is intensely carried out, and waste from industrial sites are mixed into the strait waters in various ways (Üstünada et al., 2011).

Apart from all these, large cargo ships coming to the strait, which is also an important passage in maritime transport, leave bilge and ballast waters to the strait waters off the port.

All these wastes accumulate at the bottom depending on their density, remain benthic or suspended in water, and spread to various parts of the throat with water movements such as waves and currents, and can harm on organisms.

Heavy metal concentrations in macroalgae may depend on geological characteristics of sampling areas, seasons, morphological characteristics of algae, age of algae, physicochemical parameters, and interactions with other heavy metals (Sawidis et al. 2001). Because of this feature of the algae, it is important to collect the algae species to be used in studies to compare the pollution levels in different regions and to be of similar ages.

We think that high metal concentrations in the Dardanelles affect the water quality negatively and may pose a threat to the living creatures living there, and we believe that serious measures should be taken to reduce continuous input in the Dardanelles, which has high shipping and tourism potential.

Conflict of Interest

The authors declare that they have no conflict of interest.

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