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Analysis of Metal Contents of Seaweed (*Ulva lactuca*) from Istanbul, Turkey by EDXRF

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Abstract

The aim of this study is to investigate the contents of essential and toxic trace element by the energy-dispersive X-ray fluorescence spectrometry (EDXRF) in seaweed (*Ulva lactuca*) from collected eight different regions of Istanbul (Turkey) in the years of 2006 and 2007. It has been analyzed by the samples using two annular radioactive sources and an Ultra-LEGe detector. A radioisotope excited X-ray fluorescence analysis using the method of multiple standard additions was applied for the elemental analysis of seaweed samples. In this study, the metal concentrations in the seaweed samples vary within 0.8-1.8% and 1.1-2.1% for Cl, 0.6-2.3% and 0.2-3.4% for K, 0.9-2.7% and 0.4-2.7% for Ca, 0.2-1.0% and 0.2-0.3% for Ti, 0.7-2.1% and 0.7-2.3% for Fe, 0.05-0.1% and 0.04-0.1% for Br, 0.3-0.6% and 0.3-0.4% for Sr, 0.1-0.2% and 0.1-0.2% for I, and 0.09-0.3% and 0.09-0.1% for Ba in the years of 2006 and 2007, respectively. The results demonstrated that these seaweeds contain some essential element, but no toxic element.

Keywords: Seaweed (*Ulva lactuca*), Elemental analysis, EDXRF, Ultra-LEGe detector, radioactive source, multiple standard additions.

İstanbul'dan Toplanan Deniz Yosunlarında EDXRF Yöntemi Kullanılarak Metal Konsantrasyon Analizi

Özet

Bu çalışmanın amacı, 2006 ve 2007 yıllarında İstanbul'un sekiz farklı bölgesinden toplanan deniz yosunlarında, EDXRF (enerji ayrımlı X-ışını floresans spektrometresi) tekniği kullanılarak temel ve toksit bazı elementlerin miktarlarının belirlenmesidir. Numuneler iki adet radyoaktif kaynak ve Ultra-LEGe tipi bir detektör ile analiz edilmiştir. Deniz yosunlarının element konsantrasyonun belirlemede, radyoaktif uyarımlı x-ışını floresans analizlerinde kullanılan çoklu standart ilave metodu kullanılmıştır. Çalışmada deniz yosunu örneklerindeki metal konsantrasyonunun 2006 ve 2007 yılları için sırasıyla; Cl için %0,8-1,8 ve %1,1-2,1 K için %0,6-2,3 ve %0,2-3,4, Ca için %0,9-2,7 ve %0,4-2,7, Ti için %0,2-1,0 ve %0,2-0,3, Fe için %0,7-2,1 ve %0,7-2,3, Br için %0,05-0,1 ve %0,04-0,1, Sr için %0,3-0,6 ve %0,3-0,4, I için %0,1-0,2 ve %0,1-0,2, ve Ba için %0,09-0,3 ve %0,09-0,1 aralığında değiştiği gözlenmiştir. Sonuçlara bakıldığında deniz yosunu örneklerinde toksik içerikli elementlere rastlanmamıştır.

Anahtar Kelimeler: Deniz yosunu (*Ulva lactuca*), Element analizi, EDXRF, Ultra-LEGe detektör, radyoaktif kaynak, çoklu standart ilave metodu.

Introduction

Seaweed is a mass of growth of marine algae from natural shock or from pond culture. Unlike other terrestrial and aquatic plants, seaweeds have no true roots, stems or leaves. They are widely distributed in the ocean and seas, ranging from tide level to considerable depths or attached to substrates such as sand, mud, rocks, shells, and coral inter alia. Generally, there are four main groups of marine seaweeds; (1) red algae (*Rhodophycae*); (2) brown algae (*Phaeophycae*); (3) green algae (*Chlorophycae*), and (4) blue green algae (*Cyanophycae*).

Many current and potential uses of seaweeds have been identified and these have been separated into 10 categories (1) agriculture, horticulture and agronomy, (2) uses in animal aquaculture, (3) aesthetics, (4) cosmetics, (5) environmental health, monitoring and remediation, (6) food, (7) health, thalassic and wellness, (8) industry, (9) pharmaceutical and pharmacology and (10) science, technology and biomedicine.

In Asia, seaweed is consumed as vegetable, and on average, the Japanese eat 1.4kg seaweed per person per year. France was the first European country to establish a specific regulation concerning the use of seaweeds for human consumption as non-traditional of mineral elements, macro elements and trace elements. Because seaweeds are also one of the most important vegetable sources of calcium, seaweed consumption may be useful for expectant mothers, adolescents and elderly all exposed to a risk of calcium deficiency (Burtin, 2003).

Ulva lactuca is a widespread macro algae occurring at all levels of the intertidal zone, in calm and protected harbors as deep as 10 meters and in northern climates. *Ulva lactuca* grows along rocky or sandy coasts of oceans and estuaries. *Ulva lactuca* growing in moderation has many uses. In some parts of Britain and Asia, seaweed is consumed by humans and livestock as it is considered valuable to human nutrition. Many of its nutrients include iron, protein (15 percent), iodine, vitamins (A, B1, and C) and trace elements. Because of antibacterial properties, it has been recommended by treating skin irritations typically, including burns.

The elemental analyses of seaweeds have been carried out in several countries by various techniques (Serfor-Armah *et al.*, 1999; El-Moselhy *et al.*, 2004; Carvalho *et al.*, 1996; Fu *et al.*, 2000; Lozano *et al.*, 2003; Blackmore, 1998; Mohead and Khaled, 2005). Some researchers determined the chemical composition of *Ulvaria oxsperma* (Kützing) Bliding, *Ulva lactuca* (Linnaeus) and *Ulva fascita* (Delile) (de Padua, 2004). It is known that *Ulva lactuca* is evaluated for its nutritional value as food for ruminants, and goats (Arieli *et al.*, 1993; Ventura and Castanon, 1998). A qualitative and quantitative evaluation of the seaweed diet of North Ronaldsay sheep was done on the beach shore of North Ronaldsay (Hansen *et al.*, 2003). *Ulva* and *enteromorpha*, which are the indicators of heavy metal pollution, were studied on the northwest coast of Spain (Villares *et al.*, 2001). The determination of heavy metals in environmental bio-indicators by Voltammetric and spectroscopic techniques was carried out (Locatelli *et al.*, 1999). *Ulva lactuca* L. (Chlorophyta) was analyzed to determinate the affinity, capacity and oxygen sensitivity of two different mechanisms for bicarbonate utilization (Axelsson *et al.*, 1999). Contamination of marine algae (seaweed) growing in the St. Lawrence River estuary and Gulf of St. Lawrence was analyzed for metals, iodine, and organochlorine pesticides (Phaneuf *et al.*, 1999).

XRF spectrometry (Energy and wave dispersive) is a useful tool for qualitative and quantitative element analysis of environmental, biological or industrial samples. XRF's versatility stems from its rapid, non-destructive, multi-element determinations from ppm to high weight percent of elements from Sodium (Na) through Uranium (U). In our previous works, EDXRF method has been used for the determination of trace elements of different samples such as tobacco, lichens, cole, plants, fish and moss (Aslan *et al.*, 2004; 2010; Çevik *et al.*, 2003, Tıraşoğlu *et al.*, 2005; 2006; Verep *et al.*, 2007, Özdemir *et al.*, 2010).

In this work, seaweed (*U. lactuca*) samples collected from Istanbul region in different years were analyzed quantitatively for their chlorine, potassium, calcium, titanium, iron, iodine, strontium, bromine, and barium contents.

Material and Methods

Experimental Procedure

Seaweed (*U. lactuca*) samples were collected along the coast of Istanbul region in the years of 2006 and 2007. These samples were collected at certain depths (0-3 meters). As shown in Figure 1, these stations are scattered in this area. In order to provide sample homogeneity, seaweed (*U. lactuca*) samples were taken several times from each station. Samples were attentively washed in tap water to remove salt water, sand, and particulate matter, and then these samples were put in distilled water. They were dried using ovens and then powdered using a spex mill. At the end, samples were sieved to provide particle size homogeneity using a 400 mesh sieve.

Samples were positioned according to the geometry (Tıraşoğlu *et al.*, 2006). 1.85 GBq ^{55}Fe and ^{241}Am radioactive sources excited by 5.96 and 59.54 keV respectively, photon energies were used for direct excitation. The samples were analyzed in the form of pellets to obtain their characteristic X-ray spectra by using a collimated Ultra-LEGe detector having a thickness of 5 mm and an energy resolution of 150 eV at 5.96 keV. The output from the preamplifier, with a pulse pile-up rejection capability, was fed to a multi-channel analyzer interfaced with a personal computer provided with suitable software for data acquisition and peak analysis. Spectra were analyzed using the Genie 2000 program and net peak areas were determined using the Origin 7.0 software program (Demo version). Each sample was irradiated for a time interval ranging from 2000 to 5000s. A representative example of a spectrum is given in Figure 2 for elements excited by ^{55}Fe and ^{241}Am radioactive sources.

In this study, the standard addition method was used to obtain the elemental concentration. The method involves the addition of known quantities of the analyte to the specimen. If analyte is presented at low levels and no suitable standards are available, standard addition may prove to be an alternative, especially if the analyst is interested in only one analyte element. The principle is as follows: Adding a known amount of analyte i(ΔW_i) to the unknown

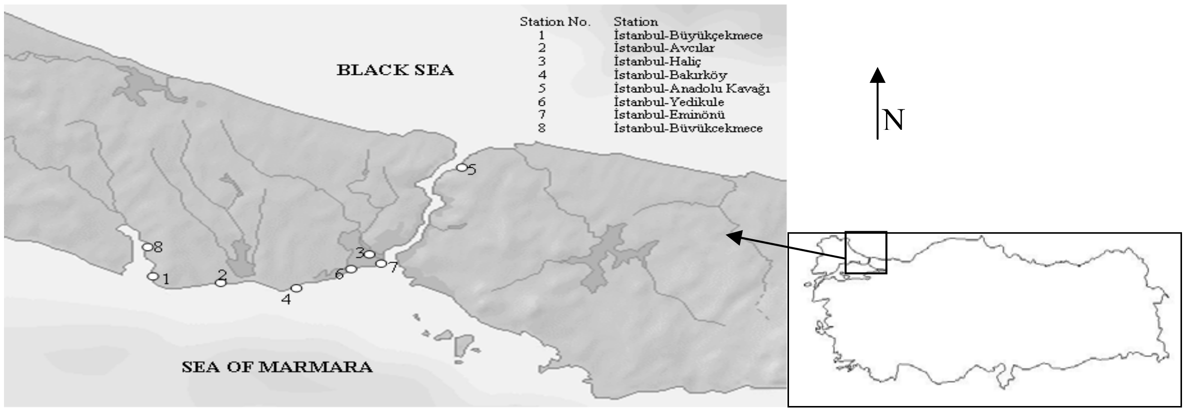

Figure 1. Study area.

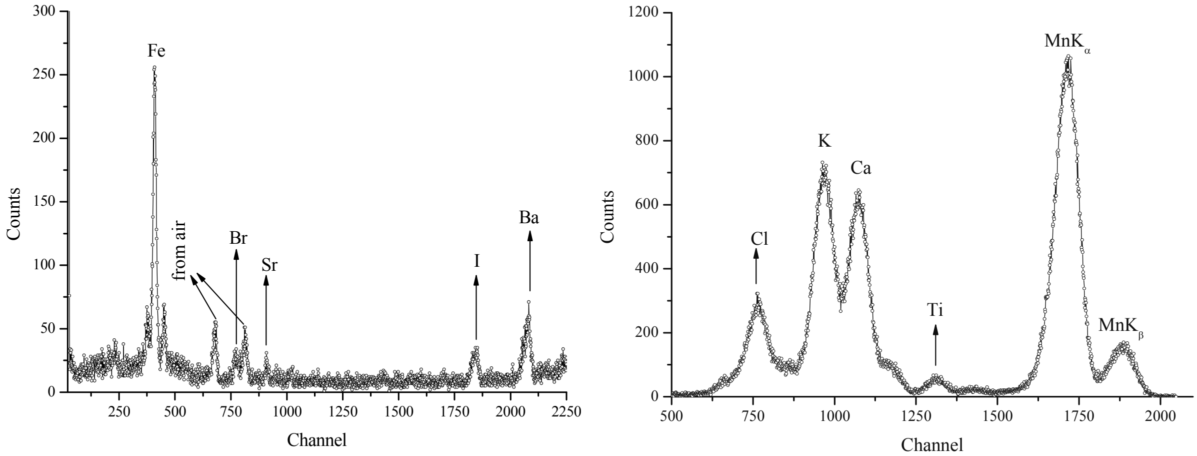

Figure 2. Typical spectra of sample recorded with Ultra-LEGe detector using (a) Fe-55 and (b) Am-241 radioisotopes.

sample gives an increased intensity $I_i + \Delta I_i$. Assuming a linear calibration, the following equations apply:

$I_i=M_iW_i$. For the original samples and $I_i + \Delta I_i=M_i(W_i+\Delta W_i)$ for the sample with the addition.

Thus, the method assumes that linear calibration is adequate throughout the range of addition because it assumes that an increase in the concentration of analyte by amount ΔW_i will increase the intensity by $M_i\Delta W_i$. These equations can be solved for the weight fraction of element $i(\Delta W_i)$. To check the linearity of the calibration, the process can be repeated by adding different amounts of the analyte to the sample and plotting the intensity measured versus the concentration axis equals W_i. The intensities used for calibration must be corrected for background and line overlap (Van Grieken, 1993). In this study, certain amounts of the element to be analyzed, called analyte, are added to the samples. The chemicals containing these addition standards as well as their purities are given in Table 1. In order to determine the amount of elements in the obtained spectrum, we used calibration curves in Figure 3.

Results and Discussion

The concentrations of nine elements examined in seaweed samples collected from Istanbul region in the years of 2006 and 2007 are shown in Table 2 for 8 stations.

Ca element concentrations in seaweed (*U. lactuca*) are almost higher than the other elements for each station except for Anadolu Kavağı and Haliç station in the year of 2006. Ca element concentration increased for Büyükçekmece, Avcılar, and Büyükçekmece Lake and decreased for Bakırköy, Haliç, Anadolu Kavağı, Yedikule, and Eminönü. In addition, High titanium and iron element concentrations were found in seaweed from Yedikule station. High K and Cl element concentrations were found in seaweed from Büyükçekmece Lake. High Ba, I, and Sr element concentrations were found in seaweed from Avcılar. Besides, high Br element concentration was found in samples from Anadolu Kavağı. These results were found in seaweed for 8 stations in the year of 2006. In the year of 2007, it was found that K element concentrations increased in seaweed from Büyükçekmece, Avcılar, Haliç,

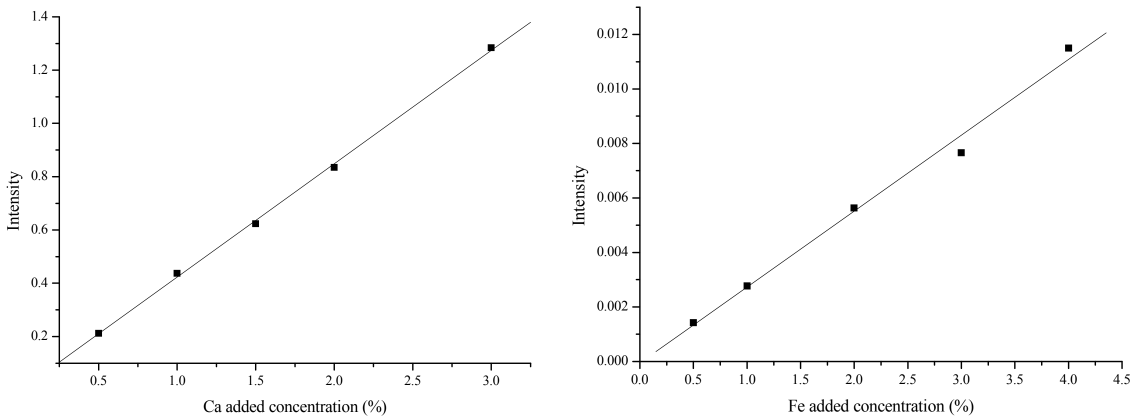

Figure 3. Calibration curve for (a) Ca and (b) Fe.

Table 1. Addition standards and rank of purities for chlorine, potassium, calcium, titanium, iron, iodine, strontium, bromine and barium

Analyte	Addition standard	Purity (%)	Percent analyte
Cl, K	$KClO_3$	99.5	28.92(Cl), 31.90(K)
Ca	CaO	99	71.47
Ti	TiO_2	99.9	59.93
Fe	Fe	99	100
I	KI	99	76.45
Ba	BaO_2	99.99	91.10
Sr	$SrCO_3$	99.9	59.35
Br	KBr	99	67.17

Table 2. Chlorine, potassium, calcium, titanium, iron, bromine, strontium, iodine and barium concentration for *Ulva lactuca*

Station No	Station	Year	Concentration (%)								
			Cl	K	Ca	Ti	Fe	Br	Sr	I	Ba
1	Büyükçekmece	2006	0.953	1.384	1.706	0.719	1.214	0.112	0.307	0.169	0.101
		2007	1.162	3.986	2.629	0.238	0.801	0.094	0.414	0.202	0.098
2	Avcılar	2006	1.656	1.126	2.654	0.814	1.392	0.095	0.686	0.245	0.151
		2007	2.187	2.825	6.285	0.284	1.932	0.082	0.452	0.226	0.117
3	Haliç	2006	0.918	0.740	1.724	0.934	2.537	0.083	0.303	0.161	0.100
		2007	1.235	3.444	1.361	0.247	1.366	0.098	0.306	0.172	0.149
4	Bakırköy	2006	1.252	0.621	2.656	0.271	0.705	0.085	0.424	0.205	0.097
		2007	1.328	0.263	1.792	0.263	2.328	0.048	0.373	0.172	0.116
5	Anadolu Kavağı	2006	1.526	1.119	0.901	0.316	1.756	0.123	0.312	0.168	0.111
		2007	1.725	0.601	0.438	0.228	0.766	0.085	0.330	0.174	0.099
6	Yedikule	2006	0.926	0.825	4.963	1.077	2.085	0.051	0.306	0.155	0.109
		2007	1.114	2.162	3.263	0.304	1.783	0.152	0.341	0.174	0.102
7	Eminönü	2006	0.831	0.857	2.709	0.871	2.145	0.117	0.361	0.155	0.103
		2007	1.164	0.342	0.631	0.235	1.967	0.083	0.312	0.163	0.100
8	Büyükçekmece Lake	2006	1.726	1.350	2.754	0.238	0.956	0.103	0.342	0.171	0.110
		2007	1.879	2.313	5.672	0.322	1.443	0.121	0.393	0.192	0.102

Yedikule, and Büyükçekmece Lake. Ca element concentration increased in samples from Büyükçekmece, Avcılar, and Büyükçekmece Lake and decreased in samples from Bakırköy, Anadolu Kavağı, Yedikule, and Eminönü. Ti and Fe element concentrations decreased in samples from Yedikule and Haliç but Fe element concentration increased in samples from Avcılar and Bakırköy. The overall error in concentrations is estimated to be 6-10%. This is attributed to the errors that incurred during sampling (6%) and EDXRF analysis (6-8%).

In nature, chlorine is found in the combined state only, chiefly with sodium as common salt (NaCl), sylvite, and carnallite. Chlorine is widely used in making many everyday products, and it is also used for producing safe drinking water over the world. Potassium is never found free in nature and it is an essential constituent for plant growth and is found in most soils. Calcium is a metallic element, fifth in abundance in the earth's crust, of which it forms more than 3%. Calcium is an essential constituent of leaves, bones, teeth, and shells. Calcium is never found in nature uncombined, but it occurs abundantly in limestone, gypsum, and fluorite. Iron is a vital constituent of plant and animal life and appears in hemoglobin. The pure iron metal is very reactive chemically and corrodes, rapidly, especially in moist air or at elevated temperatures. Titanium is not found unbound to other elements in nature, it is the ninth most abundant element in the Earth's crust (0.63% by mass) and it is present in most igneous rocks and in sediments derived from them. The titanium dioxide (TiO_2), commonly found in a black or brownish forms is known as rutile. It is not a poisonous metal and the human body can tolerate titanium in large doses. Barium is surprisingly abundant in the Earth's crust, being the 14^{th} most abundant element. High amounts of barium may only be found in soil and in food, such as nuts, seaweed, fish, and certain plants. Human activities add greatly to the release of barium into the environment because of its extensive use in the industries. As a result, barium concentrations in air, water, and soil may be higher than naturally occurring concentrations in many locations. The health effects of barium depend upon the water-solubility of the compounds. Some barium compounds that are released during industrial processes dissolve easily in water and are found in lakes, rivers, and streams. These barium compounds can spread over great distances because of their water-solubility. When fish and other aquatic organisms absorb the barium compounds, barium will accumulate in their bodies. The uptake of very large amounts of barium that are water-soluble may cause paralyses and in some cases even death. Small amounts of water-soluble barium may cause a person to experience breathing difficulties, increased blood pressures, heart rhythm changes, stomach irritation, muscle weakness, changes in nerve reflexes, swelling of brain and liver, and kidney and heart damage. Iodine is a non-metallic, solid element and it is only slightly soluble in water. Iodine is naturally present in the ocean, sea and some sea fish and water plants store it in their tissues. Iodine can be found naturally in air, water and soil. The most important sources of natural iodine are the oceans. Many medicines and cleansers for skin wounds contain iodine. Iodine is a building material of thyroid hormones that are essential for growth, the nervous system and the metabolism. If the iodine is excessively taken, it can be dangerous for human health because the thyroid gland labour too hastily. This affects the entire body; it causes disturbed heartbeats and loss of weight. Strontium reacts vigorously with water and quickly tarnishes in air. Strontium in its elemental form occurs naturally in many compartments of the environment, including rocks, soil, water, and air. Strontium compounds can move through the environment fairly easily, because many of the compounds are water-soluble. When strontium concentrations in water exceed regular concentrations, this is usually caused by human activities, mainly by dumping waste directly in the water. Excessive strontium concentrations can also be caused by settling of dust particles from air that have reacted with strontium particles from industrial processes. Bromine is the only nonmetallic element that is liquid under ordinary conditions and it is soluble in organic solvents and in water. Organic bromines are often applied as disinfecting and protecting agents, due to their damaging effects on microorganisms. When they are applied in greenhouses and on farmland they can easily rinse off to surface water, which has very negative health effects on daphnia, fishes, lobsters and algae. Bromine is corrosive to human tissue in a liquid state and its vapors irritate eyes and throat. Humans can absorb organic bromines through the skin, with food and during breathing. Bromine is a naturally occurring element that can be found in many inorganic substances but humans started the introduction of organic bromines in the environment many years ago. Through food and drinking water humans absorb high doses of inorganic bromines. These bromines can damage the nervous system and thyroid gland.

In this work, seaweed samples were collected in different years for a better investigation of mineral contents and heavy metal pollution. However; in this study the heavy metals having high toxicity were not found in the samples. Since the mineral content of *U. lactuca* varies according to the composition of sediment, sea water, sand, and rock, the absence of heavy metals in all samples suggested that most of the seaweed (*U. lactuca*) was collected from their nearly unpolluted natural habitats. In many countries, different works on heavy metal pollution and determination of metal pollution by different species of seaweeds have been carried out.

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