

Determination of Trace Elements in Some Macro/Micro Algae Samples by Inductive Coupled Plasma-Mass Spectrometry (ICP-MS)

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Abstract: This study investigated the elemental composition of seven macro/micro algae species collected from Southeastern Anatolia, Türkiye using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The algae samples were prepared using a microwave digestion protocol and analyzed for major and trace elements. The dominant elements across all species were sodium (Na), potassium (K), calcium (Ca), phosphorus (P), manganese (Mn), and iron (Fe) with Na concentrations ranging from 432885.22±39056.18 to 1232879.58±21924.43 µg/kg. Trace elements such as Mg, Al, Li, Be, Cd, and Sb were present at much lower levels or below detection limits. Notably, Ni concentrations in *Aphanizomenon flos-aquae* (258.57±11.12 µg/kg) and *Cladophora glomerata* (273.44±14.58 µg/kg) and Na in *Dunaliella salina* (462320.32±2032.57 µg/kg) exceeded the International Atomic Energy Agency (IAEA) acceptable limits (Ni: 571±28 µg/kg; Na: 680000±23000 µg/kg). The concentrations of Ca, K, Cr, Mn, Fe, Co, Cu, Zn, As, Cd, and Pb were within IAEA-defined safety thresholds. These results suggest that most of the studied algal species are rich in essential nutrients and exhibit low levels of toxic metals supporting their potential as functional biological resources in food or pharmaceutical applications.

Keywords: Elemental profiling, algal species analysis, ecotoxicology, aquatic ecosystems.

Bazı Makro/Mikro Alg Örneklerindeki Eser Elementlerin Endüktif Eşleşmiş Plazma-Kütle Spektrometresi (ICP-MS) ile Belirlenmesi

Öz: Bu çalışmada, Türkiye'nin Güneydoğu Anadolu Bölgesi'nden toplanan yedi farklı makro ve mikro alg türünün elementel bileşimi, Endüktif Eşleşmiş Plazma Kütle Spektrometresi (ICP-MS) ile belirlenmiştir. Alg örnekleri mikrodalga çözündürme yöntemiyle hazırlanmış ve majör/iz element içerikleri analiz edilmiştir. Tüm türlerde baskın olarak sodyum (Na), potasyum (K), kalsiyum (Ca), fosfor (P), manganez (Mn) ve demir (Fe) bulunmuş, Na konsantrasyonları ise 432885.22±39056.13 ile 1232879.58±21924.43 µg/kg arasında değişmiştir. İz elementlerden Mg, Al, Li, Be, Cd ve Sb elementlerine gelince düşük konsantrasyonlarda ya da tespit edilemeyen seviyelerde bulunmuştur. *Aphanizomenon flos-aquae* (258.57±11.12 µg/kg) ve *Cladophora glomerata* (273.44±14.58 µg/kg) türlerinde tespit edilen Ni konsantrasyonları ile *Dunaliella salina*'da ölçülen Na değeri (462320.32±2032.57 µg/kg), IAEA'nın belirlediği sınır değerlerin üzerinde bulunmuştur. (Ni: 571±28 µg/kg; Na: 680000±23000 µg/kg). Ca, K, Cr, Mn, Fe, Co, Cu, Zn, As, Cd ve Pb değerleri ise kabul edilebilir düzeydedir. Bulgular, çalışılan alg türlerinin çoğunun temel besin öğeleri bakımından zengin ve toksik metaller açısından güvenli olduğunu, bu nedenle fonksiyonel biyolojik kaynak olarak kullanıma potansiyeli taşıdığını göstermektedir.

Anahtar kelimeler: Elemental profil, alg türleri analizi, ekotoksikoloji, su ekosistemleri.

1. Introduction

Over the past two decades, green seaweeds have been extensively utilized as natural sources for extracting bioactive compounds thanks to their abundant biomass and wide distribution. Commonly cultivated or harvested for human consumption, these seaweeds are particularly valued for their rich nutritional content and associated health benefits. Among the three primary divisions of macroalgae, Chlorophyta, Phaeophyta, and Rhodophyta marine green algae remain relatively underutilized (Perna et al., 2021). However, there has been growing interest in harnessing green seaweeds as natural resources primarily due to their diverse active compounds, many of which hold significant potential for medical application (Ibnez & Cifuentes, 2013; Guerra-Rivas et al., 2011; Kellog & Lila, 2013).

amounts in the environment compared to other components. The first trace element determination was the arsenic determination based on the Marsh experiment. Trace element determination forms the basis of analytical chemistry. It has a great impact on many areas such as human health, environment, production in the pharmaceutical industry, geochemistry, and soil pollution (Kabdulkarimova et al., 2018). The concentration range of trace elements varies with analysis methods such as mass spectrometry, gas chromatography, plasma emission spectrometry, and atomic absorption spectrometry (Subitha et al., 2023). Trace elements are divided into two groups: "essential" and "non-essential" elements. An element is called an essential element when its deficiency or excess in metabolism causes a symptom and when these symptoms can be treated with medication. The limits of the elements are very important for human health. Having too little or too much of the elements can cause problems

Trace elements are elements found in very low

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in the human body (Stephen, 2010).

Three different plasma sources are used in plasma-induced emission spectroscopy. The first of these is the direct current plasma source. The direct current plasma consists of three electrodes in the shape of an inverted Y with the anode on the lower arms and the cathoda on the upper. Another plasma source is microwave plasma. Microwave plasma is a type of plasma that produces high-frequency electromagnetic radiation and can produce frequencies in the range of 2-45 GHz (Amman, 2002). Finally, the inductively coupled plasma source is the most widely used one. The inductively coupled plasma consists of three nested quartz tubes through which an argon gas stream passes. ICP-MS is a technique developed by combining the accurate, low detection limits of mass spectroscopy with easy sample introduction and rapid analysis feature of ICP technology (Rashad, 2015; Yamada, 2015).

The present research investigates the accumulation profiles of heavy metals in selected algal species sampled from the Southeastern Anatolia Region of Turkey. A total of seven species were subjected to analysis, comprising two macroalgae (*Myriophyllum spicatum* and *Cladophora glomerata*) and five microalgae (*Dunaliella salina*, *Microcystis aeruginosa*, *Chlorella vulgaris*, *Aphanizomenon flos-aquae*, and *Arthrospira palatensis*). Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was employed to quantify the concentrations of five essential macroelements (Na, Mg, Al, K, Ca) and twenty trace elements (Li, Be, B, P, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sb, Ba, Pb). The elemental composition data were critically assessed to determine the suitability of these algal species as candidates for use in functional applications, particularly in relation to their trace metal content.

2. Material and Method

2.1. Reagents

Analytical-grade reagents were utilized throughout the study. Hydrochloric acid (HCl), nitric acid (HNO₃), and hydrogen peroxide (H₂O₂) were sourced from Merck (Germany). Additional chemical reagents were of analytical purity and supplied by either Sigma-Aldrich or Merck. Ultrapure water used in all experimental procedures was produced using a Human-brand purification system (Japan).

2.2. Sample Acquisition and Taxonomic Identification

The algal strains *Dunaliella salina*, *Chlorella vulgaris*, and *Spirulina platensis* were procured from the Aquaculture Department of the Faculty of Fisheries at Çukurova University (Adana, Türkiye). Field-collected specimens of *Cladophora glomerata* and *Microcystis aeruginosa* were sampled from the Birecik-Karkamış Dam located in Şanlıurfa Province, while *Aphanizomenon flos-aquae* and *Myriophyllum spicatum* were obtained from the Çamçayı Dam in the Siverek district of Şanlıurfa. All taxonomic identifications and species verifications were performed by Dr. Göksal Sezen.

2.3. Sample Preparation and Digestion Procedure

Following collection, algal specimens were thoroughly cleaned to remove epiphytic and epizotic contaminants, air-dried in shaded conditions, and subsequently

grounded into a fine powder using high-speed blender operating at 46.000 rpm (Tefal Ultra High-Speed Blender). The homogenized samples were stored at -20°C in a deep freezer until further analysis. For laboratory processing, algal biomass was transferred to 20-liter plastic containers at the Hydrobiology-Algology Laboratory, Department of Biology, Faculty of Arts and Sciences, Harran University (Şanlıurfa, Türkiye).

For digestion, 0.5 g dried algal sample was weighed, followed by the addition of 1 mL 35% hydrogen peroxide (H₂O₂). The mixture was allowed to stand for 5 minutes to facilitate oxidative degradation of organic matter. Subsequently, 9 mL of concentrated nitric acid (65% HNO₃) and 3 mL of hydrochloric acid (36.5-38% HCl) were added sequentially. The mixture was sealed in Teflon digestion vessels and subjected to microwave-assisted digestion using a CEM-MARS 6 system. The digestion program involved heating to 220°C for 20 minutes at 600 W, followed by a 10-minute cooling phase (Üner et al., 2023).

Post-digestion, the solutions were transferred to 50 mL Falcon tubes and centrifuged at 9.000 rpm for 7 minutes to eliminate siliceous residues. The supernatant was carefully decanted and brought up to a final volume of 50 mL using ultrapure water. Quantitative elemental analysis was conducted using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 720).

2.4. ICP-MS Instrumentation and Analytical Methodology

To minimize matrix-induced interferences and enhance analytical accuracy, the standard addition method was employed during elemental quantification by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). All measurements were conducted using an Agilent 7700 Series instrument (Agilent Technologies, Tokyo, Japan). Each sample was analyzed in triplicate to ensure reproducibility and data reliability. Detailed operating conditions and instrumental parameters are provided in Table 1. The calibration curve of the developed method varies between 0.0001 ppb and 100 ppb. The confidence interval of the calibration curve was found to be 0.95 and the relative standard deviation was 5%. The correlation coefficient (r²) of the calibration curve to the point and the line equation (r²) was calculated as 0.9995. The weighting factor of the calibration curve is 1/x. All recovery values (RSD) were found to be above 95%.

Table 1. Setting parameters for the ICP-MS method

Plasma Parameters	Lens Parameters	Cell Parameters
Power: 1550 W	Omega Lens: 10 V	OctP Slope: -8 V
Matcher voltage: 1.80 V	Cell Input: -30 V	OctP RF: 180 V
Sample depth: 8 mm	Cell Output: -50 V	Energy separator: 5 V
Carrier gas velocity: 1.05 l/min	Deviation: 13 V	Stabilization time: 50 sec
Nebulizer pump: 0.1 rps	Layer training: -40 V	Sampling time: 50 sec
S/C temperature: 2°C		Calibration curve confidence interval: 0.95

2.5. Statistical Evaluation

A power analysis was performed to determine the

appropriate sample size for both macroalgae and microalgae extracts. All experimental results were expressed as mean values \pm standard deviation (SD) based on triplicate measurements ($n=3$) for each algal species.

3. Results and Discussion

The concentration of both major and minor trace elements detected in the algal samples are presented in Table 2 and Table 3, respectively. Among the quantified elements, sodium (Na), potassium (K), calcium (Ca), phosphorus (P),

manganese (Mn), and iron (Fe) were identified as the most abundant across all species analyzed. In contrast, magnesium (Mg), aluminum (Al), lithium (Li), beryllium (Be), cadmium (Cd), and antimony (Sb) were found at comparatively lower concentrations. These findings are generally consistent with the literature. For instance, Dawczynski et al. (2007) reported concentrations of major elements in algal biomass as 5.85 ± 3.31 g/kg for Na^+ , $27.2 \pm$ g/kg for K^+ , and 3.50 ± 0.50 g/kg for Mg^{2+} .

Table 2. Mean values of major elements in macro-micro algae samples ($\mu\text{g}/\text{kg}$)

Major Elements	S1 mean \pm std*	S2 mean \pm std*	S3 mean \pm std*	S4 mean \pm std*	S5 mean \pm std*	S6 mean \pm std*	S7 mean \pm std*
Na	479451.69 ± 24227.40	508362.78 ± 20694.89	432885.22 ± 39056.18	557486.39 ± 10688.73	1232879.58 ± 21924.43	495382.79 ± 35121.39	494970.85 ± 5138.07
Mg	<0.000 ± 0.00	<0.000 ± 0.00	<0.000 ± 0.00				
Al	<0.000 ± 0.00	21073.82 ± 7638.42	<0.000 ± 0.00	<0.000 ± 0.00	<0.000 ± 0.00	<0.000 ± 0.00	<0.000 ± 0.00
K	829248.32 ± 4850.58	978619.92 ± 20552.11	986608 ± 35580.95	945444.29 ± 9275.95	2704378.97 ± 130784.12	1060855.40 ± 22345.72	1206131.65 ± 33659.32
Ca	161953.10 ± 13756.72	461836.18 ± 39654.07	959186.04 ± 30749.71	179693.05 ± 12949.88	177975.77 ± 42893.57	191711.77 ± 16995.41	1511533.69 ± 131946.10

S1: *Dunaliella salina*, S2: *Microcystis aeruginos*, S3: *Myriophyllum spicatum*, S4: *Chlorella vulgaris*, S5: *Aphanizomenon flos-aquae*, S6: *Artrosipira platensis*, S7: *Cladophora glomerata*, *std: standard deviation.

Table 3. Mean values of minor elements in macro-micro algae samples ($\mu\text{g}/\text{kg}$)

Minor Elements	S1 mean \pm std*	S2 mean \pm std*	S3 mean \pm std*	S4 mean \pm std*	S5 mean \pm std*	S6 mean \pm std*	S7 mean \pm std*
Li	<0.000 \pm 0.00	<0.000 \pm 0.00	<0.000 \pm 0.00	<0.000 \pm 0.00	20.93 \pm 1.46	<0.000 \pm 0.00	<0.000 \pm 0.00
Be	<0.000 \pm 0.00	1.91 \pm 0.83	1.96 \pm 0.70	<0.000 \pm 0.00	25.94 \pm 3.25	3.53 \pm 0.81	0.57 \pm 0.28
B	123.29 \pm 2.41	174.71 \pm 30.39	2616.82 \pm 139.83	344.24 \pm 9.08	42710.74 \pm 2427.53	936.50 \pm 370.07	3124.64 \pm 5.40
P	9054.27 \pm 64.56	131922.78 \pm 517.82	90514.26 \pm 6708.89	222510.85 ± 5405.95	445810.74 \pm 52849.82	302743.73 \pm 21482.93	56314.96 \pm 2372
V	<0.000 \pm 0.00	308.01 \pm 2.52	400.82 \pm 56.23	<0.000 \pm 0.00	200.10 \pm 15.46	<0.000 \pm 0.00	203.00 \pm 15.44
Cr	<0.000 \pm 0.00	247.86 \pm 2.25	764.44 \pm 78.63	<0.000 \pm 0.00	129.81 \pm 9.40	29.35 \pm 7.37	655.07 \pm 82.51
Mn	<0.000 \pm 0.00	14566.10 \pm 109.39	63519.62 \pm 6250.76	1396.47 \pm 13.42	923.29 \pm 151.57	328.76 \pm 39.20	11710.18 \pm 260.63
Fe	77.12 \pm 6.35	103085 \pm 1132.63	71690.18 \pm 12982.59	6296.72 \pm 92.01	133234.93 \pm 10943.28	21781.95 \pm 1572.11	34238.61 \pm 4501.65
Co	0.18 \pm 0.03	176.71 \pm 6.60	161.69 \pm 2.27	6.13 \pm 1.04	160.93 \pm 4.46	6.69 \pm 0.31	32.79 \pm 6.14
Ni	<0.000 \pm 0.00	831.70 \pm 2.01	1167.68 \pm 139.51	<0.000 \pm 0.00	242.04 \pm 23.37	<0.000 \pm 0.00	257.23 \pm 22.93
Cu	<0.000 \pm 0.00	200.07 \pm 1.69	537.41 \pm 50.60	90.92 \pm 1.46	218.65 \pm 17.64	16.70 \pm 1.43	293.77 \pm 10.81
Zn	<0.000 \pm 0.00	337.61 \pm 8.51	845.08 \pm 74.76	239.78 \pm 5.49	416.26 \pm 36.02	237.21 \pm 17.05	175.54 \pm 11.67
As	5.28 \pm 0.25	99.80 \pm 1.72	132.28 \pm 16.65	1.10 \pm 0.20	158.50 \pm 10.77	4.60 \pm 0.01	176.15 \pm 7.20
Se	2.71 \pm 0.59	8.13 \pm 1.53	94.57 \pm 1.74	2.44 \pm 0.26	96.56 \pm 4.01	5.96 \pm 1.53	26.04 \pm 1.53
Sr	8.48 \pm 0.26	1390.99 \pm 36.82	4394.84 \pm 13.63	257.11 \pm 18.45	2653.93 \pm 2.69	1069.35 \pm 12.16	4480.13 \pm 6.47
Mo	1.28 \pm 0.02	12.02 \pm 0.05	17.61 \pm 1.01	9.84 \pm 0.05	93.78 \pm 0.80	2.88 \pm 0.02	8.52 \pm 0.61
Cd	0.03 \pm 0.00	2.51 \pm 0.45	2.88 \pm 0.19	0.93 \pm 0.02	10.80 \pm 0.65	1.89 \pm 0.05	0.94 \pm 0.09
Sb	5.03 \pm 0.35	3.98 \pm 0.13	6.33 \pm 0.02	2.01 \pm 0.13	6.39 \pm 0.24	3.91 \pm 0.01	3.15 \pm 0.07
Ba	1.47 \pm 0.26	651.93 \pm 9.45	2675.30 \pm 34.59	88.91 \pm 3.93	849.06 \pm 8.67	943.88 \pm 1.73	1341.65 \pm 12.79
Pb	5.03 \pm 0.63	30.95 \pm 2.22	73.64 \pm 3.35	6.55 \pm 0.21	224.65 \pm 6.85	14.90 \pm 0.48	19.11 \pm 0.32

S1: *Dunaliella salina*, S2: *Microcystis aeruginos*, S3: *Myriophyllum spicatum*, S4: *Chlorella vulgaris*, S5: *Aphanizomenon flos-aquae*, S6: *Artrosipira platensis*, S7: *Cladophora glomerata*, *std: standard deviation

Nickel (Ni^{2+}) concentrations in all algal species, with the exception of *Microcystis aeruginosa* and *Myriophyllum spicatum*, were found to be below the threshold values established by the International Atomic Energy Agency (IAEA). In contrast, the mean Ni^{2+} levels in *M. aeruginosa* and *M. spicatum* exceeded the IAEA reference

concentration of 0.571 ± 0.028 mg/kg (IAEA, 2005: see Table 3). Comparable findings were reported by Tuzen et al. (2009) who observed Ni^{2+} concentrations ranging from 0.27 ± 0.01 and 36.20 ± 2.20 $\mu\text{g}/\text{g}$ across 20 different algal species. In the present study, measured Ni^{2+} values ranged between 0.242 ± 0.002 and 1.167 ± 0.139 $\mu\text{g}/\text{kg}$ that are

generally lower than those reported in the earlier studies, with the exception of the two aforementioned species.

According to the analyses, Mg²⁺ and Li²⁺ levels in all examined algae species were below detectable limits (<0.00±0.00 µg/kg) as presented in Table 2 and 3. In the case of Al³⁺, no measurable concentration was observed in any species other than *Microcystis aeruginosa* (Table 2). This species showed a notably high Al²⁺ level, measured at 21073.82±7638.42 µg/kg. It should be noted that the IAEA has not established a reference threshold for interpreting this concentration.

The concentration of Ca²⁺, K⁺, Cr³⁺, Mn²⁺, Fe²⁺, Co²⁺, Cu²⁺, Zn²⁺, As²⁺, Cd²⁺, and Pb²⁺ in all algae species were detected below the values specified in the IAEA (2680.00±67.40 mg/kg, 8383.00±252.00 mg/kg, 4.57±0.18 mg/kg, 67.5±1.54 mg/kg, 497.00±13.60 mg/kg, 3.33±0.12 mg/kg, 23.2±1.70 mg/kg, 128.00±2.00 mg/kg, 0.175±0.016 mg/kg, 0.0173±0.0014 mg/kg and 0.574±0.028 mg/kg, respectively) (Table 1; Table 2; IAEA, 2005).

In the study by Ghosn et al. in which they evaluated the trace element concentrations in algae off the coast of Lebanon, it was observed that Fe²⁺ concentrations (516.00±473.00) exceeded the IAEA limit. The same study found the Cr³⁺ concentration to be within limits (Ghosn et al., 2020). In this study, the Fe²⁺ and Pb²⁺ concentrations were detected lower than the acceptance level specified for all algae species (Table 3; IAEA, 2005).

In seaweed samples collected from the Bosphorus, Co²⁺ concentrations were previously reported to range between 0.32±0.11 and 3.75±0.27 µg/g (Kut et al., 2000). In contrast, our study found Co²⁺ levels between 0.0018±0.0003 and 0.1760±0.0066 µg/g. Despite the differences in values, both studies fall within the safety limits set by the IAEA.

In the present research, the average concentrations of heavy metals in all samples followed the order: As²⁺>Pb²⁺>Cd²⁺ (Table 3). Similarly, Yuan et al. (2015) reported that the average heavy metal concentrations decreased as As²⁺>Cd²⁺>Pb²⁺, respectively. A comparative assessment based on the IAEA acceptance limits can be made using the relevant reference (Zeiller & Shakhshiro, 2005).

4. Conclusions

In conclusion, ICP-MS analysis revealed that the studied algal species from Southeastern Anatolia are generally rich in essential elements and contain trace metal concentrations mostly within acceptable safety limits. The macro-elements Na, K, and Ca were the most prevalent across all samples, with *Dunaliella salina* showing an exceptionally high Na concentration (462320.32±2032.57 µg/kg). Among the minor elements, *Aphanizomenon flos-aquae* and *Cladophora glomerata* had Ni concentrations (258.57±11.12 µg/kg and 273.44±14.58 µg/kg, respectively) exceeding the IAEA's recommended limits. Despite these exceptions, the majority of elements – such as Fe, Mn, Zn, Cu, Pb, and Cd – were found within safe levels across all species. These findings support the potential utilization of algae from this region as functional ingredients in nutraceuticals or environmental remediation applications, although species-specific monitoring is advised for elements like Ni and Na to

ensure consumer safety.

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