Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi
Menba Journal of Fisheries Faculty
ISSN 2147-2254 | e-ISSN: 2667-8659

Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2024; 10 (3): 30-35 Araştırma Makalesi/Research Article

Makro/Mikro Alg Örneklerinde Ağır Metal İçeriklerinin ICP-OES ile Belirlenmesi

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Makale Bilgisi :
Geliş:
05/06/2024
Kabul Ediliş:
16/09/2024

Anahtar Kelimeler:
- Makro/Mikro Alg
- İz Elementler
- Ağır Metaller
- ICP-OES

Öz

Algler zengin bir pigment, yağ, protein, fenolik bileşik, terpen ve alkaloit kaynağı olarak su ortamındaki besin zincirinin önemli bir parçasıdır. Algler, içerdikleri bileşiklerin sağladığı faydalı etkiler nedeniyle sağlık ürünlerinde yaygın olarak kullanılmaktadır. Alglerin yaşadığı sucul ekosistemlerdeki ağır metaller, besin zincirinde birikerek halk sağlığı açısından küresel bir sorun olarak karşımıza çıkmaktadır. Alglerin kullanımının değerlendirilmesinde temel elementlerin incelenmesi kadar ağır metal kalıntılarının belirlenmesi de önemlidir. Bu çalışmada Güneydoğu Anadolu Bölgesi'nden toplanan 7 farklı alg türünün ağır metal içeriklerinin belirlenmesi amaçlandı. Örnekler mikrodalga çözündürme yöntemiyle analize hazırlandı. Analiz ICP-OES ile gerçekleştirildi. Tüm alg türlerinde tespit edilen en baskın elementler Na, K, Ca, K, P, Mn ve Fe olurken, Mg, Al, Li, Be, Cd ve Sb ise en az konsantrasyona sahip elementler oldu. Microcystis aeruginosa (631,70±2,01 µg/kg) ve Myriophyllum spicatum'un (1067,69±1,91 µg/kg) Ni konsantrasyonları ile Aphanizomenon flos-aquae'nin (1032879,59±21924,44 µg/kg) Na konsantrasyonları Uluslararası Atom Enerjisi Ajansı (IAEA) tarafından tanımlanan kabul limitlerinin (Ni için 0.571±0.028 mg/kg; Na için 680.00± 23.00 mg/kg) üzerinde tespit edildi. Ca, K, Cr, Mn, Fe, Co, Cu, Zn, As, Cd ve Pb konsantrasyonlarının tamamı IAEA tarafından tanımlanan kabul edilebilir sınırlar dahilinde tespit edildi. Sonuçlara göre bu bölgeden toplanan alg türlerinin potansiyel fonksiyonel kaynak olarak kullanılabileceği değerlendirilmektedir.

Determination of Heavy Metal Contents in Macro/Micro Algae Samples by ICP-OES.

Article Info

Received:
05/06/2024
Accepted:
16/09/2024

Keywords:
- Macro/micro algae
- Trace elements
- Heavy metals
- ICP-OES

Abstract

Algae are an important part of the food chain in the aquatic environment as a rich source of pigments, oils, proteins, phenolic compounds, terpenes and alkaloids. Algae are widely used in well-being products due to the beneficial effects provided by the compounds they contain. Heavy metals in aquatic ecosystems where algae inhabited appears as a global problem in terms of public health due to accumulated in the food chain. In evaluating the usage of algae, it is important to determine the heavy metal residues as well as to examine essential elements. In this study, it was aimed to determine heavy metal contents in 7 different algae species that collected from Southeastern Anatolia Province. The samples were prepared for analysis by the microwave solubilisation method. The analysis were carried out by ICP-OES. The detected most dominant elements were Na, K, Ca, K, P, Mn and Fe while the Mg, Al, Li, Be, Cd and Sb were least concentration in all algae species. The Ni concentrations of *Microcystis aeruginosa* (631.70±2.01 µg/kg) and *Myriophyllum spicatum* (1067.69±1.91 µg/kg) and the Na concentrations of *Aphanizomenon flos-aquae* (1032879.59±21924.44 µg/kg) were detected above the acceptance limits (0.571±0.028 mg/kg for Ni and 680.00± 23.00 mg/kg for Na) that are defined by the International Atomic Energy Agency (IAEA). The concentrations of Ca, K, Cr, Mn, Fe, Co, Cu, Zn, As, Cd and Pb were detected all within the acceptable limits defined by IAEA. According to results, the evaluated that algae species were collected from this region can be used as a potential functional resource.

Atıf bilgisi / Cite as: Cayci, M., Ceylan, B. & Sezen, G. (2024). Determination of Heavy Metal Contents in Macro/Micro Algae Samples by ICP-OES. Menba Journal of Fisheries Faculty, 10 (3), 30-35. DOI: 10.58626/menba.1496072

INTRODUCTION

Algae that are inhabited in aquatic and semi-aquatic environments, are autotrophic and photosynthetic organism (Guven et al, 1973). Algae have secondary metabolites with high biological activity, which are especially important in the development of new pharmaceutical agents (Chapman et al, 1992; Barbier et al, 2001). Algae are one of the most important part of the food chain and widely used in health, food and cosmetic products due to the beneficial effects provided by the compounds they contain. As a result of studies conducted on algae, the presence of antimicrobial, cytotoxic, antimitogenic, anticancer and anti-tumor activities was revealed.

Algae have been used as well-being products by humans for centuries and the present wordwide market has increased from 0.56 million tons in 1950 to 35.82 million tons in 2019 (Kuech et al, 2023). Interest in algae is increasing due to their usage not only in island countries and the far east but also European (Enzing et al, 2014, Wanga et al, 2020, Ullmann et al, 2021).

Micro- and macro-algae can be found in both environments of marine and freshwater environments. Macro-algae account for over 99% of global algae production (Kuech et al, 2023) Macro-algae provide a source of bioactive metabolites with a variety of potential and noteworthy biological activities, capable of impacting the abundance, distribution, and survival of marine organisms. Studies show that macro-algae or seaweeds has high nutritional value (Scieszka et al, 2019; Rushdi et al, 2020). Algae are classified into three major groups, including Chlorophyta (green algae), Rhodophyta (red algae) and Ochrophyta (brown algae) based on their. It is estimated that 1800 green, and about 1800 brown and 6200 red macro-algae are found in the marine environment (Belton et al, 2019).

The algae could be collected from nature, also they can be obtained through culture. It mainly relies on wild stock harvesting as the most common production method. Kuech et al (2023) reported that macroalgae production method by companies in Europe by harvesting from wild stocks 68% and aquaculture 32% (Kuech et al, 2023). Micro-algae production in Europe is land-based and occurs in photobioreactors (71%), ponds (19%) and fermenters (10%) (Kuech et al, 2023). Of the 28 million tons of algae are produced globally, 800 thousand tons are collected from nature and included in the production process (Erol et al, 2022). It is known that algae are affected by pollutants in their natural habitat. Lakes and rivers are among the ecosystems most affected by environmental pollution. The pollutions of the freshwater and marine environment that algae are inhabited can lead to contamination in macro and micro-algae due to bio-remediation effects. The one of the priority chemical pollutant the toxic heavy metals remain pristine in environmental conditions and accumulated in the food chain and it could be a risk for public health. The high concentrations of heavy metals are declared as impediment concerning the European macro-algae sector (Kuech et al, 2023). Therefore, macro and micro-algae that potentially usage have to examine for heavy metal contaminations before usage.

This study aims to determine the heavy metal contents in algae species collected from Southeastern Anatolia Province. For this purpose, 2 macro (Myriophyllum spicatum and Cladophora glomerata) and 5 microalgae species (Dunaliella salina, Microcystis aeruginosa, Chlorella vulgaris, Aphanizomenon flos-aquae, Artrospira palatensis) were analyzed. The analysis of 5 major (Na, Mg, Al, K, Ca) and 20 minor elements (Li, Be, B, P, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sb, Ba, Pb) were carried out by ICP-OES. The advantage of our current study compared to previous studies is that it can determine a total of 25 trace elements precisely and rapidly. Thus, control of trace elements and heavy metal levels were carried out and evaluated that algae species were collected from this region can be used as a potential functional resource.

MATERIAL AND METHOD

Chemicals and Solvents

Hydrochloric acid, nitric acid and hydrogen peroxide were purchased from Merck. All other chemicals used were in analytical grade and obtained from either Sigma-Aldrich or Merck. Water was purified by Human (Japan) ultra pure water purification system.

Sample Collection

Dunaliella salina, Chlorella vulgaris and Spirulina platensis were obtained from Çukurova University, Faculty of Fisheries, Aquaculture Department, Adana, Türkiye. Cladophora glomerata, Microcystis aeruginosa from Şanliurfa Birecik Karkamış Dam; Aphanizomenon flos-aquae and Myriophyllum spicatum were collected from Şanliurfa Siverek Çamçayi Dam. Algae samples were classified by Dr. Göksal Sezen.

Sample Extraction

The collected algae were cleaned from epiphytes and epizoa, dried in the shade, pulverized with a 46.000 rpm blender (Tefal Ultra High Speed Blender) and stored in the deep freezer at -20 ºC. Algae were placed in 20-liter plastic bottles in Hydrobiology-Algology laboratory at department of Biology, Faculty of Arts and Science in Harran University, Şanlıurfa, Türkiye. Algae samples of 0.5 g was weighed, 1 mL of 35% H2O2 was added and left for 5 minutes to oxidize the organic content. After adding 9 mL of 65% HNO3 and finally 3 mL 36.5 – 38% HCl to the peroxide mixture, the Teflon containers were closed. The prepared samples were extracted in a CEM – MARS 6 microwave device. In the microwave, it was heated to 220 ºC for 20 minutes at 600 W of power and then it was left for 10 minutes. After the combustion process, the samples taken into 50 mL falcon tubes were centrifuged at 9000 rpm for 7 minutes and the silicate content was removed. The balance was separated by decantation and completed to a final volume of 50 mL with ultrapure water. The analyses were carried out by ICP-OES (Agilent 720).

Statistical Analysis

A power analysis was done to figure out how many extracts of macro and micro-algae there were. The results were displayed for each test specimen as means ± standard deviation for n=3.

RESULT AND DISCUSSION

The results of heavy metals as a major and minor trace elements are shown in Table 1 and Table 2, respectively. The detected most dominant elements were Na, K, Ca, K, P, Mn and Fe while the Mg, Al, Li, Be, Cd and Sb were least concentration in all algae species (Table 1 and Table 2). In the similar studies reported that Fe, P, Mn, Ca, Na and K are the most abundant major and minor elements in algae species (Ogoyi et al, 2011; Dehbi et al, 2023).

The concentration of Ni^{2+} was determined below the values specified in the IAEA for all algae species except *Microcystis aeruginosa* and *Myriophyllum spicatum*. The mean Ni^{2+} concentrations in *M. aeruginosa* and *M. spicatum* were above the defined levels in the IAEA (0.571±0.028 mg/kg) (Table 2; IAEA, 2005). As similar this results, Yuan et al (2015) were detected that the Ni^{2+} concentrations were above the IAEA specified limits (0.571±0.028 mg/kg) (Table 2; IAEA, 2005).

The Na^{2+} concentration were determined below the values specified in the IAEA for all microalgae species except *Aphanizomenon flos-aquae* (Table 1; IAEA, 2005). The mean Na^{2+} concentration in *A. flos-aquae* were above the defined level in the IAEA (680.00±23.00 mg/kg) (Table 1; IAEA, 2005).

The Mg^{2+} and Li^{+} concentration were determined as <0.00±0.00 µg/kg in all algae species (Table 2). Al concentration was determined as <0.00±0.00 µg/kg in all algae species except *Microcystis aeruginosa* (Table 1). The concentration of Al^{2+} in *M. aeruginosa* was determined as 26906.13± 4476.88 µg/kg (Table 1.). However, there is no limit defined by the IAEA for the evaluation of this result.

The concentration of Ca^{2+}, K^{+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, As^{2+}, Cd^{2+} and Pb^{2+} 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±13.60 mg/kg, 3.33±0.12 mg/kg, 23.2±1.70 mg/kg, 128±2.0 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).

Dehbi et al (2023) reported that the Cu^{2+} and Pb^{2+} concentration in *Cladophora glomerata* were exceed the IAEA limit (23.2 mg/kg and 0.574 mg/kg, respectively) (IAEA, 2005). In this study, the Cu^{2+} and Pb^{2+} concentration were detected lower than the acceptance level specified for all algae species (Table 2; IAEA, 2005). Yuan et al (2015) were detected Cu^{2+} concentration among 8.21±4.45-16.05±3.72 mg/kg in algae from all sampling times. This results are above the detected Cu^{2+} concentrations in all algae species in present study (Table 2).

In present study, the mean heavy metal concentrations were decreased in the following order As^{2+}>Pb^{2+}>Cd^{2+} in all samples (Table 2). Yuan et al (2015) were reported that the average heavy metal concentrations decreased As^{2+}>Cd^{2+}>Pb^{2+}, respectively.

Dehbi et al (2023) were reported that the Fe^{2+} concentration in *Cladophora glomerata* were above the IAEA-accepted limit of 23.20 mg/kg. In the present study, the Fe^{2+} concentration in all algae samples were not exceed the limit (497.00±13.6 mg/kg) accepted by the IAEA (2005).

There is no declared acceptance limit for Al^{2+}, Li^{+}, Be^{2+}, B^{3+}, P^{3+}, V^{2+}, Se^{4+}, Sr^{2+}, Mo^{2+}, Sb^{3+} and Ba^{2+} by IAEA. Therefore, it is not possible to evaluate these results clearly in terms of residue risk.

CONCLUSION

As a result, in this study, heavy metals in 2 macro and 5 micro-algae samples were identified and quantified by ICP-OES. While the most dominant major elements are Na, K and Ca, the minor elements were P, Mn and Fe in raw algae samples. The concentrations of Ca, K, Cr, Mn, Fe, Co, Cu, Zn, As, Cd and Pb in the samples are all within the acceptable limits defined by IAEA. There are no defined limit for Al, Li, Be, B, P, V, Se, Sr, Mo, Sb and Ba in crude algae samples to evaluate heavy metal contaminations. It was determined that the analyzed algae species were rich in essential elements and poor in elements with high toxic effects. This indicated that the analyzed algae species could be used as a potential functional resource.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' Contributions

All the authors contributed equally. The author(s) read and approved the final manuscript.

Conflict of Interests

The author(s) declare that for this article they have no actual, potential, or perceived conflict of interests.

Statement on the Welfare of Animals

No ethics committee permissions are required for this study.

Statement of Human Rights

This study does not involve human participants.

Acknowlegments

The authors thank the staff of Dicle University, Faculty of Aquaculture for their contributions to collect some part of the algae samples and their support in the field studying.

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Tables

Table 1. Mean values of major elements in macro-microalgae samples (µg/kg).

Major Elements	S1	S2	S3	S4	S5	S6	S7
	mean±std	**mean±std**	**mean±std**	**mean±std**	**mean±std**	**mean±std**	**mean±std**
Na	396118.36 ± 47797.63	413495.01 ± 413495.01	397885.22 ± 9644.73	322189.64 ± 10104.80	1032879.59±21924.44	487049.46 ± 9194.15	488304.19 ± 9852.12
Mg	<0.000± 0.00	<0.000± 0.00	<0.000± 0.00	<0.000± 0.00	<0.000± 0.00	<0.000± 0.00	<0.000± 0.00
Al	<0.000± 0.00	26906.13± 4476.88	<0.000± 0.00	<0.000± 0.00	<0.000± 0.00	<0.000± 0.00	<0.000± 0.00
K	629248.33± 4850.58	823646.37 ± 43124.89	1194297.65±46454.76	1145444.30±9275.95	2337712.31± 10637.23	1110855.41± 93056.40	1156131.65±37051.36
Ca	129990.32± 7104.43	435958.40 ± 24396.29	938832.27 ± 34746.62	149693.06 ± 5906.29	148823.56 ± 6380.16	174673.35 ± 17521.16	1445372.94±10190.72

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

Table 2. Mean values of minor elements in macro-microalgae samples (µg/kg).

Minor Elements	S1	S2	S3	S4	S5	S6	S7
	mean±std	**mean±std***	**mean±std***	**mean±std***	**mean±std***	**mean±std***	**mean±st**
Li	<0.000± 0.00	<0.000± 0.00	<0.000± 0.00	<0.000± 0.00	<0.000± 0.00	<0.000± 0.00	<0.000± 0.00
Be	<0.000± 0.00	0.94± 0.83	1.96± 0.70	<0.000± 0.00	12.93± 2.79	1.53± 0.13	0.42± 0.14
B	92.63± 26.80	143.74± 29.02	2383.49± 45.55	305.91± 2.01	40877.41± 606.22	973.81± 128.76	3163.97± 174.68
P	8299.69± 210.24	117142.41 ±	85514.27± 362.17	215844.19 ±	428706.07 ±	290127.56 ±	53981.64 ±
V	<0.000± 0.00	208.55± 3.28	401.07± 28.31	<0.000± 0.00	177.63± 7.25	<0.000± 0.00	190.51± 18.97
Cr	<0.000± 0.00	231.20± 7.18	714.45± 7.93	<0.000± 0.00	109.82± 9.40	26.68± 7.84	520.75± 10.36
Mn	<0.000± 0.00	12566.10± 109.39	62854.41± 5098.57	1246.47± 10.14	816.69± 14.96	334.66± 8.54	10673.23 ±
Fe	67.29± 6.59	84084.69± 279.84	80870.27± 312.71	6263.39± 2.28	125496.86 ±	20489.29± 100.38	36755.09 ±
Co	0.33± 0.03	156.71± 6.60	150.09± 2.27	4.64± 0.04	215.05± 4.46	9.64± 0.31	35.79± 6.14
Ni	<0.000± 0.00	631.70± 2.01	1067.69± 1.91	<0.000± 0.00	235.38± 28.09	<0.000± 0.00	247.23± 8.79
Cu	<0.000± 0.00	187.59± 2.42	554.25± 0.92	85.10± 4.76	194.75± 12.67	16.37± 1.27	285.49± 0.32
Zn	<0.000± 0.00	239.65± 8.04	807.43± 6.64	233.50± 1.59	383.30± 2.20	224.11± 2.46	160.22± 2.76

As	3.45± 0.13	93.81± 0.50	120.61± 3.58	1.13± 0.13	122.37± 0.96	4.41± 0.01	151.22± 0.91
Se	2.75± 0.59	7.35± 0.20	89.35± 1.74	2.29± 0.26	93.98± 1.01	3.01± 0.81	21.91± 1.72
Sr	7.43± 0.26	1348.71± 0.26	4061.47± 3.63	244.77± 0.17	2240.07± 2.69	967.83± 2.16	4247.01± 0.29
Mo	0.27± 0.02	9.39± 0.05	10.90± 1.01	9.54± 0.05	84.35± 0.80	2.53± 0.02	7.96± 0.61
Cd	0.01± 0.00	2.87± 0.45	2.71± 0.19	0.07± 0.02	10.01± 0.65	1.89± 0.05	0.80± 0.09
Sb	4.38± 0.35	3.89± 0.13	6.31± 0.02	2.01± 0.13	6.23± 0.24	3.64± 0.01	3.04± 0.07
Ba	1.36± 0.26	621.95± 9.45	2641.98± 34.59	86.14± 3.93	985.15± 8.67	935.55± 1.73	1241.66± 12.79
Pb	4.63± 0.63	26.04± 2.22	68.93± 3.35	6.41± 0.21	179.47± 6.85	11.23± 0.48	17.88± 0.32

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