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ELEMENTAL ACCUMULATION IN MACROALGAE *TREPTACANTHA BARBATA* (STACKHOUSE) ORELLANA & SANSÓN, 2019 FROM SINOP, TÜRKIYE

Elif Arıcı
Sinop University Vocational School of Health Services Sinop-Turkey
email: earici@sinop.edu.tr

ABSTRACT: In this study, the contamination profile of heavy metal levels (Cd, Cu, Fe, Hg, Pb, Zn) of *Treptacantha barbata* (Stackhouse) Orellana & Sansón, 2019, seawater and sediment samples were analyzed by ICP-MS and reference materials were used to determine the reliability of the analysis. All samples were collected from the Sinop coast (Türkiye) from August 2021 through April 2022. As a result of the study, metal levels were found in the following sequence: Hg < Cd < Pb < Cu < Zn < Fe for seawater and *T. barbata* and Hg < Cd < Cu < Pb < Zn < Fe for sediment. Among the metals, Cu was bio-accumulative in biota; Cd and Hg metals in the sediment are micro concentrator by *T. barbata.*

KEYWORDS: *Treptacantha barbata*, metals, ICP-MS, bio-concentration factor, biota-sediment accumulation factor, monitoring

INTRODUCTION

The Black Sea coasts can be affected by different types of pollution from industrial, agricultural, domestic, touristic, shipping, harbor and fishing activities. Metal pollution is one of the most important in the marine environment as it affects human and biota life (Bat *et al.*, 2018). Marine benthic organisms, especially macroalgae can provide information about metal contamination of seawater due to easy acquisition and accumulation features. Monitor of metal pollutants by using macroalgae is essential. The wide distribution of algae, their abundance, being immobile in a fixed position, their sedentary nature, their size suitable for analysis, and their easy recognition allow them for monitoring studies (Arıcı and Bat, 2017; Bat *et al.*, 2020).

The widely spread brown algae are the most common flora in rocky coastlines' intertidal zones. It is known that they are higher resistance to metals. Therefore, they are the most preferable to determine coastal environmental situation that can reflect indirectly the average levels and temporal variations of contaminants (Kravtsova *et al.*, 2014). Macroalgae are key links for benthic food webs; therefore, they act as time-integrators of pollutants (Topçuoğlu *et al.*, 2003).

Treptacantha barbata (Stackhouse) Orellana & Sansón, 2019 (previously known as *Cystoseira barbata* (Stackhouse) *C. Agardh*, 1820), has been evaluated by many researchers in monitoring pollution from different coastal ecosystems in the Black Sea since 1992 (Güven *et al.*, 1992, Topçuoğlu *et al.*, 2003; Altuğ *et al.*, 2005; Tüzen *et al.*, 2009; Çulha *et al.*, 2010, 2013; Arıcı and Bat, 2016; Bat and Arıcı, 2016; Arıcı, 2017; Arıcı *et al.*, 2019). However, monitoring studies are scarce for the Black Sea countries. For this reason, this present study aims to identify (i) current metal uptake capacities; (ii) determine relationships with water and sediment; and (iii) compare the variabilities of metal levels with similar studies which have been carried out along Sinop coasts and other regions.

MATERIALS AND METHODS

Study area: Sinop is located on the Black Sea side of Türkiye with its natural beauties. Sinop coasts are important potential fishing areas and macroalgae are key links for benthic food webs. However, coastal tourism activities, local fishing activities and domestic sewages cause environmental problems in this region (Topçuoğlu *et al.*, 2003; Bat *et al.*, 2018).

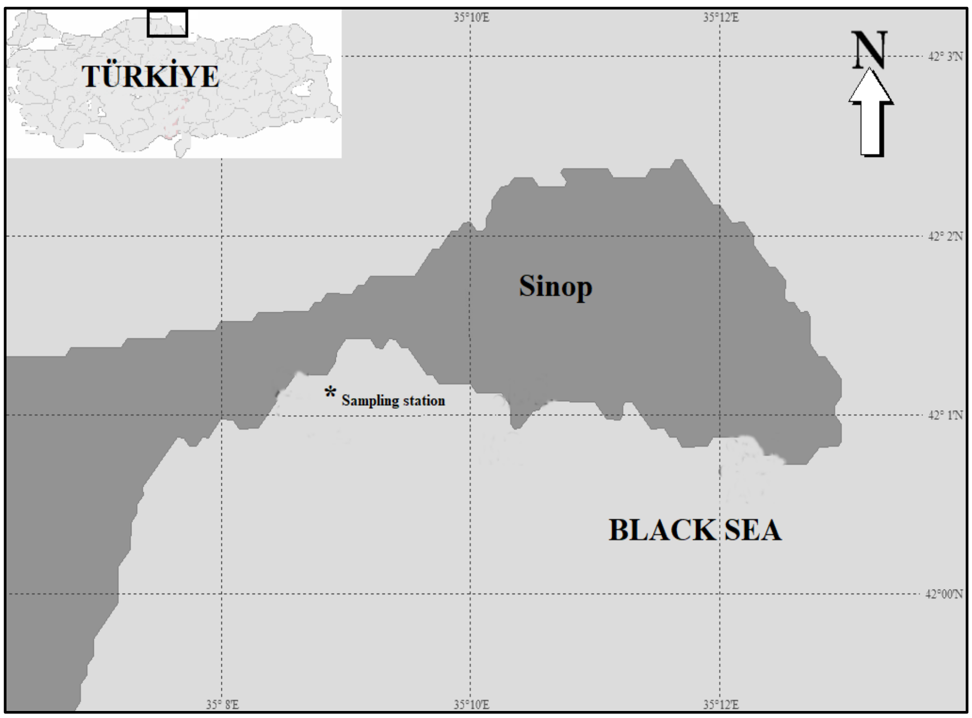

Fig. 1. Sampling station.

Korucuk Denizler Locality (Fig. 1) is the most preferred beach in the summer season and this station is under the pressure of rapid construction, tourism activities, sewage wastes of the industrial zone, human-induced water and beach pollution. For this reason, this station was chosen for monitoring metal capacities, which was under intense pressure. The brown algae *T. barbata*, seawater and sediment samples were taken from there in the sandy and rocky coastlines' upper littoral zone from August 2021 to April 2022 seasonally.

Sampling procedures: Macroalgae samples were collected by hand and first washed in seawater to be cleaned from epiphytes, sediment fragments and other organic particles. Then they were put in polyethylene bags, labeled and transported to the laboratory. In the laboratory, algae were washed again with bi-distilled water and dried at 65°C for 24 hours in an oven (BINDER). Dried samples were placed in vacuum packaging packages until digestion. A microwave digestion system (Milestone Systems, Start D 260) with Suprapur® HNO_3 (nitric acid) according to the Aquatic plant HPR-FO-08 method (Milestone) was used.

Surface water samples were taken from the area where the seaweed sample was collected in to evaluate the correlation between algae and water. Water samples were taken in 250 cc polyethylene bottles with 2 replications. 1 ml of Merck 65% extra pure HNO_3 was added, labeled and stored until analysis.

A surface sediment samples transported to the laboratory in polyethylene bags, dried at 105°C for 24 hours. Dried samples were passed through a motorized sieve shaker with different mesh sizes according to the method of Buchanan (1984) sea benthos studies. Particle sizes of 63 µm and smaller were digested with Seawater Sediment HPR-EN-33 methodology (Milestone).

Analytical procedures: Metal analyzes were measured using the Agilent 7700XICP-MS system. ICP-MS measurements were executed under the following conditions: RF power: 1550 W; Plasma gas flow rate: 15 L min^{-1}; Sampling depth: 8 mm; Sampling rate: 0.1 mL min^{-1}; Auxiliary gas flow rate: 1.0 L min^{-1}; Carrier gas flow rate: 0.95 L min^{-1}; He gas flow rate: 4.3 mL min^{-1}; Atomizing chamber temperature: 2°C. The calibration values and average results of the elements were made at the level of ppb (µg solute / kg solution).

To check the accuracy of the method, Certified Reference Material BCR 279 - *Ulva lactuca* was used in the analysis of macroalgae samples; NIST SRM 2702 was used for the standardization of sediment measurements and TUBITAK National Metrology Institute (UME) CRM 1201 was used for the accuracy of metal measurements of water samples and the recovery efficiencies were checked (Table 1).

Table 1. Recovery rates (%) of reference materials.

Metals	Certified Reference Materials		
	BCR 279	**NIST SRM 2702**	**(UME) CRM 1201**
	Recovery rates (%)		
Cd	98.54	98.53	96.20
Cu	99.24	102	99.15
Fe	99.33	99.46	99.12
Hg	100	97.54	111
Pb	96.22	101	98.63
Zn	98.63	97.44	99.03

Calculation of Bioaccumulation Factors:

Biota-Sediment Accumulation Factor (BSAF) is a parameter that defines the biological accumulation between biota and sediment (Kleinov *et al.*, 2008). A BSAF value less than 1 indicates that the metal in the sediment does not accumulate in the biota. BSAF > 2 is macro-concentrator; 1 < BSAF < 2 is micro-concentrator and BSAF <1 is de-concentrator.

Bio-Concentration Factor (BCF) shows how much the metal in the water passes into the living organism. It is expressed as the ratio of the metal concentration in the organism to the surrounding environment concentration (Geyer *et al.*, 2000). If the BCF value is higher than 5000, it is indicated to be very bio-accumulative.

BSAF and BCF parameters of metals were calculated as follows using equations:

BCF = Cb/ Cw and BSAF = Cb/ Cs; where: Cb = concentration of metal in macroalgae (mg kg^{-1} dw); Cw = concentration of metal in seawater (mg/L) and Cs = concentration of metal in sediment (mg kg^{-1} dw).

Statistical analysis: Statistical calculations were performed by IBM_SPSS ver. 21. One-way analysis of variance (ANOVA) was used to evaluate interspecific significance between metal levels in different seasons. P-values of less than 0.05 were considered as statistically significant. All metal values were determined on a dry weight basis.

RESULTS AND DISCUSSION

In this study, cadmium (Cd), copper (Cu), iron (Fe), mercury (Hg), lead (Pb) and zinc (Zn) levels were determined from the most intensely polluted beach (Korucuk Denizler) of Sinop. The brown algae *Treptacantha barbata*, which is found in all seasons was used to evaluate biota-sediment and biota-seawater interactions.

The seawater temperature, salinity, dissolved oxygen and pH results in the sampling station are given in Table 2. Temperature values were measured as 13°C, 25.2°C, 17.1°C and 8.8°C from spring to winter. Salinity values ranged from 17.34 (summer) to 17.66 (winter). The highest dissolved oxygen (8.81 mg/L) was measured in winter. The lowest value was 5.60 mg/L in the same station in summer. pH values were between 8.33 and 8.47.

Table 2. Some physico-chemical parameters of seawater at the sampling station.

PARAMETERS	SEASONS			
	Summer	**Autumn**	**Winter**	**Spring**
Temperature (°C)	25.2	17.1	8.8	13
Salinity (‰)	17.34	17.62	17.66	17.35
Dissolved oxygen (mg/L)	5.60	7.54	8.81	5.80
pH	8.43	8.33	8.34	8.47

The obtained results give that, the mean element concentrations of seawater were as follows: Hg (8.8E ± 9.380), Cd (0.0025 ± 0.0005), Pb (0.007 ± 0.001), Cu (0.0075 ± 0.005), Zn (0.018 ± 0.016) and Fe (0.840 ± 0.437) mg/L, respectively. Concentrations of metals exhibited no variations depending on seasons in seawater ($p < 0.05$).

The most abundant metals of sediment are the following in order of: Hg (0.007 ± 0.004), Cd (0.25 ± 0.031), Cu (5.37 ± 0.721), Pb (8.87 ± 5.245), Zn (17.88 ± 6.274) and Fe (13963.99 ± 7236.08) mg kg^{-1}dry dw. A statistical difference in the sediment was found for essential elements of Fe and Zn in autumn season ($p \leq 0.05$). This is due to the high amount of coal fuel used by the surrounding houses, hotels and restaurants/cafes in the autumn. However, the fact that Zn concentrations do not exceed 100 mg/kg indicates that there is no sustained anthropological contamination (Storelli *et al.*, 2001). In addition, Pb which can be toxic even in small amounts, showed a sudden increase in the spring season. Önen and Öztürk (2017) were also found Pb values in seawater peaked in spring. Since the sampling area is considered as a picnic area, in the spring season, it is exposed to urban waste. The Pb level is especially related to the discharges and nutrient level increases to the sea (Rubio *et al.*, 2017). It is not desired that the amount of Pb in biota exceed 5 mg/kg (CEVA, 2019). Therefore, serious Pb pollution cannot be mentioned in the study area.

The average element concentrations sequence in *T. barbata* showed the similarity to seawater as: Hg < Cd < Pb < Cu < Zn < Fe. The metal concentrations in algae ranged from 0.13 to 0.75 mg $kg^{-1}$for Cd, 2.13 to 9.32 mg kg^{-1} for Cu, 301.12 to 495.82 mg kg^{-1} for Fe, 0.011 to 0.014 mg kg^{-1} for Hg, 0.38 to 3.14 mg $kg^{-1}$for Pb and 9.90 to 47.63 mg kg^{-1} for Zn (Table 4). According to Kravtsova *et al.*, (2014) study, heavy metal concentrations in *T. barbata* are 3-4 times higher than in sea water. Similar findings were obtained in our study as well.

Table 4. Average element concentrations (mean ± standard deviations) in *Treptacantha barbata.*

METALS (mg kg^{-1} dry wt.)	**SEASONS**				**Mean**
	Summer	**Autumn**	**Winter**	**Spring**	
Cd	0.59	0.75	0.13	0.20	0.42±0.30
Cu	9.32	8.05	2.13	6.40	6.47±3.13
Fe	301.12	495.82	359.41	389.24	386.40±81.61
Hg	0.010	0.012	0.011	0.014	0.012±0.010
Pb	3.14	1.67	0.38	0.78	1.49±1.22
Zn	46.29	47.63	9.90	15.38	29.80±19.94

The European Commission Regulations (629/2008, 488/2014, 420/2011), CEVA (2019) and Codex standard (FAO/WHO, 1995) indicate that maximum levels of Pb, Hg and Cd are 5, 0.50 and 3 mg kg^{-1}dry dw, respectively. In our results, no toxic value exceeds the allowable values.

BSAF value greater than 2 indicates that the species is a macro-concentrator for that element. A greater bioaccumulation of 1.71 was detected for Hg. The BSAF values were found to be less than 2, which indicates that the metal in the sediment does not have much effect on the algae tissue. The mean BSAF values are: Hg; 1.71 > Cd; 1.68> Cu; 1.22 > Zn; 0.86 > Pb; 0.17 > Fe; 0.02. Sediments in general act as a sink for heavy metals in the aquatic environment (Tessier and Campbell, 1987). Studied metals did not exhibit seasonal variations ($p > 0.05$), but were found higher values the measured metal values in water column. According to the data, *T. barbata* species is a micro-concentrator for Hg, Cd and Cu metals. Copper, which is found in the water column and near the surface, combines with sulfur and sinks rapidly to the bottom (Fowler and Knauer,1986). The horizontal and vertical distribution of copper depends on ships and human activities (Jalkanen *et al.*, 2021).

The resulting BCF values of Cu and Zn were above the bioaccumulation threshold (BCF > 500), it is concluded that the macroalgae, *T. barbata* takes these elements from seawater and accumulates them in its body. But other metals are considered not accumulative with respect to the bioaccumulation criteria. BCF threshold values are between 500 and 5000 according to regulatory laws (Table 5).

Table 5. Bio-Concentration Factor threshold values.

Regulations	Bio-accumulative	Very bio-accumulative
OSPAR Convention, 1972	500	
Toxic Substances Control Act - American Chemistry Council, 1976	1000	5000
National Industrial Chemicals Notification and Assessment Scheme, 1989	2000	5000
Canadian Environmental Protection Act, 1999	5000	
Stockholm Convention on Persistent Organic Pollutants, 2001	5000	
REACH Regulation, 2006	2000	5000
EU CLP 1272 Regulation, 2008		
Legislation on Plant Protection Products, 2009	2000	5000
Biocidal Products Regulation, 2012	2000	5000
Globally Harmonized System of Classification and Labelling of Chemicals (GHS), 2021	500	

Table 6. Comparison of previous studies in Turkish Black Sea waters (µg/g).

Stations	Cd	Cu	Fe	Pb	Zn	Ref.
Sinop	2.4	7.9	3414	12.8	85.8	1
Sinop	1.4	5.7	654	6.8	64.4	1
Sinop	1.3	4.2	446	5.3	12.1	1
Sinop	0.02	5.7		0.1	43.9	2
Sinop	0.2	6.0		0.1	191.5	2
Sinop	0.09	1.7		3.5	6.5	2
Sinop	1.02	16.4	560	2.10	48.0	3
Sinop		2.47	242		6.62	4
Sinop		3.91	373	0.01	10.97	5
Sinop		5.33	81	0.01	20.47	5
Sinop		2.01	455	0.01	0.08	5
Sinop		4.42	184	0.01	13.22	5
Sinop		5.87	991	0.01	6.84	5
Sinop		6.03	272	0.01	4.65	5
Samsun	0.01	6.53	534.43	0.01	0.20	6
Kastamonu	0.01	16.33	283.67	0.01	15.3	6
Ordu	0.01	5.06	632.55	0.01	5.83	6
Sinop		4.02	536	0.01	10.28	6
Sinop			327			7
Samsun					65.0	7
Kastamonu	0.23	37.0	1151	1.4	58.0	7
Sinop	0.13	5.0	327	1.0	44.0	7
Samsun	0.32	25.0	1250	1.3	65.0	7
Sinop	1.20	4.80	748	8.0	65.0	8
Sinop		3.10	308		13.0	8
Sinop	1.30	7.00	2143	10.0	76.0	8
Sinop	0.09	1.30	261	1.5	5.0	8
Sinop	0.86	4.05	865	6.5	39.75	8
Sinop	0.20	10.2	481.4	1.44	59.53	9
Sinop	0.20	10.2	481.4	1.44	59.53	10
Sinop	0.50	6.79	549.0	1.95	33.71	This study

- [1]Güven *et al.*,1992; [2]Topçuoğlu *et al.*, 2003 ; [3] Altuğ *et al.*, 2005 [4]Tüzen *et al.*, 2009[5] Çulha *et al.*, 2010[6] Çulha *et al.*, 2013 [7]Arıcı and Bat 2016 [8]Bat and Arıcı 2016 [9]Arıcı 2017[10]Arıcı *et al.*, 2019
- Hg levels were determined by only Arıcı 2017 and Arıcı *et al.*, 2019 as 0.01 mg/kg

Algae, generally accumulate Cu and Zn readily from seawater (Ho, 1988). In this study, the BCF values of *T. barbata* were decreasing as flows: Cu; 862.67 > Zn; 854.44 > Fe; 460 > Pb; 212.86 > Cd; 168 > Hg; 15.The reason for the high BCF values for zinc and copper metals is that they are metals that occur naturally in water and precipitate into the sediment over time (Huang *et al.*, 2020).Our recorded BCF values of Zn and Fe were found similar in the eastern Aegean Sea conducted by Önen and Öztürk (2017).

Accumulation of metals by macroalgae depends on many abiotic and biotic factors such as: speciation, salinity, intensity of water exchange, seawater temperature, light, metabolism rate of plants, morphological characteristics, taxonomy, physiological state *etc*. (Sawidis *et al.*, 2001, Kravtsova *et al.*, 2014). Previous studies on heavy metal differences of *T. barbata* in Turkish Black Sea waters were given in Table 6.

As for *T. barbata*, the overall mean concentrations (mg kg^{-1}) of the heavy metals followed decreasing trend: Fe > Zn > Cu > Pb > Cd > Hg. According to the results, the essential metals (Fe, Zn and Cu)in biological function were presented in relatively high amounts. As a result, it is very significant to continuously monitor the metal levels of the marine algae to assess them ecologically.

CONCLUSION

Treptacantha barbata, which is widely distributed on the Black Sea coasts, is a good indicator of heavy metal pollution. Toxic metals such as Cd, Pb and Hg could pose a danger to marine coastal environments, their high toxicity restricts the metabolic growth of algae. These toxic metals are not biodegradable and concentrate high amounts in algal tissues. Also, it has a great importance for understanding the exchange of metals between the seawater column and sediments. Among the studied metals, Cu was bio-accumulative in biota; Cd and Hg metals in the sediment are micro concentrators by *T. barbata*. Although the sampling area Korucuk Denizler Locality is the most preferred and crowded beach in summer and is under pollution pressure, the results did not exceed the permissible limits set for metals by European Commission Regulation, CEVA and Codex standards.

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