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RESEARCH PAPER

Heavy metal accumulation in molluscs associated with *Cystoseira barbata* in the Black Sea (Türkiye)

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Abstract

Species that share the same habitat in the aquatic environment are affected by the pollution they are exposed to and this pollution can accumulate in various amounts in each species. This preliminary study focuses on heavy metal accumulation in different mollusc species associated with a macroalga. Accumulation levels of ten heavy metals (Cd, Cu, Zn, Ni, As, Pb, Sn, Mn, Fe, Se) in five molluscs (*Mytilus galloprovincialis*, *Tricolia pullus pullus*, *Bittium reticulatum*, *Tritia neritea*, *Rissoa splendida*) and *Cystoseira barbata* were investigated in Karakum (Sinop Peninsula/Black Sea) on May 2019. Heavy metals determinations were performed by the Inductively Coupled Plasma – Mass Spectrometer (ICP-MS). The amount of heavy metal accumulated in the species in decreasing order is Fe> Ni> Mn> Zn> As> Cu> Cd> Se> Pb> Sn. The level of As accumulation in *C. barbata* was described according to the quality criteria for edible algae sold in France. Cd, Ni, As, and Zn concentrations in different species of mollusca were above either national or international limits. The total metal accumulation of each species was also examined using the Metal Pollution Index (MPI). Sorting between species according to MPI value was determined as *C. barbata* >*B. reticulatum* > *R. splendida* > *T. neritea* > *T.pullus pullus* > *M. galloprovincialis*. The highest metal accumulation was in *C. barbata*. The high detection of metal pollution in the work area shows that the aquatic environment is polluted from many sources.

Introduction

Metals and radionuclides constitute a large part of anthropogenic pollutants in the marine ecosystems of the Black Sea. The main sources of pollution in the Black Sea are atmospheric fallout, large rivers flowing into the Black Sea (Danube, Dnieper, Dnester, etc.), and local polluting emissions (Strezov, 2012). Wastewater outflows, industrial sludge, the atmosphere, the transition of terrestrial inputs to water by precipitation, and anthropogenic activities are the most significant factors in this pollution (Idris, 2008). Heavy metals are constantly being released to coastal ecosystems from man-made sources (Arıcı & Bat, 2016 a,b). The amounts of metal pollution in the marine environment were estimated by analyzing the seawater, the organisms living in the seawater, and the sediment (Karbowska, 2016). Marine species are one of the most reliable indicators for identifying sources of biologically available metal contaminations (Bazzi, 2014). There are many species used to research such adverse effects in aquatic ecosystems and to identify the pathogens. These species, called bioindicators, are used extensively, especially in aquatic ecosystems. The most important of these species are algae and molluscs, that are widely distributed in the seas (Zorita et al., 2006; Hamed & Emara, 2006; Yap et al., 2002; Vlahogianni et al., 2007; Maanan, 2008; Akcali & Kucuksezgin, 2011). Because marine macroalgae are the basis of the bottom ecosystems in the seas and provide habitats for nutrition, protection, and reproduction for countless species (Milazzo et al., 2000; Filimon et al., 2016; Lolas et al., 2018; Bitlis, 2019; Mancuso et al., 2021). Heavy metals may accumulate in algae and be transferred to other trophic levels, including molluscs, crabs, fish, and ultimately, humans (Pinto et al., 2003). Excessive metal accumulation in algae may create a toxic effect by transitioning to other living species through biomagnification.

The aim of this study is to determine the heavy metal content in *Cystoseira barbata*, a brown alga that is common in the Black Sea coastal area, and in mollusc species (*Mytilus galloprovincialis*, *Tricolia pullus pullus*, *Bittium reticulatum*, *Tritia neritea*, *Rissoa splendida*) that live dominantly at this type of algae. Heavy metal concentrations were determined for the first time in *Tricolia pullus pullus* and *Rissoa splendida* gastropod mollusc species.

Materials and Methods

Samples of *M. galloprovincialis* (Bivalve-filter feeding), *T. pullus pullus, B. reticulatum, R. splendida* (Gastropod-herbivore), *T. neritea* (Gastropod-carnivore) and *C. barbata* (Brown macroalgae) were collected from 3 different stations in Karakum (Sinop Peninsula-Black Sea) on May 2019 (Figure 1). Karakum beach is overpopulated in summer due to tourism. There are hotels and residential areas around. In the laboratory, macro benthic organisms were divided into main taxonomic groups using a binocular stereomicroscope (Olympus SZX7, Tokyo, Japan) and then identified and counted at the species level. We followed the methods of Rosioru et al., 2012; Öztürk et al., 2014, and the Check List of European Marine Mollusca (Clemam, 2020) for the systematic status of the species. In the study, 300 mollusc species were examined. Since the sizes of mollusk species are very small, heavy metal measurements were carried out by using all their tissues, including the shell. Cd (Cadmium), Cu (Copper), Zn (Zinc), Ni (Nickel), As (Arsenic), Pb (Lead), Sn (Tin), Mn (Manganese), Fe (Iron), Se (Selenium) heavy metal concentrations in molluscs and brown algae were determined by ICP-MS (Agilent 7800-Inductively Coupled Plasma–Mass Spectrometer, United States). Heavy metal concentrations in the samples were analyzed according to the method described by Bernhard (1976) and Yap et al. (2002, 2004). Certified Reference Material NCS-ZC73034 (Prawn Trace Elements) was used for calibration. The results showed good agreement between certified and analytical values (recovery rates 98-104%). All analyses were carried out as two parallels and two replicates. The metal results obtained were also compared with internationally acceptable values (TGK, 2005; SÜY, 2008; EC, 2006; US FDA, 2007; FAO, 1983, CEVA, 2019).

Figure 1. Karakum Beach, Sinop Penninsula (Black Sea).

Statistical analysis of data was carried out using Minitab 19.0 statistical package program. The Shapiro-Wilk test was used to determine the normality of the data. Since the parameters other than Mn, Fe, Ni and Cu did not have a normal distribution, Kruskal-Wallis test was applied to these data. One-way ANOVA was used for Mn, Fr, Ni and Cu data. In addition, Spearmen correlation analysis was performed with the available data. The metal absorption of each specimen was interpreted by the Metal Pollution Index (MPI) that was calculated using the equation shown below:

$$MPI = (Cf_1, x\ Cf_2, . . . Cf_n)^{1/n}$$

(Cf_i = concentration for the metal in the sample, mg kg^{-1}dry weight, n= number of metals. If this combined index is above 1 the concentrations of trace metals would be considered elevated and the ecosystem could be regarded as "polluted" (Teodorovic et al., 2000).

Results and Discussion

The results of one-way analysis of variance (ANOVA) with the metal levels of the alga and mollusc species are shown in Table 1. For each metal concentration (Cd, Cu, Pb, As, Sn, Se, Mn, Ni, Zn and Fe), heavy metal accumulation between the species showed statistically significant differences ($p<0.05$). The average accumulation of heavy metals in all species was ordered as Fe>Ni>Mn>Zn>As>Cu>Cd>Se>Pb>Sn.

According to the results, Fe, Ni, Mn, and Zn were the most dominant among the 10 metals selected for all species. Looking at the distribution of metals in species, Cu, Pb, As, Sn, Ni, Fe in *C. barbata*; Mn in *B. reticulatum*; Se in *M. galloprovincialis*; Cd in *R. splendida* and Zn in *T. neritea* had the highest concentrations. This metal accumulation, which occurs at different rates in algae, bivalves, and gastropods, is due to the changing nutritional regime and living conditions depending on the species, and changes in the biological activity of the living species (Jeng et al., 2000; Özden et al., 2009). *M. galloprovincialis* a filter-feeding bivalve, and *T. neritea* (meat-eating) feeds on herbivores. In this study, Se, As and Sn were the elements measured for the first time in gastropod species. Se is the basic trace element in organisms and participates in important biochemical processes (Ralston et al., 2008; Retondario et al., 2019). One of the important properties of Se is the antagonistic effect of this element against a wide variety of compounds, especially heavy metals. A significant number of studies conducted on humans, vertebrates and plants have shown that the application of Se protects against Hg, As and Cd toxicity by reducing metal-induced oxidative stress and increasing the antioxidant capacity of exposed organisms (Trombini et al., 2022).

Table 1. Heavy metal concentrations in macroalga and mollusc species and MPI values (mg kg^{-1} dw; mean ± SE).

Species	Cd	Cu	Pb	As	Sn	Se	Mn	Ni	Zn	Fe	MPI
C. barbata	0.24±0.00^{a}	18.15±0.52^{a}	1.05±0.00^{a}	62.20±1.88^{a}	0.32±0.02^{a}	3.05±0.01^{a}	39.04±1.19^{a}	388.81±10.33^{a}	16.66±0.38^{ab}	750.95±21.68^{a}	11.79
T. pullus pullus	0.11±0.00^{b}	11.49±0.14^{b}	0.40±0.01^{bf}	2.14±0.03^{b}	0.22±0.01^{b}	4.39±0.10^{bcdef}	28.35±0.38^{b}	273.42±3.39^{b}	17.49±0.22^{b}	472.48±9.10^{b}	6.08
B. reticulatum	0.55±0.01^{c}	14.92±0.02^{a}	0.73±0.01^{ce}	3.04±0.02^{cdf}	0.16±0.01^{cdef}	4.41±0.06^{cdef}	47.04±0.26^{a}	56.72±0.11^{c}	40.60±0.03^{c}	668.47±0.87^{a}	7.86
M.galloprovincialis	0.22±0.00^{d}	5.58±0.39^{c}	0.57±0.01^{d}	3.24±0.26^{def}	0.14±0.02^{def}	4.55±0.25^{def}	12.19±0.83^{c}	32.02±2.12^{d}	11.16±0.81^{de}	441.42±30.26^{b}	4.41
R. splendida	41.03±0.30^{e}	6.77±0.29^{c}	0.70±0.01^{e}	3.89±0.19^{e}	0.17±0.01^{ef}	4.48±0.07^{ef}	18.95±0.85^{d}	85.67±3.65^{e}	11.99±0.60^{e}	335.63±13.34^{c}	7.19
T. neritea	1.14±0.05^{f}	10.82±0.74^{b}	0.41±0.01^{f}	2.76±0.22^{f}	0.15±0.01^{f}	4.51±0.49^{f}	13.34±1.50^{c}	104.34±9.48^{e}	59.83±4.46^{f}	418.41±37.74^{bc}	7.10

Vertically letters ^{a,b,c,d,e,} and ^{f} show statistically significant differences (p<0.05). MPI: Metal Pollution Index (MPI>1, polluted by heavy metal).

The correlation coefficients of the metals are shown in Table 2. In the correlation analysis performed, the correlations between Se and other heavy metals were found to be negative, while positive and negative changes were observed in the correlation of other metals with each other. Although the correlation between Mn and the other metals (Fe, Cu, Sn, and Pb) was positive and the relationship between Fe and the other metals (Ni, Cu, As, Sn and Pb) was also positive, Se and Cd were negative. The relationships between Ni and Cu, As and Sn were positive, while the relationship between Ni and Se was negative. The correlation between Cu and As, Sn and Pb was positive, while the correlation between Cu and Se was negative. As with Sn and Pb were positive and with Se negative correlation. Both Fe and Zn were positive and statistically significant ($p \leq 0.05$). Sn was also associated strongly and positively with Pb ($p \leq 0.01$). The strongly associated relationships between Se and Sn and Pb were negative ($p \leq 0.01$).

Variations expressed in different standard deviations occurring in individual metals were the expected results. The results of the ANOVA test demonstrated the complex relationship between environmental concentrations of metals and bioaccumulation in the species (Gündoğdu et al., 2020). The results showed that these metals have the same origin or result from synergistic interactions among themselves. The identified positive correlations can be explained by a common origin of the metals or by the synergistic interaction between them (Haritonidis & Malea, 1999). Negative correlations may be due to different origins, environmental behaviors, or competition for the metal's uptake site (Villares et al., 2005).

Table 2. Correlation coefficient (r) between heavy metal contents in five species.

	Mn	Fe	Ni	Cu	Zn	As	Se	Cd	Sn
Mn	1								
Fe	.848**								
Ni	.0.407	.539*							
Cu	.834**	.902**	.684**						
Zn	.039	.071	-.244	.267					
As	.427	.707**	.789**	.693**	-.242				
Se	-.383	-.527*	-.681**	-.574**	.271	-.836**			
Cd	-.263	-.540*	-.254	-.460	-.332	-.188	.185		
Sn	.488*	.647**	.942**	.711**	-.307	.870**	-.682**	-.177	
Pb	.580*	.689**	.437	.569**	-.339	.831**	-.702**	.107	.625**

** Correlation significant at the 0.01 level (two-tailed)
* Correlation significant at the 0.05 level (two-tailed)

Heavy Metal in *Cystoseira barbata*

It is well known that the rate of accumulation of trace elements of marine macroalgae depends on many factors. The most important abiotic factors are the concentration and speciation of elements in the environment, salinity, intensity of water exchange, water temperature and light. Among the biotic factors, the metabolic rates, morphological characteristics, taxonomic identities, ontogenesis stages and physiological states of plants are considered important (Ryabinin et al., 2011; Saenko et al., 1976). Brown algae, which are commonly found in the sea, contain compounds with biological activity, such as laminarin, which is a metabolic product. In addition, sulfated polysaccharides are also present in the cell wall, other than cellulose and alginic acid. These substances contained in algae offer functional groups such as hydroxyl, carboxyl, sulfate, and amino. These functional groups serve as the binding sites responsible for biosorption (Sun et al., 2016). The bonding of functional groups in the algal cell wall with metal ions varies according to the type of algae and the type of metal (Ramakrishna et al., 2005). *C. barbata* is a very good source of heavy metal concentration due to these characteristics in its structure (Bonanno & Orlando-Bonaca, 2018). In terms of this characteristic, it is one of the algae that can provide the necessary elements for human metabolism and can be consumed as human food (Circuncisão et al., 2018). Also macroalgaea may acculumate toxic elements such as arsenic (As), aluminum (Al), cadmium (Cd), lead (Pb) and mercury (Hg) and these elements may pose a health risk to life (Ak et al., 2021). Due to high phosphate concentrations, *Cystoseira* sp. accumulates heavy metals greater in brown maritime macroalgae (Phillips, 1990). Because of this feature, brown algae is a good indicator of As heavy metal. According to our results, Cu, Pb, As, Sn, Ni, and Fe were the highest trace elements in *C. barbata* in terms of metal content. Many authors have described that brown algae accumulate high levels of arsenic (Morita and Shibata, 1990; Kut et al., 2000; Tukai et al., 2002). Many macroalgae species, including *C. barbata*, were studied in and around Sinop (Tuzen et al., 2009; Türk Çulha et al., 2010; Culha et al., 2013; Arıcı & Bat, 2016a, Bat & Arıcı, 2016, Arici et al., 2019). However, there was an increase in Ni and As heavy metal levels in the *C. barbata* species sampled by this latest study. When the studies conducted in the seas around Turkey are examined (Table 3), the Cd, As and Sn levels in the brown algae sampled in the Marmara Sea and the Cd, Pb and Zn heavy metal levels in the algae sampled from the Aegean Sea are higher than the results we determined. In this study, Sn heavy metal *C. barbata* was also measured for the first time and its value is 0.32 mg kg^{-1}. Again in this study, Se heavy metal in algae was measured for the first time after the study of Tüzen et al. (2009). Se heavy metal value measured by Tuzen et al. (2009) was much lower. When we compare the *C. barbata* results sampled from coastal areas in the world's oceans, it is seen that the heavy metal results of Zn, Mn in Varna, Mn and Fe in Sevastopol, Mn and Syria, Pb, As and Zn in Italy, and Zn, Mn and Fe in Russia have similar or high levels than our results. The characteristics of the surrounding terrestrial environments, the species sampled from the various stations, the various nutrient content resulting from the sediment structures in the regions, the physiological attitudes of living species, the active metal concentrations in the sampling regions, the input from sea traffic, and anthropogenic factors are all responsible for these regional differences (Türk Çulha et al., 2017). It is thought that all these factors cause the accumulation of heavy metals in living species in different ways.

Table 3. Comparison of heavy metal content of alga and mollusca species with other studies in different Seas (mg kg^{-1}; dw; Nd: not detectable; *µg kg^{-1}).

***Cystoseira barbata* (Alga)**											
Site	**Cd**	**Cu**	**Pb**	**Ni**	**As**	**Sn**	**Se**	**Zn**	**Mn**	**Fe**	**Ref.**
Sinop/Black Sea	0.55±0.04*	2.47±0.18	4.62±0.32*	2.05±0.14	--	--	91±7*	6.62±0.26	14.9±1.3	242±15	1
Sinop/Black Sea	--	5±0.7	--	0.8±0.01	--	--	--	--	--	327±18	2
Sinop/Black Sea	0.20±0.1	10.2±7.3	1.44±0.7	4.44±2.6	18.17±9.9	--	--	59.5±24.9	21.3±10.9	481.4±376	3
Sinop/Black Sea	1.20 ± 0.10	4.80 ± 0.90	8.00 ± 2.00	3.80 ± 0.60	--	--	--	65.0 ± 6.0	33.00 ± 4.0	748.0 ± 29.0	4
Sinop/Black Sea	2.53	2.01	20.01	0.79	--	--	--	0.08	--	455	5
Samsun/Black Sea	<0.01	6.53	<0.01	4.60	--	--	--	0.20	--	534.43	6
Kastamonu/Black Sea	<0.01	16.33	<0.01	14.30	--	--	--	15.30	--	283.67	6
Ordu/Black Sea	<0.01	5.06	<0.01	3.37	--	--	--	5.83	--	632.55	6
Black Sea Coasts	0.14-1.71	5.43-12.7	0.62-6.89	1.92-199	--	--	--	20-212	26.2-340	1169-12.918	7
Foça/Aegean Sea	17.8±5.67*	5.29±0.68	2.96±0.14*	--	--	--	--	23.8±5.79	--	96.8±0.95	8
Aegean Sea	0.03-7.23	ND-6.64	0.90-62.1	0.90-33.0	--	--	--	80.3-295.0	15.9-414.0	570.0-6288.0	9
Cape Rusalka/Black Sea	--	27±1	--	6±0.5	48±1	--	--	130±4	252±16	620±13	10
Black Sea	0.55-1.38	4.2-7.5	<0.03-4.2	--	52.6-69.1	--	--	12.4-35.6	--	210-615	11
Venice Lagoon/Italy	0.1±0.0	7±9	3.2±2.0	1.8±0.6	242±104	--	--	38±33	--	444±198	12
Tartou/Syria	<0.05	0.99±0.4	0.28±0.03	--	131±1	--	--	11.17±0.33	514±28	4421±121	13
Varna/Bulgaria	0.12	7.7	0.6	1.1	7.2	--	0.5	14.3	47.7	334.3	14
Sevastopol/Black Sea	--	--	--	2.7-3.45	11.2-24.7	--	--	30.1-35.0	15.9-43.2	209-722	15
Dardanos district in Çanakkale	1.45±0.26	0.43±0.09	0.50±0.02	0.75±0.04	110.91±1.82	0.80±0.09	0.04±0.01	0.79±0.08	5.26±0.09	116.86±1.42	16
C.barbata	**0.24**	**18.15**	**1.05**	**388.81**	**62.20**	**0.32**	**3.05**	**16.66**	**39.04**	**750.95**	**This study**

***Mytilus galloprovincialis* (Bivalve Mollusca)**											
Site	**Cd**	**Cu**	**Pb**	**Ni**	**As**	**Sn**	**Se**	**Zn**	**Mn**	**Fe**	
Sinop/Black Sea	0.03-0.27	0.10-1.89	0.11-1.18	--	--	--	--	1.58-7.28	--	--	17
Sinop/Black Sea	1.79 ± 0.01	8.01 ± 0.02	0.01 ± 0.19	4.02 ± 0.19	--	--	--	256.4 ±1.3	22.8 ± 0.11	598 ± 7.0	18
Sinop/Black Sea	6.83 ± 0.30	1.21 ± 0.25	--	--	--	--	--	150.3 ±17.4	--	--	19
Sinop/Black Sea	2.53 ± 0.19	6.98 ± 0.31	--	--	--	--	--	228 ±16.8	--	--	20
Sinop/Black Sea	0.27-0.98	2.41-4.82	2.10-4.10	--	--	--	--	79-163	--	--	21
Sinop/Black Sea	0.55	25.5	0.51	--	--	--	--	70	--	--	22
Sinop/Black Sea	1.79 ± 0.01	8.01 ± 0.02	0.01 ± 0.19	4.02 ± 0.19	--	--	--	256.4 ±1.3	22.8 ± 0.11	598 ± 7.0	23
Sinop/Black Sea	0.07-0.11	--	0.14-0.21	--	--	--	--	--	--	--	24
Sinop/Black Sea	0.006-0.011	0.52-0.93	0.13-0.45	--	--	--	--	16.4-21.8	--	--	25
NW Black/ Russia	ND-7.7	0.67-14.9	0.16-4.8	0.38-9.0	6.7-21.8	0.09-0.7	4.3-17.5	12.5-636	0.91-13.7	48-305	26

Ionian Sea/Italy	0.44	10.09	1.37	---	15.92	--	--	--	--	--	27
M.galloprovincialis	**0.22**	**5.58**	**0.57**	**32.02**	**3.24**	**0.14**	**4.55**	**11.16**	**12.19**	**441.42**	**This study**
					Tritia neritea **(Gastropod Mollusca)**						
Site	**Cd**	**Cu**	**Pb**	**Ni**	**As**	**Sn**	**Se**	**Zn**	**Mn**	**Fe**	
Sinop/Black Sea	<0.2	--	<0.4	--	--	--	--	--	--	--	28
Sacca del Canarin lagoon /Adriatic Sea	0.75	61.3	13.03	--	--	--	--	369	--	--	29
Paleochori Bay/Portugal	--	--	--	--	19.7-40.05	--	--	--	--	--	30
Suez Canal/ Mediterranean Sea	1.72 ± 0.07	--	3.93 ± 0.50	--	--	--	--	4.44 ± 0.80	--	--	31
T. neritea	**1.14**	**10.82**	**0.41**	**104.34**	**2.76**	**0.15**	**4.51**	**59.83**	**13.34**	**418.41**	**This study**
					Bittium reticulatum **(Gastropod Mollusca)**						
Site	**Cd**	**Cu**	**Pb**	**Ni**	**As**	**Sn**	**Se**	**Zn**	**Mn**	**Fe**	
Sinop/Black Sea	<0.2	--	<0.4	--	--	--	--	--	--	--	28
B. reticulatum	**0.55**	**14.92**	**0.73**	**56.72**	**3.04**	**0.16**	**4.41**	**40.60**	**47.04**	**668.47**	**This study**

1: Tuzen et al. (2009); 2: Arici and Bat (2016a); 3: Arici et al. (2019); 4: Bat and Arıcı (2016); 5: Türk Çulha et al. (2010); 6: Culha et al. (2013); 7: Türkmen and Aydın (2021); 8: Akçalı and Küçüksezgin (2011); 9: Aydın-Önen and Öztürk (2017); 10: Manev and Petkova (2021); 11: Kut et al. (2000); 12: Caliceti et al. (2002); 13: Al-Masri et al. (2003); 14: Panayotova and Stancheva (2013); 15: Kravtsova et al. (2014); 16: Ak et al. (2021); 17: Bat et al. (1999); 18: Topcuoğlu et al. (2002); 19: Culha et al. (2007); 20: Culha et al. (2008); 21: Bat et al. (2012); 22: Belivermiş et al. (2016); 23: Türk Çulha et al. (2017); 24: Bat et al. (2018); 25: Bat et al. (2021); 26: Kapranov et al. (2021); 27: Giandomenico et al. (2016); 28: Bat and Şahin (2018); 29: Camusso et al. (1998); 30. Ruiz-Chancho et al. (2013); 31: Sharaf and Shehata (2015).

Heavy Metal in Mollusca

For many invertebrates, including bivalves and gastropods, diet is the main way of metal intake. These creatures are considered to be potentially good bioindicators of environmental trace metal contamination and provide chemical analysis of an indirect estimate of the concentration of such substances in their immediate environment (Rainbow, 1995; Chong & Wang, 2000; Luoma & Rainbow, 2005). Since molluscs are considered as food for humans, fish and other sea creatures, and even for birds, it is important to control pollution with toxic elements (Brown et al., 1991). Many studies have proven that living molluscs were able to store various heavy metals in their tissues in high concentrations and adapt to environmental conditions (Usero et al., 1997; Ananthan et al., 2006; Kesavan et al., 2013). In this study, while *R. splendida*, *T. pullus pull*us, one of the mollusc species living in *C. barbata*, are the species whose heavy metal accumulation in the tissue was studied for the first time, *T. neritea* is one of the gastropods sampled from the Karakum region for the first time and whose heavy metal concentration was measured in terms of metals other than Cd and Pb. When bivalve and gastropod species are examined in Table 1, it is seen that *M. galloprovincialis* has a high value of Se, *B. reticulatim* has a high value Mn, *R. splendida* has a high value Cd, and *T. neritea* has a high value Zn. The least metal accumulation was observed in the *T. pullus pullus* gastropod. Since As, Sn and Se have not been studied in gastropod and mussel species in the sampling conducted on the shores of Sinop before, our results were compared with studies conducted on the coasts of neighboring countries (Table 3). Accordingly, it is observed that the As and Se values detected in *M. galloprovincialis* species were low (Kapranov et al., 2021; Giandomenico et al., 2016), and the Sn value was high (Kapranov et al., 2021). We can also see that the Cd and Pb values detected in gastropods *T. neritea* and *B. reticulatum* were higher than the data of Bat and Şahin (2018). When the data of the countries located in the Mediterranean basin were examined, Sn, Se, Ni, Mn and Fe values were detected for the first time in *R. splendida*, *T. pullus pullus*, *T. neritea* and *B. reticulatum,* except for As in *T. neritea* sampled off the coast of Portugal. A metal accumulation of different proportions were determined in bivalve and gastropod species. Differences in terms of metal accumulation were observed in mollusc species. It is believed that this difference is due to the fact that each species has different feeding regimes and living conditions, as well as changes in the biological activity depending on the seasons (Jeng et al., 2000; Özden et al., 2009). In order to determine the heavy metal pollution on the shores of Sinop, studies were carried out on various living species, sediment and seawater samples (Bat et al., 1999,2020; Gundogdu et al., 2016; Gündoğdu et al., 2020; Culha et al., 2007, 2008, 2013; Çulha et al., 2016; Türk Çulha et al., 2010, 2017). The absence of enterprises that can cause industrial pollution in and around the Sinop Peninsula implies the source of metal accumulation in this region to a different point. This situation, which is determined especially in molluscs, shows that the shores of Sinop are more affected by household waste. Due to the lack of treatment facilities in Sinop province, 95% of the city's sewage is discharged to the sea by the wastewater sewage system. The population of the province, which has a population of over 30.000, increases 2-3 times in summer. The coasts of Sinop are affected by intensive land-based pollution and organic matter pollution caused by domestic discharges (Bat et al., 2015; Culha et al., 2007; Bat et al., 2009; Bat et al., 2012). In particular, untreated household waste and human activities along the coastline increase during the summer months, possibly leading to high concentrations of metals (Bat et al., 1999).

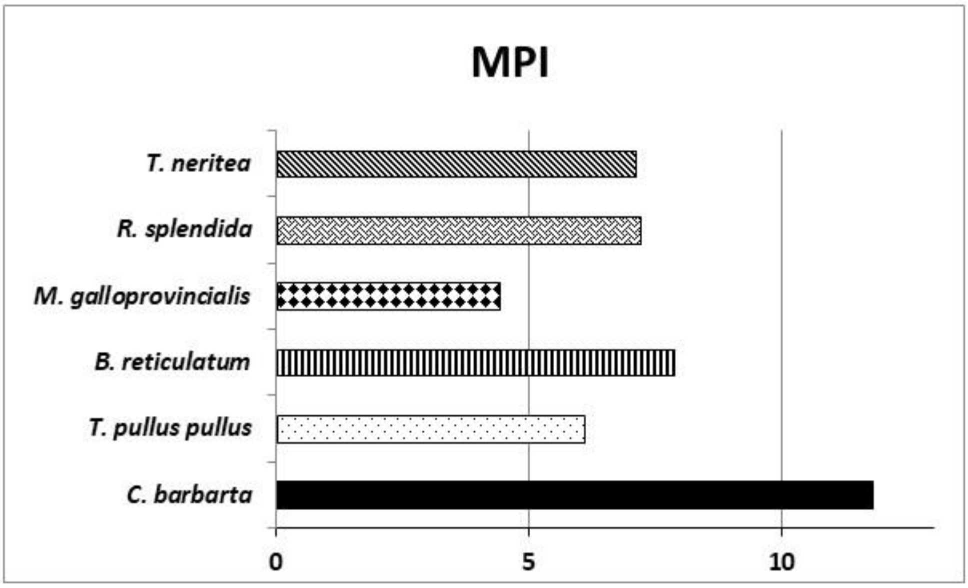

Figure 2. Metal Pollution Index (MPI) levels determined in macroalgae and molluscan species.

The MPI values calculated from the average concentrations of the analyzed heavy metals are given in Figure 2. The MPI values determined according to the species are; *C. barbata* > *B. reticulatum* > *R. splendida* > *T.neritea* > *T. Pullus pullus* > *M. galloprovincialis*. In the study, it was found that brown algae accumulate much more heavy metals than mollusc species. The group with the highest accumulation after algae is gastropod species. The heavy metal concentrations detected in five different mollusc species and brown algae were also compared with the maximum elemental values determined according to national and international food standards. The highest metal detected in all living things was Fe, while the lowest metal was Sn. There is very little legislation on seaweed in the European Union, so there are very few controls on potentially harmful metals. France is the first European country to introduce regulations on the use of seaweed for human consumption (Besada et al., 2009). Due to the lack of national and international guidelines on Se, Sn, Mn, and Fe in algae and mollusc species, it is debatable whether the levels observed in this study are actually safe for human consumption. The other heavy metal results obtained in the study were compared with the consumable limit levels of the different organizations mentioned in Table 4. The high amount of As accumulation in *C. barbata* was identified according to the quality criteria applied to edible algae sold in France. According to CEVA (2019), the highest metal detected in *C. barbata* was arsenic, which exceeds the limit value set by France. The highest concentration of Cd was measured at 41.03 mg kg^{-1} in *R. splendida* and at 1.14 mg kg^{-1} in *T. neritea*. This concentration was above the specified US FDA, EC, SÜY and TGK limits. Zn was detected in almost all samples and the highest concentration (59.83 mg kg^{-1}) was detected in *T. neritea*. This value was slightly above the limit set by the TGK. The highest Ni concentration was detected in *R. splendida* (85.67 mg kg^{-1}), *T. neritea* (104.34 mg kg^{-1}) and *T. pullus pull*us (273.42 mg kg^{-1}). Ni values in three gastropod species were well above the stated legal limit of the USFDA. According to TGK, SÜY and FAO criteria, arsenic was detected above legal limits in all mollusc species.

Table 4. Comparison of heavy metal concentrations and international standards in algae and molluscs (mg kg^{-1} dw).

Organization	Cd	Cu	Pb	As	Sn	Ni	Zn	Reference
For Algae								
Edible seaweed and microalgae – Regulatory status in France and Europe	0.5	--	5.0	3.0	5.0	--	--	CEVA (2019)
C.barbata	0.24	18.15	1.05	**62.20**	0.32	388.81	16.66	This study
For The Molluscs								
Food and drug administration (US)	4.0	--	1.7	86.0	--	80.0	--	USFDA (2007)
Turkish Food Codex	1.0	20.0	1.0	1.0	--	--	50.0	TGK (2005)
Aquaculture Regulation	1.0	--	1.5	--	200.0	--	--	SÜY (2008)
Food and Agriculture Organization	10.0	50.0-100.0	1.0	0.5-25.0	--	--	200.0-500.0	FAO (1983)
European Commission	1.0	--	1.5	--	--	--	--	EC (2006)
T. pullus pullus	0.11	11.49	0.40	**2.14**	0.22	273.42	17.49	This study
B. reticulatum	0.55	14.92	0.73	**3.04**	0.16	56.72	40.60	This study
M. galloprovincialis	0.22	5.58	0.57	**3.24**	0.14	32.02	11.16	This study
R. splendida	**41.03**	6.77	0.70	**3.89**	0.17	85.67	11.99	This study
T. neritea	**1.14**	10.82	0.41	**2.76**	0.15	104.34	59.83	This study

Conclusions

In this study, the concentrations of heavy metals in the brown marine algae *C. barbata* and 5 mollusc species living in it were investigated. Among the species studied, *R. splendida* and *T. pullus pullus* are gastropod species that were monitored for heavy metals for the first time. The gastropod species selected in terms of comprehensive metal examination were carried out for the first time for the Karakum station. As a result of the study, it was found that there is an accumulation of metals in different proportions. The highest accumulation in brown algae was also observed and the As value was found to be very high. It is thought that *M. galloprovincialis* may be a good bioindicator for determining Se and Ni and *C. barbata* for As, Ni, Fe, Pb, Cu, Mn and Sn heavy metals, especially in terms of Fe, Ni, As, Fr, Mn of gastropod species. Algae and *M. galloprovincialis* species are used as human food, while other mollusc species are used as food by many aquatic species. According to the determined legal limit values, it was concluded that these species, which have ecological importance, may be objectionable when consumed in terms of other living species in terms of bioconcentration and biomagnification. It is suggested that future research on seafood should include broader research that will survey other commonly consumed marine species, sediments, and different types of algae.

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Ethical approval

The author declares that this study complies with research and publication ethics.

Data availability statement

The authors declare that data are available from authors upon reasonable request.

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Author Contribution

Saniye Türk Çulha: Conceptualization, Formal analysis, Methodology, Writing-original draft, Review and editing

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