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ASSESSMENT OF ELEMENTAL UPTAKES BY *ULVA* (CHLOROPHYTA) SPECIES COLLECTED FROM SINOP COASTS OF THE BLACK SEA

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ABSTRACT: Seaweeds are proposed as possible bio-indicators by Marine Strategy Framework Directive (MSFD) is aimed to ensure Good Environmental Status (GES) of the seas by 2020 in the EU. For this purpose, selected metals Al, As, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb and Zn levels were analysed in *Ulva* species, and their surrounding water and sediments in Sinop Province, using an ICP-MS. The heavy metal concentrations of species were found As < 3, Cd < 0.5, Hg < 0.1, Pb < 5 and Cu < 30 mg/kg dry wt., varied seasonally and regionally. Zinc levels ranged from 16.6 to 268.6 mg/kg; the average value of Zn exceeds maximum permissible limit (50 mg/kg) without *Ulva lactuca* (32.5 mg/kg). Zn was determined as macro concentrator (BSAF > 2) and Al, Mn and Fe were found as bio-accumulative elements (BCF > 200). The mean concentration data measured from the eight sites (Türkeli, Ayancık, Inceburun, Akliman, Tersane, Karakum, DSİ and Gerze) were found on the order of Fe > Al > Zn > Mn > Cu > Ni > As > Pb > Co > Cd > Hg. The sea lettuce species are cosmopolitan and tolerant towards salinity and pollution. The fact that the results are below the limit values indicates that these species can be regarded as nutrients and flavourings. The effects of anthropogenic pollution in this region must be regularly monitored by bio-monitor species.

KEYWORDS: *Ulva* spp., Chlorophyta, Sinop, heavy metal, biomonitoring.

INTRODUCTION

The pollution of the coastal marine ecosystem mainly heavy metals has become one of the most critical environmental problems of the century. High concentrations of metals are accumulated by marine resources, incorporated into food chain and affecting human health. Bio-monitors are used in the assessment of contaminants in the environment; water, sediments and organisms and have been used to describe the ecological behaviour of pollutants and also monitoring the levels of contamination (Phillips and Rainbow, 1994). Macroalgae are recognized as bio-monitors to assess the metal pollution in seawater due to their sedentary lifestyle, easy uptake and accumulation capacities (Chakraborty *et al*. 2014).

Ulva spp. have been suggested as useful indicators of metal contamination (Topçuoğlu *et al*. 2001) and were used as a bio-indicator to monitor in the Black Sea Turkish coasts since 1992 (Cevher 1991; Öztürk 1991, 1994; Güven *et al*. 1992, 1998; Öztürk *et al*. 1994a, b; Toğçuoğlu *et al*. 2002; Altuğ *et al*. 2005; Candan and Taş 2009; Türk Çulha *et al*. 2013; Bat and Arıcı 2016; Arıcı and Bat 2016). However, there is a relative lack of detail information about the impact of increased anthropogenic stress related to heavy metal concentrations of *Ulva* species for the Black Sea countries.

Marine macroalgae have been commercially collected and consumed generally in Asian countries for decades with official regulations. In Europe, seaweeds are considered as novel food and a specific regulation was established in 1990 for edible seaweeds including *Ulva* spp. (CEVA, 2014). In Turkey there is no legal regulation for macroalgae, but Marine Strategy Framework Directive (MSFD) is aimed to ensure Good Environmental Status (GES) of the seas by 2020 in the EU, using 11 qualitative descriptors (MSFD, 2008). For this purpose, metals (Al, As, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb and Zn) were analysed in *Ulva flexuosa*, *U. intestinalis*, *U. lactuca*, *U. linza* and *U. rigida*, and their surrounding water and sediments in Sinop Province to describe ecological situation that has been subjected to an increasing anthropogenic pressure owing to the growing population.

The aim of this study was to determine the variability of metal concentrations in *Ulva* spp.; to identify metal uptakes; and to choose a good accumulator species along Sinop coast.

MATERIALS AND METHOD

Sinop Province is situated in the middle part and also at the outermost point on the Turkish coastline of the Black Sea and is one of the Black Sea natural harbours. The coastal configuration and inputs displays locally and seasonally variabilities.

Samples of *Ulva* species (*U. flexuosa*, *U. intestinalis*, *U. lactuca*, *U. linza* and *U. rigida*), seawater and sediments were collected during September 2015 and July 2016 seasonally from Türkeli, Ayancık, Inceburun, Akliman, Tersane, Karakum, DSİ and Gerze stations covering all Sinop coasts (Fig.1).

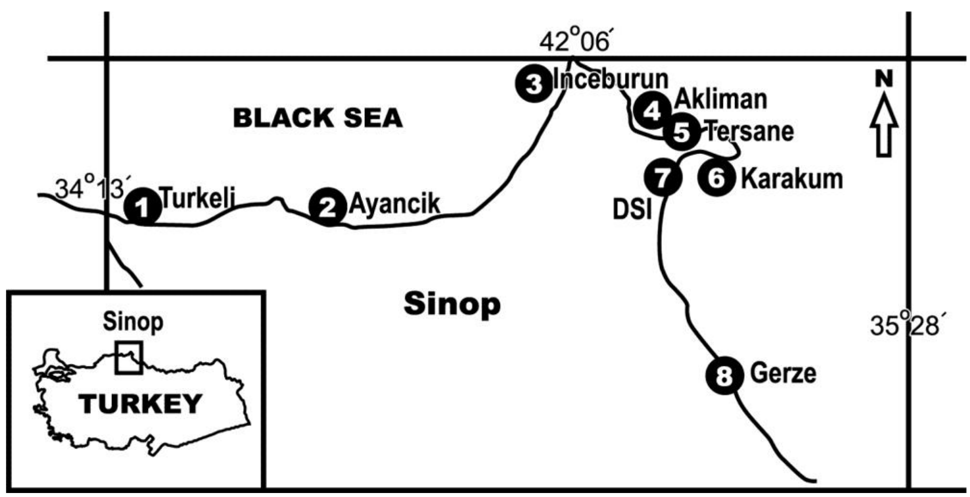


Fig. 1. Study area (Sinop coast)

Macroalgae samples were rinsed with water for removal of external particles, labelled, and transported to the laboratory. Algae were washed again and dried at 70^{o}C for 48 hours. According to Aquatic plant HPR-FO-08 method (Milestone), dried samples digested with Suprapur® HNO_3 (nitric acid) using a microwave digestion system (Milestone Systems, Start D 260) for analysis.

For each 250 cc water sample, a 1 ml of concentrated ultrapure HNO_3 was added and stored in polyethylene bottles. Sediment samples dried at 105^{o}C-24 h and the less than 63 µm was digested with Seawater Sediment HPR-EN-33 methodology (Milestone Systems, Start D 260).

Selected metals (Al, As, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb and Zn) in samples were determined with ICP-MS (Agilent Technologies, 7700X), used three replicates and results of mean concentrations were detected as mg/kg dry wt. in algae and sediments, and ppb in water samples. The methodology was validated by the Certified Reference Materials (CRMs) (BCR 279- *Ulva lactuca*) (Table 1). Results of analytical precision for sample replicates obtained were in agreement with the certified values at 99% confidence level.

Biota-Sediment Accumulation Factor (BSAF) and Bio-concentration factor (BCF) are parameters to describe bioaccumulation of sediment and water-associated metals into the ecological receptors (Kleinov *et al*. 2008; Geyer *et al*. 2000) that are calculated according to the following formulas: $BSAF = [C]_{biota} / [C]_{sediment}$; $BCF = [C]_{biota} / [C]_{water}$.

SPSS ver. 21.0 was used for statistical calculations, and the p value ≤0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Metal concentration levels in seawater:

Heavy metal concentrations in seawater from eight sampling sites are presented in Table 1. In the main, the concentrations of the metals followed the order of Hg < Cd < Co < As = Pb < Cu < Ni < Mn < Al < Zn < Fe. Concentrations of Co, Ni and Pb exhibited significant variations ($p \leq 0.05$) depending on seasons. The range of Cu concentrations was wide (1.39-17.56 ppb).

Heavy metal concentrations in sediments:

Table 1 shows the mean concentrations in sediments. Mn, Cd, and Hg levels displayed statistical significance depending upon locations ($p \leq 0.05$). In general, metal concentrations of sediments were increased in the order of Hg < Cd < Pb < Co = As <Cu <Ni < Zn < Mn < Al <Fe.The Mn was higher and ranged between 85.9 and 516 mg/kg dry wt.

The collected predominant *Ulva* spp. from Sinop coast are shown in Table 2. Their minimum and maximum results are presented in Table 3. Mean concentrations of the algae are shown in Fig. 2 (a-k).

BSAFs for macroalgae are in the range of 1 to 2; BSAF>2 macro concentrator, 1<BSAF<2 micro concentrator and BSAF<1 deconcentrator are named as (Nenciu *et al*. 2016). However, there is no criteria have been explicitly developed for evaluating the quality of BCF by regulatory agencies. BCFs are varied in the range between 1000 and 5000 (Table 4) (Arnot and Gobas, 2006).

Table 1. Mean concentrations of heavy metals in seawater (ppb) and sediments (mg/kg dry wt.).

	Al	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Hg	Pb
Seawater											
Türkeli 1	251.88	23.11	457.58	1.53	6.84	3.25	274.33	1.61	0.38	0.03	2.71
Türkeli 2	206.82	33.52	348.87	1.31	5.08	3.72	224.25	1.82	0.32	0.03	1.81
Ayancık	446.12	20.06	511.33	1.65	4.8	1.71	191.86	1.65	0.41	0.05	1.41
Akliman	208.55	12.91	285.13	1.53	4.23	2.88	368.58	1.56	0.46	0.04	6.2
Karakum	171.55	8.55	180.26	1.48	3.22	1.39	181.2	1.5	0.41	0.04	1.23
Tersane	258.94	13.22	260.19	1.58	4.69	17.56	508.71	1.55	0.64	0.04	6.61
DSİ	1117.74	64.76	1557.78	2.14	5.62	2.04	344.46	3.56	0.44	0.27	3.46
Gerze	378.35	23.79	472.58	1.5	4.34	1.58	224.62	1.64	0.37	0.03	1.73
İnceburun	164.01	7.33	151.19	1.47	3.66	1.58	217.92	1.54	0.4	0.04	1.48
Sediment											
Türkeli 1	5724	516	10393	4.5	16.9	4.6	17.1	7.4	0.08	0.03	3.7
Türkeli 2	4321	325	7041	3.5	18.2	9	16.7	4.1	0.05	0.02	3
Ayancık	5205	420	10617	4.5	16.4	4.4	18.7	7	0.05	0.02	3.7
Akliman	3584	333	7260	2.7	7.5	4.6	21.1	5.1	0.1	0.01	39.3
Karakum	400	12.2	613.7	0.22	0.33	0.54	0.79	0.42	0.003	0.005	0.19
Tersane	1348	85.9	2533	0.9	2.9	1.9	6	2.3	0.02	0.01	2.2
DSİ	2157	97.2	3783.8	2	6.5	1.5	5.4	3.6	0.02	0.01	1.9
Gerze	5010	231	8300	3.3	13	5.7	13.2	3.6	0.04	0.01	1.7

Variability of the concentration of metals in the algae

Heavy metal accumulation of *U. flexuosa* was determined for the first time in the Black Sea of Turkey. The mean values of heavy metal accumulation in this species increased in the order of Hg < Cd < Co < As < Ni < Cu < Pb < Zn < Mn < Fe < Al. Bioaccumulation potential of filamentous algae are high (Littler *et al*. 1983), and in this species mean Co, Cu, Mn,Ni and Pb levels of *U. flexuosa* were accumulated more than other *Ulva* species. There were no significant differences between seasons and stations ($p > 0.05$, ANOVA). Essential elements (Mn, Fe and Al) were accumulated from seawater (BCF > 2000). But Zn showed different tendency (BSAF=5) due to their accumulation in the sediments over time (Table 5). The mean accumulation of Pb (6.05 mg/kg dry wt.) and Zn (67.95 mg/kg dry wt.) observed above the guideline values (Table 4). Storelli *et al.* (2001) suggested that Zn concentrations were in excess of 100 mg/kg in benthic algae is derived from anthropogenic pollution. In Türkeli station, Zn level was 125.6 mg/kg caused by marsh area and also intense agricultural activities. In addition, population in DSİ station was 3 times higher in summer.

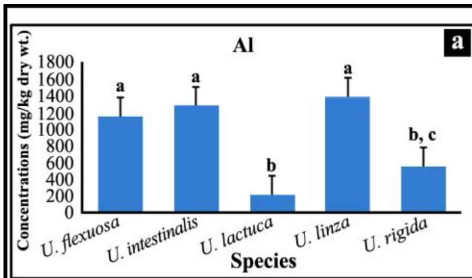
Table 2. Sampled *Ulva* species on Sinop coasts.

	Türkeli	Ayancık	Inceburun	Akliman	Tersane	Karakum	DSİ	Gerze
U. flexuosa	X			X			X	
U. intestinalis	X	X	X			X		
U. lactuca	X				X			
U. linza	X	X	X			X	X	X
U. rigida	X			X	X		X	

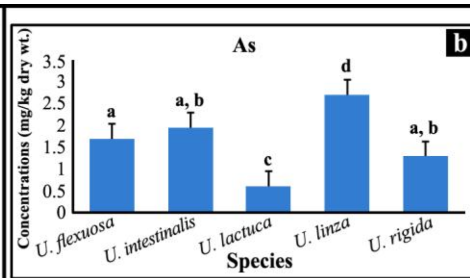
Table 3. Minimum and maximum heavy metal concentrations of *Ulva* species (mg/kg dry wt.)

	U. flexuosa		***U. intestinalis***		***U. lactuca***		***U. linza***		***U. rigida***	
	min	**max**	**Min**	**max**	**min**	**max**	**min**	**max**	**min**	**max**
Al	582.4	1726.3	490.5	2080	50.3	376.5	310.7	2465.6*	7.3	1111**
As	0.6	2.8	1.2	2.7	0.4	0.8	0.6	4.8	0.5	2.1
Cd	0.02	0.05	0.04	0.3	0.02	0.05	0.037	0.54	0.061	0.37
Co	0.1	2.1	0.2	1.1	<0.00	0.5	0.04	1.36	<0.00	0.4
Cu	0.96	10.1	1.6	5.1	1.9	3	1.2	7.6**	1.3	5.3
Fe	500.5	2013.8	526.6	2751.8	101.7	465.3	319.2	3039.3	12.4	1156**
Hg	0.007	0.029	0.01	0.031	0.002	0.008	0.006	0.048**	0.002	0.012
Mn	5.7	937.9	14.5	71.7	2.7	44.7	6.4	162	1.4	35
Ni	1.19	6.4	1.5	5.7	0.2	3.5	1.7	2.8	0.7	3.8
Pb	0.6	12.7	0.5	2	0.4	0.6	0.4	2.5	0.1	5.9
Zn	25.5	125.6	16.6	268.6*	20.2	44.8	33.8	167.1*	18.1	167.2

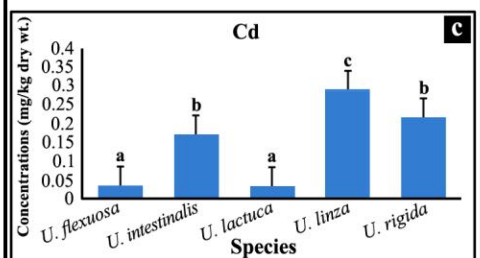
($p \leq 0.05$. α:0.05. ANOVA); *differentiation depending on seasons; ** differentiation depending on stations.



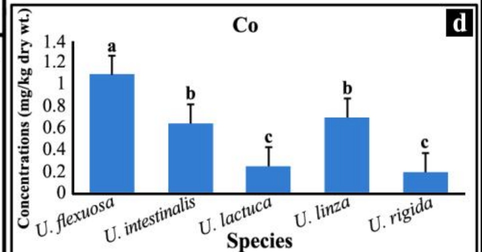
The order of Al accumulation was *U. lactuca* < *U. rigida* < *U. flexuosa* < *U. intestinalis* < *U. linza*. According to all stations and seasons. Mean Al values accumulated at least in *U. lactuca* with 213.4 mg/kg dry wt. This essential element in *U. flexuosa*. *U. intestinalis* and *U. linza* showed similar statistically variations (p>0.05). The maximum Al accumulation in *U. linza* was 1388.2 mg/kg dry wt.



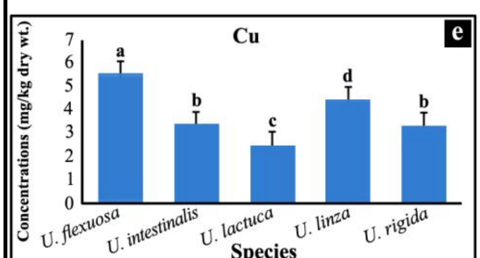
The As values were increased in the order of *U. lactuca* < *U. rigida* < *U. flexuosa* < *U. intestinalis* < *U. linza.* This non-essential element was accumulated minimum value by *U. lactuca* (0.6 mg/kg dry wt.). As concentrations were high levels in *U. linza* with 2.7 mg/kg dry wt.



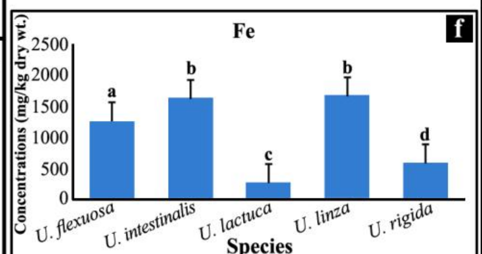
Cd was accumulated in *Ulva* species in the order *U. lactuca* = *U. flexuosa* < *U. intestinalis* < *U. rigida* < *U. linza.* The maximum Cd content in *U. linza* was 0.29 mg/kg dry wt. that was also accumulated from sediment and the minimum content was 0.035 mg/kg dry wt.



The order of Co content was *U. rigida* < *U. lactuca* < *U. intestinalis* < *U. linza* < *U. flexuosa*. *U. flexuosa* showed statistically difference (p > 0.05) between groups. The maximum accumulation was in *U. flexuosa* with 1.1 mg/kg dry wt. and the minimum accumulation was in *U. rigida* with 0.2 mg/kg dry wt.



The Cu values were found in the order *U. lactuca* < *U. rigida* < *U. intestinalis* < *U. linza* < *U. flexuosa.* There is a statistically difference in maximum level of *U. linza* level dependent upon stations.



The Fe contents increased in the order of *U. lactuca* < *U. rigida* < *U. flexuosa* < *U. intestinalis* < *U. linza*. The maximum content of Fe was found in *U. linza* with 1679.3 mg/kg dry wt. There was a direct relationship between accumulation rate of Fe in *U. linza* and water concentration.

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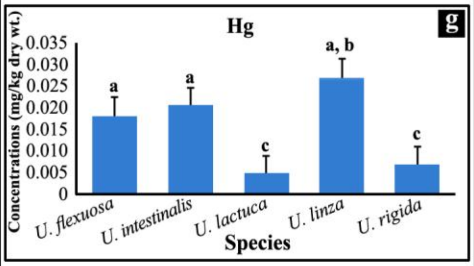
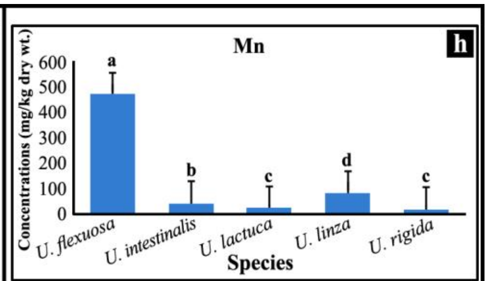
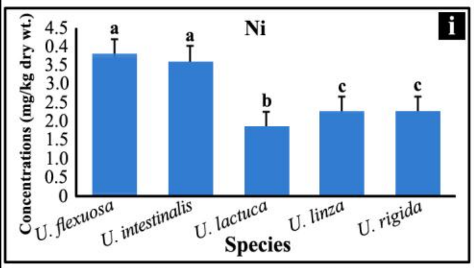


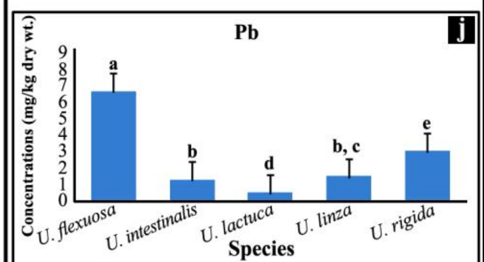
The non-essential Hg levels in *Ulva* species were found in the order *U. lactuca* < *U. rigida* < *U. flexuosa* < *U. intestinalis* < *U. linza*. The minimum content was 0.005 mg/kg dry wt. in *U. lactuca*. All of Hg values were lower than 1 mg/kg dry wt.



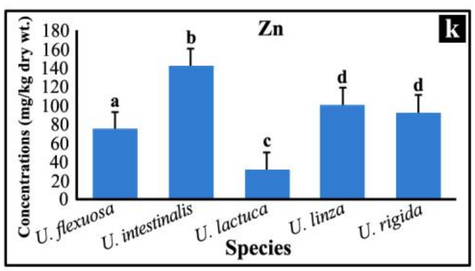
Mn contents showed significant intraspecific variability as *U. rigida* < *U. lactuca* < *U. intestinalis* < *U. linza* < *U. flexuosa*. The lowest value was 18.2 mg/kg dry wt. and the highest value was 471.8 mg/kg dry wt. This essential element had a linear relationship with water and sediment concentrations.



The mean Ni levels were found in the order *U. lactuca* < *U. linza* = *U. rigida* < *U. intestinalis* < *U. flexuosa*. This essential element was accumulated at least in *U. lactuca* with 1.85 mg/kg dry wt.



The Pb contents increased in the order of *U. lactuca* < *U. intestinalis* < *U. linza* < *U. rigida* < *U. flexuosa*. The concentrations were varied from 0.5 to 6.65 mg/kg dry wt. The maximum level was in *U. flexuosa* with 12.7 mg/kg dry wt.



Zn levels of *Ulva* species were found in the order *U. lactuca* < *U. flexuosa* < *U. rigida* < *U. linza* < *U. intestinalis*. The lowest concentration was 32.5 mg/kg dry wt. and the highest concentration was 142.6 mg/kg dry wt.

Fig. 2. a-k, Mean heavy metal concentrations of *Ulva* species

Table 4. An overview of bioaccumulation endpoint criteria.

Program	Criteria
Toxic Substances Control Act (TSCA) and Toxic Release Inventory (TRI) (USEPA, 1976) [24]	1000-5000 (bio-accumulative) ≥5000 (very bio-accumulative)
Canadian Environmental Protection Act (CEPA), 1999 [25]	≥5000
Registration, Evaluation and Authorization of Chemicals (REACH) Annex XII (European Commission 2001) [26]	≥2000 (bio-accumulative) ≥5000 (very bio-accumulative)
Stockholom Convention on Persistent Organic Pollutants (UNEP 2001) [27]	≥5000

Table 5. Mean BSAF and BCF values of species.

	U. flexuosa		***U. intestinalis***		***U. lactuca***		***U. linza***		***U. rigida***	
	BSAF	**BCF**	**BSAF**	**BCF**	**BSAF**	**BCF**	**BSAF**	**BCF**	**BSAF**	**BCF**
Al	0.3	**4817.6**	0.2	**5522.3**	0.1	853.6	0.4	**3928.4**	0.1	731.6
As	0.4	817.1	0.4	1395.8	0.1	375	**6.7**	**8694.6**	0.4	625
Cd	0.7	83.4	2.7	290	0.6	79.2	**20.8**	969.2	**3.8**	352.8
Co	0.3	558.2	0.1	370	0.1	333.3	0.2	355.7	0.1	177.1
Cu	0.7	1416.4	0.7	1641.8	0.8	522.7	0.8	1174.3	1.7	944.3
Fe	0.2	**2918.1**	0.2	**6314.9**	0.0	712.6	0.2	**4092.9**	0.1	821.5
Hg	1.2	445.6	1.1	411.6	0.2	156.9	1.6	562.4	0.5	139.6
Mn	1	**9710.1**	0.1	**3616.9**	0.1	1252.5	0.2	**2253.2**	0.1	797.7
Ni	0.3	591	0.4	751.5	0.1	278.6	0.3	527.9	0.3	392.1
Pb	0.4	1055.4	0.3	592.8	0.2	141.4	0.6	387.3	0.6	188.8
Zn	**5**	274.9	**7.8**	352	**3**	101.5	**6.5**	217.5	**9.7**	267.4

BSAF > 2 (macro concentrator) and BCF > 2000 (bio-accumulative) values are shown in bold.

The heavy metal content in *U. intestinalis* were Hg < Cd < Co < Pb < As < Ni < Cu < Mn < Zn < Al < Fe. Zinc values were nearly increased 16 times higher in Karakum station in winter (268.6 mg/kg dry wt.) than spring (16.6 mg/kg dry wt.). Therefore, Zn accumulation had differentiation between seasons (p=0.01, ANOVA). Moreover, Zn concentrations were translocated to roots efficiently (Nicolaidou and Notti 1998) and this species was macro concentrator for Zn element (BSAF > 2) (Table 5). Akliman station showed high Pb values in summer and the Pb results were found similar in Öztürk *et al.* (1994b) and Bat and Arıcı (2016) studies. Because, Akliman is touristic recreation area in summer seasons where people have a picnic and swim in.

U. lactuca collected in autumn and winter seasons in this study. There was no statistically significant between seasons and stations ($p > 0.05$). However, when the essential elements of the two stations (Tersane and Türkeli) were compared. There was a statistical difference between the levels of Al, Mn, Fe and Zn. Tersane is settlement site and fishing vessels conduct into a harbour. With the beginning of fishing season in autumn ship traffic increases the load of Zn, Ni and Cu. Nevertheless, the concentrations were lower than the recommended legal limits (Table 5). The order of accumulation in *U. lactuca* was Hg = Cd < Co = Pb < As < Ni < Cu < Mn < Zn < Al < Fe. Zinc concentration was accumulated from sediments (BSAF=3). Unlike other species, Zn levels do not exceed maximum permissible limit (32.5 mg/kg dry wt., <50 mg/kg dry wt.) and also other heavy metal concentrations were below the values proposed by regulatory agencies (Table 6). This species was studied since 1985 (Tuncer 1985) and the results of trace elements varied depending on seasons and sampling areas. Comparision of resukts from other studies indicated that concentrations of *U. lactuca* were found highest levels in the Marmara Sea due to intensive industrial activities (Ergül *et al*. 2010).

Table 6. Comparisons of heavy metal concentrations.

	Heavy metals						References
	As	**Cd**	**Hg**	**Pb**	**Zn**	**Cu**	
				0.5	30	30	FAO, 1983 [35]
				1	50	20	Anonymous, 1995 [36]
				2	7	2	WHO/FAO 1999, 2004 [37,38]
	3	0.5	0.1	5			CEVA, 2014 [17]
U. flexuosa	1.5	0.03	0.02	**6**	**68**	3.7	This study
U. intestinalis	2.1	0.11	0.02	1.1	**75.2**	3.3	This study
U. lactuca	0.6	0.03	0.01	0.5	32.5	2.4	This study
U. linza	2.2	0.11	0.02	1.3	**74.2**	3.4	This study
U. rigida	1.3	0.12	0.01	1.2	**81**	4.2	This study

U. linza was observed in all seasons and was the most common along Sinop coasts. The measured heavy metal levels were found at least in Inceburun. Since there is no settlement area. The highest anthropogenic and industrial effects were observed in Gerze in the summer. The mean values of heavy metal levels were Hg < Cd < Co < Pb < As <Ni < Cu < Mn < Zn < Al < Fe. There are several studies in Sinop (Öztürk 1991, 1994; Öztürk *et al*. 1994a; Altuğ *et al*. 2005; Türk Çulha *et al*. 2013; Bat and Arıcı 2016; Arıcı and Bat 2016b); however only Arıcı and Bat (2016) found the similar order of elements.

Variable population and shipping activities have an effect upon metal uptakes. The level of Cu was significantly different in Inceburun and Tersane ($p \leq 0.05$, $p=0.01$ and 0.03). Arsenic was an indicator element for Tersane station (BSAF=31.4 and BCF=46562.5) that increased in winter. Besides, Cd was found high value in sediments (BSAF=20.8), because Cd fall down to sediments and settles relatively faster than organisms (Fowler and Knauer 1986). Uptake of Al, As, Cd, Hg, Fe and Zn elements were highest amount in this species. The essential elements Al, Fe and Mn were bio-accumulative (BCF > 2000) (Table 4).

The heavy metal levels of the elements in *U. rigida* was in the order Hg < Cd < Co < Pb < As < Ni < Cu < Mn < Zn < Al < Fe. Previous studies in Turkey coasts (Güven *et al*. 1992, 1998; Altuğ *et al*. 2005; Özden and Tuncer 2015; Bat and Arıcı 2016) found similar pattern. In this study, this species was found only in autumn season in Akliman and Gerze, and Al and Fe levels showed statistically significant ($p=0.02$) depending upon these stations. Cd and Zn metals were accumulated from sediments (BSAF= 3.8 and 9.7) (Table 5).

CONCLUSION

Ulva species (sea lettuce) belonging to Chlorophyta division are tolerant to salinity and pollution, and generally members of this species are cosmopolitan. Therefore, *Ulva* species are most preferred for assessment of heavy metal pollution as indicators. Sea lettuce and aonori are consumed particularly in Asian countries (Japan, Korea, China, Vietnam, Indonesia and Thailand). However, in Europe seaweeds are evaluated as novel food. The heavy metal concentrations of sampled *Ulva* species on Sinop coasts observed below the proposed values by WHO/FAO, EC, EPA and Turkish standards. This result has been a sign that although it is not preferred in our country, *Ulva* species can be regarded as a nutrient and flavouring. In aquatic environments, accumulation of heavy metals may affect all marine organisms and through the food chain can affect human health. In this study, the most common heavy metal levels were determined to describe ecological situation of Sinop coasts for ensure GES in accordance with MSFD. Macroalgae have been widely used for biological monitoring of environmental quality. This region is must be regularly monitored by predominant *Ulva* species due to high anthropogenic inputs.

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