

# Assessment of trace metal contamination in the sea cucumber (*Holothuria tubulosa*) and sediments from the Dardanelles Strait (Turkey)

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**Abstract** This study was performed to determine the concentrations of some trace metals (Cd, Cu, Pb, Ni, Zn, and Fe) in *Holothuria tubulosa* (Gmelin, 1788) belonging to *Echinoderm* species and in sediments that they live at three different stations (Gelibolu, Umur Bey/Lapseki, and Dardanos) on Dardanelles Strait between April 2013 and March 2014. The mean trace metal concentrations determined in *H. tubulosa* and sediment were as follows: Cd 0.18 mg/kg, Cu 2.43 mg/kg, Pb 2.09 mg/kg, Ni 14.58 mg/kg, Zn 16.86 mg/kg, and Fe 73.46 mg/kg and Cd 0.70 mg/kg, Cu 5.03 mg/kg, Pb 14.57 mg/kg, Ni 27.15 mg/kg, Zn 54.52 mg/kg, and Fe 3779.9 mg/kg, respectively. It was detected that the statistical difference between trace metals determined seasonally in muscle tissue of *H. tubulosa* was significant ( $p > 0.05$ ). As a result of the study, it was detected that *H. tubulosa* is a bioindicator species in determining Ni trace metal in sediment. The results were compared to the limit values of National and International Food Safety, and it was detected that Cd and Ni concentrations measured in sediment were above LEL of Ni and Cd concentrations according to Sediment Quality Guidelines.

**Keywords** Sea cucumber · *Holothuria tubulosa* · Trace metals · Sediment · Dardanelles Strait · Marmara Sea

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## Introduction

The sea cucumbers are obtained by fishing in marine areas of Turkey. Their commercial importance gradually increases because of their excessive exporting (Sicuro and Levine 2011) in recent years and being a new species in the aquaculture. Populations of local sea cucumber have been reduced and new stock regions are sought (Conand 2004; González-Wangüemert et al. 2014; Purcell et al. 2012) because of increases in demand of these species in Asian markets, thereby especially increases in fishing pressure in Indo-Pacific Oceans (Chen 2004; Toral-Granda et al. 2008). One of these regions is Turkey's seas. There are 22 sea cucumber species in Turkey's seas (Öztoprak et al. 2014). Commonly found and commercially valuable sea cucumbers are *Holothuria tubulosa*, *H. mammata*, *H. polii*, *H. sanctori*, and *Stichopus regalis* (Aydın, 2008; Gonzales-Wangüemert et al., 2014). This living organism which is only used in hand-line fishing as a fish meal in Turkey is commercially fished and exported recently to far east countries, thereby providing foreign exchange inflow to the country (Gonzales-Wangüemert et al. 2014). It is used as a food material in many countries, particularly in China, and it is effective in treating diseases (Bodbar et al. 2011).

The sea cucumbers play an important role in reusing the bottom sediment by the effect of bioturbation due to the fact that the sea cucumbers living on and inside the sediment mix the sediment and use organic and inorganic substances in the sediment as a food (Massin 1982; Bulteel et al. 1992). They improve the living space of benthic organisms and help in reducing organic substance burden owing to such effect (Purcell 2010; MacTavish et al. 2012; Massin and Jangoux 1976; Massin 1982; Bruckner et al. 2003). Benthos has a potential to accumulate (bioaccumulate) heavy metals from surrounding water bodies in excessive amounts (Shulkin et al. 2003; Silva et al. 2006). It was reported that *H. tubulosa*

was an effective bioindicator species as a benthos for monitoring the trace metals and other contaminations (Warnau et al. 2006; Sicuro and Levine 2011). However, information about heavy metal behavior of this species is limited (Warnau et al. 2006).

The object of this study was to determine the metal concentrations in edible muscle tissue of *H. tubulosa* sampled from the Dardanelles Strait. Heavy metal pollution in gulfs and inland seas is more important and effective than the pollution in open seas. The Dardanelles Strait which is an important transition point of polluted water that comes from the Black Sea and Marmara Sea and reaches Mediterranean via upper currents is under the influence of both under currents and upper currents (Türkoğlu et al. 2004). In particular, the heavy metal pollution carried from the Dardanelles Strait to the Mediterranean via upper currents is higher than the pollution carried to the Black Sea via under currents. It is specified that increases in heavy metal (trace metals) in the Dardanelles Strait are caused by wastewater disrupting optimal stability of aquatic environment (Süren et al. 2007; Altug et al. 2009). Studies about trace metal accumulation in marine animals in the Dardanelles Strait are mostly focused on algae, mollusca, and fishes. There is no study relating to heavy metal accumulation in the sea cucumbers living in Turkey's seas. This study is very important in the sense of being the first in Turkey.

## Material and methods

### Study area

This study was performed at three different stations in Gelibolu (40° 22' 04,70" N–26° 37' 57,35" E) located in the Dardanelles Strait (south of Marmara sea), Umur bey/Fener (Lapseki) (40° 15' 09,39" N–26° 32' 53,14" E), and Dardanos (40° 04' 24,52" N–26° 21' 10,77" E) (Fig. 1). The reason for choosing these regions is the fact that they represent the coastal zone; there are numerous areas from which abundant samples are obtained, particularly in a depth of 0–30 m for land pre-studies; and that metal studies were previously done in bivalve (Mollusca) species in these regions. The number of residential areas at Gelibolu called S1 is very few; rather, there are agricultural areas. In addition, there is a low-flow freshwater inflow from an area near the station. There is also a ship maintenance, repair, and construction site in the area (seaside) near that region. The station S2 which is chosen as Umur Bey/Fener region is a residential area bound to Lapseki. Settlement in that area which also includes a military zone is along the shore. Touristic activities increase during summer time. Dardanos station selected as S3 is also area in which touristic activities take place. This region located in camping site is among the regions which have the highest pollution due to human-induced inflows (Ateş et al. 2014).

### Sample collection

*H. tubulosa* and sediment samples were collected monthly from a depth of 0–30 m at three stations via scuba diving between April 2013 and March 2014. From each station, 15–30 *H. tubulosa* samples and 0.5 kg of sediment samples from the habitat of the species were taken such that they represented all the size groups. In the sampling of December 2013, no sampling was performed due to lack of *H. tubulosa* in S2 station. The samples that were provided to laboratory with a cold chain were stocked in cold storage at –21 °C until analyses were done. In addition, temperature, saltness, oxygen, and pH values of the surface water were measured in situ using a hand-held portable WTW Multi 3420 Model Multiparameter device during sampling from the stations.

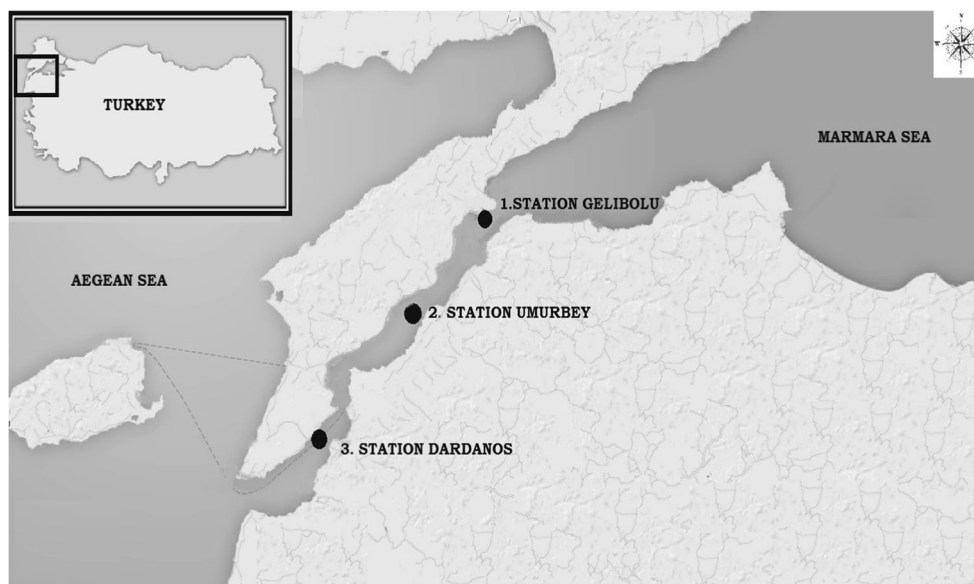
### Analytical procedure

*H. tubulosa* samples were washed with tap water first and then bidistilled water before the analyses. The samples were dissected using a stainless steel scissors and a lancet after filtering water on blotting papers. Visceral organs of the organism as well as eggs and sperms thereof that exist in its body at certain months were completely removed. The edible muscle tissue was homogenized for use in the analysis and dried in an oven at 105 °C for 24 h until it had a fixed weight. Of HNO<sub>3</sub>, 10 ml was added per dry weight of 1 g, and the mixture was left at room temperature overnight with a watch glass thereon. Then, these Erlenmeyer flasks were heated firstly at a low temperature (40 °C) for 1 h and then at a slowly increasing temperature (140 °C) for 3 h in a fume hood on a heat-adjustable hot plate until colored vapor of the samples disappeared. Of HNO<sub>3</sub>, 1 ml was added to samples upon completion of organic degradation and the volume was brought to 25 ml with bidistilled water (Bernhard 1976; Yap et al. 2004).

The sediments sampled from the stations were classified according to the particle size (Buchanan 1984), and the sediment samples smaller than 125 to 63 µm were used in order to determine the trace metals in sediment. The sediment samples of 0.5 g were added from 4:1 HNO<sub>3</sub>/HClO<sub>4</sub> aqua regia mixture and thereafter, burned firstly at a low temperature (40 °C) for 1 h and then at a slowly increasing temperature (140 °C) for 3 h in a fume hood on a heat-adjustable hot plate until colored vapor of the samples disappeared. The samples were brought to 25 ml with bidistilled water upon completion of organic degradation (Bernhard 1976; Yap et al. 2004). In addition, burnable substance amounts (%) in sediments sampled from each station were calculated (Buchanan 1984).

The metal concentrations of the samples were measured using ICP-OES Perkin Elmer Optima 8000 device. The wavelengths for metal reading were 214.440 nm for Cd, 220.353 nm for Pb, 231.604 nm for Ni, 327.393 nm for Cu, 213.857 nm for Zn, and 238.204 nm for Fe, respectively. The

**Fig. 1** Study area and stations from the Dardanelles Strait (Marmara Sea)



concentration values for the sea cucumber and sediment were expressed in mg/kg dry weight. The Standard Reference Material (SRM) used to test accuracy and precision of the device and the calibration curve was analyzed initially and after analysis of each sample in triplicate. In this study, “SRM 1566b (NIST-Oyster tissue)” supplied for determining trace metals in *H. tubulosa* and “RTC-CRM016” supplied for the sediment samples are certified reference materials (Table 1).

### Data analysis

Data from *H. tubulosa* and sediment analyses were grouped according to seasons and stations, and conformity of trace metal values to normal distribution was tested using Anderson-Darling test. Homogeneity of variances between the stations and seasons was tested using Levene’s statistic test. Two-way analysis of variance (ANOVA) was used to determine the interaction between stations and period. Significance test of homogenous groups was evaluated with one-way ANOVA followed by Tukey’s HSD test. Spearman’s correlation analysis was applied to verify existing relationships (between sediment metal concentrations and *H. tubulosa*

metal levels with physicochemical variables). Biota-sediment accumulation factor (BSAF) was also calculated. BSAF is the ratio between the metal concentration in biota and that found in sediment (Abdallah and Abdallah 2008). The formula is  $BSAF = C_{org}/C_{sed}$ , where  $C_{org}$  is the heavy metal concentration in the organism (mg/kg dry weight (dw)) and  $C_{sed}$  is the heavy metal level in sediment (mg/kg dw). BSAF was calculated for concentrations recorded for *H. tubulosa* and sediment. Statistics were performed using SPSS v21 software.

### Results

Values of physicochemical parameters measured at the stations are provided in Table 2. Accordingly, temperature values measured in the seawater was minimum 8.7 °C at S2 station in winter (January 2014) and maximum 25.1 °C at S1 (June 2013) and S2 (August 2013) stations in summer. Minimum saltness value measured at the stations was 22.4‰ in summer (June and July 2013), whereas maximum saltness value was 32.3‰ in autumn at S3 (November 2013) station. Minimum pH was measured as 8.27 at S3 station in summer (June 2013), while maximum pH was measured as 8.74 at S2 station

**Table 1** Certified and measured values of trace metal concentrations in reference materials NIST-Oyster tissue SRM1655b-sediment and RTC-CRM016 (mean ± SD)

Trace metals	NIST-Oyster tissue SRM 1655b	Measured (mg/kg dw; n = 3)	RTC-CRM016 Sediment	Measured (mg/kg dw; n = 3)
Cd	2.48	2.27 ± 0.006	0.3–0.6	0.79 ± 0.01
Cu	71.6	72.40 ± 0.33	16.8–18	16.58 ± 4.71
Pb	0.308	0.31 ± 0.002	12.7–15.5	13.12 ± 0.07
Ni	1.04	1.09 ± 0.05	15.8–17.7	15.95 ± 0.13
Zn	1424	1358 ± 8.02	77.8–82.2	74.56 ± 0.41
Fe	205.8	203.5 ± 17.45	16.8–18	16.83 ± 0.68

**Table 2** The physicochemical variables at each sampling sites

Stations	Periods	Temperature (°C)	Salinity (%)	pH	DO (mg/l)
S1	Spring	13.9–22.3	23.2–24.4	8.34–8.50	9.91–13.18
	Summer	24.5–25.1	22.4–22.5	8.28–8.36	7.31–8.48
	Autumn	15.1–24.2	22.9–24	8.59–8.59	9.02–10.32
	Winter	8.7–10.4	25.7–26.4	8.39–8.50	11.05–11.70
	Mean ± SE	17.63 ± 1.85	24.10 ± 0.43	8.44 ± 0.03	10.06 ± 0.52
S2	Spring	13.2–21.1	24.0–26.4	8.33–8.52	10.08–13.19
	Summer	24.4–25.1	22.7–22.9	8.33–8.36	8.18–8.80
	Autumn	15.7–24.9	22.9–24.5	8.61–8.74	9.53–13.94
	Winter	9.4–10.7	26.9–27.2	8.45–8.60	11.82–12.42
	Mean ± SE	17.79 ± 1.76	24.68 ± 0.51	8.49 ± 0.04	11.01 ± 0.55
S3	Spring	14.1–23.1	28.9–31.1	8.31–8.51	9.98–12.50
	Summer	21.6–22.2	29.7–30.5	8.27–8.32	8.63–9.82
	Autumn	17.5–24.0	26.1–32.3	8.49–8.55	8.54–9.29
	Winter	10.4–12.9	31.5–32.1	8.35–8.50	10.32–12.69
	Mean ± SE	17.92 ± 1.38	30.33 ± 0.49	8.41 ± 0.03	10.04 ± 0.40

(November 2013) in autumn. Minimum dissolved oxygen value was 7.31 mg/l at S1 station in summer (June 2013) and 13.94 mg/l at S2 (November 2013) station in autumn.

When the particle analysis results of the sediments sampled from the stations were examined, it was found that S1 (85.45 %) and S2 (65.68 %) stations were contained in the medium sand class with a size of 250 µm, whereas S3 station (38.13 %) was contained in the fine sand class with a size of 125 µm (Table 3). Another parameter examined in sediment samples is percent burnable substance (BS) value. These values were determined as 3.79 % for S1 station, 3.80 % for S2 station, and 6.33 % for S3 station. It was found that the difference between S1 and S2 stations was insignificant,

whereas the difference between S1 and S3 stations, and S2 and S3 stations, was significant ( $p > 0.05$ ). When BS% values were examined, seasonally maximum values were determined in autumn (4.91 %) for S1 station, spring (4.68 %) for S2 station, and summer (7.63 %) for S3 station. It was detected that BS% values showed statistically significant differences seasonally at the stations ( $p < 0.05$ ; Table 3).

**Trace metal concentrations in sediments**

Trace metal concentrations in sediment samples were 0.28–1.96 (0.70 ± 0.04) mg/kg for Cd, 0.38–10.93 (5.03 ± 0.47) mg/kg for Cu, 2.58–48.01 (14.57 ± 1.27) mg/kg for Pb, 7.94–77.04 (27.15

**Table 3** The trace metal concentrations (mg/kg dw; mean ± SE) and burnable substance (BS; %) and grain size (GS; %) values in sediments during sampling periods

Stations	Periods	Cd	Cu	Pb	Ni	Zn	Fe	BS (%)	GS (%)
S1	Spring	0.47 ± 0.04 <sup>a</sup>	3.17 ± 0.37 <sup>a</sup>	6.45 ± 0.95 <sup>ab</sup>	28.24 ± 5.04 <sup>a</sup>	41.18 ± 8.09 <sup>ab</sup>	5019.1 ± 374.36 <sup>a</sup>	4.78	84.45 medium sand (250 µm)
	Summer	0.50 ± 0.04 <sup>a</sup>	4.63 ± 0.42 <sup>a</sup>	6.04 ± 0.21 <sup>a</sup>	18.55 ± 1.13 <sup>a</sup>	36.96 ± 3.21 <sup>a</sup>	2993.8 ± 263.07 <sup>b</sup>	3.93	
	Autumn	0.45 ± 0.03 <sup>a</sup>	ND	8.29 ± 0.79 <sup>b</sup>	19.96 ± 2.35 <sup>a</sup>	71.95 ± 11.69 <sup>b</sup>	3211.8 ± 379.29 <sup>b</sup>	4.91	
	Winter	0.68 ± 0.15 <sup>a</sup>	ND	6.20 ± 1.64 <sup>ab</sup>	14.65 ± 2.39 <sup>a</sup>	32.44 ± 7.31 <sup>a</sup>	2755.4 ± 292.24 <sup>ab</sup>	1.92	
S2	Spring	1.32 ± 0.25 <sup>a</sup>	3.09 ± 1.09 <sup>a</sup>	28.79 ± 6.41 <sup>abc</sup>	16.14 ± 1.19 <sup>a</sup>	56.73 ± 11.39 <sup>a</sup>	4647.1 ± 647.82 <sup>a</sup>	4.68	65.68 medium sand (250 µm)
	Summer	1.19 ± 0.10 <sup>a</sup>	4.31 ± 0.33 <sup>a</sup>	40.82 ± 1.50 <sup>a</sup>	11.06 ± 0.74 <sup>b</sup>	90.63 ± 2.73 <sup>b</sup>	2828.7 ± 222.48 <sup>a</sup>	3.71	
	Autumn	1.05 ± 0.10 <sup>ab</sup>	5.92 ± 1.00 <sup>a</sup>	33.62 ± 1.01 <sup>b</sup>	10.52 ± 0.12 <sup>b</sup>	128.9 ± 11.99 <sup>c</sup>	2719.4 ± 369.56 <sup>a</sup>	4.57	
	Winter	0.66 ± 0.09 <sup>b</sup>	ND	7.80 ± 1.59 <sup>c</sup>	20.51 ± 2.53 <sup>ab</sup>	33.72 ± 6.25 <sup>a</sup>	2973.8 ± 307.21 <sup>a</sup>	2.48	
S3	Spring	0.59 ± 0.04 <sup>a</sup>	9.94 ± 0.57 <sup>a</sup>	9.91 ± 0.53 <sup>a</sup>	40.95 ± 4.03 <sup>a</sup>	46.24 ± 3.84 <sup>a</sup>	4016.8 ± 115.97 <sup>a</sup>	4.44	38.13 fine sand (125 µm)
	Summer	0.50 ± 0.05 <sup>a</sup>	9.42 ± 0.24 <sup>a</sup>	10.43 ± 0.27 <sup>a</sup>	45.87 ± 2.57 <sup>a</sup>	49.10 ± 7.76 <sup>a</sup>	4226.4 ± 126.73 <sup>a</sup>	7.63	
	Autumn	0.38 ± 0.01 <sup>b</sup>	ND	7.63 ± 0.26 <sup>b</sup>	44.96 ± 3.21 <sup>b</sup>	38.10 ± 1.51 <sup>b</sup>	5110.7 ± 151.36 <sup>b</sup>	6.90	
	Winter	0.59 ± 0.01 <sup>a</sup>	3.96 ± 0.57 <sup>a</sup>	8.87 ± 0.64 <sup>ab</sup>	54.38 ± 5.94 <sup>a</sup>	28.28 ± 1.53 <sup>b</sup>	3861.8 ± 165.68 <sup>a</sup>	6.28	

ND: not detected

<sup>a,b,c</sup>  $p < 0.05$

$\pm 1.65$ ) mg/kg for Ni, 10.17–177.60 ( $54.52 \pm 3.41$ ) mg/kg for Zn, and 1162.7–6714.1 ( $3779.9 \pm 117.42$ ) mg/kg for Fe, respectively. Mean trace metal distribution in sediments was determined as  $\text{Fe} > \text{Zn} > \text{Ni} > \text{Pb} > \text{Cu} > \text{Cd}$ . When seasonal concentration distribution in sediment was examined for each station, it was found that values of Cd, Ni, and Fe for S1 station (maximum in spring), Pb (maximum in summer) for S2 station, and Cu and Zn (maximum in winter) for S3 station were detected at maximum concentrations. It was found that seasonal Cu concentrations accumulated in sediments were not statistically significant ( $p > 0.05$ ). However, it was detected that Cd concentrations for S2 and S3 stations except for S1 station, Ni concentration for S2 and S3 stations, Fe accumulated for S1 and S3 stations except for S2 station, and Pb and Zn concentrations at all stations showed significant differences ( $p < 0.05$ ; Table 3).

As a result of a correlation analysis between physicochemical parameter values measured in seawater and trace metal concentrations determined in sediment, a positive correlation was found between Zn in sediment and temperature ( $r = 0.620$ ,  $p < 0.05$ ), whereas a negative correlation was found with saltness ( $r = -0.686$ ,  $p < 0.05$ ). In addition, a strong correlation was found between Zn and Pb in sediment ( $r = 0.804$ ,  $p < 0.01$ ), while Pb in sediment had a positive correlation between both Cd ( $r = 0.703$ ,  $p < 0.05$ ) and Cu ( $r = 0.576$ ,  $p < 0.05$ ).

#### Trace metal concentrations in *H. tubulosa*

Ranges for annual mean concentrations of trace metals determined in all body tissues of *H. tubulosa* were 0.04–1.66 ( $0.18 \pm 0.02$ ) mg/kg for Cd, 0.01–6.84 ( $1.43 \pm 0.32$ ) mg/kg for Cu, 0.48–5.80 ( $2.09 \pm 0.15$ ) mg/kg for Pb, 6.99–33.76 ( $14.58 \pm 0.65$ ) mg/kg for Ni, 9.27–29.17 ( $16.86 \pm 0.36$ ) mg/kg for Zn, and 24.97–178.30 ( $73.46 \pm 3.22$ ) mg/kg for Fe, respectively. Concentration order in muscle tissue was found as  $\text{Fe} > \text{Zn} > \text{Ni} > \text{Pb} > \text{Cu} > \text{Cd}$ . When seasonal concentration distribution in *H. tubulosa* for each station was examined, it was determined that at S2 station, Cd (maximum in spring), Pb (maximum in summer), and Zn (maximum in autumn) were maximum, and at S3 station, Ni (maximum in winter) and Fe (maximum in autumn) were maximum. It was detected that Ni concentrations accumulated in *H. tubulosa* muscle tissue for S2 and S1 stations and Fe concentration for S2 station showed statistically significant differences ( $p < 0.05$ ). Pb, Cd, Cu, and Zn concentrations at all stations showed significant differences (Table 4).

As a result of a correlation analysis between physicochemical parameter values measured in seawater and trace metal concentrations determined in *H. tubulosa*, a negative correlation was found between Zn in *H. tubulosa* and temperature ( $r = -0.591$ ,  $p < 0.05$ ), whereas a positive correlation was found with dissolved oxygen ( $r = 0.607$ ,  $p < 0.05$ ). In addition, a positive correlation was found between Zn in *H. tubulosa*

and both Cu ( $r = 0.667$ ,  $p < 0.05$ ) and Pb ( $r = 0.610$ ,  $p < 0.05$ ), while Fe in *H. tubulosa* had a positive correlation with Ni ( $r = 0.592$ ,  $p < 0.05$ ). Nevertheless, a strong positive correlation was found between Cu and Pb in *H. tubulosa* ( $r = 0.857$ ,  $p < 0.001$ ), while a strong positive correlation was found with Cd in sediment ( $r = 0.761$ ,  $p < 0.01$ ). Moreover, a positive correlation was found between Fe in *H. tubulosa* and Ni in sediment ( $r = 0.666$ ,  $p < 0.05$ ).

Two-way ANOVA was used to determine the interaction between stations and period. It was detected that Cd and Ni concentrations in sediment for periods showed that statistically significant differences are not obtained ( $p > 0.05$ ; Table 5).

#### Sediment and *H. tubulosa* accumulation factor (BSAF)

BSAF values determined seasonally at the all stations are provided in Table 6. BSAF  $> 1$  was determined for Ni concentration at S1 station in winter and at S2 station in summer, for Cu concentration at S3 station in winter, and for Cd concentration at S1 station in spring. Minimum BSAF values were determined for Fe concentrations.

## Discussion

#### Physicochemical variables

A variety of factors (e.g., temperature, dissolved oxygen, pH, saltness, and particle size distribution in sediment) have an effect on accumulation of metals passing from the water column to the sediment (Chen and Jiao 2008). In this study, water temperatures measured in seawater ranged between 8.7 and 25.1 °C. Minimum temperature was measured for all stations in winter, while maximum temperatures were measured for S1 and S2 stations (25.1 °C) in summer but for S3 stations (24.0 °C) in autumn. In further studies, when the results obtained with similar stations in the Dardanelles Strait were compared, Tuncer et al. (2007) determined as 8.52–26.59 °C for Gelibolu, 10.53–24.26 °C for Lapseki, and 10.8–19.38 °C for Dardanos, and Ateş et al. (2014) determined as 8.70–25.10 °C for Gelibolu, 9.40–25.10 °C for Lapseki, and 10.4–24.00 °C for Dardanos. According to these results, it was detected that the temperature values were low at S1 and S2 stations; however, the temperature values were high at S3 station.

Saltness values at the stations range between 22.40 and 32.30‰. It is reported that lower saltness values during summertime were caused by the effect, duration, and speed of prevailing winds (northeastern and southwestern) that influence the surface currents and undercurrents of the Dardanelles Strait (Polat and Tugrul 1996). It is thought that although surface seawater is under the influence of Marmara Sea, higher saltness values at S3 station were caused by the

**Table 4** The trace metal concentrations (mg/kg dw; mean ± SE) in *H. tubulosa* during sampling periods

Stations	Periods	Cd	Cu	Pb	Ni	Zn	Fe
S1	Spring	0.63 ± 0.23 <sup>ac</sup>	1.70 ± 0.21 <sup>a</sup>	2.18 ± 0.43 <sup>a</sup>	24.16 ± 2.62 <sup>a</sup>	19.67 ± 0.74 <sup>a</sup>	117.63 ± 3.29 <sup>a</sup>
	Summer	0.15 ± 0.02 <sup>abc</sup>	0.52 ± 0.17 <sup>b</sup>	0.71 ± 0.03 <sup>b</sup>	12.40 ± 1.08 <sup>b</sup>	15.19 ± 0.53 <sup>b</sup>	102.93 ± 18.44 <sup>a</sup>
	Autumn	0.13 ± 0.01 <sup>b</sup>	ND	0.74 ± 0.06 <sup>b</sup>	15.66 ± 1.28 <sup>ab</sup>	15.03 ± 0.70 <sup>b</sup>	78.46 ± 13.17 <sup>a</sup>
	Winter	0.17 ± 0.01 <sup>c</sup>	ND	1.50 ± 0.08 <sup>ab</sup>	17.07 ± 0.48 <sup>a</sup>	19.03 ± 0.90 <sup>a</sup>	99.71 ± 9.13 <sup>a</sup>
S2	Spring	0.24 ± 0.04 <sup>a</sup>	0.35 ± 0.02 <sup>a</sup>	3.61 ± 0.55 <sup>ac</sup>	11.63 ± 0.77 <sup>a</sup>	17.95 ± 0.56 <sup>a</sup>	67.13 ± 7.29 <sup>ab</sup>
	Summer	0.13 ± 0.01 <sup>b</sup>	0.02 ± 0.02 <sup>b</sup>	5.34 ± 0.10 <sup>b</sup>	14.62 ± 3.60 <sup>abc</sup>	13.43 ± 0.88 <sup>b</sup>	55.31 ± 4.00 <sup>a</sup>
	Autumn	0.15 ± 0.01 <sup>ab</sup>	1.51 ± 0.02 <sup>c</sup>	3.82 ± 0.16 <sup>c</sup>	8.31 ± 0.36 <sup>b</sup>	12.40 ± 1.00 <sup>b</sup>	50.86 ± 6.80 <sup>a</sup>
	Winter	0.15 ± 0.01 <sup>ab</sup>	ND	1.83 ± 0.08 <sup>a</sup>	14.84 ± 0.69 <sup>c</sup>	17.02 ± 0.23 <sup>a</sup>	83.73 ± 4.91 <sup>b</sup>
S3	Spring	0.14 ± 0.00 <sup>a</sup>	2.27 ± 0.07 <sup>a</sup>	1.47 ± 0.08 <sup>a</sup>	14.19 ± 0.69 <sup>a</sup>	19.79 ± 0.46 <sup>a</sup>	63.55 ± 6.82 <sup>a</sup>
	Summer	0.09 ± 0.01 <sup>b</sup>	0.19 ± 0.04 <sup>b</sup>	0.99 ± 0.05 <sup>b</sup>	18.26 ± 3.81 <sup>a</sup>	16.86 ± 0.37 <sup>b</sup>	54.00 ± 7.55 <sup>a</sup>
	Autumn	0.09 ± 0.01 <sup>b</sup>	ND	1.31 ± 0.21 <sup>ab</sup>	11.23 ± 0.79 <sup>a</sup>	14.78 ± 0.36 <sup>c</sup>	55.55 ± 5.23 <sup>a</sup>
	Winter	0.14 ± 0.01 <sup>a</sup>	6.60 ± 0.12 <sup>ac</sup>	1.51 ± 0.11 <sup>a</sup>	12.66 ± 1.13 <sup>a</sup>	21.27 ± 2.04 <sup>ab</sup>	56.07 ± 3.03 <sup>a</sup>

ND: not detected

<sup>a,b,c</sup> *p* < 0.05

Mediterranean-derived salty water which enters the strait from Aegean Sea as an undercurrent. The study belonging to Türkoglu et al. (2004) supports this idea.

pH values determined in the study range between 8.27 and 8.74 (8.44 for S1, 8.49 for S2, and 8.41 for S3) and are between characteristic values of seawater. As a consequence of a seasonal evaluation, it is detected that for all stations, maximum pH values were measured in autumn, whereas minimum values were measured in summer. Ateş et al. (2014) reported that minimum pH values were measured in spring, but maximum pH values were measured in autumn. Tuncer et al. (2007) detected that pH value at S3 station was minimum in winter (8.1) but maximum in spring and autumn (8.3).

Concerning dissolved oxygen (DO), the standard for sustaining aquatic life is stipulated at 5 mg/l (Chapman 1992). When the concentration values of dissolved oxygen obtained from the study were evaluated, it was detected that maximum oxygen values were found at S1 station in spring and at S2 and S3 stations in winter. Seasonal DO results of Ateş et al. (2014) at similar stations were as follows: maximum values for S1 and S2 in winter and for S3 in autumn and minimum DO for all stations in autumn. In summer with increases in water temperature, biological degradation increases due to bacterial activity; thereby, amount of oxygen is reduced depending on biochemical reactions (Türkoğlu et al. 2004). A similar case was also observed in this study. This difference could be caused by the effect of alterations in speed of biotic events (photosynthesis and respiration) as well as by the effect of abiotic factors (water temperature, saltness, and surface layer) (Türkoğlu et al. 2004).

BS amounts (%) were calculated for each station by classifying the sediment samples from three stations according to particle size, and an analysis was performed for the particle

size (Table 3). It was detected that BS values (%) determined seasonally at the stations were statistically significant (*p* < 0.05). These differences are caused by the factors consisting of meteorological conditions and terrestrial inputs along with surface water currents of Black Sea and undercurrents coming from North Aegean Sea. %BS values (0.87–4.50 % for S1, 1.37–9.86 % for S2, and 2.32–7.29 % for S3) determined by Ateş et al. (2014) at similar stations were very high compared to our results. Ateş et al. (2014) stated that in particular, the organic substance change in sediment increased in autumn and winter months, which was caused by the death of phytoplankton species due to decreases in temperature. Aydın and Sunlu (2004) found that BS values in sediments sampled from a depth of 5 m in South Aegean Sea were 16.8 %. It is reported that in the region between Chios and Çeşme, the organic substance accumulated in sediment due to heavy ship traffic, human being activities, fish farms located in Gulf of Ildır, terrestrial inputs, and wind and water movements in that region. Our study in the Dardanelles Strait is similar with studies of Aydın and Sunlu (2004), in that the organic substance accumulation in that region varies depending on the region, excessive different pollution sources (such as industry and agriculture), human being activity, and heavy ship traffic.

#### Trace metal concentration in sediment

*H. tubulosa* is a living organism which has a close relationship with sediment and provides its food requirement from detritus and organic substances contained in sediment and plays an important role in reusing bottom sediment (Warnau et al. 2006). Lifetime of that organism having a sedentary characteristic is from 4 to 8 years (Warnau et al. 1998, 2006). Based

**Table 5** Results of the two-way ANOVA for heavy metals in *H. tubulosa* and sediment from three stations and periods

Response variable	Source of variation	Sum of squares	df	Mean squares	F value	P value
<i>H. tubulosa</i>						
Cd	Stations	0.451	2	0.226	5.362	0.006
	Periods	0.851	3	0.284	6.745	0.000
	Interaction	0.818	6	0.136	3.242	0.006
Pb	Stations	122.325	2	61.162	131.015	0.000
	Periods	10.403	3	3.468	7.428	0.000
	Interaction	52.909	6	8.818	18.889	0.000
Ni	Stations	437.626	2	218.813	6.954	0.002
	Periods	344.798	3	114.933	3.653	0.015
	Interaction	804.449	6	134.075	4.261	0.001
Zn	Stations	154.609	2	77.305	11.268	0.000
	Periods	544.031	3	181.344	26.433	0.000
	Interaction	24.259	6	4.043	0.589	0.738
Fe	Stations	36,835.645	2	18,417.822	27.242	0.000
	Periods	7,265.076	3	2,421.692	3.582	0.017
	Interaction	5,307.020	6	884.503	1.308	0.261
Cu	Stations	5.862	2	2.931	2.443	0.092
	Periods	8.381	3	2.794	2.328	0.080
	Interaction	28.068	6	4.678	3.898	0.002
Sediment						
Cd	Stations	6.952	2	3.476	37.556	0.000
	Periods	0.490	3	0.163	1.763	0.159
	Interaction	2.291	6	0.382	4.125	0.001
Pb	Stations	9,500.019	2	4,750.010	121.770	0.000
	Periods	1,963.077	3	654.359	16.775	0.000
	Interaction	3,545.715	6	590.952	15.149	0.000
Ni	Stations	20,906.942	2	10,453.471	121.395	0.000
	Periods	456.416	3	152.139	1.767	0.159
	Interaction	1,585.108	6	314.185	3.649	0.003
Zn	Stations	29,010.268	2	14,505.134	29.069	0.000
	Periods	33,055.638	3	11,018.546	22.082	0.000
	Interaction	24,467.630	6	4,077.938	8.172	0.000
Fe	Stations	18,543,080.373	2	9,271,540.186	10.144	0.000
	Periods	23,429,688.287	3	7,809,896.096	8.545	0.000
	Interaction	29,614,446.011	6	4,935,741.002	5.400	0.000
Cu	Stations	8.999	2	4.499	0.662	0.518
	Periods	106.166	3	35.389	5.210	0.002
	Interaction	50.723	6	8.454	1.245	0.291

Significance levels are  $p < 0.05$ 

on this long lifetime, they are thought to be a good indicator for determining the accumulation of sediment trace metals in the organism as they use sediments in their habitats for feeding. Likewise, trace metal concentrations in sediment are not much variable in a short time and show the environment changes very well in a long term (Rubio et al. 2000). The mean trace metal (Cd, Cu, Pb, Ni, Zn, and Fe) concentrations in sediments from the stations were determined as 0.70, 5.03, 14.57, 27.15, 54.52, and 3779.9 mg/kg, respectively. In the

studies, it is reported that the organic substance concentration increased with reducing particle size and clay particles of sediment contained more organic substance, thereby containing more metal concentration (Förstner and Wittmann 1983). According to particle size analysis at the stations, the particle size of sediments was 250  $\mu\text{m}$  at S1 and S2 stations, whereas the particle size at S3 station (Dardanos) was 125  $\mu\text{m}$  and % BS values were higher at S3 station than the other stations. Distribution of trace metals by stations was as follows:

**Table 6** Mean biosediment accumulation factor (BSAF) values

Stations	Periods	Cd	Cu	Pb	Ni	Zn	Fe
S1	Spring	1.34	0.54	0.34	0.86	0.48	0.02
	Summer	0.30	0.11	0.12	0.67	0.41	0.03
	Autumn	0.29	–	0.09	0.79	0.21	0.02
	Winter	0.15	–	0.24	1.17	0.59	0.04
S2	Spring	0.18	0.11	0.13	0.72	0.32	0.02
	Summer	0.11	0.01	0.13	1.32	0.15	0.02
	Autumn	0.14	0.26	0.11	0.79	0.10	0.02
	Winter	0.23	–	0.24	0.72	0.51	0.03
S3	Spring	0.24	0.23	0.15	0.35	0.43	0.02
	Summer	0.18	0.02	0.10	0.40	0.34	0.01
	Autumn	0.24	–	0.17	0.25	0.39	0.01
	Winter	0.24	1.67	0.17	0.23	0.75	0.02

maximum values for Cd, Pb, and Zn at S2 station and maximum values for Cu, Ni, and Fe at S3 station. At S3 station, the coastal structure mainly consists of medium sand while the predominant bottom structure consists of fine sand and mud toward deeper regions (Tuncer et al. 2007; Ateş et al. 2014). The difference of sediment structure effects metal accumulation features. This is because particle size fractions smaller than 63 µm (silt-clay) are the most important carriers of natural and anthropogenic components. Again, these fractions are able to be transferred to very long distances (Balkis and Algan 2005). In particular, as sources of trace metals determined in sediment samples from Dardanos station, it is thought that this increase is caused by being a touristic area, increased population during summertime, sewage discharged from coastal region, and ship waste caused by transit transport yearly as well as human-induced inputs. The results of studies to Süren et al. (2007) support this situation. Ilgar (2011) reported that current played an active role in trace metal concentration of the Dardanelles Strait sediment. It is expressed that the dynamic effect from current allows for high sedimentation ratio and high metal concentrations. In the study, rather high Fe and Ni concentrations were observed. One reason of this increase is thought to be caused by increased organic substance quantity in sediment. In a study executed in a coastal region of Aegean Sea, it was detected that trace metal contamination amount was higher at the points to which the sewage was discharged (Aloupi and Angelidis 2001). Yet, in this region, it is predicted that strong undercurrents from Aegean Sea caused fine and metal-enriched sediments to become resuspended and to disperse into open seas.

There are few studies relating to trace metal accumulation in sediment at selected stations in the Dardanelles Strait. Ilgar (2011) measured mean trace metal concentration in sediment as maximum for Fe and minimum for Pb and Cu in the study performed in the Dardanelles Strait. These results were quite higher than current study values. In the study of Ilgar and Sari

(2008) performed at 13 stations, quite higher concentration values than current data results were detected. In the studies in Marmara Sea when the results of Topçuoğlu et al. (2004) were evaluated, it was observed that Cd and Zn values were high in the current study; only Cd values were high but lower than the results of Okay et al. (2008) compared to data of Ünlü et al. (2008). In Table 7, trace metal concentrations in sediment samples from different regions of Turkey and world were compared to the results of this study, and similarity and differences between pollution statuses of the regions were represented. These trace metal differences could be caused by terrestrial environment surrounding the study areas and industrial, agricultural, and urban activities.

In order to determine whether trace metal in sediment samples obtained from the Dardanelles Strait have detrimental effects, values in Sediment Quality Guidelines (SQGs) were compared to current results (Violintzis et al. 2009; Burton 2002; Persaud et al. 1993). Accordingly, Ni concentration for S1 and S3 stations was slightly higher than lowest effect level (LEL). Cd concentration for S2 station was found to be slightly above LEL and threshold effect level (TEL). Given general mean values, a TEL and LEL increase in Cd and a LEL increase in Ni were observed. It is seen that metal concentrations in the other stations are not detrimental (Table 8).

**Trace metal concentration in *H. tubulosa***

It has been reported that the sea cucumbers could be used as a bioindicator species for determining metal levels in marine environments (Warnau et al. 2006; Coulon and Jangoux 1993; Papadopoulou et al. 1976; Massin 1982; Bulteel et al. 1992). Although it has an economic and ecological importance, there is less information about the sea cucumbers (Ramon et al. 2010). Bechtel et al. (2013) stated that information on trace metal contents in the sea cucumbers was not adequate. One of the first and the most important study belongs to Chang-Lee et al. in 1989. Study data obtained from bivalve species were compared and interpreted by the researchers.

In this study, the mean trace metal (Cd, Cu, Pb, Ni, Zn, and Fe) concentrations in muscle tissues of *H. tubulosa* sampled from three stations were 0.18, 1.43, 2.09, 14.58, 16.86, and 73.46 mg/kg dry weight, respectively. There is no study about accumulation of trace metals in the sea cucumbers living in Turkey’s seas heretofore. Trace metal concentrations determined by different countries in *H. tubulosa* and different sea cucumber species are provided in Table 9. Warnau et al. 2006 specified that metal accumulation changes in *H. tubulosa* varied depending on sampling time, sampling region, and sampling depth. In this study, metal changes varied depending upon stations and seasons. When compared to the other studies, the obtained results showed similarity to the results of Warnau et al. (2006), whereas in another study data belonging



**Table 7** Investigated trace metal levels in sediment from previous studies (mg/kg dw)

Area	Cd	Cu	Pb	Ni	Fe	Zn	References
Dardanelles Strait	0.70±0.04	5.03±0.47	14.57±1.27	27.15±1.65	3,779.9±117.42 0.38 %	54.52±3.41	This study
Dardanelles Strait	–	9–22	8–20	25–55	1.42–3.40 %	34–76	Ilgar 2011
Dardanelles Strait	–	6–50	7–328	6–75	1–3.4 %	21–2.211	Ilgar and Sarı 2008
Marmara Sea	3.3–8.9	60.6–139	23.8–178	–	–	500–1.190	Pekey 2006
Marmara Sea	<0.02–0.5	12.7–30.6	21.6–31.9	20.53–53.88	5.956–14,896	34.1–51	Topçuoğlu et al. 2004
Marmara Sea	<0.02	22–58	<0.1–67	35–165	3.5–6.3 %	88–185	Ünlü et al. 2008
Marmara Sea	2.0	57	92	128	128	128	Okay et al. 2008
Aegean Sea	–	19	22	60	2.79 %	73	Sarı and Çağatay 2001
Pago Gulf	<0.15	10.1	14.4	11.2	9.12	13.0	Denton and Morrison 2009
Kavala Gulf	0.3	21.3	40.1	15.7	–	258	Kamidis et al. 2004
Thermaikos Gulf	0.3–8.4	32–130	38–190	63–130	32–57	84–537	Violintzis et al. 2009

to Papadopoulou et al. (1976) obtained from Gulf of Saronikos, it was observed that Fe had similar concentration and Zn had lower concentration, but when compared to *H. tubulosa* data sampled by Sicuro et al. (2012) from Adriatic Sea, it was observed that Cd, Pb, and Fe concentrations were high; Cu concentration was low; and Zn had similar concentration values to the current study. In the sea cucumbers, Fe and Zn were present in high concentrations, but Cu was present in low concentrations (Chang-Lee et al. 1989). Chang-Lee et al. (1989) compared trace metal contents of the sea cucumbers to specified trace metal studies. Accordingly, they reported that in contrast to mollusca species, Cu concentrations in the sea cucumbers were very low and the differences between metals varied depending on geographical and seasonal changes. In this study, they also reported that Cu concentration was very low in that organism. In these regions, when compared to trace metal values obtained from the other mussel species previously, Cu, Zn, and Fe were found to be low (Çolakoğlu et al. 2012; Çayır et al. 2012). It is seen that only Zn value was high and the other results showed similarity when the results

obtained from this study compared to the results of Chang-Lee (1989). In the studies of Denton et al. (2006), Cd, Pb, and Ni values were lower than the concentrations in the current study samples when trace metal data were compared in the current study, whereas Cu, Fe, and Zn were higher and Ni concentration was lower than the values obtained when evaluated according to the results obtained by Bechtel et al. (2013). Again, from the study of Mageswaran and Balakrishnan (1985), it is seen that Pb, Cu, and Fe concentrations were high, but Cd and Zn concentrations were low; when compared to the results of Matsumoto et al. (1964), it was seen that Pb concentration was high but Fe and Zn concentration were low. Trace metal concentrations obtained from different regions of the world have similarities and differences depending upon pollution statutes and geographical features of the regions and anthropogenic factors. It is thought that this difference is due to the fact that species is sampled from different stations as well as features of terrestrial environment surrounding the regions; food content status based on sediment structure at the stations; metal density for sampled regions; road and maritime traffic-originated

**Table 8** Sediment Quality Guidelines (SQG; mg/kg dw)

Trace metal	Sediment									
	NOAA mg/kg <sup>a</sup>				OMEE mg/kg <sup>b</sup>		This study			
	ERL	ERM	TEL	PEL	LEL	SEL	S1	S2	S3	Mean
Cu	34	270	18.7	108.2	16	110	3.90	4.10	7.77	5.03
Zn	150	410	124	271	120	820	45.63	77.50	40.43	54.52
Cd	1.2	9.6	0.68	4.21	0.6	10	0.52	1.06	0.52	0.70
Pb	46.7	218	30.24	112.18	31	250	6.75	27.76	9.21	14.57
Ni	–	–	–	–	16	75	20.35	14.56	46.54	21.75
Fe (%)	–	–	–	–	2	4	0.37	0.32	0.43	0.38

<sup>a</sup>“Sediment Quality Guidelines NOAA ” (Violintzis et al. 2009; Burton 2002)

<sup>b</sup>“OMEE ” (Persaud et al. 1993)

**Table 9** Comparison of trace element concentrations in the sampled sea cucumbers in the world’s different seas (mg/kg dw)

Species	Cd	Pb	Cu	Ni	Fe	Zn	References
<i>H. tubulosa</i>	0.18±0.02	2.09±0.15	1.43±0.32	14.58±0.65	73.46±3.22	18.86±0.36	This study
<i>H. tubulosa</i>	0.07±0.00	1.16±0.04	2.50±0.14	–	24.50±0.57	17.40±0.42	Sicuro et al. 2012
<i>H. polii</i>	0.07±0.00	0.65±0.03	2.50±0.14	–	19.40±0.28	14.90±0.57	Sicuro et al. 2012
<i>H. tubulosa</i>	0.38–2.84	1.23–18	0.76–5.78	–	15–191	10.1–26	Warnau et al. 2006
<i>H. tubulosa</i>	–	–	–	–	74	36	Papadopoulou et al. 1976
<i>H. floridana</i>	2.54–2.92	0.52–3.56	0.91–1.18	–	–	–	Medina et al. 2004
<i>H. atra</i>	0.04–0.07	<0.26–<0.63	0.71–2.51	<0.15–<0.34	–	12.6–21.2	Denton et al. 2006
<i>Parastichopus californicus</i>	–	–	3.5±0.2	4.0±1.3	184.2±151.4	40.4±4.3	Bechtel et al. 2013
<i>H. scabra</i>	3.0	19.2	9.2	35.5	40–90	6.7	Mageswaran and Balakrishnan 1985
<i>H. atra</i>	1.4	7.2	2.3	14.0	146–452	8.6	Mageswaran and Balakrishnan 1985
<i>Holothuria sp.</i> (ww)	–	14.4	1.9	–	50.0	8.7	Matsumoto et al. 1964 (ed. Eisler 2010)
<i>P. parvimensis</i>	–	–	1.3±0.4	–	21.5±3.3	2.9±2.2	Chang-Lee et al. 1989
<i>P. californicus</i>	–	–	1.0±0.2	–	71.6±9.2	2.3±0.1	Chang-Lee et al. 1989
<i>H. leucospilota</i>	0.040	0.020	–	–	–	–	Noël et al. 2011
<i>H. atra</i>	<0.14	<0.28	1.62	0.37	39.5	17.8	Denton and Morrison 2009
<i>H. atra</i>	72.52	97.52	3.18	–	11.72	24.38	Jinadasa et al. 2014
<i>H. scabra</i>	41.62	34.71	3.45	–	5.03	3.68	Jinadasa et al. 2014
<i>H. floridana</i>	2.731	2.047	1.047	–	–	–	Gonzales et al. 2004
<i>Isostichopus badionotus</i>	0.795	4.311	1.010	–	–	–	Gonzales et al. 2004
<i>Astichopus multifidus</i>	0.416	0.146	0.226	–	–	–	Gonzales et al. 2004

inputs; and pollution arising from industrial, agricultural, and urban activities. There are a variety of reasons for heavy metal pollution in the Dardanelles Strait. First of them is that this region is one of the important trade routes through which freighters pass. Over 600 freighters in average pass through the Dardanelles Strait annually. Transfer of Pb contained in fuels and bilge discharged from ships into water increases metal pollution in the strait. Olgunoğlu and Polat (2007) reported that heavy metals such as Fe, Cu, Pb, and Cd accumulated in high amounts since industrial waste contained high amounts of these metals and that there was a metal pollution arising from transfer of Pb contained in bunker fuel into water because of heavy ship traffic in the region of interest. Moreover, in certain studies, it was reported that increase in Pb concentrations in living organisms was caused by Pb passing to atmosphere from exhaust gases of motor vehicles and thus, elevated atmospheric Pb that reaches marine environment (Maanan 2007). Mol and Üçok Alakavuk (2011) examined the concentration values of Zn, Cu, Cd, Hg, and Pb in tissues of *Mytilus galloprovincialis* sampled from Marmara Sea. It was reported that major pollution factors in Erdek, Gemlik, and South Marmara were originated from transporting of geological metals to rivers, textile, refinery, chemical industry, and anthropogenesis. Üstünada et al. (2011) stated that pollution in this region was increased by

the effect of industrial enterprises, small businesses, and sewage in the Dardanelles Strait. Altuğ et al. (2009) specified that high urbanization rate, industrialization, and marine activities caused the Dardanelles Strait to become dirty and chemical pollution in the seas was carried to the strait by two strong currents arising from saltness difference; thus, water quality in this region is affected adversely.

Seasonal variations seen in metal concentrations were evaluated in a variety of species including *Echinoderms* (Phillips, 1980; Warnau et al. 1998). When seasonal metal distribution was evaluated, maximum metal accumulation was seen in spring (March, April, and May) for Cd, Pb, Ni, and Fe, whereas maximum Cu and Zn levels were observed during winter (December, January, and February). Seasonal study of Warnau et al. (2006) indicated that maximum Zn and Cu concentrations were seen in summer, maximum Pb and Cd concentrations were seen in winter, and maximum Fe concentration was observed in autumn. Seasonal changes in metal concentrations were resulted from changes in available food amounts and reproduction cycle including maturation of gonads and gametes (Bryan 1976; Usero et al. 1997). Yet, reproduction and reproduction time are the ones that are the most important factors having an effect on metal accumulation in organisms (Adami et al. 2002; Ansari et al. 2004; Türkmen and Türkmen 2005; Mubiana et al. 2006; Cardellicchio et al. 2008). Bayne

et al. (1993) reported that seasonal metal changes in mussel tissue varied, which was caused by changes in metabolic activity of the organism. It is reported that some metals in soft tissues of *Mytilus edulis*, a bivalve species, reached their peak levels in spring (Mubiana et al. 2005; Maanan 2008). In another study, it is reported that many of metals accumulate in mussels in high concentrations during spring, autumn, and summer except for Cd (Vlahogianni et al. 2007). Phillips (1976) who has carried out a study in a different mussel species specified that Zn, Cd, Pb, and Cu had seasonal changes and these metals reached their maximum levels at the end of winter. Türk Çulha et al. (2014) reported that in *H. tubulosas* species in the Dardanelles Strait, gonad development accelerated with increases in water temperature during spring (April–May) and spawning took place in August and September. It was thought that metabolic activities of the sea cucumbers were seen mostly in pre-reproduction period and the organism deposited metals in muscle tissue thereof depending on nutrition in pre-spawning period. This process includes March, April, and May (spring). When study data was evaluated, Pb, Cd, Fe, and Ni concentrations were found high in these months. Organic substance amount detected in sediment was found maximum in autumn. Borchardt et al. (1988) reported that low condition values in mussels cause high metal concentrations, resulting in maximum levels especially at the end of winter and in the beginning of spring. Bayne et al. (1993) specified that there was a decrease in mussel meat yield with food amount and temperature decreases in the environment during winter, and in this period, Zn and Cu concentrations were high in tissues of the organism. Although Zn and Cu metals were present in mollusca tissue (Apeti et al. 2005), Zn and Cu concentrations in *H. tubulosa* were not detected in high amounts. It is thought that the reason of high amounts of Zn and Cu determined especially in winter is because of charcoal and vehicle fuels (atmospheric originated input) used as fossil fuel such as canalization and domestic wastewater discharged without any refinement. Yet, metals bound to the organic substance can be mixed with water by dissolving as terrestrial and coastal pollution is increased during summertime and heavy rains are seen in spring.

The obtained results were compared to the national and international limit values in fish and mollusca species. Accordingly, Cd and Pb values obtained from muscle tissue of *H. tubulosa* were determined above suggested “acceptable” trace metal values when compared to the limit values determined for fish species by Turkish Food Codeks (TFC) (2005) (0.05 mg/kg for Cd, 0.30 mg/kg for Pb, 20 mg/kg for Cu, and 50 mg/kg for Zn). It was seen that only Pb exceed the limit value when values determined for mollusca species by TFC (2005) (1.00 mg/kg for Cd, 1.50 mg/kg for Pb, 20 mg/kg for Cu, and 50 mg/kg for Zn) were compared. When compared to tolerable values specified for fish species by international organizations (Rouane-Hacene et al 2012; Papagiannis et al.

2004; Çevik et al. 2008), it was detected that Cd and Pb values determined among metal concentrations in muscle tissues of *H. tubulosa* were high according to EU (2010) (0.05–0.1 mg/kg for Cd and 0.2–0.4 mg/kg for Pb) and FAO (2009) (0.05 mg/kg for Cd and 0.30 mg/kg for Pb), and when compared to tolerable values determined for mollusca species, it was detected that Cd concentration was higher than limit values (1 mg/kg for Cd, 1 mg/kg for Pb, 6 mg/kg for Cu, and 75 mg/kg for Zn) determined by Spain (<1–1.4 mg/kg for Cd, 1–3 mg/kg for Pb, 5–7 mg/kg for Cu, and 176–316 mg/kg for Zn) and USA (1 mg/kg for Cd, 1 mg/kg for Pb, 6 mg/kg for Cu, and 75 mg/kg for Zn), whereas Pb concentration was found higher than tolerable values determined by FDA (2001) (0.2 mg/kg for Cd, 1.5 mg/kg for Pb, 100 mg/kg for Cu, and 150 mg/kg for Zn) and USA.

### Metal comparisons of sediment and tissue

Bioaccumulation of metals by organism occurs if the BSAF is >1 (Aydin-Onen et al. 2015). The efficiency of metal bioaccumulation in *H. tubulosa* was evaluated by calculating the BSAF, which is defined as the ratio between the metal concentration in the organism and that in sediment (Lau et al. 1998). Maximum BSAF values determined in the study were found for Ni at S1 and S2 stations, for Cd at S1 station, and for Cu at S3 stations (Table 6). In the study, as Ni accumulated in all body tissues of the organism in high amounts, we can say that *H. tubulosa* is a good indicator in determining the trace metal Ni in the environment.

### Conclusions

As a result, the obtained data suggest that working area is becoming dirty slowly. It was detected that the sea cucumbers are successful species which could be used in pollution monitoring studies as well as mollusk species. High Cd and Pb concentrations determined in muscle tissue of *H. tubulosa* sampled from the Dardanelles Strait indicate the presence of trace metals in that working region. High Ni values determined in muscle tissue of *H. tubulosa* according to BSAF suggest that this organism is an invertebrate which can be used as a “bioindicator species” in pollution monitoring studies. Given long lifetime of the sea cucumbers, it is considered that metal compounds in sediment passes not only via food but also adsorption onto organism’s body; thus, this accumulation inside the organism’s body can cause commercial and economic problems.

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