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Heavy Metal Concentrations in Razor Clam (*Solen marginatus*, Pulteney, 1799) and Sediments from Izmir Bay, Aegean Sea, Turkey

Elif Çağrı Taş[1*], Uğur Sunlu[2]

[1*]*Aquaculture Department of Basic Sciences, Faculty of Fisheries, Ege University, 35100 Bornova/Izmir, Turkey*
Corresponding author, E-mail: e.cagri.tas@ege.edu.tr, ORCID: https://orcid.org/0000-0001-6478-9205
[2]*Aquaculture Department of Basic Sciences, Faculty of Fisheries, Ege University, 35100 Bornova/Izmir, Turkey*
E-mail: ugur.sunlu@ege.edu.tr, ORCID: https://orcid.org/0000-0001-8759-4929

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A B S T R A C T

This study was carried out to determine the concentrations of some heavy metals (Cd, Cu, Pb, Zn, Cr and Fe) in *Solen marginatus* (Pulteney, 1799) and sediments in the middle region of Izmir Bay. Metal concentrations in *S. marginatus* vary in the one-year period between summer 2005 and summer 2006. The order of accumulation of metal concentrates in soft tissue of razor clam was determined as Cd < Pb < Cr< Cu < Zn < Fe. Metal accumulations in the soft tissue of *S. marginatus* were compared with Provisional Tolerable Weakly Intakes (PTWI) and Provisional Tolerable Daily Intakes (PTDI) for human consumption. The results show that the maximum concentrations of metals were markedly below the limits of the FAO (Food and Agriculture Organization), WHO (World Health Organization) and TFC (Turkish Food Codex) for human consumption. The order of the metal concentrations detected in the sediment samples was Cd < Pb < Cu< Cr < Zn < Fe. In this study, the maximum heavy metal values determined in the sediment are below the criteria values of the stated for international sediment quality guidelines in the NOAA (The National Oceanic and Atmospheric Administration), OMEE (The Ministry of Environment and Energy of Ontario), ANZECC (The Australia and New Zealand Environmental on Conservation Council), CCME (The Canadian Council of Ministers of Environment). There was a statistically significant but weak correlation between concentrations of Cu and Cr in sediment and *S. marginatus.* The significant correlations have shown that Solen species can be used as a bioindicator species, such as mussels due to their ability to accumulate heavy metals.

Introduction

Heavy metals are major anthropogenic pollutants of estuarine and coastal water. As they live in close contact with the sediment for long periods and due to their feeding activity, benthic macroinvertebrate species can accumulate as large amounts of environmental contamination and offering the possibility of use as bioindicators of metal pollution in coastal areas (Rainbow, 1997, Freitas et al. 2012). Marine and estuarine animals for instance mollusk inclined to collect heavy metals in their body tissue from food, water and ingestion of particulate matter and so transferred the heavy metals through the food chain (Netpae and Phalaraksh 2009; Yap et al. 2009). Bivalves accumulate metals such as copper and zinc and can tolerate very high metal concentrations without apparent signs of any detrimental effect (Lin and Hsieh, 1999). Razor clams are members of Class Bivalvia, characterized by two long, narrow shell valves gaping at both ends but connected by hinges, two siphons and one strong foot. Razor clam in Izmir Bay belongs to the genus *Solen*. *Solen marginatus* lives buried in sand and/or muddy sand substrate in low intertidal and subtidal areas and they are distribution from Norway to the Mediterranean and Black Sea, and West Africa (Hayward and Ryland, 1998). It is used as a food material in many countries, particularly in Japanese, China, Taiwan, Malaysia, France, Spain, Italy, England.

Some papers have been published concerning heavy metal concentrations observed in mollusk (Egemen et al. 1994; Sunlu et al. 1998, Sunlu 2002, 2006; Ozsuer and Sunlu, 2013), fish samples (Sunlu et al.2001; Kucuksezgin et al. 2011) polychaetes samples (Taş et al. 2009, 2018) at the Izmir Bay. However, there hasn't been published study on the heavy metal levels in razor clam (*Solen marginatus*) sample. On the other hand, numerous studies have been published on the heavy metal levels in sediment samples (Kucuksezgin 2001, Kucuksezgin et al. 2006, 2008; Özkan, 2012; Atalar et al. 2013, Sert, 2018).

In this study it is aimed to investigate the seasonal variation of selected heavy metals (Cd, Pb, Cu, Cr, Zn and Fe) in the soft tissues of *Solen marginatus* and the sediment their inhabit as well as to determine its usability as a bioindicator. In this study it is aimed to investigate the seasonal variation of selected heavy metals (Cd, Pb, Cu, Cr, Zn and Fe) in the sediment and in the soft tissues of *Solen marginatus* as well as its to determine usability as a bioindicator.

Materials and Methods

Sampling Location

Izmir Bay is located in the eastern Aegean Sea between latitudes of 38º 20' and 38º 42' N and longitudes of 29º 25' and 27º10' E and is one of the great natural bays of the Mediterranean. The bay has a total surface area of over 500 km^2, water capacity of 11.5 billion m^3, a total length of 64 km and opens in the Aegean Sea (Kucuksezgin et al., 2006). Izmir Bay is naturally divided into three sections; Inner, middle and outer bays. The middle bay is separated from the inner bay by a 13 m deep sill the Yenikale Strait. The maximum depth of the middle Bay is 45m. (Aksu et al., 1998). The study area is between Degaj Shipyard and Çilazmak Lagoon in the north of Pelikan Lighthouse located in the middle bay part of Izmir Bay. This region is dominated by the *Cymodocea nodosa* (Phanerogamae) facies. The depth is between 0.50-1.00 m and the bottom structure is in the muddy sand feature.

Sample Collection, Preparation and Digestion Procedure

The samples were collected seasonally (Summer 2005-Summer 2006) from 2 stations at old shipyard area in Izmir Bay. *S. marginatus* samples (n = 90) of similar size (shell length = 6-10 cm), were collected randomly by hand picking from each sampling area.

All specimens were kept in sediment and transported to the laboratories in the Faculty of Fisheries, Ege University. The razor clam samples were washed with tap water and distilled water to remove sediment and debris. The soft tissue of razor clams were carefully dissected from the shell using clean stainless steel equipment. Then soft tissues were pooled, mixed and homogenized. Triplicate analyses were conducted for all tissues and each sampling location. The tissues homogenates were prepared according to international standard methods. The composite samples of each tissue were measured as wet weight by XB 22OA, Precisa (Zurich, Switzerland) (with a precision of 0.0001 g) and stored in a freezer at -20°C until analysis and digested with conc. HNO_3: $HClO_4$ (5:1) (extra pure Merck) under reflux and filtered (Bernhard,1976). Inter-calibration homogenate samples (IAEA-142/ TM) for mollusks and (IAEA-314) for sediment from the IAEA, Monaco Laboratory were used as a quality control for the analytical methodology. Homogenate samples were digested and analysed along with each series of samples. Surface sediment samples were collected using a Van Veen grap from each station around the area inhabited by the razor clams. Sediment samples were placed in polyethylene bags, transported to laboratory and kept in freezer at -20 C until analysis. One gram (<160 μ) of oven-dried sediment samples was digested for 12 hours with freshly prepared mixture 10 ml. HCl : HNO_3 (3:1) (extra pure Merck) using hot plate digester (Arnoux et al.. 1981). Digested tissue and sediment samples were diluted to 50 ml with distilled water and filtered through filter paper. Heavy metal concentrations (Cd, Cr, Cu, Pb, Zn, Fe) were analysed using Inductively Couppled Plasma-Optical Emission Spectrometer, (ICP-OES), (Perkin Elmer 2000 DV). All samples were analysed in triplicate and the results were expressed as mg/kg weight.

Data Analysis

Normality of data and homogeneity of variances were verified using the Levene test. In order to find the differences among sampling sites (in terms of level of heavy metal in *S. marginatus* and its sediment), one-way ANOVA was performed, followed by the least significant difference (LSD) post hoc test. Pearson's correlation coefficients were used to test the relationships between the concentrations of metals in sediments with the soft tissue of *S. marginatus*. All statistical analysis was performed according to Zar (1999). Statistically significant differences were expressed as $P \leq 0.005$.

Health Risk Assessment

The risk to human health as a result of mollusks consumption was evaluated by calculating the weekly heavy metal exposures and comparing the values with the respective provisional tolerable weekly intake (PTWI). The permissible weekly intake of heavy metals as Provisional Tolerable Weekly Intake (PTWI), are set by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA) (FAO/WHO, 2010; 2011). The PTWI is defined as the estimated amount of a substance in food or drinking water, expressed on a body weight basis (mg/kg or μg/kg of body weight), that can be ingested weekly over a lifetime without appreciable health risk (FAO/WHO, 2010). The average daily mollusks and other seafood consumption in Turkey is 1 g/person (FAO, 2010). Multiplying this value by the mean level of the heavy metals in edible tissues of other seafood species, the mean daily intake of metals from mollusks can be calculated. The EWI and EDI values were calculated by considering that a 70-kg person will eat 1 g seafood/day, which correspond to 7 g seafood/week. The estimated weekly intakes (EWI) were calculated from daily intake (EDI) (Türkmen et al. 2008, 2009).

Results and Discussion

Heavy Metal Concentrations in Solen marginatus

Razor clams live buried in sediment and capable to accumulate high concentrations of heavy metal in tissues as they are filter-feeders (Hassan and Kanakaraju, 2013). When seasonal concentration distribution in *S. marginatus* was examined for each station, it was found that values of Cd and Fe were maximum in Autumn 2005 and maximum Zn concentration was detected in Winter 2006. Cu for both stations were detected at minimum concentrations at Spring 2006 (Figure 1 and 2). It was found that Cd, Cr, Cu and Zn concentrations accumulated in soft tissue of *S. marginatus* showed significant differences ($P \leq 0.05$) at two stations. However, Pb and Fe concentrations at two stations showed statistically insignificant differences ($P \geq 0.05$). In this study, the minimum and maximum heavy metal concentrations in *S. marginatus* samples were 0.04–0.19 mg/kg d.w. for Cd, 0.010-0.202 mg/kg d.w. for Pb, 0.427-4.330 mg/kg d.w. for Cu, 0.110-1.387 mg/kg d.w for Cr, 7.25-13.70 mg/kg d.w for Zn, and 39.00-134.00 mg/kg d.w for Fe, respectively. Concentration order in soft tissue was found as Cd < Pb < Cr< Cu < Zn < Fe.

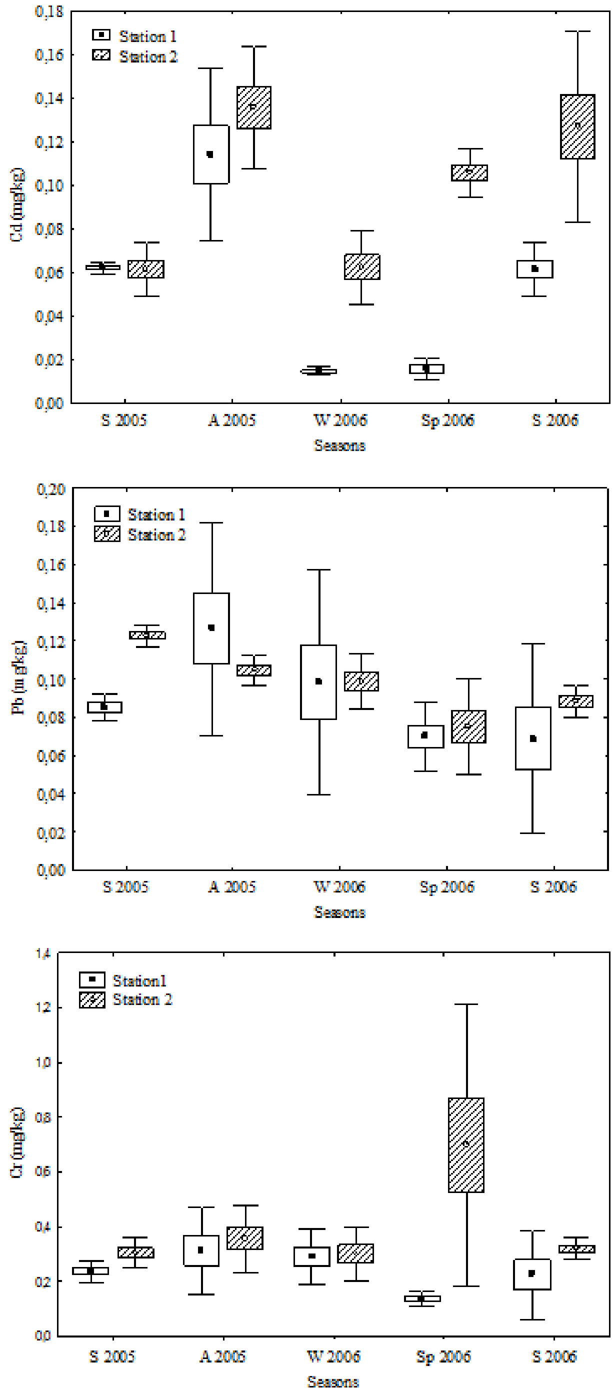

Figure 1 Mean, standart eror and confidence interval (±1.96* σȳ) in *S. marginatus* for Cd, Pb, Cr

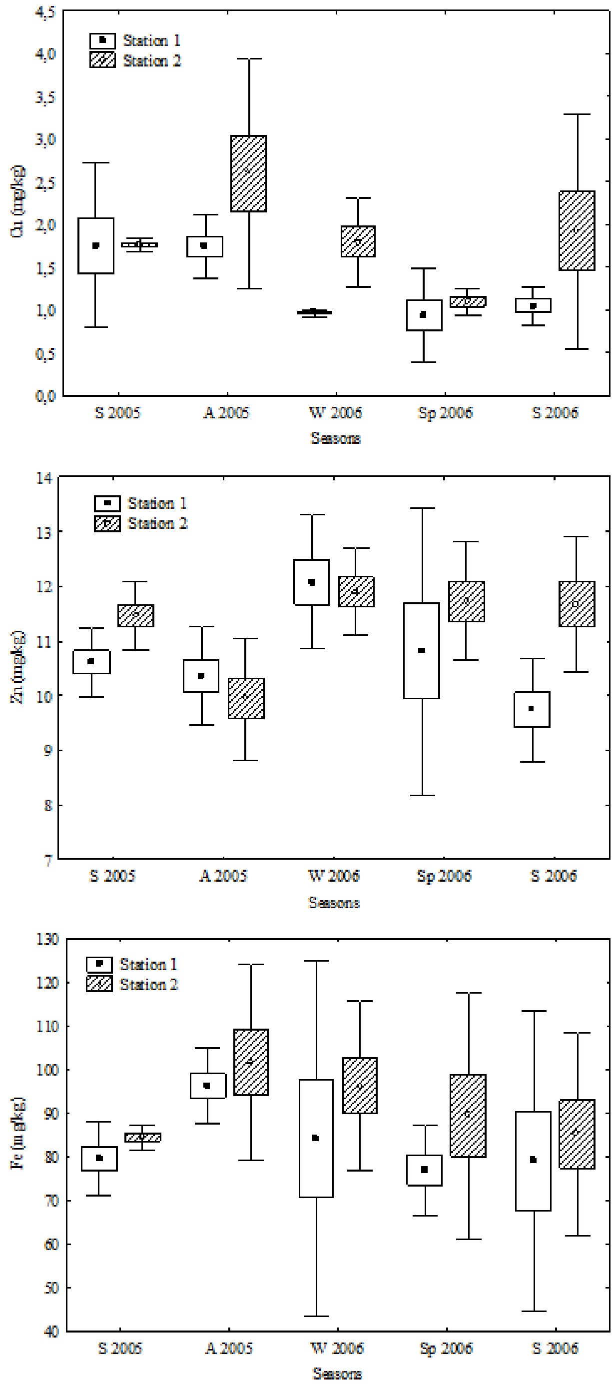

Figure 2 Mean, standart eror and confidence interval (±1.96* σȳ) in *S. marginatus* for Cu, Zn, Fe.

There is no published data in scientific literature about heavy metal levels of *S. marginatus* from the Izmir Bay (Aegean Sea, Turkey). For this reason, it is impossible to compare any conclusion about metal levels in the *S. marginatus* in Turkey. Various researchers have addressed measurements of Cd, Pb, Cr, Cu, Zn and Fe in *Solen sp.* from different regions of the world, and some of these data are summarized in Table 1. When compared to the other studies, there were differences between metal concentration in this study and those of previous studies. Heavy metal concentrations in soft tissues of *S. marginatus* were lower than all previous other studies. This result also showed that Izmir Bay at Turkish coast was less polluted than any different location of the world.

Heavy Metal Concentrations In Sediments

Sediment samples were collected from each sampling stations around the area inhabited by the razor clam. Heavy metal concentrations in sediment samples were 0.043–0.199 mg/kg d.w. for Cd, 2.51-5.45 mg/kg d.w. for Pb, 1.95-15.3 mg/kg d.w. for Cu, 14.5-32.8 mg/kg d.w for Cr, 28.2-36.6 mg/kg d.w for Zn, and 5460–10700 mg/kg d.w for Fe, respectively. The order of the mean metal concentrations found in sediment was Cd < Pb < Cu< Cr < Zn < Fe. Fe was also the most abundant element at both sampling stations. When seasonal concentration distribution in sediments were examined for each station, it was found that values of Cd, Pb, Cr and Fe were minimum in Autumn 2005. For both stations, Cu and Zn were detected at minimum concentrations in Spring 2006 (Figure 3 and 4). According to our analysis, Pb, Cu, Cr, and Fe concentrations in sediments were significantly different than each other in two stations (P≤ 0.05). It was found that Cd and Zn concentrations accumulated in sediment at the stations were not statistically significant (P≥0.05). When compared to the other studies, there were differences between metal concentration in this study and those of previous studies. Heavy metal concentrations in sediment were lower than all previous other studies in Izmir Bay (Table 2). This is because that the works carried out in the previous years include the inner part of the Izmir Bay as well as the middle part. Izmir inner bay area contains more intense pollution than the middle part.

The Ministry of Environment and Energy of Ontario (OMEE, 1993), the National Oceanic and Atmospheric Administration (NOAA, 1999), the Australia and New Zealand Environmental on Conservation Council (ANZECC, 2000), the Canadian Council of Ministers of Environment (CCME, 2001), published the "Sediment Quality Criteria" regulation to determine the impacts of pollutants on sediment for the protection of life. Although these values are not intended to be used as regulatory rules, they may be useful for determining the potential toxicity of contaminant sediments and classifying sediments as "reference" or "degraded" (Miller et al., 2000). There is no national regulation on heavy metal quality criteria in sediments. The values determined in the regulation and the maximum values determined in the study are given in Table 3. In this study, the maximum heavy metal values determined in the sediment are below the criteria values stated in the international sediment quality guidelines.

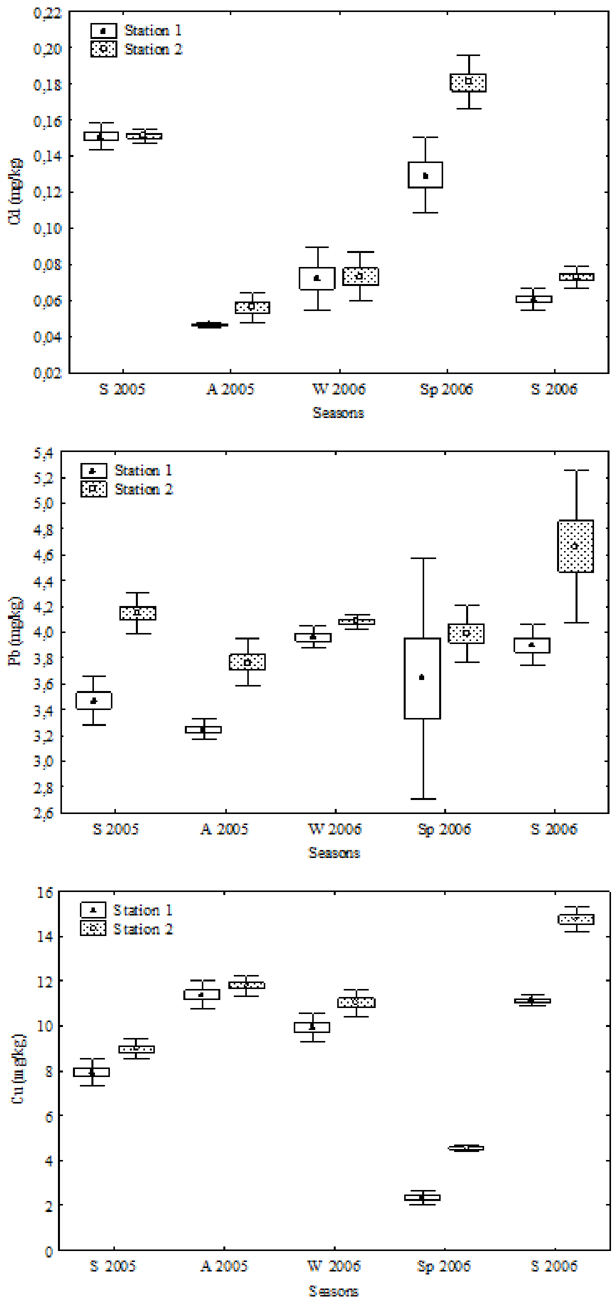

Figure 3 Mean, standart eror and confidence interval (±1.96* σȳ) in sediment for Cd, Pb, Cu.

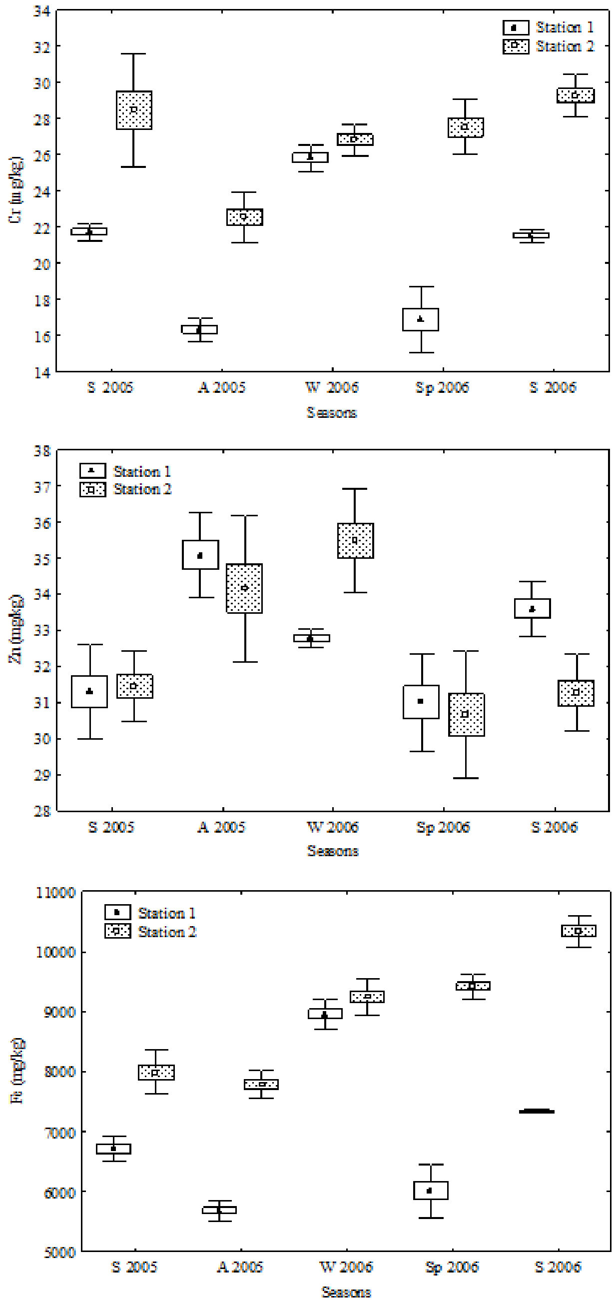

Figure 4 Mean, standart eror and confidence interval (±1.96* σȳ) in sediment for Cr, Zn, Fe.

Table 1 Heavy metals concentrations in the soft tissue of *Solen* species from the different regions of the world (mg/kg).

Study Area	Species	Cd	Pb	Cr	Cu	Zn	Fe	R
Taiwan	*S.strictus*	0.54	ND	-	6.00	55.5	-	(1)
Sarawak	*Solen spp*	0.67-2.83	9.50-16.17	-	2.17-10.67	72.17-97.00	-	(2)
Sarawak	*S. regularis*	0.7	-	30.5	6.50	-	614.7	(3)
Tanjung Lumpur	*S. brevis*	0.67	1.61	-	8.64	87.74	415.2	(4)
Sungai Sarawak	*S. regularis*	2.35	4.85	8.12	2.21	27.08	177.82	(5)
Persian Gulf	*S. dactylus*	ND	3.00-27.0	3.00-8.00	10.00-24.00	55.0-76.0	500.0-1900.0	(6)
Persian Gulf	*S. brevis*	0.67	4.38	-	-	63.3	-	(7)
K. Selangor	*S. sarawakensis*	21.08	-	23.8-31.8				(8)
Izmir Bay	*S.marginatus*	0.09-0.19	0.01-0.20	0.11-1.38	0.47-4.33	7.25-13.70	39.0-134.0	(9)

R: References, 1: Jeng et al. 2000, 2: Kanakaraju et al. 2008, 3: Kanakaraju et al. 2008, 4: Kamaruzzaman et al. 2010, 5: Yusoff and Long 2011, 6: Saeedi et al. 2012, 7: Salahshur et al. 2012, 8: Hassan and Kanakaraju (2013), 9: This study

Table 2 Heavy metals concentrations in the sediment from the Izmir Bay (mg/kg).

Study Area	Cd	Pb	Cr	Cu	Zn	Fe	R
Izmir Bay	-	13.2-304.9	-	16.1-213.4	60.7-888.9	14898-48770	(1)
Izmir Bay	1.6-3.7	24.1-54.5	-	7.5-28.5	11-68.2	-	(2)
Izmir Bay	0.22	36.0	210.0	32.0	120.0	53700	(3)
Izmir Bay	-	6.7-103	-	4.1-79	14-311	-	(4)
Izmir Bay	0.005-0.82	14-113	29-316	-	-	-	(5)
Izmir Bay	0.005-0.82	3.10-119	19-316	2.20-109	14-412	-	(6)
Izmir Bay	0.35	128.92	259.43	45.98	177.03	-	(7)
Izmir Bay	0.0043-0.199	2.51-5.45	14.45-32.80	1.95-15.30	28.20-36.60	5460-10700	(8)

R: References, 1: Gey and Mordoğan, 1988, 2: Egemen et al., 1998, 3: Atgin et al. 2000, 4: Küçüksezgin. 2001, 5: Küçüksezgin et al. 2006, 6: Küçüksezgin et al. 2011, 7: Özkan, 2012, 8: This study

Table 3 Criteria values according to marine sediment quality regulations (mg/kg d.w.)

Marine Sediment (mg/kg d.w.)		Heavy metals					
		Cd	Cr	Cu	Pb	Zn	Fe
CCME*	ISQG	0.7	52.3	18	30.2	124	-
	PEL	4.2	160	108	112	271	-
NOAA**	TEL	0.676	52.3	18.7	30.24	124	-
	ERL	1.2	81	34	46.7	150	-
	PEL	4.21	160.4	150	112.18	271	-
	ERM	9.6	370	270	218	410	-
OMEE***	LEL	0.6	26	16	31	120	20000
	SEL	10	110	110	250	180	40000
ANZECC****	STV	1.5	80	65	50	200	-
This study	Max.	0.199	32.80	15.30	5.45	36.60	10700

* Canadian Council of Ministers of the Environment (CCME), 2001; ** National Oceanic & Atmospheric Administration (NOAA), "Sediment Quality Guidelines" 1999; *** Ontario Ministry of Environment & Energy (OMEE), 1993; **** Australian and New Zealand Environment on Concervation Council (ANZECC), 2000.

Table 4 Estimated Weekly Intake (EWI) and Estimated Daily Intake (EDI) of heavy metals in clams.

Metals	PTWI[a]	PTWI[b]	PTDI[c]	EWI[d]	EDI[e]	TFC[f]
Cd	0.007	0.49	0.07	0.00133	0.00019	1
Pb	0.025	1.75	0.25	0.00141	0.00020	1.5
Cu	3.5	245	35	0.03031	0.00433	20
Cr				0.00970	0.00138	-
Zn	7	490	70	0.0959	0.0137	50
Fe	5.6	392	56	0.938	0.134	-

[a]PTWI (Provisional Tolerable Weekly Intake) mg/week/kg body wt; [b]PTWI for 70 kg adult person (mg/week/kg body wt.); [c]PTDI (Permissible Tolerable Daily Intake) (mg/day/70 kg body wt.); [d]EWI (Estimated Weekly Intake) mg/week/kg body wt.; [e]EDI (Estimated Daily Intake) mg/week/kg body wt.; [f]TFC (Turkish Food Codex) The tolerable values in mollusca.

Correlation of Sediment and Solen marginatus

Marine mollusks are often used as indicators of metal pollution, because a positive (linear) relation is assumed between the concentrations of the pollutant in the environment and the animal (Bryan, 1980, Bryan, 1984, Bryan and Langston, 1992). According to correlation analysis between heavy metal concentrations in *S. marginatus* and its sediment, a positive correlation was found for Cu (r= 0.32 P≤0.05) and for Cr (r= 0,36 P≤0.05). Concentration of Cu and Cr in *S. marginatus* correlated significantly with their respective concentrations in sediment (Figure 5). On the contrary, no significant correlations were found between Cd, Pb, Zn and Fe levels in the razor clam tissues and its sediment. Yap et al. (2002) reported significant correlations between Cu in the total soft tissue of *Perna viridis* and the total Cu (r = 0.66, P≤0.05) in the sediment. However, Hummel et al. (1997)

found insignificant correlation between copper concentration in sediment and soft tissue of mussels or cockles. The Cu concentration of clams reflects the Cu concentration of the sediment, whereas that of the cockles and mussels is not related to the sediment concentrations (Hummel et al. 1997). Similarly, Absil et al. (1996) observed that the accumulation of copper by clams was influenced by the sediment type. The bioavailability of metal level in different geochemical fractions of sediment is not same, leading different bioavailability of metal to organisms (Salahshur et al. 2014).

Health Risk Assessment Heavy Metals in Solen marginatus

The PTWI is the maximum level of an element to which a person can eat per week over a length of life with no risk of health effects. The average daily mollusks and other seafood consumption in Turkey is 1 g/person (FAO, 2010). The estimated weekly intake (EWI) and estimated daily intake (EDI) values were calculated by considering that a 70-kg person will eat 1 g seafood/day, which correspond to 7 g seafood/week, respectively. The EWIs and EDIs were given in Table 4. As it is understood from Table 4, when the amounts of weekly (EWI) and daily (EDI) consumption were compared with the amounts of internationally usable weekly intake (PTWI) and daily intake (PTDI) and Turkish Food Codex (TFC) results were within normal limits. As a result, the calculated EWIs and EDIs of the heavy metal values are usually beneath the recommended PTWIs and PTDIs or TFCs and specified no bad-tempered effects to the consumers.

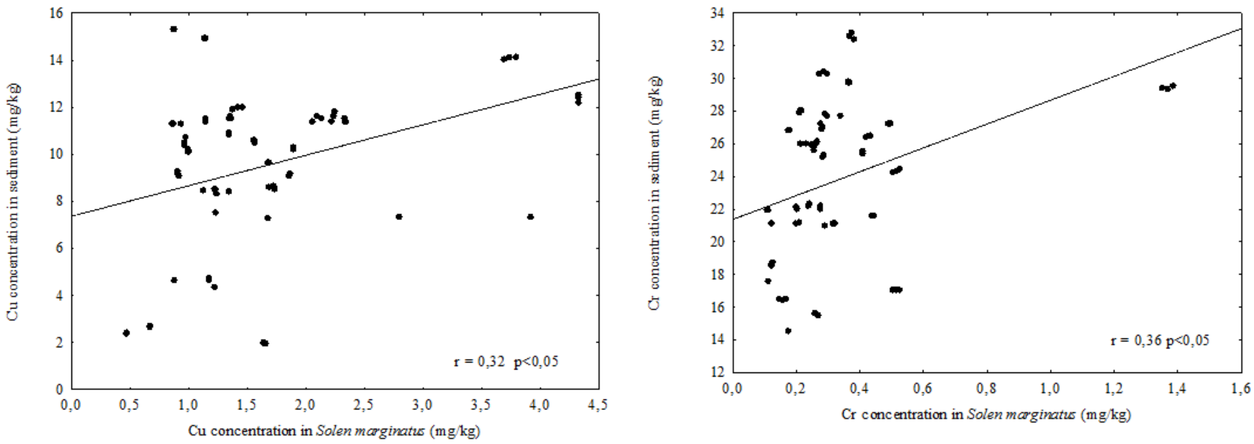

Figure 5 Correlation of Cr,Cu concentrations between sediment and *S. marginatus* (n=90).

Conclusion

The results presented above clearly demonstrated that the maximum heavy metals concentrations in Solen species from Izmir Bay were lower than from the different regions of the world. The heavy metal levels in *Solen marginatus* are below the proposed limit values for human consumption. As it is understood from conclusions, the calculated EWIs and EDIs of the heavy metal values are usually beneath the recommended PTWIs and PTDIs or TFCs and specified no bad-tempered effects to the consumers. The significant correlations between Cu and Cr in the total soft tissue of *S. marginatus* and the total Cu and Cr in the sediment has shown that Solen species can be used as a bioindicator species, such as mussels due to their ability to accumulate heavy metals. In this study, the maximum heavy metal values determined in the sediment are below the criteria values stated in the international sediment quality guidelines. When evaluated in this respect, Izmir middle bay sediments are not under threat of heavy metals but should be monitored periodically.

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