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RESEARCH ARTICLE

SOME MORPHOLOGICAL TRAITS AND HEAVY METAL ACCUMULATION IN MUSCLE TISSUE OF *Ruditapes decussatus* (Linnaeus, 1758)

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ABSTRACT

This study aimed to investigate some morphological characteristics and heavy metal accumulation in muscle tissue of *Ruditapes decussatus* (Linnaeus, 1758) in the spring and autumn seasons of 2018 in the Yumurtalık Coastline of İskenderun Bay located in the northeastern Mediterranean Sea. To this end, shell length (SL), total weight (TW), shell height (SH), shell inflation (SI), shell weight (SW), roundness index (RI), cup index (CI) and total length-weight relationship (LWR) of Bivalvia and heavy metal accumulation in the muscle tissue of Bivalvia were measured for two seasons. Minimum and maximum values of SL, TW, SH, SI, SW, RI and CI were determined as 21.50-39.00 mm, 0.80-22.15 g, 10.50-28.70 mm, 9.11-20.90 mm, 3.08-4.66 g, 1.03-2.14 and 0.58-0.67 respectively. The relationship between total length and total weight of *R. decussatus* was calculated to be $W=0.0052*SL^{2.54}$ for spring and $W=0.0031*SL^{2.88}$ for autumn. The growth type of Bivalvia specimens was determined as isometric growth (2.971). Heavy metal accumulation in the muscle tissue in spring and autumn seasons was found as statistically significant only for zinc and copper in different seasons ($P<0.05$). Biometric data of Bivalvia speciemens and muscle tissue heavy metal level are given and discussed in comparison with the results obtained from other studies. Considering the height-weight relationship, growth type and tissue heavy metal accumulation, it can be said that this area is ecologically suitable for *R. decussatus*.

Keywords: Bivalvia, *Ruditapes decussatus*, Morphological traits, Heavy metal

1. INTRODUCTION

Biometric measurements based on the morphology of the studied Mollusca species are very important in terms of giving information about the population. The shells of Mollusca, which have an important place among the living groups where morphological studies are carried out, can show a variable structure by being affected by environmental and climatic changes [1, 2]. The bivalves are in some ways the most highly modified of all the mollusks [3] and shell part of mollusks has variable structure in the chaotic area [1]. It has been reported in various studies that growth changes in bivalves are used to obtain information about the conditions that cause global and local environmental changes [4, 5]. *R. decussatus* is a member of the family Veneridae. This family (common name: venus clams) is a very large family of marine bivalve mollusks, and hence, makes up a significant proportion of bivalves. A great proportion of Veneridae family is exploited as food sources in other countries such as Portugal, Egypt, and Spain [6, 7, 8, 9]. It has been suggested to grow members of this family in wastewater from aquaculture [9]. Additionally, it has been reported that some species belonging to Veneridae family prefer suspension, endogenous, and coralligen beds as their habitat and can go down to 700 m [10]. Veneridae family lives in areas protected from possible strong waves such as sheltered bays and sea lakes because it is a rare and sensitive bivalve species; also, it belongs to the group of endogenous suspensivores, burying itself at a certain depth in loose sediments [10, 11]. Distribution of *R. decussatus* has been reported along the Atlantic coast, from Norway to Congo and on the West and South coasts of the British Islands, and in the North Sea only in Norway and Denmark and the Atlantic Ocean. In addition, it can be found through the Mediterranean Sea as well as in the Red Sea where this species migrated through the Suez Canal. *R. decussatus* also exists from the south to western Morocco and Senegal, West Africa [12, 13]. Since mussels and oysters feed by filtering the water, they can

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absorb the metals in the water. They can accumulate heavy metals in the processes of exchanging and binding dissolved ions in water with their absorption in water to get nutrients. Therefore, Mollusca has a high potential for monitoring heavy metals uptake from the water along with the sediments [14]. Heavy metals are toxicants that disrupt the ecological balance and are carried to living beings through the food chain or waterway in the aquatic environment and have the potential to damage all life activities of the living beings and change this metabolic structure [15, 16]. According to the literature review, there are no detailed morphological measurement studies for *R. decussatus* in the Yumurtalık Lagoon, which is located in the northeastern part of the Mediterranean and is very rich in terms of both fish and invertebrate species [17, 18]. Therefore, giving the morphological characteristics of the mussel in terms of length, width, height and weight is important for the comparison of mussel morphology in future studies. Although there are a few studies which attempt to determine the distribution areas of the *R. decussatus* species on the Yumurtalık coast [17, 19, 20], there are not enough studies on morphological characteristics and heavy metal accumulation together. This study firstly aims to examine shell composition of the Yumurtalık coast and depending on this, investigates shell evaluations about some morphological parameters and heavy metal levels of *R. decussatus* sampled from the Yumurtalık coast.

2. MATERIALS AND METHODS

This study was carried out at the Yumurtalık Coastline of İskenderun Bay in the spring and autumn seasons of 2018 (Figure 1). The temperature and salinity levels of the sea water in the spring and autumn seasons were measured by YSI EcoSense. A total of 602 shells were collected from the sea coast and sediment of the sea (about 0-15 m). Samples were stored in labelled plastic container for being subjected to morphometric measurements. Species names were updated according to the checklist of species-group taxa of the Taxonomic Database on Marine Mollusca [21]. For heavy metal analysis, the samples kept in the deep freezer were taken out and washed by removing salt water. The muscle was completely removed from the shell and other parts, taken into jars to be dried, and then dried in an oven at 70 ºC for 48 hours. The dried samples were divided into small pieces by a microwave homogenizer and homogenized. Nitric acid and hydrogen peroxide were used for mussel muscle samples (1:1). All samples were completed in 10 ml polypropylene tubes with distilled water and the levels of heavy metals (Zn, Cu, Cd, Pb) were measured using inductively coupled plasma mass spectrometry (ICP-MS) [22]. Standard solutions of the analyzer were calibrated with chemicals prepared from Merck. Standard reference material (SRM, Dorm-2) was used to analyze the accuracy and precision of our results. Allometry was examined for morphometry (LWR) [23] and shape indices of individual bivalves were determined [24]. Shell length (maximum antero-posterior distance), shell height (maximum distance from hinge to ventral margin), shell inflation (maximum distance between outer edges of two valves), roundness index [RI=SL/SH] and cup index [$CI=SI/(SL*SH)^{0.5}$] of individual organisms were measured accurately to 0.01 mm using digital calipers [24]. The total weight of individual mussels and their shell weight were determined by digital balance (precision of 0.001 g). Ricker's [25] length-weight equation and Pauly's *t*-test [23] were applied. Independent samples *t*-test was applied for evaluation of heavy metal analysis in muscle tissue by using the SPSS software (SPSS Statistics V 27.0.1.0, IBM, Corp., USA).

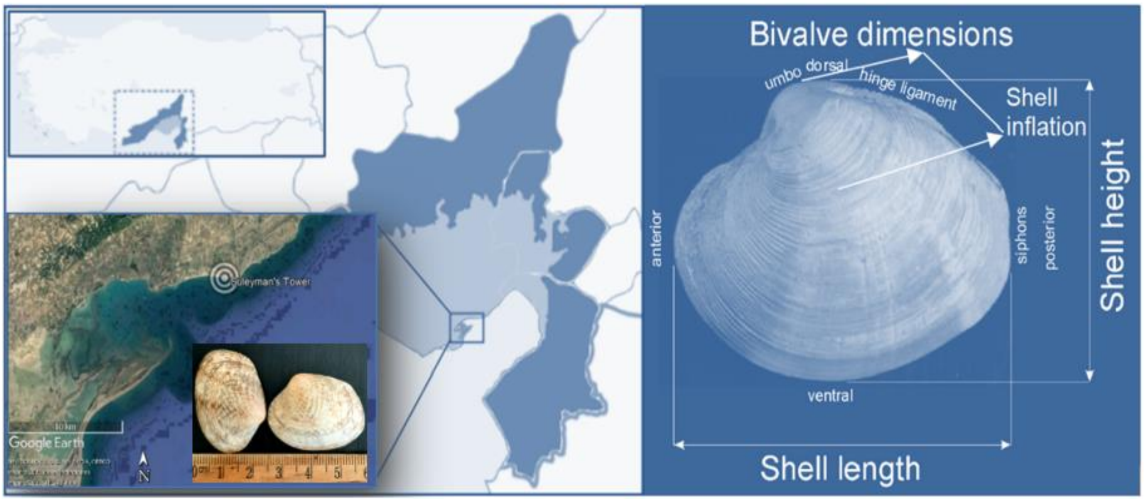

Figure 1. In the left side; The sampling site (Yumurtalık Coastline in İskenderun Bay) and *Ruditapes decussatus,* in the right side; bivalve dimensions can be viewed at wileyonlinelibrary.com

3. RESULTS AND DISCUSSION

Morphometric analysis and measurement of the heavy metal accumulation in the muscle tissue of *R. decussatus* were carried out at the Yumurtalık Coastline in İskenderun Bay. The mean sea water temperatures were measured to be 23.45±1 and 19.77±1 in spring and autumn, respectively. The salinity levels, however, were 37.10±0.16 and 35.65±0.33 ppt in spring and autumn, respectively. Biotope characteristics of the sample area were determined as gravelly, sandy, muddy and silt bottoms. All the 602 shells were collected from the Yumurtalık coast. A classification of them showed that they belonged to Anomioidea (0.99%), Arcidae (1.99%), Cardiidae (5.81%), Chamidae (1.32%), Glycymerididae (11.29%), Lucinidae (1.49%), Mytilinidae (2.82%), Nassariidae (1.16%), Ostreidae (11.46%), Pectinidae (0.99%), Pteriidae (1.99%), Semelidae (0.99%), Strombidae (0.83%), Spondylidae (0.83%), Tellinidae (1.22%), Veneridae (1.34%) and other (53.48%) families. These results were in line with the findings of previous systematic studies [17,19,20]. The descriptive statistics of the body size measurement of *R. decussatus* are presented in Table 1. For both seasons, the arithmetic means and standard error of measurements of the SL, TW, SH, SI, SW, RI, CI were obtained as 29.05±1.01 cm, 8.59±0.84 g, 19.97±1.13 mm, 14.53±0.70 mm, 3.65±0.10 g, 1.52±0.07 and 0.62±0.01, respectively. The minimum and maximum values of the SL, TW, SH, SI, SW, RI, and CI of both seasons were calculated as 21.50-39.00 mm, 0.80-22.15 g, 10.50-28.70 mm, 9.11-20.90 mm, 3.08-4.66 g, 1.03-2.14 and 0.58-0.67 respectively. The arithmetic means and standard error values of Bivalvia measurements according to the seasons were found to be 32.45±1.10 mm, 15.57±1.04 g, 22.35±1.20 mm, 16.9±0.70 mm, 4.02±0.11 g, 1.48±0.08 and 0.62±0.00 for spring and 25.65±0.70 mm,1.60±0.18 g, 17.60±1.60 mm, 12.16±0.40 mm, 3.27±0.02 g, 1.56±0.11 and 0.61±0.00 for autumn. The minimum and maximum values were calculated as 28.50-39.00 mm, 7.12-22.15 g, 17.30-28.70 mm, 13.9-20.90 mm, 3.37-4.66 g, 1.06-1.90 and 0.60-0.67 for spring and 21.50-28.0 mm, 0.80-2.75 g, 10.50-25.50 mm, 9.11-14.21 mm, 3.08-3.38 g, 1.03-2.14 and 0.58-0.67 for autumn. The coefficient R^2 of determination for bivalves was found to be 0.879 for both seasons. Similarly, the values of *a* and *b* were found as 0.011 and 2.971. The growth type of Bivalvia specimens was determined to be isometric growth. A positive correlation was reported in the relationship between the total weight and shell length ($P<0.01$; 0.8795; Figure 2). A similar situation was observed between the cup index and roundness index, as well ($P<0.01$; 0.9898; Figure 2). In general, descriptive features in bivalves were measured mostly in the spring season (Figure 3), except for the roundness index (1.48±0.08) and *b* (2.540) value (Table 1). The growth type of Bivalvia specimens was determined as negative allometry in spring and isometric growth in autumn (Table 1). *R. decussatus* has a wide distribution in the world and an important ecological and economic value in terms of its place in the trophic chain of lagoons and marine systems. One may study it as a bioindicator in these areas in terms of its place in the culture of shellfish [6]. Therefore, the morphometric indices and length-weight relationships of *R. decussatus* have been estimated widely in several areas as an indicator to compare the differences between ecosystems (Table 1). Environmental factors such as water temperature and nutrient availability are primary factors for the development of Bivalvia while salinity and photoperiod have a secondary effect [26].

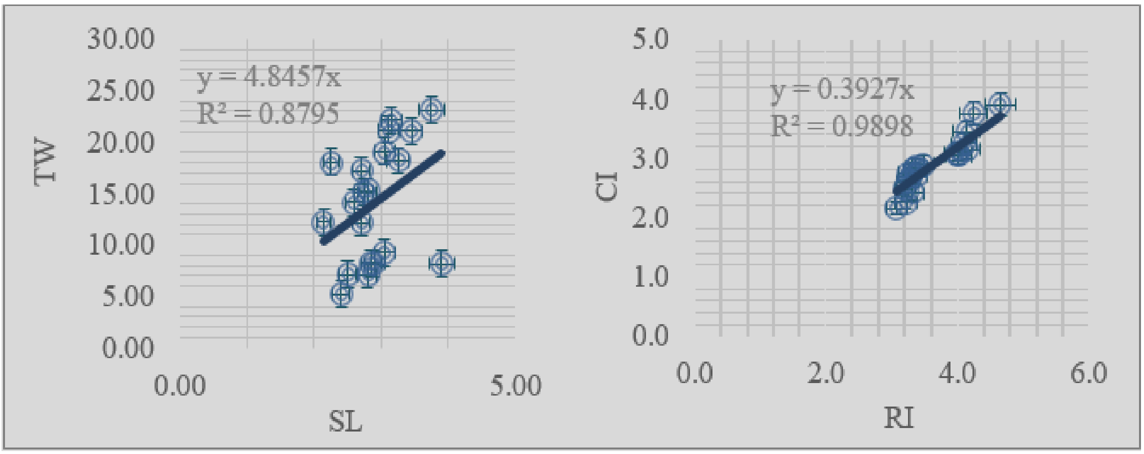

Figure 2. The relation of *R. decussatus*'s total weight (TW, g), shell length (SL, cm), cup index (CI) and roundness index (RI).

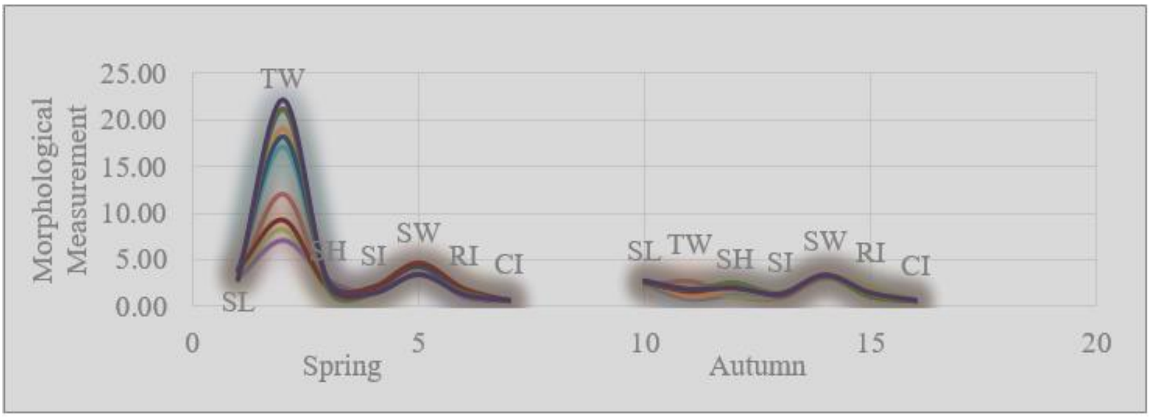

Figure 3. Morphological traits of *R. decussatus*'s in spring and autumn season

Table 1. The shell length (mm), total weight (g), shell height (mm), shell inflation (mm), shell weight (g), roundness index and cup index with growth parameters (LWR) of *Ruditapes decussatus* (n=18).

Season	Descriptive statistics							LWR			
	SL x̅ ±SEM Min-Max	TW x̅ ±SEM Min-Max	SH x̅ ±SEM Min-Max	SI x̅ ±SEM Min-Max	SW x̅ ±SEM Min-Max	RI x̅ ±SEM Min-Max	CI x̅ ±SEM Min-Max	*a*	*b*	R^2	*GT*
Spring	32.45±1.10	15.57±1.04	22.35±1.20	16.90±0.70	4.02±0.11	1.48±0.08	0.62±0.00				
	28.50-39.00	7.12-22.15	17.30-28.70	13.90-20.90	3.37-4.66	1.06-1.90	0.60-0.67	0.005	2.540	0.877	-A
Autumn	25.65±0.70	1.60±0.18	17.60±1.60	12.16±0.40	3.27±0.02	1.56±0.11	0.61±0.00				
	21.50-28.0	0.80-2.75	10.50-25.50	9.11-14.21	3.08-3.38	1.03-2.14	0.58-0.67	0.003	2.888	0.863	I
In this study (∑)											
x̅ ±SEM	29.05±1.01	8.59±0.84	19.97±1.13	14.53±0.70	3.65±0.10	1.52±0.07	0.62±0.01				
Min-Max	21.50-39.0	0.80-22.15	10.50-28.70	9.11-20.90	3.08-4.66	1.03-2.14	0.58-0.67	0.011	2.971	0.879	I
In other studies											
Moroccan Coast [27]	25.86-49.36	5.30-22.01	20.29-33.94	14.18-24.23	ns	ns	ns	ns	ns	ns	ns
Lake Timsah, Egypt [28]	26.36±1.51 11.70-40.60	3.95±0.99 0.31-10.51	17.84±1.73 8.40-27.00	11.7±1.32 4.90-17.3	2.66±0.67 0.23-6.71	ns	ns	0.000	2.864	0.980	I
Pag Bay - Eastern Adriatic Sea [29]	31.9±5.5 17.7-43.5	ns	ns	ns	ns	ns	ns	ns	2.910	0.965	I
Port Said-Egypt [30]	≅16-35.9	≅0.70-5.80	≅0.70-2.25	ns	≅0.70-5.50	ns	ns	0.091	3.350	0.857	+A
Lake Timsah, Egypt [31]	22.00	0.86-1.15	ns	ns	1.27-1.65	ns	ns	3.55	2.81	0.979	I
Aquaculture, Turkey [32]	32.10±3.01	11.46±1.57	33.67±1.61	ns	8.88 ± 1.37	ns	ns	ns	ns	ns	ns
Galicia, N.W. Spain [33]	48.79±2.42	26.47±3.99	34.92±1.71	23.27± 1.26	14.17±2.24	ns	ns	ns	ns	ns	I

LWR: total length and weight relationship, x̅ ±SEM: arithmetic mean± standard error of the mean, Min-Max: the range of variability of the linear and mass indices, *a* and *b*, coefficients of the equations; R^2: coefficient of determination, *GT*: growth type (I: isometric growth, -/+A: negative or positive allometry), ns: not specified.

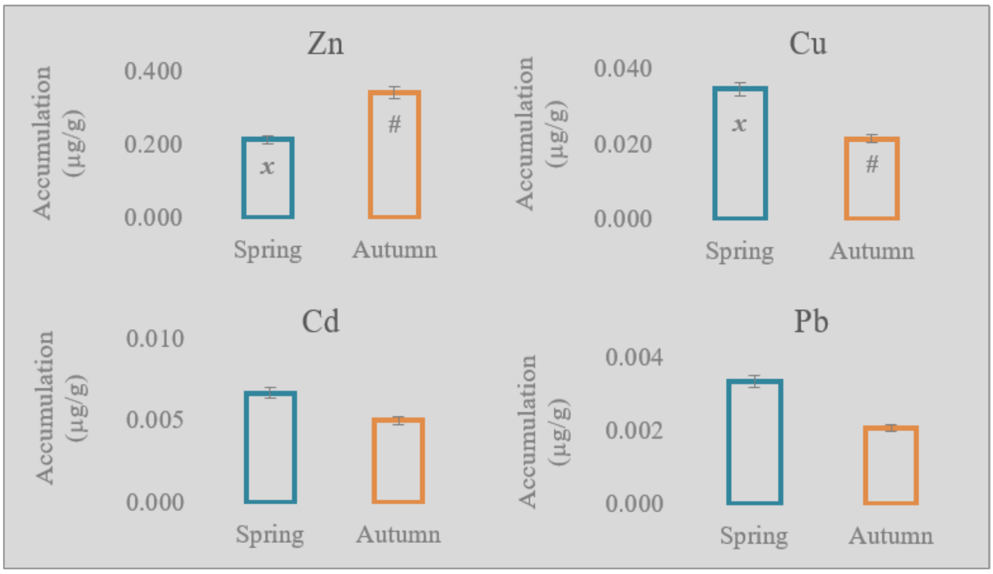

Figure 4. Accumulation level (µg/g) of metals in muscle tissue of *R. decussatus.* The sign of cross and sharp (*x, #*) in bars within season indicate statistically significant differences (*P*<0.05).

In our study, negative allometry (*b*=2.540, R^2=0.877) and isometry (*b*=2.888, R^2=0.863) were observed in the Yumurtalık coast in the spring and autumn seasons, respectively. The growth characteristics of Bivalvia species sampled from the Urdaibai Estuary (North Spain) also showed isometric characteristics (*b*=2.900, R^2=0.903) [34]. The dominance of isometries and negative allometries over positive allometries is an important phenomenon in bivalve growth types. This may be related to the creature's continuous egg-laying strategy. A similar situation was observed in different studies (e.g.: [31]). In a study by Sherif [30], it was reported that the shell growth of bivalve *V. decussatus* was mainly affected by temperature. In another study conducted with *R. decussatus*, it was reported that shell development slowed down in the winter season [35]. Descriptive measurement values of bivalves in the Yumurtalık coast were calculated mostly in the spring season, and found to be 23.45±1 ºC and 37.10±0,16 ppt. It has been reported that bivalves develop well when the water temperature is 20-24 ºC and the salinity is between 32-40 ‰ [36]. When Bivalvia samples from different regions are compared with the Bivalvia samples in our study in terms of the height-weight relationship and growth patterns, we can conclude that this species has average morphometric characteristics and this area is ecologically suitable for the species. The average Zn, Cu, Cd, and Pb concentrations in the muscle tissue of *R. decussatus* in this study and some species belonging to the Veneridae family in different localities are given in Table 2.

Table 2. Compare with the average Zn, Cu, Cd, and Pb concentrations in muscle tissue of *R. decussatus* in this study and other species belonging to Veneridae family in different study (µg/g dry weight).

Species	Zn	Cu	Cd	Pb	References Year
Ruditapes decussatus Yumurtalık Coast, Turkey	0.278±0.001	0.028±0.001	0.006±0.000	0.003±0.000	In this study
			In other studies		
Circe scripta Region: Daya Bay, China	9.79±0.82	0.90±0.03	0.07±0.03	0.34±0.10	[37] 2020
Gafrarium divaricatum Region: Daya Bay, China	10.64±0.52	1.10±0.59	1.14±0.25	0.24±0.13	[37] 2020
Ruditapes decussatus Region: Sardinian (Calich) Coast, Italy	16.00±0.85	1.2±0.05	0.010±0.0013	0.059±0.0063	[38] 2018
Ruditapes philippinarum Region: Laizhou Bay (Southern), China	53.47±30.21	12.13±3.40	1.98±0.86	1.06±0.28	[39] 2017
Ruditapes decussatus Region: Alexandria coast, Egypt	ns	ns	2.063±0.26	8.090±0.19	[40] 2013
Paphia undulata, Region: Alexandria coast, Egypt	ns	ns	0.903±0.10	3.120±0.17	[40] 2013
Tapes decussata Region: Egyptian Mediterranean coast	14.13±0.02	3.46±0.32	0.115±0.002	0.29±0.003	[41] 2012
Paphia undulata Region: Egyptian Mediterranean coast	9.72±0.06	1.92±0.32	0.133±0.002	0.14±0.003	[41] 2012
Venerupis decussata Region: Egyptian Mediterranean coast	8.35±0.06	1.54±0.12	0.036±0.003	0.05±0.004	[41] 2012
Gafrarium pectinatum Region: Egyptian Mediterranean coast	51.83±0.02	11.85±0.32	0.131±0.001	0.43±0.002	[41] 2012
Tapes decussata Region:Varano Lagoon, Italy	ns	ns	0.22±0.06	0.14±0.006	[42] 2001

The levels of Zn, Cu, Cd, and Pb found in muscle tissue of *R. decussatus* indicated statistically significant differences for only zinc and copper accumulation within seasons ($P<0.05$; Figure 4). The arithmetic means and standard error values of heavy metals in the muscle tissue for the sum of the two seasons were reported to be 0.278±0.001 µg Zn/g, 0.028±0.001 µg Cu/g, 0.006±0.000 µg Cd/g, 0.003±0.000 µg Pb/g (Table 2). Copper and zinc are trace elements that are normally required for metabolic cellular activities, but cadmium and lead are non-essential heavy metals that pose a threat to life even at low concentrations [38]. Essential metal levels (Zn-Cu) and non-essential metal levels (Cd-Pb) in the investigated coastal bivalves were found to be in the range of 0.19-0.35 µg Zn/g; 0.019-0.039 µg Cu/g and 0.004-0.009 µg Cd/g; 0.0019-0.005 µg Pb/g. These concentrations were observed below the concentrations determined in the species in China, Italy, and Egypt (Table 2). Heavy metals cause accumulation in the tissues of bivalves far above normal levels. At non-lethal toxic stressor levels for bivalves, situations such as escaping from the environment (withdrawal by siphoning) and closing of the valve to maintain its internal balance have been observed; in contrast, inhibition of byssal yarn production, impaired burrowing behavior in the dune field, inhibition of respiration, inhibition of filtration rate, inhibition of protein synthesis and suppressed growth have been observed in cases where toxicity exposure was fatal [43]. It has been reported that small organisms show more heavy metal accumulation than others [44] but *V. senegalensis* was reported in their sensitivity to heavy metal accumulation and their resistance to metal in another study [45]. *R. decussatus* has a high filtration capacity and is therefore viewed as an important biological model for an indication of environmental pollution and contamination [6]. The molybdenum intolerance of *V. senegalensis* belonging to Veneridae was investigated, and it was concluded that molybdenum was not toxic at the levels encountered in the marine environment. In addition, the lethal and non-lethal effects of the copper were examined, and it was reported that *V. senegalensis* had a high tolerance to heavy metals and a high recovery rate [45]. As we can see in Table 2, where comparisons were made with the species belonging to the Veneridae family sampled from different regions, heavy metal levels did not seem to be at high levels in the samples collected from the Yumurtalık coastal area. This was a very important and positive situation for the area from an ecological point of view. Some bivalve catcher was found to be naturally important in the predation of shorebirds of some species belonging to Veneridae family [46]. It is suggested that the average heavy metal levels in the wet tissue of bivalves should be below 50.0 mg kg^{-}1 for Zn, 1.0 mg kg^{-}1 for Cu and Cd, and 0.50 mg kg^{-} 1 for Pb [47]. Accordingly, we can conclude that the metal pollution in the area where the samples were collected was not at a high level.

4. CONCLUSION

In this study, in which morphological characteristics and muscle tissue heavy metal concentrations of *R. decussatus* were examined, statistically significant differences were found only for zinc and copper accumulation over the seasons. As a consequence of these values, this species has average morphometric properties and this area is ecologically suitable for the species. In addition to its ecological importance, the species *R. decussatus* has an important socio-economic role in all regions. Nevertheless, intense fishing pressure can lead to the extinction of the natural population stock. The coastal area of Yumurtalık is very important in terms of fish species and shorebird richness. A commercial evaluation of the area within ecological disciplines in crustacean cultivation, in which this species is included, may be of importance for development.

CONFLICT OF INTEREST

The author stated that there are no conflicts of interest regarding the publication of this article.

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