APPLICATION OF MULTI-METAL BIOACCUMULATION INDEX AND BIOAVAILABILITY OF HEAVY METALS IN *Unio sp.* (UNIONIDAE) COLLECTED FROM TERSAKAN RIVER, MUĞLA, SOUTH-WEST TURKEY

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ABSTRACT

In the present study, some heavy metals (Cd, Co, Cr, Cu, Fe, Mn, N, Pb and Zn) were seasonally determined in some tissues of mussel *Unio sp.* from Tersakan River, which is an important agricultural, a visiting area and, a water source for irrigation. Generally, higher concentrations of the measured metals were found in the winter and autumn compared with those during spring and summer. High levels of heavy metals were found in shell samples, while low levels in muscle. The measured metal concentrations in tissues is not differed significantly for all metals except Fe between autumn and spring but significant differences for Cd, Co, Cr, Cu, Mn and Pb were observed between summer and autumn ($p<0.05$). Individual Multi-Metal Bioaccumulation Index (MBI) values varied from 0.01 to 0.44, 0.00 to 0.47, and 0.00 to 0.43 in muscle, gill, and shell respectively. Generally, IMBI values calculated in muscle were higher than gill and shell. According to the acceptable values for human consumption designated by various health organizations, concentrations of some metals such as Cd and Pb were more than the designated limits, especially in summer.

KEYWORDS: Bioaccumulation, heavy metals, multi-metal bioaccumulation index, *Unio* sp.

1. INTRODUCTION

Effluents from textile, leather, tannery, electroplating, galvanizing, pigment and dyes, metallurgical and paint industries and other metal processing and refining operations contain considerable amounts of toxic heavy metal ions which are both potential hazards for human health and for other life forms. The river systems may be excessively contaminated with toxic heavy metals released from domestic, industrial, mining and agricultural effluents. The contamination of freshwaters with a wide range of pollutants has become a matter of great concern over the last few decades, not only because of the threat to public water supplies, but also with of the damage caused to the aquatic life [1-5].

Bivalves are well known for their biological features of concentrating some substances especially heavy metals in their tissues [6]. Because of their main characteristics such as being sedentary organisms, their longevity, their abundance and availability throughout the year, their environmental tolerance, they are often chosen for biomonitoring studies. Besides, bivalves have good net accumulation capacities. The importance of bivalves in pollution impact studies has been shown within the scope of the International Mussel Watch Programme [7-8]. Although all heavy metals are potentially harmful to most organisms at some level of exposure and absorption [9], some heavy metals such as Zn, Cu and Co are essential in trace amounts for normal growth and development. However, others such as Hg, Cd and Pb have no known biological role [10, 11]. Since it is edible and marketed commercially, the determination of contaminant levels in mussel species provides the assessment of possible toxicant risk to public health [12].

This study includes the metal bioaccumulation of Cd, Co, Cr, Cu, Fe, Mn, N, Pb and Zn in *Unio sp.* tissues and evaluating Individual Multi-Metal Bioaccumulation Index for influence of heavy metal pollution.

2. MATERIALS AND METHODS

2.1 Study Area

In this study, heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) contents in *Unio sp.* were investigated. The mussels were collected from the Tersakan River polluted by agricultural and domestic wastes. The Tersakan River is a temperate stream (36°45'51"N, 28°49'20"E), which is im-

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pacted by unpredictable environmental conditions associated with a Mediterranean climate. Its length is 30 km and this stream has temporal and spatial water flow variations throughout the water course (48–780 m^3/s) [13]. The lower section of the stream was channelized by local authorities to prevent seasonal floods. The stream flows into Mediterranean Sea. Vegetation is usually abundant throughout the stream banks and depth varies between 0.5–2 m. The sampling site was characterized by muddy substrate, limited vegetation and slow flow velocity. It had recently been affected by heavy floods, which occur seasonally because of high annual precipitation [14].

2.2 Collection of Mussel and Physical and Chemical Analyses

Freshwater mussels *Unio sp.* were caught by bucket. 20 freshwater mussels were selected from each caught in the Tersakan River in every season of 2010. Physico-chemical parameters (pH, temperature, conductivity and dissolved O_2) of freshwater on sampling days were determined by a Hach Lange HQ40D model. The samples were brought to the laboratory at the same day, weighed, measured and dissected. During the sampling, physico-chemical parameters of the water were analyzed on the selected station using LCK cuvette test on Hach Lange DR 2800 model Spectrophotometer. LCK codes used were as follows: LCK 305 for (NH_4N), LCK 339 for (NO_3), LCK 342 for (NO_2), LCK 348 for (PO_4P), LCK 328 for (K), LCK 327 for (Ca) and (Mg). The analysis of each parameter was performed as described on standard cuvette tests.

2.3 Digestion and Heavy Metal Determination

Tissues of the same species from the same station were pooled to make 20 subsamples. They were transferred to an oven set (120 °C) to dry. Drying continued until all the wet tissues reached a constant weight. Dry tissue samples were put into digestion flasks and 2 ml hydrogen peroxide and 8 ml nitric acid (Merck) were added. The digestion flasks were then put on a microwave digestion unit at 120 °C (gradually increased) until all the materials were dissolved. After digestion, the digested samples were diluted with distilled water appropriately in the range of standards which were prepared from stock standard solution of the metals (Merck). The extraction procedure for the tissues included multi-acid digestion (HNO_3-$HClO_4$-HCl) and analysis for Cd, Co, Cr, Cu, Fe, Mn, N, Pb and Zn, by ICP-AES (Perkin-Elmer Corp., Nonvalk, CT, USA). Verification of the method was demonstrated by analysis of an independent standard reference material (DORM-2, certified dogfish muscle tissue, National Research Council, Ottowa, Ontario, Canada).

2.4 Data Analyses

Kruskal-Wallis following Man-Whitney-U test were used to determine whether metal concentrations differ significantly between tissues and season at a level of a = 0.05. Paired relationships between environmental parameters and metal concentrations were revealed using Pearson's correlation tests. These statistical calculations were performed with SPSS 20.0 for Windows. The individual mean (multi-metal) bioaccumulation index (IMBI) was calculated as [15]:

$$\mathrm{IMBI} = \frac{\sum_{\mathrm{i}}^{\mathrm{n}} = {}_1 C_{\mathrm{i}} / C_{\mathrm{imax}}}{\mathrm{n}}$$

With N the total member of metals, C_i the individual metal concentration of heavy metal *i*, C_{imax} the maximal observed concentration of heavy metal *i* and 0<IMBI<1.

3. RESULT AND DISCUSSION

3.1 Environmental Parameters and Shell Allometric Parameters

Some physicochemical parameters of water in the study area and also shell allometric parameters of samples are presented in Table 1 and Table 2, respectively.

TABLE 1 - Seasonally water parameters of Tersakan River (average±SEM, n=3)

	Autumun	Winter	Spring	Summer
Ammonium (NH_4N) mg/L	0.16±0.1	0.32±0.1	0.21±0.2	0.01±0.1
Nitrate (NO_3) mg/L	0.55 ±0.2	1.85±0.2	0.27±0.1	0.71±0.1
Nitrite (NO_2) mg/L	0.03±0.1	0.03±0.1	0.01±0.1	0.04±0.1
Orthophosphate (PO_4P) mg/L	0.35±0.1	0.37±0.1	0.10±0.1	0.06±0.1
Potassium (K^+) mg/L	0.35±0.1	1.71±0.2	3.30±0.1	0.23±0.1
Total hardness dH	21.80±0.1	40.20±0.3	49.4±0.3	22.80±0.2
Calcium (Ca) mg/L	54.40±0.2	41.10±0.3	31.70±0.2	46.20±0.2
Magnesium (Mg) mg/L	61.40±0.3	149±0.4	195±0.4	70.90±0.3
Dissolved Oxygen (mg/L)	8.50±0.1	6.83±0.2	10.24±0.3	9.54±0.2
Temperature T ºC	21.9±0.2	15.60±0.2	22.4±0.2	26.30±0.2
pH	7.41±0.2	7.30±0.2	7.48±0.1	7.12±0.2
Conductivity (µS cm^{-1})	889±0.4	887±0.3	820±0.3	920±0.4
BOI_5	4.02±0.2	0.96±0.1	2.50±0.2	4.14±0.2

TABLE 2 – The seasonal averages of some allometric parameters in *Unio sp.* from Tersakan River (average±SEM, n=60)

	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)	Mussel Weight (g)
Autumn	66.30±0.2	26.63±0.1	42.33±0.1	40.150±0.4
Winter	66.51±0.3	26.61±0.1	42.24±0.1	40.70±0.6
Spring	55.97±0.6	20.35±0.1	34.04±0.5	42.05±0.7
Summer	54.08±0.6	20.36±0.2	33.53±0.5	37.05±1.0

It is well documented that salinity of waters is an important factor influencing concentrations of selected metals in the soft tissue of *Unio sp.* in the Tersakan River. The mean concentrations of Cd, Co, Cr, Mn, Ni, Pb and Zn are the higher in autumn and summer than winter and spring. Similarly, the values of conductivity, NO_3, NO_2 and Ca^{+2} in autumn and summer are also higher than the values in the other seasons.

According to Broman et al. [16], the bioavailability of Cd in soft tissues of mussels is dependent on salinity of the adjacent water. The tissue Cd levels of *Unio sp.* collected from the Tersakan River in summer and winter were high when salinity levels were high. However Co, Cr, Mn, Ni, Pb and Zn concentration were determined higher in autumn and summer when salinity was high. Broman et al. [16] observed that there was no relationship between Zn levels and salinity. However, our results showed a positive relationship between Zn levels and salinity. Although some other environmental factors such as salinity, pH, hardness and temperature play significant roles in metal accumulation, other studies showed that accumulation of heavy metals in a tissue is mainly dependent upon water concentrations of metals and exposure period [17].

There were also seasonal differences between mussels of different size classes from the sampling site. The longest shell length of *Unio sp.* (70.58 mm) was measured in autumn while the shortest length (50.3 mm) was measured in summer. The longest shell width (40.35 mm) was in summer while the shortest shell width (24.3 mm) was in winter. The maximum mussel weight was determined in spring as 57 g and minimum weight of mussel also was determined in summer as 29 g. The longest shell height of mussel (43.82 mm) was measured in autumn while the shortest height (20.11mm) was measured in summer.

There was a statistically significant negative relationship between the metal content of the tissues and the mussel length, width, height and weight for all metals in autumn ($p < 0.05$). Significantly positive relationships were found in winter between only Cr and weight ($r < 0.509$), while negative relationships were found between Pb-width and Cd-width ($r < -0.511$ and $r < -0.598$). Additionally in spring, almost all metals except Fe, Mn and Ni have negative correlation with length, width, height and weight ($r < -0.334$). Finally, in summer Ni showed positive correlations with weight and length (respectively, $r < 0.287$ and $r < 0.335$). Other trace metals showed weak relationships.

The amount of heavy metal bioaccumulation in the tissues may vary depending on length and weight of samples [18, 19]. Our data showed that almost all trace metals have negative relationships with mussel size in every season; only some of the metals such as Cr (in winter) and Ni (in summer) had positive correlations.

3.2 Seasonal Variations of Metal Accumulation

Mean concentrations of all metal accumulation in autumn, winter, spring and summer is shown in Table 3. Investigated concentrations of heavy metals in tissues have shown seasonal variation and the following maximum concentrations were found: 2.660 µg/g dry weight (d.w.) for Cd, 4.786 µg/g d.w. for Co, 24.430 µg/g d.w. for Cr, 291.179 µg/g d.w. for Cu, 1921.090 µg/g d.w. for Fe, 752.840 µg/g d.w. for Mn, 112.440 µg/g d.w. for Ni, 15.960 µg/g d.w. for Pb and 460.330 µg/g d.w. for Zn. We have reported that almost all metals displayed the maximum values during the winter, except Fe, Cd and Co. The maximum metal concentration for Fe was determined in spring while the highest Cd and Co concentrations were found in summer.

TABLE 3 - Mean concentrations (µg/g dry weight) of metal accumulation of *Unio sp.* on seasonally (average±SEM, n=60)

Metals	Autumn	Winter	Spring	Summer
Cd	0.131±0.01^{a}	0.255±0.02^{b}	0.081±0.03^{c}	1.075±0.07^{d}
Co	0.501±0.08^{a}	0.526±0.09^{a}	0.488±0.07^{ab}	1.551±0.12^{b}
Cr	4.274±0.56^{a}	5.618±0.74^{a}	2.891±0.40^{a}	8.404±0.33^{b}
Cu	6.973±0.83^{a}	17.999±5.52^{a}	6.084±0.94^{a}	2.800±0.85^{b}
Fe	533.804±66.73^{a}	575.360±65.48^{a}	373.690±47.43^{a}	352.141±38.92^{a}
Mn	490.940±183.84^{a}	206.590±23.04^{a}	179.472±24.22^{ab}	114.236±15.44^{b}
Ni	46,060±9.42^{a}	12.937±2.35^{a}	10.738±1.51^{a}	10.910±1.54^{a}
Pb	15.960±1.91^{a}	5.870±1.98^{a}	1.892±0.34^{a}	3.938±0.34^{b}
Zn	460.300±35.75^{a}	135.560±21.01^{a}	17.752±4.59^{a}	17.907±4.11^{a}

The same superscript letters in the same row are not significantly different at p<0.05

TABLE 4 - Mean concentrations (µg/g dry weight) values of metals in tissues of *Unio sp.* in Tersakan River (average±SEM, n=240)

Tissue	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Muscle	0.296±0.04[a]	0.284±0.06[a]	2.284±0.39[a]	3.070±1.97[a]	77.879±7.41[a]	12.975±6.26[a]	4.646±1.13[a]	1.016±0.23[a]	4.215±2.13[a]
Gill	0.478±0.05[b]	0.861±0.11[b]	6.206±0.41[b]	6.420±3.61[b]	627.184±38.887[b]	231.742±13.358[b]	7.536±0.77[b]	2.123±0.3[b]	56.627±7.28[b]
Shell	0.382±0.06[a]	1.155±0.08[c]	7.403±0.54[b]	15.902±1.24[c]	671.182±55.05[b]	268.291±18.24[b]	20.821±1.77[c]	4.157±0.21[c]	8.475±2.48[c]

Result show that the highest accumulation of heavy metal for each season was Fe. Fe is generally the most abundant metal in all of the reservoirs because it is one of the most common elements in the earth's crust [20]. According to Cabrera et al. [21] pyrite oxidation produces sulphate and the Fe^{2+} ion, which is oxidized to Fe^{3+} by microorganisms such as *Thiobacillus ferrooxidans*. Results shown that all metal concentrations decreased from winter to spring, but again increased from spring to summer.

According to the correlations of heavy metal concentrations in *Unio sp.* from the Tersakan River, in autumn the data showed very high levels of correlation with mostly positive values for Cr and Fe ($p < 0.05, r < 0.812$). These two metals were in positive correlation with all metals. Additionally, Ni and Pb also showed positive correlations with all metals except for Cd and Zn in autumn ($r < 0.961$). There are significant positive correlations between the tissues in winter and spring ($r < 0.642$). In this season, Co and Cr are positively correlated with almost all metals. Significantly positive relationships were found in summer between Cu-Fe, Cu-Ni, Mn-Cr, Mn-Zn (respectively, r<0.482, 0.459, 0.457, 0.535). On the other hand, in summer there was a significant high level correlation between Fe and Cr. ($p < 0.05, r < 0.943$).

The heavy metal accumulation of Cu, Mn, Ni, Pb and Zn in winter was found higher when comparing seasons. There were significant differences between the seasons and the tissues for each of the heavy metals ($p < 0.05$). The concentrations of Fe, Ni and Zn in *Unio sp.* from the Tersakan River were not found to be statistically significant among all seasons ($p < 0.05$). The heavy metal concentrations in tissues did not differed significantly for all metals except for Fe between autumn and spring ($p < 0.05$) but significant differences for Cd, Co, Cr, Cu, Mn and Pb were observed between summer and autumn. All heavy metal elements were not significantly different in tissues between autumn and winter ($p < 0.05$).

In general, Cd and Pb have been found at higher levels in the tissues in *Unio sp.* in almost every season. A number of studies have shown that the concentrations of Cd and Pb in aquatic organisms depend mainly on their environmental levels [22-24]. The present study shows that levels of all metals were significantly higher in tissues in autumn and summer. High levels of metals in tissues on different season could be originated from different sources around the study area. These sources are motor oil and ballasts water for Pb and Cd in summer when tourist activities are increased. Also Cd could occur from phosphorus fertilizer used in agriculture in summer and autumn. Moreover, other heavy metals, such as Zn as composed fertilizer, Mn and Cu as micro elements fertilizer, are used in citrus fruits plantations and green-houses around the Tersakan River where agricultural activities become more intense especially in autumn and winter.

3.3 Metal Accumulations in Tissues

The mean concentrations of all metals in the gill, shell and muscle of *Unio sp.* collected from Tersakan are given in Table 4. We determined that maximum metal concentration of Co, Cu, Mn, Pb and Zn were the highest in gill tissues but the mean metal concentration of these metal except Zn were highest in shell tissues. Maximum metal concentrations of Cr were observed in muscle while mean concentration for this metal were highest in shell tissues. The highest mean and the maximum concentration of Ni were determined in shell tissue. The maximum metal concentration for Cd was observed in shell tissues while the mean concentration of Cd was highest in gill tissues.

The accumulation of investigated heavy metals in muscle tissue was in order as; Fe>Mn>Ni>Zn>Cu>Cr>Pb>Cd>Co. This order was as; Fe>Mn>Zn>Ni>Cu>Cr>Pb>Co>Cd in gill tissue while it was Fe>Mn>Ni>Zn>Cu>Cr>Pb>Co>Cd in shell tissues. The significant differences were not found for Cd between gill and muscle ($p < 0.05$). The differences in Fe, Cr and Mn were not statistically significant between gill and shell ($p < 0.05$) but significant differences were observed for other metals. Results show that the concentrations of Fe, Mn, Zn and Ni have shown high levels when their mean concentrations were compared with the mean concentration levels of other metals. The highest Co, Cr, Cu, Fe, Mn and Ni concentrations were found in shell tissues. General evaluation of the heavy metal accumulation in tissues could be ordered as shell>gill> muscle. The highest Co, Cr, Cu, Fe, Mn and Ni concentrations were found in shell tissues whereas the least metal concentrations were found in muscle tissues. These results are similar to those reported by Yilmaz [25] who indicated the heavy metal accumulations on economically important fish inhabiting nearly to Tersakan River. According to the results of Yilmaz [25], the lowest metal contents were determined in edible parts (muscle) of all species.

Yap et al. [12] investigation regarding *P. viridis* at the west coast of Peninsular Malaysia revealed that *P. viridis* contained metal concentrations in the soft tissues which ranged from 0.68 to 1.25 µg/g d.w. for Cd, 7.76 to 20.1 d.w. µg/g for Cu, 2.51 to 8.76 d.w. µg/g for Pb and 75.1 to 129 d.w. µg/g for Zn. These metal concentrations were relatively higher for Cd, Cu, but less for Pb and Zn compared to the concentrations in *Unio sp.* in the Tersakan River. Yarsan and Bilgili [26] measured heavy metal concentrations in tissues of *Unio stevenianus* of Van lake 1.43±0.81 µg/g d.w. for Pb, 0.09±0.02 µg/g d.w. for Cd, 5.83±0.73 µg/g d.w. for Cu, 15.93±3.26 µg/g d.w. for Zn. Our results were generally higher for all metals compared to their findings. Kır and Tuncay [27] investigated the accumulation of some heavy metals in muscle, liver and carapace tissues of crayfish (*Astacus leptodactylus*) inhabiting Kovada Lake. They showed that the metal concentrations found in the liver and carapace were higher than those in the muscle. These results were relatively similar with our results, but our results showed that mussels accumulate the heavy metals at a higher level than their surroundings. The studies on Cu and Zn in *Crongon crongon* [28] and *Humarus americanus* [29] present a marked ability on the part of crustaceans to regulate internal copper and zinc concentrations. In the present study, it has been shown that essential metals such as Zn, Cu, Mn and Fe were the most abundant metals in different tissues analyzed throughout the year, whereas Co was the least abundant in *Unio sp.* inhabiting Tersakan River. Zn and Fe play a role in the enzymatic and respiratory processes [30] and also Cu and Zn are apparently essential metals for crustaceans [31, 32]. The gill was also the main target organs for Pb and Zn accumulation which is in accordance with the findings of Manly and George [33] and Van-Balogh [34]. Adams et al. [35] showed Zn was predominantly accumulated by the gill and the mantle in *Amblema perplicata*. Undoubtedly, the unionid gills play a distinct role in metal uptake and storage. As the gills serve also as breeding chambers, its involvement in metal storage may have harmful effects on the reproductive success of freshwater unionids [36].

Our result showed that some metals were found in higher concentration on shell tissue (Fig. 1). But as there is a lack of available information on shell metals for the adjacent regions of southwestern Turkey, concentration data related to other geographical zones were taken from literature and were compared with the data reported in the present study [37-40]. Yap [37] analyzed distributions of Cu and Zn in the shell lipped part periostracum and soft tissues of *Perna viridis* sampled from 17 geographical sites. The concentrations of Cu in the periostracum and the soft tissues of *P. viridis* were 7.41- 42.63 µg/g d.w. and 3.49-31.1 µg/g d.w., respectively. Meanwhile, the concentrations of Zn in the periostracum and soft tissues of *P. viridis* were 4.90-39.79 µg/g d.w. and 65.75-144.9 µg/g d.w., respectively. General accumulations of Cu and Zn in the periostrcum in *Perna viridis* were higher than in shell tissue in our study. Yap et al. [38] measured distributions of Cu, Zn, and Pb concentrations in the selected soft tissues (foot, cephalic tentacle, mantle, muscle, gill, digestive caecum, and remaining soft tissues) and shells of the mud-flat snail *Telescopium telescopium* were determined in snails from eight geographical sites in the south-western intertidal area of Peninsular Malaysia. The shell demonstrated ten times higher concentrations of Pb (41.23± 1.20 digestive caecum) when compared to our result. Yap et al. [39] determined trace metals (Fe, Cu, and Ni) in the Mangrove Area of Peninsular Malaysia using different soft tissues of flat tree oyster *Isognomon alatus*. The mean concentrations of Fe, Cu and Ni in muscle were 797.43 µg/g d.w., 4.31 µg/g d.w. and 0.90 µg/g d.w., respectively. Their study show that highest Fe and Cu concentrations were found in the muscle but the concentrations of Ni in our study were higher than their findings. They also determined Fe, Cu and Ni (1129.71 µg/g d.w., 7.83 µg/g d.w. and 1.21 µg/g d.w., respectively) concentrations in mantle plus gill and these results were relatively similar with Fe concentrations but our study showed higher concentrations of Cu and Ni. Kraak et al. [40] made evident that the Cu regulation capacity of *U. pictorum* is organ-specific: gill, digestive gland and shell showed higher accumulation capacity for Cu than gonads and kidney. Bourgoin and Risk [41] found a considerably higher concentration of Pb in the recent shells of a bivalve *Mya truncata* than that in fossilized shells. This indicated a temporal increased concentration of Pb in the environment. They suggested that the bivalve shell could be used as a 'benchmark' to monitor the anthropogenic inputs of Pb to the environment. High degrees of heavy metal variability in the total shells of *Unio sp.* suggested it to be generally a more sensitive and precise biomonitoring material for heavy metals than the total soft tissues of *Unio sp*. Our study suggests that mussel shells can be used as a biomonitoring material as the metals directly absorb onto the shell surfaces of the mussels. This obviously could be due to the outer layer of the shell which is directly contacted with water.

Levels of heavy metal accumulation found in all tissues of the analyzed mussel varied greatly. The distribution of total metal load IMBI values varied from 0.01 to 0.44, 0.00 to 0.47, and 0.00 to 0.43 in muscle, gill, and shell respectively. In summer, total metal load values in muscle and gill calculated high concentration when compared with other seasons. In contrast, the highest total metal loads were determined on winter for shell. Generally, IMBI values calculated in muscle were higher than gill and shell.

IMBI concentrations were calculated in many articles for European eel *Anguilla anguilla* as a good monitoring tool [15, 42, 43]. However, the freshwater mussels are more useful in heavy metal studies to monitor polluted streams. Also, they are abundant and widely distributed along the Aegean coastal streams. This reason increases the importance of freshwater mussels. IMBI values were calculated for all tissues and were then compared with seasonally. It was shown that higher IMBI values were determined in muscle and summer. Our data showed that IMBI values can be successfully calculated for bivalves.

Accumulation of heavy metals in mussels may be considered as an important warning signal for health and human consumption. Dietary standards and guidelines applicable for heavy metals in crustaceans are summarized by Turkish Food Codes (TFC) [44] and EU [45]. The limit values for human consumption of metals are shown Table 5. Consequently, it can be concluded that the levels of heavy metals in muscle are not at acceptable levels for all of the studied

TABLE 5 - The limit values for human consumption of metals for Crustaceans (TFC [44] and EU [45])

Cadmium (Cd)			Lead (Pb)		
T.F.C (Max.limit) (μg/g)	EU (Max.limit) (μg/g)	Periodic Average (μg/g)	T.F.C (Max.limit) (μg/g)	EU (Max.limit) (μg/g)	Periodic Average (μg/g)
0.50	0.50	0.047±0.00	0.50	0.50	0.151±0.08
		0.197±0.04			1.031±0.32
		0.150±0.08			0.149±0.06
		0.789±0.09			2.734±0.73

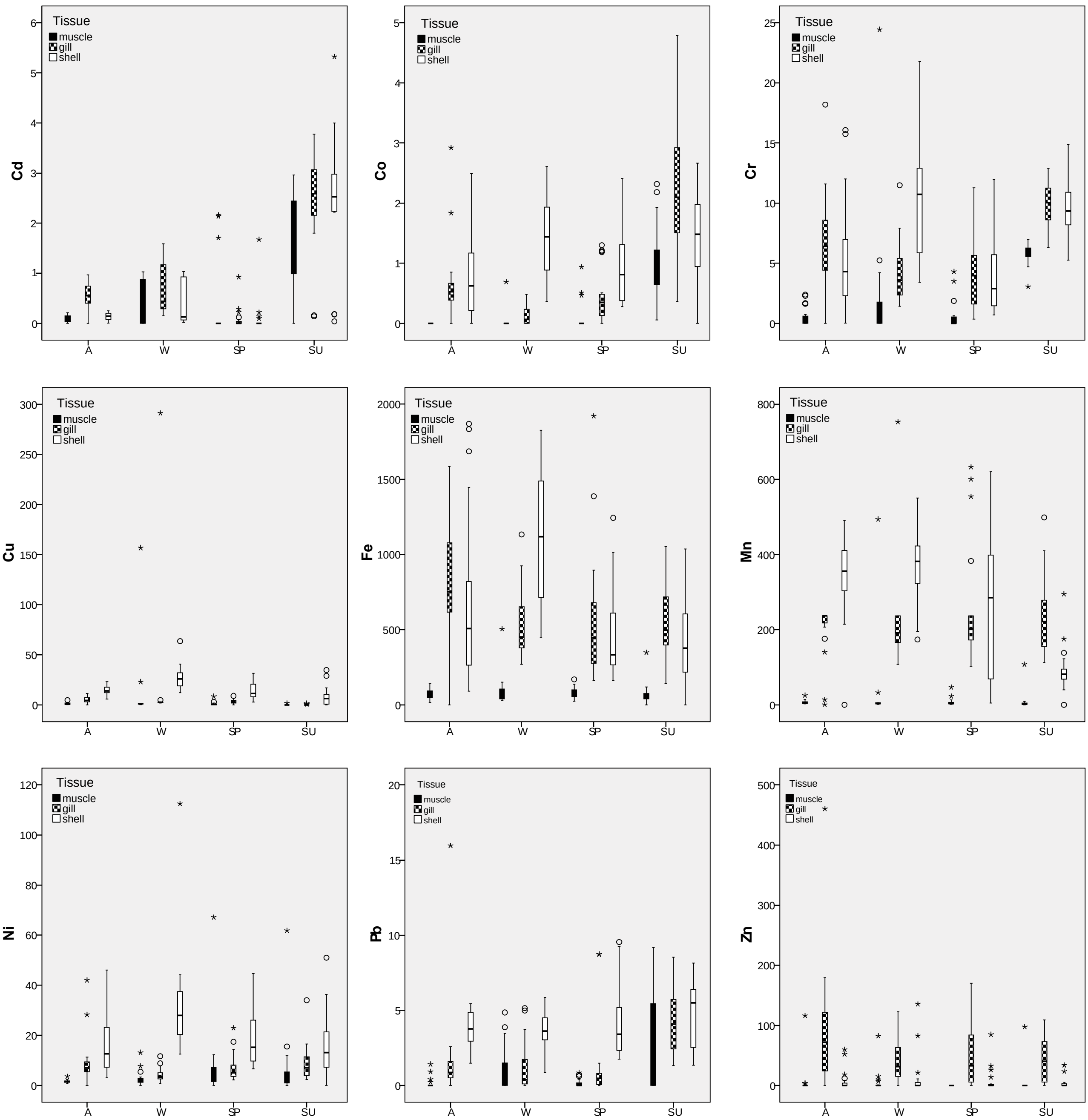

FIGURE 1 - Average heavy metal accumulation in tissues on seasonally (A:Autumn; W:Winter; Sp:Spring; Su:Summer).

samples in this region. Mean concentration of Cd and Pb levels in muscle were higher than the acceptable values for human consumption designated by various health organizations [44, 45]. The concentration of Cd was higher in summer and Pb concentration was determined higher in winter and summer. The means of heavy metal concentrations were beneath Turkish legal standards, which are 1.0 µg/g for Cd, 20.0 µg/g for Cu and 50.0 µg/g for Zn (wet weight) in bivalve mollusks [46].

4. CONCLUSION

When the data were compared with the acceptable values for human consumption designated by various health organizations, concentrations of some metals such as Cd and Pb were higher than the designated limits, especially in summer. In general, the measured heavy metals were found in higher concentrations in winter and autumn than the concentrations in spring and summer. No seasonal differences were found for the concentrations of Fe, Ni and Zn, while the concentrations of other heavy metals had seasonal differences, generally as a result of the measured concentrations in summer ($p < 0.05$). On the other side, considering the tissues together, the highest levels of heavy metals were found in shell samples, while the lowest levels in muscle. It has been also shown that IMBI were useful for determining metal accumulations in *Unio sp.*

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REFERENCES

[1] Malik, A. (2004) Metal bioremediation through growing cells. Environment International, 30, 261–278.

[2] Leland, H.V., Luoma, S.N. and Wilkes, D.J. (1978) Heavy metals and related trace elements. Water Pollution Control Federation, 50, 1469-1514.

[3] Corbett, R.G. (1977) Effects of coal mining on ground and surface water quality Monongalia County-West Virginia. Science of the Total Environment, 8, 21.

[4] Mance, G. (1987) Pollution threat of heavy metals in aquatic environment. Elsevier- London, 363.

[5] Langston, W.J. (1990) Toxic effects of metals and the incidance of marine ecosystems In: Furness, R.W. and Rainbow, P.S. (ed). Heavy Metals in the Marine Environment, CRC Press, New York, 256.

[6] Avelar, W.E.P., Mantelatto, F.L.M., Tomazelli, A.C., Silva, D.M.L., Shuhama, T. and Lopes, J.L.C. (2000) The marine mussel *Perna perna* (Mollusca, Bivalvia, Mytilidae) as an indicator of contamination by heavy metals in the Ubatuba bay, Sao Paula, Brazil. Water Air and Soil Pollution, 118, 65-72.

[7] Goldberg, E. D. (1975) The Mussel Watch a first step in global marine monitoring. Marine Pollution Bulletin, 6, 111.

[8] Kavun, V.Y., Shulkin, V. M. and Khristoforova, N. K. (2002) Metal accumulation in mussels of the Kuril Islands, north-west Pacific Ocean. Marine Environmental Research, 53, 219–226.

[9] Larsson, A., Haux, C. and Sjöbeck, M. (1985) Fish pshysiology and metal pollution: results and experiences from laboratory and field studies. Ecotoxicology and Environmental Safety, 9, 250-281.

[10] Kalay, M. and Canlı, M. (2000) Elimination of essential (Cu, Zn) and nonessential (Cd, Pb) metals from tissue of a freshwater fish *Tilapia zilli*. Turkish Journal of Zoology, 24, 429-436.

[11] Nemesok, J.G. and Hughes, G.M. (1988) The effects of copper sulphate on some biochemical parameters of rainbow trout. Environmental Pollution, 49, 77-85.

[12] Yap, C.K., Ismail A. and Tan, S.G. (2004) Heavy metal (Cd, Cu, Pb and Zn) concentrations in the green-lipped mussel *Perna viridis* (Linnaeus) collected from some wild and aquacultural sites in the west coast of Peninsular Malaysia. Food Chemistry, 84, 569–575.

[13] Barlas, M., Dirican, S. and Özdemir, N. (2001), Fish fauna of Tersakan River. Proceedings of the National of XI. National Symposium on Fisheries-Hatay, Turkey, 309–318.

[14] Önsoy, B., Filiz, H., Tarkan, A.S., Bilge, G. and Tarkan, A.N. (2011) Occurrence of non-native fishes in a small man-made lake (Lake Ula, Muğla): past, present, future perspectives. Turkish Journal of Fisheries and Aquatic Sciences, 11, 209–215.

[15] Maes, J., Belpaire, C. and Goemans, G. (2008) Spatial variations and temporal trends between 1994 and 2005 in polychlorinated biphenyls, organochlorine pesticides and heavy metal in European eel (*Anguilla anguilla*) in Flanders-Belgium. Environmental Pollution. 153, 223-237

[16] Broman, D., Lindqvist, L. and Lundbergh, I. (1991) Cadmium and zinc in *Mytilus edulis* L. from the Bothnian Sea and the northern Baltic proper. Environmental Pollution, 74, 227–244

[17] Canlı, M. and Atlı, G. (2003) The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six mediterranean fish species. Environmental Pollution, 121, 129–136.

[18] Barghigiani, C. and Ranieri D,S. (1992) Mercury content in different size of important edible species of the northern Tyrrhenian sea. Marine Pollution Bulletin, 24, 114-116.

[19] Zyadah, M.A. (1999) Accumulation of some heavy metals in *Tilapia zilli* organs from lake Manzalah, Egypt. Turkish Journal of Zoology, 23, 365-372.

[20] Usero, J., Izquierdo, C., Morillo, J. and Gracia, I. (2003) Heavy metals in fish (*Solea vulgaris, Anguilla anguilla* and *Liza aurata*) from salt marshes on the southern Atlantic Coast of Spain. Environmental International, 1069, 1–8.

[21] Cabrera, F., Vlemente, L., Diaz Barrientos, E., Lŏpez, R. and Murillo, H. M. (1999) Heavy metal pollution of soil affected by the Guadiamar toxic Hood. Science of the Total Environment, 242, 117–129.

[22] Amiard, J.C., Amiard-Triquet, C., Berhet, B. and Metayer, C. (1987) Comparative study of the patterns of bioaccumulation of essential (Cu-Zn) and non-essential (Cd-Pb) trace metals in various estuarine coastal organisms. Journal of Experimental Marine Biology and Ecology, 106, 73-79.

[23] Heath A.G. (1987) Water Pollution and Fish Physiology. CRC Press, Florida USA, 245.

[24] Bryan G.W. and Langston, W.I. (1992) Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries, review. Environmental Pollution, 76, 89-131.

[25] Yilmaz, F. (2009) The comparison of heavy metal concentrations (Cd, Cu, Mn, Pb, and Zn) in tissues of three economically important fish (*Anguilla anguilla*, *Mugil cephalus* and *Oreochromis niloticus*) Inhabiting Köycegiz Lake-Mugla (Turkey). Turkish Journal of Science & Technology, 4 (1), 7-15.

[26] Yarsan, E. and Bilgili, A. (2000) Heavy Metal Levels in Mussels (*Unio stevenianus* Krynicki) Obtained from Van Lake. Turkish Journal of Veterinary & Animal Science, 24, 93-96.

[27] Kır, İ. And Tuncay, Y. (2010) The Investigation of some Heavy Metals in Crayfish (*Astacus leptodactylus*) Inhabiting Kovada Lake, Sdu Journal of Science, 5 (2), 179-186.

[28] Amiard J.C., Amiard-Triquet, C. and Metayer, C. (1985) Experimental study of bioaccumulation, toxicity and regulation of some trace metals in various estuarine and coastal organism. Symposia Biologica Hungarica, 29, 313-323.

[29] Chou, C.C., Uthe, J.F., Castell, J.D., Kean, J.C. and Lores, E.M. (1987) Effect of dietary on growth survival and tissue concentration of cadmium, zinc, copper and silver injuvenile American lobester (Homarus americanus). Canadian Journal Of Fisheries & Aquatic Sciences, 44, 1443-1450.

[30] Bryan, G.W. (1968) Concentration of zinc and copper in tissues of decapod crustacean. Journal of the Marine Biological Association of the United Kingdom, 48, 303-321.

[31] Voutsinou-Taliadouri, F. (1982) Monitoring of some metals in some marine organisms from the Saronikos Gulf. J. Etud. Pollut., 6, 329-333.

[32] Rainbow, P.S. and White, S.L. (1989) Comparative strategies of heavy metal accumulation by crustaceans: zinc, copper and cadmium in a decapod, an amphipod and a barnacle. Hydrobiologia, 174, 245-262.

[33] Manly, R. and George, W.O. (1977) the occurrence of some heavy metals in populations of the freshwater mussel *Anodonta anatina* (L.) from the River Thames. Environmental Pollution, 14, 139-154.

[34] Van-Balogh, K. (1988) Heavy metal pollution from a point source demonstrated by mussel (*Unio pictorum* L.) at Lake Balaton, Hungary. Bulletin of Environmental Contamination and Toxicology, 41, 910-914.

[35] Adams, T.G., Atchinson, G.J. and Vetter, R.J. (1981) the use of the three ridge clam (*Amblema perplicata*) to monitor trace metal contamination. Hydrobiologia, 69, 187-194.

[36] Gundacker, C. (2000) Comparison of heavy metal bioaccumulation in freshwater molluscs of urban river habitats in Vienna. Environmental Pollution, 110, 61-71.

[37] Yap, C.K. (2012) Distribution of Cu and Zn in the shell lipped part periostracum and soft tissues of *Perna viridis*: The potential of periostracum as a biomonitoring material for Cu. Pertanika Journal of Tropical Agricultural Science, 35 (3), 413–426.

[38] Yap, C.K., Noorhaidah, A., Azlan, A., Nor Azwady, A.A., Ismail, A., Ismail, A.R., Siraj, S.S. and Tan, S.G. (2009) *Telescopium telescopium* as potential biomonitors of Cu, Zn, and Pb for the tropical intertidal area. Ecotoxicology and Environmental Safety, 72, 496–506.

[39] Yap, C.K., Azmizan, A.R. and Hanif, M. S. (2011) Biomonitoring of trace metals (Fe, Cu and Ni) in the mangrove area of Peninsular Malaysia, using different soft tissues of flat-tree oyster Isognomon alatus. Water, Air and Soil Pollution, 218, 19-36.

[40] Kraak, M.H.S., Toussaint, M., Bleeker, E.A.J. and Lavy, D. (1993) Metal regulation in two species of freshwater bivalves, Ecotoxicology of Metals in Invertebrates (SETAC Special Publications). Lewis, Chelsea, MI, 175-186.

[41] Bourgoin, B. P. and Risk, M. J. (1987) Historical changes in lead in the eastern Canadian Arctic, determined from fossil and modern *Mya truncata* shells. Science of the Total Environment. 67, 287-291.

[42] Esteve, C., Alcaide, E. and Urena, R. (2012) The effect of metals on condition and pathologies of European eel (*Anguilla anguilla*): In situ and laboratory experiments. Aquatic Toxicology, 109, 176-184.

[43] Maes, G.E., Raeymaekers, J.A.M., Pampoulie, C., Seynaeve, A., Goemans, G., Belpaire, C. and Volckaert, F.A.M. (2005) The catadromous European eel *Anguilla anguilla* (L) as a model for fresh water evolutionary ecotoxicology: relationship between heavy metal bioaccumulation, condition and genetic variability. Aquatic Toxicology, 73, 99-114

[44] TFC. (2008) Turkish Food Codes, Official Gazette, Communique on Maximum Limits for Contaminants of Food Ingredients (Communique Number: 2008/26).

[45] EU. (2006) Commission Regulation Directive, (EC) No 1881/2006 of 19 December.

[46] Republic of Turkey, Ministry of Food, Agriculture and Livestock (1996) Guidebook of fisheries quality control services. General Directorate for Protection and Control, Ankara (*in Turkish*).

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