

THE ASSESSMENT OF HEAVY METAL CONTENT BY USING BIOACCUMULATION INDICES IN EUROPEAN CHUB, *Squalius cephalus* (Linnaeus, 1758)

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Abstract: The concentrations of nine metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn), individual total metal load (IMBI) values and metal pollution index (MPI) were determined in water, sediment and European chub, *Squalius cephalus* (Linnaeus, 1758) inhabiting Sarıçay Stream Turkey. A total of sixty European chub samples and twelve sediment and water samples were taken and analysed seasonally between June 2011 and May 2012. Heavy metals were analysed by ICP-AES. The distribution of the IMBI values ranged from 0.040 to 0.418. Distribution patterns of IMBI in seasons and stations of European chub follow the sequence: spring> winter> summer> autumn, Station III> I> II, respectively. Result of high IMBI values in all seasons and stations can be explained by the fact that increasing MPI value of Zn, Fe and Mn. Among the heavy metals studied Cd and Co were below the detection limits in most seasons. The heavy metal concentrations in the edible tissue of European chub were compared with the tolerable national and international values in fish. The results obtained, showed that the heavy metal concentrations in edible tissue were excessive and were not safe within the limits for human consumption.

Keywords: *Squalius cephalus*, IMBI, MPI, Heavy metal pollution, Sediment, Water.

1. INTRODUCTION

Freshwater environments are rapidly polluted due to activities such as the production of technology, continued industrialisation, population growth, and migration from rural areas to urban areas, unplanned urbanisation and destruction of natural areas (Eisler, 1993; Nimmo et al., 1998; Al-Yousuf et al., 2000; Alam et al., 2002; Demirak et al., 2006). Waste and wastewater released after industrial activities include the heavy metals. Heavy metals which enter surface and underground waters have potential risks on organisms and this has become an important issue in recent years. Heavy metals tend to accumulate in living organisms. Metals are particular concern among environmental pollutants, because of potential toxic effect and ability to accumulate in aquatic ecosystems (Censi et al., 2006; Dalman et al., 2006). Significant quantities of heavy metals are discharged into rivers, which can be strongly accumulated, and biomagnified along water, sediment and aquatic food

chains, resulting in sub lethal effects or death in local fish populations (Megeer et al., 2000; Jones et al., 2001; Almeida et al., 2002; Xu et al., 2004). Fish might be considered as one of the most significant indicators in water, because they are often at the top of the aquatic food chain and may concentrate large amounts of some metals from the water (Rashed, 2001; Mansour & Sidky, 2002). Also, fish are a regular component of the human diet; therefore they can represent a dangerous source of certain heavy metals (Barak & Mason, 1990; Papagiannis et al., 2004; Özparlak et al., 2012).

European chub was chosen to investigate because they are most abundant in small rivers and large streams and widely tolerant to most conditions (Andres et al., 2000). Adults are solitary while juveniles occur in groups. Feeding larvae and juveniles live in very shallow shoreline habitats and feed on a wide variety of aquatic and terrestrial animals and plant material; large individuals prey predominantly on fish and breed in fast-flowing

water above the gravel bottom, rarely among submerged vegetation (Kottelat & Freyhof, 2007).

The aim of this study is to determine concentrations of cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron(Fe), manganese (Mn), nickel (Ni), lead (Pb), zinc (Zn) in sediment, water and edible tissue (muscle) of European Chub, taken from Sarıçay stream in the South-West of Turkey by applying the individual mean (multi-metal) bioaccumulation index, condition factor and also MPI was used to find IMBI values which got affected by these metals.

2. MATERIALS AND METHODS

2.1 Study area

Sarıçay stream is about 50 km in length. There are two dams on Sarıçay which are called Akgedik and Geyik. Three sampling points were selected (Fig. 1) in the research area. The first station (station I) was chosen upstream in front of the dam, where there is agricultural activity. The second station (station II) was located in the area where the water slows down. This sampling point is rich in the view of aquatic organism's biodiversity. The third station (station III) was chosen after the industrial companies which produce animal nutrition, olive oil and concrete plant. This sampling point was also affected by the fertilisers and pesticides used for agricultural activity.

2.2. Sample collection and preparation

During the study period, four fishing expeditions were carried out in the Sarıçay Stream between September 2011 and August 2012. The

samples were collected by electrofishing, using Deca-Lord-12 V generator which produces 250–600 V. Total of sixty European chub samples were transported to the laboratory in a thermos-flask with ice on the same day in each study period. Approximately 5 g of muscle from each sample were dissected, washed with deionised water, packed in polyethylene bags, and stored at -20°C prior to analysis. Sediment and water samples for metal analysis were also collected from the same 3 stations, which were chosen for fish sampling. Water samples were collected in polyethylene bottles. Samples were acidified with 10% HNO_3 , put in an ice bath and brought to the laboratory. Then samples were filtered through a $0.45\ \mu\text{m}$ micro pore membrane filter and kept at -20°C until analysis. The surface sediments were collected with sediment collector with an acid-washed plastic scoop and returned to the laboratory in polyethylene bags. The samples were sieved through a 2-mm sieve.

The Standard length (L) and weight (W) of all sixty fish were immediately measured and estimated (mean and standard deviation) as 135.4 ± 17.4 mm and 383.1 ± 203.6 g respectively; their muscle tissues were put into polyethylene containers.

2.3. Analytical procedures

For muscle tissue samples, 0.5g wet tissue samples taken of each fish were put into digestion flasks and 30% hydrogen peroxide and 70% nitric acid (Merck) were added. The digestion flasks were then put on a microwave digestion unit (speedwave four microwave digestion system) to 120°C (gradually increased) until all the materials were dissolved.

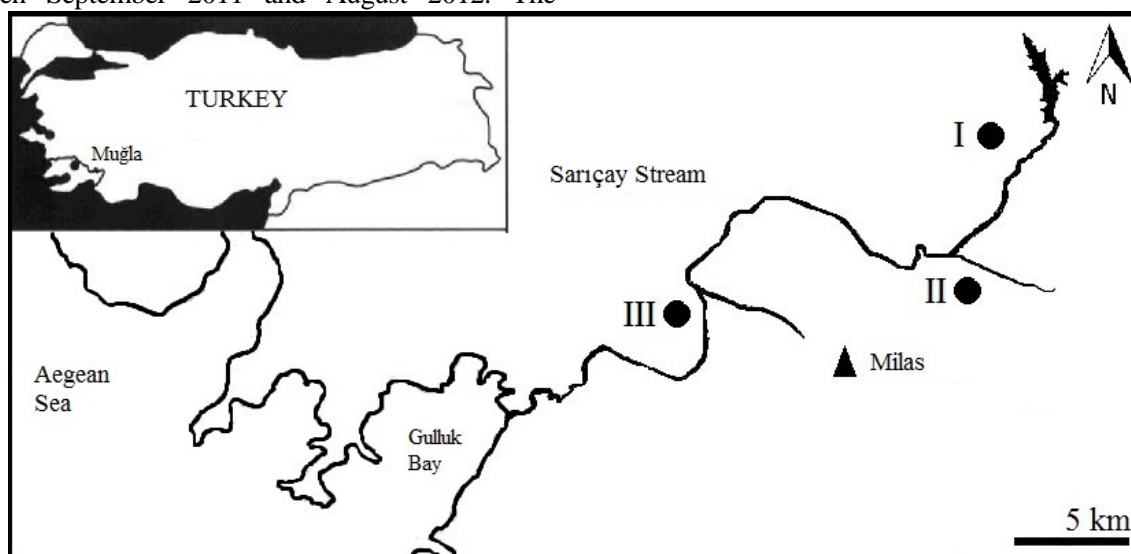


Figure 1. Sarıçay Stream and sampling sites.

After digestion the digested samples were diluted with ultra-distilled water appropriately in the range of standards which were prepared from stock standard solution of the metals (Merck). The solution was transferred, diluted and filtered through 0.45µm nitrocellulose membrane filter (Alam et al., 2002). All reagents were of analytical reagent grade. Deionised water was used throughout the study. High purity argon was used as inert gas (Karadede et al., 2004). For sediment, 0.5 g dry sediment samples were placed in a Teflon vessel (100 ml capacity) and digested with HNO₃ (4 ml), HF (4 ml), and HCl (2 ml). Teflon vessels were then put on a microwave digestion unit and follow the same order as fish sample. All samples were analysed simultaneously two times for Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn by ICP-AES Optima 2000-Perkin Elmer (Inductively Coupled Plasma-Atomic Emission Spectrometry). Detection limits (µg l⁻¹) were as follows: Cd (0.001), Co (0.001), Cr (0.007), Cu (0.014), Pb (0.001), Zn (0.006), Ni (0.004), Mn, (0.005), Fe (0.003). Standard reference materials were as follows: for water SRM-143d, National Institute of Standards and Technology; for sediment CRM-277, Community Bureau of Reference; for fish DORM-3 (National Research Council Canada, Ottawa Ontario, Canada) used and replicate analysis of these reference materials showed good accuracy, with recovery rates for metals between 91% and 109% for fish, 92% and 104% for sediment and between 94% and 102% for water.

2.4. Statistical analyses of condition and heavy metal data

The linear regression analyses were applied to data to compare the relationships between length and weight. Condition index (CI) was calculated as $CI = 100W/L^b$, where respectively L and W relate to standard length in centimetres and body weight in grams, b = the value obtained from the length-weight equation (King, 1995). IMBI has been used in many investigations for European eel *Anguilla anguilla*, Blue crab *Callinectes sapidus* and fresh water mussels *Unio sp.* as a good monitoring tool (Maes et al., 2008; Esteve et al., 2012; Genç & Yilmaz 2015; Genç et al., 2015).

Relative bioaccumulation index was calculated by dividing (standardizing) the individual concentration of heavy metal *i* (C_i) by the maximum observed concentration (C_{imax}) and averaging over all metals, to relate heavy metal bioaccumulation to condition. Thus, the individual mean (multi-metal) bioaccumulation index (IMBI) was defined as:

$$IMBI = \frac{\sum_{i=0}^n C_i / C_{imax}}{n}$$

with *n* the total number of metals, C_i the individual concentration of heavy metal *i*, C_{imax} the maximal observed concentration of heavy metal *i* and $0 < IMBI < 1$ (Maes et al., 2005).

The Metal Pollution Index (MPI) was used, obtained with the equation (Usero et al., 1997);

$$MPI = (Cf_1 \times Cf_2 \dots Cf_n)^{1/n}$$

where, Cf_i = concentration for the metal 'i' in the sample.

Statistical analysis of data was carried out using Stat soft and SPSS 20.0 statistical package program. The nonparametric Kruskal–Wallis and Mann-Whitney test was used to assess whether metal concentrations varied as significantly between stations. Also, Pearson's correlation matrix was used between fish and sediment water heavy metal values.

3. RESULTS AND DISCUSSION

The mean of metal concentrations of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in water and sediment are shown in table 1. The table is generated by comparing measured concentrations of elements with water quality standards currently effective in Turkey (TEG, 1988; USEPA, 1999). Water quality regulations in Turkey divide inland waters into four classes. Class I indicates to clean water that can be used for domestic purposes after simple disinfection, for recreational purposes or for irrigation. Class II indicates to fairly clean water that can be used as domestic water after treatment, for recreational purposes or for fishing, farming, etc. Class III indicates to polluted water that can only be used as industrial water after treatment. Class IV indicates to heavily polluted water that should not be used at all (TEG, 1988; Demirak et al., 2006). In water, Cd was below the detection limit in all seasons and stations. The highest mean values for all analysed metals, except Ni, were found in stations III, and lowest metal concentrations of all metals were found in stations II. According to TEG (1988) only the average Fe concentration was found Class II. The other metal average concentrations were found Class I. When heavy metal levels of water compared with the priority toxic pollutants (USEPA, 1999) average metal concentrations of Sarıçay stream waters were observed lower than the criteria maximum concentrations (CMC) and criterion continuous concentration (CCC) values of the US EPA water quality criteria (Table 1). The background levels for metals in sediments and the probable effect concentrations for sediments were given in table 1.

Table 1. Comparison of overall metal concentrations in water ($\mu\text{g l}^{-1}$) and sediment ($\mu\text{g g}^{-1}$) of Sariçay Stream with guidelines

		Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Present study (Sediment ^a)	Mean \pm SE	0.80 \pm 0.08	35.9 \pm 3.75	38.24 \pm 3.17	47.12 \pm 2.89	8068 \pm 388.49	265.98 \pm 15	32.01 \pm 1.94	32.56 \pm 3.29	123.6 \pm 11.9
	Min-max	BDL-1.83	7.54-92.12	12.70-99.78	8.74-102.56	2617-14062	43.36-417.8	13.22-68.91	5.92-84.85	9.09-347.55
PEC ^b	max	5	-	111	149	-	-	49	128	459
BC ^c	max	0.4	-	17	8	-	-	11	14	67
Present study (Water)	Mean \pm SE	BDL	1.53 \pm 0.18	6.70 \pm 0.46	2.25 \pm 0.23	649.79 \pm 74.49	51.01 \pm 2.33	7.37 \pm 0.31	0.57 \pm 0.05	32.2 \pm 1.53
	Min-max	-	BDL-4.08	3.00-14.15	BDL-6.23	109.34-2287.17	29.71-87.49	4.20-12.14	BDL-1.04	14.00-52.60
Water quality criteria ^d	CMC	4.3	-	16	13	-	-	-	65	120
	CCC	2.2	-	11	9	-	-	-	2.5	120
Turkish Environmental Guidelines ^e	Class I	3	10	20	20	300	100	20	10	200
	Class II	5	20	50	50	1000	500	50	20	500
	Class III	10	200	200	200	5000	3000	200	50	2000
	Class IV	>10	>200	>200	>200	>5000	>3000	>200	>50	>2000

^a Number of examined samples 12; SE: Standard error; Min-max: minimum-maximum levels; BDL: Below the detection limit.

^b PEC: probable effect concentrations (MacDonald et al., 2000).

^c BC: background concentration (Bervoets & Blust, 2003).

^d Turkish Environmental Guidelines, TEG (1988).

^e CCC: Criterion Continuous Concentration; CMC: Criteria Maximum Concentrations, (USEPA, 1999).

Table 2. The mean heavy metal concentrations ($\mu\text{g g}^{-1}$ wet weight) in muscles of European chub in Sariçay Stream

Period	Station	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Autumn	1.	BDL**	BDL	0.93 \pm 0.17 ^a	1.19 \pm 0.29 ^a	29.17 \pm 6.19 ^a	12.87 \pm 3.12 ^a	0.78 \pm 0.15 ^a	0.18 \pm 0.16 ^a	49.02 \pm 9.030 ^a
	2.	BDL	0.044 \pm 0.0 ^{a*}	0.36 \pm 0.07 ^b	0.94 \pm 0.19 ^a	17.83 \pm 3.34 ^a	4.19 \pm 1.17 ^b	0.94 \pm 0.37 ^a	0.04 \pm 0.03 ^a	33.27 \pm 6.670 ^a
	3.	BDL	0.007 \pm 0.0 ^a	0.58 \pm 0.15 ^{ab}	0.46 \pm 0.12 ^a	17.79 \pm 5.2 ^a	3.92 \pm 1.29 ^b	2.56 \pm 1.28 ^a	0.25 \pm 0.14 ^a	40.42 \pm 11.16 ^a
Winter	1.	BDL	BDL	0.20 \pm 0.13 ^a	1.10 \pm 0.39 ^a	64.55 \pm 30.32 ^a	7.06 \pm 0.74 ^a	1.36 \pm 1.17 ^a	1.61 \pm 0.65 ^a	51.61 \pm 13.50 ^a
	2.	BDL	BDL	0.96 \pm 0.96 ^a	1.40 \pm 0.90 ^a	27.57 \pm 19.71 ^{ab}	4.28 \pm 1.02 ^{ab}	1.09 \pm 0.47 ^a	2.15 \pm 0.77 ^{ab}	16.97 \pm 4.110 ^b
	3.	BDL	0.499 \pm 0.5	0.50 \pm 0.50 ^a	1.54 \pm 0.49 ^a	12.44 \pm 4.09 ^b	3.65 \pm 0.63 ^b	2.21 \pm 0.62 ^a	3.74 \pm 0.48 ^b	20.07 \pm 1.790 ^{ab}
Spring	1.	BDL	BDL	0.88 \pm 0.51 ^a	2.98 \pm 0.45 ^a	0.18 \pm 0.17 ^a	2.64 \pm 0.54 ^a	1.80 \pm 0.13 ^a	5.15 \pm 0.70 ^a	12.71 \pm 1.370 ^a
	2.	0.057 \pm 0.04 ^a	BDL	1.85 \pm 1.53 ^a	2.44 \pm 0.61 ^a	9.56 \pm 8.97 ^{ab}	3.93 \pm 1.87 ^a	1.78 \pm 0.65 ^a	2.48 \pm 1.16 ^{ab}	22.32 \pm 7.840 ^a
	3.	0.121 \pm 0.01 ^a	BDL	2.39 \pm 0.99 ^a	3.06 \pm 0.37 ^a	26.19 \pm 4.19 ^b	11.52 \pm 2.25 ^b	2.15 \pm 0.76 ^a	0.44 \pm 0.08 ^b	31.53 \pm 7.520 ^a
Summer	1.	BDL	BDL	0.54 \pm 0.19 ^a	2.17 \pm 0.25 ^a	23.83 \pm 3.60 ^a	7.33 \pm 1.84 ^a	0.49 \pm 0.20 ^a	0.35 \pm 0.13 ^a	96.64 \pm 67.85 ^a
	2.	BDL	BDL	0.65 \pm 0.10 ^a	1.90 \pm 0.28 ^a	13.81 \pm 2.04 ^b	4.61 \pm 1.14 ^a	0.26 \pm 0.05 ^a	0.42 \pm 0.06 ^a	26.72 \pm 2.660 ^a
	3.	BDL	BDL	1.78 \pm 0.51 ^b	1.39 \pm 0.38 ^a	12.84 \pm 2.43 ^b	5.29 \pm 0.53 ^a	0.84 \pm 0.23 ^a	0.88 \pm 0.25 ^a	43.70 \pm 9.540 ^a

Statistical differences of heavy metal accumulation on tissues are presented as letters (a,b,c), the statistically significant difference ($p<0.05$).

*Mean values and \pm standard deviation.

**BDL: Below the detection limit.

The background levels are proposed by Bervoets & Blust (2003), based on total metal values measured at reference sites. The probable effect concentration (probable, severe and toxic effect levels of some metals) for sediment levels were reported by MacDonald et al. (2000).

Mean concentrations of Fe, Mn, Ni and Zn ($10164 \mu\text{g g}^{-1}$, $363.12 \mu\text{g g}^{-1}$, $42.30 \mu\text{g g}^{-1}$, $168.84 \mu\text{g g}^{-1}$, respectively) in the sediment from Sarıçay stream were found higher in station I, while for Cd, Co, Cr, Cu and Pb ($1.13 \mu\text{g g}^{-1}$, $53.04 \mu\text{g g}^{-1}$, $53.00 \mu\text{g g}^{-1}$, $54.63 \mu\text{g g}^{-1}$, respectively) the highest concentrations were determined in station III. The mean concentrations of Cd, Cr, Cu, Ni, Pb and Zn in sediment from the Sarıçay stream were less than the probable effect concentrations (Table 1). However, the average values of Cd, Cr, Cu, Ni, Pb and Zn in sediment from the Sarıçay stream exceeded the background levels. Demirak et al., (2006) reported that average values of Cd, Cu, Pb, Zn and Cr never exceeded the probable effect concentrations, while average Cd, Pb and Cr concentrations were found higher than background levels. According to Bervoets & Blust (2003) Cd and Zn concentrations were found higher than background levels at all sites and Pb and Cu were determined higher than background levels at most stations, while the average values of Cr and Ni were not exceeded the background levels.

As found for water and sediment, the concentrations of heavy metals in sediments and water were higher in station III and I than station II. Therefore, this stream is affected mainly by anthropogenic activities waste discharges from producing industrial, agricultural. Increase of concentrations of heavy metals in station I might be explained by the agricultural activity land flushing that enriches rivers with continental heavy metals and increase of concentrations of heavy metals in station III may be explained by the wastewaters impact responsible of high levels of metals by the industrial activities (animal nutrition factory, oil factory and concrete plant) and agricultural activity.

The present study focused on the accumulation of heavy metals in European chub and showed differences in the accumulation of heavy metals during the seasons, in each station. The mean accumulation of heavy metals of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in seasonally and in muscle of European chub is shown in Table 2.

The highest metal accumulation was determined (Zn $368.01 \mu\text{g g}^{-1}$) in station I in summer on a chub. Average Zn concentrations ranged from $12.71 \mu\text{g g}^{-1}$ to $96.64 \mu\text{g g}^{-1}$. In the present study, values were found higher than in the previous study

of Sarıçay stream (Yilmaz et al., 2007).

The highest concentrations of Cu was determined $4.96 \mu\text{g g}^{-1}$ in winter and station II, while lowest concentrations of Cu was found $0.074 \mu\text{g g}^{-1}$ in autumn in stations III. Agtas et al., (2007) found highest Cu concentration $3.79 \mu\text{g g}^{-1}$ in the winter, while lowest Cu concentration was found $1.01 \mu\text{g g}^{-1}$ in the summer in the muscle tissue of chub in Yildiz River. Cu concentrations are similar to reported earlier in fishes. According to Agtas et al., (2007), Cu and Zn concentrations were determined high in winter, while Fe concentration was found high in spring.

Cu and Zn carefully regulated by physiological mechanisms in most organisms; accumulate in porphyrins and enzymes (Bowen, 1979). Besides, the activity of numerous enzymes (Cu-Zn superoxide dismutase, catalase, tyrosinase etc.) depends on the presence of Cu. The main role of Zn is that as a co-factor in many enzymatic systems, it is involved in the utilisation of almost all nutrients. Manganese acts either as a co-factor for numerous enzymes involved in nitrogen, lipid and carbohydrate metabolism or as an integral part of enzymes (private carboxylase, lipase) (Schlenk & Benson, 2001). However, they are regarded as potential hazards that can endanger both animal and human health. Similar to the route of essential metals, non-essential ones are also taken up by fish and accumulate in their tissues (Allen-Gil & Martynov, 1995; Canli & Atli, 2003; Yilmaz, 2006). Cr values ranged from $0.15 \mu\text{g g}^{-1}$ to $7.89 \mu\text{g g}^{-1}$ in spring and station II. The highest concentration of Pb was determined $6.781 \mu\text{g g}^{-1}$ in spring in station I. Pb values were higher than reported previously in European chub (Yilmaz et al., 2007). Yilmaz et al. (2007), found Fe concentrations between $15.67-135 \mu\text{g g}^{-1}$ in European chub. In this study, concentration values of Fe were found between $2.40 \mu\text{g g}^{-1}$ in spring, station II and $183.60 \mu\text{g g}^{-1}$ in winter in station I. Fe values were found higher than previously reported values in same fish species in Sarıçay stream. Concentration of Mn was found between $0.096 \mu\text{g g}^{-1}$ in station III and $20.79 \mu\text{g g}^{-1}$ in station I both in autumn. Duman & Kar (2012) found Mn concentration is higher in winter than the other season. In this study, Mn concentrations were found higher in autumn than in the other seasons. Our results of Mn content in the samples were similar the results reported by Yilmaz et al., 2007. According to Duman & Kar (2012), Cr, Mn and Cu concentrations were found high in the winter in muscle tissue of European chub. Mostly, the amount of heavy metals increased after the rainy season, as shown in spring and winter. High metal accumulation in winter and spring might be due to heavy rainfall during these

seasons, which increases the metal content of water by washing down (Dural et al., 2007). Some of the heavy metal concentrations are higher in station III (downstream) and the reason might be the factory that produces animal nutrition, oil factory, concrete plant and agricultural activity, which are situated near the station III. Regarding Co, Cd, Cu and Ni concentrations in European chub, there were no significant differences between.

Correlation was applied to determine the relative importance of the different environmental compartments contributing to the variation in metal levels in the tissues. The Pearson's correlation coefficient matrix for the element pairs was performed, as there was a linear relationship among the element pairs. Only Pb and Zn were found to have positive correlation coefficients between fish and environmental compartments (for Pb, $r = 0.627$, $p < 0.01$ between sediments and muscle, for Zn, $r = 0.268$, $p < 0.05$, between sediments and muscle). Also Demirak et al., (2006) reported high correlations between muscle, gills with sediment in Zn and Cu concentrations. Zn and Cu concentrations in the gills of *S. cephalus* can be used as a bio-indicator for monitoring the degree of the pollution in the study area (Demirak et al., 2006).

The distribution of the IMBI values ranged from 0.040 to 0.418 (Fig. 2). Increase of IMBI shows that individuals are more polluted. According to Maes et al., (2005) index value assessment was defined before 0.22 "low" and after 0.25 "high" polluted individuals.

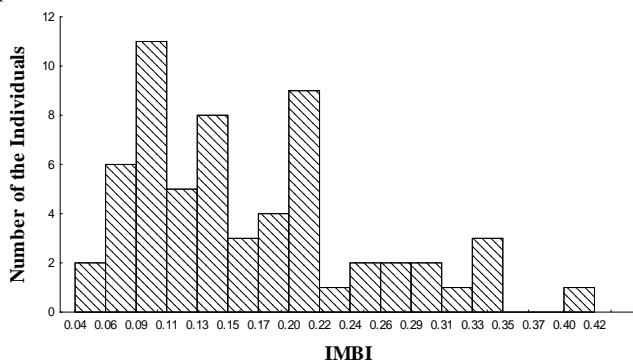


Figure 2. The distribution of individual mean bioaccumulation index (IMBI) values in muscle of European chub. Increase of x-axis defines the pollution of individuals ($N=60$).

The distribution of the results of individual mean (multi-metal) bioaccumulation index (IMBI) according to seasons and stations were given in the figure 3. According to IMBI, heavy metals pollution increases in spring in all stations. The highest metal concentrations of individuals were found to be in spring, at the station III. IMBI was found lower in

the whole of station II, compared to stations I and III. Distribution patterns of IMBI in seasons of European chub follow the sequence: Spring > winter > summer > autumn (significant difference by Kruskal–Wallis and Man-Whitney, $p < 0.05$).

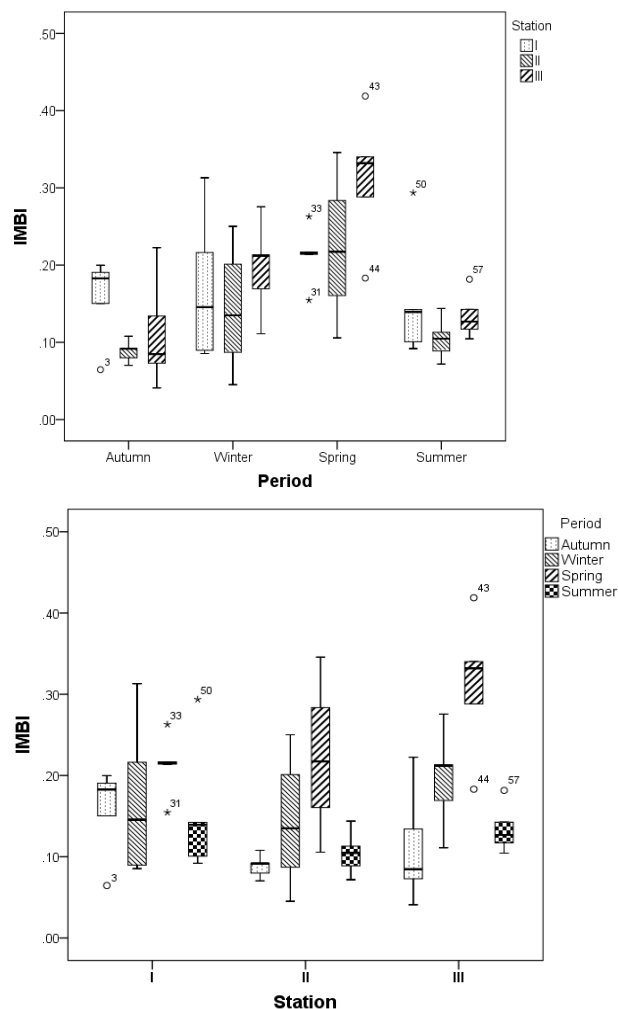


Figure 3. Comparison of individual mean bioaccumulation index values in muscle of European chub in different season from the stations. A line within the box marks the median. Whiskers above and below the box indicate maximum and minimum (excluding outliers). The numbers of individual outliers are indicated on the graph. ($N=60$).

IMBI may explain distribution of total metal load seasonally but cannot introduce which metals are affecting these values. IMBI values of most of the metals varied notably depending on the MPI values seasonally. The results of high IMBI values in all season except Fe value in spring may explain the fact that increase MPI values of Zn (37.31, 23.66, 16.96, 36.89), Fe (18.75, 12.60, 0.45, 15.33) and Mn (4.27, 4.46, 3.67, 5.09 all values in autumn, winter, spring and summer, respectively) and also may explain the high IMBI values in every station because of the

highest MPI values of Zn (32.92, 21.39, 29.07), Fe (5.11, 3.44, 14.86) and Mn (5.81, 3.19, 4.44 all values in station I, II and III, respectively).

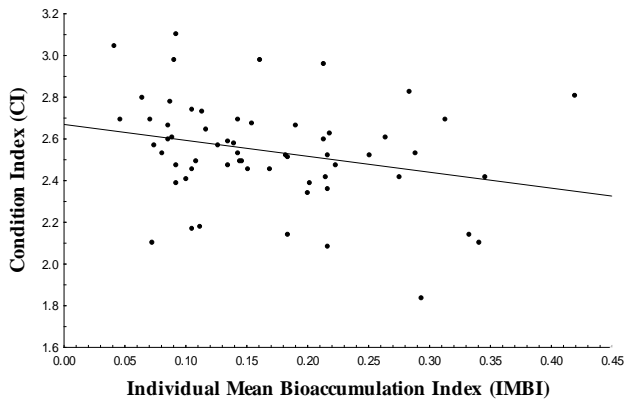


Figure 4. Linear regression ($R = -0.26$; $p = 0.04$) of heavy metal bioaccumulation (IMBI) and condition indices (CI) of European chub ($N = 60$).

The regression equation between length and weight was $\log(W) = 2.798 \log(L) - 1.606$ (R Value 0.918, $N = 60$, $p < 0.001$). The relative condition index (CI) was calculated as $CI = 100(W/L^{2.798})$ (Fig. 4). Significant negative correlations between the CI and heavy metal concentrations were determined only for Zn (r value -0.352, $p < 0.01$) and Cu (r value -0.255, $p < 0.05$). The significant negative correlations between CI and the IMBI were determined $r = -0.265$,

$p < 0.05$. There is no significant correlation between condition index and heavy metals (except Zn and Cu) on chubs that are length and weight ranges between 10.5-22 cm and 19-173g, respectively.

According to Maes et al., (2005), regression analysis revealed a strong negative correlation between individual bioaccumulation and condition indices in *Anguilla anguilla* (European eel). According to the Tekin-Özan & Aktan (2012) study, negative relationships were found between fish weight and length and Ni in muscle. Also Tekin-Özan & Aktan (2012) found positive relationships between Cr, Cu, Fe, Mn, Zn and weight, length of *Scomber japonicas* (chub mackerel). Yi & Zhang (2012) found positive correlations between fish size and Zn, Cd, Pb in grass carp, *Coreius heterodom* and *Cyprinus carpio* (common carp), while negative correlations were found in *Silurus asotus* (catfish) and *Pelteobagrus fulvidraco* (yellow-head catfish). Also, similar results between heavy metal concentrations and individual conditions factors were found. Their study showed that there are positive relationships between fish sizes and metal levels in most cases. Authman (2008) found a negative correlation between condition factors and iron, manganese, lead, copper and zinc on *Oreochromis niloticus* (Nile tilapia).

Table 3. Heavy metal concentrations ($\mu\text{g g}^{-1}$) in muscle tissue of *European chub* according to guidelines and other studies.

	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn	References
Sarıçay Stream, Turkey (ww*)	0.01±0.01**	0.97±0.19	1.72±0.15	21.32±3.55	5.94±0.57	1.36±0.19	1.48±0.24	37.09±6.10	Present study
Sarıçay Stream (I and II) (ww)	0.02 and 0.001	BDL***	0.19 and 0.57	4.47 and 4.24	0.11 and 1.2	BDL	0.07 and 0.30	6.35 and 9.66	Yılmaz et al., (2007)
Beyşehir Lake, Turkey(dw**)	0.58	0.24	-	-	-	-	0.34 (dw)	-	Altındağ & Yiğit (2005)
Beyşehir Lake, Turkey(ww)	-	BDL	BDL	4.41	BDL	-	BDL	8.49	Tekin-Özan & Kir (2006)
Dipsiz Stream, Turkey(ww)	0.01	2.54	0.79	-	-	-	0.23	11.06	Demirak et al., (2006)
Enne Dam Lake, Turkey(ww)	0.07	BDL	0.87	11.32	0.38	1.11	BDL	16.31	Uysal et al., (2009)
Menzelet dam lake, Turkey(ww)	0.32	-	3.17	-	-	-	-	-	Erdoğan & Ateş (2006)
Almus Lake, Turkey (ww)	0.15	1.1	-	-	-	-	1.1	30.85	Mendil et al., (2005)
Amous UC and DC, France(ww)	0.005 and 0.005	-	0.36 and 0.38	-	0.22 and 0.20	-	0.04 and 0.09	8.5 and 6.9	Casiot et al., (2009)
Yamula Dam, Turkey (ww)	4.40	2.78	5.28	-	8.38	3.23	8.88	27.83	Duman & Kar (2012)
FAO/WHO	0.50	-	30	-	-	-	0.50	40	FAO/WHO (1989)
EC	0.05	-	-	-	-	-	0.30	-	EC (2006)
IAEA (Wyse et al.,2003)	0.19	0.73	3.28	146	3.52	0.60	0.12	67.10	Wyse et al., (2003)
USEPA	4.00	-	-	-	-	-	-	-	USEPA (2000)
UNEP	0.30	-	-	-	-	-	0.30	-	UNEP (1985)
TFC	0.05	-	20	-	-	-	0.20	50	TFC (2002)

*ww wet weight, **dw dry weight, ** Mean values and ± standard deviation, ***BDL: Below the detection limit.

Also Farkas et al., (2003) noticed that there is negative relationship between the heavy metal concentrations of organs and the condition factor of *Abramis brama* (Common bream). According to Canli & Atli (2003), there were negative relationships between heavy metal levels and fish sizes in most cases. Nussey et al. (2000) recorded negative correlations between the length of the specimens and the Mn concentrations in the gills and liver as well as for Pb concentrations in the gills, liver and skin. These results indicate that the longer fish usually have the lower tissue metal concentrations. Canpolat & Çalta (2003) expressed that smaller fish are more active and need more oxygen to supply more energy. It is obvious that fish development is affected by heavy metals in water and juveniles are more sensitive than the mature stages (Heath, 1987; Weis & Weis, 1989; Friedmann et al., 1996; Canli & Atli, 2003; Tekin-Özan & Aktan, 2012).

A significant increase of heavy metal concentrations (except Cd) was observed in Sarıçay stream in five years (Table 3). Heavy metal concentrations increase in Sarıçay stream has showed the impact of industrial organisation. This increase in short time indicates to the high level heavy metal accumulation in the near future. Cd, Co, Cu, Fe, Zn mean concentration values were all lower than the limits of FAO/WHO, EC, IAEA, USEPA, UNEP and TFC. According to IAEA (Wyse et al., 2003) limits, Cr values in the present study were found high. Average Pb values were also higher than the limits for fish proposed by FAO/WHO, EC, IAEA, UNEP and TFC. According to the priority list of Hazardous Substances established by the agency for toxic substances and disease registry (ATSDR, 2013), the descending order of heavy metals threatening to human health were As>Pb>Cd>Ni>Zn>Cr>Cu>Mn. According to our results, the examined fish were associated with enhanced metal content in their muscle and were not safe within the limits for human consumption.

5. CONCLUSION

The study will be helpful for biomonitoring of aquatic environment in the context of heavy metal pollution in relation to human health hazard and also give information on IMBI, MPI and concentration of metals in European chub tissue in Sarıçay Stream. The study indicated that IMBI and MPI indices might be suitable for European chub. Also this study was carried out to provide information on heavy metal concentrations in European chubs inhabiting Sarıçay stream which were widely consumed by

local people. As a result, it carries importance in the view of human health and food safety. Based on the samples analysed, metal concentrations found in the muscles of European chub proved to be higher than the tolerance levels for human consumption. It is obvious that heavy metals pollution in tissue in Sarıçay stream is mainly due to the surface water runoff of agricultural activities and wastewater occurred by industrial activities.

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