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RESEARCH PAPER

Accumulation of Metals and Metalloids in Aegean Chub, Inhabiting in Two Middle Sized Streams in Southwest Anatolia

Burak Oglu[1,*], Bulent Yorulmaz[2]

[1] Estonian University of Life Science, Department of Hydrobiology, Institute of Agricultural and Environmental Science, Tartu 51014, Estonia.
[2] Mugla Sitki Kocman University, Science Faculty, Department of Biology, Kötekli 48000, Turkey.

* Corresponding Author: Tel.: +90 372 5822 9866;
E-mail: ogluburak@gmail.com

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Abstract

Essential and non-essential total eleven metals and metalloids (Al, B, Cr, Co, Cd, Cu, Fe, Pb, Mn, Ni and Zn) concentrations were determined by ICP-AES in muscle of *Squalius fellowesii* (Günther, 1868), between November of 2013 and June of 2014, on chosen four stations on Tersakan and Sarıçay streams. Average concentrations (µg g^{-1} wet weight) in Tersakan: Al (60.81±58.51), B (27.13±12.42), Co (0.07±0.05), Cd (0.01±0.02), Cu (1.44±0.51), Cr (0.75±0.54), Fe (33.49±30.69), Mn (2.19±1.35), Ni (0.98±1.27), Zn (20.71±20.16) and in Sarıçay: Al (33.82±37.19), B(8.15±8.90), Co (0.04±0.035), Cd (0.01±0.01), Cu (0.90±0.44), Cr (0.40±0.27), Fe (12.97±8.17), Mn (3.75±2.045), Ni (0.54±0.82), Zn (19.05±10.65) were found. Lead (Pb) was found below detection limits in all stations or seasons while Al accumulation was found highest. The effects of water quality parameters on essential and non-essential metal-metalloid accumulations in fish tissue were indicated by PCA and Co-inertia analyses. Concentrations of observed non-essential metals did not exceed the consumption limits. The evaluation of the data obtained from the study, in Tersakan and Sarıçay streams, reveal that the ecological balance can be changed in a negative way in case of continuation of the pollution of these two streams.

Keywords: *Squalius fellowesii*, heavy metals, metalloids, Co-inertia, muscle.

Introduction

In addition to natural resources, increase of industrial, agricultural activities and domestic sewage leads to increase the heavy metal pollution in environment (Demirak, Yılmaz, Tuna, & Özdemir, 2006; Dvořák, Andreji, Dvorakova-Liskova, & Vejsada, 2014; Ivanović, Janjić, Baltić, Milanov, Bošković, Marković, & Glamočlija, 2016; Nazir, Khan, Masab, Rehman, Rauf, Shahab, Ameer, Sajed, Ullah, & Rafeeq, 2015; Samad, Mahmud, Adhikary, Rahman, Haq, & Rashid, 2015). Main sources of the contaminants in small rural rivers are agricultural runoff, small industrial facilities and municipal wastes (Dragun, Tepić, Krasnići, & Teskeredžić, 2016). Heavy metals have become an important issue in recent years due to its tendency to persistent in the aquatic ecosystems and accumulate inhabiting organisms (Dragun *et al*., 2016; Ivanović *et al*., 2016; Öztürk, Özözen, Minareci, & Minareci, 2009; Papagiannis, Kagalou, Leonardos, Petridis, & Kalfakakou, 2004).

Heavy metal concentrations in fish and solubility of the metals are affected by the physicochemical parameters and other environmental factors (pH, salinity, hardness, temperature, dissolved oxygen) in the water (A. Akbulut & Akbulut, 2010; N. E. Akbulut & Tuncer, 2011; Ashraf, Maah, & Yusoff, 2011; Barlas, Akbulut, & Aydoğan, 2005; Başyiğit & Tekin-Özan, 2013; Dvořák *et al*., 2014; Ivanović *et al*., 2016; Yi & Zhang, 2012).

Metals such as iron, copper, nickel, manganese, zinc are essential for the metabolism, whereas non-essential metals like cadmium, lead are toxic even at low concentrations for organisms (Fernandes, Fontaínhas-Fernandes, Cabral, & Salgado, 2008; Krasnići, Dragun, Erk, & Raspor, 2013). However, high concentrations of essential metals might be dangerous for the organisms (Dvořák, Andreji, Mráz, & Líšková, 2015). Therefore, besides non-essential metals, it is important to examine the essential metals.

Due to the fact that the fishes are the top of organisms of the food chain in the aquatic system, they can accumulate heavy metals in large quantities (Dvořák *et al*., 2015; Rashed, 2001). Studies on tissues of fishes carry importance because fish has an indicator role for identifying the ecological status of the water and determining heavy metals that threaten human health (Andreji, Dvorak, Dvorakova-Liskova, Massányi, Stranai, Nad, & Skalicka, 2012; Ashraf *et*

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al., 2011; Dural, Göksu, & Özak, 2007; Özparlak, Arslan, & Arslan, 2012).

Aegean chub, *Squalius fellowesii* was chosen because it is omnivorous and widely tolerant to most conditions (Andres, Ribeyre, Tourencq, & Boudou, 2000) and one of the most common fish in Muğla area (Giannetto, Pompei, Lorenzoni, & Tarkan, 2012).

In the last decade, the environmental issues especially heavy metal studies in Muğla area took attention from researchers because of both tourism and economic (fisheries, agriculture) importance (Genç & Yılmaz, 2016; Genç, Yılmaz, İnanan, Yorulmaz, & Ütük, 2015; Kasımoğlu, 2014; Öğlü, Yorulmaz, Genç, & Yılmaz, 2015; Tuna, Yılmaz, Demirak, & Özdemir, 2007; Yılmaz, Özdemir, Demirak, & Tuna, 2007; Yorulmaz, Yılmaz, & Genç, 2015).

The aim of this study was to observe the present concentration of metals and metalloids in edible tissue (muscle) of Aegean Chub, inhabiting Sarıçay and Tersakan streams in the South-West of Turkey. For this purpose present concentrations of aluminium (Al), boron (B), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron(Fe), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) were determined Further aim of our study was to determine the relationships between heavy metal accumulation in fish and physical-chemical parameters of water.

Materials and Methods

Study Area

Sarıçay and Tersakan streams are about 50 km and 30 km in length, respectively. Two stations were chosen from each stream (Figure 1). First stations in Sarıçay (S-I) (37°19'23.4" N, 27°48'53.3" E) and Tersakan (T-I) (36° 48' 17.9" N, 28° 50' 19.4" E) were chosen upstream of the rivers and thought to be partially unpolluted where there were less agricultural activity and non-industrial site. The second station in Sarıçay (S-II) (37°17'7.2" N, 27°42'39.7" E) was located in near the industrial site area (factory that produces animal nutrition, concrete plant and gravel pit). The second station of Tersakan (T-II) (36° 42' 54.1" N, 28° 47' 46.9" E) was chosen downstream of the river where the water flows slowly, after agricultural land, domestic discharge point.

Sample Collection and Analytical Procedures

Water temperature (°C), pH, electrical conductivity (µS cm^{-1}) and dissolved oxygen (mgO_2 L^{-1}) at the stations were measured with the Hach Lange HQ 40d brand multimeter, during the field work. Other physical and chemical parameters were measured by using HACH LANGE DR 2800 spectrophotometer in the laboratory with given kits; Total hardness (˚dH), calcium and magnesium with LCK 327 ammonium (NH_4-N, mg L^{-1}) with LCK 304, nitrate (NO_3-N, mg L^{-1}) with LCK 339, nitrite (NO_2-N, mg L^{-1}) with LCK 342 and Ortho-Phosphate (PO_4-P, mg L^{-1}) with LCK 348 Fish samples were collected by electrofishing, using Deca-Lord-12 V generator, between November 2013– June 2014 from four sites of the Tersakan and Sarıçay streams. Sampling periods were selected according to seasons. In each season twenty, total eighty Aegean chub were caught. Approximately 0.5 g of edible tissue from each fish sample were dissected, washed with deionized water, packed in polyethylene bags, and kept at -20°C until to analysis. The standard length (*L*) and weight (*W*) of all examined fish were measured and estimated as 160.3±29.40 mm and 580.2±259.1 g, respectively. 30% hydrogen peroxide and 70% nitric acid (Merck) were applied to wet muscle tissue of fish samples. The solution and tissue were put into the microwave digestion unit (speedwave four microwave digestion system) to 120°C (gradually increased) until all the materials were dissolved. The solution was transferred and diluted by Ultra-distilled water and filtered through 0.45µm nitrocellulose membrane filter (Alam, Tanaka, Allinson, Laurenson, Stagnitti, & Snow,

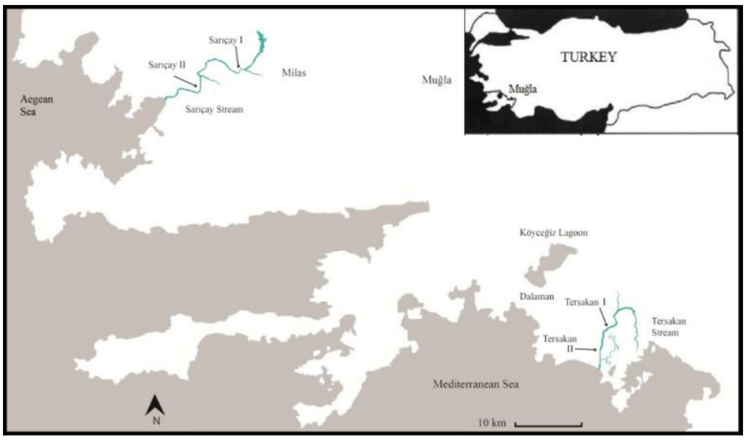

Figure 1. Map of Sarıçay, Tersakan streams showing the sampling sites. Arrows are indicated stations.

2002). All samples were analysed simultaneously two times for Al, B, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometry). Detection limits (mg L^{-1}) were as follows: Al (0.0005), B (0.011), Cd (0.0004), Co (0.001), Cr (0.001), Cu (0.007), Pb (0.06), Zn (0.005), Ni (0.018), Mn, (0.002), Fe (0.0017). Standard material DORM-3 was used for analyse for each elements.

Statistical Analyses

Statistical analyses of data were carried out using R project and SPSS 20.0 statistical package program (IBM.Corp, 2011; R.Core.Team, 2016). The nonparametric Kruskal–Wallis and Man-Whitney tests were used to assess whether metal concentrations varied as significantly between stations. In addition, to assess statistical associations between metal-metalloids accumulation on fish tissue and physicochemical parameters of water, Spearman correlation analyses were applied (Wei & Simko, 2016). Principal component analysis (PCA) was used to determine the similarities and differences among metals-metalloids and also plotted and colour-coded based on stations and seasons by using the ade4 package in R (Chessel, Dufour, & Thioulouse, 2004) Finally the co-inertia analysis (Dray, Chessel, & Thioulouse, 2003) was carried out to discover common patterns among water quality and metal-metalloid data. The overall concordance, measuring the correlation between tables of water quality and metals-metalloids, was estimated in the form of the RV-coefficient, followed by the permutation test for statistical significance. The Co-inertia analysis was performed with R package ade4 (Dray, Dufour, & Chessel, 2007).

Result and Discussion

The mean accumulations of Al, B, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in muscle tissues of Aegean chub and the comparison among sampling sites in Tersakan and Sarıçay were shown in Table 1.

The result shows that the mean concentrations of metals-metalloids, except Cd, in muscle tissues were different among stations (Table1). According to the results of the Mann-Whitney test, there was no statistical difference between stations in terms of Cd metal accumulation ($P>0.05$). Demirak *et al*. (2006) found similar results and reported that Pb and Cd concentrations were undeterminable. Cd level in the muscle of *Squalius cephalus* caught from Lake Beyşehir was found higher than our values (Altındağ & Yiğit, 2005). Lead and cadmium are toxic elements which do not take part in any metabolic activity in animal (Le, Shirai, Nguyen, Miyazaki, & Arai, 2009; Wren & Stephenson, 1991).

Cobalt has beneficial such as joining to enzymatic systems and harmful effects on organisms (ATSDR, 2004; Özparlak *et al*., 2012). Co content in the muscle of Aegean chub varied from 0.004 to 0.17 µg g^{-1} and increase of Co value was obtained in Tersakan II ($P<0.05$). Yılmaz *et al*. (2007), reported that Co value were ranging from 0.010 µg g^{-1} to 0.076 µg g^{-1} in Sarıçay II. Co may enter the environment by natural or anthropogenic resources such as smelting facilities, airport traffic, highway traffic, and other industrial waste (ATSDR, 2004). The increase of Co concentration in Tersakan II may be due to the airport located near the station.

The highest B concentration was found in Tersakan II as 43.82 µg g^{-1}, while the overall average was 17.64 µg g^{-1} in two streams. Significant differences in B accumulation among sites were detected in stream based ($P<0.05$). Emiroğlu, Çiçek, Arslan, Aksan, and Rüzgar (2010), reported that B concentrations ranges from 7.85 to 100.71 mg kg^{-1} in chub muscle from Seydi stream. B element was used in agricultural fertilizer and pesticide (ATSDR, 2010) Intensive agricultural activities, carried out in downstream basin of Tersakan river can be the reason of B concentration increases in Tersakan II. .

Water sediment is conservative for chromium compounds and some of chromium compounds (Cr III) has an important role as a glucose tolerance factor (Goyer & Clarkson, 1996; Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014). Cr concentrations in muscle of Aegean chub ranged from 0.056 to 2.74 µg g^{-1} in both streams. The seasonal average Cr level reached the highest level in the spring (0.84 µg g^{-1}, $P<0.05$).). An increase in Cr values were found in the second station in Tersakan ($P<0.05$), and there was no statistical difference in Sarıçay ($P>0.05$). Dvořák *et al*. (2015), reported that Cr values were found between 0.11-0.21 mg kg^{-1} on chub from Morava Lake. Concentrations of Cr were determined between 9.85-42.4521 mg kg^{-1} by Özparlak *et al*. (2012).

For humans and animals, Cu is the essential metal of the part of the enzymes (Yılmaz *et al*., 2007). In Tersakan, the maximum Cu concentration was found 2.54 µg g^{-1} in second station, while the maximum Cu concentration was found 1.79 µg g^{-1} in second station of Sarıçay stream. When present data compared with previous study on the same fish and in the same river, current Cu values were found higher than previous study (Yılmaz *et al*., 2007). Duman and Kar (2012) reported higher values on same fish than current study.

Manganese is an essential metal for animals and used in many manufacturing (ATSDR, 2012). The highest individual and average Mn levels were determined in Sarıçay I, while the lowest Mn level was found in Tersakan II. These values were lower than those reported in other studies (N. E. Akbulut & Tuncer, 2011; Duman & Kar, 2012). Aluminium and iron are most common elements in the earth's crust (Jaishankar *et al*., 2014). An increase in the concentrations of Ni, Al and Fe were observed in

Table 1. The mean concentrations of metal-metalloid (µg g^{-1} wet weight) in muscles of Aegean chub in Tersakan and Sarıçay streams.

	Co	Cd	Cr	Cu	Ni
Tersakan I	0.06±0.05[a*]	0.02±0.03[a]	0.56±0.39[a]	1.51±0.51[a]	0.69±1.11[a]
Tersakan II	0.09±0.05[b]	0.01±0.01[a]	0.95±0.68[b]	1.37±0.51[a]	1.28±1.43[b]
Sarıçay I	0.05±0.04[a]	0.01±0.01[a]	0.33±0.20[c]	0.89±0.45[b]	0.39±0.31[a]
Sarıçay II	0.04±0.03[a]	0.01±0.01[a]	0.47±0.34[ac]	0.92±0.42[b]	0.69±1.33[a]
	Al	**B**	**Fe**	**Mn**	**Zn**
Tersakan I	53.51±65.76[ab]	28.93±12.12[a]	18.97±8.73[a]	2.72±1.15[a]	31.03±31.97[a]
Tersakan II	68.10±51.26[b]	25.34±12.73[a]	48.03±52.65[b]	1.66±1.55[b]	10.39±8.35[b]
Sarıçay I	27.85±28.31[a]	9.98±11.69[b]	13.49±11.18[c]	4.52±2.28[c]	20.99±14.62[ac]
Sarıçay II	39.79±46.07[a]	6.31±6.11[b]	12.46±5.16[c]	2.98±1.81[a]	17.11±6.67[c]

Statistical differences of metal-metalloid accumulation on tissues are presented as letters (a,b,c), the statistically significant difference (P<0.05). Number of observation is 20 for each station (N=80 for each metal-metalloid).
*Mean values and ± standard deviation.

Tersakan II (P<0.05). The highest concentrations of Al, Fe and Ni were detected 276.45 µg g^{-1}, 253.04 µg g^{-1} and 6.72 µg g^{-1}, respectively, all in Tersakan stream. Ni concentrations were lower than those which were reported in the literature (N. E. Akbulut & Tuncer, 2011; Duman & Kar, 2012). Ni was not determined by Yılmaz *et al.* (2007) in Sarıçay stream. The average Al concentrations were 78.4 µg g^{-1} in February, 60.4 µg g^{-1} in May, 788.33 µg g^{-1} in August observed in Delice River (N. E. Akbulut & Tuncer, 2011). Fe values determined in present study, were lower than those reported by N. E. Akbulut and Tuncer (2011), and higher than the previous study which was carried on the same stream and on the same fish by Yılmaz *et al.* (2007).

In addition to the role of co-factor in many enzymes, Zn is involved in intermediary metabolism, DNA metabolism and repair, reproduction (Maret & Sandstead, 2006; Yılmaz, 2009). The highest Zn accumulation was determined 154.20 µg g^{-1} in Tersakan I in summer. Also, the highest average Zn concentration was found in Tersakan I (P<0.05). Zn values of current study, show similarity to the given results in other studies (Dvořák *et al.*, 2015; Özparlak *et al.*, 2012). However, Zn concentrations of present study were found higher than previous studies in the same region. (Demirak *et al.*, 2006; Yılmaz *et al.*, 2007).

No differences were observed, except Mn level, between stations in Sarıçay stream whereas significant increases were found in Co, Cr, Ni, Al and Fe levels between stations in Tersakan stream. Mn and Zn levels were determined higher in the first station of Tersakan stream. A likely explanation is that these variations in metal and metalloid levels between stations in Tersakan might be a result of local point discharges (domestic waste), agricultural activities carried out in the stream basin and airport traffic near to downstream of Tersakan. Many comparisons on the metal and metalloid levels in the muscle of *Squalius* sp. were done. Papagiannis *et al.* (2004) and Yılmaz *et al.* (2007) mentioned the difficulties of comparing metal concentrations on two different fishes even in same tissue. However, Kasımoğlu (2014) investigated Co, Cr, Cu, Fe, Mn, Ni, Zn (0.07 µg g^{-1}, 1.73 µg g^{-1}, 1.66 µg g^{-1}, 31.21 µg g^{-1}, 2.04 µg g^{-1}, 1.29 µg g^{-1}, 22.11 µg g^{-1}) on European eel in Tersakan stream and found similar results to current study.

Principal component analysis (PCA) of among metal-metalloid concentrations and PCA scores at different seasons and stations were illustrated in Figure 2. First two PCA components were describing 44.8 % of metal-metalloid data. ANOVA was applied to the results of the PCR analysis and a significant difference was found between the stations (P<0.001), while there was no difference between the seasons (P>0.05). In scores of PCA, the significant difference was found between rivers. Figure 2 shows that plot of the sample scores position of station 1 and 2 (Tersakan) and station 3 and 4 (Sarıçay) located separately. Random distribution of plot indicates that metal and metalloid accumulation in fish poorly depends on seasons.

The direction of the arrows indicates similarity of variables. According to first two PCA, Cd was not included in any group, while Mn and Zn were found significantly relative to each other. (Demirak *et al.*, 2006) reported that positive correlation was present between Zn and Cu. Agtas, Gey, and Gul (2007) recorded positive correlations between copper and zinc concentrations in muscle of chub too. Dvořák *et al.* (2015) observed positive correlations among analysed metal Hg, Pb, Cd, Cr, Zn in chub.

According to correlation graph, the highest positive correlation was found between B accumulation and nitrate concentration in water (r=0.68**) followed by B and magnesium (r=0.64), Fe and magnesium (r=0.62**) (Figure 3). High level of calcium in water has negative effect to B (r=-0.70**), Cu (r=-0.50*), Fe (r=-0.53*) accumulation in fish tissue while positive correlation between calcium in the water and Mn concentration in fish was found (r=0.55*). Positive correlation was found between conductivity of water and Al accumulation in fish (r=0.55*) while negative correlation was found between conductivity and Mn concentration. Phosphate values of water and Zn accumulation in

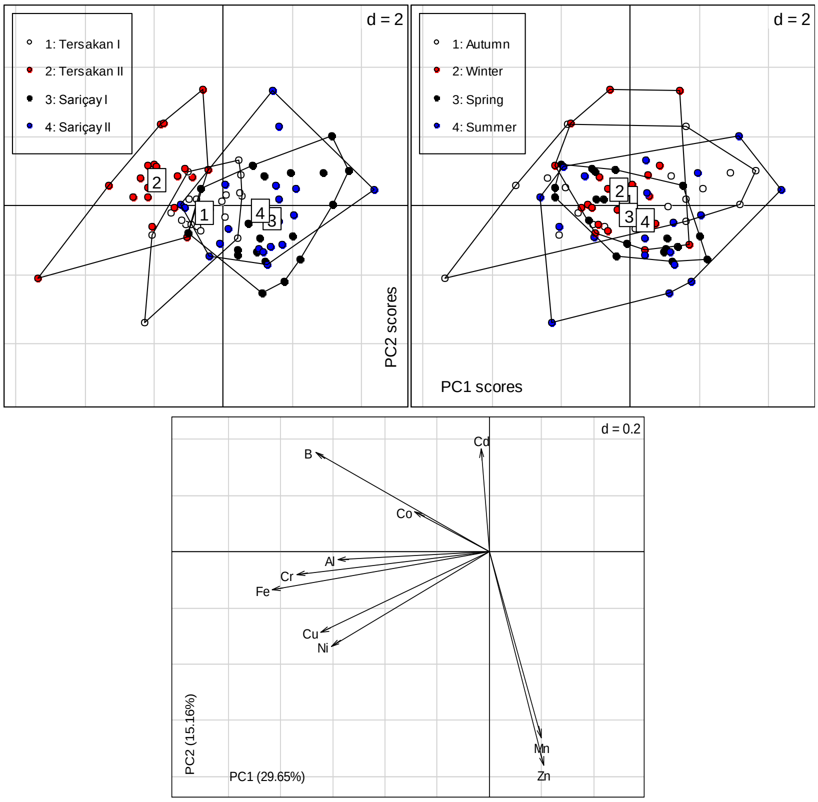

Figure 2. Score plot of first two component PCA model of metal-metalloid concentrations in *Squalius fellowesii* tissue from four sampling sites and four seasons.

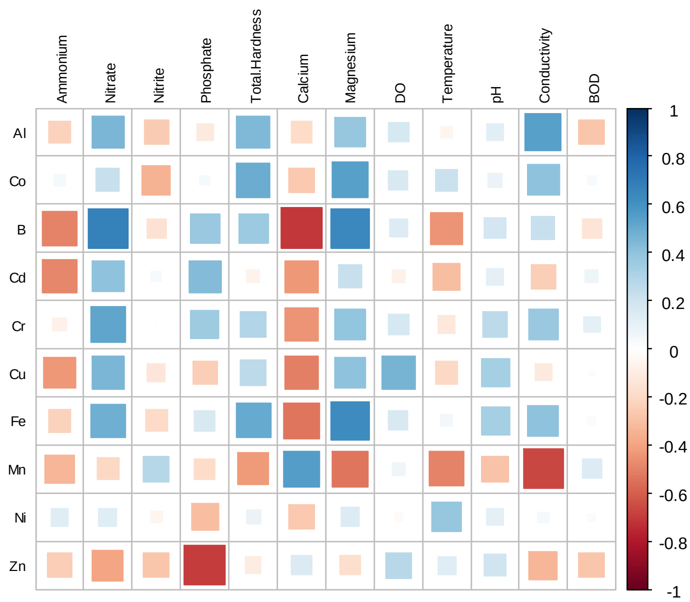

Figure 3. Spearman correlation between metal-metalloid concentrations in fish tissue and measured physicochemical parameters in water. Colour of the boxes indicates the relative of the parameters (correlation coefficients). Blue colours are specifying positive correlations while red colours are specifying negative correlations. Colours shades indicated the weakness of the correlation.

fish have negative correlation (r=0.70**). There were no relationships between metal accumulation and nitrite, dissolved oxygen, temperature, pH and BOD_5 parameters of water.

The Co-inertia analyses revealed intermediate but statistically significant relationship between water quality and metals-metalloids (RV=0.48, P=0.010; Figure 4). The weights of initial variables in common patterns indicate, that the water samples with higher temperature and ammonium values correspond to

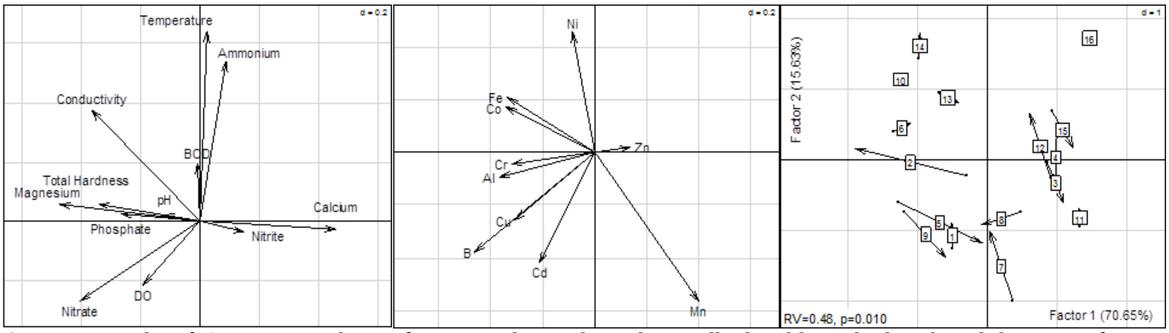

Figure 4. Results of Co-inertia analysis of water quality and metals-metalloids tables. The length and direction of arrows in the first and the second figure denote the weights of initial variables concerning the first two factors. The last figure represents the concordance between water quality and metals-metalloids: the beginning and end of arrows denote the relative location of samples according to their water quality and metals-metalloids data, respectively; the numerical measure of concordance is given by RV-coefficient, which p-value is evaluated by permutation test; percentage values indicate the proportion of the overall covariance between water quality and metals-metalloids tables described by the first two factors.

stations and seasons with higher values of Ni and with lower values of B, Cd; the higher conductivity of water was negatively related with Mn and positively related with Fe and Co; the total hardness and magnesium content of water are positively and calcium content negatively related with Co, Cr and Al. Dissolved oxygen content of water was found positively related with Cd concentration in muscles of fish. In the last graph of the Figure 4, numbers 1-16 denote different stations and seasons combinations (number 1, 5, 9, 13 defines Tersakan I and combinations of autumn, winter, spring, and summer, respectively). Beginning of the arrows indicating the metals and metalloids in fish and end of the arrow indicates water quality parameters. Environmental parameters are known to have an impact on the accumulation of heavy metals in fish (Dragun *et al*., 2016). Also heavy metal accumulation may show differences between different species because of metabolic activity, physiology; feeding habits of the fish (Papagiannis *et al*., 2004; Yılmaz *et al*., 2007).

The order of heavy metals threatening to human health, according to agency for toxic substances and disease registry is Pb>Cd>Co>Ni>Zn>Cr>Cu> Mn>Al (ATSDR, 2015). The most toxic metals Pb and Cd according to ATSDR (2015), were not determined or found in low levels in present study (Table 2). Mean of Al values were determined far above, while Ni, Cr and Mn concentrations slightly exceeded the recommended values of IAEA (Wyse *et al*., 2003). Al, Mn, Cr and Ni accumulate in muscle tissue of fish. These metals make organometallic compounds. According to Dragun, Marijić, Vuković, and Raspor (2015) Mn occurs with age in gastro intestinal cytosol of european chub. A. Akbulut and Akbulut (2010) found that Zn, Cr, Cu Pb and B accumulated in muscle tissue of European chub in relatively high concentrtaions. Agricultural activity and domestic wastes which were carried by surface waters to these two rivers maybe the reason of high concentrations of these metals. When it was compared with previous study in Sarıçay stream, a significant increase of Fe, Mn, Zn, Cu levels were found while Cd remains constant in *Squalius fellowesii* (Yılmaz *et al*., 2007). Those metals do not present a danger in low levels as it is essential nutrients that need to be taken daily. However essential metals can be dangerous when they are accumulated in organisms by over consumption. Local people and amateur fishers consume Aegean chub in the research area, so essential metals can accumulate over the acceptable limits in edible tissues of chub and can be dangerous for human as well as non-essential metals. Therefore, besides non-essential metals, it is important to examine the essential metals. This situation may cause to rise over the carrying capacity in the future and may result damage to living organisms in the river as well as the risk that it may threaten public health by contaminating the irrigation water used for agriculture to human food taken from Sarıçay stream. Minor sized rivers have small dilution capacity and it can lead high accumulation in the river, even if the amount of pollution is insignificant (Dragun *et al*., 2016).

Conclusion

This study emphasises that agricultural activities, as well as industrial activities, may have an impact on metal-metalloid pollution in the stream. As a result, metal and metalloid levels determined in fish samples obtained from sampling points in Sarıçay were relatively low when the values were compared with the levels detected in Tersakan stream except Mn. The reason of higher metal-metalloid accumulation Tersakan stream might be, 4-season agricultural activity, citrus fruit production around the river, intensive fertilisation to increase yield, sewerage of the settlements. All the concentrations of metals and metalloids in edible tissues were below the limits of FAO/WHO, EC, USEPA and TFC. Nonetheless, the consumption of fish caught from these two rivers can reach threatening levels for human health in near future.

Table 2. Metal-metalloid concentrations (µg g^{-1}) in muscle tissue of Aegean chub compare to guidelines

	Al	B	Co	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Ref.
Tersakan Stream	60.81	27.13	0.08	0.02	0.75	1.44	33.50	2.19	0.98	BDL*	20.72	a
Sarıçay Stream	33.82	8.15	0.04	0.01	0.40	0.90	12.98	3.75	0.55	BDL	19.05	a
FAO/WHO	-	-	-	0.30	-	-	-	-	-	0.30	40	b
EC				0.05	-	-	-	-	-	0.30	-	c
IAEA 407	13.8	-	0.10	0.19	0.73	3.28	146	3.52	0.60	0.12	67.10	d
TFC	-	-	-	0.05	-	20	-	-	-	0.20	50	e

*BDL: Below the detection limit; [a] Present study; [b](FAO/WHO, 2011); [c] (EC, 2008); [d](Wyse, Azemard, & Mora, 2003) [e](TFC, 2002)

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