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The Effect of Fish Size, Age and Condition Factor on the Contents of Seven Essential Elements in *Anguilla anguilla* from Tersakan Stream Mugla (Turkey)

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Abstract

The correlation coefficients between age, weight, total length and condition factor (K) and trace metals (Co, Cr, Cu, Fe, Mn, Ni and Zn) in *Anguilla anguilla* were determined. Totally 160 fish samples were collected from Tersakan Stream (Mugla) between September 2011 and August 2012. Highly significant (P<0.01) relationship between trace metal concentrations in fish muscle and age, weight, total length and condition factor (K) was found. Significantly positive relationship among trace metals (Co, Cr, Cu, Fe, Mn, Ni and Zn) and fish age, weight and total length was observed, while all trace metal concentrations were negatively correlated with fish condition factor (K). The comparison of trace metal concentrations in the muscle of *Anguilla anguilla* with the tolerable values indicated that the trace metal concentrations in muscle tissue did not exceed the WHO/FAO, EPA and IAEA–407 guidelines.

Keywords: *Anguilla anguilla*; Trace metals; Food safety; Mugla

Introductıon

Trace elements may exert benefical or harmful effects on plant, animal and human life depending upon the concentration [1]. These elements are introduced into the environment through various routes such as smelting processes, fuel combustion and industrialization [1,2]. They find their way into rivers, lakes or oceans through atmospheric fallout, dumping wastes, accidental leaks, runoff of terrestrial systems (industrial and domestic effluents) and geological weathering [1,3,4].

In aquatic environment, fishes reflect the effects of numerous interacting biotic and abiotic factors. In many cases the variation in elemental concentration in fish tissue has been attributed to variation in size and age [5]. Therefore, many studies have focused attention on the dependence between the contents of metals and fish biometry (weight and length) and age [6-10]. Variation of elemental concentration in fish tissue due to size has been reported [11-15].

As a consequence, pollutants discharged in the aquatic environment are likely to accumulate in fish and represent a potential risk not only to the fish, but also to other fish consumers, particularly humans. Catadromous eels, widely distributed throughout the world, are one of the top predator in freshwater ecosystem [16]. Due to the long-life cycle and the spesific biological and ecological features of anguillid eels, they are vulnerable to adverse impacts from nature and human activities [17,18]. Accordingly, the eel populations have declined dramatically in recent years and causes are attributed to over-fishing, construction, climate change, other enviromental factors, especially enviromental pollution [19,20]. Hence, anguillid eels has been widely used as bioindicator for environmental monitoring to assess the aquatic system quality in many countries [21,22].

This study investigates relationship of fish size and nutritional quality, occurrence of trace metals and further identifies the optimum size, i.e. total length and body weight, of this species suitable for human consumption.

Material and Method

Study area

Dalaman- Tersakan Stream (36°45’51”N, 28°49’20”E) is located in province Mugla in the southwest of Turkey (Figure 1). The sampling site (Tersakan Stream) is a temperate stream which is impacted 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). The lower section of the stream was channelized by local authorities to prevent seasonal floods. The stream flows into the Mediterranean Sea [23] (Figure 1). There are eight known species inhabiting the stream of which the most abundant are *Mugil cephalus* (Linnaeus), *Leuciscus cephalus* (Linnaeus), *Gambusia affinis* (Baird and Girard) and *Anguilla anguilla* (Linnaeus) [23].

Sampling and analysis

Water and fish samples were collected 4 times (from September 2011 to August 2012) for every 3 months at four stations in the Tersakan Stream. The sampling bottles were pre-conditioned with 5% nitric acid and later rinsed thoroughly with distilled de-ionized water. At each sampling site, the polyethylene sampling bottles were rinsed at least three times before sampling was done. Pre-cleaned polyethylene sampling bottles were immersed about 10 cm below the water surface. About 0.5 L of the water samples were taken at each sampling site. Samples were acidified with 10% HNO_3, placed in an ice bath and brought to the laboratory. The samples were filtered through a 0.45 μm micropore membrane filter and kept at 4°C until analysis. A total of 160 samples of eel (Anguilla anguilla) were captured by backpack electrofishing with a battery-powered unit (550 V,5-100 Hz) at four different stations along the tersakan stream in the period of Semptember 2011 to August 2012). Fish samples were immidiately transported to the laboratory in a thermos flask with ice. The fish species selected were based on their

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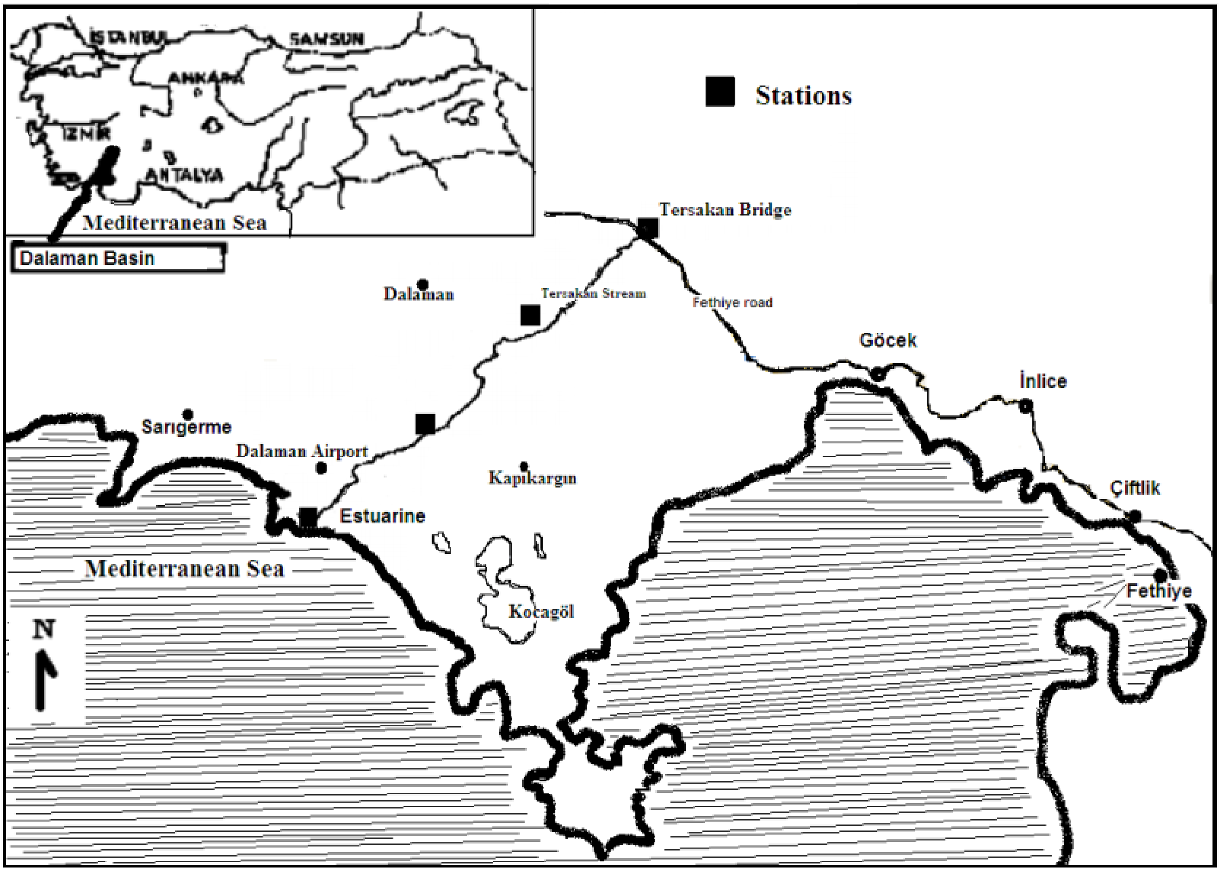

Figure 1: Sampling area of *Anguilla anguilla* specimens in Mugla province (Turkey).

Element	Wavelength (nm)	Detection limit (mg/L)
Co	228.616	0.0070
Cr	267.716	0.0071
Cu	327.393	0.0097
Fe	238.204	0.0046
Mn	257.610	0.0014
Ni	231.604	0.0150
Zn	202.548	0.0040

Table 1: Spectral lines used in emission measurements and detection limit for the elements measured by ICP-AES.

abundance along the river watercourse and frequency in use as food by the inhabitants of these areas.

The samples were carefully cut opened using a plastic knife in order to remove the muscle, and freeze dried and pulverized into a uniform particle size prior to analysis. The small sized particles were subjected to acid digestion using nitric acid. Approximately 5 g of muscle from each sample were dissected, washed with deionized water, weighed, packed in polyethylene bags, and stored at -20°C prior to analysis.

The muscle tissue samples were digested with concentrated nitric acid. Dissected samples were transferred to a 100 mL teflon beaker. Thereafter, 10 mL ultrapure concentrated HNO_3 (Merck) was added, and the sample heated at (100, 150, 210 and 280°C on a hot plate for 0.5, 0.5, 0.5 and 2 hours) with a DK-20 Heating Digester respectively. Two mL of 1 N HNO_3 was added to the residue, and the solution continuously evaporated on the hot plate, until it was digested in every sample. After cooling, a further 10 mL of 1 N HNO_3 was added. The solution was transferred, diluted and filtered through 0.45 µm nitrocellulose membrane fitler [24].

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All samples were analysed in duplicates for Co, Cr, Cu, Fe, Mn, Ni and Zn by ICP/AES (Optima 2000-Perkin Elmer), which is a fast multi-element technique with a dynamic linear range and moderate-low detection limits [25]. Detection limits are given in Table 1. The detection limit is defined as the lowest analytical signal to be distinguished qualitatively at a specified confidence level from the background signal [26]. The accuracy of analytical procedure was checked by analyzing the standard reference materials (Water: SRM-143d, National Institute of Standards and Technology; Fish: DORM–2, National Research Council). Recovery rates ranged from 79 to 96% for all the elements analysed.

Statistically analysis

Statistical analysis of data was carried out using SPSS 14.0 statistical package program. One-way analysis of variance (ANOVA) was used to assess whether metal concentrations varied as significantly among tissues. Relationship of metal levels in muscle tissue with metal levels

Age (years)	Fish Number (N)	Total length (mm)	Body weight (g)	K
3	61	216,70 ± 39,23 A	42,02 ± 8,65 A	0,42 ± 0,12 A
4	53	279,45 ± 56,37 B	49,12 ± 4,46 B	0,22 ± 0,02 B
5	26	352,65 ± 51,57 C	54,53 ± 7,45 C	0,12 ± 0,01 C
6	12	443,58 ± 77,77 D	58,20 ± 9,52 D	0,06 ± 0,01 CD
7	8	571,37 ± 64,272 E	63,93 ± 9,77 E	0,03 ± 0,007 D
Total	160	372,75 ± 89,54	53,56 ± 8,454	0,26 ± 0,11

[A,B,C,D,E]: Different letters in each column indicate statistically significant differences (p<0.05).

Table 2: Total length, weight, age and condition factor (K) (mean ± SE) of *Anguilla anguilla.*

Trace metals in water		Co	Cr	Cu	Fe	Mn	Ni	Zn
		LOD	0.3 ± 0.34	1.15 ± 0.49	27.62 ± 6.1	0.63 ± 0.24	0.48 ± 0.14	1.78 ± 0.53
FAO [30]		0.05	0.1	0.2	5	0.2	0.2	2
TWPCR [16]	I	10	20	20	300	100	20	200
	II	20	50	50	1000	500	50	500
	III	200	200	200	5000	3000	200	2000
	IV	>200	>200	>200	>5000	>3000	>200	>2000

I: High quality water, II: weakly polluted water, III: Polluted water, IV: High polluted water
LOD: Limit of Detection

Table 3: Concentrations of trace metal in water of Tersakan Stream (mean±standard error (SE)).

Trace metals in muscle	Co	Cr	Cu	Fe	Mn	Ni	Zn
	0.07 ± 0.02	1.73 ± 0.27	1.66 ± 0.28	31.21 ± 6.75	2.04 ± 0.65	1.29 ± 0.25	22.11 ± 4.67
FAO/WHO limits [32]	-	-	5	5	100	-	40
EPA [33]	27	4,1	54	410	190	27	410
IAEA-407 [31]	-	0,73	3,28	146	3,52	0,60	67,10
TFC [25]	-	-	20	-	20	-	50

Table 4: Trace metal concentrations (µg/g wet weight, mean±standart errors (SE)) in muscle tissue of *Anguilla anguilla.*

Parameters	*Anguilla anguilla*
Cr muscles/ Cr water	.73**
Cu muscles/ Cu water	.82**
Fe muscles/ Fe water	.94**
Mn muscles/ Mn water	.89**
Ni muscles/ Ni water	.90**
Zn muscles/ Zn water	.96**

Table 5: Correlation coeffitients between metal concentration in water and muscle tissue.

in water samples and fish biometric parmeters was performed using the Pearson's correlation analysis.

Results and Discussions

Fish biometric parameters, total length, weight and condition factor (K), are shown in Table 2. The range of mean values of eel's was (216.70-571,37 mm), body-weight 42.02-63.93 g and condition factor 0.03-0.42.

Sampled fish individuals belonged to 5 age groups (3, 4, 5, 6, 7 years old). Fish total length, body weight and condition factor among different age groups were found statistically significant different (p<0.05) (Table 2).

The average concentrations of trace metals in water samples from the Tersakan Stream are presented in Table 3. According to the findings, pH value in water varied between 7.55–8.06. Metal concentrations in water followed the order: Fe > Zn > Cu >Mn > Ni > Cr, while Co levels were below limit of detection. İron is one of the most abundant elements in the earth's crust [27,28]. In water Fe may occur as complex and diverse mixture of soluble and insoluble forms such as inorganic and organic complex and or associated with colloids and suspended particulate matter. Similar finding was observed by Bamishaiye et al [29]. High metals concentrations in water can retard the growth and development in fish, particularly the developmental stages, resulting in possible changes in fish size.

Metal concentrations in Tersakan Stream's water samples were compared with international standards. The obtained results showed that the concentrations of Cr, Cu, Fe, Mn and Ni in water exceeded the values of FAO [30] (Table 3). Accordingly, the water taken from Tersakan Stream is not proper for irrigation.

Metal concentrations in Tersakan Stream's water samples were compared with international standards. The obtained results showed that the concentrations of Cr, Cu, Fe, Mn and Ni in water exceeded the values of FAO [30] (Table 3). Accordingly, the water taken from Tersakan Stream is not proper for irrigation. Trace metal concentrations in water samples were determined to be class I according to Turkish Water Pollution Control Regulations (TWPCR) [31]. According to our results, Tersakan Stream might be classified as a stream of good water quality but, periodically impacted by pollution exposure. Especially the use of fertilizers and pesticides in agriculture, domestic and urban sewage and industrial waste is believed to would increase the metals concentrations. Regular environmental monitoring in the stream is also very important.

All measured metal concentrations in fish muscle tissue were higher than total dissolved metal concentrations in water (Table 4). The trace metal concentrations in muscle of *Anguilla anguilla* were lower than the concentrations issued by WHO/FAO [32], EPA [33], IAEA–407 [34] and TFC [35] guidelines. Accordingly, there is a little risk for the human consumption of these fish species. Thus, these fish can be considered for now to be fit and safe for consumption. Because the levels of heavy metals are below the permissible limits (Table 4). On the other hand, this study shows that a potential danger may occur in the future depending on the agricultural development.

Age Total length (mm) weight (g)	Co	Cr	Cu	Fe	Mn	Ni	Zn
3 (175–265 mm) (34,2–49,5 gr)	0,01 ± 0,14 B	1,11 ± 0,23 E	1,05 ± 0,24 C	20,3 ± 2,14 C	1,11 ± 0,15 C	0,89 ± 0,05 E	15,64 ± 2,48 C
4 (248–326 mm) (45–53,5 gr)	0,03 ± 0,18 B	1,65 ± 0,25 D	1,67 ± 0,36 B	29,04 ± 3,63 C	1,91 ± 0,39 C	1,29 ± 0,09 D	20,56 ± 3,67 C
5 (304–400 mm) (51,5–59 gr)	0,06 ± 0,82 B	2,37 ± 0,36 C	2,4 ± 0,28 A	45,01 ± 6,8 B	2,97 ± 0,46 B	1,59 ± 0,06 C	30,29 ± 6,22 B
6 (400–490 mm) (54,5–61,5 gr)	0,16 ± 0,07 A	2,83 ± 0,45 B	2,65 ± 0,62 A	47,51 ± 5,89 B	3,54 ± 0,91 B	2,03 ± 0,16 B	32,02 ± 4,77 B
7 (518–635 mm) (60–67,5 gr)	0,18 ± 0,09 A	3,26 ± 0,13 A	2,43 ± 0,38 A	59,42 ± 4,76 A	4,72 ± 0,98 A	2,29 ± 0,23 A	40,31 ± 7,48 A

[A,B,C,D,E]: Different letters in each column indicate statically significant difference in metal levels of different age groups ($p<0.05$).

Table 6: Trace metal concentrations in muscle of *Anguilla anguilla* (μg/g wet weight) sorted according to fish age groups.

	Age	Length	Weight	K
Co	.67**	.68**	.66**	-.65*
Cr	.83**	.81**	.84**	-.68*
Cu	.61**	.55**	.68**	-.73**
Fe	.79**	.78**	.81**	-.92**
Mn	.63**	.61**	.68**	-.96**
Ni	.76**	.75**	.80**	-.87**
Zn	.61**	.57**	.63**	-.76**

*; P < 0.05 **; P < 0.01 ***; P < 0.001

Table 7: Correlation matrix between total lengths, weight, age and condition factor and Heavy metal concentrations in muscle of *Anguilla anguilla*.

In order to understand interrelationship between the metal concentration in water and fish muscle tissues, Pearson's correlation analysis was carried out (Table 5). It is evident that metal concentration in water and in eel's muscle tissue were highly significantly positively correlated, which may indicate that the increased concentration of heavy metals in water is reflected in increased metal concentration in the fish muscle. In consistence to the present findings, Bird [36] reported increased heavy metal concentrations in water and accordingly in fish. Higher contamination of trace metals in fish tissues were correlated with agricultural runoff entering the water bodies. Chaisermartin [37] also reported that agricultural runoff with fertilizers contains heavy metals and cause pollution in the receiving water bodies, contaminating their fish. Bioconcentration factor, expressed as the ratio of trace metals Cr, Cu, Fe, Mn, Ni and Zn in the fish muscle tissue and water samples were high. This led to the conclusion that metal concentration (Cr, Cu, Fe, Mn, Ni and Zn) in the muscle tissues of fish accumulated from the river water.

The distribution of trace metal concentrations in muscle tissues of *Anguilla anguilla* according to age groups (3-7 years old), total length and body weight are given in Table 6.

An increasing trend in Co, Cr, Cu, Fe, Mn, Ni and Zn values in muscle tissue was observed with increasing fish length, weight and age (Table 6). The effect of fish age on heavy metal accumulation showed a similar pattern to those of fish weight and length. Generally, the growth-dependent variations of trace element levels are known to be influenced by various factors such as metabolic rate and growth dilution of the elements [5,22]. The concentrations of all analysed trace metals in the muscle tissues were statistically significant with eel's age ($p<0.05$), i.e. trace metal concentrations were generally higher in tissue of older and bigger compared to younger and smaller fish. This was more conspicuous when the correlations coeffitients were considered, which showed a highly significant ($p<0.01$) positive correlations between concentrations of Co, Cr, Cu, Fe, Mn, Ni, Zn and body length and weight (Table 7). Ziyadah [38] reported that the tissues tend to accumulate high concentrations of heavy metals with the increase of fish size. Many studies have also demonstrated a positive relationship between body size (length, weight and age) and trace metal concentration [9,13,39,40].

Correlation analysis resulted in negative correlation of all trace metal concentrations and fish condition factor (K). On the other hand, it was found that the correlations between the trace metal concentrations of muscles and the condition factor (K) of fish samples varied with characteristic opposite trends compared to those related to, length, weight and age (Table 6). The negative relationship between the trace metal concentration and the condition factor of fish suggests the relative dilution effect of the lipid content of tissues [41].

İt is generally accepted that trace element accumulation in living organisms, which is controlled by spesific uptake, detoxification and elemination mechanisms, depends significally also on the size-specific metabolic rate of organisms [42]. Some metals do not increase in concentrations with age or size because they are thought to be under homeostatic control [43]. So, in present study, the positive correlation between some metals and fish age and sizes may be due to loss of homeostasis capacity of *Anguilla anguilla* under chronic metal exposure leading to bioaccumulation. This assumption is supported well also by the fact that lipid as a percent of body weight is usually lower in younger fish, decreases during spawning and reaches its peak at the end of the main feeding period [44]. These variation may explain also the opposite correlations observed in present study between heavy metals-size and heavy metals-condition factors of fish [20].

It could be concluded that, *Anguilla anguilla* is a good species for biomonitoring pollution as it could withstand the adverse conditions in the ecosystem. The concentrations of heavy metals in fish muscle vary significantly not only as function of fish sizes and age, but is influenced in a remarkable degree by the fish condition [41].

Conclusions

Fishes are suitable indicators of heavy metal contamination in aquatic ecosystem because they occupy different trophic levels and are of different sizes and ages. Since fishes are located at the end of the aquatic food chain they reflect the water quality status and represent indicators of water pollution, particularly heavy metals.

The trace metal concentrations in muscle of *Anguilla anguilla* were compared with the tolerable values in fish. The result obtained showed that the trace metal concentrations in muscle tissue did not exceed the WHO/FAO, EPA, IAEA–407 and TFC guidelines. Therefore, according to the results of this study, the consumption of *Anguilla anguilla* from Tersakan Stream can be safe for human health in spite of possible contamination with heavy metals. The knowledge on heavy metal concentrations in fish is important and necessary for the management water plans involving polluted aquatic systems and for the evaluation of potential risk to human health.

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