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Toxic metals (Cd and Pb) levels in the muscle of commercially important fish, *Anguilla Anguilla* from Tersakan Stream Mugla (Turkey): Correlations between Cd and Pb concentrations with biological features

Muğla Tersakan Çayı'nda yaşayan ekonomik önemi olan *Anguilla Anguilla*'nın kas dokusundaki toksik metal (Cd ve Pb) seviyeleri: Biyolojik özelliklerle Cd ve Pb konsantrasyonları arasındaki korelasyonlar

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ABSTRACT

In this study, two toxic metals (cadmium and lead) were analyzed in tissues of 160 fish samples of Anguilla anguilla caught from the Tersakan Stream (Muğla) between August–2011 and September–2012. Cd and Pb for Anguilla anguilla were found upper than the World Health Organization (WHO), European Units, Food and Agriculture Organization (FAO) and Turkish Food Codex (TFC) limits for human consumption in edible parts (muscle) of Anguilla anguilla samples and posed a risk for human health. Metal concentration differences among tissues of Anguilla anguilla were statistically significant ($p < 0.05$) and various degrees of correlations coefficient were found between Cd and Pb with Biological Features (age, body size and body weight).

Keywords: *Anguilla anguilla,* Toxic metal, Food safety, Mugla

ÖZET

Bu çalışmada Ağustos 2011- Eylül 2012 tarihleri arasında Tersakan Çayın'dan (Muğla) yakalanan 160 Anguilla anguilla örneğinin dokularında iki toksik metal (kadmiyum ve kurşun) analiz edilmiştir. Anguilla anguilla örneklerinin yenilebilir kısımlarında (kas) Cd ve Pb değerleri, insan tüketimi için Türk Gıda Kodeksi, Avrupa Birliği ve Dünya Sağlık Örgütü tarafından belirlenen limitlerin üstünde olup, sağlık açısından risk oluşturmaktadır. Anguilla anguilla' nın dokuları arasındaki metal konsantrasyonları arası fark istatistiki açıdan önemli olup ($p<0.05$), biyolojik özelliklerle (yaş, boy ve vücut ağırlığı) Cd ve Pb arasında değişen oranlarda korelasyon katsayıları bulunmuştur.

Anahtar sözcükler: *Anguilla anguilla,* Toksik metal, Gıda güvenliği, Muğla

1. Introduction

Fishes are ideal indicator of heavy metal contamination in aquatic ecosystem because they occupy different trophic levels and are of different sizes and ages (11,18). Since fishes are located at the end of food chain in the aquatic ecosystem, they reflect the status of water quality and are indicator of water pollution particularly heavy metals.

The European eel (*Anguilla anguilla*) is receiving world-wide attention as a result of the decline observed in

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its abundance. Natural and anthropogenic perturbations in the continental and oceanic environments where it lives are suspected to be the reasons for this decline. Metal accumulation in fish is, therefore, important for public health concerns because eels are used for human consumption in many areas. Eel is a long-lived fish and its relative resistance to toxic substances emphasizes the obvious danger of accumulation of heavy metals at sublethal doses which can lead to acute toxic effects at higher trophic levels, including man (10). Eels which are abundantly caught and consumed by the local people in Mugla region are the species of high commercial value to be exported abroad.

Cadmium and lead, as a non-essential metal, has become an important pollutant in aquatic ecosystem with the increasing use of this metal in industry, agriculture and anthropogenic activities (38, 14).

Lead is found in small amount in the earth's crust. It can be found in all parts of our environment. Most of it came from human activities, like mining, manufacturing and the burning of fossil fuels. The principal source of Pb in the marine environment appears to be the exhaust of vehicles run with leaded fuels that reaches the sea water by a way of rain and wind blown dust (8). Lead accumulate in aquatic biomass, they are concentrated and passed up the food chain to human consumers. Lead is also known to damage the brain, the central nervous system, kidneys, liver and the reproductive system (2).

Cd contamination in fresh water is particularly alarming because it is known to be highly toxic to the exposed organisms leading to lethal and sublethal damage. Cd causes direct morality in fish and other aquatic fauna (6, 20, 22), reduces hatching rates (25), retards growth and development (33) and causes deformities and behavioural abnormalities. Cd is known to disrupt physiological and biochemical processes in fish (32, 41). Cd also accumulates in organs and tissues (42, 40) and therefore often results in biomagnification (9).

The aim of this study was to determine whether the residues of toksic metals like Cd and Pb in edible muscle tissue of *Anguilla anguilla* is safe for human consumption, and explore the relationship between metal accumulation and biological features (body size, body weight and age) of the European eel *Anguilla anguilla*, in order to evaluate the potential risk of metal pollution in eel populations in the Tersakan Stream.

2. Material and Methods

2.1. Study Area

Dalaman- Tersakan Stream, (36°45'51"N, 28°49'20"E) is located in province Mugla in the southwest of Turkey. 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) (5). The lower section of the stream was channelized by local authorities to prevent seasonal floods. The stream flows into Mediterranean Sea (Figure 1). 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. There are eight known species inhabiting the stream while *Mugil cephalus*, *Leuciscus cephalus, Gambusia affinis* and *Anguilla anguilla* (Linnaeus, 1758) are the most abundant fish species (5).

2.2 Sampling and 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 August to september 2012. Fish samples were transported to the laboratory in a thermos flask with ice on the same day. The fish species selected were based on abundance along the course of the river and widely eaten by the inhabitants of these areas.

The samples were carefully cut opened using a plastic knife in order to remove the organs (muscle, liver and gill), they were freze 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, gill and liver from each sample were dissected, washed with deionized water, weighed, packed in polyethylene bags, and stored at -20°C prior to analysis.

The tissue samples were digested with conc. nitric acid. Dissected samples were transferred to a 100 mL Teflon beaker. Thereafter, 10 mL ultrapure conc. HNO_3 (Merck) was added, and the sample heated at 100, 150, 210, and 280 ^{0}C on a hot plate for 0.5, 0.5, 0.5 and 2 hours

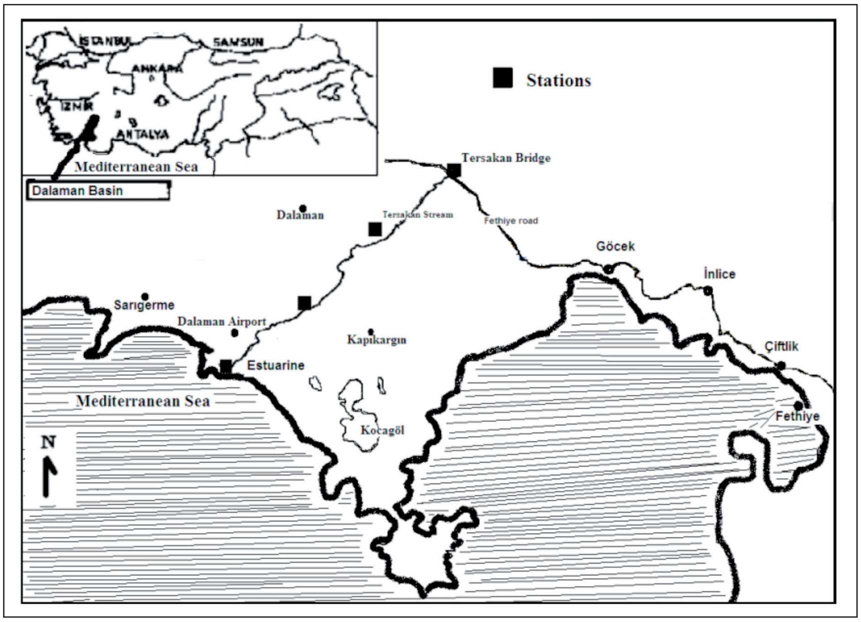

Figure 1: Sampling area of *Anguilla anguilla* specimens in Mugla province (Turkey).

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 filter (3).

All samples were analysed two times for Cd and Pb by ICP/AES (Optima 2000-Perkin Elmer), which is a fast multi-element technique with a dynamic linear range and moderate-low detection limits (35). Standard solutions were prepared from stock solutions (Merck, multi element standard). Standard reference materials, DORM-2 (for muscle) and DOLT-2 (for liver)–National Research Council Canada, were analysed for each of the Cd and Pb elements. Replicate analysis of these reference materials showed good accuracy, with recovery rates for metals between 92-107 % for fish. The absorption wavelengths and detections limits were 228.804 nm and 0.001 ppm for Cd, 220.35 nm and 0.0042 ppm for Pb respectively.

2.3. 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 sigificantly among tissues. Also, to determine the correlation between the elements in tissues, the Pearson's correlation coefficient matrix for the elements was done.

3. Results and Discussion

The variation of weight and total length of *Anguilla anguilla* samples according to age is shown in Table 1. The highest mean total length value of *A. anguilla* was 571,37 mm and lowest mean total length was value 216,70 mm. The highest mean weight was 63,93 g and the lowest mean weight was 42,02 g. Significant differences between total length and weight according to age were found ($p<0.05$) (Table 1).

The distribution of cadmium concentrations in tissues of *Anguilla anguilla* according to age (Length and weight) are given in Table 2 and Figure 2. The obtained results showed that the mean concentrations of Cd were highest in the gill of 7 age group, followed by the liver. İn

Table 1. The mean weight (g) and mean total length (mm) of studied *Anguilla anguilla* samples. (Ranges are given in parentheses).

Age	Fish Number (N)	Mean Length (mm) (range)	Mean Weight (g) (range)
3	61	216,70 ^{A} (175–265)	42,02 ^{A} (34,2–49,5)
4	53	279,45 ^{B} (248–326)	49,12 ^{B} (45–53,5)
5	26	352,65 ^{C} (304–400)	54,53 ^{C} (51,5–59)
6	12	443,58 ^{D} (400–490)	58,20 ^{D} (54,5–61,5)
7	8	571,37 ^{E} (518–635)	63,93 ^{E} (60–67,5)

The different word in Each column is statically different (p<0,05) contrast, the lowest concentrations of Cd were recorded in the muscle of 4 age group. The Cd concentrations in livers and gill were higher than those in the muscle.

Cadmium burdens in muscle and other tissues of *Anguilla anguilla* elevated with the increase in age (body size and body weight). There were no significant differences of cadmium burden among tissues of 3 age groups (p>0.05) however there were significant differences of cadmium burden among tissues of other age groups (p<0.05) (Table 2).

There were no significant differences of cadmium burden among muscles with the increase in age (body size and body weight) (p>0.05) while there were significant differences of cadmium burden among gill and liver with the increase in age (body size and body weight) (p<0.05).

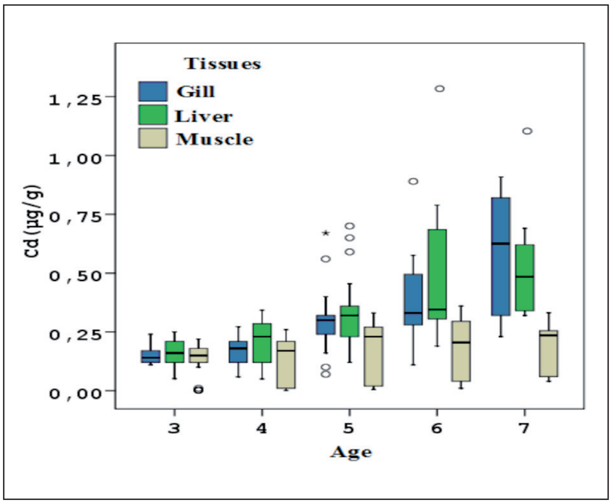

Figure 2: The distribution of mean cadmium concentrations (µg/g in wet weight basis) in tissues of Anguilla anguilla according to age.

There was a significant difference (P< 0.05) between the concentrations of Cd in gill and liver organs of the eel. Gills seemed to be the organ which accumulates the highest value of Cd. There was no a significant difference (P>0.05) between the concentrations of Cd in muscles of all eel age group. Muscles showed the lowest levels of Cd. The concentration of Cadmium is rarely found in natural water (19). It is considered to be toxic if its concentration exceeds 0.01 mg/ L both in drinking and irrigation water (36). Cadmium with some other heavy metals lead and mercury are of no biological function in human system and they are potentially toxic even at trace concentrations (31). The effects of acute cadmium are high blood-pressure, kidney damage, destruction of testicular tissue as well as destruction of red blood cells (17).

The distribution of lead concentrations in tissues of *Anguilla anguilla* according to age (Length and weight) are given in Table 3 and Figure 3. The obtained results showed that the mean concentrations of Pb were highest in the gill of 7 age group. İn contrast, the lowest concentrations of Cd were recorded in the muscle of 3 age group. The Pb concentrations in livers and muscle were higher than those in the liver but except in muscle of 3 age group.

Lead burdens in muscle and other tissues of *Anguilla anguilla* elevated with the increase in age (body size and body weight). There were no significant differences of lead burden among tissues of 7 age groups (p>0.05) however there were significant differences of cadmium burden among tissues of other age groups (p<0.05) (Table 3). There were significant differences of lead burden among muscles and other tissues with the increase in age (body size and body weight) (p<0.05).

Table 2. Mean Cd concentrations (µg/g in wet weight basis) in tissues of *Anguilla anguilla* according to age (body size and body weight).

Age (length (mm)- weight (g))	**Gill (range)**	**Liver (range)**	**Muscle (range)**
3 (175–265 mm–34,2–49,5 gr)	0,15 [D] (0,11–0,24)	0,16 [D] (0,01–0,25)	0,13 (0,001–0,22)
4 (248–326 mm–45–53,5 gr)	0,17 [Da] (0,01–0,31)	0,21 [Da] (0,05–0,38)	0,12 [b] (0,001–0,26)
5 (304–400 mm–51,5–59 gr)	0,29 [Ca] (0,07–0,67)	0,33 [Ba] (0,12–0,7)	0,16 [b] (0,005–0,33)
6 (400–490 mm–54,5–61,5 gr)	0,4 [Ba] (0,11–0,89)	0,51 [Aa] (0,19–1,4)	0,18 [b] (0,01–0,36)
7 (518–635 mm–60–67,5 gr)	0,59 [Aa] (0,23–0,98)	0,55 [Aa] (0,32–1,22)	0,19 [b] (0,04–0,41)

[a,b,c]: The different word in each row is statically different (p<0,05).
[A,B,C,D,E]: The different word in each column is statically different (p<0,05).

Table 3. Mean Pb concentrations (µg/g in wet weight basis) in tissues of *Anguilla anguilla* according to age (body size and body weight)

Age (length (mm)- weight (g))	Gill (range)	Liver (range)	Muscle (range)
3 (175–265 mm–34,2–49,5 gr)	0,82 [Ea] (0,21–1,41)	0,69 [Db] (0,1–1,16)	0,61 [Eb] (0,01–1,31)
4 (248–326 mm–45–53,5 gr)	1,21 [Da] (0,47–1,85)	1,04 [Cb] (0,19–1,31)	1,09 [Db] (0,66–1,69)
5 (304–400 mm–51,5–59 gr)	1,68 [Ca] (1,02–2,95)	1,3 [Bb] (0,57–1,72)	1,51 [Ca] (1,2–2,25)
6 (400–490 mm–54,5–61,5 gr)	2,04 [Ba] (1,41–3,68)	1,46 [Bc] (1,25–1,86)	1,79 [Bbc] (1,35–3,01)
7 (518–635 mm–60–67,5 gr)	2,35 [A] (1,58–2,98)	1,84 [A] (1,59–2,27)	2,14 [A] (1,36–3,68)

[a,b,c]: The different word in each row is statically different (p<0,05).
[A,B,C,D,E]: The different word in each column is statically different (p<0,05).

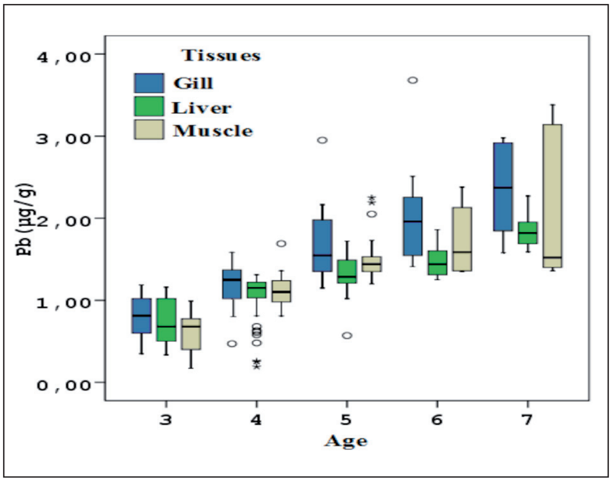

Figure 3: The distribution of mean lead concentrations (µg/g in wet weight basis) in tissues of Anguilla anguilla according to age.

Our results show that metal accumulation is highest in liver and in gills, whereas it is low in muscle in eel, except Pb. The liver tissue is highly active in the uptake and storage of heavy metals. It is well known that large amount of metallothionein induction occurs in the liver tissue of fishes. The gills are uptake site of waterborne ions, where metaconcentrations increase especially at the beginning of exposure, before the metal enters other parts of organism (23). Many studies reported that on a number of fish species, which show that muscle is not an active tissue in accumulating heavy metals (4, 15, 34).

The length, age and weight of a fish is known as determining factor of its metal burden (KOJADINIVIC et al. 2006). Statistical analyses shows positive significant correlation between body size, age and body weight of *Anguilla anguilla* with Cd and Pb concentrations in muscle tissue.

The correlations between the levels of accumulated metals in the tissues and biological features (Total Leight, Weight and age) were examined (Table 4). There were different correlations between the toksic metal (Cd and Pb) levels in muscle tissue and biological features. İn the muscle tissue, two non-essential metals, Cd and Pb, pozitively correlated with age (r=0.19, p<0.05 and r=0.78, p<0.01 respectively), TL (r=0.14, p<0.05 and r=0.78, p<0.01 respectively) and W (r=0.23, p<0.05 and r= 0.83, p<0.01 respectively) (Table 4).

A highly significant positive correlation between total length and body weight was found (r=0.91, p<0.01). Also there was significant positive correlation between Cd and Pb levels in muscle tissue. Significant correlations between cd and pb with body size and age were found however the highest positive significant correlation showed between Cd and Pb with body weight (Table 4). Trend of metal levels such as cd and pb were increased with increasing of biological features.

Table 4. Pearson correlations of Cd and Pb concentrations in muscle tissue of *Anguilla anguilla* species with their length, weight and age.

	Age	Total Length	Weight	Cd	Pb
Age	-				
Length	.95**	-			
Weight	.91**	.91**	-		
Cd	.19*	.14*	.23*	-	
Pb	.78**	.78**	.83**	.27**	-

*P < 0.05, **P < 0.01, ***P < 0.001

Highly significant positive correlation (r= 0.83, P<0.01) was found when comparing individual body weight of *Anguilla anguilla* and concentration of Pb in muscle tissue. Also statistical analyses shows a significant correlation (r=0.23, P<0.05) between Cd concentration in muscle tissue and body weight. Pb is a cumulative toxin. In human beings, it binds with SH group of proteins, apart from that, Pb damages blood circulation, central nervous system, liver and kidneys.

Cd and Pb for *Anguilla anguilla* were found higher than the World Health Organization (WHO), European Units, Food and Agriculture Organization (FAO) and Turkish Food Codex (TFC) limits for human consumption in edible parts (muscle) of *Anguilla anguilla* samples and posed a risk for human health (Table 5) (12,13). These levels of toksic heavy metals (cadmium and lead) accumulated by *Anguilla anguilla* might be due to the increase in agricultural influx waters and some anthropogenic activities. With the passage of time as the fishes grow, these metals accumulate in various internal organs like liver, muscles and gill to a significantly high concentration not suitable for human consumption. On the other hand, high levels of metals in tissues of *A. anguilla* could be originated from different sources around the study area. These sources are; i) for Pb and Cd, boat traffic, motor oil and ballasts water, also Cd can be occuring from phosphorus-fertilizer used in agriculture.

4. Conclusions

Fish species living in contaminated waters tend to accumulate heavy metals in their organs and tissues. Various heavy metals are accumulated in fish body in different amount (21). Fish species mostly absorbed heavy metals from its feeding diets, sediments and surrounding waters resulting to their accumulation in reasonable amounts (MCCARTHY & SHUGART 1990).

The accumulation of heavy metals in fish species are found to be influenced by several factors like temperature pH of water, conductivity, rainfall, hardness, salinity and also by biotic community interactions (1). Microhabitat utilization, feeding habits, age, sex and fish species also determine the accumulation pattern of heavy metals (24). The levels of Cd and Pb in muscle tissue of *Anguilla anguilla* were high when compared with the maximum permissible limits by (TFC), (FAO/WHO) and EU limits in fish. Considering the results of this study, *Anguilla anguilla* is adequate and most suitable species for use as bio-monitors of toksic metals (Cd and Pb) pollution in the Tersakan Stream. Consequently, we recommend the use of these species as biological indicators as a tool for future monitoring programs, to evaluate the evolution of Heavy metal pollution in this area.

Consequently, the results indicated that Tersakan Stream have been polluted by heavy metals (Cd and Pb) due to anthropogenic activities and from the legal standpoint, the muscle of *Anguilla anguilla* caught was not suitable for human consumption and posed a risk for human health. The differences in the level of heavy metals accumulated by the fish species respectively could be attributed to the differences in their metabolic rates, feeding habits, age, sex and fish species (24). Body size and health which are closely related to growth and

Table 5. Cd and Pb concentrations (μg/g in wet weight basis) in muscle tissues of *Anguilla anguilla* according to age compared to the maximum permissible limits by (TFC), (FAO/WHO) and EU in fish.

Age (length (mm)- weight (g))	Mean Cd (μg/g) Concentration (range)	Mean Pb (μg/g) Concentration (range)
3 (175–265 mm–34,2–49,5 g)	0,13	0,61
4 (248–326 mm–45–53,5 g)	0,12	1,09
5 (304–400 mm–51,5–59 g)	0,16	1,51
6 (400–490 mm–54,5–61,5 g)	0,18	1,79
7 (518–635 mm–60–67,5 g)	0,19	2,14
Turkish Guidelines (2002)	0,10	0,30
FAO/WHO limits (1989)	0,5	0,5
EU limits (2001)	0,1	0,1

metabolism has been shown to attribute most of the variations in heavy metals content of fishes (27). *Anguilla anguilla* being a bottom feeder may have accumulated Cd and Pb from the sediment in the Tersakan Stream.

This study clearly indicates that the fishes living in water bodies receiving industrial effluents and city waste water containing various toxicants particularly heavy metal ions, even at low concentration, absorb and accumulate these heavy metals in their various tissues like gill, liver and muscle. Also the present study shows that precaution measures need to be taken in order to prevent future heavy metal pollution.

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