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The Accumulation of Some Heavy Metals in Northern Pike *(Esox lucius* L., 1758*)* Inhabiting Sıdıklı Küçükboğaz Dam Lake (KIRŞEHİR)

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ABSTRACT

Pike (*Esox lucius*) samples were obtained from the designated locations of Sıdıklı Küçükboğaz Dam Lake between March 2012 and February 2013. Some heavy metal concentrations (Cu, Fe, Mn, Zn, Cr and Al) in the muscle, liver, skin, intestine and gills of this fish were measured by Flame Atomic Absorption Spectrometry (AAS) device and it was observed that heavy metals accumulated at different levels in different tissues of pike. Heavy metal concentration in different fish tissues varied as dry weight Cu: 0.1123-0.3764, Fe: 4.3135-9.505, Mn: 0.5442-0.1684, Zn: 3.3065-2.475, Cr: 0.1889-2.517, Al: 2.17-1.045 µg g^{-1}. When the heavy metal levels in the tissues were compared with the national and international permissible limits, it was seen that the values of all metals in the muscles of the fish were below the determined limit values according to the analysis results. Seasonal changes in metal (Cu, Fe, Mn, Zn, Cr and Al) concentrations were observed in pike tissues, but it was concluded that these differences would not negatively affect the consumption recommendations based on the levels permitted by FAO/WHO (2020). According to the analysis, it was concluded that the fish collected from this region can be safely consumed by humans and the lake water can be used for irrigation purposes in agriculture.

Keywords: *Esox lucius,* heavy metal, Sıdıklı Küçükboğaz Dam Lake, Kırşehir

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Sıdıklı Küçükboğaz Baraj Gölü (Kırşehir)'nde Yaşayan Turna Balığı (*Esox lucius* L., 1758)'nda Bazı Ağır Metallerin Birikimi

Öz: Turna (*Esox lucius*) numuneleri, Mart 2012 ile Şubat 2013 tarihleri arasında Sıdıklı Küçükboğaz Baraj Gölü'nün belirlenen yerlerinden temin edilmiştir. Turna'nın kas, karaciğer, deri, bağırsak ve solungaçlarındaki bazı ağır metal konsantrasyonları (Cu, Fe, Mn, Zn, Cr ve Al) Alev Atomik Absorpsiyon Spektrometresi (AAS) cihazı ile ölçülmüş ve farklı dokularında farklı seviyelerde ağır metallerin biriktiği gözlemlenmiştir. Farklı balık dokularındaki ağır metal konsantrasyonu, kuru ağırlık olarak; Cu: 0.1123-0.3764, Fe: 4.3135-9.505, Mn: 0.5442-0.1684, Zn: 3.3065-2.475, Cr: 0.1889-2.517, Al: 2.17-1.045 mg/kg şeklinde değişiklik göstermiştir. Dokulardaki ağır metal seviyeleri ulusal ve uluslararası izin verilen sınırlarla karşılaştırıldığında, balıkların kaslarındaki tüm metal değerlerinin analiz sonuçlarına göre belirlenen sınır değerlerinin altında olduğu belirlenmiştir. Turna dokularında metal (Cu, Fe, Mn, Zn, Cr ve Al) konsantrasyonlarında mevsimsel değişiklikler gözlemlenmiş, ancak bu farklılıkların FAO/WHO (2020) tarafından izin verilen seviyelere göre tüketim önerilerini olumsuz etkilemeyeceği sonucuna varılmıştır. Yapılan analize göre, bu bölgeden toplanan balıkların insanlar tarafından güvenle tüketilebileceği ve göl suyunun tarımda sulama amaçlı olarak kullanılabileceği sonucuna varılmıştır.

Anahtar kelimeler: ***:*** *Esox lucius*, ağır metal, Sıdıklı Küçükboğaz Baraj Gölü, Kırşehir

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Introduction

It is known that heavy metals are among the leading toxic substances that pollute the natural environment. Pollution when combined with water has become a serious health problem in recent years as it creates a widespread threat by increasingly accumulating through the food chain in all organisms in the ecosystem (Yilmaz et al. 2016; Polat and Akkan 2016; Uncumusaoglu and Akkan 2017; Akkan et al. 2018; Mutlu et al. 2018). Therefore, they are easily soluble in water resulting that they can be absorbed very easily by aquatic organisms and their binding to the proteins of living things is quite strong (Kalay et al. 2004; Muşlu 2008). It eventually causes structural dysfunctions at cellular and molecular levels in aquatic organisms and increases in the frequency of DNA breaks (Kalay and Karataş 1999; Levesque et al. 2002; Akkan et al. 2013; Giordano et al. 1989).

When low ambient concentrations are examined, heavy metals seem to accumulate in metabolically active tissues such as liver, kidney and spleen at high levels and this accumulation is associated with metal detoxification and disfunction of metabolic events (Sharma 1983; Cicik 2003).

The aquatic environment is highly susceptible to the harmful effects of heavy metal contamination because aquatic organisms are in close and prolonged contact with soluble metals. Increasing heavy metal accumulation in fish constituting a ring of the biological cycle and consumed as an important protein source, causes both toxic effects on fish and negative effects on human health (Dural et al. 2007; Canbek et al. 2007; Sipahi et al. 2013). Therefore, it can be considered as one of the most important indicators of the impact of metal pollution in water ecosystems. The release of industrial and agricultural wastes has recently increased heavy metal concentrations in lake ecosystems, which leads aquatic organisms to be exposed to these metals at high levels (Kalay and Canlı 2000; Sankar et al. 2006; Said et al. 2014). When heavy metals released into the aquatic environment with different types of wastes exceed the legal limits, especially when they are used as drinking water or irrigation water, they reach more dangerous dimensions and cause many problems in living organisms, community-environmental health and agriculture (Segar and Pellenbarg 1973). Therefore, an accurate water pollution assessment and monitoring is of great importance because of its direct effects on aquatic life and human health (Saha et al. 2017). Lead (Pb), cadmium (Cd) (Kumar et al. 2013), nickel (Ni) (Kaaber et al. 1978, 1979), chromium (Cr) (U.S.EPA 1999), mercury (Hg) and arsenic (As) (Sankhla et al. 2016) are among the most important heavy metals that cause water pollution and pose a great threat due to their toxic effects.

The aim of this study is to determine the heavy metal pollution level of this lake by analyzing some heavy metal accumulation levels in the muscle, liver, skin, intestine and gills of Northern Pike collected from different locations of Sıdıklı Küçükboğaz Dam Lake.

Materials and Methods

Study Area and Sampling

Sıdıklı Küçükboğaz Dam Lake is located in the Central Basin of Kızılırmak River, in the Southwest of Kırşehir Province, on the Körpeli Boğaz Stream, in the north of Hirfanlı Dam Lake. It is connected to a small river known as Kepez Özü. The reservoir volume of the lake is 28,500 m^3, the reservoir area is 1.65 km^2 and the area to be irrigated is 4,945 hectares. It is 30 km away from Kırşehir city center (Figure 1). In the dam lake, there are *Cyprinus carpio* (European carp), *Silurus glanis* (Wels catfish), *Tinca tinca* (Tench), *Capoeta tinca* (Anatolian khramulya), *Squalius cephalus* (chub), and *Esox lucius* (Northern Pike).

Figure 1. Satellite Map Image of Sıdıklı Küçükboğaz Dam Lake (Taken from Google Earth.)

Field and Laboratory Studies

Study materials consist of 15 pike samples (*E. lucius*) collected by sampling every month in a one-year period from different parts of the Sıdıklı Küçükboğaz Dam Lake between March 2012 and February 2013.The samples were caught using fanned nets and fishing lines with 12x12, 16x16, 18x18 and 22x22 mm mesh sizes and brought to the laboratory in suitable sized bags. While water samples were taken into brown bottles of 500 ml, sediment samples were taken 3 m from the beach and the lake floor with a plastic shovel placed in plastic bags and brought to the laboratory.

Preparation of Tissues and Organs of Northern Pike (*E. lucius*) for Analysis

Approximately 0.5 g of tissue samples (muscle, gill, liver, skin, intestine) from each fish were excised, washed with distilled water, weighed, packed in polyethylene bags and stored at -20°C until metal analysis. All tissue samples were transferred to 100 ml teflon tubes and samples (0.5 g) using the microwave solubilization program (pressure 200 psi, ramp time 15 mins. Temperature 210°C, maximum power 450 W, retention time 10 mins.) in a microwave oven (CEM MARS -5 Closed Cup Microwave Digestion System) was dissolved with 5 mL HNO_3 (65% (m/m)). Teflon tubes were sealed with a watch glass and heated at 50-100°C on a hot plate for 4 hours until the solution slowly evaporated to almost dryness. 2 ml of HNO_3 (65% (m/m)) and H_2O_2 (30% (m/m)) were added to the residue and the solution was evaporated again on the hotplate. After cooling, 2.5 ml of 1N HNO_3 was added to the digested residue and transferred to 10 ml graduated flasks, then diluted to level with deionized water (Ciftci et al. 2011). Samples were filtered through a 0.45 μm syringe filter (Sartorius) prior to analysis.

Preparation of Sediment for Analysis

Sediment samples were taken from the shore of Sıdıklı Küçükboğaz Dam Lake with a plastic scoop, placed in 500 ml colored bottles and brought to the laboratory for analysis. It was placed in a petri dish, dried in an oven at 60ºC and pounded in a mortar until it was pulverized. 0.1 g was weighed and put into a teflon tube and 5 ml of concentrated HNO_3 and then 1 ml of concentrated $HClO_4$ were added and left for one hour. Solubilization was carried out for one hour and allowed to cool at room conditions. Excess acids in the environment were evaporated at 50-100 ºC. It was completed to 10 ml with deionized water, and kept in a cool place without light until it was analyzed in glass tubes by filtering.

Sediment solubilization was done in a microwave oven. Microwave solubilization method provides great advantages in terms of short time, less acid consumption and preventing possible metal loss (Karadede et al. 2004).

Preparation of Water Samples for Analysis

The pH of the water samples taken from the Sıdıklı Küçükboğaz Dam Lake was first measured during the field, taken in 500 ml brown bottles, brought to the laboratory and the pH was measured and recorded again. Then it was filtered and taken into another bottle of the same size, 1 ml of concentrated HNO_3 was added (to prevent bacteria that may occur) and stored in a cool and dark environment until the analysis.

Analysis Method

In this study the accumulation of heavy metals copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), chromium (Cr) and aluminum (Al) were analyzed. Analyzes were carried out with a Flame Atomic Absorption Spectrometer (AAS) (Analytik Jena AG, Jena, Germany) equipped with a 50 mm burner head and an injection module (SFS-6). All absorption lines of an element in the 185-900 nm spectral range were analyzed analytically using a Xe short arc lamp as a continuous lamp source. The spectral background of the sample in AAS is always corrected simultaneously, independently and directly on the analysis line. All measurements were made under optimum conditions in triplicate using an injection module SFS-6, which provides computer-controlled aspiration of cavities, analytical solutions and samples (Ciftci et al. 2011; Ciftci and Er 2013). All solutions were prepared using ultrapure water (specific resistance 18 M/cm) from a MilliQ purification system (Millipore Corporation, Massachusetts, USA). Standard analysis solutions were prepared from 1000 mg L-1 stock solutions (Merck). Results are expressed in μg g^{-1}. One-way ANOVA and Tukey tests were used to test the differences in metal levels between samples (significance level $p < 0.05$). All statistical calculations were made through SPSS 16.0 for Windows.

Results

This study was carried out on the Northern Pike found in Sıdıklı Küçükboğaz Dam Lake and provides information on the metal accumulation levels in the studied tissues. According to the results obtained in this study, it was determined that the heavy metals that accumulated the most in the liver were Cu and Fe. It was observed that Mn, Cr and Al accumulated in the gills at the highest rates. Compared to other tissues, Zn accumulated mostly in the intestines. Cu is least found in muscle tissue and the order of change in other tissues is gill > skin > intestine. In the analysis for Fe, the least accumulation was observed in the skin. Accordingly, the relationship between other tissues was in the form of intestine > gill > muscle tissue. Mn is mostly found in the skin and intestines after the gills, and its level is very low in the muscle tissue. Metal accumulation rates for Zn are gill > liver > skin > muscle tissue. Looking at the Cr levels, the least accumulation was observed in the skin, and the order in other tissues was liver> muscle tissue> intestine. Finally, in the results obtained for the Al analysis, no significant accumulation was observed in the muscle tissue, while the Zn ratio in the intestines was found to be higher than in the liver.

Mean concentrations of Cu, Fe, Mn, Zn, Cr and Al metals accumulating in different tissues of Northern Pike (*E. lucius*) are given in Table 1. During the study, research was carried out on 15 fish materials collected in monthly periods. The results of the analysis show that heavy metals are mostly accumulated in the liver and gills. While Fe, Zn and Al values were determined to be the most abundant metals in the liver with concentrations of 10.551 µg/kg, 2.6935mg/kg and 0.801 mg/kg, respectively, the second highest metal concentration was found to be the most abundant in the gills with Zn, Fe and Al values of 4.686 mg/kg, 2.804 µg/kg and 1.548 mg/kg respectively. Metal concentrations accumulated in the muscles, on the other hand, showed the lowest concentration level throughout the study. Except for Mn and Al metals, all metal concentrations accumulating in the liver were measured at higher levels than others. Considering the different tissues of pike, the lowest Cr metal level was recorded as 0.048 µg/kg in the skin, and the highest value was measured with 0.689 mg/kg in the liver.

Table 1. Concentrations of heavy metals in various tissues of Northern Pike (gill, intestine, skin, muscle tissue and liver) (one year period) (mg/kg)

	Mean.±SE (Min.-Max.)				
Metal (mg/kg)	**Gill** X± SE Min.–Max	**Intestine** X± SE Min.–Max	**Skin** X± SE Min.–Max	**Muscle** X± SE Min.–Max	**Liver** X± SE Min.–Max
Cu	0.187±0.0045^{a,x} (0.0588-0.3898)	0.205±0.0083^{a,x} (0.1178-0.2536)	0.165±0.0036^{a,x} (0.0788-0.3575)	0.2726±0.0231^{a,xy} (0.0553-0.7616)	0.572±0.0305^{b,z} (0.3764-1.0527)
Fe	2.804±0.2560^{b,y} (1.7021-4.3135)	3.227±0.3347^{a,y} (1.9015-4.879)	1.063±0.0698^{a,y} (0.588-1.888)	1.231±0.0589^{a,yz} (0.7413-1.7027)	10.551±0.1694^{c,w} (9.505-11.65)
Mn	0.7005±0.0032^{b,x} (0.5442-0.8164)	0.222±0.0039^{a,x} (0.1501-0.4247)	0.3316±0.0070^{a,xy} (0.1771-0.5946)	0.1166±0.0055^{a,x} (0.0208-0.3575)	0.1096±0.0407^{a,x} (0.0502-0.1684)
Zn	4.686±0.2546^{a,x} (3.3065-5.7035)	9.762±0.2430^{a,y} (8.175-10.39)	2.8385±0.0320^{a,z} (2.305-3.2105)	1.6521±0.0440^{a,z} (1.054-2.05)	2.6935±0.0905^{a,yz} (1.913-3.4295)
Cr	0.5976±0.2608^{a,x} (nd-2.2015)	0.1527±0.0037^{a,x} (0.047-0.3384)	0.048±0.0020^{a,xy} (nd-0.1259)	0.058±0.0038^{a,xy} (nd-0.17)	0.689±0.3252^{a,xy} (nd-2.517)
Al	1.548±0.0766^{a,x} (0.7318-2.17)	0.862±0.0625^{a,x} (0.325-1.5185)	0.804±0.0224^{a,xy} (0.555-1.287)	0.712±0.0143^{a,xy} (0.56-1.099)	0.801±0.0070^{a,xy} (0.5419-1.045)

Horizontally, the letters a, b and c show statistically significant differences ($p < 0.05$).
The letters x, y, z and w vertically show statistically significant differences ($p < 0.05$).
nd: not detected.
X ± SE: Mean ± standard error. Number of samples taken annually n:15

According to the results of the analysis, while Fe has the highest accumulation, Cr lowest and the highest accumulation rate of Fe and Cu metals is in the liver. In addition, it was determined that the relationship between heavy metal concentrations was listed as Fe> Zn> Al> Cu> Mn> Cr. Similarly, in the study of Tekin-Özan and Kır (2007), while it is known that the pike (*E. lucius*) living in Lake Işıklı detected Cu, Mn and Cr in the liver below the analysis limit, they also determined the Fe and Zn values.

Since fish is a strong source of protein, the metal concentrations accumulated in the muscle tissues are very important. The result shows that the heavy metal concentrations accumulated in the muscle tissue of Northern Pike were found to be lower than the maximum values recommended and allowed by FAO (2005) and WHO (1995), which is very important. Accordingly, the mean heavy metal concentrations in the muscles, Cu; 0.2726 mg/kg, Fe; 1.231 mg/kg, Mn; 0.1166 mg/kg, Zn; 1.6521 mg/kg, Cr; 0.058 mg/kg, and Al; 0.712 mg/kg calculated as. In the studies (Türkmen et al. 2009; Company et al. 2010), the researchers similarly found that the muscle was not an active tissue in accumulating heavy metals. In addition to analyzes made in the muscle due to its importance in nutrition, analyzes were also performed on the gills and skin, especially the liver, due to its high capacity in accumulating heavy metals.

According to the results of the metal analyzes in the water of the Sıdıklı Küçükboğaz Dam Lake, the Cu (0.0120 mg/L) and Cr (0.583 mg/L) accumulation levels were measured below the analysis limit. It was determined that the Zn (0.769 mg/L) accumulation level was insignificant, while the Fe (0.489 mg/L) and Mn (0.491 mg/L) accumulation levels were significant. According to the results of the metal analyzes made in the sediment of the Sıdıklı Küçükboğaz Dam Lake, it was seen that the Cu (0.1651 mg/kg), Fe (21.7495 mg/kg), Zn (0.8997 mg/kg) and Cr (0.1701 mg/kg) accumulation levels were insignificant, while the Mn (5.9197 mg/kg) accumulation levels were significant.

Heavy metal deposition levels of the study were evaluated statistically by One Way ANOVA and Tukey tests. Cu and Fe values in liver and other tissues; Zn and Al values in the gill, intestine and other tissues were determined to be statistically significant ($p < 0.05$). Considering the differences between all tissues, the Cr value was not considered statistically significant ($p < 0.05$). In the liver, the differences in Cu and Fe accumulations were found to be statistically significant, while the values obtained in terms of other metals were insignificant ($p > 0.05$). While Mn value in gill and skin, values in Zn and Al accumulation in intestines and gills were statistically significant, changes in accumulation of other metals were statistically insignificant ($p > 0.05$).

Discussion

In aquatic ecosystems, sediment is constantly addressed in monitoring studies, as it acts as a natural reservoir of pollutants that tend to precipitate and is the natural memory of the aquatic ecosystems in which it is located (Mutlu et al. 2020). In the heavy metal analysis carried out in the sediment of Sıdıklı Küçükboğaz Dam Lake, Cr was measured below the analysis limit except for the months of May-June and July. Cu, Fe, Mn, Zn and Al were detected every month. It has been determined that the metal that accumulates the most in the sediment is Al in the Spring-Summer-Autumn and Winter seasons. According to the study carried out in the study area covering a 28 km coastline along the Black Sea, it was determined that the heavy metals that accumulated most in the sediment were Fe and Al (Fikirdeşici Ergen et al. 2018).

According to the analysis made in Kapulukaya Dam Lake (Kırıkkkale), it has been shown that Al is present in the sediment to a significant extent (Binici and Pulatsü 2018). The presence of heavy metals in ionic form in water can cause an increase in toxicity effects. The presence of heavy metals in the form of toxic ions in waters can directly harm organisms and their consumers. Some of the heavy metals found in this way are taken directly by living things after they enter the water, while others are carried to the sediment (Kaptan 2014). For this reason, the analyzes to be made are of great importance. As a result of heavy metal analysis in the water of Sıdıklı Küçükboğaz Dam Lake, Cr (0.583 mg/L) was measured below the analysis limit in all seasons. Cu (0.0120 mg/L) has only been detected in August. Fe (0.489 mg/L), Mn (0.491 mg/L) and Zn (0.769 mg/L) were detected between June and September. Al has been detected between May and September. Al (1.001 mg/L) was detected at the highest rate in Spring, Al, Zn and Cr in Summer. It has been determined that the most common metals in water are Al and Zn. According to the results of heavy metal analysis carried out in Ulubat Lake Water, it was determined that the highest level of accumulation was Zn (6.13mg/L) metal in Spring (Berber et al. 2021). Studies have shown that Al (35.28 mg/L) in the Kovada lake in the Antalya basin, Al (27.59 mg/L) in the Karakuyu Marshes, and Al (9.57 mg/L) and Zn (309.86 mg/L) in the Burdur Basin Lakes show the highest level of accumulation (Oruçoğlu and Beyhan 2019). As a result of heavy metal analysis in some tissues and organs of Northern Pike living in Sıdıklı Küçükboğaz Dam Lake; Fe and Cu were detected in the liver, and Zn in the intestine and gill in the samples collected in the spring. In the samples collected during the summer months, Cu and Fe were detected in the liver, Mn in the gill and skin, and Zn and Al in the intestine Cu and Fe were detected in the liver in the samples collected in autumn, Zn in the intestine and gills, and Mn in the gills in the samples collected in the winter months. Cu, Fe, Zn, Cr, Al do not differ between tissues. In the study conducted in Ladik Lake (Samsun), Al, Cr, Mn and Zn levels in the tissues of fish were measured at the level of gill>liver>muscle in all seasons. Cu and Fe levels were found in the order of liver>gill>muscle (Erdoğan et al. 2021). Zn, Cu, Pb and Cd levels were investigated in the liver, gill and muscle tissues of economically important species (*Caranx rhoncus, Scomber japonicus, Pegusa lascar*) in Mersin Bay and it was determined that Cu accumulates at the highest level in the liver (Karayakar et al. 2017). These results showed parallelism with our study. At the end of the study, it was determined that heavy metals accumulated mostly in the liver and gills. The liver is an important organ where metals are stored, both because it plays an active role in metabolism and because it is the organ where metalloproteins, which are metal-binding proteins, are produced (Al-Yousuf et al. 2000; Liu et al. 2012). Metals taken up with respiratory water adhere to the mucus in the gills, and the metals remain between the lamellae as respiratory water passes through the gill lamellae. Thus, the metal concentration in the gills can reach high levels (Heath 1995). The low level of accumulation of metals in the muscle tissue can be explained by the fact that the muscle is not a metabolically active organ (Karadede et al. 2004) however, researches are carried out with the concern that the metals accumulated in this tissue can reach the human by joining the food chain (Wang et al. 2010).

This study was carried out to determine the heavy metal levels accumulated in the muscles, liver, skin, intestines and gills of the Northern Pike living in the Sıdıklı Küçükboğaz Dam Lake. A natural advantage of this study is it enables us to obtain information about heavy metal pollution of the reservoir. Although the metal levels determined in Sıdıklı Küçükboğaz Dam Lake are not excessively high in general, a metal pollution tending to increase has been detected. According to the results, significant differences were found in the heavy metal accumulation levels in the tissues and organs of Northern Pike. According to these results, it was concluded that the heavy metal levels accumulated in the muscle tissue of Northern Pike in this region were not significantly found, which poses no danger in consuming them as food. In addition, the results underline the importance of controlling the commercial activity on the Sıdıklı Küçükboğaz Dam Lake and the streams feeding it, in addition to preventing the leakage of pesticides and chemical fertilizers used in agricultural activities into the lake. Considering that the water of the dam lake is used for agriculture and irrigation purposes, it is very important to take precautions against all kinds of dangers thought to increase when fish consumption is taken into account in terms of protein content. In addition, metal pollution of the lake water and regular inspections are of great importance. In addition to these, tourism activities should be arranged by considering the pollution capacity of the lake. It should not be forgotten that it is an inevitable fact that wastes left to nature consciously or unconsciously will return to humans.

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