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The Seasonal Variations of Heavy Metal Levels in Muscle, Liver and Gill of Pike (*Esox lucius* L., 1758) Inhabiting Işıklı Lake (Turkey)

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ABSTRACT

This study was carried out between October 2012 and July 2013 and the aim of the current study is to investigate to determine levels of some heavy metals in muscle, liver and gills of pike in Işıklı Lake, Turkey. To determine the accuracy of our device, metal levels were analyzed in DORM-3 and DOLT -4 standard reference materials, the resulting values were compared with values given by the National Council of Canada. Except Cr, all metals were determined in all tissues in all seasons. Cr was below detection limit in liver in autumn and summer and in muscle in summer. Generally, higher metal levels were found in liver, while the lowest were determined in muscle tissue of pike. Heavy metal levels in tissues increased in summer and winter, but decreased in autumn and spring. Some of the results were above the limits for fish given by WHO, EC and Turkish Food Codex. This study shows that a potential danger may occur in this region in the future depending on the agricultural development.

Keywords: Heavy metal, Muscle, Liver, Gill, Pike, Işıklı Lake, Turkey

Işıklı Gölü'nde Yaşayan Turna Balığı (*Esox lucius* L., 1758)'nın Kas, Karaciğer ve Solungaçlarındaki Ağır Metal Seviyelerinin Mevsimsel Değişimi

Öz

Bu çalışma Ekim 2012 ve Temmuz 2013 tarihleri arasında yapılmış ve Işıklı Gölü'nde yaşayan turna balığı'nın kas, karaciğer ve solungaçlarındaki bazı ağır metallerin seviyelerinin belirlenmesi amaçlanmıştır. Cihazımızın çalışma doğruluğunu saptamak amacıyla DORM-3 ve DOLT-4 standart referans materyallerinden de metal analizi yapılarak, elde edilen değerler Kanada Ulusal Konseyi tarafından verilen değerlerle kıyaslanmıştır. Cr dışındaki tüm metaller her mevsimde her dokuda tespit edilmiştir. Cr'un karaciğerde yaz ve sonbaharda, kaslarda yaz mevsiminde analiz limitinin altında olduğu tespit edilmiştir. Genel olarak, metallerin en fazla karaciğerde biriktiği, kaslarda ise en düşük seviyede olduğu belirlenmiştir. Ağır metal seviyeleri yaz ve kış mevsimlerinde artmış, sonbahar ve ilkbahar mevsimlerinde azalmıştır. Sonuçların bazıları WHO, EC ve Türk Gıda Kodeksi tarafından verilen limit değerlerin üstünde bulunmuştur. Bu çalışma, gelecekte tarımın gelişmesine bağlı olarak bu bölgede bir tehlikenin oluşabileceğini göstermektedir.

Anahtar Kelimeler: Ağır metal, Kas, Karaciğer, Solungaç, Turna Balığı, Işıklı Gölü, Türkiye

INTRODUCTION

Pollution of the aquatic environments is one of the serious problems in the world. Different materials like toxic metals, acids, pesticides, fossil fuels, nitrates, sulfates, microorganisms, hot water, radioactive substances are cause water pollution (Göksu 2003). From these, heavy metals are treated as one of the most serious pollutants of the aquatic environment and these metals may pose a serious threat to aquatic life because of their toxicity, bioaccumulation, long persistence and biomagnification (Harte et al. 1991; Schüürmann and Markert 1998; Iqbal and Shah 2014). Heavy metals are diffuse to aquatic environment from different natural and anthropogenic sources like industrial effluents, agricultural runoffs, transport, burning of fossil fuels, geological structure, mining activities and atmospheric deposition (Adnano 1986; Dawson and Macklin 1998; Kalay and Canlı 2000).

Low levels of some heavy metals are essential for the development of living organisms, but some of them such as Pb, Hg and Cd are non-essential and very toxic. And also, essential metals may be toxic when they are presents above the permissible concentration (Puttaiah and Kiran 2008). Heavy metals can be taken up by fish, both through food chain and from water and end up in fish, where they accumulate in various organs and tissues (Hadson 1988). Fish are a good indicator for the estimation of water pollution because fish could be sampled more frequently and easily in comparison with other water organisms (Moiseenko and Kudryavtseva 2001). Concentrations of heavy metal levels in fish depend on different factors such as ecological needs, size and age of individuals (Newman and Doubet 1989), their life cycle and life history, feeding habits, season of capture and physico-chemical parameters of water (Zhong and Wong 2007). Heavy metal concentrations in fish tissues is self-regulated and high levels of metals can be toxic. Heavy metals not only affect the productivity and reproductive capabilities of fish (EPA 2009) but cause structural damage in fish at cellular and molecular levels (Kalay et al. 2004; Fulladosa et al. 2006). Essentially, fish assimilate the metals by three possible ways (body surface, gills or digestive tract) (Dallinger et al. 1987; Pourang 1995). The body surface is generally assumed to play a minor role in heavy metal uptake of fish (Varanasi and Markey 1978; Dallinger et al. 1987; Pourang 1995), whereas the gills are regarded to be the important site for direct uptake from the water (Hughes and Flos 1978; Thomas et al. 1983; Dallinger et al. 1987).

Gill, liver and muscle were chosen as target organs for assessing metal accumulation. We selected muscle because of its importance for human consumption and as a primary site of metal (Kotze et al. 1999). And liver was

analyzed because this organ tends to accumulate metals (Tepe et al. 2008), specialized in metal storage and detoxification (Kotze et al. 1999). The aim of this study are these: (1) to determine seasonal variations of heavy metal concentrations in the fish gills, muscle and liver (2) and compare with the acceptable metal levels in fish muscle given by different institutions.

MATERIAL AND METHODS

Study Area

Işıklı Lake (29° 92' E, 38° 22' N), located in the southwest of Turkey (Figure 1) and used for irrigation. The lake is approximately 7 m depth, its area is 9749 ha and fed by Büyük Menderes Stream, Karanlık Stream and Kufi Stream. There are small rush islands in the lake. There are a lot of apply, cherry and peach gardens, grains fields, restaurants and hotels around it (Aygen and Balık 2005; Akarsu et al. 2006). The fish species found most commonly in the lake are *Cyprinus carpio*, *Esox lucius*, *Tinca tinca* and two endemic fish species (*Aphanius anatoliae*, *Chondrostoma meandrense*) (Akarsu et al. 2006). The lake is polluted by industrial waste from agricultural though rainwash, agricultural runoffs and domestic effluents.



Figure 1. Map of Işıklı Lake (Turkey) (Taken from maps.google.com) and different localities from where the samples were taken.

Sampling and sample preparation

This study was carried out at October-2012, January-2013, April-2013 and July-2013 at three sampling stations (Figure 1) from the Işıklı Lake. Fish samples were collected and brought to the laboratory and dissected. For analysis, 2-5 g of the epaxial muscle on the dorsal surface, the entire liver and four gill racers each sample

were dissected, weighed and dried at 70 °C for 24-48 h until they reached a constant weight. All samples were placed in decomposition beakers and 5 ml HNO₃ (%65) added to each, were kept at room temperature for 24 h. Then they were heated at 120 °C on hot plate for 2 h, until the solution evaporate slowly to near dryness. After cooling, added 1 ml H₂SO₄ (%30) and diluted to 25 ml with deionized water, then added 1-2 drop HNO₃.

Analytical procedures

All samples were analyzed for three times for Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se and Zn by using for ICP-AES Vista. Two standard material DORM-3 and DOLT-4 (National Research Council Canada) were analyzed for each ten elements. The absorption wavelength were 228.802 nm for Cd, 267.716 nm for Cr, 324.754 nm for Cu, 259.940 nm for Fe, 257.61 nm for Mn, 202.03 nm for Mo, 352.454 nm for Ni, 220.353 nm for Se, 196.026 nm for Pb and 213.856 nm for Zn, respectively. The analysis limits were 0.4 µg/L for Cd, 0.5 µg/L for Cr, 0.3 µg/L for

Cu, 0.35 µg/L for Fe, 0.05 µg/L for Mn, 0.8 µg/L for Mo, 1.3 µg/L for Ni, 3 µg/L for Pb, 5 µg/L for Se and 0.3 µg/L for Zn.

Statistical procedures

All metal concentrations were determined as on dry weight basis as milligrams per gram for fish tissues. However, we gave the results as milligrams per kilogram for fish tissues. Statistical analysis of data was carried out using SPSS 18 statistical package programs. One-Way ANOVA and Duncan's Multiple Comparison Test were used to compare the data among seasons at the level of 0.05.

RESULTS AND DISCUSSION

The accuracy and precision were checked by analyzing standard reference materials (DORM-3, DOLT-4) under the same conditions (Table 1).

Table 1. Concentrations of metals found in certified reference material DORM-3 and DOLT-4 .

| Metals | DORM 3 Certified | DORM 3 Observed | Recovery (%) | DOLT 4 Certified | DOLT 4 Observed | Recovery (%) |
|--------|------------------|-----------------|--------------|------------------|-----------------|--------------|
| Cd | 0.290±0.020 | 0.24±0.01 | 82 | 24.3±0.8 | 22.45±0.12 | 92 |
| Cr | 1.89±0.17 | 1.72±0.11 | 91 | - | - | - |
| Cu | 15.5±0.63 | 13.21±1.69 | 85 | 31.2±1.1 | 35.12±2.36 | 112 |
| Fe | 347±20 | 400.78±8.25 | 115 | 1833±75 | 1698±22.1 | 92 |
| Mn | - | - | - | - | - | - |
| Mo | - | - | - | - | - | - |
| Ni | 1.28±0.24 | 1.12±0.47 | 87 | 0.97±0.11 | 0.99±0.05 | 102 |
| Pb | 0.395±0.05 | 0.41±0.09 | - | 8.3±1.3 | 7.97±1.12 | 96 |
| Se | - | - | 105 | - | - | - |
| Zn | 51.3±3.1 | 57.14±8.47 | 111 | 116±6 | 125.78±4.54 | 108 |

Replicate analysis of these reference materials showed good accuracy, with recovery rates for metals between 82% and 115% for DORM 3, 92% and 112% for DOLT 4.

Heavy metal levels of muscle, liver and gill of *Esox lucius* and its seasonal variations were given in Table 2. Cr was below detection limit (<0.0005) in liver in spring and summer, in muscle in summer. The distribution patterns of Cd, Cu, Fe, Ni, Pb and Se in tissues of *Esox lucius* follows the order: Liver>gill>muscle, Cr and Mo levels follow the order: Liver >muscle>gill, Mn and Zn levels follow the order: Gill>liver>muscle. In this study, results show that the highest levels of heavy metals were found in the liver and gill, while lowest levels were found in the muscle. These results are agreement with some other studies about heavy metal accumulation in fish (Tekin-Özan 2008; Karadede-Akın 2009; Mohammadi et al. 2011; Başıyigit and Tekin-Özan 2013; Canpolat 2013). Liver is a vital organ in vertebrata and has a major role

in metabolism (Liu et al. 2012). The accumulation of metals in liver could be due to the greater tendency of the elements to react with the oxygen carboxylate, amino group, nitrogen, and/or sulphur of the mercapto group in the metallothionein protein, whose level is highest in the liver (Al-Yousuf et al. 2000). Metal levels in the gills could be due to the element complexing with the mucus, which is impossible to remove completely from between the lamellae before tissue is prepared for analysis (Heath 1987). In addition, muscle tended to accumulate low metals because of inactive tissue accumulating heavy metals (Karadede et al. 2004).

In this study, we also aimed to determining the seasonal variation of heavy metals in liver, gill and muscle. In muscle, maximum metal levels were 0.10 mg/kg for Cd, 0.36 mg/kg for Pb in summer, 1.76 mg/kg for Cr, 0.63 mg/kg for Mo in spring, 2.17 mg/kg for Cu, 18.68 mg/kg for Fe, 5.25 mg/kg for Mn, 1.69 mg/kg for Se, 25.65 mg/kg for Zn in autumn, 1.66 mg/kg for Ni in winter. Minimum

metal levels were 15.21 mg/kg for Fe, 3.72 mg/kg for Mn, 0.81 mg/kg for Se, 0.69 mg/kg for Ni in summer, 0.019 mg/kg for Cd, 1.11 mg/kg for Cu, 15.06 mg/kg for Zn,

0.04 mg/kg for Pb in spring, 1.072 mg/kg for Cr in autumn, 0.18 mg/kg for Mo in winter. Cd, Cr, Fe, Mn, Mo, Ni, Pb and Zn levels were found significant from some seasons to some seasons (<0.05).

Table 2. Heavy metal concentrations (mg kg⁻¹) in different organs of *Esox lucius* from the Işıklı Lake

| Season | Tissue | Cd | Cr | Cu | Fe | Mn | Mo | Ni | Pb | Se | Zn |
|--------|--------|-------------------------|-------------------------|-------------------------|----------------------------|--------------------------|-------------------------|--------------------------|-------------------------|-------------------------|----------------------------|
| Autumn | Muscle | 0.05±0.013 ^b | 1.072±0.91 ^b | 2.17±1.24 ^a | 18.68±5.41 ^b | 5.25±3.43 ^b | 0.22±0.10 ^a | 1.30±0.17 ^a | 0.09±0.08 ^a | 1.69±0.77 ^a | 25.65±11.16 ^b |
| | Liver | 0.20±0.18 ^a | 1.004±0.91 ^a | 11.25±9.26 ^a | 377.48±79.29 ^a | 2.76±1.17 ^a | 1.08±0.73 ^a | 8.50±5.04 ^a | 0.92±0.77 ^a | 15.75±4.23 ^b | 90.83±5.26 ^a |
| | Gill | 0.05±0.01 ^b | 1.124±0.53 ^b | 1.80±0.17 ^a | 45.68±12.75 ^a | 34.76±13.26 ^a | 0.17±0.04 ^a | 0.85±0.44 ^a | 0.12±0.78 ^{ab} | 1.42±0.77 ^{bc} | 198.25±47.66 ^a |
| Winter | Muscle | 0.015±0.01 ^a | 1.74±0.50 ^c | 1.37±0.90 ^a | 15.91±3.94 ^a | 4.65±2.01 ^a | 0.18±0.08 ^a | 1.66±0.99 ^b | 0.14±0.09 ^a | 0.89±0.63 ^a | 20.33±14.27 ^a |
| | Liver | 0.14±0.07 ^a | 21.45±9.97 ^b | 13.38±9.54 ^a | 453.55±288.72 ^a | 8.48±5.87 ^a | 1.79±1.10 ^{ab} | 19.45±12.12 ^b | 1.02±0.67 ^a | 6.49±4.11 ^a | 177.46±117.58 ^b |
| | Gill | 0.13±0.01 ^a | 2.17±0.75 ^c | 2.99±3.10 ^a | 128.15±47.66 ^b | 55.90±24.13 ^b | 0.24±0.05 ^{ab} | 2.81±1.79 ^b | 0.19±0.03 ^a | 0.18±0.20 ^a | 345.46±162.55 ^b |
| Spring | Muscle | 0.019±0.02 ^b | 1.76±0.43 ^c | 1.11±0.93 ^a | 18.21±6.70 ^a | 4.86±1.59 ^a | 0.63±0.32 ^b | 1.55±0.40 ^b | 0.04±0.02 ^a | 0.99±0.46 ^a | 15.06±1.48 ^a |
| | Liver | 0.92±0.16 ^b | BDL** | 15.72±2.31 ^a | 353.18±77.96 ^a | 19.45±2.81 ^b | 2.04±0.37 ^{ab} | 7.99±2.15 ^a | 3.79±1.26 ^b | 4.28±1.20 ^a | 76.45±7.39 ^a |
| | Gill | 0.12±0.02 ^c | 0.17±0.09 ^a | 2.84±0.91 ^a | 72.19±17.34 ^a | 35.05±6.03 ^a | 0.34±0.11 ^{bc} | 1.18±0.48 ^a | 0.39±0.18 ^c | 2.47±1.46 ^b | 332.29±21.34 ^b |
| Summer | Muscle | 0.10±0.03 ^c | BDL | 1.76±0.56 ^a | 15.21±4.97 ^a | 3.72±0.76 ^a | 0.25±0.14 ^a | 0.69±0.17 ^a | 0.36±0.16 ^b | 0.81±0.52 ^a | 15.89±4.41 ^a |
| | Liver | 1.16±0.32 ^c | BDL | 16.96±3.64 ^a | 428.60±146.59 ^a | 25.66±7.84 ^c | 2.71±1.19 ^c | 7.05±1.53 ^a | 4.52±2.48 ^b | 5.62±3.02 ^a | 102.82±17.43 ^a |
| | Gill | 0.13±0.02 ^c | 0.46±0.47 ^a | 2.77±0.69 ^a | 75.49±13.40 ^a | 32.82±6.04 ^a | 0.39±0.15 ^c | 0.70±0.10 ^a | 0.39±0.25 ^{bc} | 3.03±1.37 ^b | 459.92±62.06 ^c |

* Means with the same superscript in the same row are not significant different according to Duncan's multiple range test ($p<0.05$)

** Below Detection Limit

In liver, maximum metal levels were 1.16 mg/kg for Cd, 16.96 mg/kg for Cu, 25.66 mg/kg for Mn, 2.71 mg/kg for Mo, 4.52 mg/kg for Pb in summer, 15.75 mg/kg for Se in autumn, 21.45 mg/kg for Cr, 453.55 mg/kg for Fe, 19.45 mg/kg for Ni, 177.46 mg/kg for Zn in winter. Minimum levels were 7.05 mg/kg for Ni in summer, 1.004 mg/kg for Cr, 11.25 mg/kg for Cu, 2.76 mg/kg for Mn, 1.08 mg/kg for Mo, 0.92 mg/kg for Pb in autumn, 0.14 mg/kg for Cd in winter, 4.28 mg/kg for Se, 76.45 mg/kg for Zn, 353.18 for Fe in spring. Cd, Cr, Fe, Mn, Mo, Ni, Pb, Se and Zn levels were found significant from some seasons to some seasons (<0.05).

In gill, maximum metal levels were 0.13 mg/kg for Cd, 2.17 mg/kg for Cr, 2.99 mg/kg for Cu, 55.90 mg/kg for Mn, 2.81 mg/kg for Ni in winter, 0.39 mg/kg for Pb in spring, 0.13 mg/kg for Cd, 128.15 mg/kg for Fe, 0.39 mg/kg for Mo, 0.39 mg/kg for Pb, 3.03 mg/kg for Se, 459.92 mg/kg for Zn in summer. Minimum levels were 0.05 mg/kg for Cd, 1.80 mg/kg for Cu, 45.68 mg/kg for Fe, 0.17 mg/kg for Mo, 0.12 mg/kg for Pb, 198.25 mg/kg for Zn in autumn, 0.18 mg/kg for Se in winter, 0.17 mg/kg for Cr in spring, 32.82 mg/kg for Mn, 0.70 mg/kg for Ni in summer. Cd, Cr, Fe, Mn, Mo, Ni, Pb, Se and Zn levels were found significant from some seasons to some seasons (<0.05).

In general, the metal levels were highest in autumn in muscle, in summer in liver and in gill. Başyigit and Tekin-Özcan (2013) found the highest levels of some metals in muscle in autumn, in liver in summer, in gill in winter. Iqbal and Shah (2014) reported that the metal levels in *Cyprinus carpio* from Rawal Lake had found during the summer were comparatively higher than the winter. Fidan et al. (2008) reported that Co, Cr, and Fe levels in

liver of *Carassius carassius* in winter, Al, Cd, Li, Ni and Pb levels in summer. Canpolat and Calta (2003) determined the highest heavy metal level in some tissue and organs of *Capoeta capoeta umbra* in spring and summer.

A number of studies have shown that various factors such as ecological needs of fish, metabolism, feeding habit, their life cycle and physico-chemical parameters as salinity, temperature and pH value (Newman and Doubet 1989; Philips 1990) can play a role in the tissues accumulation of metals. The increase of heavy metal levels in summer could be because of the increased physiological activity of fish during summer primarily induced by the increasing water temperature (Canpolat and Çalta 2003).

CONCLUSIONS

Işıklı Lake is one of the most important water sources of the region because of its use for irrigation and having great potential fisheries activity. In this study we also compared our results with permissible levels in fish tissues for heavy metals given by some different institutes. Fish generally accumulate contaminants from aquatic environments, and have been largely used in food safety studies (Başyigit and Tekin-Özcan 2013). Fish muscle is the main edible part of fish and directly influence human health (Coulibaly et al. 2012). Turkish Food Codex established maximum levels for four of the metal studied, above which human consumption is not permitted: 0.2 mg/kg for Pb, 0.05 mg/kg for Cd, 20 mg/kg for Cu and 50 mg/kg for Fe (Republic of Turkey Ministry of Food, Agriculture and Livestock, 2002). The levels of Pb and Cd in muscle were higher than these maximum levels.

The acceptable levels given by World Health Organization reported as Pb: 0.10, Zn: 30, Cr: 1.0, Fe: 2.0 and Mn: 1.0 mg kg⁻¹ (UNESCO, WHO, UNEP, 1992). Pb levels in winter and summer, Cr levels in autumn, winter and spring, Fe and Mn levels in all seasons were higher than these limits given by World Health Organization. In spite of these, the levels of all studied metals except Pb in summer were below the legal values for fish and fishery products proposed by European Commission (EC, 2006). Although levels of heavy metals are not high, this study shows that precautions need to be taken in order to prevent future heavy metal pollution.

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