Bioaccumulation of Some Heavy Metals in the Muscle of *Cyprinus carpio* from Three Locations in Dukan Lake

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**KEY WORDS:**
heavy metals, Muscle, *Cyprinus carpio*, Dukan Lake

**ABSTRACT**

Because of their toxicity, when present in concentrations beyond the allowable limit, heavy metals in our environment have been a source of great concern. These metals are released into the environment in a variety of ways that favor consumption, such as through industrial processes. Fish are the most popular type of seafood. This study's purpose was to determine the concentrations of heavy metals (Cu, Pb, Cd, Ni, and Co) in the muscles of *Cyprinus carpio* from three distinct areas of Dukan Lake (the center of Dukan Lake, Ranya, and Qaladze) of Sulaimani province. The mean values of the elements examined in fish muscles were measured in the descending sequence of Cu > Pb > Cd > Ni > Co. All the discovered heavy metals were within the permitted FAO levels, except for cadmium, whose value (0.136 ppm) was higher than the 0.05 ppm threshold set by the European Union and government (EC, 2006). In conclusion, consuming such fish poses no dangers to humans based on detected amounts of targeted heavy metals except cadmium. The concentration of Co is affected by the location, sex, and season of fish captured, while the concentration of Cu and Ni are affected by fish sex.
التكاثر الحيوي لبعض المعادن الثقيلة في عضلات الكارب الشائع من ثلاثة مواقع لبحيرة دوكان

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2 قسم علوم الحيوان، كلية علوم الهندسة الزراعية، جامعة السليمانية، السليمانية، العراق
3 قسم الأحياء المجهرية، كلية الطب البيطري، جامعة السليمانية، السليمانية، العراق

الخلاصة

بسبب سميتها عند وجودها بتركيزات تتجاوز الحد المسموح به، كانت المعادن الثقيلة في بيئتنا مصدر قلق كبير. يتم إطلاق هذه المعادن في البيئة بعدة طرق، مثل العمليات الصناعية. الأسماك هي أشهر أنواع المأكولات البحرية التي تستهلك من قبل البشر. كان الغرض من هذه الدراسة هو تحديد تراكيز المعادن الثقيلة (النحاس، الرصاص، الكادميوم، النيكل، الكوبالت) في عضلات أسماك الكارب الشائع من ثلاث مناطق من بحيرة دوكان (مركر بحيرة دوكان، رانيا، فلازنز)، محافظة السليمانية. كانت القيم المتوسطة للعناصر التي تم فحصها في عضلات الأسماك في التسلسل التنازلي: النحاس> الرصاص> الكادميوم> النيكل> الكوبالت. كانت جميع المعادن الثقيلة المكتشفة ضمن مستويات منظمة الأغذية والزراعة المسموح بها، باستثناء الكادميوم، الذي كانت قيمته (0.136 جزء في المليون) أعلى من الحد المسموح به (0.05 جزء في المليون) الذي أُقرّ عليه الاتحاد الأوروبي والحكومة (المفوضية الأوروبية، 2006). في الختام، لا يمكن استهلاك مثل هذه الأسماك في التنازل للنحاس والرصاص والكادميوم. يتأثر تركيز الكوبالت بالموقع والجنس، بينما يتأثر تركيز النحاس والنيكل بنجس الأسماك.

الكلمات المفتاحية: المعادن الثقيلة، الكارب الشائع، بحيرة دوكان.

INTRODUCTION

Fish are freshwater and saltwater finfish, crustaceans, mollusks, and other aquatic animals intended for human consumption, according to the United States Food and Drug Administration (FDA). The food industry and international trade both heavily rely on the fishing sector. Overall, fish production in 2016 hit an all-time high of 171 million tons, of which 88 percent was used for direct human consumption, thanks to relatively stable capture fisheries production, reduced wastage, and continued aquaculture growth. A record-breaking per capita consumption of 20.3 kg in 2016 resulted from this production. The sector is contributing to both economic expansion and the battle against poverty. Rising demand and rising prices pushed global fish exports to reach $152 billion in 2017, 54% of which came from developing countries (Food and Agriculture Organization, 2018).

Pollutants and hazardous metals: Since rain mixes up chemicals in the soil and washes pollutants from the environment onto the land, practically any type of chemical produced on Earth ends up in rivers and seas, where fish and shellfish can store these poisons in their bodies and organs (Gautam et al., 2014). The most frequent and potentially deadly contaminants in fish are heavy metals and chemical compounds, particularly dioxins and polychlorinated biphenyls, or PCBs. Heavy metals, such as lead, mercury, cadmium, and copper, bond with oxygen to send messages to the
neurological system, and they are thought to have contributed to brain damage in humans. Chemical contaminants in laboratory animals cause cancer, liver disruption, and hormone disruption and cause the deposition of fat within the body. Salmon and trout in the Great Lakes have quantities of contaminants large enough that the government actively warns against consuming them. The United States Food and Drug Administration advises limiting children's and pregnant women's consumption of swordfish, dolphin, tilefish, and royal mackerel to 12 ounces (335 g) of fish per week because it was found that common ocean fish contained excessive amounts of mercury. Additionally, tuna, which is the second most popular marine product in the US after shrimp, is only occasionally allowed on the list of fish that are recommended for eating, because fish organs are small and short-lived and dwell in the open sea, and on farms with a clean water supply, mercury and other contaminants are less likely to accumulate in fish bodies and organs (Agency for Toxic Substances and Disease Registry, 2004).

The use of agrochemicals that release a variety of hazardous heavy metals into agricultural soils and water bodies, including arsenic, mercury, cadmium, copper, nickel, cobalt, zinc, and copper, is one of the main causes of environmental pollution from toxic metals and minerals. Other causes include industrial development, mining, milling, and the combustion of fossil fuels (Gupta et al., 2019; Kumar et al., 2019). Our hypothesis suggests that human activity and agricultural uses of some materials made of some heavy metals may lead to the accumulation of heavy metals in the environment around Dukan Lake. So, the aims of this study were the quantification of heavy metal accumulation, including Nickel, copper, lead, cadmium, and cobalt, in the muscles of Cyprinus carpio from three locations in Dukan Lake in two seasons.

MATERIALS AND METHODS

The Study Region

*Cyprinus carpio* fish were sampled in the winter and spring from Dukan Lake. Dukan Lake, located in Sulaymaniyah's northwest and about 76 kilometers from the city center, is considered to be the largest lake in the Iraqi Kurdistan region. The lake's boundaries are found between latitudes 34°17’N and 36°33’N and longitudes 43°17’E and 46°24’E, with a full-pool operational altitude of 515 m above mean sea level. The lake has a volume of 6.8 billion cubic meters and a surface area that would be roughly 270 km² at high tide and 48 km² at low tide, respectively. The drainage region has a total area of approximately 11,690 km², of which 1080 km² are within the plains of Qaladze and Ranya. The Dukan dam, constructed in 1959 upstream of the same-named town, creates the lake. Rainfall and snowfall feed the rivers, resulting in peak discharge in the spring and low water in the summer and early falls. A larger lake to the north and a smaller lake to the south are separated by a curving valley, which divides the lake itself into two sections (Toma, 2013).
Fish Samples

Ninety fish samples with a weight between 1020 and 2000 g and a length between 31 and 45 cm were taken from three distinct areas of Dukan Lake. Each of the fishing areas (the center of Dukan Lake, Ranya, and Qaladze) received 30 samples (15 for winter and 15 for spring) (Figure 1). The samples were put in labeled and sterile plastic containers and transported in cool boxes to the college of veterinary medicine at Sulaimani University.

Figure 1: Sampling locations include Ranya, Qaladze, and the center of Dukan Lake.

Samples Processing

Before dissection, all of the dissecting tools, including scalpels, forceps, scissors, knives, a mortar and pestle, and glassware, were sterilized. Dissection was used to get samples of fish muscle in an aseptic manner. The skin was removed from the right side of the fish behind the dorsal fin after removing the scales with a sterilized knife. Muscle (flesh) samples were collected through an incision on the same side and section of the fish body. All samples obtained were prepared for the intended inspections.

Extraction of Heavy Metals

Determination of Ash: Ash was estimated in the samples by taking 10 g of dried samples, and then the crucibles were placed inside a muffle furnace at 600 °C for 18 h until a white or gray powder was obtained (AOAC, 1990).

Heavy Element Estimation: After the samples were burned at 600 °C in a muffle furnace, the heavy metal concentrations in the samples were determined using atomic absorption. Nitric acid (15–20 mL) was added to 1 g of removed ash in a 100 mL beaker to create a 1:1 (v/w) suspension before being covered with a watch glass. In a water bath, the mixture was heated until the ash dissolved and then cooled to room temperature. The samples were then quantitatively transferred to a volumetric flask with a 100 ml capacity, finished with distilled water, and used to create a model that was
prepared for estimation by an atomic absorption spectrometer (Shimadzu AA-6200) in the lab of the Department of Chemical Engineering at Tikrit University. Standard solutions: In a volumetric flask, intermediate standards were diluted with 1% nitric acid and kept in plastic bottles (AOAC, 1990).

Statistical Analyses

The data for this study were subjected to a one-way analysis of variance (ANOVA) using XL Stat for Windows. The variances between the means were calculated using Duncan's multiple range tests. The mean and standard error of the data were provided, and the significance threshold was set at P0.05 (Stell et al., 1980).

RESULTS AND DISCUSSION

Analyzing the bioaccumulation of metals in aquatic species is crucial because the majority of people rely on seafood as a source of nourishment. The mean values of the elements examined in fish muscles were in the descending sequence of Cu > Pb > Cd > Ni > Co, as shown in tables 1, 2, 3, 4, and 5.

According to our findings in Table 1, the highest copper concentrations recorded in male fish muscle from Dukan and Rayna areas in winter and spring were 5.39 and 5.27 ppm respectively, while the lowest concentration was discovered in female fish muscle from Dukan Lake in spring, at 1.09 ppm. Locations and seasons have no significant effect on copper concentration (P > 0.05), and the descending order for concentration by sex and sex effect on copper concentration was Male > Female, with 3.17 and 2.04 ppm, respectively.

Because it is a vital component of metabolic enzymes, copper serves as a necessary trace metal and micronutrient for cellular metabolism in living organisms (Monteiro et al., 2009). It is a plentiful element that naturally occurs as a mineral and has a large number of applications (Sfakianakis et al., 2015). The primary sources of copper emissions into the environment include copper mining, smelting, and refining; industries that use copper to make products like wire, pipes, and sheet metal; and the burning of fossil fuels (Mahurpawar, 2015).

The range of copper concentration was 5.39-1.09 ppm, and all locations, tissues, and sexes were within the FAO's 30 mg/kg fresh weight limit for copper concentration (Agency for Toxic Substances and Disease Registry, 2004), and Jaber et al. (2021) found the Cu concentration in Cyprinus carpio from the Euphrates and Tigris rivers were 425.05 ppm and 192.25 ppm, respectively. The differences between these two studies may be caused by differences in the aquatic environment (Tigris and Euphrates), season, fish species, age, physiological status, fish weight, and feeding habits of the fish (Khidhir, 2022).
Table 1: Bioaccumulation of Copper (ppm) from Cyprinus carpio tissues in three different locations in Dukan Lake (Mean± Standard error)

<table>
<thead>
<tr>
<th>Location</th>
<th>Season</th>
<th>Male</th>
<th>Female</th>
<th>The average effect of location</th>
<th>The average effect of the season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dukan</td>
<td>Winter</td>
<td>5.39 ± 1.30 a</td>
<td>1.92±0.64 c</td>
<td>2.70± 0.49 a</td>
<td>Winter</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>2.41 ±0.72 bc</td>
<td>1.09±0.16 c</td>
<td></td>
<td>2.18± 0.33 a</td>
</tr>
<tr>
<td>Ranya</td>
<td>Winter</td>
<td>0.98± 0.12 c</td>
<td>1.35± 0.08 c</td>
<td>3.10± 0.62 a</td>
<td>Spring</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>5.27± 0.82 a</td>
<td>4.79± 0.195ab</td>
<td></td>
<td>3.03± 0.44 a</td>
</tr>
<tr>
<td>Qaladaza</td>
<td>Winter</td>
<td>2.62± 0.62bc</td>
<td>1.66± 0.33 c</td>
<td>2.01± 0.24 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>2.33± 0.61 bc</td>
<td>1.44± 0.22 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The average effect of sex</td>
<td>3.17± 0.39a</td>
<td>2.04± 0.38b</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The averages of the cells with the same (identical letters) do not differ statistically (P 0.05).

As shown in Table 2, the highest concentration of cobalt recorded in male fish muscle from Dukan in winter was 0.130 ppm, while the lowest concentration was found in female fish meat from Ranya in spring, which were 0.10 ppm. Cobalt concentrations were significantly (P ≤ 0.05) affected by fishing location, with the highest concentration of cobalt measured in Qaladaza fish muscle (0.075 ppm), whereas the lowest measured concentration in Ranya fish muscle was 0.033 ppm. Also, the season effect was significantly (P≤0.05) on the concentration of cobalt; the muscles of fish collected in the winter contained the highest concentration of cobalt, which was 0.073 ppm, in comparison to the lowest cobalt concentration recorded in the muscles of fish collected in the spring, which was 0.042 ppm. The sex of fish effects significantly (P≤0.05) cobalt concentration, with the highest concentration found in male muscle, (0.077 ppm), and the lowest concentration recorded in female muscle (0.038 ppm).

Cobalt and its compounds are ubiquitous in nature and are part of numerous human activities. Cobalt is found in high concentrations in soil and water as it is used in the production of artificial fertilizers. Additionally, it is used in medicine to treat anemia that cannot be cured with iron (Leyssens et al., 2017). Cobalt salts can induce benign dermatitis, and long-term inhalation of cobalt dust can irritate the respiratory tract and lead to chronic bronchitis (Packer, 2016).

Our findings support those of another study by Jia et al. (2018), which found that the four fish species (Carassius auratus, Squaliobarbus curriculus, Pelteobagrus fulvidraco, and Silurus asotus) collected in the Xiang River, a river in southern China that has been impacted by mines, were within
the range of cobalt (0.008–0.088 ppm). It also agrees with the findings of the Anandkumar et al. (2018) study, which found cobalt concentrations in tissues of two pelagic and five demersal fish species collected from the Miri coast in Sarawak with a range of 0.10 to 9.20 ppm. Even though cobalt plays a biologically important role as a metal component of vitamin B12, the cobalt concentration in the studied fish's muscles can be regarded as a good source of the mineral for humans. However, excessive exposure to cobalt has been shown to cause several harmful health effects.

Table 2: Bioaccumulation of Cobalt (ppm) from Cyprinus carpio tissues in three different Dukan Lake locations (Mean± Standard error)

<table>
<thead>
<tr>
<th>Location</th>
<th>Season</th>
<th>Male</th>
<th>Female</th>
<th>The average effect of location</th>
<th>The average effect of the season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dukan</td>
<td>Winter</td>
<td>0.130±0.057 a</td>
<td>0.041±0.021 b</td>
<td>0.065±0.019 ab</td>
<td>Winter 0.073±0.013 a</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>0.062 ±0.039 ab</td>
<td>0.026±0.016 b</td>
<td>0.033±0.009 b</td>
<td>Spring 0.042±0.009 b</td>
</tr>
<tr>
<td>Ranya</td>
<td>Winter</td>
<td>0.087 ±0.027 ab</td>
<td>0.026±0.00 b</td>
<td>0.075±0.011 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>0.010±0.000 b</td>
<td>0.026±0.010 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qaladaza</td>
<td>Winter</td>
<td>0.092±0.025 ab</td>
<td>0.080±0.014 ab</td>
<td>0.077±0.014 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>0.083±0.029 a</td>
<td>0.046±0.017 b</td>
<td>0.038±0.007 b</td>
<td></td>
</tr>
</tbody>
</table>

The averages of the cells with the same (identical letters) do not differ statistically (P<0.05).

According to the finding in table 3, the highest cadmium concentration recorded in female fish muscle from the Qaladaza area in spring was 0.136 ppm, while the lowest concentration found in female fish muscle from the Qaladaza area in winter was 0.010 ppm. Locations, sex, and season don’t significantly affect (P > 0.05) the concentration of cadmium. The level of cadmium measured above the 0.05 ppm standard established by the European Union and government (EC, 2006), and this might be related to an increase in industrial activity last year that led to increased Cd contamination of Iraqi water (Al-Hussaini et al., 2018). Exposure to Cd has several detrimental impacts on both human and animal health, with particular attention paid to the kidney, liver, and vascular systems. Low Cd concentrations are associated with bone effects, such as an increased risk of fractures and osteoporosis, and other consequences related to Cd exposure, including cancer, can also be taken into consideration (Åkesson et al., 2014). The results of our study are in agreement with those of Khidhir (2022) who used samples taken from the Tigris, Al Uzym, and Tharthar rivers and lakes to evaluate the concentration of cadmium (Cd) in the muscles of the fish Kattan (Luciobarbus xanhopterus) and Xashni (Planiliza abu). The range of cadmium was 0.054–0.074 ppm. Khidhir (2011) tested the wild fish in Sulaimani city markets for the presence of cadmium and
discovered that all five types had levels that ranged from 0.451 to 0.475 ppm. This indicates that the natural water sources where the fish were harvested were tainted with heavy metals.

**Table 3:** Bioaccumulation of Cadmium (ppm) from *Cyprinus carpio* tissues in three different locations in Dukan Lake (Mean± Standard error)

<table>
<thead>
<tr>
<th>Location</th>
<th>Season</th>
<th>Male</th>
<th>Female</th>
<th>The average effect of location</th>
<th>The average effect of the season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dukan</td>
<td>Winter</td>
<td>0.094±0.020 abc</td>
<td>0.029±0.013 c</td>
<td>0.062±0.008 a</td>
<td>Winter</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>0.062 ±0.013 abc</td>
<td>0.061 ±0.007 abc</td>
<td></td>
<td>0.063±0.013 a</td>
</tr>
<tr>
<td>Ranya</td>
<td>Winter</td>
<td>0.057± 0.002 abc</td>
<td>0.063± 0.006 abc</td>
<td>0.058±0.006 a</td>
<td>Spring</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>0.033± 0.049 c</td>
<td>0.078±0.014 abc</td>
<td></td>
<td>0.069±0.010 a</td>
</tr>
<tr>
<td>Qaladaza</td>
<td>Winter</td>
<td>0.125 ±0.071 ab</td>
<td>0.010 ±0.00 c</td>
<td>0.078±0.023 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>0.042 ±0.007 bc</td>
<td>0.136± 0.015 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The average effect of sex</td>
<td>0.069±0.013 a</td>
<td>0.063±0.010 a</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The averages of the cells with the same (identical letters) do not differ statistically (P > 0.05).

According to the results in Table 4, the highest concentration of nickel was detected in male fish muscles from Rayna areas during the winter (0.138 ppm), while the lowest concentration was detected in female fish muscles from Qaladaza locations during the same time period (0.018 ppm). According to sex concentration, location and season have no significant effect on nickel concentration (P > 0.05). The effect of sex on nickel concentration was a decrease in order of male > female, 0.084 ppm and 0.051 ppm, respectively. All results are lower than the Maximum Permissible Limit of Ni, which was 0.5–1 ppm (WHO, 1989). Various health effects, including nickel allergy, contact dermatitis, and organ system toxicity, can result from nickel exposure. The Institute of Medicine claims that nickel is carcinogenic and can lead to respiratory issues (Belsito et al., 2008). Numerous studies have shown that nickel compounds have the potential to cause cancer in experimental animals (Palaniappan and Karthikeyan, 2009).

Our results are higher than the results found by Baharom and Ishak (2015), who discovered that in all samples, Ni values between 0.06 and 0.07 ppm were found in the tissue of six freshwater fish species (*Hampala microlepidota*, *Barbonymus schwansenfeldii*, *Mystacoleucus marginatus*, *Hemibagrus nemurus*, *Cyclocheilicthys apogon*, and *Oreochromis niloticus*) from Galas River. Despite the fact that our findings were lower than those of Ahmed (2020), who studied the accumulation of heavy metals Ni in the muscles of *Cyprinus carpio* (*Cyprinus carpio*) with the mean concentration (Ni 2.75 ppm), the research indicates that the tested fish muscle was not safe for human
consumption and that heavy metals could contaminate groundwater in the Khor al-Zubair region of Basrah province, Iraq.

Table 4: Bioaccumulation of Nickel (ppm) from Cyprinus carpio tissues in three different locations in Dukan Lake (Mean± Standard error)

<table>
<thead>
<tr>
<th>Location</th>
<th>Season</th>
<th>Male</th>
<th>Female</th>
<th>The average effect of location</th>
<th>The average effect of the season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dukan</td>
<td>Winter</td>
<td>0.123±0.022 ab</td>
<td>0.033±0.019 de</td>
<td>0.080±0.011 a</td>
<td>Winter</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>0.079±0.017 bc</td>
<td>0.087±0.021 bc</td>
<td></td>
<td>0.065±0.010 a</td>
</tr>
<tr>
<td>Ranya</td>
<td>Winter</td>
<td>0.138±0.004 a</td>
<td>0.026±0.016 e</td>
<td>0.061±0.011 a</td>
<td>Spring</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>0.052±0.020 cde</td>
<td>0.029±0.007 de</td>
<td></td>
<td>0.070±0.007 a</td>
</tr>
<tr>
<td>Qaladaza</td>
<td>Winter</td>
<td>0.055±0.021 cde</td>
<td>0.018±0.004 e</td>
<td>0.061±0.009 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>0.058±0.015 cde</td>
<td>0.113±0.010 ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The average effect of sex</td>
<td>0.084±0.009 a</td>
<td>0.051±0.008 b</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The averages of the cells with the same color (identical letters) do not differ statistically (P>0.05).

According to the results in Table 5, the highest lead concentration recorded in female fish muscle from the Qaladaza area in the spring was 0.153 ppm, while the lowest concentration found in female fish muscle from the Qaladaza area in the winter was 0.054 ppm. Locations, Sex, and season don’t affect significantly (P>0.05) on Concentration of Lead. And this is lower than the lead level range revealed in Khidhir (2011) data, which showed that five fresh species from the province of Sulaimani had Pb concentrations ranging from 0.306 to 0.364 ppm. The lead concentration measurements in our study were lower than the lead's maximum legal limit, as stated by the EC (2014) and FSSAI (2015). Although the level of lead in our study is below the maximum legal limit, lead is a persistent heavy metal that has been classified as a priority hazardous chemical, so long-term ingestion of this fish may result in health issues (Sfakianakis et al., 2015). Pb is a naturally occurring substance, but anthropogenic sources, such as base metal mining, battery manufacturing, Pb-based paints, and leaded gasoline, considerably raise its environmental concentrations (Monteiro et al., 2011).

Table 5: Bioaccumulation of Lead (ppm) in three different locations in Dukan Lake from the tissues of Cyprinus carpio (Mean± Standard error)
CONCLUSION

Consuming such fish poses no possible dangers to humans based on detected amounts of targeted heavy metals except for cadmium, whose value (0.136 ppm) was higher than the 0.05 ppm threshold set by the European Union and government. The concentration of Co is affected by the location, sex, and season of fish captured, while the concentration of Cu and Ni are affected only by fish sex.

REFERENCES


