DETERMINATION OF Cd Cu, Fe, Mn AND Pb IN EUROPEAN CARP (Cyprinus carpio carpio)

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ABSTRACT

The concentrations of five elements (Cd, Cu, Fe, Mn, and Pb) were determined in the muscle, liver, gills, bones and skin of cultured carp (Cyprinus carpio carpio) caught at Tzonevo Dam in region Varna, Bulgaria. The samples analyzed for Cd, Cu, Fe, Mn and Pb by Atomic Absorption Spectrometer. The highest levels of Pb, Mn and Cd were found in the skin of the fish specie (5.26 mg/kg w.w., 8.74 mg/kg w.w. and 0.34 mg/kg w.w., respectively) while Cu and Fe had been accumulated predominantly in the gills (2.68 mg/kg w.w. and 55.54 mg/kg w.w. respectively). Among the metals analyzed Fe was the most abundant in the different tissues, while Cd and Cu where the least abundant. The results obtained in this study were compared with those reported in other studies. The concentration of these five elements in the European carp samples found in the literature showed a similar tendency except for Pb. Metal concentration in the edible part of the examined fish (muscle) was in the safety permissible levels for human consumption set by various health organizations.

Key words: Heavy metals, cultured carp, liver, gills, AAS, Bulgaria

INTRODUCTION

Fish is the final chain of aquatic food web and an important food source for humans. Water pollution leads to contamination of fish species with toxic metals, from many sources, e.g. industrial and domestic waste water, natural runoff and contributory rivers (16,14). In the water basin, pollutants are potentially accumulated in organisms and sediments, and subsequently transferred to man through the food chain (17). For this reason, determination of chemical quality of aquatic organisms, particularly the contents of heavy metals in fish is extremely important for human health (5). A well known fact is that fish muscle is not an active tissue in accumulation of heavy metals (18). On the contrary liver is a good monitor of water pollution with metals since their concentrations are proportional to those present in environment. Dam lake ecosystems are vulnerable to heavy metal pollution. Tzonevo Dam is situated in the Valley of Luda Karmenia River and ranks third in size in region Varna, Bulgaria. It is biodiversity is of importance for the local residents. Recently, agricultural and industrial developments as well as increase in population have substantially increased the contamination of Tzonevo dam.

The aim of this study is to investigate the distribution of selected metals (Cd, Cu, Fe, Mn and Pb) in different tissues (muscle, skin, gills, liver and bones) of the cultured carp (Cyprinus carpio carpio) collected from Tzonevo Dam by using atomic absorption spectrophotometer (AAS).

MATERIAL AND METHODS

Biology and ecology of the fish sample

Cultured carp dwells in middle and lower reaches of rivers and lakes and shallow confined waters and can survive cold winter periods. Carp are omnivorous, with a high tendency towards the consumption of benthic organisms. Zooplankton consumption is dominant in fish ponds where the stocking density is high. Additionally, the carp consumes the stalks, leaves and seeds of aquatic and terrestrial plants, decayed aquatic plants, etc.

Sampling collection

Samples of the fishes were acquired from three locations along Tzonevo Dam. All the fish species were sampled in July 2009. Total length and weight of the samples (total number 9) brought to laboratory on ice after collections were measured to the nearest millimeter and gram before dissection. The biometric data of the fish sample are as follows (mean ±SD): weight 1662.0 ±43.0 g; length 45.5 ±8.0 cm. Special care was taken to prevent metal contamination of the samples by the laboratory equipment. After biometric measurements, the fishes were immediately dissected, washed
with distilled water, weighted, packed in polyethylene bags and stored at frozen at -18 °C until chemical analysis. Approximately 1 g sample of muscle, gills, skin and bones and entire liver were dissected, washed with distilled water, weight, packed in polyethylene bags and stored at -18 °C until chemical analysis.

**Analytical procedure**

All reagents were of analytical reagent grade unless otherwise stated. Double deionised water (Milli-Q Millipore 18.2 MΩ cm⁻¹ resistivity) was used for all dilutions. HNO₃ was of suprapur quality (Merck, Darmstadt, Germany). The elemental standard solutions used for calibration were produced by diluting a stock solution of 1000 mg/l of the given element supplied by Sigma Chem., Co., USA. All tissues samples were transferred to a 100 mL Teflon beaker. Thereafter, 10 mL ultrapure concentrated nitric acid was added slowly to the sample. The Teflon beaker was covered with a watch glass, and heated at 200 °C on a hot plate for 3 h, until the solution evaporate slowly to near dryness. Two milliliters of 1 N HNO₃ was added to the residue and the solution was evaporated again on the hot plate. By repeating the additional digestion twice, all organic materials in each sample were completely digested. After cooling, 2.5 mL of 1 N HNO₃ was added to digested residue and was transferred to 25 mL volumetric flasks, then diluted to level with deionized water. Before analysis, the samples were filtered through a 0.45 μm nitrocellulose membrane filter (1). All samples were analyzed three times for Cd, Cu, Fe, Mn and Pb by Atomic Absorption Spectrometer (Varian Model Spectrometer AA-240). Deuterium background corrector was used. Copper, manganese, cadmium, iron and lead were determined in air-acetylene flame. The operating parameters for working elements were set as recommended by manufacture given in Table 1.

**Table 1. Instrumental analytical conditions of investigated elements**

<table>
<thead>
<tr>
<th>Working conditions</th>
<th>Mn</th>
<th>Cu</th>
<th>Cd</th>
<th>Fe</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>279.5</td>
<td>324.8</td>
<td>228.8</td>
<td>248.3</td>
<td>283.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>217.0</td>
</tr>
<tr>
<td>Slit width (nm)</td>
<td>0.2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Lamp current (mA)</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Ar flow (ml/min)</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>Acetylene/air ratio</td>
<td>3.5/1.5</td>
<td>3.5/1.5</td>
<td>3.5/1.5</td>
<td>3.5/1.5</td>
<td>3.5/1.5</td>
</tr>
</tbody>
</table>

The analytical measurements were based on peak height. Standard solutions were prepared from stock solutions (Merck, multi element standard).

**RESULTS AND DISCUSSION**

The results of the analyses for trace metals in European carp in muscle, skin, gills, liver and bond tissues are shown in Table 2 expresses as mg/kg wet weight (mg/kg w.w.).

**Table 2. The mean heavy metal concentration (mg/kg wet weight ±SD) in the different tissues of cultured carp (Cyprinus carpio)**

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cu</th>
<th>Pb</th>
<th>Mn</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>0.09±0.01</td>
<td>1.03±0.1</td>
<td>0.60±0.06</td>
<td>0.52±0.05</td>
<td>16.41±1.6</td>
</tr>
<tr>
<td>Skin</td>
<td>0.34±0.03</td>
<td>1.15±0.11</td>
<td>5.2±0.53</td>
<td>8.74±0.8</td>
<td>10.63±1.0</td>
</tr>
<tr>
<td>Gills</td>
<td>0.25±0.02</td>
<td>2.68±0.25</td>
<td>2.53±0.25</td>
<td>4.83±0.5</td>
<td>55.54±5.5</td>
</tr>
<tr>
<td>Liver</td>
<td>&lt;0.01</td>
<td>0.74±0.07</td>
<td>0.45±0.05</td>
<td>0.05±0.01</td>
<td>17.18±1.7</td>
</tr>
<tr>
<td>Bones</td>
<td>0.19±0.02</td>
<td>0.72±0.07</td>
<td>4.1±0.41</td>
<td>2.67±0.3</td>
<td>9.02±0.90</td>
</tr>
</tbody>
</table>

Cadmium is accumulated in human tissues. It is known that most human exposure to Cd is from food and a serious contaminant, a highly toxic element, which is transported in the air. The lowest and highest cadmium levels in studied sample were 0.09 mg/kg w.w. for muscle tissues and 0.34 mg/kg w.w. for skin, respectively. The concentration for Cd in liver was below the limit of detection for this element. Unfortunately, maximum permitted level of Cd is set only for seafood (especially for muscle tissues) as 0.05 mg/kg fresh wet weight according to European Union (6) and Bulgarian Food Authorities (3). The World Health Organization (WHO) sets permissibly tolerable weekly intake (PTWI) for Cd as 0.007 mg/kg body weight (7). Altındağ and Yiğit (2005) reported Cd values of 0.543 μg/g for muscle tissues and 0.658 for gilt of Cyprinus carpio from Lake Beyşehir, Turkey (2). Cd values varies from 0.23 μg/g to 0.54 μg/g in muscle of Cyprinus carpio from Yesliılmak.
River in Tokat, Turkey (10); from 0.03 μg metal/g dry weight to 0.11 μg metal/g dry weight for muscle tissues and 0.02 μg metal/g dry weight to 0.35 μg metal/g dry weight for liver tissues of three economically important fish species captured from the Tuzla lagoon (5). Our values are within the data stated in the literature.

As copper is essential part of several enzymes and for the synthesis of hemoglobin, most organisms have evolved mechanisms to regulate concentrations of this metal in their tissues in the presence of variable concentrations in the ambient water, sediments and food (19). The minimum and maximum copper concentration in this study were found in bones (0.72 mg/kg w.w.) and gills (2.68 mg/kg w.w.), respectively. There are no guidelines on acceptable levels of Cu in the edible parts (muscle) of fish suggested by FAO/WHO (7). According to literature, our data are in the limits reported by various authors - 1.1 ± 1.7 mg/g for muscle tissues in Cyprinus carpio from Yeşılırmak River in Tokat, Turkey (10); from 3.08 μg/g dry weight (for muscle), 10.03 μg/g dry weight (for gonads) and 15.13 μg/g dry weight (for liver) for Cyprinus carpio from Lake Parnotis, Greece (12); 1.2-1.4 μg/g for Cyprinus carpio from Bedrikale Lake, Turkey and 1.3-2.8 μg/g for the same fish species from Avara Lake, Tokat, Turkey (9).

Manganese is essential trace element and its metabolic role includes manganese containing enzyme systems. A deficiency of that essential element can lead to skeletal and reproductive abnormalities in mammals (15). Our results show maximum and minimum level of Mn in skin (8.74 mg/kg w.w.) and liver (0.05 mg/kg w.w.), respectively. Manganese has been reported in the range of 9.6-64.3 μg/g in muscle tissues of Cyprinus carpio in Lake Tokat, Turkey (9); 1.04 to 8.77 μg/g w.w. in muscle tissues of different fish species from Iskenderun Bay, Turkey (19); and 0.3599 mg/kg for liver, 1.5256 mg/kg for intestine, 0.1766 for muscle of cultured carp from Lake Kasumigaura (1). Totally daily intake varies from 2.5 to 7 mg for humans (11). Manganese contents in the analyzed samples (especially the one for muscle tissues) are smaller than reported by literature.

Average iron concentration of the fish sample varied from 9.02 mg/kg w.w. for bones to 55.54 mg/kg w.w. for gills. These values are in the range measured in some edible fish species by other authors. Mendil & Ülüler (2007) (9) obtained Fe concentrations between 88.8-158 μg/g in muscle

![Fig. 2. The mean heavy metal concentration in skin of cultured carp (Cyprinus carpio)](image)

![Fig. 3. The mean heavy metal concentration in gills of cultured carp (Cyprinus carpio)](image)
tissues of *Cyprinus carpio* in six different lakes in Turkey; between 14.4 and 37.2 µg/g in muscle tissues of cultured carp in Yeşilırmak River in Tokat, Turkey (19) and from 4.12 mg/kg (muscle) up to 179 mg/kg (liver) for cultured Lake Kasumigaura carp (1). This discrepancy might be because iron content depends on species, individuals, and sampling period.

CONCLUSIONS

Iron present the highest concentrations followed by Mn, Pb, Cu and Cd. The highest concentrations of the analyzed elements were found in skin, gills and bones while the lowest concentrations of detected metals were in muscle and liver. The last statement is a controversial to all the data stated in the literature. Many studies showed that heavy metals accumulate mainly in metabolic organs such as liver that stores metals to detoxicate by producing metallothioneins.

In the present study, the concentration of Pb in all tissues of carp collected from Tzonevo dam were higher than those stated by various health organizations the data reported in the literature. Thus they may affect or alter the ecosystem structure and food webs of the lake. In Tzonevo Dam, the concentrations of heavy metals in fish where high, possibly due to contamination of the water by metals or the geochemical structure of the region.

ACKNOWLEDGMENTS

The authors would like to thank the National Science Fund, Ministry of Education and Science of Bulgaria for their financial support (Project DVU 440 / 2008).

REFERENCES


