ECOLOGIA BALKANICA

2014, Vol. 5, Special Edition April 2014 pp. 15-20

Determination of Heavy Metals in Roach (Rutilus rutilus) and Bleak (Alburnus alburnus) in Zhrebchevo Dam Lake

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Abstract. The aim of this study was to examine the concentration of iron (Fe), nickel (Ni), lead (Pb), manganese (Mn), copper (Cu), chromium (Cr), cadmium (Cd) and zinc (Zn) in roach (*Rutilus rutilus*) and bleak (*Alburnus alburnus*) as species for human consummation. Two fresh water fish species, roach and bleak were caught from Zhrebchevo Dam Lake in Bulgaria. Determination of heavy metals (Fe, Cu, Ni, Pb, Zn, Mn, Cr and Cd) in muscle samples were performed with electro thermal atomic absorption spectrometry (ETAAS). The heavy metal content in the meat of roach and bleak were found to be 0.59±0.032 - 0.69±0.128 mg kg^{-1} for Cu, 6.59±0.224 - 7.34±0.142 mg kg^{-1} for Fe, 0.03±0.025 - 0.04±0.012 mg kg^{-1} for Ni, 0.06±0.044 - 0.07±0.031 mg kg^{-1} for Pb, 4.05±0.263 - 5.46±0.388 mg kg^{-1} for Zn, 0.49±0.060 - 0.72±0.080 mg kg^{-1} for Mn, 0.09±0.036 - 0.1±0.045 mg kg^{-1} for Cr, 0.01±0.002 - 0.01±0.003 mg kg^{-1} for Cd. The data show that the differences between the content of Fe, Cu, Mn, Zn, Cr and Ni in roach and bleak are significant, while these ones of Pb and Cd are not significant. The significant differences in the content of heavy metals in muscles of roach and bleak are as a result of multiple factors, including season, food, chemical properties of water or sediment**.** The data established during the investigation show that the edible part of fish do not carry heavy metals loads and concentrations are below the legal value for fish and fish products established by the Food and Agriculture Organization and national legislation. This paper is helpful to consumers and academics concerning the mineral of body composition of roach (*Rutilus rutilus*), and bleak (*Alburnus alburnus*).

Key words: Fish, roach, bleak, *Rutilus rutilus*, *Alburnus alburnus*, heavy metals, ETAAS.

Introduction

The roach widely distributed in Europe fresh water is species mostly caught together with bleak (OKGERMAN *et al*., 2009). The fish inhabit nearly every type of water bodies, from large lakes, coastal brackish lagoons and rivers, to small oxbow lakes and clay-pits (RACZYŃSKI *et al*., 2008). The roach and bleak represent one of the most commonly found and most widely distributed fishes in Bulgaria – river Danube and many dam lakes as Dospat, Batak, Koprinka, Zhrebchevo wetlands along the Bulgarian Black Sea coast (Durankulak and Shabla Lake). The species also represent and important component of recreational catches, carried out by amateur anglers (BRYLIŃSKA, 2000; PETROVA & STOYKOV, 2002; 2009).

The content of minerals in fish tissue has an important role in human metabolism (ÖZDEN, 2010). The levels of contaminants,

especially toxic trace minerals in fish tissue are of particular interest because of potential risk to human. These minerals can be classified as potentially toxic (aluminum, arsenic, cadmium, lead, mercury etc.), probably essential (nickel, vanadium, cobalt) and essential (cooper, zinc, selenium) (CANLI & ATLI, 2003). The effects of toxic metals, presented in aquatic food products upon human health and environment are of great interest today (ÖZDEN *et al.*, 2013; YORDANOVA *et al.*, 2008, STANCHEVA *et al.*, 2013a, b). The accurate determination of mineral elements in fish meat is therefore important in nutrition studies, particularly because meat, as a biological material, exhibits natural variations in the amounts of nutrients contained (GREENFIELD & SOUTHGATE, 2003).

Overall, the most widely used trace elements determination techniques in fish meat are UV-Vis spectrophotometry (BLAND *et al.*, 1999), ETAAS (ANGELOVA *et al.*, 2006; DOSPATLIEV *et al.*, 2008), X-ray fluorescence, ICP-OES (DOSPATLIEV *et al.*, 2010; DOSPATLIEV *et al.*, 2011) and ICP-MS (FORRER *et al.*, 2001; MATSUURA *et al.*, 2001).

The aim of this study was to examine the concentration of iron (Fe), nickel (Ni), lead (Pb), manganese (Mn), copper (Cu), chromium (Cr), cadmium (Cd) and zinc (Zn) in roach (*Rutilus rutilus*) and bleak (*Alburnus alburnus*) as species for human consummation.

Materials and Methods

Study area. The Zhrebchevo Dam Lake is located in Southeastern Bulgaria, geographic coordinates (42° 36′ 56″ N, 25° 51′ 33″). Surface area 25 km², maximum depth is 50.50 m. The dam water is used as a source of irrigation water and as a recreational area. There are more than 15 freshwater fish species, including roach and bleak.

Sample preparation. The study was carried out in February-March 2013. Roach and bleak were caught by recreational anglers' using fishing rod. A freshly caught fish (27 fish per group) should be anaesthetized (el stunning) and stored on ice in an insulated box at a temperature 0-4° C and transported to the Central laboratory of Trakia University, Stara Zagora on the next day. The mean weight and length of the roach were 127.15±43.73 g and 206.90±26.20 mm, of bleak were 32.63±4.73 g and 156.60±7.00 mm respectively. The meat samples (35 g), without skin from all fish specimens were taken and examined. They were prepared for the experiment after dried at 105° C in a fan oven and stored in dark plastic bottles.

Reagents. Reagents were qualified as pure (Merck® and Fluka®). The standard solutions for ETAAS determination of Cu, Fe, Ni, Pb, Zn, Mn, Cr and Cd, with concentration of 1000 mg.l^{-1} were supplied by Merck (Darmstadt, Germany). Double-distilled water was used for all procedures. To determine the pH of H_2O was used pH-meter (Consort C932, Belgium).

Mineralization of samples. For the determination of Cu, Fe, Ni, Pb, Zn, Mn, Cr and Cd, 3.0 g (weighted to the nearest 0.01 g) of each air-dried muscle sample was put in a round-bottomed 100 ml flask 22.5 ml of HCI and 7.5 ml of HNO_3 were added. After that the flask was connected to a reflux condenser and let it stand for no less than 16 hours at room temperature, then heated gently to boiling for 2 hours. After cooling and flushing the condenser with 25 ml of 12.5 % nitric acid, the sample was filtered and 100 ml of 12.5 % nitric acid was added to the part of it in liquid phase. For analyzed of the muscle samples were used Perkin-Elmer AAnalyst 800 atomic absorption spectrometer (Norwalk, CT).

Statistical analysis. Statistical analyses were performed using STATISTICA 6 (StatSoft Inc., 2002). The accuracy of the measurements was assessed by standard deviation (SD) for n=10.

Results and Discussion

The iron content of these two species was very low compared to that of mammals (WATANABE *et al.*, 1997) and another fish like salmon (ATANASOFF *et al.*, 2013). Mean concentrations of iron in the samples of roach were 6.59±0.224 mg kg^{-1} and bleak 7.34±0.142 mg kg^{-1} respectively (Table 1). A

joint effort of the FAO/WHO (1999) has set a limit for iron intake based on human body weight. For an adult weighting (60 kg), the provisional tolerable daily intake for iron is 48 mg (MITEV *et al.*, 2013). The statistical analysis of the iron data showed that significant differences ($p \leq 0.001$) were found between two studied fish species.

Copper is essential trace element for fish metabolism and important micronutrients in the human diet (AMES, 1998). This element is a cofactor in a wide range of enzymes, including cytochrome oxidase, superoxide dismutase and lysyl oxidase (WATANABE *et al.*, 1997). Copper is essential for good health, but very high intake can cause adverse health problems, such as liver and kidney damage (IKEM & EGEIBOR, 2005; VELCHEVA *et al.*, 2006). Muscle copper levels in roach were 0.69±0.128 mg kg^{-1} and bleak 0.59±0.032 mg kg^{-1} respectively. The statistical analysis of the cooper data showed that significant differences ($p \leq 0.001$) were found between two fish species (Table 1). The established maximum level for copper in fish tissue according to Bulgarian legislation, above which fish consumption is not permitted, is 10 mg kg^{-1} (REGLAMENT 31/2004).

Zinc is another important essential element and it presents active site of many enzymes of fish. Its deficiency can lead to loss appetite, growth retardation and skin changes in marine species (ÖZDEN, 2013). The muscle concentration of zinc in roach was found to be 5.46±0.388 mg kg^{-1} and in bleak 4.05±0.263 mg kg^{-1} respectively (Table 1). The FAO (1983) set a limit daily human intake for Zn 30 mg kg^{-1}. The established maximum level for zinc in Bulgarian legislation above which fish consumption is not permitted is 50 mg kg^{-1} (REGLAMENT 31/2004). The statistical analysis of the zinc data showed that significant differences ($p \leq 0.001$) were found between two species.

Lead exists in water mostly in particulate form. The WHO suggests a maximum tolerable weekly intake 25 µg kg^{-1} body weight for adult per week for lead (MITEV *et al.*, 2013). Lead content of catching fish were determined in roach as 0.07±0.031 mg kg^{-1} and bleak 0.06±0.044 mg kg^{-1} (Table 1). The established maximum level for lead in Bulgaria legislation, above which fish consumption is not permitted, is 0.20 mg kg^{-1} (REGLAMENT 31/2004).

Cadmium is a non-essential element in food and in natural waters it can cause kidney and liver damages at any concentration. The EU has set the upper limit of cadmium concentration in fish for human consummation at 1.0 mg kg^{-1} (ÖZDEN *et al.*, 2009). In our study were determined in roach 0.01±0.002 mg kg^{-1} and in bleak 0.01±0.003 mg kg^{-1} (Table 1). The established maximum level for cadmium, according Bulgaria legislation above which fish consumption is not permitted, is 0.05 mg kg^{-1} (REGLAMENT 31/2004).

Nickel concentration in roach was found as 0.04±0.012 mg kg^{-1} and in bleak as 0.03±0.025 mg kg^{-1}. Many trace amounts, could be beneficial to activate some human enzymes systems. The WHO (1984) recommends 100-300 µg kg^{-1} body weight nickel for daily intake. However, nickel toxicity in humans is not very common occurrence because the absorption of nickel is very low (ÖZDEN, 2008). The statistical analysis of the nickel data showed that significant differences ($p \leq 0.01$) were found between two studied species. The established maximum level for nickel, in Bulgaria legislation above which fish consumption is not permitted is 0.05 mg kg^{-1} (Reglament 31/2004).

Manganese is recognized as essential trace element for human, but the human requirements or levels of absorption from the diet have not been clearly determined (ÖZDEN *et al.*, 2010). The manganese content was 0.49±0.060 mg kg^{-1} in roach and in bleak was 0.72±0.080 mg kg^{-1} (Table 1). The statistical analysis of the manganese data showed that significant differences ($p \leq 0.001$) were found between these two species.

Chromium concentrations in selected biological materials are elevated in the vicinity of industrial operations and municipal waste treatment facilities (ATANASOFF, 2014). Chromium content was also determined in this study (Table 1). The fish samples that had the higher concentration of chromium were from roach

muscles 0.10±0.045 mg kg^{-1} and they were 10 % more than in these ones of bleak ($p<0.05$). The established maximum level for chromium, in Bulgaria legislation above which fish consumption is not permitted is 0.3 mg kg^{-1} (REGLAMENT 31/2004).

Table 1. Heavy metals content (mg kg^{-1}) in roach and bleak meat.

Elemental concentration (mg.kg^{-1})						
Element	n	Roach		Bleak		Reference (REGLAMENT 31/2004)
		Mean	SD	Mean	SD	
Fe	10	6.59	±0.224	7.34***	0.142	-
Pb	10	0.07	±0.031	0.06^{ns}	0.044	0.20
Mn	10	0.49	±0.060	0.72***	0.080	-
Cd	10	0.01	±0.002	0.01 ^{ns}	0.003	0.05
Zn	10	5.46	±0.388	4.05***	0.263	50
Cu	10	0.69	±0.128	0.59***	0.032	10
Ni	10	0.04	±0.012	0.03*	0.025	0.05
Cr	10	0.10	±0.045	0.09**	0.036	0.3

ns - no significant correlation; *correlation is significant ($p<0.01$); **correlation is significant ($p<0.05$); *** correlation is significant ($p<0.001$)

It is know that the variation in the mineral composition of fish meat is closely related to seasonal and biological differences (species, size, dark/white muscle, age and sex), food source and environmental conditions. As can be seen from the results of both two fish, legal limitations for toxic metals were content not exceeded.

Conclusion

Fish are often at the top of aquatic food chain and may concentrate large amounts of metals from the water. Multiple factors, including season, food, chemical properties of water or sediment can play a significant role in toxic metal accumulation.

This investigation provides practical and useful information on the heavy metal content of roach and bleak for first time in Zhrebchevo Dam Lake. Based on the samples analyzed, the edible parts of fishes do not carry heavy metals loads and concentrations are below the legal value for fish and fish products established by the Food and Agriculture Organization (1983).

It can be concluded that this study contributes to a description of the investigated elements of fish meat which could be use to extend existing information. These results will be important for the nutritionists and researchers for improving processing of fish. It is also helpful for similar academic studies and to prepare tables of compositions of food.

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Received: 26.11.2013
Accepted: 02.03.2014