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Determination of Bioaccumulation of Heavy Metals and Selenium in Tissues of Brown Trout *Salmo trutta macrostigma* (Duméril, 1858) from Munzur Stream, Tunceli, Turkey

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Abstract The objective of the present work was to determine the bioaccumulation of arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg), uranium (U) and selenium (Se) in gill, liver, and muscle tissues of the fresh water fish *Salmo trutta macrostigma* (Duméril, 1858) in Munzur Stream, Tunceli, Turkey. The highest concentrations of U (1.83 μg kg^{-1}), Pb (119.84 μg kg^{-1}) and Se (1.31 μg kg^{-1}) were recorded in the gills of *S. t. macrostigma*. Concentrations of As (46.27 μg kg^{-1}), Cd (109.19 μg kg^{-1}), Hg (16.40 μg kg^{-1}), Cu (18.19 μg kg^{-1}) were recorded at highest levels in the liver. The results showed that there were significant differences in concentrations of As, Cd, Cu, Pb, Se, U and Hg in gill, liver and muscle tissue ($p < 0.05$). Heavy metals were within the edible parts of the investigated fish were in the permissible safety levels for human uses.

Keywords *Salmo trutta macrostigma* · Bioaccumulation · Brown trout · Heavy metals · Munzur Stream

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Heavy metals have the tendency to accumulate in various organs of aquatic organisms, which in turn may enter into the human metabolism through consumption causing serious health hazards (Puel et al. 1987; Raja et al. 2009). Moreover, heavy metal contamination can have devastating effects on the ecological balance of the recipient environment (Farombi et al. 2007; Vosyliene and Jankaite 2006). Domestic, industrial and other human activities result in contamination of natural aquatic systems with heavy metals (Conacher et al. 1993; Velez and Montoro 1998).

In aquatic systems, fish have been considered to be good indicators of heavy metal contamination (Burger et al. 2003). Furthermore, fish are important for the human diet and are widely consumed in the world (Zhang et al. 2007). Many studies have been performed on metal pollution in different species of edible fish (Unlü et al. 1996; Prudente et al. 1997; Karadede-Akin and Unlu 2007; Erdogrul and Erbili 2006; Zhong et al. 2007; Vinodhini and Narayanan 2008; Raja et al. 2009; Rauf et al. 2009; Olowu et al. 2010).

Fish tissue is known to be able to accumulate large amounts of toxic contaminants from their living environment (Suhaimi et al. 2005). Sub-lethal effects of heavy metals are of concern as they accumulate and are transferred through food-chains to humans (Yılmaz and Yılmaz 2007).

Essential trace element, selenium, is important for mammals, birds and fish. Selenium compounds also are

capable of protecting from the toxicity of heavy metals such as cadmium and mercury (Watanabe et al. 1997; Rayman 2000).

Salmo trutta macrostigma is a salmonid species with high economic value in the region (Kocabas et al. 2011). The Munzur Stream is an important water supply in Turkey and also a fishing area for commercial and amateur fishing. Munzur Stream has been contaminated by domestic effluents and agricultural products (Ural, et al. 2011). In this framework, the objective of this study was to determine the bioaccumulation of heavy metals and selenium in gill, liver, muscle tissue of the brown trout *S. t. macrostigma* in Munzur Stream, Tunceli, Turkey.

Materials and methods

Brown trout (n = 30, 11.50 ± 0.40 cm; 35.70 ± 0.60 g) were collected by electrofishing at site (between 39°21′ 14.25″ N, 39°13′ 10.33″ E and 39°21′ 11.05″ N, 39°13′ 43.95″ E) in Munzur stream, Ovacık, Tunceli (Fig. 1). Sample preparation and analyses were carried out by Bornova Veterinary Control and Research Institute, İzmir. About 30 fish from sampling site were selected and analyzed for heavy metals. The collected fish were immediately put into an ice compartment and transported to the laboratory where the fish were kept for 24 h at 10°C. Samples of fish tissues (gills, liver and muscle) were homogenized thoroughly in a laboratory blender with stainless steel blades. For each homogenized sample 0.5 g homogenate (wet weight) was weighed and placed in a polytetrafluorethylene (PTFE) vessel with 5 mL of nitric acid (65% suprapur Merck) and digested in a programmable microwave digester for 38 min at temperatures ranging from 100 to 190°C. The digest was finally made up with 2% nitric acid (65% suprapur Merck), 0.5% hydrochloric acid (30% Suprapur Merck) solution to 50 mL in acid-washed volumetric flasks, transferred to 50 mL polypropylene centrifuge tubes.

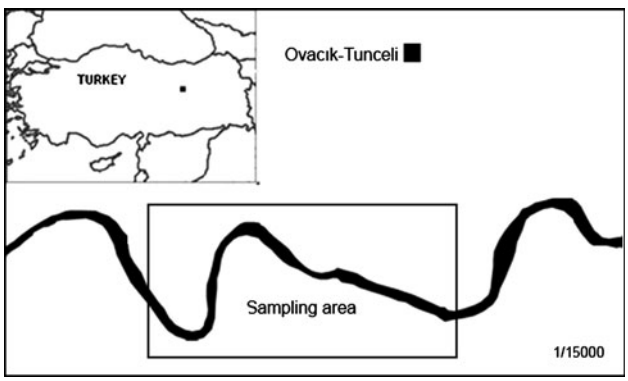

Fig. 1 Location of sampling area for fish samples

The filtrate was analyzed for As, Cd, Cu, Pb, Hg, U and Se concentrations by using an inductively coupled plasma-mass spectrometer (ICP-MS (Agilent 7700×, Agilent Technologies, Inc., Santa Clara, CA 95051, USA)) with an autosampler (Agilent ASX-500). Multi-element calibration solutions of all investigated elements were prepared daily by dilution of a mixed element standard stock solution (AccuTrace MES-21-1, AccuStandard, Inc., New Haven, CT, USA) and 10 ppm mercury standard stock solution (AccuTrace MES-21-HG-1). Interferences on the ICP-MS analyses were inhibited by using an internal standard mix for ICP-MS systems (Agilent, 5,188–6,525, containing: 100 ppm of 6-Li, Sc, Ge, Rh, In, Tb, Lu, Bottle of 100 ml and Bi in 10% HNO^3). Trout samples were spiked with 1,000 μg kg^{-1} concentration of heavy metals for the recovery repeatability tests. Ten homogenized blank and spiked samples were carried through the microwave wet digestion procedures. Then, digested samples were analyzed using ICP-MS. Recovery percentage was calculated as R (%) = 100 * C/concentration of spike (1,000 μg kg^{-1}). C = S − B, where C: Real spiked concentration, S: mean spiked concentration of the studied element, and B: mean blank concentration of the studied element. Data are expressed as mean metal concentrations (±SD). One-way analysis of variance and the Tukey–Kramer test were applied to determine statistical differences in metal concentrations between tissues (Steel et al. 1996).

Results and Discussion

Recovery percentages of heavy metals are presented in Table 1. Recoveries of all studied metals were over 90 %.

Mean metal concentrations in the various tissues are presented in Table 2. The liver contained the highest mean values of As, Cd, Hg and Cu; whereas gill tissue contained the highest mean values for U, Pb and Se. Pb concentrations were below the detection limit of 0.10 ± 0 μg kg^{-1} in liver and muscle tissue, and Hg concentrations were below the detection limit 0.01 ± 0 μg kg^{-1} in gill and muscle tissue. The results showed that there were significant differences among gill, liver and muscle tissue in the concentrations of As, Cd, Cu, U and Hg ($p < 0.05$). Dural et al. (2007) and Ploetz et al. (2007) reported that the highest levels of Cd, Pb, Cu, Zn and Fe were in the liver and gills of fish species viz. *Sparus aurata, Dicentrachus labrax, Mugil cephalus* and *Scomberomorus cavalla*. Yilmaz et al. (2007) reported that in *Leuciscus cephalus* and *Lepomis gibbosus*, Cd, Co and Cu accumulations in the liver and gills were at the highest levels, while these accumulations were at lower levels in the fish muscle.

Table 1 Recovery percentages of heavy metals

Element	Blank mean (n = 10) ± SD* (μg kg^{-1})	Spike mean (n = 10) ± SD (μg kg^{-1})	Recovery (%)
Arsenic (As)	295 ± 28.0	1,273 ± 99.0	98.3
Cadmium (Cd)	1.08 ± 0.16	1,003 ± 74.0	100
Copper (Cu)	132 ± 13.4	1,105 ± 77.0	97.6
Uranium (U)	0.22 ± 0.64	910 ± 61.0	91.0
Lead (Pb)	65.4 ± 2.91	989 ± 61.0	92.8
Selenium (Se)	404 ± 60.2	1,485 ± 105	106
Mercury (Hg)	7.60 ± 0.89	1,079 ± 27.0	107

* *SD* Standard deviation

According to Allen-Gill and Martynov (1995), low levels of Cu and Zn in fish muscles appear to be due to low levels of binding proteins in the muscles. Canli and Kalay (1998) determined the concentrations of Cd and Cr were in the gills, liver and muscles of *Cyprinus carpio*, *Barbus capito* and *Chondrostoma regium* caught at five stations in the Seyhan River in Turkey. Liver and gill tissues showed higher metal concentrations than muscles tissue. Zhang et al. (2007) reported that the concentrations of heavy metals in intestinal tissues were higher than in muscle in 19 fish species. Rauf et al. (2009) stated that fish liver exhibited highest tendency to accumulate both Cd and Cr. In this study, the highest concentrations of heavy metals in brown trout *S. t. macrostigma* were found in liver and the lowest in muscles. According to Turkish Food Codex (2002), heavy metals were within the edible parts of *S. t. macrostigma* were in the permissible safety levels for human uses. The maximum acceptable levels (μg kg^{-1} wet weight) in Turkish Food Codex (2002) for fish are presented in Table 2.

Metal accumulation levels in various organs of brown trout *S. t. macrostigma* were different and are arranged in a decreasing order: U – gills > liver > muscles; Pb – gills > liver = muscles; Cd – liver > gills > muscles; Hg – liver > gills > muscles; Cu and As – liver > gills > muscles; Se – gills > muscle > liver. Vinodhini and Narayanan (2008) determined that the order of heavy metal accumulation in the gills and liver was Cd > Pb > Ni > Cr and Pb > Cd > Ni > Cr, respectively. In this study, the order of heavy metal accumulation in the gills and liver was Cu > Pb > As > Cd > U > Hg and Cu > Cd > As > Hg > U > Pb, respectively. Furthermore the order of selenium accumulation in tissues was gills > muscle > liver.

Natural U in freshwater ecosystems is considered to be both a radiological and a chemical hazard (Cooley et al. 2000; Khune et al. 2002). Uranium is a naturally occurring metal whose natural abundance in freshwater may be increased as a result of various anthropogenic contributions such as the different stages of the nuclear fuel cycle (mines in particular), agricultural use (phosphate based fertilizers), research laboratories, and military use of depleted uranium (Colle et al. 2001). In *S. t. macrostigma,* U bioaccumulation was detected in their soft tissues. It could be explained due to geologic formation and the high content of metal-bearing ore of the region (URL-1 2012). Also, the highest concentration of uranium was determined in gills. U could be accumulated in gills at a significant level related to their osmoregulation function (Simon and Garnier-Laplace 2005)

In conclusion, high levels of heavy metals were found in liver of the brown trout, while the levels were the lower in muscle tissue, with the exception Pb and U. The examined fish were within the limits for human consumption. The studies of these organisms as biological indicators of heavy metal contamination in local waters will require further investigations to develop the protocols for their use.

Table 2 Mean (±S.D.) concentrations μg kg^{-1}, (wet weight) of metals in gill, liver and muscle tissues, and maximum acceptable levels in edible tissue μg kg^{-1} according to the Turkish Food Codex (2002)

Element	Gill	Liver	Muscle	Max acceptable Level
Arsenic (As)	32.4 ± 14.1^{a}	46.3 ± 30.7^{b}	26.9 ± 16.4^{c}	1,000
Cadmium (Cd)	22.5 ± 10.8^{a}	109 ± 35.7^{b}	4.08 ± 2.83^{c}	50.0
Copper (Cu)	732 ± 6.80^{a}	18,185 ± 8058^{b}	518 ± 97.0^{c}	20,000
Uranium[1] (U)	1.83 ± 0.55^{a}	0.60 ± 1.052^{b}	0.14 ± 0.20^{c}	–
Lead (Pb)	119 ± 151^{a}	0.10 ± 0.00^{b}	0.10 ± 0.00^{b}	200
Selenium[1,2] (Se)	1,313 ± 178^{b}	24.7 ± 38.5^{a}	514 ± 96.0^{c}	–
Mercury (Hg)	0.01 ± 0.00^{a}	16.4 ± 40.2^{b}	0.01 ± 0.00^{a}	500

ND Not detected

[a,b,c] Different letters in the same row indicate significant differences in metal concentration between tissues ($p < 0.05$)

[1] There is no information about maximum levels of this element in fish samples in Turkish standards

[2] The suggested maximal daily safe intake is 0.007 μg Se kg^{-1} body weight day by the World Health Organisation (WHO 1996)

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