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Distribution and Relationships of Eleven Trace Elements in Muscle of Six Fish Species from Skadar Lake (Montenegro)

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Abstract

Concentrations of eleven trace elements (Zn, Mn, Cu, Fe, Cr, Ni, Co, Hg, Pb, Cd and As) as well as the principal components of fish flesh (water%, dry matter%, fat%, protein%, ash% and Ca and Mg concentration) were determined in muscles of six fish species (*Scardinus knezevici*, *Alburnus scoranza*, *Cyprinus carpio*, *Rutilus prespensis*, *Anguilla anguilla* and *Perca fluviatilis*) collected from Skadar Lake in 2016. This study, being the first of its kind in fish from Montenegrin lakes, gave the following overall ranking of the investigated elements in fish muscle: Zn > Fe > Mn > Cu > Hg > Cr > As > Ni > Pb > Cd. Several significant inter-metal correlations were observed, while significant relationships between metal concentrations and fish age and size were established predominantly for essential elements. Significant inter-species differences in accumulation of analyzed trace elements were confirmed for Zn, Mn, Fe and As. The total metal accumulation was the highest in roach and the lowest in perch, while European eel was found to accumulate more arsenic than other fish species. The consumption of fish from Skadar Lake doesn't pose a risk to human health.

Key words: bioaccumulation, heavy metals, fish muscle, pollution, Skadar Lake

Introduction

Among the various pollutants, trace metals and metalloids, if occurring in higher concentrations, can become severe poisons for all living organisms due to their high persistence, toxicity and tendency to accumulate in water and especially in sediment (Förstner & Wittmann, 1981; Maceda-Veiga, Monroy, Navarro, Viscor, & de Sostoa, 2013). Since heavy metals are non-biodegradable, they can be accumulated by various aquatic organisms including fish. There are two main routes of trace elements uptake for fish: aqueous uptake by the gills (and to a lesser extent by skin) and dietary uptake by ingestion of contaminated food (Wood, Farell, & Brauner, 2011; Kamunde & MacPhail, 2011). Being the top predators in aquatic food chains, fish have been widely used as bioindicators of metal pollution (Dorea, 2008; Has-Schon et al., 2008; Matasin, Ivanusic, Orescanin, Nejedli, & Gaiger, 2011; Noel *et al*, 2013; Küpeli, Altundağ, & İmamoğlu 2014; Ahmad & Sarah, 2015). Although trace elements accumulate in different fish organs (liver, kidney, muscle, gills), fish muscle is the most frequently used tissue for the analysis because it is the main edible part of the fish.

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The aim of this study was to determine the accumulation level of eleven selected trace elements (Zn, Cu, Fe, Mn, Cr, Co, Ni, Hg, Pb, Cd and As) as well as the main chemical constituents in the muscle of six fish species from Skadar Lake, with different feeding habits (European eel, bleak, common carp, rudd, roach and perch). We also examined the dependence of metal concentration on age and biometric characteristics of fish (size, weight and condition factor) as well as correlation among different trace metals and among trace metals and the main chemical constituents in fish muscle. There are only two previous studies (Filipovic, Vukovic, & Knezevic, 1980; 1981) of trace metals in fish from Skadar Lake. These investigations comprised only four metals (Fe, Cu, Cr and Cd) in muscle of three fish species (common carp, bleak and rudd) and gave only data about metals concentrations. Thus, this is the first investigation of trace elements in fish from Skadar Lake after gap of more than 30 years and majority of the trace elements selected for this study, were analyzed for the very first time. Having in mind that Skadar Lake lies on a border of two countries (Montenegro and Albania) and that fishery is one of the main sources of income and food for majority of local inhabitants in both countries (CEED, 2007), we found important to assess whether there is potential human health risk due to the consumption of fish from this lake.

Materials and Methods

Study Area

Skadar Lake is situated on the border of Montenegro and Albania (two thirds of the lake belongs to Montenegro, one third belongs to Albania). With the surface area that fluctuates seasonally from approximately 370 to 540 km^2, Skadar Lake is the largest lake on Balkan Peninsula (Figure 1) with the mean depth of 5 m (Beeton, 1981). The main tributary is Moraca River, which provides more than 60% of the lake water. The outlet river - Bojana creates connection between the lake and Adriatic Sea. The Montenegrin part of the lake was declared a national park in 1983. and included in the Ramsar list of wetlands of international importance in 1996. However, lake tributary Moraca River (the main recipient of communal and industrial wastewater from capitol Podgorica), run-off from Zeta valley (the main agricultural area of Montenegro) and groundwater contaminated by wastewater from the surrounding settlements are the potential sources of Skadar Lake pollution (e.g. artificial fertilizers, protective agents, heavy metals from industrial wastewater etc) (ROYAL HASKONING, 2006).

The lake represents one of the most important centers of biodiversity for Western Balkan and South-Eastern Europe with more than 100 species of water birds (Vizi, 1981) and 50 fish species (Maric & Milosevic, 2011). Fish are the lake's most significant natural resource in terms of contribution to the local economies. Annual catch in Skadar Lake is 520 tones of fish, mainly *Cyprinus carpio* and *Alburnus scoranza* (CETI & APAWA, 2007). Beside these two commercially most important species, our investigation included *Rutilus prespensis* (the endemic species of Skadar Lake and Prespa Lake), *Scardinius knezevici* (the endemic species of Skadar-Ohrid-Drim system), *Perca fluviatilis* (the most abundant alochtonous species introduced to the lake in late 80-s) and migratory fish *Anguilla anguilla.* Furthermore, these fish species were selected for research because of the different ecological features, primarily different feeding habits (Table 1).

Sampling and Chemical Analyses

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The field work was conducted in April 2016. at locality Radus (common place for commercial fishing). Fish were collected with multi mesh gillnet and fyke net (255 individuals in total) and the body-wet weight (g) and the total body length (cm) of each fish specimen were measured. For each analyzed species, samples were divided into size categories that consisted of a 4 − 50 specimens of the same fish species, depending on fish size. Only in case of a very large specimen (e.g. carp) one fish represented one size category. The fish age for bleak, rudd, carp, roach and perch was determined by examining the scales according to Lagler (1952). The condition factor (CF) was calculated according to the following equation (Lombardi, Peri, & Verrengia Guerrero, 2010): $CF = W_t/L^3 \times 100$ (W_t is the total weight of the fish in grams and L is the total length in cm).

Tissue samples of the dorsal muscle were taken, than milled using an electric grinder and stored in air tight polyethylene bottles at -18 °C until chemical analyses. All chemical analyses were performed in laboratory of Center for Eco-toxicological Research (CETI, Podgorica). The chemical composition of fish muscle was determined according to AOAC (1997). Dry matter content was determined by drying the samples to a constant weight at 105°C (AOAC 952.08). Ash content was quantified using incineration in a muffle furnace at 550 °C for 24 h (AOAC 938.08). Total fat content was determined by Soxhlet extraction method for 6 h (AOAC 948.15), while crude protein content was determined by Kjeldahl method (AOAC 981.10). Three parallel determinations were carried out for each parameter.

For elements analyses, each fresh fish sample (500 mg ± 0.1 mg) was weighted and digested using closed microwave assisted digestion (Berghof MWS-4, Germany) with 5 cm^3 of nitric acid (≥69%, puriss. *p.a.*, Sigma Aldrich) and 2 cm^3 of hydrogen peroxide (≥30%, for trace analyses, Sigma Aldrich). Digested samples were filtered and transferred in volumetric flasks by adding deionised water (MEST EN 13804, 14084). Blanks with no fish tissue were run with each batch of samples to resolve potential contamination of reagents used in digestion. All analyses were carried out in triplicates. Working standards for measurements of elements were prepared from Sigma Aldrich solutions of 1000 mg dm^{-3} each. For analyses of Fe (248.3 nm), Mn (279.5 nm), Zn (213.9 nm) and Cu (324.8 nm), flame atomic absorption spectrophotometry was used on Shimadzu AA-6800 (Japan) and for analyses of Pb (220.3 nm), Cd (228.8 nm), As (189.0 nm), Co (228.6 nm), Ni (231.6 nm), Cr (267.7 nm), Ca (315.9 nm) and Mg (285.2 nm) ICP-OES Thermo iCAP 6300 (UK) was used. Mercury content was determined using Advanced Mercury Analyser (AMA-254, Lecco). Concentrations of analyzed trace elements were expressed on a wet weight basis, as mg kg^{-1}. The limits of quantification (LOQ) were: 0.1 mg kg^{-1} for Pb; 0.01 mg kg^{-1} for Cd; 0.06 mg kg^{-1} for As; 0.025 mg kg^{-1} for Co and Cr; 0.02 mg kg^{-1} for Ni; 0.2 mg kg^{-1} for Fe, Mn, Cu and Zn; 0.001 mg kg^{-1} for Hg; and 5 mg kg^{-1} for Ca and Mg. The reliability of the analytical method was evaluated using Certified Standard Reference Material IAEA-407. The recovery rates were in the range of 95-104% compared to the certified values. For some samples, concentrations were below the limits of quantification (LOQ). In such cases, in order to calculate mean concentrations, values bellow LOQ were replaced by half of the LOQ (GEMS/Food, 1995).

Statistical Analyses

Since the data did not show a normal distribution (Kolmogorov-Smirnov test) and the examined groups were of unequal size, in order to test the differences in concentration of trace elements between different fish species, the non-parametric Kruskal-Wallis test was applied, followed by post hoc test based on nonparametric multiple

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comparisons. The same method was used to test differences between predatory and non-predatory groups of fish. To estimate the total metal content in the different fish species, the Metal Pollution Index (MPI) was used as proposed by Usero, Gonzalez-Regalado, and Gracia (1996, 1997). Relations between the fish age, fish biometric data (size, weight and condition factor) and concentrations of trace elements in fish muscle were evaluated by Spearman correlation. The same method was used to estimate inter-correlations among different trace elements as well as among trace elements and the main chemical constituents in fish muscle. All statistical analyses were performed using the statistical software package STATISTICA 7 (StatSoft, Tulsa, USA). The obtained metal concentrations were compared to maximum permitted concentrations (MPC) for human consumption established by Montenegrin national legislation and some international and national guidelines (Table 1).

Results

The feeding habits, age and biometric characteristics of the analyzed fish (body weight, total body length and condition factor) are listed in Table 2. From six fish species analyzed in this study, rudd is predominantly herbivorous (aquatic vegetation), bleak is a water-column dweller, feeding mainly on plankton, common carp and roach are omnivorous bottom feeders, European eel is bottom-dwelling predator and perch is water-column dweller, mainly piscivorous (Kottelat & Freyhof, 2007). The mean CF values varied among the fish species and ranged between 0.18 ± 0.03 in European eel and 1.58 ± 0.22 in rudd.

Chemical Composition of Fish Muscle

The list of analyzed chemical constituents of muscles from six fish species is given in Table 3. Average amount of water varied between 64% in European eel to 79% in perch, mean protein% ranged from 15.5% in European eel to 19.3% in rudd, while mean fat content showed the greatest variation: from 0.72% in carp to 15% in European eel. Highest mean ash% was observed in bleak (2.68%) and the lowest in European eel (1.05%). Average calcium and magnesium concentrations were lowest in European eel (5.31 mg kg^{-1} and 1.84 mg kg^{-1} respectively), while the highest mean Ca concentration was recorded in bleak (53.45 mg kg^{-1}) and the highest mean Mg concentration in roach (9.29 mg kg^{-1}). The Kruskal-Wallis test revealed significant differences between fish species for all analyzed chemical constituents. Post hoc nonparametric multiple comparisons showed that European eel had significantly higher content of fat than all other fish species ($P < 0.00001$), higher content of dry matter ($P = 0.0001$) and lower content of water ($P = 0.0001$) than rudd, carp and perch and significantly lower content of proteins ($P = 0.001$) and ash ($P = 0.001$) than rudd, roach and bleak. Carp had significantly lower content of fat than eel, rudd and bleak ($P = 0.00001$) and lower content of ash than roach and bleak ($P = 0.001$). Roach and bleak had significantly higher concentration of Ca and Mg than all other fish species ($P = 0.001$).

Concentrations of Trace Elements

Concentrations of trace elements in fish muscle and MPI (Metal Pollution Index) values are shown in Table 4. The MPI values were computed in order to normalize and compare the whole metal contamination between the

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different fish species. Metal pollution index ranged from 0.165 to 0.338 and the sequence of MPI in different fish species was as following: Roach > Bleak > Eel > Carp > Rudd > Perch.

Calculation of the overall average concentrations of investigated trace elements in the muscles of fish from Skadar Lake gave the following ranking: Zn > Fe > Mn > Cu > Hg > Cr > As > Ni > Pb > Cd. The accumulation patterns somewhat differed among analyzed fish species depending on their ecological features, but the concentrations of essential metals Zn, Fe, Mn and Cu were the highest, while that of Cd was the lowest in all analyzed fish species.

The general order of muscular Zn bioaccumulation was: Bleak > Carp > Roach > Rudd > Eel > Perch. Observed differences in Zn level were significant only among perch and bleak (P = 0.008) and perch and carp (P = 0.03). The general order of muscular Mn bioaccumulation in this study was: Roach > Bleak > Rudd > Eel > Carp > Perch. Mn concentrations in muscle of carp and perch were significantly lower (P = 0.0001) than in other analyzed fish species. The distribution pattern of Fe concentrations in muscle of six fish species was: Roach > Bleak > Carp > Rudd > Perch > Eel. Highly significant difference in Fe level was observed between eel and all other fish species (P = 0.00001). The general order of muscular Cu bioaccumulation was: Eel > Perch > Carp > Rudd > Bleak > Roach, but differences in Cu level among fish species were not statistically significant. The distribution pattern of Cr concentrations in muscle of six fish species was: Roach > Carp > Bleak > Rudd > Eel > Perch, with no significant differences among analyzed fish species. Co concentrations were below the LOQ in muscle samples of all analyzed fish species. The general order of muscular Ni bioaccumulation was: Carp > Perch > Roach > Eal > Rudd > Bleak, but observed differences in Ni level among fish species were not statistically significant. The distribution pattern of Hg concentrations in muscle of six fish species was: Eel > Perch > Rudd > Carp > Roach > Bleak, with no significant differences among analyzed fish species. Pb and Cd concentrations in fish muscles were below LOQs in most of analyzed samples (Table 4). Arsenic was not detectable in any of the muscle samples of carp and perch. The general order of muscular As bioaccumulation was: Eel > Bleak > Roach > Rudd. Statistical analyzes of differences in As level in the muscles of fish species showed that European eel had significantly higher content of As than other analyzed fish species (P = 0.0001).

Correlation Analyses

Growth-dependent variations were observed in concentrations of several trace elements in muscle of studied fish. For Fe, Cr, Pb, Cd and Hg there were no significant correlations with age nor size (length and weight) in any of the analyzed fish species. Significant correlations ($P < 0.05$) with age were found for Cu, Ni, Zn and Mn in rudd (R = 0.80; R = 0.72; R = –0.60; R = –0.69) and for Zn in carp (R = 0.80) and roach (R = 0.85). Furthermore, significant correlations ($P < 0.05$) with body weight were found for Cu, Ni, Zn and Mn in rudd (R = 0.80; R = 0.70; R = –0.64; R = –0.77), for Zn in roach (R = –0.94) and for As in eel (R = 0.73). Significant correlations ($P < 0.05$) with body length were found for Cu, Ni, Zn and Mn in rudd (R = 0.79; R = 0.65; R = –0.61; R = –0.73) and for Zn and Ni in roach (R = –0.94; R = –0.81). Finally, overall CF was in negative correlation with Zn, Mn and As ($P < 0.05$), but in positive correlation with Ni ($P < 0.05$).

Several significant correlations were observed among the metals and main chemical constituents of fish muscle. Significant negative correlations with water% in fish muscle were observed for concentrations of Zn, Mn, As and fat% ($P < 0.01$), while positive correlation was found for Ni and Cr ($P < 0.05$). Protein% was negatively

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correlated with Cu, As and water%, but positively with concentrations of Fe, Cr, Ca and Mg ($P < 0.01$). Fat% was positively correlated with Mn and As, but negatively with Fe and Ni ($P < 0.01$). Concentrations of Ca and Mg were positively correlated with Zn, Fe, Mn, Cr and protein%, but negatively correlated with Cu.

Several significant correlations were observed among studied trace elements. Significant positive correlations among trace elements were found for Mn-Cr, As-Mn, As-Cu, Zn-Fe, Zn-Mn and Zn-Cd, while significant negative correlations were observed for Mn-Ni, As-Fe, As-Ni, Zn-Cu, Zn-Ni and Hg-Cr.

Discussion

Fish muscle of six species from Skadar Lake contained in average 75% water, 18% protein and 4% fat, what was in agreement with usual findings (Nair & Suseela, 2000; FAO/INFOODS, 2013). Generally, there is an inverse relationship between the water, protein and fat contents of fish flesh, but in our study, statistically significant negative correlation was observed only between water and fat ($P < 0.01$). Fat content in fish species varies with climate, season, available food and physiological status of fish. Condition factor is a measure of general fish's health and it reflects recent feeding conditions and environmental quality (Froese, 2006; Rennie & Verdon, 2008). The mean CF values for fish analyzed in this study were in agreement with common findings for the selected fish species. Although CF value is generally related to accumulation of fat, significant positive correlation among fat% and CF in our study was observed only in eel and bleak ($P < 0.01$).

There is no information about maximum permitted levels of Zn, Fe, Mn, Cu, Cr, Ni, Co and As in fish tissues in National Regulation of Montenegro. For that reason, concentrations of these trace elements were compared to other international and national standards (Table 1). That comparison showed that average concentrations of these elements in six fish species from Skadar Lake were below the maximum permitted levels for human consumption. Concentrations of Hg, Pb and Cd were below the maximum permitted levels in the all analyzed muscle samples according to both international legislation and Montenegrin standards for fish. Furthermore, calculated values of metal pollution index (MPI) for all fish species were far below critical value of 1, what confirms that concentrations of analyzed metals in fish muscle are not elevated. These findings suggest that Skadar Lake could be still regarded as unpolluted ecosystem. In previous investigations of fish from Skadar Lake (Filipovic *et al.*, 1980; 1981) the average concentrations of four analyzed metals (Zn, Fe, Cu and Cd) were very similar to those of our study. That indicates that in spite of different sources of pollution around Skadar Lake, metals concentrations didn't increase over the period of last 30 years.

The general order of muscular trace elements bioaccumulation in this study (Zn > Fe > Mn > Cu > Hg > Cr > As > Ni > Pb > Cd) showed that essential elements had the highest concentration in fish muscle what is in agreement with common findings (Andreji *et al*, 2006, Yilmaz, Ozdemir, Demirak, & Tuna, 2007; Maceda-Veiga, Monroy, & de Sostoa, 2012; Petkovšek, Grudnik, & Pokorny, 2012; Dvorak, Andreji, Dvorakova-Liskova, & Vejsada, 2014). Zn, Fe, Mn and Cu have a vital functions in all living organisms and their concentration in fish is usually the highest (Kennedy, 2011). In our research, Zn showed the highest concentrations from all analyzed metals in muscles of all fish species. The observed sequence of Zn accumulation in six analyzed fish species from Skadar Lake suggests that Zn accumulation tendency decrease with the trophic position of fish, what is in agreement with some other studies (Cheng, De Schamphelaere, Lofts, Janssen, Allen, 2005; Andreji *et al*. 2005, 2006; Kensova, Celechovska, Dobravova, & Svobodova,

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2010). Mn was the second most abundant metal in muscle of analyzed fish. This element can be significantly bioaccumulated by aquatic organisms, but has no tendency to biomagnify along the food chains and the last was confirmed by our results. Cu concentrations from our investigation were in similar range as in some other studies (Mendil *et al*, 2005; Andreji *et al*, 2006). Although differences in Cu level among analyzed fish species were not statistically significant, highest Cu level in predatory fish species (European eel and perch) observed in our research, suggests that Cu accumulation might have tendency to increase with the trophic position of fish. On the other side, accumulation of Fe and Cr in fish from Skadar Lake showed the opposite trend: higher levels of these metals were observed in non-predatory species in comparison to predators. From analyzed essential metals, Co was the only one that was not detected in any of the muscle samples. Although significant sites of Co accumulation are mainly soft tissues, generally, this element have low accumulation rate in the fish (Bird, Mills, & Schwartz, 1999), probably due to its high affinity to organic particles in solution.

Unlike essential metals, Pb, Hg, Cd and As have no biological function and hence their concentrations in fish are generally low, but they could be harmful to living organisms even in trace amounts. Aquatic microorganisms have the ability to transform elementary Hg and its compounds to methyl-Hg which is the chemical form of most concern because of its high toxicity. This metal predominantly accumulates in fish muscle (Wiener, Krabbenhoft, Heinz, & Scheuhammer, 2003). It is one of the few metals known to biomagnify through food webs; therefore, carnivorous fish species usually contain the highest amounts of Hg as a top predators in the aquatic food chains (Dusek *et al*, 2005; Chen, 2012; Noel *et al*. 2013). The last was confirmed by our results since the highest Hg concentrations were recorded in fish species from the highest trophic level (European eel and perch).

Pb and Cd are toxic metals that could be harmful to aquatic organisms even at very low concentrations. In our research, Pb and Cd concentrations in fish muscle were below detection limit in most of analyzed samples and the mean concentrations of these metals were similar to those of some unpolluted areas (Dusek *et al*, 2005; Yilmaz *et al*, 2007; Petkovsek *et al*, 2012; Yancheva, Stoyanova, Velcheva, Petrova, & Georgieva, 2014). That is in accordance to very low Pb and Cd concentrations recorded in sediments of Skadar Lake (Kastratovic, Jacimovic, Bigovic, Djurovic, & Krivokapic , 2016).

As is metalloid able to accumulate in fish, but there is no considerable biomagnification trough the food web (Williams, Schoof, Yager, & Goodrich-Mahoney, 2006; Leeves, 2011). The last was partly confirmed by our findings. European eel, as one of the top predators in aquatic food chains, had significantly higher content of As (P = 0.0001) than other analyzed fish species from Skadar Lake, but at the same time, in muscle of the other analyzed predatory fish (perch) As was not detected in any of the samples. Highly significant differences in As level observed between two predatory species, eel and perch, could be attributed to their different ecological features: perch is solely piscivory fish that lives and feeds mainly in the water column, while eel is bottom-dwelling species that burrow in sediments and feeds on various organisms (e.g. snails, polychaetes and fish). Described features imply higher likelihood of As accumulation from the surrounding environment (and food) in eel in comparison to perch. Furthermore, significant positive correlation among As level and fat% (P < 0.01) observed in our research, could be associated with the highest As accumulation rate in European eel (i.e. species with the highest fat% of all analyzed fish). The mean As concentration obtained in this study was generally comparable to the levels reported in some similar studies (Noel *et al*, 2013; Has-Shon, Bogut, & Strelec, 2006),

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but higher in comparison to As level in the muscle of the fish from some unpolluted areas (Has-Shon *et al*, 2008; Petkovsek *et al*, 2012).

Metal bioaccumulation is species–specific, therefore some differences observed in the accumulation pattern among analyzed fish species might be a result of different ecological needs, metabolism and feeding patterns (Andres, Ribeyre, Tourencq, & Boudou, 2000; Qiao-qiao, Guangwei, & Langdon, 2007; Monikh, Safahieh, Savari, & Doraghi, 2013). Sediment is a remarkable source of trace elements for fish and a degree of contact with sediment highly depends on feeding habits. In other words, bottom-dwelling species are expected to accumulate more trace metals in comparison with water-column dwellers. However, our expectation that perch and bleak, as water-column dwellers, would show lower load of trace elements than rudd, carp, roach and eel was only partly confirmed (i.e. for Zn, Mn, Cr, As, Pb and Cd in perch and for Ni, Hg and Pb in bleak). These findings suggest that, in Skadar Lake, the fish's relation to sediments is probably not crucial in the metal load variation among fish species. For instance, perch and bleak are active and fast swimmers what is usually associated with high metabolic rate and that may result in higher accumulation rate of certain metals in comparison to bottom-dwelling species (Canli & Atli, 2003).

Generally, accumulation level of trace elements in fish depends on relation between specific uptake (through the gills, skin and the digestive system), metabolization and elimination mechanisms. These processes are usually related with environmental factors (e.g. water temperature, pH, hardness, salinity), but also with the age/size-specific metabolic rate of organisms (Jezierska & Witeska, 2006; McKinley, Taylor, & Johnston, 2012). Although some authors (Burger et al. 2002; Canli & Atli 2003; Scerbo *et al*. 2005; Yi & Zhang, 2012; Merciai, Guasch, Kumar, Sabater, & García-Berthou, 2014; Dvorak *et al*., 2014) reported clear relation between the concentrations of trace elements in fish muscle and the age and size of a fish, only a few significant correlations between metals accumulation and fish age and size were observed in our study. The possible reason could be the absence of some age categories, but regarding Pb and Cd, primary reason was the small number of muscle samples where these metals were detectable. Higher concentrations of Zn and Mn, observed in younger fish in our study, may be linked to higher metabolic rate in younger fish and to superior bioregulation mechanism for these metals in older fish in comparison to younger ones (De Wet, Schoonbee, De Wet *et al*., 1994).

Positive correlations between metal accumulation and age/size of the fish, like higher Cu, Ni and As concentrations observed in older and larger fish in our study, may result from the longer time of exposure or slower excretion (Di Giulio & Hinton, 2008). On the contrary, observed negative correlation between fish size and Zn and Mn accumulation might be caused by tissue growing more rapidly than metals intake. That imply a higher potential uptake of trace metals in smaller than larger fish (Authman, 2008). Concentrations of other analyzed trace metals (Fe, Cr, Hg, Pb and Cd) were relatively independent on fish age and size (no correlation) and that usually occurs when the uptake and excretion rates of the metals balance. Moreover, the relationship among metal level and fish age and size may not exist when food is limiting and fish stop growing, but continue to accumulate contaminants (Burger & Campbell, 2004).

Significant inter-correlations among trace elements in fish tissue may reflect a common source of occurrence and similar biogeochemical pathways in fish body (Jezierska & Witeska, 2001). In our study, significant positive correlations among trace elements were found for Mn-Cr, As-Mn, As-Cu, Zn-Fe, Zn-Mn, Zn-Cd, while negative correlations were observed for Mn-Ni, As-Fe, As-Ni, Zn-Cu, Zn-Ni and Hg-Cr. Many authors reported positive correlations among metals (e.g. Burger & Campbell 2004; Mendil *et al*., 2005; Andreji, Stranai, Kačániová, Massanyi, & Valent 2006; Has-Shon *et al*., 2015), but in some cases, even in the same fish species, metal-relationships were the opposite or there were no significant relations (Burger *et al*., 2002, Andreji, Stranai, Massanyi, & Valent, 2005, Andreji, Stranai, Massanyi, & Valent, 2006). There are many reasons for such contrasting results since inter-metal relations depend on many factors, such as diet, trophic level, metabolism, health status, exposure time, pollution level of the environment etc.

Conclusions

The results for the eleven analyzed trace elements in muscle of six fish species from Skadar Lake showed variation in a range that seems to be typical for fish of waters without considerable anthropogenic impact. The investigated trace elements (especially those of highest priority: Hg, Pb, Cd and As) were below maximum permitted levels of many recognized international standards. Therefore, all six analyzed fish species could be recommended for human diet. Concentrations of essential metals, Zn, Fe, Mn and Cu were the highest, while that of Pb and Cd were the lowest in all analyzed fish species.

Statistically significant inter-species differences in accumulation of analyzed metals were confirmed only for Zn, Mn, Fe and As. Perch was the species with the lowest MPI, while roach had the highest overall concentration of the trace elements (i.e. the highest MPI).

Significant relationships between heavy metal concentrations and fish age and size were established predominantly for essential elements, while for non-essential ones there were no definite or established relationships. Similar situation was observed in case of inter-metal relationships. Considerably low and uniformly distributed concentrations of majority of non-essential elements as well as very small percentage of samples where some of these metals were detectable (i.e. Pb and Cd), might substantially contribute to the absence of clear relationships between non-essential metals and both fish biometric characteristics and other studied metals.

Although results of this investigation showed no risk for fish consumers, further studies are needed for better understanding of the trace metals bioaccumulation mechanisms in fish of Skadar Lake. Having in mind all potential contaminants in Skadar Lake hinterland, it is recommended to conduct continuous monitoring of fish to ensure that the concentrations of metals remain within the prescribed worldwide limits.

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Table 1. Permitted levels of trace elements in fish (mg kg^{-1} wet weight) established by different guidelines

References[1]	Country	Hg	Pb	Cd	As	Cu	Zn	Fe	Mn	Ni	Cr
EC (2006)	Europe	0.50	0.30	0.05	–	–	–	–	–	–	–
FAO (1983)	Worldwide	0.50	0.50	0.05	0.1-4	30	40-100	–	–	–	–
FAO/WHO (1989)	Worldwide	0.30	0.50	0.10	–	30	50	–	–	–	1.0
MAFF (2000)	UK	0.50	2.0	0.20	1.0	20	50	–	–	–	1.0
Official Gazette RS (2011)	Serbia	0.50	1.0	0.10	2.0	30	100	30	–	–	–
Anonymous (2005)	Croatia	0.50	0.20	0.05	2.0	30	100	30	–	–	–
Anonymous (2004)	Bulgaria	0.50	0.40	0.05	1.0	10	50	–	-	0.5	0.3
TFC (2002)	Turkey	0.50	1.0	0.1	–	20	50	–	20	–	–
Official Gazette MN (2009)	Montenegro	0.50	0.30	0.05	–	–	–	–	–	–	–

"–" data were not available in publication or variable was not studied

[1]For references, see list of references

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Table 2. List of examined fish species, feeding habits, age and biometric data given as mean ± SD and range (N – number of collected specimens, TW – total body weight, TL – total body length and CF – condition factor)

Species	Common name	Feeding habits	Age	N	TW (g)	TL (cm)	CF
Scardinius knezevici	Rudd	herbivorous	2-8	40	491.9 ± 291.6 (26.2 – 910.2)	29.4 ± 7.9 (12.8 – 39.7)	1.58 ± 0.22 (1.25 – 1.77)
Alburnus scoranza	Bleak	planctivorous	1-4	85	20.3 ± 12.6 (4.8 – 39.3)	13.1 ± 3.2 (7.2 – 16.7)	0.79 ± 0.09 (0.64 – 0.95)
Cyprinus carpio	Common carp	omnivorous	2-6	15	2823 ± 1583 (107 – 7600)	57.2 ± 12.7 (19.6 – 79.6)	1.34 ± 0.11 (1.20 – 1.55)
Rutilus prespensis	Roach	benthophagous	1-3	80	42.6 ± 36.7 (12.5 – 105.1)	13.9 ± 3.9 (9.5 – 19.5)	1.23 ± 0.16 (1.01 – 1.42)
Perca fluviatilis	Perch	predator	2-4	15	155.3 ± 122.7 (70.1 – 296.3)	21.3 ± 5.1 (17.3 – 27.2)	1.39 ± 0.10 (1.33 – 1.50)
Anguilla anguilla	European eel	predator	/	20	327.5 ± 254.8 (85.8 – 902.3)	53.7 ± 10.9 (37.3 – 74.5)	0.18 ± 0.03 (0.15 – 0.22)

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Table. 3. Content of main chemical constituents (mean ± SD and range) in muscle of fish collected from the Skadar Lake

	Scardinius knezevici	*Alburnus scoranza*	*Cyprinus carpio*	*Rutilus prespensis*	*Anguilla anguilla*	*Perca fluviatilis*
Water %	76.85 ± 3.19	71.68 ± 5.84	78.13 ± 2.19	74.96 ± 3.85	64.82 ± 4.52	79.51 ± 1.23
	(71.1 – 80.2)	(66.7 – 79.3)	(73.1 – 80.6)	(70.3 – 79.8)	(59.2 – 71.4)	(78.6 – 80.9)
Dry matter %	23.15 ± 3.19	28.33 ± 5.82	21.87 ± 2.18	25.04 ± 3.85	35.17 ± 4.52	20.51 ± 1.21
	(19.8 – 28.9)	(20.7 – 33.3)	(19.4 – 26.9)	(20.2 – 29.7)	(28.6 – 40.8)	(19.1 – 21.4)
Ash %	1.51 ± 0.72	2.68 ± 0.68	1.07 ± 0.10	2.48 ± 0.96	1.05 ± 0.12	1.36 ± 0.24
	(0.9 – 3.1)	(1.2 – 3.3)	(0.9 – 1.4)	(1.3 – 3.5)	(0.9 – 1.2)	(1.2 – 1.6)
Protein %	19.31 ± 1.82	18.95 ± 3.01	17.97 ± 0.84	19.51 ± 2.37	15.46 ± 1.14	18.07 ± 0.35
	(16.7 – 23.1)	(14.7 – 23.3)	(16.6 – 19.3)	(16.7 – 22.3)	(13.7 – 17.1)	(17.7 – 18.4)
Fat %	2.44 ± 1.03	6.21 ± 3.04	0.72 ± 0.53	1.75 ± 0.46	15.79 ± 4.16	1.10 ± 0.36
	(1.1 – 4.6)	(2.1 – 10.36)	(0.05 – 1.7)	(1.4 – 2.4)	(9.4 – 23.9)	(0.8 – 1.5)
Ca (mg kg^{-1})	19.73 ± 23.42	53.45 ± 21.74	6.08 ± 2.49	38.48 ± 27.91	5.31 ± 1.95	10.93 ± 8.99
	(2.5 – 65.1)	(5.3 – 79.1)	(4.1 – 13.7)	(5.3 – 69.8)	(3.4 – 9.3)	(5.4 – 21.3)
Mg (mg kg^{-1})	4.33 ± 3.23	8.07 ± 4.98	2.33 ± 1.14	9.29 ± 5.05	1.84 ± 1.23	2.74 ± 1.05
	(2.1 – 12.4)	(2.4 – 14.9)	(0.3 – 4.2)	(2.6 – 16.1)	(1.6 – 2.1)	(2.6 – 2.9)

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Table. 4. Metal pollution index values (MPI) and concentrations (mg kg[-1]) of eleven examined trace elements (mean ± SD and range) in muscle of six fish species collected from Skadar Lake

	Scardinius knezevici	*Alburnus scoranza*	*Cyprinus carpio*	*Rutilus prespensis*	*Anguilla anguilla*	*Perca fluviatilis*
MPI	0.211	0.284	0.233	0.313	0.247	0.169
Hg	0.118 ± 0.037 (0.059 – 0.170)	0.096 ± 0.040 (0.064 – 0.146)	0.112 ± 0.040 (0.034 – 0.187)	0.099 ± 0.040 (0.054 – 0.143)	0.138 ± 0.035 (0.114 – 0.206)	0.121 ± 0.019 (0.102 – 0.141)
Pb	0.114 ± 0.002 (<0.1 – 0.115)	<0.1	0.112* <0.1 – 0.112	<0.1	<0.1	<0.1
Cd	0.018 ± 0.002 (<0.01 – 0.020)	0.020 ± 0.001 (<0.01-0.021)	0.012* <0.01 – 0.012	<0.01	<0.01	<0.01
As	0.091 ± 0.003 (<0.06 – 0.13)	0.098 ± 0.074 (<0.06 – 0.20)	<0.06	0.062 ± 0.049 (<0.06 – 0.15)	0.288 ± 0.069 (0.17 – 0.38)	<0.06
Cu	0.64 ± 0.19 (0.29 – 0.86)	0.61 ± 0.24 (0.30 – 0.93)	0.58 ± 0.24 (0.27 – 1.16)	0.57 ± 0.26 (0.38 – 0.92)	0.84 ± 0.12 (0.70 – 1.00)	0.81 ± 0.05 (0.76 – 0.85)
Zn	16.29 ± 11.40 (6.20 – 42.17)	26.12 ± 7.91 (11.02 – 32.78)	20.88 ± 7.25 (8.51 – 28.73)	19.47 ± 10.71 (7.77 – 34.77)	15.088 ± 2.142 (12.70 – 19.00)	5.83 ± 1.46 (4.30 – 7.20)
Fe	5.66 ± 1.36 (4.30 – 8.85)	7.90 ± 1.23 (5.98 – 10.01)	6.95 ± 1.96 (2.62 – 10.01)	14.48 ± 7.41 (6.10 – 23.36)	3.23 ± 0.778 (2.40 – 4.30)	4.77 ± 0.21 (4.60 – 5.01)
Mn	1.03 ± 1.12 (0.12 – 3.53)	1.76 ± 0.69 (0.30 – 2.43)	0.20 ± 0.08 (0.12 – 0.46)	5.12 ± 3.96 (0.37 – 9.40)	0.64 ± 0.071 (0.55 – 0.72)	0.22 ± 0.05 (0.18 – 0.27)
Ni	0.032 ± 0.009 (0.02 – 0.05)	0.028 ± 0.006 (0.02 – 0.04)	0.152 ± 0.18 (0.03 – 0.67)	0.041 ± 0.015 (0.02 – 0.06)	0.038 ± 0.027 (0.02 – 0.10)	0.071 ± 0.012 (0.06 – 0.08)
Cr	0.074 ± 0.048 (0.025 – 0.106)	0.12 ± 0.06 (0.06 – 0.24)	0.078 ± 0.064 (0.025 – 0.217)	0.178 ± 0.106 (0.070 – 0.31)	0.069 ± 0.21 (0.05 – 0.102)	0.063 ± 0.023 (0.05 – 0.09)
Co	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025

* Single sample value

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Figure 1. Location map of the Skadar Lake in Montenegro (locality Radus is marked with "**x**")