Age and diet-specific trace element accumulation patterns in different tissues of chub (*Squalius cephalus*): juveniles are useful bioindicators of recent pollution

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Highlights

- Three different feeding groups of chub from the River Szamos were analyzed.
- Feeding groups were differed based on the trace element patterns of tissue.
- Generally, the highest concentrations of elements were found in the juveniles.
- Only the hepatic copper concentrations showed positive correlation with fish age.
- The juveniles of chub can be used as a bioindicators of the recent metal pollution.

Abstract

Chub (*Squalius cephalus* L. 1758) specimens of three age groups with different types of diet were collected in November 2013 in the River Szamos/Someş, Hungary. The Ca, K, Mg, Na, Cd, Cr, Cu, Fe, Mn, Pb, Sr and Zn concentrations were analyzed in the muscle, gills and liver samples of chub by microwave assisted plasma-atomic emission spectrometry (MP-AES). The Kruskal–Wallis test revealed significant differences among different age groups based on the trace element concentrations in the liver, muscle and gills. The trace element concentration pattern in muscle and liver of different age groups differed, may be according to the different diet types of the groups. Meanwhile no differentiation among the age groups based on the trace element concentration in the gills was observed, probably because the pattern of trace elements in the gills is related to the habitat preference, which does not differ during the life-span of chub. In contrast to expectations, trace element concentrations in juveniles were the highest in most cases, certainly because of their specific diet, relatively fast metabolic rate and inadequately developed detoxification system. Only the copper concentrations in liver increased with fish age. Considerable concentrations of trace elements in the tissues of juveniles were observed in the case of elements whose concentrations in the River Szamos were higher in 2013 than in previous years. According to this phenomenon, trace element patterns in the tissues of juveniles may be good indicators of recent pollution of watercourses.

Keywords: Fish, Muscle, Liver, Gills, Heavy metals, MP-AES

Introduction

Nowadays, the heavy metal pollution of aquatic ecosystems is a burning issue all over the world, mainly due to these elements' toxicity, long persistence, bioaccumulation and biomagnification in the food web (Miracle and Ankley, 2005; Carrasco et al., 2011, Carneiro et al., 2014). Two sources of heavy metals are in water ecosystems: the natural geological background and anthropogenic activities, such as industrial and agricultural emissions and atmospheric deposition (MacDonald et al., 2000; Lenhardt et al., 2009). Since trace elements can be accumulated in living organisms in different concentrations and their bioavailability is not equal, systematic monitoring of the concentrations of pollutants in organisms to predict environmental risks should be given priority (Nakata et al., 2005; Zhou et al., 2008; Rašković et al., 2018).

Fish communities are widely used in monitoring impacts of aquatic contaminations (Hermenean et al., 2015; Jia et al., 2016; Liu et al., 2016). Fish living in polluted waters can take up various amounts of trace elements by several routes; i.e. through the skin or gills, or into the digestive system by feeding (Yilmaz et al., 2007; Lenhardt et al., 2012). Metal accumulation in fish depends on both the chemical properties of the elements and different factors such as ecological needs, and the physiological state, size and age of individuals, as well as their life cycle, life history and feeding habits (Newman and Doubet, 1989; Canpolat and Çalta, 2003; Zhang and Wong, 2007; Lenhardt et al., 2015).

The accumulated trace element patterns are generally different among the different tissues, primarily due to the various physiological features (Subotić et al., 2013a, 2013b). Gills and liver, as metabolically active organs, are target organs for trace element accumulation (Yilmaz et al., 2007; Lenhardt et al., 2012). Therefore gills and liver are frequently used as target organs for metal accumulation assessments (Djikanović et al., 2016; Jia et al., 2017). Generally, the trace element accumulation in muscle tissue is lower; but fish muscle is the main part of the fish consumed by humans (Jia et al., 2017; Subotić et al., 2013a). For this reason, monitoring the trace element pattern of fish muscle is equally important (Yancheva et al., 2015, Traina et al., 2019).

Chub [*Squalius cephalus* (Linnaeus, 1758) previously *Leuciscus cephalus*] is one of the most common and widespread cyprinid species in Europe and is a very popular game fish (Kottelat and Freyhof, 2007). Chub is most abundant in small rivers and large streams, but it also occurs in slow-flowing lowland rivers and very small mountain streams (Kottelat and Freyhof, 2007). Adults are predominantly piscivorous predators of freshwater ecosystems (Czeglédi and Erős, 2013). Chub is a generalist fish species; it also survives in more polluted water (Machala et al., 2001; Dragun et al., 2012; 2016). Therefore, chub is increasingly used to assess the contamination levels of water bodies and give relevant information regarding the endangered status (vulnerability) of aquatic organisms (e.g. Dragun et al., 2007; Triebskorn et al., 2007; Yılmaz et al., 2007; Krasnići et al., 2013).

In recent years, considerable research has focused on the concentrations of trace elements in adult fish (e.g. Subotić et al., 2013a, 2013b; Hermenean et al., 2015; Djikanović et al., 2018). However, the accumulation and distribution of trace elements in fish tissues are influenced by size, age and feeding behavior (Jia et al., 2017; Ndimele et al., 2017). The diet of a fish species can alter considerably during its life-span; therefore the pattern of accumulated trace elements can vary among the different age groups of the same species (Handy et al., 2003;

Dragun et al., 2009; Subotić et al., 2013b). During the ontogenesis of the chub, there is a rather pronounced diet shift: there are three different types of diet (Balestrieri et al., 2006; Marković et al., 2007; Sasi and Ozay, 2017). Chub juveniles (age 0+) have a plankto-phytophage diet; they feed mainly on diatoms, green algae, and zooplankton (Marković et al., 2007). The older, sexually immature chub (age 1+, 2+) have a plankto-phyto-zoophage diet, feeding on diatoms, green algae, and macrophytes, and also on animals, such as larvae and imagines of Odonata, Trichoptera, Diptera and Ephemerophtera insects (Marković et al., 2007). The sexually mature adult chub (age 3+, and thereafter) have a mainly carnivorous diet. They prey predominantly on fish, but also feed on insects, molluscs, and amphibians (Ünver and Erk'akan, 2011). These significant shifts in diet can lead to different bioaccumulation and biomagnification features in the different age groups of chub (Merciai et al., 2014). Furthermore, several studies have highlighted that trace elements can be accumulated in the tissues of juveniles in the highest concentrations as a result of various mechanisms: e.g. the specific diet, the higher relative metabolic rate, and the inadequately developed detoxification system of young specimens (Merciai et al., 2014; Jia et al., 2017; Ndimele et al., 2017). Therefore, the pollution indicator capability of different age groups of the same species can be different (Jia et al., 2017).

The River Szamos (Romanian: Someş) is one of the most polluted rivers in Europe (Kraft et al., 2003; 2006). In early 2000, both its water and a huge amount of sediment were contaminated with cyanide and heavy metals entering the River Szamos (Lakatos et al., 2003). These events caused an ecological disaster in the River Szamos and in its recipient watercourse, the River Tisza; moreover the sediment of these rivers were strongly enriched by contaminants compared to the local geochemical background concentrations (Van der Veen et al., 2002; Fleit and Lakatos, 2003; Kraft et al., 2003; Lakatos et al., 2003). Thereafter, trace element concentrations decreased in both the Szamos and the Tisza (Óvári et al., 2004). However, recent pollution of the River Szamos with Pb, Cd, Cu and Zn has occurred due to intensive mining activities in Romania (Simon et al., 2017).

In this paper, we analyzed concentrations of twelve elements in the muscle, gills and liver of different age groups of chub living in the River Szamos. Different trace element concentrations in varying age groups of chub were hypothesised, because of their different type of diet and other physiological features. It was also hypothesised that trace element concentration would be higher in older groups than in younger ones, due to the bioaccumulation and biomagnification of these elements. Diverse patterns of accumulated trace elements in the chub groups which differed by age, size, and diet were analyzed to test these hypotheses; at the same time, the potential risks of contaminated fish consumption on human health were evaluated. Furthermore, this study aims to understand which age group of chub performs better as a bioindicator of the trace element contamination of freshwater ecosystems.

Material and methods

Study area and sample collection

The River Szamos is the second largest tributary of the River Tisza. Its catchment basin collects waters over a 15 882 km^2 area of Romania and Hungary. Its total length is 415 km, but the Hungarian section is only 50 km long. Fish were caught at the village of Csenger, near the Hungarian-Romanian border, where the water depth varies between a few centimeters to 2-3 meters, and the main sediment types are pebbles and sand. The coordinates of the sampling site are 47°50'17.89"N, 22°41'37.48"E (Fig. 1).

A total of 33 chub specimens were collected in November 2013 by electrofishing (Hans Grassl IG200/2b, PDC, 75–100 Hz, 350–650 V, max. 10 kW, Hans Grassl GmbH, Germany). All chub specimens were captured alive and killed immediately by spinal severance. Measurements of the standard length (SL) and body weight (W) of each specimen were made to the nearest 0.1 mm and 0.01 g, respectively. Samples were stored at -18 °C until sample processing. We confirm that all procedures were performed in compliance with the relevant laws and institutional guidelines, and that the appropriate institutional committees have approved them (permission number: HBH/01/00971-2/2013).

Scales were used to determine the age of each specimen. Scale samples were taken below the dorsal fin, from the area above the lateral line (Tesch, 1968). Preparations of scales were assessed using a binocular microscope (10× magnification). Age assessment was applied in accordance with Tesch (1968). Age estimations were validated by a second independent assessment.

The feeding groups

Several studies showed that there are three different types of diet of chub, depending on their age (Balestrieri et al., 2006; Marković et al., 2007; Caffrey et al., 2008). Therefore, three different feeding groups of chub were created. Each specimen was assigned to one of the three different feeding groups, according to age and size:

First feeding group: the juveniles (age 0+; N = 8);

Second feeding group: the sexually immature individuals (age 1+ and 2+; N = 16);

Third feeding group: the sexually matured adults (age 3+ onwards; N = 9).

Sample processing and element analysis

Muscle, gills and liver samples were removed with plastic tools in order to avoid any metal contamination of the samples. Gills were sampled by removing the whole branchial apparatus and collecting the second gill arch from the left side. The dissected samples were then rinsed with double deionised water (Milli-Q) and were weighed into glass beakers using an analytical balance (Precisa 240A). They were dried overnight at 105 °C until reaching constant weight and reweighed to determine their dry mass. Samples were digested on an electric hot plate using 4.0 mL 65% (m/m) nitric acid (reagent grade, Merck) and 1.0 mL 30% (m/m) hydrogen-peroxide (reagent grade, Merck) in the same container at 80 °C for 4 h. Digested samples were diluted with 1 % (m/m) nitric acid (reagent grade, Merck and Milli-Q water) up to a final volume of 10 mL (Braun et al., 2009; 2012). The concentrations of Ca, K, Mg, Na, Cd, Cr, Cu, Fe, Mn, Pb, Sr and Zn were measured by a microwave plasma-atomic emission spectrometer (MP-AES 4100, Agilent Technologies) system. The values of the limits of detection (LOD) in µg l^{-1} were: Ca, 0.070; K, 0.270; Mg, 0.080; Na, 0.484; Cd, 1.863; Cr, 0.489; Cu, 0.414; Fe, 0.856; Mn 0.057; Pb, 1.235; Sr, 0.051; and Zn, 3.125, respectively. The limit of detection of each sample was verified based on the instrumental detection limit, sample mass and volume to which it had been diluted. The average detection limits for each of the assessed elements were (mg kg^{-1} wet weight): Ca, <0.001; K, 0.002; Mg, 0.001; Na, 0.004; Cd, 0.009; Cr, 0.004; Cu, 0.003; Fe, 0.006; Mn <0.001; Pb, 0.009; Sr, <0.001; and Zn, 0.023, respectively. The following wavelength lines of the MP-AES analysis were used: Ca 422.673 nm, K 766.491 nm, Mg 285.213 nm, Na 588.995 nm, Cd 228.802 nm, Cr 425.433 nm, Cu 324.754 nm, Fe 371.993 nm, Mn 403.076 nm, Pb 405.781 nm, Sr 407.771 nm, and Zn 481.053 nm.

An auto sampler (Agilent SPS3), a Meinhard type nebulizer and a double pass spray chamber were used. We applied a five-point calibration procedure prepared from the multi-element standard solution (Merck ICP multi-element standard solution IV). Certified reference material was used (ERM-BB422, fish muscle) during the measurement. The recoveries were within the 10% of the certified values for the metals. The wavelengths and measuring parameters were chosen on the basis of suggestions provided by the instrument’s software (MP Expert). Concentrations of all elements were expressed as mg kg^{-1} wet weight (ww).

Bioconcentration factor and metal pollution index

The bioconcentration factor (BCF) of a chemical compound is defined as the ratio between the concentration of that chemical in an organism (or in a certain tissue of the organism) and the concentration of the chemical in the aqueous environment (Ivanciuc et al., 2006):

$$BCF = C_{fish}/C_{water},$$

where C_{fish} means the concentration of the chemical in the whole body or tissue of the organism, expressed as mg kg^{-1} wet weight, and C_{water} is the concentration of the chemical in the aqueous environment in which the respective organism lives, expressed as mg l^{-1}. Data relating to concentrations of the trace elements of water were obtained from the data base of the National Environmental Information System of Hungary (OKIR in Hungarian). These were compared with criterion chronic concentrations (CCCs) for freshwater of the National Recommended Water Quality Criteria (USEPA, 2017).

Metal pollution index (MPI) was assessed to compare the total content of trace elements excluding the macroelements (Cd, Cr, Cu, Fe, Mn, Pb, Sr, Zn) for the different tissues of feeding groups of chub. The MPI formula is the following (Usero et al., 1997; Ju et al., 2017):

$$MPI = (C_1 \times C_2 \times C_3 \times \ldots C_n)^{1/n},$$

where C_n is the mean concentration of trace element *n* in the analyzed tissue (mg kg^{-1} wet weight).

Statistical analysis

IBM SPSS Statistics for Windows (Version 20.0) (IBM, 2011) and Past 3.03 (Hammer et al., 2001) software packages were used for statistical analysis. The normal distribution was tested with the Shapiro-Wilk test. The homogeneity of variances was tested with the Levene's test. Since the variables lacked a normality of distribution (Shapiro-Wilk test, $p < 0.05$), the non-parametric Kruskal–Wallis test was used to display the differences among the concentrations and BCF values of elements in three different tissues (muscle, gills, and liver) of three feeding groups of chub. LSD Multiple Comparison test as a post hoc test was used to explore the significant differences between the groups. Spearman's non-parametric correlation test was used to check for significant relationships between trace element concentrations and feeding groups of chub. Principal component analysis (PCA) was used to assess the differentiation of feeding groups among the analyzed fish tissues, based on the concentration of trace elements (Cd, Cr, Cu, Fe, Mn, Pb, Sr, Zn), except for the macroelements.

To evaluate the health risks of chub for human consumption, trace element concentrations of muscle (fillet) were compared with the maximum acceptable concentrations (MACs) set by the European Union (EU, 2008) and the Food and Agriculture Organization of the United Nations (FAO, 1983).

Results

Age analysis

Age analysis indicated that fish belonged to age classes from 0+ to 4+. The average standard length and weight of the analyzed chub specimens per feeding group are shown in Table 1. The Kruskal–Wallis test revealed significant differences (standard length: H = 27.27, $p < 0.001$; weight: H = 27.27, $p < 0.001$) in mean standard length and mean weight among the three feeding groups.

Characterization of the study area

The descriptive statistics of the concentrations of elements in the water, obtained from the the data base of the National Environmental Information System of Hungary (OKIR in Hungarian) are presented in Table 2. The mean concentrations of Cu, Pb and Zn were above the criterion chronic concentrations (CCCs) for freshwater of National Recommended Water Quality Criteria prescribed by the USEPA (2017) (Table 2).

Macroelements

Mean values of the concentrations of macroelements in muscle, gills and liver are presented in Table 3. The highest concentrations of Ca, Mg and Na were observed in gills, while the highest concentrations of K were measured in muscle.

The Kruskal–Wallis test revealed significant differences among the feeding groups with regard to element concentrations in muscle for Ca, K and Mg ($p < 0.05$), in gills for Ca, K, Mg and Na ($p < 0.05$), and in liver for K, Mg and Na ($p < 0.05$). The LSD Multiple Comparison test revealed that the concentrations of these elements were significantly lower ($p < 0.05$) in the case of juveniles than in older groups (Table 3).

There was a significant ($p < 0.05$) increase in concentrations of certain macroelements with fish age (Table 4). Both K and Mg concentrations in all examined tissues, and Na concentrations in gills and liver, were positively correlated with fish age (Table 4).

Essential and non-essential trace elements

Mean values of the concentrations of the trace elements in muscle, gills and liver are shown in Table 3. The concentration of Cd in the muscle of the oldest group (age 3+≥) was below the detection limit. The highest concentrations of Cd, Cr, Cu, Fe and Pb were detected in liver, while Mn, Sr and Zn reached the highest concentrations in gills. The lowest concentrations of most of the studied trace elements were observed in the muscle tissue.

The Kruskal–Wallis test revealed significant differences among the feeding groups with regard to trace element concentrations in muscle for Cr, Cu, Fe, Mn, Pb, Sr and Zn ($p < 0.05$), in gills for Cr and Pb ($p < 0.05$), and in liver for Cr, Cu, Pb and Sr ($p < 0.05$).

The LSD Multiple Comparison test indicated that concentrations of Cr, Fe, Pb, Sr and Zn in muscle were significantly higher in the cases of juveniles than in older groups ($p < 0.05$) (Table 3). The highest concentrations of Pb in gills and liver were also observed in fish from the youngest age class (0+) (LSD Multiple Comparison test, $p < 0.05$) (Table 3). The Cu concentration measured in the liver was significantly higher in the oldest group (3+≥) than in the younger groups (LSD Multiple Comparison test, $p < 0.05$).

In the case of trace elements, only the concentrations of Cu in the liver showed a significant increase with fish age ($p < 0.05$) (Table 4). On the contrary, opposite trend was observed for some trace elements, which had a significant trend, showing decreasing concentrations with age ($p < 0.05$). We experienced a significant ($p < 0.05$) decrease in concentrations of Pb with fish age, in all studied tissues (Table 4). In addition, Cr, Fe, Mn and Zn concentrations in muscle were negatively correlated with fish age (Table 4).

The PCA showed separations among the different feeding groups of chub based on trace element concentrations in the muscle (Fig. 2) and the liver (Fig. 3). In the case of muscle, the first component (PCA 1) contributed 88.03% of the total variance, while the second (PCA 2) contributed 7.34% of the total variance. Based on the trace element pattern, juveniles were completely separated from the other groups (Fig. 2). The PCA showed less separation between the 1+ year-old, 2+ year-old and older chub (age 3+ onward) (Fig. 2).

According to trace element concentrations in liver, the PCA showed less differentiation among the different feeding groups (Fig. 3); the first component (PCA 1) contributed 36.56% of the total variance, while the second (PCA 2) contributed 31.19% of the total variance. Juveniles were clearly separated from the other groups, especially from the older chub (age 3+ onward) (Fig. 3). The 1+ and 2+ year-old chub showed notable overlaps with other groups (Fig. 3). There were no notable separations among the feeding groups based on the trace element concentration in the gills, according to the PCA.

Bioconcentration factor and metal pollution index

The bioconcentration factor values are given in Table 5. The highest BCF values for Cd, Cr, Cu, Fe and Pb were observed in the cases of liver tissues. The highest BCF values for Mn and Zn were recorded in gills. The BCF values also showed that for most of the trace elements accumulation was more higher in the tissues of juveniles.

The highest MPI values were observed in juveniles in all examined tissues (Fig. 4).

Human health implications

MACs set by the European Union (EU) for Cd and Pb in fish meat are 0.05 and 0.30 mg kg^{-1} ww, respectively. MACs prescribed by the FAO (1983) for Cr, Cu, Fe, Mn and Zn in fish meat are 1.0, 30.0, 43.0, 1.0 and 40.0 mg kg^{-1} ww, respectively.

Both the concentrations of Cd in juveniles (0+) and the 1+, 2+ age groups, and the concentrations of Pb in juveniles (0+) were over the limit permitted by the EU (EU 2008) in the muscle samples. The concentrations of the other analyzed elements were below the MAC levels prescribed by the EU (2008) and the FAO (FAO, 1983) in all of the muscle samples.

4. Discussion

Both our results and earlier studies have demonstrated that metals deriving from industrial, mining and agricultural activities have serious impacts in the River Szamos. Woelfl et al. (2006), Málnás et al. (2014) and Simon et al. (2017) reported that the contamination level of Szamos is moderately with trace elements, especially with Cu, Cr, Mn, Pb, Sr, and Zn than adjacent rivers. At the same time, our present study and the water chemistry data of the National Environmental Information System of Hungary have also revealed continuous pollution of the River Szamos. Moreover, Cu, Pb and Zn concentrations in the water were above the criteria for chronic concentrations set by the USEPA (2017), indicating a considerable degree of contamination with these elements.

The distribution of macroelements (Ca, K, Mg, Na) were found according to their functions in the organism. Generally, the lowest concentrations of macroelements were in the tissues of juveniles, because of various physiological features (Venugopal and Shahidi, 1996; Uysal et al., 2008). For example, in the case of Ca and Mg

- elements which accumulate mainly in the bony structures of gills (e.g. arches and rakers) (Mayer-Gostan et al., 1983; Playle, 1998) - lower concentrations of these elements were observed in the gills of juveniles. This is related to ontogenetic development features, namely that the bones of juveniles were not fully developed (Witten et al., 2001). Similar results were found in a study based on laboratory experiments (Harangi et al., 2016).

Seventeen trace elements are required by some terrestrial and aquatic organisms (Yılmaz et al., 2017), such as Cu, Cr, Fe, Mn, and Zn. For organisms trace amounts of these elements are essential for normal growth and development. However, at higher levels of exposure and adsorption all trace elements can potentially be harmful for most aquatic organisms (Yılmaz et al., 2017). Contrary to our hypothesis, only the Cu concentrations in the liver showed an increase with fish age. Higher Cu concentrations in the liver of older chub (age 3+ onward) may be the result of their predominantly piscivorous feeding (Djedjibegovic et al., 2012). Milošković et al. (2016) also revealed a higher concentration of Cu in the liver of older chub, which is probably caused by the biomagnification features of Cu. In the muscle of chub Cr, Fe, Mn and Zn were negatively correlated with fish age, and differed significantly among feeding groups. Earlier studies demonstrated a considerable degree of continuous pollution of these elements along the River Szamos (e.g. Óvári et al., 2004; Woelfl et al., 2006, Málnás et al., 2014, Simon et al., 2017). According to the water chemistry data of the National Environmental Information System of Hungary, there are recent contaminations involving Cr, Fe and Zn, because concentrations of these elements were about 2–10 times higher than they had been in previous years. Similarly to our results, negative correlations between Mn and Zn concentrations in muscle and fish size/age were also found in the study by Djikanović et al. (2016), in the case of nase (*Chondrostoma nasus*) from the Medjuvršje Reservoir (Republic of Serbia). The Medjuvršje Reservoir had been contaminated by Mn and Zn in the year of the study (Djikanović et al., 2016), which implies a potential bioindicator capacity of juvenile fish in monitoring recent environmental trace element pollution events.

Strontium (Sr) is a rarely investigated element in fish. The beneficial effects of Sr are questionable, but it can also be bioaccumulated in the body, and can replace Ca in the organism. Woelfl et al. (2006), Málnás et al. (2014) and Simon et al. (2017) also reported that the River Szamos is contaminated with a high amount of Sr which may beoriginated mainly from agricultural activities. The highest concentrations of Sr were measured in the gills of chub. Farrel and Campana (1996) found that water was a more important source of bioaccumulation for Sr than consumed food. Dragun et al. (2016) observed that the Sr concentrations in the soluble gill fractions of chub have not reflected the exposure level in water, because Sr mainly accumulated in the bony structures of gills (e.g. arches and rakers) by Ca replacement, rather than in soft tissues (e.g. soluble gill fractions) (Hermenean et al., 2017).

Neither vital nor beneficial effects caused by non-essential trace elements on organisms have been established yet; these elements (e.g. Cd and Pb) are toxic to all organisms at low concentrations (Eisler, 1985). In all examined tissues of chub, Pb was negatively correlated with fish age, and its concentration in juveniles was significantly higher than that of older groups. Similar results were found in different fish species by several authors (e.g. Demirak et al., 2006; Merciai et al., 2014; Ndimele et al., 2017). The occurrence of higher concentrations of Pb in juveniles could be assigned to both high metabolic rate and inadequately developed toxic metal neutralization mechanisms in younger fish (Kljaković Gašpić et al., 2002; Jia et al., 2017). The higher Pb concentration in juveniles might be also caused by a recent pollution event. The Pb concentration of the water of the Szamos was approximately 14 times higher in 2013 than that in previous years, based on data from the National Environmental Information System of Hungary. This recent pollution incident affected all fish, but that effect on juveniles was the most pronounced due to the phenomena mentioned above. According to this phenomenon, the microchemistry pattern of the tissues of juveniles can be used as a bioindicator of recent pollution events, not only for essential, but also for non-essential trace elements.

Earlier studies and our results also showed that muscle is the tissue with the lowest concentration of the majority of trace elements (Djikanović et al., 2016; Jia et al., 2017), respectively. According this phenomenon, fish muscle is not sufficient to indicate trace elemental contamination in the whole fish body, due to its lower accumulation potential (Jia et al., 2017). In spite of this, in recent years, a considerable number of studies on trace element contamination in fish muscle have been published, since fish muscle is the main part of the fish consumed by humans (Jia et al., 2017; Subotić et al., 2013a, 2013b; Yancheva et al., 2015). Furthermore, this present study demonstrated that the trace element pattern of muscle is the most variable among the different age groups of fish, as a results of several mechanisms (e.g. specific diet). Therefore, monitoring the trace element pattern of fish muscle is equally important.

Although the accumulation of trace elements in muscle tissue is also relevant, the main target tissue of the bioaccumulation of trace elements is the main detoxifying organ, the liver (Dragun et al., 2012; Lenhardt et al., 2012). Once the trace elements cross biological barriers and enter into the bloodstream, they will reach the liver and will accumulate there (Yancheva et al., 2015); therefore, fish liver is the most important bioindicator organ of water contamination (Jovanović et al., 2011). Concentrations and BCF values of the majority of examined trace elements (Cd, Cr, Cu, Fe and Pb) were the highest in the liver, which implied that an examination of the liver is also important. In spite of this, trace element concentrations in the liver did not differ among the feeding groups of chub, as much as they did in the case of muscle.

A few differences in the trace element concentrations were observed in gills among the feeding groups, as the pattern of trace elements in gills is accounted for by the habitat preference (Subotić et al., 2013a). Although the diet of chub alters during its life-span, the habitat preference is permanent, because chub is a pelagic fish species of riverine habitats (Kottelat and Freyhof, 2007). The highest concentrations and BCF values of Mn, Sr and Zn were observed in gill samples because these elements are mainly taken up from water via gills, rather than by feeding (Jia et al., 2017; Subotić et al., 2013a, 2013b). This phenomenon indicated that an examination of gills is also important in monitoring accumulated trace elements in fish.

We hypothesized that trace element concentrations in different feeding groups of chub may be different. This hypothesis was confirmed: the trace element concentration pattern of different feeding groups of chub differed, especially in the cases of muscle and liver. We also hypothesized that the trace element concentrations would be higher in older groups than in younger ones. However, this hypothesis was not confirmed by the results: concentrations of most trace elements were higher in juveniles, and the concentrations of certain elements also showed negative correlations with fish age, especially in the case of muscle. Higher trace element concentrations in juveniles, and therefore a significant negative correlation between fish size and accumulated element concentrations, have also been reported by several authors (Merciai et al., 2014; Jia et al., 2017; Ndimele et al., 2017). There are several mechanisms which could cause this phenomenon. Chub is a relatively large fish species, and its growth is relatively fast in the first years (Vlach et al., 2005), therefore tissue growth could be more rapid than trace element intake (Ndimele et al., 2017). On the one hand, several authors hypothesized that the lipid content of tissues has a relative dilution effect (Braune et al., 1999; Farkas et al., 2003), therefore the lower fat content of juveniles tissue could cause higher trace element concentrations in their body than is the case with adults (Merciai et al., 2014). On the other hand, lipid as a percentage of body weight reaches its peak at the end of the main feeding period – autumn - and so in November this relative dilution effect in adults can also explain this phenomenon (Farkas et al., 2003). Furthermore, the metabolic rate of fish is size-specific and the relative metabolic rate of juveniles is higher (Newman and Doubet, 1989), therefore both the food intake and the relative quantity of respiratory water passing through the gills (as the main sources of trace elements) are higher in juveniles. Higher trace element concentrations in juveniles may also occur as a result of the inadequately developed detoxification mechanisms in young fish (Ndimele et al., 2017). The specific diets of different feeding groups of chub are also important. Juveniles feed mainly – and continuously - on phyto- and zooplankton, and according to published data, high amounts of trace elements can accumulate in green algae and diatoms (Bácsi et al., 2015; González-Dávila et al., 2000; Novák et al., 2014). According to data from the National Environmental Information System of Hungary, the trace element pollution in the River Szamos was higher in 2013 than in the previous years, especially in the case of Cr, Fe, Pb and Zn. Furthermore, the relative trace element exposure of juveniles was higher than that of adults due mainly to higher metabolic rate. In addition, other factors could also have influenced this phenomenon, e.g. changes in body surface-volume ratio during growth, or even faster short-term uptake by juveniles (Merciai et al., 2014; Jia et al., 2017). Finally, many of these factors are likely to interact (Merciai et al., 2014). According to the phenomena mentioned above, the bioaccumulation patterns of trace elements in juveniles can be utilized as an effective bioindicator of recent environmental trace element contamination (Jia et al., 2017). A recent pollution event can cause a relatively higher exposure over the entire life-span of juveniles, than is the case in older specimens (Merciai et al., 2014). Moreover, juvenile chub are primary/secondary consumers in the aquatic food web, because they feed mainly on phytoplankton and zooplankton, therefore trace elements can be accumulated earlier in their organs than is the case in piscivorous, tertiary consuming adults (Croteau et al., 2005).

In the case of muscle, the mean concentrations of Cd and Pb in juveniles were over the prescribed MACs. The trace element concentrations in the muscle of larger specimens did not exceed MACs, and the minimum size limit of chub in Hungary is 25 cm standard length, so the consumption of fillets of older specimens does not seem to be harmful. Nevertheless, higher concentrations of Cd and Pb in the muscle of younger (age 0+, 1+, and 2+) specimens require further examinations. It can be dangerous to both the ecosystem (e.g. due to biomagnifications) and to human health (e.g. due to poaching), thus the regular consumption of chub from the River Szamos can be potentially hazardous for human health. Trace element concentrations in the gills and liver exceeded the prescribed MACs several fold in the cases of all feeding groups; consequently, consumption of the inner organs (e.g. gill and liver) of chub from the River Szamos is not recommended, according to prescribed MACs.

Conclusions

In conclusion, the results of the present study indicate a significant differentiation among the feeding groups of chub based on metal accumulation patterns. The differences observed could be explained by the specific physiological characteristics and diets of different age classes of chub.

Contrary to our hypothesis, the highest trace element concentrations were measured in juveniles. The reasons for this phenomenon could be found in various mechanisms: e.g. the specific diet, higher relative metabolic rate, and inadequately developed detoxification system of young specimens. Consequently, the prescribed MACs were exceeded mainly in juveniles. Our results also show that the trace element pattern of juveniles was a good indicator of the recent pollution events of the river in question, because all environmental factors (such as trace element contaminations) which had an effect on the young of the year chubs over a season, were integrated in their different tissues. Based on the investigation of trace element pattern of juvenile chubs in 2013, we detected recent contaminations in the cases of several elements, which are also confirmed by water chemistry data from the National Environmental Information System of Hungary.

Trace element concentrations in fillets of adult chub, which can be considered for consumption by humans, did not exceed the prescribed MACs, but inner organs (e.g. gills and liver) contained higher concentrations of trace elements than the prescribed MACs in several cases. Therefore, consumption of the inner organs of chub living in the River Szamos is not recommended.

Chub is one of the most abundant fish species in relatively fast flowing sub-mountain, highland and lowland streams and rivers; it has an important role in the food web and angling of these habitats, and furthermore, it is widespread in the rivers of Europe. In view of these facts, and following our results, the juveniles of chub can be used as a widespread, effective bioindicators in monitoring the recent trace element pollution of aquatic ecosystems. However, further studies are needed to identify such patterns in other habitats.

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Table 1

Number of sampled individuals (n) of chub (*Squalius cephalus*) caught in the River Szamos/Someş, and their minimum, maximum, mean and standard deviation of standard length (SL, mm) and weight (W, g) per feeding group.

Feeding group	n	SL ± SD (mm)	Min.	Max.	W ± SD (g)	Min.	Max.
Age 0+	8	65.8 ± 8.2	54.2	75.5	5.11 ± 1.73	2.49	6.97
Age 1+, 2+	16	119.2 ± 18.0	93.6	140.5	34.09 ± 15.23	15.64	53.19
Age 3+≥	9	171.9 ± 17.3	152.0	212.3	99.93 ± 30.50	65.54	169.55

Table 2

Descriptive statistical data of total trace element concentrations in water from the River Szamos/Someş obtained from the the data base of the National Environmental Information System of Hungary (OKIR in Hungarian).

	Trace element concentration (μg l^{-1})			
Element	**Min**	**Max**	**Mean ± SD**	**Threshold values (μg l^{-1})[a]**
Cd	0.2	1.4	0.58 ± 0.44	0.72
Cr	1	12	3.18 ± 3.68	74
Cu	5	20	9.47 ± 5.72*	3.1
Fe	45	658	365.78 ± 226.59	1000
Mn	1.8	388	145.98 ± 110.72	–
Pb	3	20	6.92 ± 6.25*	2.5
Zn	1	270	136.22 ± 93.55*	120

[a] Criterion chronic concentrations (CCCs) for freshwater of National Recommended Water Quality Criteria (USEPA 2017)

* Mean concentration of trace element in the water was higher than the threshold value.

Table 3

Concentrations of trace elements in the muscle, gills, and livers (in mg kg^{-1} wet weight) of different feeding groups of chub in the River Szamos/Someş (mean value ± standard deviation).

Tissue	Element	Age 0+	Age 1+, 2+	Age 3+≥
Muscle	**Ca**	150.70 ± 156.86^{a}	307.07 ± 89.64^{b}	263.30 ± 114.23^{b}
	K	1063.41 ± 1130.07^{a}	2745.29 ± 154.38^{b}	3070.33 ± 227.90^{c}
	Mg	105.29 ± 129.10^{a}	236.26 ± 15.36^{b}	264.11 ± 33.99^{c}
	Na	185.55 ± 207.07^{a}	363.83 ± 71.76^{a}	320.63 ± 41.11^{a}
	Cd	0.12 ± 0.19^{a}	0.05 ± 0.09^{a}	BDL
	Cr	0.27 ± 0.34^{a}	0.07 ± 0.01^{b}	0.06 ± 0.01^{b}
	Cu	0.33 ± 0.13^{a}	0.16 ± 0.06^{b}	0.25 ± 0.10^{a,b}
	Fe	8.27 ± 3.19^{a}	1.62 ± 0.54^{b}	1.58 ± 0.77^{b}
	Mn	0.33 ± 0.16^{a}	0.25 ± 0.07^{a}	0.17 ± 0.11^{b}
	Pb	0.30 ± 0.25^{a}	0.02 ± 0.01^{b}	0.01 ± 0.01^{c}
	Sr	1.25 ± 0.64^{a}	0.33 ± 0.14^{b}	0.49 ± 0.26^{b}
	Zn	10.48 ± 4.92^{a}	3.40 ± 0.68^{b}	3.21 ± 0.77^{b}
Gills	**Ca**	4253.80 ± 5131.17^{a}	9283.52 ± 1273.91^{b}	9014.05 ± 1337.04^{b}
	K	529.26 ± 553.50^{a}	1676.47 ± 260.34^{b}	1688.21 ± 236.43^{b}
	Mg	155.25 ± 174.05^{a}	418.39 ± 70.92^{b}	439.64 ± 72.62^{b}
	Na	220.30 ± 243.82^{a}	796.50 ± 168.31^{b}	905.11 ± 168.73^{b}
	Cd	0.16 ± 0.35^{a}	0.07 ± 0.13^{a}	0.01 ± 0.01^{a}
	Cr	0.29 ± 0.10^{a}	0.14 ± 0.03^{b}	0.23 ± 0.01^{a}
	Cu	0.66 ± 0.27^{a}	0.51 ± 0.18^{a}	0.62 ± 0.20^{a}
	Fe	30.29 ± 18.69^{a}	22.35 ± 8.05^{a}	25.24 ± 11.88^{a}
	Mn	8.37 ± 5.15^{a}	7.64 ± 3.11^{a}	6.20 ± 1.92^{a}
	Pb	1.36 ± 1.92^{a}	0.08 ± 0.04^{b}	0.06 ± 0.03^{b}
	Sr	40.22 ± 36.56^{a}	18.81 ± 3.22^{a}	21.33 ± 3.80^{a}
	Zn	61.78 ±9.12^{a}	60.97 ± 10.26^{a}	68.88 ± 9.48^{a}
Liver	**Ca**	97.40 ± 63.12^{a}	165.50 ± 87.64^{a}	165.87 ± 95.00^{a}
	K	496.23 ± 637.97^{a}	1760.90 ± 370.74^{b}	2110.18 ± 397.94^{c}
	Mg	66.51 ± 114.83^{a}	139.03 ± 44.13^{b}	161.54 ± 38.14^{b}
	Na	200.89 ± 297.06^{a}	557.94 ± 117.29^{b}	744.42 ± 128.18^{c}
	Cd	0.18 ± 0.35^{a}	0.17 ± 0.49^{a}	0.07 ± 0.17^{a}
	Cr	0.81 ± 0.40^{a}	0.41 ± 0.15^{b}	1.21 ± 1.32^{a,b}
	Cu	2.03 ± 1.41^{a}	2.54 ± 1.03^{a}	4.33 ± 1.36^{b}
	Fe	52.32 ± 43.23^{a}	66.15 ± 35.52^{a}	70.46 ± 11.64^{a}
	Mn	2.54 ± 2.08^{a}	2.13 ± 1.45^{a}	2.00 ± 1.47^{a}
	Pb	1.48 ± 1.02^{a}	0.29 ± 0.24^{b}	0.04 ± 0.03^{c}
	Sr	1.85 ± 0.90^{a}	0.69 ± 0.55^{b}	1.07 ± 0.67^{a,b}
	Zn	36.52 ± 29.58^{a}	23.56 ± 9.36^{a}	18.59 ± 6.79^{a}

BDL: Below detection limit.

^{a,b,c} The values with different letters in the same row are significantly different (LSD Multiple Comparison test, $p < 0.05$).

Table 4

Correlation coefficients between trace element concentrations in tissues of chub from the River Szamos/Someş and feeding groups, significant at $p < 0.05$ (N = 33).

Elements	Tissues		
	Muscle	**Gills**	**Liver**
K	0.834	0.575	0.708
Mg	0.625	0.564	0.526
Na	n.s.	0.688	0.693
Cr	-0.749	n.s.	n.s.
Cu	n.s.	n.s.	0.549
Fe	-0.643	n.s.	n.s.
Mn	-0.395	n.s.	n.s.
Pb	-0.791	-0.624	-0.888
Zn	-0.643	n.s.	n.s.

n.s.: Non-significant.

Table 5

Values of the bioconcentration factor (BCF) based on the concentration of trace elements (mg kg^{-1} wet weight) in the specific tissue and the concentration of trace elements (mg l^{-1}) in the water of the River Szamos/Someş (mean value ± standard deviation).

Elements	Tissue	Age 0+	Age 1+, 2+	Age 3+≥
Cd	**Muscle**	203.40 ± 336.81^{a}	88.05 ± 159.41^{a}	–
	Gills	275.12 ± 600.70^{a}	123.12 ± 232.64^{a}	12.40 ± 28.21^{a}
	Liver	319.63 ± 609.11^{a}	303.77 ± 851.01^{a}	126.67 ± 288.73^{a}
Cr	**Muscle**	85.96 ± 108.46^{a}	20.68 ± 4.05^{b}	19.07 ± 2.25^{b}
	Gills	92.39 ± 32.59^{a}	43.52 ± 8.07^{b}	72.41 ± 5.74^{a}
	Liver	253.62 ± 126.2^{a}8	130.40 ± 46.42^{b}	380.88 ± 416.36$^{a, b}$
Cu	**Muscle**	34.75 ± 14.08^{a}	17.24 ± 5.99^{b}	26.44 ± 10.54^{a,}
	Gills	69.28 ± 28.22^{a}	53.59 ± 19.28^{a}	65.63 ± 21.42^{a}
	Liver	214.54 ± 149.01^{a}	268.41 ± 109.13^{a}	457.36 ± 143.25^{b}
Fe	**Muscle**	22.62 ±8.71^{a}	4.42 ± 1.48^{b}	4.31 ± 2.11^{b}
	Gills	82.82 ± 51.09^{a}	61.11 ± 22.01^{a}	69.02 ± 32.48^{a}
	Liver	143.04 ± 118.19^{a}	180.84 ± 97.11^{a}	192.64 ± 31.83^{a}
Mn	**Muscle**	2.26 ± 1.06^{a}	1.69 ± 0.47$^{a, b}$	1.19 ± 0.75^{b}
	Gills	57.33 ± 35.27^{a}	52.32 ± 21.27^{a}	42.46 ± 13.16^{a}
	Liver	17.38 ± 14.27^{a}	14.56 ± 9.94^{a}	13.70 ± 10.08^{a}
Pb	**Muscle**	42.69 ± 36.55^{a}	2.36 ± 1.50^{b}	1.17 ± 0.85^{c}
	Gills	196.14 ± 277.06^{a}	12.26 ± 6.19^{b}	8.74 ± 4.65^{b}
	Liver	214.18 ± 147.40^{a}	41.25 ± 35.39^{b}	6.42 ± 4.06^{c}
Zn	**Muscle**	76.90 ± 36.13^{a}	24.95 ± 5.02^{b}	23.57 ± 5.67^{b}
	Gills	453.54 ± 66.92^{a}	447.54 ± 75.35^{a}	505.63 ± 69.61^{a}
	Liver	268.12 ± 217.18^{a}	172.97 ± 68.71^{a}	136.45 ± 49.83^{a}

Notation: The Cd concentration was below the detection limit in the case of the muscle samples of the older chubs (age 3+ and thereafter).

[a,b,c] The values with different letters in the same row are significantly different (LSD Multiple Comparison test, $p < 0.05$).

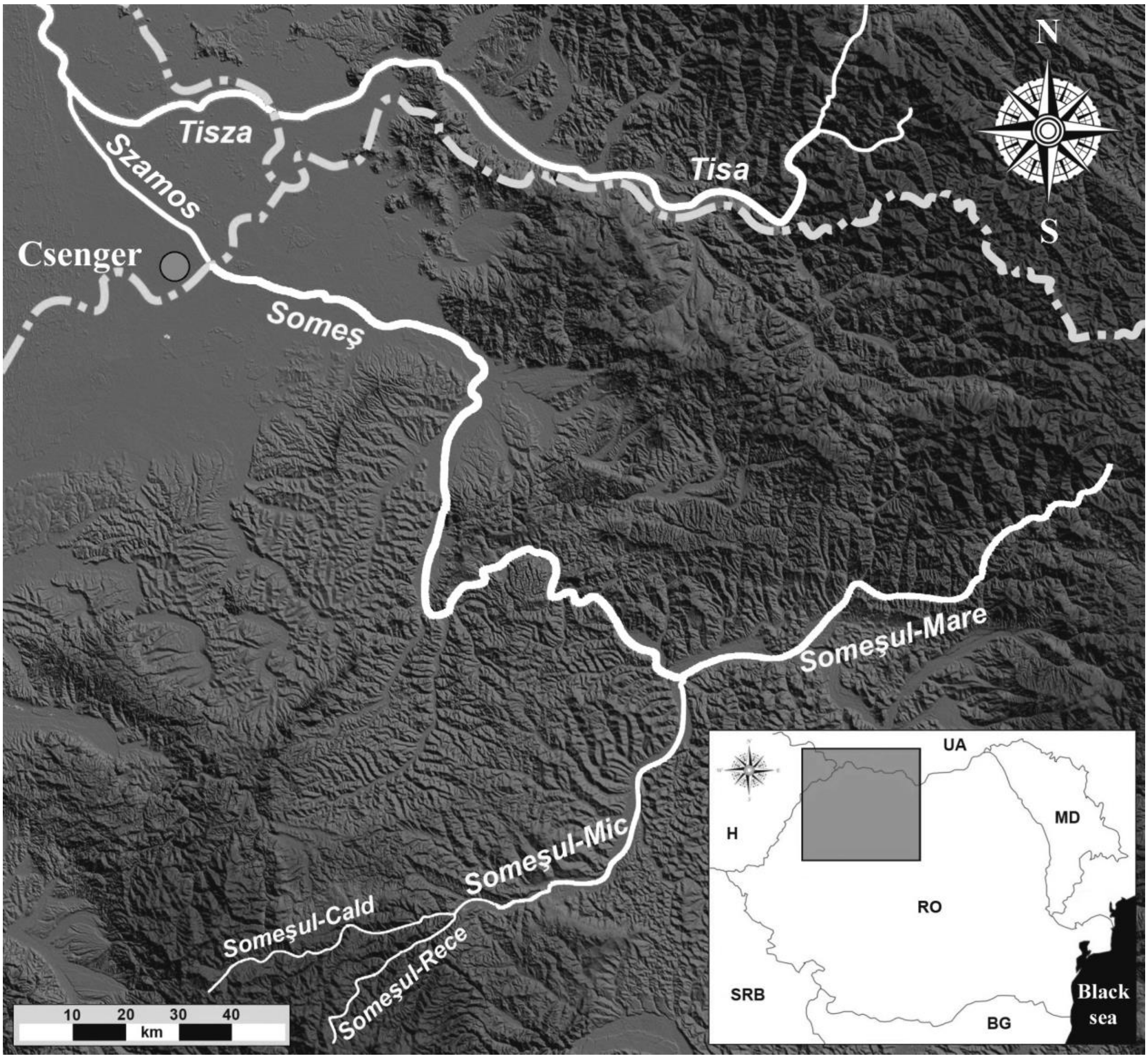

Fig. 1. Map of the Water System of River Szamos/Someş and the sampling area at the village of Csenger

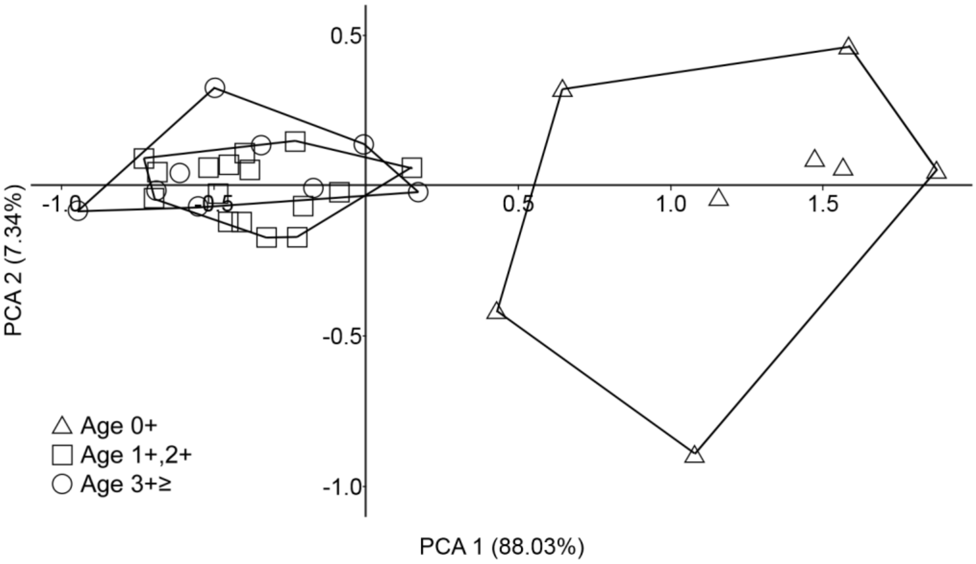

Fig. 2. Principal component analysis of trace element concentrations (mg kg^{-1}, wet weight) in the muscles of the three feeding groups of chub in the River Szamos/Someş.

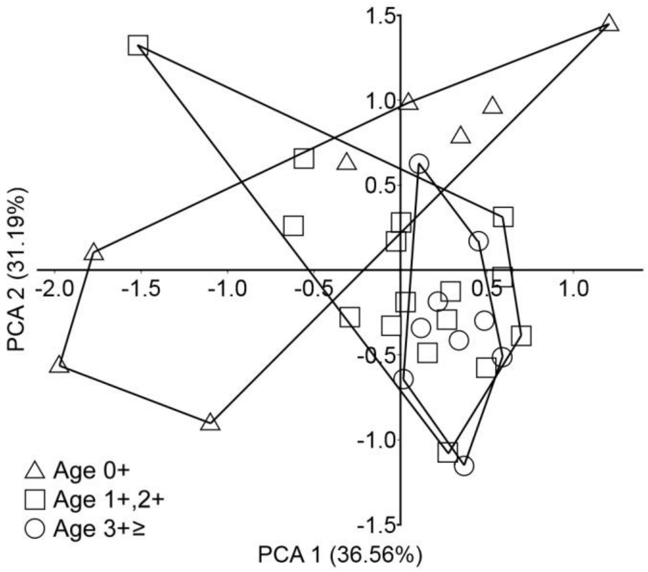

Fig. 3. The PCA plot element concentrations (mg kg^{-1}, wet weight) in the livers of the three feeding groups of chub in the River Szamos/Someş.

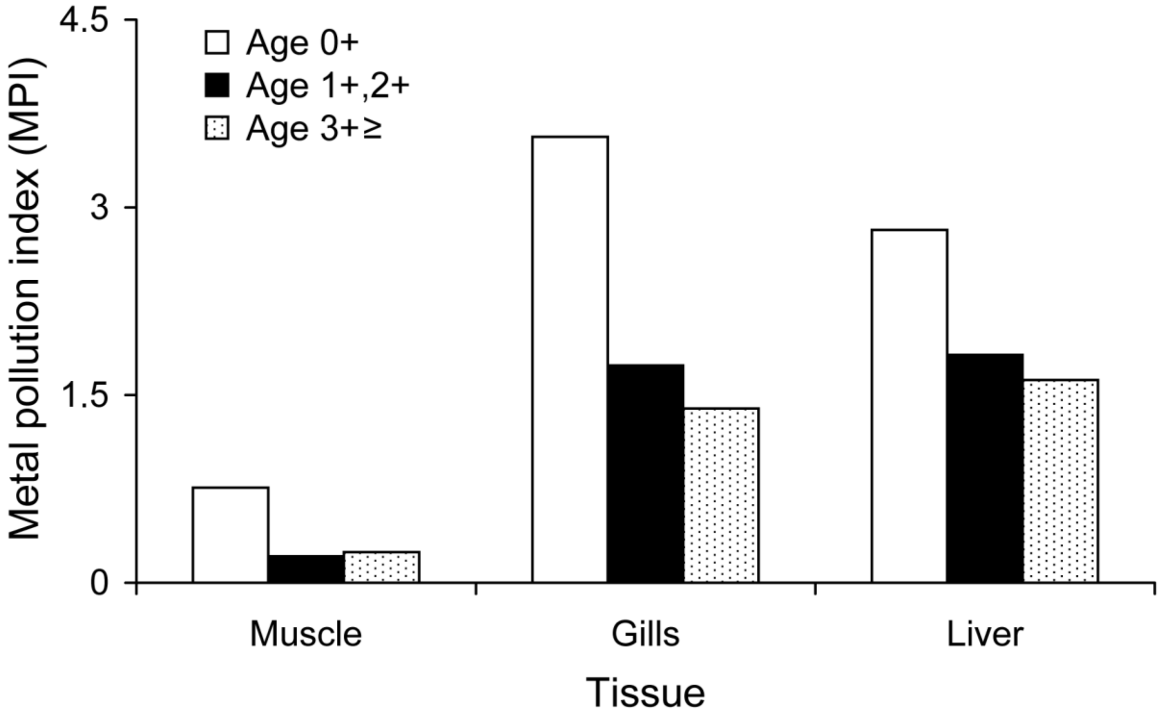

Fig. 4. Mean metal pollution index (MPI) for various tissues of different feeding groups of chub in the River Szamos/Someş.