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Research Article

Accumulation and seasonal variation of toxic and trace elements in tissues of *Cyprinus carpio* from semi-intensive aquaculture ponds

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Abstract – The aim of this study was to assess the accumulation and seasonal variations of 20 toxic and trace elements in the abdominal and dorsal muscle (DM), liver and gut contents (GCs) of *Cyprinus carpio,* and in the sediments and zooplankton from semi-intensive aquaculture ponds during a six-month production season (from May to October). Sediments showed the highest concentrations of the measured elements, followed by zooplankton, fish GCs and livers, while the lowest levels were present in fish muscles. Correspondence analysis revealed that the elemental composition of the fish GCs was mostly similar to that of the sediment and zooplankton. The element concentrations of carp liver and muscles were more similar to each other. Overall, the results indicate that fish liver was the main target organ accumulating Cu, Se and Zn, regardless of their initial amounts in the environment. A seasonal decrease of a range of elements in fish liver (Al, Co, Fe, Li, Mn, Ni, Pb and Sr) was evident towards the end of the investigation period. In addition, the fish DM showed a seasonal accumulation of Sr and Li. However, toxic elements *i.e.* As, B, Ba Hg and V did not show such a seasonal variation, and were evenly distributed between fish liver and muscles. The concentrations of some elements, such as As, in fish tissues were similar or even higher than in literature data from polluted sites, but none of them exceeded the maximum acceptable concentrations prescribed by Serbian legislation or by FAO or EU regulation.

Keywords: common carp / toxic elements / semi-intensive aquaculture ponds / sediment / zooplankton

1 Introduction

Pollution of freshwater ecosystems with toxic and trace elements has been rising over the past few decades worldwide and is now being considered as a major problem in developing and undeveloped countries due to their low levels of wastewater treatment (Kazi *et al.*, 2009; Ozden, 2010). Toxic and trace elements are entering rivers, lakes and other aquatic ecosystems, including semi-intensive fish pond systems, from anthropogenic sources, posing serious threats to the environment and to humans. They are characterised by long-term persistence in the environment, bioaccumulation in animal and plant tissues and sediments, as well as biomagnification through the food chain (Chen *et al.*, 2000; Schenone *et al.*, 2014). Even at low concentrations, toxic elements can be very harmful when ingested over a long period. Some essential metals such as Zn can also produce toxic effects when their concentration is excessively elevated in animals and humans (Celik and Oehlenschlager, 2007).

Due to the low level of wastewater treatment in Serbia, most agricultural, industrial and municipal contaminants are directly introduced into the Danube and Sava rivers, especially near large cities *i.e.* Belgrade and Novi Sad (Terzić *et al.*, 2008; Teodorović, 2009). Additionally, linking the Danube and Tisza rivers, some parts of a large multi-purpose canal system – the Danube – Tisza – Danube canal system (DTD), located in Province of Vojvodina – are identified as areas with high heavy metal contamination (ICPDR, 2005; Sakan *et al.*, 2007). The majority of fish farms in Serbia are located in Vojvodina Province, covering a surface of around 14 000 hectares (Marković and Poleksić, 2013). More than 90% of these semi-intensive fish farms use the DTD canal system as the water supply for fish ponds and therefore present a potential entry point for

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contamination of the aquaculture environment. Common carp (*Cyprinus carpio*) is one of the most commercially important farmed fish in the world (FAO, 1983). In Serbia, common carp is the main species produced in semi-intensive fish farms, with the annual production estimated at around 11 000 tones (Marković and Poleksić, 2013). In semi-intensive aquaculture, common carp feeds on different natural food resources from the pond *i.e.* zooplankton and zoobenthos, and is additionally fed with supplemental feed, according to the fish biomass and estimated natural food availability (Tacon and De Silva, 1997). Due to the sediment-dwelling behaviour linked to their feeding habits, carp can ingest and accumulate elements from the sediments, which are sites with higher metal concentrations (Chen *et al.*, 2000; Alam *et al.*, 2002).

Recent studies on toxic element pollution in Serbia have mainly focused on wild fish populations from the River Danube and its tributaries (Poleksić *et al.*, 2010; Jarić *et al.*, 2011; Lenhardt *et al.*, 2012; Subotić *et al.*, 2013a), while there is only one paper focusing on bioaccumulation of heavy metals in the tissues of the great cormorant (*Phalacrocorax carbo*) that feeds on farmed common carp as prey (Skorić *et al.*, 2012). To the best of our knowledge, assessment of elemental pollution in the aquaculture environment involving fish, zooplankton, and sediment has not been investigated in this area to date. Therefore, due to the potential introduction of polluted water from the rivers into the DTD system that is used as the water supply for the majority of fish farms in the area and the associated implications for consumer health, an assessment of the current state of farmed fish, their natural food and the aquaculture environment in Serbia is very important.

The main objectives of this study were to investigate the elemental content of the tissues and gut of *Cyprinus carpio* and the potential risk for human consumption; to assess the level of the same toxic and trace elements in the pond sediments; and also to measure their concentration in zooplankton, the dominant natural food of carp. We selected two types of edible fish tissues for sampling – abdominal and dorsal muscles (DMs) – in order to reveal any differences in the element contents between them; and a non-edible tissue, the fish liver as an indicator of chronic exposure to toxic and trace elements. We also sampled the carp gut contents (GCs) that usually consist of a high percentage of pond sediments due to their benthic feeding habits (Adámek *et al.*, 2003) in order to indicate the potential pool of elements present in the fish body. Additionally, we monitored these semi-intensive fish ponds continuously during the 6 month production cycle, from May to October, to reveal any seasonal variations in the elemental concentrations. We hypothesised that the highest accumulation of toxic and trace elements would be in the sediment and zooplankton of fish ponds. Accordingly, we expect an increase in the concentration of these elements in fish tissues towards the end of the rearing period, due to continuous ingestion of sediments by carp searching for benthic food items and the prominent bioturbation process that is distinctive for carp ponds (Adámek and Maršálek, 2013).

2 Materials and methods

2.1 Study area

The "Despotovo" fish farm is located in the village of Despotovo, in Vojvodina Province (North of Serbia). The coordinates of the fish farm are 45°26.336′ and 19°32.493′. The carp farm consists of six earth fish ponds that cover a surface area of 234 ha. Four neighbouring fish ponds were selected for the study, covering a surface of 166 ha. Before the beginning of every production cycle, the ponds are filled with water from the DTD canal system that is connected to the Danube and Tisza Rivers. The DTD canal used for filling the ponds runs through the fish farm, between the investigated ponds, supplying them evenly with water, with the aid of pumps at the beginning of the rearing season. There was no refilling from the canal into the ponds during the investigation period. The water depth in fish ponds was approximately 1.5 m. Each fish pond was stocked with around 550 individuals per hectare of two-year-old carp with a mean weight of 0.9 kg. At the end of the production cycle the mean carp weight was 2.85 kg. Fish were fed daily with commercial pelleted feed "BAFI" (Futog, Serbia) containing 25% proteins (plant origin) and 7% fat.

2.2 Sample collection and preparation

Fish samples were collected monthly, from May to October 2013, using a regular seine net for semi-intensive carp ponds. Five individuals per fish pond, of similar size, (in total 20 fish per sampling) were selected and sacrificed by a blow in the head and dissected.

Samples of liver, dorsal and abdominal muscle were quickly removed, washed with distilled water, and stored at −18 °C prior to analysis. The samples of GCs were taken before feeding with pelleted feed, *i.e.* approximately 6 h since the last feeding, so that the pelleted feed comprised a negligible amount of the sampled GCs under the temperature conditions of the study (Garcia and Adelman, 1985).

The entire GCs of fish were removed from digestive tract after dissection. One part was preserved in 4% formaldehyde for analysis of food items while the remainder was packed in polyethylene bags and stored at −18 °C prior to elemental analysis. The GCs were analysed using a stereo microscope. In order to estimate the proportion of the main food items in the GC of fish, the numerical method was used (Hyslop, 1980).

Samples of sediment and zooplankton were taken monthly from May to October 2013, from three points (near the inlet, in the middle, and near the outlet) in every pond. Sediment samples were taken with a stainless Van Veen grab sampler, at a depth from 0 to 10 cm. Zooplankton samples were collected with a 250 μm mesh plankton net, with an outer cone diameter of 30 cm, using 5 m horizontal hauls at each station. This mesh size was chosen to ensure the collection of the majority (>90%) of large crustacean zooplankton species, the preferred prey for two-year-old carp, and to minimise the collection of phytoplankton. Soon after collection, zooplankton was filtered through a 0.45 μm (pore size) Millipore filter, washed with distilled water and placed in a plastic bottle.

2.3 Microwave digestion

In the laboratory, the sediment samples were dried in an oven and stone pieces were removed. Sediment samples were digested at 220 °C for 20 min using a mixture of 15 ml HCl (35%), 2 ml HNO_3 (65%) and 2 ml HF (48%) (Suprapur®, Merck KGaA, Darmstadt, Germany) per 0.1 g.

All zooplankton, pelleted feed and fish samples (~2 g) were dried in a lyophiliser (Christ Alpha 2-4 LD, Harz, Germany). The digestion was performed using an advanced microwave digestion system (ETHOS 1, Milestone, Italy) using an HPR-1000/10S high-pressure segmented rotor. In the digestion, a precisely weighed lyophilised sample of about 0.5 g was mixed in clean vessel with 10 ml HNO_3 (65%) and 2 ml H_2O_2 (30%) (Suprapur®, Merck KGaA, Darmstadt, Germany) and then heated with microwave energy for 20 min. The temperature was controlled by using a predetermined power program. The temperature was typically raised to 200 °C in the first 15 min, to a peak temperature of 200 °C for the next 20 min, and then cooled down rapidly. After cooling and without filtration, the solution was diluted to a fixed volume (volumetric flask, 25 ml) with ultra-pure water of conductivity 0.055 μS/cm (Barnstead™ GenPure™ Pro, Thermo Scientific, Germany).

2.4 Instrumental analysis

The content of elements (Al, B, Fe, Li, Mn, Sr, and Zn) in solution samples was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). ICP-OES measurements were performed using a Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, United Kingdom) spectrometer equipped with a RACID86 charge injector device (CID) detector, a concentric type nebuliser, a quartz torch and an alumina injector. The optical system was purged with argon and the Echelle polychromator thermostated at 38 °C. The instrumental conditions were optimised to obtain sufficient sensitivity and precision.

The contents of As, Ba, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Se and V in solution samples was determined by inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS measurements were performed using a Thermo Scientific iCAP Qc ICP-MS (Thermo Scientific, Bremen, Germany) spectrometer using the Qtegra operational software. The instrument was optimised for optimum performance in He KED (Kinetic Energy Discrimination) mode and H_2/He mixture gas in CCT (Collision Cell Technology) mode using the supplied autotune protocols. The instrumental operating conditions for ICP-OES and ICP-MS are shown in Table 1.

Standards for instrument calibration were prepared on the basis of multi element plasma standard solutions SS-Low Level Elements ICV Stock (10 mg/L) and ILM 05.2 ICS Stock 1 (500 mg/L) and mono element plasma standard solutions Hg Calibration Stock (10 mg/L Hg) (VHG Labs, Inc. – Part of LGC Standards, Manchester, NH 03103 USA). One multi-elemental plasma standard solution, Multi-Element Plasma Standard Solution 4, Specpure®, 1000 μg/ml certified from Alfa Aesar GmbH and Co KG, Germany, was used to prepare calibration solutions for B and Sr measurements.

The analytical process quality control, performed by the use of certified fish protein reference material (CRM) for trace metals DORM 4 (NRCC, National Research Council Canada), indicated that the resulting concentrations were within 91–107%. The concentrations of all metals were expressed as mg/kg dry weight (dw). The mean concentrations of toxic and trace elements in the investigated fish were recalculated to the wet tissue weight (mg/kg ww) in order to compare them with the maximum acceptable concentrations (MAC) in fish meat for human consumption, as established by the European Union (European Commission Regulation, 2001), the Food and Agriculture Organisation (FAO, 1983), and Serbian national legislation (Official Gazette RS No. 28/2011).

Table 1. Experimental conditions used on ICP-MS and ICP-OES equipment to determine inorganic elements in all samples.

Parameter	Experimental conditions
ICP-OES	
Radio frequency power (RF)	1350 W
Plasma view	Axial
Nebulizer	Concentric nebulizer
Spray chamber	Standard glass cyclonic
Ceramic centre tube	2 mm
Purge gas	Argon
Nebulizer argon flow rate	0.50 L/min
Auxiliary argon flow rate	0.5 L/min
Coolant argon flow rate	12 L/min
Sample flush time	30 s
Analysis pump rate	50 rpm
Analysis mode	Speed
Software	iTEVA
ICP-MS	
Radio frequency power (RF)	1550 W
Nebulizer argon flow rate	0.95 L/min
Auxiliary argon flow rate	0.80 L/min
Coolant argon flow rate	14.0 L/min
KED (He) or CCT (H_2/He)	6.0 mL/min
Dwell time	10 ms
Extraction	−50.00 V
Sample uptake rate	0.40 mL/min
Spray chamber	Cyclonic
Nebulizer	Meinhard ESI MicroFlow PFA-ST
Software	Qtegra

2.5 Statistical analysis

All data were expressed as mean ± standard error. Correspondence analysis (CA) was used to examine associations among the analysed samples with respect to the studied element concentrations. It was performed with the aid of XLSTAT software version 7.5.2 (Addinsoft). The samples were compared using unpaired t-tests at the 5% level of significance ($P < 0.05$). In order to measure the strength of the associations among pairs of variables, the Pearson product moment correlation, with a $P < 0.05$ level of significance, was used. The unpaired t-test and Pearson product moment correlations were performed with the aid of Sigma Plot 11 software (Systat Software Inc., USA). For the determination of the level of similarity between element contents in liver and those in DM, abdominal muscle and GCs for all sampling dates, as well as between the May liver sample and the rest of the liver samples we used the Bray–Curtis similarity index (Bray and Curtis, 1957).

In order to quantify the differences between the main sources of the elements – sediment and zooplankton – and GC more accurately, we calculated the deviation of the measured

Table 2. Element concentrations in sediment, zooplankton, pellets, and carp gut content, liver, dorsal and abdominal muscle (mean ± standard error, $n = 24$, except for pellets where $n = 5$). Concentrations are expressed in mg/kg dry weight.

	Sediment	Zooplankton	Pelleted feed	Gut content	Liver	Dorsal muscle	Abdominal muscle
Al	4434 ± 306^{a}	2512 ± 311	83.31 ± 5.76	4922 ± 367^{a}	39.84 ± 13.74	4.772 ± 0.480^{f}	4.448 ± 0.374^{f}
As	4.79 ± 0.91	0.763 ± 0.097^{b}	0.091 ± 0.009	1.01 ± 0.15^{b}	0.621 ± 0.055^{b}	0.401 ± 0.030^{f}	0.365 ± 0.032^{f}
B	27.48 ± 0.57	0.141 ± 0.007^{b}	0.122 ± 0.002^{c}	0.157 ± 0.020^{b,d}	0.156 ± 0.041^{b,c,d,e}	0.0788 ± 0.0007	0.140 ± 0.053^{b,c,d,e}
Ba	12.5 ± 1.1	33.17 ± 4.36	1.502 ± 0.132	0.147 ± 0.015	0.0436 ± 0.0004	0.0527 ± 0.0002	0.0534 ± 0.0001
Cd	0.08 ± 0.02	0.141 ± 0.017	0.043 ± 0.005^{c}	0.281 ± 0.041	0.040 ± 0.005^{c}	0.0097 ± 0.0004^{f}	0.0093 ± 0.0004^{f}
Co	10.02 ± 0.30	1.35 ± 0.13	0.620 ± 0.019	2.38 ± 0.36	0.073 ± 0.015	dl	dl
Cr	13.58 ± 1.73^{a}	3.95 ± 0.47	0.316 ± 0.040	14.75 ± 2.27^{a}	dl	dl	dl
Cu	14.70 ± 2.41^{a}	11.70 ± 1.06^{a,b}	7.802 ± 0.741	17.48 ± 1.82^{a,d}	15.10 ± 1.64^{a,b,d}	0.542 ± 0.036^{f}	0.518 ± 0.025^{f}
Fe	16941 ± 602	2536 ± 315	178.4 ± 6.3	5181 ± 563	138.50 ± 28.51	7.471 ± 1.176^{f}	8.70 ± 1.93^{f}
Hg	0.179 ± 0.024	0.113 ± 0.007	0.012 ± 0.001^{c}	0.005 ± 0.000	0.014 ± 0.001^{c,e}	0.0111 ± 0.0005^{c,e,f}	0.0109 ± 0.0007^{c,e,f}
Li	46.63 ± 1.85	3.15 ± 0.39	0.148 ± 0.006^{c}	5.62 ± 0.74	0.168 ± 0.054^{c,e}	0.497 ± 0.059	0.108 ± 0.015^{e}
Mn	506 ± 19	70.61 ± 8.75^{b}	49.50 ± 1.93^{c}	57.18 ± 7.15^{b,c}	2.24 ± 0.27	0.495 ± 0.021	0.404 ± 0.034
Mo	0.090 ± 0.062^{a}	0.833 ± 0.047	1.199 ± 0.108	0.098 ± 0.013^{a}	0.374 ± 0.015	0.116 ± 0.003^{a,f}	0.118 ± 0.004^{a,f}
Ni	21.79 ± 1.32	2.98 ± 0.38^{b}	1.767 ± 0.107	4.27 ± 0.61^{b}	0.072 ± 0.027	0.016 ± 0.004^{f}	0.017 ± 0.006^{f}
Pb	16.72 ± 4.43	2.84 ± 0.22	dl	0.737 ± 0.153	0.026 ± 0.012	dl	dl
Sb	0.388 ± 0.113^{a}	0.484 ± 0.056^{a}	dl	dl	dl	0.011 ± 0.005^{f}	0.015 ± 0.001^{f}
Se	dl	0.457 ± 0.085^{b}	0.124 ± 0.007	0.039 ± 0.017	1.356 ± 0.064	0.472 ± 0.031^{b,f}	0.406 ± 0.024^{b,f}
Sr	97.13 ± 9.18	33.92 ± 6.24^{b}	10.51 ± 0.99	21.93 ± 2.08^{b}	0.232 ± 0.083	0.827 ± 0.198	0.069 ± 0.016
V	83.54 ± 3.18	6.28 ± 0.71	1.984 ± 0.076	0.007 ± 0.006^{d}	0.013 ± 0.005^{d,e}	0.017 ± 0.001^{d,e,f}	0.012 ± 0.004^{d,e,f}
Zn	36.92 ± 2.27^{a}	72.99 ± 4.55^{b}	58.09 ± 3.56^{c}	63.71 ± 4.38^{b,c}	520 ± 35	32.25 ± 2.44^{a,f}	40.87 ± 3.68^{a,f}

dl - detection limit; ^{a,b,c,d,e,f} differences between values within the same row sharing a superscript letter are not statistically significant ($P > 0.05$).

element concentrations in GC from the expected gut content (EGC). EGC was obtained as the weighted average of the sediment and zooplankton concentrations.

3 Results

ANOVA showed that there was no significant difference between the sites (fish ponds) during the investigation period regarding fish weight and the analysed samples, thus the results were pooled, and the average values were used in further analysis. The average values of all investigated elements are presented in Table 2.

The CA of the studied elements from all samples is presented in Figure 1A. Two axes describing 76% of the variance were retained. Carp tissues are clearly separated along the F1 axis from the rest of samples, which are separated along the F2 axis. Since the F1 axis explains a much greater part of the variance (55%) compared with the F2 axis (21%), it is clear that difference in studied element concentrations between carp tissues and the rest of samples is much greater than differences within these two groups. Along the F1 axis, the GCs are placed between sediment and zooplankton that are its main constituents according to the analysis. Analysis of the GCs showed that it mainly consisted of detritus and organic sediment (60%), zooplankton, mainly Cladocerans (30%), and around 10% chironomid larvae. Along the F2 axis, sediment and zooplankton are very close, while GCs is largely displaced towards the positive end, indicating that sediment and zooplankton element contents are more similar to each other compared with GCs. CA showed that with the exception of Al, Cd, Cr and Cu, all other elements are less represented in the GC compared with both zooplankton and sediment. The EGC, (Fig. 1A inset) showed the greatest reduction of Sb, which was below the detection limit. Additionally, the concentrations of V, B, Ba, Hg, Pb, Li and Mn were reduced in GC with respect to EGC by 8273, 131, 115, 29, 15 and five times, respectively. On the other hand, Al, Cr, Cu, Fe and Zn were present in concentrations near to the EGC, while Cd was 2.74 times more highly represented than expected. The concentrations of the rest of elements in GC were two to three times smaller than expected. CA of element concentrations in GCs and carp tissues only partially confirmed these assumptions (Fig. 1B). Two axes describing 98% of the variance were retained. Of the elements whose concentrations were expected to be low in carp tissues, Al, Cd, Co, Cr, Fe, Li, Mn, Ni, Pb and Sr are grouped at the negative end of the F1 axis, near the GC, thus confirming the starting assumption. On the other hand, Zn is placed at the opposite end of F1 axis, and Cu near the middle, showing that Zn concentration in liver is much greater than in the GCs, and that the Cu concentration is similar to that in the GC. Of the elements expected to accumulate in carp tissues, only Se, Mo, Hg and V are placed at the positive end of F1 axis and thus their concentrations in carp tissues are greater than in GC. However, the concentrations of Hg and V, although greater in tissues than in GC, are still much lower than in sediment and zooplankton (Table 2). The rest of the elements, As, B and Ba are placed near to the middle of F1 axis, (Fig. 1B), pointing out that their concentrations in carp tissues are comparable to those in GC.

The concentrations of some elements in carp tissues, unlike those in sediment, zooplankton and GC, showed statistically significant seasonal changes. CA analysing the changes in

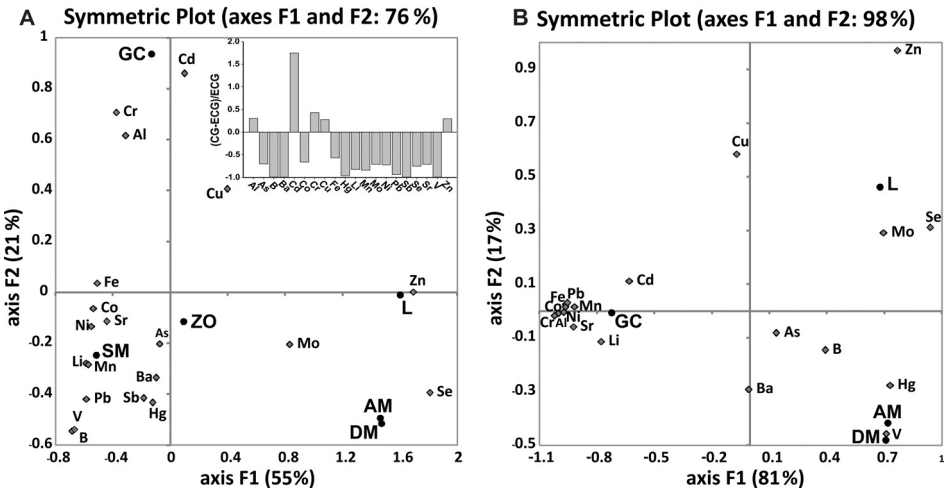

Fig. 1. (A) The CA biplot showing relationships between carp tissues, dorsal (DM) and abdominal (AM) muscle and liver (L), fish gut content (GC), zooplankton (ZO) and fish pond sediment (SM) based on their element concentrations. Inset: the deviation of the measured element concentrations in GC from the expected ones (EGC). (B) The CA biplot showing relationships between carp tissues (dorsal and abdominal muscle and liver), and gut content.

element concentrations of carp tissues during the 6 month investigation are shown in Figure 2. Two axes describing 72% of variance were retained. Along the F1 axis, which explains the greater part of the variance (47%), liver element content is placed in the positive part, clearly separated from those of dorsal and abdominal muscle, which are grouped at the negative end of F1 axis. Since most of the elements are placed at the positive part of F1 axis, their concentration is greater in liver than in muscles (Fig. 3A and B). The exceptions are As, B, Ba, Hg and V, which are uniformly distributed among the tissues (Fig. 3C); and Li and Sr, which are accumulated in the DM (Fig. 3D). The F2 axis, explaining a smaller, but important part of the variability (26%) accounts for the seasonal changes.

The most notable difference was between the May liver sample and the later liver samples (July to October). The concentrations of all elements placed at the positive part of F2 axis (Al, Co, Fe, Li, Mn, Ni, Pb and Sr) show a statistically significant decrease ($P < 0.001$) between the May and June samples (Fig. 4A), followed by a slight increase (paired t test, $P = 0.01$) in July and a constant but small decrease until the end of rearing season (between all pairs of consecutive months paired t tests showed statistically significant differences, $P < 0.05$). A similar but much less pronounced pattern, both in terms of intensity and proportion of elements (only Al and Fe), followed a significant decrease after the June sample in abdominal and DM (Fig. 4A). As a result, the concentration of Al and Fe decreased in October compared with May by 9 ± 4% in liver, 27 ± 8% in abdominal muscle (AM) and 50 ± 8% in DM. From June to October the decrease of the elements in liver was much greater than in AM and DM ($P < 0.001$), while between AM and DM t tests did not produce a statistically significant difference. A paired t-test confirmed a significant difference between the October muscle samples ($P = 0.014$), which indicates that the decrease is somewhat more pronounced in AM compared to DM. Unlike liver samples characterised by an exponential decrease ($r^2 = 0.987$, $F = 112$, $P = 0.0015$), the decrease in AM samples is linear ($r^2 = 0.81$, $F = 17$, $P = 0.014$). The concentration of elements located at the

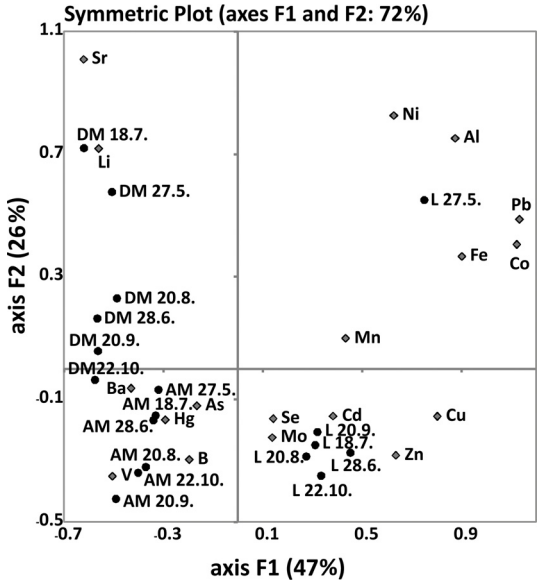

Fig. 2. The CA biplot showing relationships between studied carp tissues (dorsal and abdominal muscle and liver) for each sampling date, based on their element concentrations. Note: For abbreviations see Figure 1.

negative part of the F2 axis (As, B, Ba, Cd, Cu, Hg, Mo, Se, V and Zn) did not change over time (Fig. 2, Fig. 4B), either in the case when they were uniformly distributed among tissues (As, B, Ba, Hg and V, Fig. 3C), or when they were accumulated in the liver (Cd, Cu, Mo, Se and Zn, Fig. 3D). Of those accumulated in the liver, Se and Zn increased to concentrations three and 15 times greater, respectively, than in any available source including the GCs.

Due to the large differences in most element concentrations between the GCs and the liver as well as between the May liver sample and the rest of the liver samples, CA analysis was not sufficient to distinguish delicate seasonal differences between

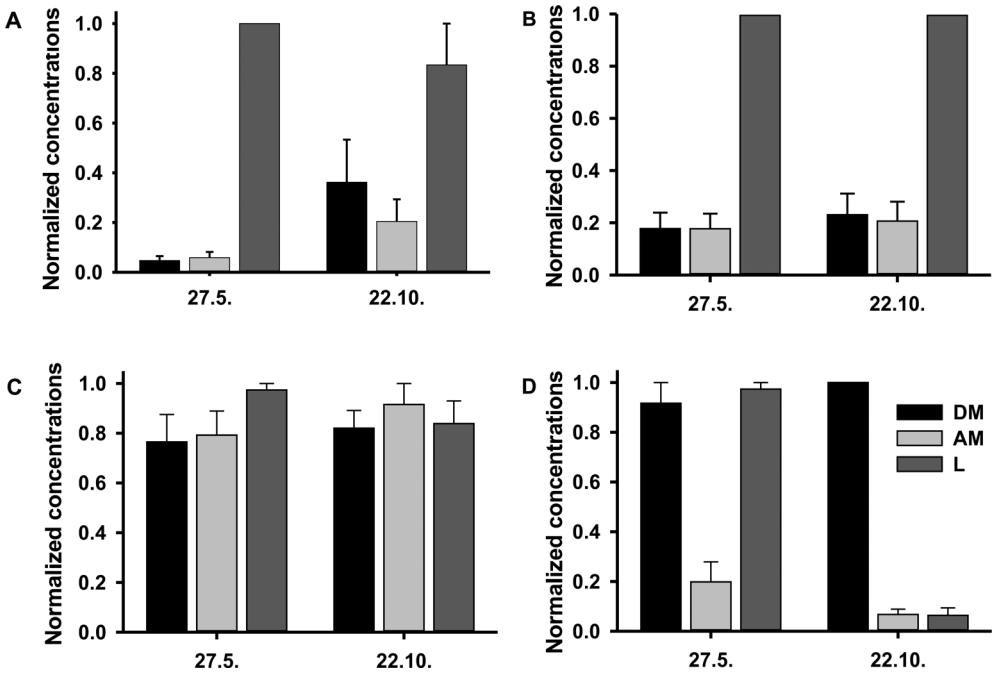

Fig. 3. Comparison between May and October normalized averaged concentrations of elements in investigated fish tissues: (A) Al, Co, Fe, Mn, Ni and Pb; (B) Cd, Cu, Mo, Se and Zn; (C) As, B, Ba, Hg and V; (D) Sr and Li. Note: For abbreviations see Figure 1.

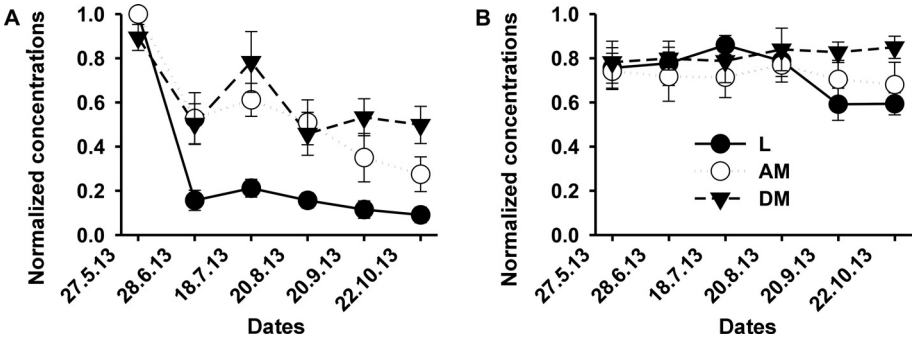

Fig. 4. Seasonal variation of elements during the whole study period: (A) Al, Co, Fe, Li, Mn, Ni, Pb and Sr; (B) As, B, Ba, Cd, Cu, Hg, Mo, Se, V and Zn. Prior to averaging concentrations for all sampling dates, each metal is normalized to the maximal concentration. Note: For abbreviations see Figure 1.

the samples (Fig. 3, data not shown in the GC − liver case). Therefore the Bray-Curtis similarity index was used to analyse the difference between liver on one side and GCs, DM and AM on the other for all sampling dates, as well as between the May liver samples and the rest of liver samples (Fig. 5). It is clear that the concentrations of the investigated elements clearly separate the liver from the other samples. However, in May, the liver samples were significantly ($P < 0.05$) more similar to the GCs than to the muscle tissues. The situation considerably changed in June, due to the already described decrease in liver concentrations of Al, Co, Fe, Li, Mn, Ni, Pb and Sr, so that the liver becomes significantly more similar to the muscle tissues compared with the GCs. There was one more significant increase in similarity between the muscle tissues and liver in September (Fig. 5).

To investigate the cause of the significant decrease in liver elemental concentrations, the average carp total weight and concentration of elements was correlated (Table 3). Correlations were negative and significant for most of the analysed elements in the liver. The exceptions were the toxic elements As, B, Hg, Pb and V. This was much less pronounced in abdominal muscles and particularly DMs.

4 Discussion

4.1 Concentrations of elements in sediments, zooplankton and fish

In this study sediments have been considered as an important accumulation site for elements in aquatic ecosystems, where they can build up to concentrations several times higher than those in the water (Mendil *et al.*, 2010; Zrnčić *et al.*, 2013). Sediment (mostly detritus and plant debris) was a major part of the carp GC (60%), and was passively ingested

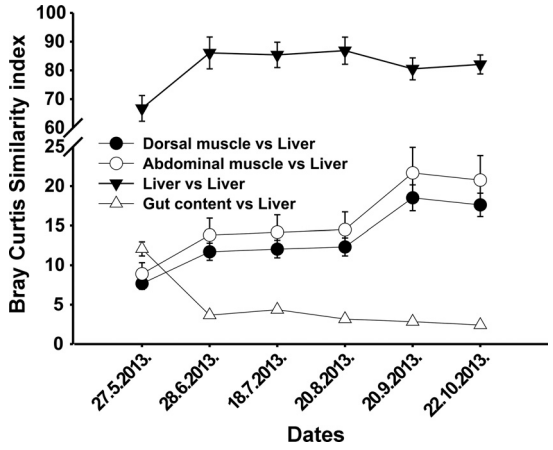

Fig. 5. Bray Curtis similarity index between liver and gut content, dorsal and abdominal muscles for all sampling dates, as well as between May liver sample and the rest of liver samples.

by fish searching for natural food – zoobenthos (Adámek *et al.*, 2003). The rest of the food items in the GCs were zooplankton, comprising large species of cladocerans and zoobenthos, mostly as chironomid larvae (30% and 10%, respectively).

The results of this study show that aluminium, iron and manganese were the most abundant elements in the fish pond sediments, showing similar concentrations with polluted aquatic ecosystems documented in the literature. The level of iron (16 941 mg/kg dw, Table 2) was similar to that found for Manchar Lake (17 712.0 mg/kg dw, Arain *et al.*, 2008) while the concentration of Pb (16.7 mg/kg dw) was found to be similar to polluted sites of the Yesilırmak River (17.3 mg/kg dw, Mendil *et al.*, 2010) and Velenjsko lake (19.7 mg/kg dw, Mazej *et al.*, 2010). Additionally, elevated levels of toxic elements such as arsenic (4.79 mg/kg dw) and lead (16.79 mg/kg dw) were found in the sediments of the investigated fish ponds, revealing a potential threat for accumulation and transfer through the food web *via* several pathways as: sediment/zoobenthos – fish; zooplankton – fish or directly to fish from resuspended sediments in the water column. Resuspension of sediments and the return of toxic and trace elements into the water column in carp ponds can happen by wave action, by burrowing activities of zoobenthos (chironomid larvae and oligochaetes), or by stirring of sediments by carp foraging for food (Gyllström and Hansson, 2004). To the best of our knowledge there is only one study about elemental composition of sediment in the DTD canal system performed some 20 km away from the "Despotovo" fish farm (Medbø *et al.*, 2005). Concentrations of Ni, Zn, Cd, Cr, Pb and Hg were several times higher in the DTD canal compared to the fish farm, concentration of Fe was in the same range, while fish farm sediment contained about two times more Mn compared to sediment in the DTD canal. Such a large difference is probably a consequence of waste water influx in the DTD channel from the neighbouring settlements and industrial facilities which is absent in the protected area of the fish farm.

In semi-intensive carp ponds the dominant groups of zooplankton are Rotifers, small Cladocerans and Copepods (Marković *et al.*, 2016). However, when zoobenthos is scarce as in the case of this study, two-year-old carp feed mainly on large crustacean zooplankton species of Cladocera and Copepoda (Rahman *et al.*, 2010; Marković *et al.*, 2016). The ingestion rate of these organisms is usually proportionate to their abundance and size, due to selective carp feeding (Maszczyk and Gliwicz, 2014). This was confirmed in our study since Cladocerans were the only zooplankton representatives found in the carp GC forming a significant proportion of the total food items ingested by the fish, (30%). Therefore, the collection of zooplankton for elemental analysis was done with a 250 μm mesh plankton net to ensure the collection of large crustacean zooplankton species and to minimise the collection of Rotifers and phytoplankton.

As in sediments, the highest concentration of elements in zooplankton was found for Al, Fe and Mn, probably derived from the bioturbation effect of carp moving particulate matter back from the sediments into the water column (Milstein and Svirsky, 1996; Komárková, 1998). However, the level of Zn was two times higher in zooplankton than in the sediments, most likely from ingested phytoplankton that are able to accumulate high levels of Zn even when there are low amounts present in the water (Mazej *et al.*, 2010). Some elements like Cu, Pb and Zn had higher concentrations in the zooplankton in our study (5.76–19.63 mg/kg dw, 0.87–5.45 mg/kg dw, 38.74–120.97 mg/kg dw, respectively) than those reported for the natural lake Balaton (3.7–12.5 mg/kg dw, 0.65–2.10 mg/kg dw, 37.0–70.0 mg/kg dw; Farkas *et al.*, 2003). During this study the level of Sr in zooplankton reached high levels (142.4 mg/kg dw), particularly during the summer period. Generally, zooplankton exoskeleton (carapax) shows a high affinity for Sr, Pb and other positively charged metal ions (Cd, Co, Ni, Zn, Cu) that bind to the carapax possibly by exchange with Ca^{2+} (Robinson *et al.*, 2003). Some studies even suggest that the surface-associated contaminants of prey (*i.e.*, zooplankton) may be more bioavailable to predators than those accumulated in tissues, due to the suitable conditions found in the gut of most animals such as low pH and a high degree of ion complexation (Robinson *et al.*, 2003). Therefore, due to their bioaccumulation properties, we believe that analysing the levels of elements in zooplankton can be used as a good indicator of the level of potentially harmful elements in the aquatic environment of carp ponds, probably providing a better indicator than the simple concentration of elements in the water.

Recent studies have raised the importance of diet born uptake of elements by aquatic animals especially in low-level contaminated waters (Tulonen *et al.*, 2006; Mazej *et al.*, 2010; Filipović Marijić and Raspor, 2012). The concentration of elements in the GCs of benthivorous fish mostly reflects the levels of elements in the sediment and in the natural food. Therefore, the GCs can provide evidence of the accessible pool of elements and their potential bioavailability for further transfer across the gastrointestinal tissue and accumulation in different tissues. Carp GCs in our study resembled the investigated external sources of elements – sediments and zooplankton – sharing the same, most prominent elements, Al, Fe and Mn. Similar results were reported by Filipović Marijić and Raspor (2012) regarding the GCs of European chub (*Squalius cephalus*). Furthermore, these elements accumulated at high concentrations in carp liver probably due to their high levels in the GCs and the accumulation properties of the liver. Similar to other studies analysing the concentrations of

Table 3. Pearson correlation coefficients (r) and their P values for the relationships between carp total weight and element concentrations in carp liver, dorsal and abdominal muscle ($n = 24$). Metal concentrations and carp total weight were log10-transformed. Average carp total weight was 1.96 ± 0.15 kg.

	Liver		Dorsal muscle		Abdominal muscle	
	r	P	r	P	r	P
Al	−0.672	***	−0.430	*	−0.479	*
As	−0.131	0.541	0.164	0.444	−0.0589	0.785
B	−0.189	0.377	0.122	0.569	0.104	0.628
Ba	−0.523	**	−0.118	0.583	0.310	0.628
Cd	−0.691	***	0.012	0.962	0.399	0.054
Co	−0.631	***	dl	–	dl	–
Cu	−0.733	***	−0.522	**	−0.659	***
Fe	−0.765	***	−0.631	***	−0.809	***
Hg	−0.008	0.970	0.507	*	0.441	*
Li	−0.593	**	−0.231	0.278	−0.771	***
Mn	−0.537	**	−0.311	0.138	−0.523	**
Mo	−0.482	*	0.201	0.347	−0.290	0.169
Ni	0.500	*	−0.186	0.384	−0.379	0.068
Pb	−0.245	0.248	dl	–	dl	–
Sb	dl		−0.104	0.628	0.236	0.268
Se	−0.679	***	0.080	0.707	0.005	0.980
Sr	−0.481	*	0.211	0.177	−0.494	*
V	−0.025	0.972	0.211	0.323	0.168	0.432
Zn	−0.650	***	−0.420	*	−0.608	**

dl - detection limit; *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

elements in common carp tissues (Lenhardt *et al.*, 2012; Skorić *et al.*, 2012; Subotić *et al.*, 2013a), we found exceptionally high levels of Zn in carp liver (around 15 times higher) compared with any external sources (sediment and zooplankton) and the carp GCs. Analysis of this phenomenon showed that the concentration of Zn in carp liver is highly related to the physiological properties of common carp liver tissue that has a higher affinity for binding Zn than that of other fish (Liao *et al.*, 2006). The same holds for Cu and Se that were present in higher concentrations in liver compared with external sources. Overall, the results indicate that liver was the target organ most capable of accumulating Cu, Se and Zn, regardless of their initial amounts in available sources.

Even though their level decreased from their concentration in the GCs, some toxic elements *i.e.* As, B, Ba Hg and V were evenly distributed between liver, dorsal and abdominal muscles of fish. Our findings are in line with some other studies concerning arsenic and mercury (Subotić *et al.*, 2013b), showing the potential of these elements to transit through protection barriers (intestinal tissue and liver) into the edible part of fish, the muscles. Toxic elements that are ingested in food can be absorbed through the gut epithelia into blood, from where they first enter the liver and later subsequently build up in the muscles (Edwards *et al.*, 2001).

The content of As in the liver of our carp samples (0.62 mg/kg dw) was higher than in carp from polluted sites of the Serbian part of the Danube River (0.48 mg/kg dw, Subotić *et al.*, 2013a; detection limit, Lenhardt *et al.*, 2012) and Ečka fish ponds (detection limit, Skorić *et al.*, 2012). The same holds for the level of As in muscles of carp from our study (0.40 mg/kg dw) that was much higher than in carp muscle from polluted sites of the Serbian part of the Danube River and the Ecka fish farm where they were below the detection limit (Lenhardt *et al.*, 2012, Skorić *et al.*, 2012). Arsenic is a highly toxic element, and similar levels in fish liver and muscles tissues can indicate its constant presence and gradual build up in the aquatic environment, particularly in sediments in the case of the investigated fish ponds.

Higher concentrations of Sr in the DM compared to the abdominal muscle and liver of our carp samples might be due a higher affinity of this element to muscle tissues (Chowdhury and Blust, 2002). Similar results were reported by Otachi *et al.* (2014) showing higher levels of Sr in fish (blue spotted tilapia, *Oreochromis leucostictus*) DM than in liver. In our study, Sr was present in sediments and consequently in zooplankton feeding on the particulate matter in the water column. Data on the levels of Sr in sediments of aquatic ecosystems are rather scarce in the literature. Otachi *et al.* (2014) reported significantly lower concentrations of Sr in the sediment of Lake Naivasha in Kenya (63.3 mg/kg dw,) compared to the sediment of the fish ponds in the present study (97.13 mg/kg dw).

4.2 Seasonal variations of element concentrations in carp liver, and dorsal and abdominal muscle

Contrary to our hypothesis, during the investigation period, we observed a seasonal decrease of some elements (Al, Co, Fe, Li, Mn, Ni, Pb and Sr) in the studied fish tissues, particularly liver. Several mechanisms can explain this trend of rapid (from May to June in liver) and gradual decrease of elements in fish tissues towards the end of the production cycle. This process

could happen due to the growth dilution effect caused by fish tissue growing faster than element intake (Farkas *et al.*, 2003), or by the activation of liver detoxification mechanisms *i.e.* depuration present in older fish that spend more metabolic energy on this process than younger fish. Juvenile fish invest most of their energy into tissue growth, and thus have higher food consumption implying greater uptake of elements (Merciai *et al.*, 2014). In this study we investigated two-year-old carp that were stocked in May with an average weight of 0.9 kg and six months later reached almost 3 kg. However, this threefold increase in average weight of fish did not affect the concentrations of all investigated elements in the same way. The rest of the investigated elements did not change over time, but, on the contrary, were parallel and consistent in all tissues throughout the production cycle. Some of them accumulated in liver *i.e.* Cu, Mo, Se, Zn, while others, *i.e.* As, B, Ba, Hg and V, were uniformly distributed between tissues. Sr and Li were exceptions since their concentrations in May were equal in the liver and DM and much lower in abdominal muscle. These elements significantly decreased in liver over time and reached concentrations similar to those in the abdominal muscles, but in the DMs they continuously built up towards the end of the season.

It is important to note that none of the investigated elements exceeded the maximal accepted concentration limits prescribed by the National Regulations of the Republic of Serbia (Official Gazette RS No28/2011), the FAO (FAO, 1983), or the EU (European Commission Regulation, 2001).

5 Conclusions

Monitoring of aquaculture ponds during a six month production cycle indicated the accumulation of a range of elements in the sediments and zooplankton of the investigated fish ponds. Of particular importance are the levels of As and Pb in the sediments that are similar to literature data from polluted sites. This information confirms our assumption that sediments of fish ponds connected to polluted rivers can become a pool of toxic elements that may be ingested by farmed carp together with the sediments during feeding.

Analysing the GCs of fish in semi-intensive production can provide important information on the available pool of elements from the environment, and on potential transfer across the intestinal tissue into blood and to different, mostly target organs. The element concentrations in the GCs resembled those in both sediments and zooplankton.

Additionally, the concentration of As in the liver and in both types of sampled muscles in carp from this study were elevated and higher than As levels at polluted sites of the Serbian part of the Danube river. Accordingly, caution is necessary when rearing carp in environments enriched with arsenic due to its potential to become uniformly distributed in fish organs, and thus build up in edible parts, posing a threat to human consumption.

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