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Metals in the Tissues of Two Fish Species from the Rare and Endemic Fish Nature Reserve in the Upper Reaches of the Yangtze River, China

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Abstract Concentrations of copper, zinc, mercury, and arsenic were measured in the muscle, gill and liver tissues of *Coreius heterodon* and *Pelteobagrus vachelli* collected from the rare and endemic fish nature reserve in the upper reaches of the Yangtze River. The concentrations of copper and zinc in the tissues of these two fish species were higher than those of mercury and arsenic. Highest metal concentrations were generally found in fish samples from Yibin. The concentrations of copper, zinc, mercury, and arsenic in *C. heterodon* were higher than that in *P. vachelli*. The fish from this study area were not safe for human consumption.

Keywords Metals · Concentration · Fish · Nature reserve

The accumulation of metals in the freshwater ecosystems has become a problem of increasing concern. These metals may accumulate to a very high toxic level and cause severe impact on the aquatic organisms without any visible sign (Gupta et al. 2009). Metals like Cu and Zn are essential elements and are carefully regulated by physiological mechanisms in most organisms, however, they are regarded as potential hazards that can endanger both animal and human health (Papagiannis et al. 2004; Yarsan et al. 2007), whereas others, such as Hg and As have no known roles in biological systems and can be toxic even at quite low levels (Canli and Atli 2003; Diaz et al. 2006; Sprocati et al. 2006). Fish samples are considered as one of the most indicative factors in aquatic ecosystems for the estimation of trace metals pollution potential (Rashed 2001; Burger et al. 2002).

The purpose of the present study is to evaluate four metals concentration in different tissues of two fish species *Coreius Heterodon* and *Pelteobagrus Vachelli* from the rare and endemic fish nature reserve in the upper reaches of the Yangtze River, which is located in the west of China. The length of rivers in this conservation area is approximately 1,163 km, covering an area of 331.74 km^2. One hundred and sixty fish species live in this area. This nature reserve is established in 2006 to protect the resource of these fish species, as well as prevent water environmental contamination under the tendency of intense industrial development. Although the population of all of the fish species in this area are currently in rapid decline because of environmental degradation due to human activities, it appears that not much attention has been paid towards contamination studies of fish inhabiting these waters. The data dealing with metals pollution in this area are extremely limited. Only a few monitoring studies have measured metal concentration in fish species from this area, however, they are focused only on the Banan region (Zhang et al. 2007). *C. heterodon* and *P. vachelli* are two important commercial and edible species in the upper reaches of the Yangtze River. It is necessary to assess the safety of fish for human consumption. The baseline data for metals concentration in fish from the nature reserve was provided in this study. It will be helpful for river management and fish conservation.

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Materials and Methods

Two fish species (*Coreius heterodon* and *Pelteobagrus vachelli*) which appear to have great economic and ecological importance in the Yangtze River were taken from special fishermen in December 2010 at three different sampling stations (Fig. 1). All the fish samples used in this study were measured to the nearest 0.1 cm and weighed to the nearest 0.1 g (Table 1). Accurate weighed samples (0.35–0.55 g) of muscle (under dorsal fin without skin), gill and liver were taken from each fish and stored in glass vials at 4°C before analysis. They were digested with 4 mL HNO_3 24 h, transferred in high pressure Teflon digestion vessel and rinsed the glass vial with 4 mL HNO_3, then put into a microwave digestion system and set the temperature control procedure (Table 2). After cooling and transfer into a 25 mL glass tube and rinsing with ultra pure water, samples underwent analysis of Cu, Zn, Hg and As.

Levels of Cu and Zn were analyzed using a Varian AA240FS atomic absorption spectrophotometer. The Hg and As concentrations of samples were measured by the KCHG AFS-3100 atomic fluorophotometer. Wavelengths for Cu, Zn, Hg and As were 324.8, 213.9, 253.7 and 193.7 nm, respectively. The detection limits of Cu, Zn, Hg, and As were 1, 1, 0.01, and 0.2 µg/L, respectively. Recovery rates ranged from 95 to 99% for all investigated elements. The accuracy of the applied analytical procedure was tested using certified reference materials (GBW08573, standard sample of yellow croaker constituent) provided by the National Research Center for Certified Reference Materials of China. They were analyzed at regular time intervals throughout the measurement together with the studied fish samples (Table 3).

Table 1 Number, length and weight ranges of the two fish species

Fish	Number	Length ranges (cm)	Weight ranges (g)
C. heterodon	33	16.8–23.0	78.0–217.1
P. vachelli	39	10.3–18.9	17.1–125.7

Table 2 Temperature control procedure of microwave digestion

Step	Temperature (°C)	Power (w)	Time (min)
1	120	800	10
2	160	900	6
3	180	1000	4

Table 3 Measurements of standard sample of yellow croaker constituent (mg/kg wet wt)

	Cu	Zn	Hg	As
Certified values	1.36 ± 0.13	28.8 ± 1.4	0.169 ± 0.018	5.08 ± 0.39
Measured values	1.35 ± 0.01	28.7 ± 0.8	0.179 ± 0.002	5.31 ± 0.02

All statistical calculations were performed using SPSS 13.0 for Windows. One-way analysis of variance (ANOVA) and Duncan's Multiple Comparison Test ($p = 0.05$) were used to access whether metal concentrations varied

Fig. 1 Map of the rare and endemic fish nature reserve in the upper reaches of the Yangtze River in southwest of China with the sampling stations

significantly between fish species. Pearson's correlation coefficient (r) was used to examine the relationship between metals concentration and fish length.

Results and Discussion

The metal concentration in the tissues of *C. heterodon* and *P. vachelli* from the different sampling stations are showed in Table 4. The concentrations of Cu and Zn in the tissues of these two fish species were higher than those of Hg and As. The mean concentrations of Cu, Zn, Hg, and As in the muscle of the two fish species were 1.76 ± 1.22, 2.64 ± 1.54, 0.29 ± 0.12, and 0.31 ± 0.12 mg/kg, respectively, while concentrations in the gill were 3.23 ± 1.99, 14.07 ± 2.97, 0.49 ± 0.18, and 0.49 ± 0.21 mg/kg, respectively. The concentration of Cu in the liver of *C. heterodon* was 593.98 ± 100.04 mg/kg, and that of *P. vachelli* 5.70 ± 3.90 mg/kg. The concentrations of Zn, Hg, and As in the liver of the two fish species were 17.53 ± 3.26, 0.36 ± 0.11, and 0.41 ± 0.18 mg/kg, respectively. The most possible reason for that may be related to different metals capabilities of accumulation, as well as with the background values in the aquatic ecosystems.

The concentration of Cu in the muscle and gill of *C. heterodon* in Yibin were significantly higher than that in Luzhou and Banan ($p < 0.05$). Yibin displayed the highest concentration of Cu in the tissues of *P. vachelli*. The concentration of Zn in the muscle of *C. heterodon* and *P. vachelli* was highest in Yibin, and was significantly higher than in Luzhou ($p < 0.05$). The highest concentrations of Hg were shown in the muscle of *C. heterodon* and *P. vachelli*. The concentrations of As were significantly higher in the muscle and liver of *P. vachelli* in Yibin compared to the values measured in fish tissues from Luzhou and Banan ($p < 0.05$). The results of this study indicated that the highest metal concentrations were generally found in fish samples from Yibin, where is in the upper reaches of the study area. It has been demonstrated that the background concentrations of elements in soil of the upper reaches of the Yangtze River were naturally higher (Chen et al. 1991). Although industrial activities and domestic disposal are present in the reserve area, discharging wastewater is forced to meet the national environmental standards, and therefore the main source of metals could be attributed to agricultural activities and surface runoff. Metal concentrations of fish samples in

Table 4 Concentrations of four metals (Cu, Zn, Hg, and As) in tissues (muscle, gill, and liver) of two fish species (*C. heterodon* and *P. vachelli*) from three study areas (mean ± SD, mg/kg wet wt)

	Fish species	Stations	Tissues		
			Muscle	Gill	Liver
Cu	*C. heterodon*	Yibin	5.65 ± 1.75[a]	8.75 ± 0.48[a]	615.44 ± 65.01
		Luzhou	1.91 ± 0.87[b]	2.58 ± 1.06[b]	572.93 ± 138.90
		Banan	1.72 ± 0.89[b]	2.87 ± 0.77[b]	614.40 ± 2.47
	P. vachelli	Yibin	1.62 ± 0.64[a]	3.22 ± 0.67	8.64 ± 5.09
		Luzhou	1.16 ± 0.14[b]	2.05 ± 0.39	5.55 ± 2.92
		Banan	1.30 ± 0.15[ab]	2.39 ± 0.53	3.68 ± 1.16
Zn	*C. heterodon*	Yibin	4.41 ± 1.86[a]	14.23 ± 1.05	20.03 ± 1.30
		Luzhou	1.75 ± 1.33[b]	15.71 ± 3.14	18.77 ± 1.45
		Banan	3.09 ± 1.32[ab]	14.08 ± 4.20	21.71 ± 2.51
	P. vachelli	Yibin	3.86 ± 1.64[a]	14.84 ± 1.48	16.18 ± 2.29
		Luzhou	1.48 ± 0.66[b]	12.18 ± 0.89	12.97 ± 0.37
		Banan	2.18 ± 0.69[b]	11.92 ± 2.86	14.91 ± 1.90
Hg	*C. heterodon*	Yibin	0.37 ± 0.05[a]	0.64 ± 0.19	0.38 ± 0.04
		Luzhou	0.36 ± 0.08[ab]	0.59 ± 0.02	0.41 ± 0.13
		Banan	0.22 ± 0.16[b]	0.38 ± 0.18	0.31 ± 0.14
	P. vachelli	Yibin	0.35 ± 0.10[a]	0.40 ± 0.14	0.38 ± 0.10
		Luzhou	0.20 ± 0.11[b]	0.40 ± 0.23	0.26 ± 0.05
		Banan	0.25 ± 0.10[ab]	0.43 ± 0.07	0.36 ± 0.11
As	*C. heterodon*	Yibin	0.30 ± 0.09	0.60 ± 0.29	0.52 ± 0.06
		Luzhou	0.33 ± 0.14	0.58 ± 0.20	0.41 ± 0.20
		Banan	0.34 ± 0.17	0.34 ± 0.04	0.38 ± 0.26
	P. vachelli	Yibin	0.40 ± 0.11[a]	0.33 ± 0.15	0.70 ± 0.02[a]
		Luzhou	0.30 ± 0.08[b]	0.45 ± 0.07	0.34 ± 0.01[b]
		Banan	0.21 ± 0.06[b]	0.49 ± 0.25	0.28 ± 0.08[b]

Different letters indicate significant differences between sampling sites ($p < 0.05$)

Table 5 Relationships between tissues metal concentrations and fish length for *C. heterodon* and *P. vachelli* (Pearson's correlation coefficient, *r*)

Fish species	Tissues	Cu	Zn	Hg	As
C. heterodon	Muscle	−0.04	−0.25	0.54	−0.38
	Gill	−0.14	0.09	0.10	0.00
	Liver	0.10	−0.33	−0.26	0.65*
P. vachelli	Muscle	−0.58**	0.21	0.25	−0.31
	Gill	0.76*	0.39	0.19	−0.66
	Liver	−0.30	0.57	0.70*	-0.61

* $p < 0.05$, ** $p < 0.01$

Luzhou and Banan were lower than that in Yibin, what could be the result of the recycling of metals in the river and their deposition into the sediments (Johnson et al. 2005; Tipping et al. 2006).

The relationships between metal concentrations and fish length in different tissues of *C. heterodon* and *P. vachelli* are showed in Table 5. No identical relationships between metal concentrations and fish length were showed in present study. Except in a few cases, significant relationships between metal concentrations and fish size were negative (Canli and Atli 2003; Papagiannis et al. 2004). The possible explanation for the higher metal concentrations found in smaller sized of fish might be explained by the fact that younger individuals appear to have higher growth rate and metabolic activity (Leonardos et al. 2005). In our study, significant negative correlation was found between concentration of Cu in muscle of *P. vachelli* and fish length ($p < 0.01$), while significant positive correlations were observed between fish length and concentrations for As in liver of *C. heterodon*, Cu in gill, and Hg in liver of *P. vachelli* ($p < 0.05$). Positive relationships between metal concentrations in fish tissues and length were also reported in other studies (Pourang 1995; Farkas et al. 2003). Fish often demonstrate increasing levels of toxicants with size or age, which appears to be consistent with the longer exposure of older animals (Jureša and Blanuša 2003). Although negative or positive correlations were observed between metal contents in some tissues and length of the species, metal concentrations in some other tissues showed no significant correlations with fish length (Henry et al. 2004; Ploetz et al. 2007), what is in agreement with the results of our study where the concentrations of Zn, Hg, and As in muscle and gill of *P. vachelli* displayed no significant correlations with fish length.

Metal concentrations in fish are usually species specific (Gaspic et al. 2002). Differences in metal concentrations between fish species might be a result of different habitat environment, feeding habits, swimming behavior, ecological needs and metabolic activities (Karadede and Unlu 2000; Mdegela et al. 2009). *C. heterodon* is an omnivorous fish living in the bottom of river, its diet consisting of mollusks, organism detritus, and some kinds of diatoms. *P. vachelli* has similar habitat environment and feeding habits to *C. heterodon*. The concentrations of Cu, Zn, Hg, and As in *C. heterodon*, however, were higher than that in *P. vachelli* (Fig. 2). Furthermore, the significant differences were found in the concentration of Cu in muscle and liver and Zn in liver between these two fish species ($p < 0.05$). This might be related to the differences in metabolic activities and metals accumulation abilities between them. Differences in geographic distribution of life stages could be additional possible explanations for the variation observed in metal concentration between different fish species (Allen-Gil and Martynov 1995).

It is generally accepted that the muscle tissue is recommended as a target tissue for estimating the safety for human consumption (Begum et al. 2005). Metal concentrations in gill reflect the metal concentration in water where fish live, while the concentrations in liver represent metals storage capacity (Romeoa et al. 1999; Yilmaz 2003). Concentrations of the four metals were lower in the muscle compared to the gill and liver (Fig. 2). Similar results were reported for some fish species in other studies, showing that the muscle were not an active tissue for metals accumulation (Tekin-Özan and Kir 2008; Alam et al. 2002). The highest concentrations of Cu and Zn were observed in liver, what is in agreement with reports in literature (Canli et al. 2001). Fish can retain Cu and Zn owing to specific binding proteins known as metallothioneins in liver tissue (Allen-Gil and Martynov 1995). Liver tissues of fish is often recommended as an environmental indicator of water pollution (Licata et al. 2005). However, higher concentrations of Hg and As were observed in gill compared to liver in the present study. This could possibly be attributed to the fish contaminated with Hg and As mainly from high volumes of water being filtered through the gill. It is interesting that the concentration of Cu in the liver of *C. heterodon* was about 200 times as high than in muscle and gill. Whether or not gene mutation occurred in the liver of *C. heterodon* which induced Cu hyper accumulation needs to be studied further.

The concentrations of Cu, Zn, and As in muscle were below the tolerance limit levels (Cu: 50 mg/kg; Zn: 50 mg/kg; As: 0.5 mg/kg) assigned by the Ministry of health of China. The concentrations of Hg in *C. heterodon* from Yibin and Banan and in *P. vachelli* from Yibin, however,

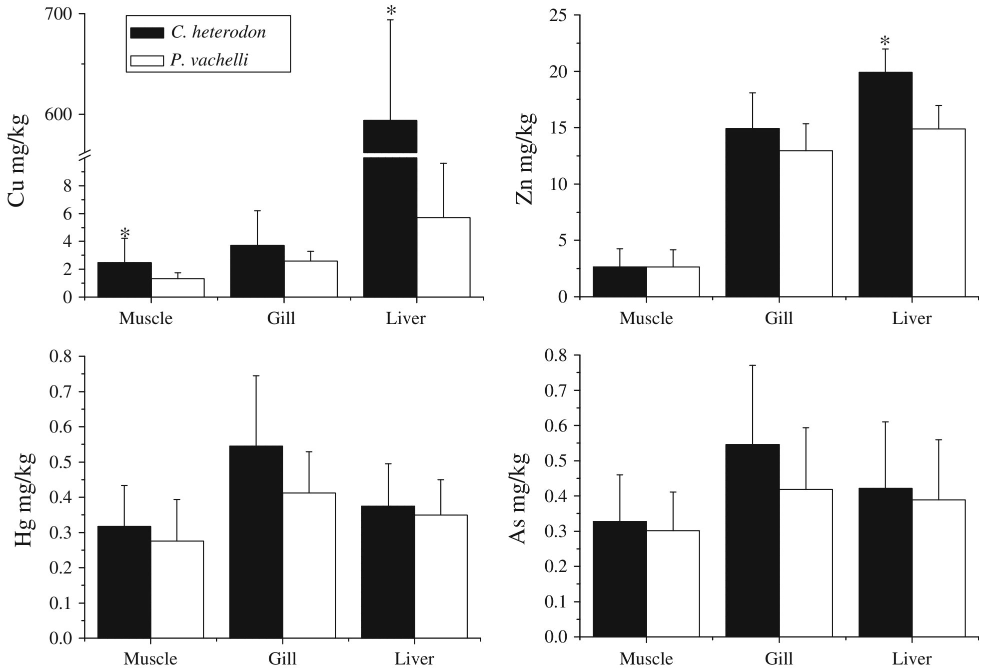

Fig. 2 Comparison of mean concentrations of Cu, Zn, Hg and As (mg/kg wet wt.) in the muscle, gill and liver between *C. heterodon* and *P. vachelli*. *$p < 0.05$, significant difference between the two fish species

were above the tolerance limit level (0.3 mg/kg). These results indicate that the fish from this study area are not safe for human consumption. The present study provide baseline data for metals accumulation in fish species from nature reserve in the upper reaches of the Yangtze Rive, and also indicate that long-term monitoring is needed in this region for metal pollution.

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