

Heavy Metal Contamination in Indochinese Molluscivorous Catfish (*Helicophagus leptorhynchus* Ng & Kottelat, 2000) from Mun River Basin, Ubon Ratchathani Province

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ABSTRACT

Concentrations of heavy metals (cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn) and zinc (Zn)) were determined in the liver, kidney, and muscle of *Helicophagus leptorhynchus* found in the Mun River, the longest Mekong tributary in Thailand. Sampling was conducted in three sites, covering the area from up- to down-streams. The concentration of Zn in the fish tissues was significantly higher than those of other heavy metals. Variation in concentrations of heavy metals was observed, and they varied according to space and time. The patterns of metal allocation in the studied organs were (a) liver: Zn > Cu > Cr > Cd > Mn > Pb, (b) kidney: Zn > Cr > Cd > Cu > Mn > Pb and (c) muscle: Zn > Cr > Mn > Cu > Cd > Pb.

Keywords: *Helicophagus leptorhynchus*, fish tissues, heavy metals

INTRODUCTION

Concern on the effect of anthropogenic pollution to freshwater ecosystems is becoming vital since large amounts of the pollutants are run-off from roads, communities, industrial and agricultural areas worldwide (Langston *et al.*, 1999). Among anthropogenic pollutants, heavy metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate, i.e. accumulate in the organism's tissues and aquatic ecosystems (Censi *et al.*, 2006). The distribution of potentially toxic elements in different organs of aquatic living things depends on the element, the species, time of exposure, and

exposure route (Marcussen *et al.*, 2007). They are, then, eventually accumulated in the food chain and cause ecological damage as well as threaten human health either directly or indirectly.

Risk posed by heavy metals to humans is commonly by means of consumption, in which fish are the major food resource from freshwater ecosystems. Moreover, fish are often at the top of the aquatic food chain and may concentrate large amounts of heavy metals from water (Mansour and Sidky, 2003; Priprem *et al.*, 2007). Potentially toxic heavy metals may accumulate in fish because of dietary exposure or absorption through the

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gills (Ruangsomboon and Wongrat, 2006). Thus, commercial and edible species have been widely investigated in order to check for those hazardous to human health (Begüm *et al.*, 2005). Moreover, the metal concentrations in fish can act as an environmental indicator for the integrity or state of the system (Wildianarko *et al.*, 2000). Also, they have the advantage of allowing the comparison of metal concentrations among sites and seasons, where water samples are near or below the detection limit (Ramelow *et al.*, 1989).

Consumption of freshwater fish in the Mekong riparian countries is the highest in the world. The average consumption of freshwater fish per person is approximately 14 kg/ person/year, which is six times higher than the world average (Hortle, 2007). Among these fish species, the Pangasiid catfish is one of the dominating fish families being harvested. The Pangasiid catfishes are skin fish, i.e. no scale covers the body and the size of adults varies from less than 40 cm in *Pseudolaia pleurotaenia* to larger than 200 cm in *Pangasianodon gigas* (Ferraris, 2007). The fishes in this family are also known as migratory riverine fish species that move between upstream refuge and spawning habitats and downstream feeding and nursery habitats. The Pangasiid catfish is omnivorous, feeding on algae, higher plants, zooplankton and insects, while larger specimens also take fruit, crustaceans and fish (Rainboth, 1996). The most common and commercial fish species of this family are *Pangasianodon hypophthalmus*, *Pangasius macronema*, *Pangasius bocourti* and *Helicophagus leptorhynchus* (Valbo-Jorgensen *et al.*, 2009; Jiwyam *et al.*, 2010). Among these Pangasiid fishes, *H. leptorhynchus* is of particular concern in terms of heavy metal

contamination since this species is benthic feeding which feed exclusively on mollusks (Poulsen *et al.* 2004). Generally large amounts of heavy metals could accumulate in sediments, which become their main reservoir in the wetlands (Svobodova *et al.*, 2002). Heavy metals accumulating in sediments can affect the concentration of heavy metals in organisms which dwell in these sediments, especially mollusks (Kim and Kim, 2006).

Diets are the main source of toxic bioaccumulation which could be transferred within the aquatic ecosystem and mostly accumulate highly in the top consumers (Ruangsomboon and Wongrat, 2006). Most of the studies in the contamination of heavy metals in the Mekong countries, so far, are focused either on water, sediment or mollusks. Surprisingly, very few studies have been done relating contamination of heavy metals with freshwater fish in the basin because fish are top consumers in the aquatic food web, though they are among the major protein sources (for example Ruangsomboon and Wongrat, 2006; Marcussen *et al.*, 2007; Pripem *et al.*, 2007). The objective of the study, then, is to evaluate heavy metal concentrations (i.e. Zn, Mn, Cu, Pb, Cr and Cd) in *H. leptorhynchus* from different organs and compare them in terms of spatio-temporal approach as well as the differences among organs *per se*.

MATERIALS AND METHODS

Sampling locations and samples

The Mun River is the largest Mekong tributary in Thailand (117,000 km² or 75%

of the Khorat Plateau) and the longest (641 km) in northeastern Thailand. A run-of-the-river hydropower dam called the Pak Mun Dam is located 6 km upstream from the confluence with the Mekong mainstream, in addition to a number of irrigation dams along the river, creating a cascade (Jutagate *et al.*, 2005). Three sites were selected in the lowland portion of the river from the upstream to downstream in Ubon Ratchathani Province, namely (A) Wangyang, Warin Chamrap ($15^{\circ} 10' 769.0''$ N $104^{\circ} 43' 117.0''$ E), (B) Buatha, Sawang Weerawong ($15^{\circ} 14' 30.4''$ N $104^{\circ} 57' 17.9''$ E), and, (C) Bandan, Khong Chiam ($15^{\circ} 19' 10.6''$ N $105^{\circ} 29' 47.1''$ E).

The three sampling sites reflected three separated areas (Fig. 1). The first site represents the most upper reach of the study at the confluent between Mun and Chi Rivers, before the run through the city of Ubon Ratchathani. The second site is downstream

of the city of Ubon Ratchathani, and the third site is at the confluence of the Mun River to the Mekong mainstream. Fish were sampled 3 times, representing the 3 seasons (summer, rainy and winter) between October 2009 and September 2010. A total of 397 specimens were collected, i.e. 96 from Wangyang, 173 from Buatha and 128 from Bandan. The size range of the samples was between 13.2 and 44.0 cm SL. Fish samples at very fresh condition were dissected to obtain the targeted tissue separately, i.e. liver, kidney and muscle. These tissues were then kept separately in cleaned plastic zip bags and stored in -80°C freezer, until analysis was carried out in the laboratory.

Laboratory

The tissues were analyzed by a modified procedure from the Association of Official Analytical Chemists (AOAC, 1995). One gram of the tissue, i.e. each for liver,

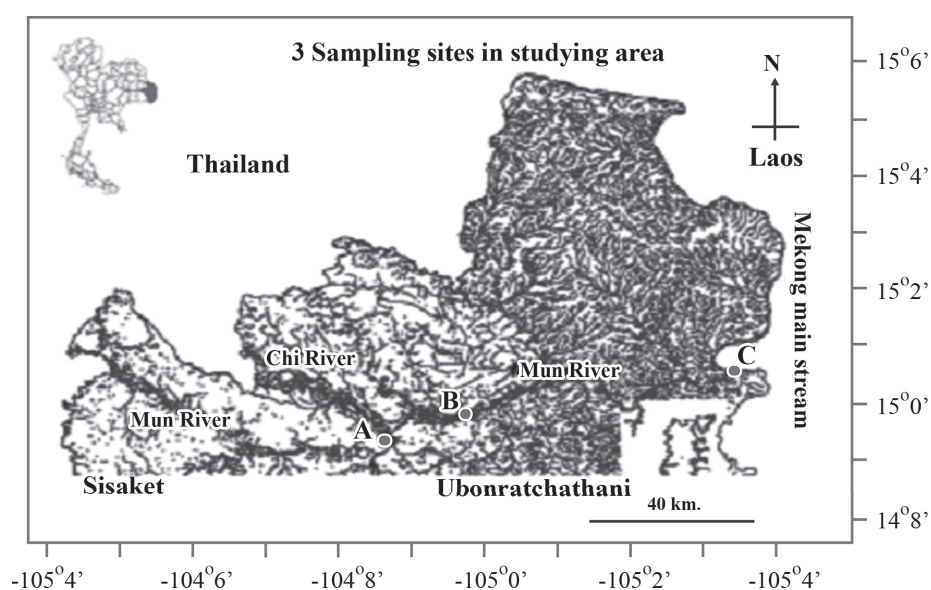


Figure 1. Three sampling sites in the study area. (A) Wangyang, (B) Buatha and (C) Bandan.

kidney and muscle, was dried for 8 hours at 60°C. Then, 0.2 g of each dried sample was digested by Nitric acid and Perchloric acid. The digested samples were cooled at room temperature, filtered through Whatmann No. 5 and finally the volume was made to 50 ml with distilled water. The concentration was presented as milligram of heavy metal in one kilogram of sample dry weight ($\text{mg kg}^{-1} \text{DW}^{-1}$). Analysis of the heavy metals (Zn, Mn, Cu, Pb, Cr and Cd) has been done by using Atomic absorption spectrophotometer (flame technic) model: GBC AVANTA.

Data analyses

The obtained values of the heavy metals were statistically analyzed, through firstly, one-way analysis of variance (ANOVA) to compare the differences in (a) concentrations of the heavy metals in the samples, (b) the concentrations of each heavy metal by the effect of sampling sites and organs. Secondly, two-way ANOVA, with sites and seasons as factors, was used for determine the effect to concentrations of each heavy metal. Duncan's multiple range post-hoc tests were applied when ANOVA revealed significant differences. Thirdly, the multivariate analysis of variance (MANOVA) was applied to multivariate heavy metals data to determine the effect of sites, seasons and their combinations. Lastly, the correspondence analysis (CA) was applied to graphically characterize the sites and combinations between sites and seasons in relation to the concentration heavy metal. All statistical analyses were carried out with R software (R Development Core Team, 2012).

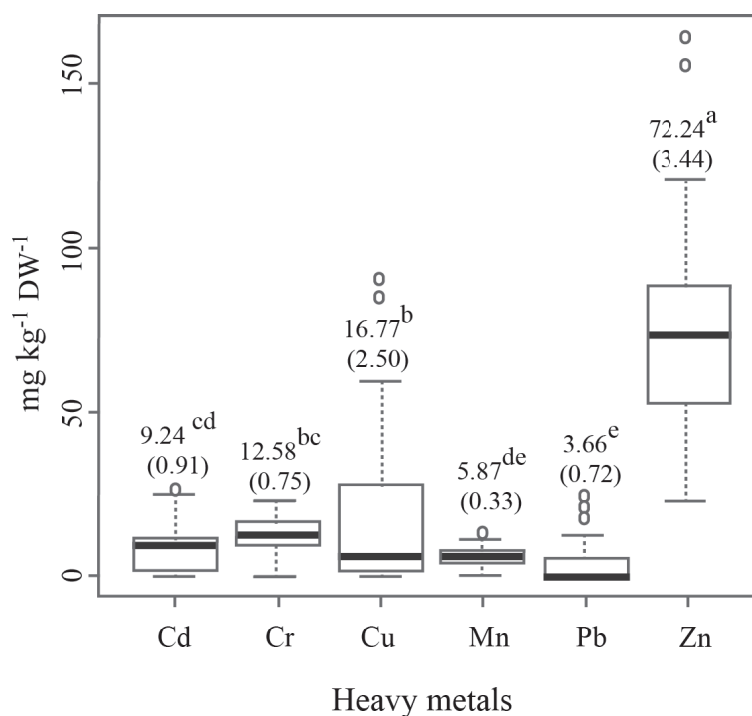
RESULTS

The average concentrations of the six selected heavy metals (Zn, Mn, Cu, Pb, Cr and Cd), in the samples of *Helicophagus leptorhynchus* from the Mun River, were mostly lower than $20 \text{ mg kg}^{-1} \text{DW}^{-1}$, except for Zn ($72.24 \pm 3.44 \text{ mg kg}^{-1} \text{DW}^{-1}$), which was significantly higher (F -test, $F_{5,414} = 201.40$, P -value $< 2 \times 10^{-6}$) than the others (Fig. 2), followed by Cu ($16.77 \pm 2.50 \text{ mg kg}^{-1} \text{DW}^{-1}$). The lowest contaminant level was Pb ($3.66 \pm 0.72 \text{ mg kg}^{-1} \text{DW}^{-1}$) but it was not significantly different from Mn. It is also worthy to note that extreme concentrations (i.e. outliers) were observed in all heavy metals except Cr.

The multivariate analysis of variance (MANOVA) revealed significant differences in the overall concentration of the heavy metals depending on stations (*Pilai's test*, $F_{2,61} = 7.56$, P -value $= 3.92 \times 10^{-10}$) and its combination to seasons (*Pilai's test*, $F_{4,61} = 1.74$, P -value $= 0.019$; Table 1). The correspondence analysis (CA) illustrated the effect of stations (Fig. 3) to each heavy metal. The two axes (first and second CAs) explained 100% and 87.4% of the total inertia in the first and second CAs, respectively. In the first CA, i.e. effected by stations (Fig. 3), Pb dominated in the tissues from Wangyang samples, meanwhile Cd and Cu did for Bandan samples. The short arms of the three remaining heavy metals, on the other hand, implied that they were prevalent in the tissues of *H. leptorhynchus* from all sampling stations. Although prevalent, statistical differences among stations (Fig. 4) were observed in Zn (F -test, $F_{2,67} = 3.18$,

Table 1. MANOVA results on the concentrations of the six heavy metals in *Helicophagus leptorhynchus* from Mun River.

Source of variation	d.f.	Pilai's-value	F-value	P-value
Stations	2	0.887	7.569	3.92×10^{-10}
Seasons	2	0.289	1.606	0.099
Stations x Seasons	4	0.603	1/744	0.020

Figure 2. Boxplots presenting the distribution of concentration of six heavy metals in *H. leptorhynchus* from the Mun River. The average associated with standard error, in parenthesis, of each heavy metal is presented above the box. Different letters, behind the averages, indicate statistical different at $\alpha = 0.05$.

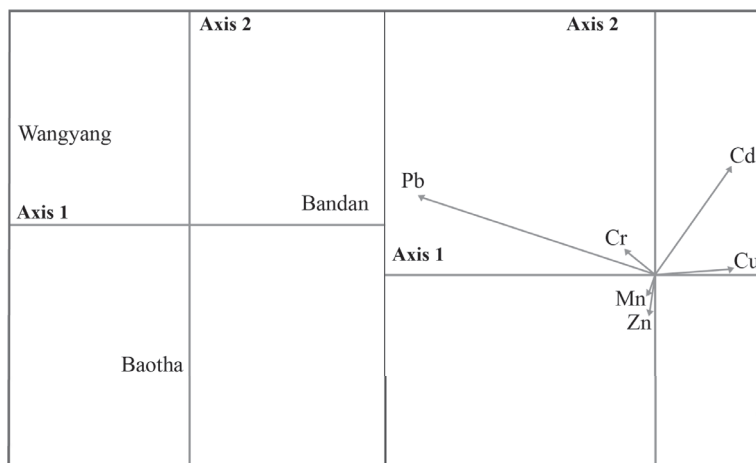


Figure 3. Ordination diagram of the correspondence analysis showing the relationship of sampling stations and selected heavy metals.

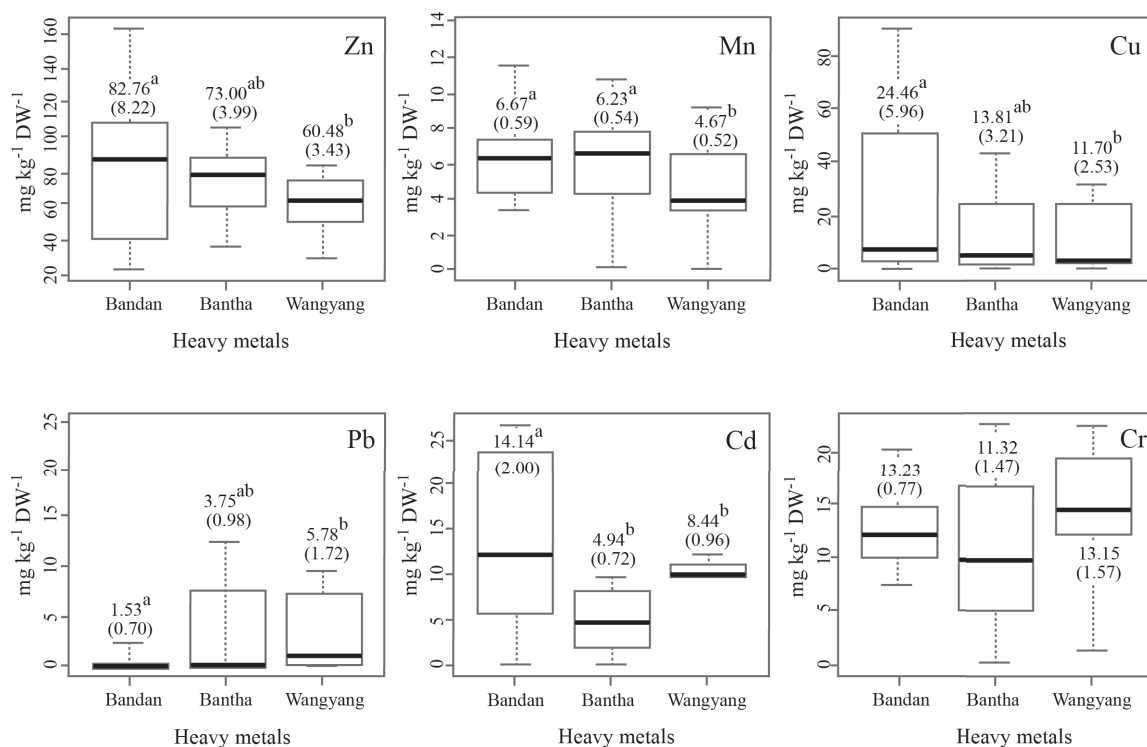


Figure 4. Boxplots presenting the distribution of concentration of each heavy metal, among the sampling stations, in *H. leptorhynchus* from the Mun River. The average associated with standard error, in parenthesis, of each heavy metal is presented above the box. The different letters, behind the averages, indicate statistical different at $\alpha = 0.05$.

P -value = 0.027) and Mn (F -test, $F_{2,67} = 3.61$ P -value = 0.033) but not for Cr (F -test, $F_{2,67} = 0.68$, P -value = 0.51). The second CA, i.e. the effect combinations of stations and seasons (Fig. 5), refined the results of the first CA and showed that the domination of Pb in Wangyang samples was only in the winter, meanwhile Cd and Cu dominated in the Bandan samples all year round. Fluctuations in heavy metals used to discriminate the combinations of stations and seasons were confirmed by means of ANOVA (Table 2). Non-statistical significant difference in concentrations of Zn and Mn, among the combination, made the arrow arms of both metals in Fig. 5. However, a significant difference was found in Mn, which could be attributed by the low variation of the contaminant level to the tissue of *H. leptorhynchus*.

The MANOVA results indicated varied significant differences in the overall concentration of the heavy metals among

organs (P ilai's test, $F_{2,67} = 1.23$, P -value = 2.26×10^{-16}). Range of the concentration of each heavy metal in the tissues of the three organs, i.e. kidney, liver and muscle, are shown in Fig. 6. ANOVA showed that the concentration levels of each heavy metal significantly varied organ by organ. Muscles contained relative less heavy metals compared to the other two organs. The significantly higher (P -value < 0.05) concentrations of Zn, Mn and Cu were encountered in the liver. The contamination level of Pb was also high in the liver but significantly different only with muscle (P -value < 0.05) and not kidney (P -value > 0.05). Meanwhile, kidney contained more quantities (P -value < 0.05) of Cd and Cr than the other two selected organs. The sequences, in terms of concentration, of the heavy metal in each selected organ are listed as (a) liver: Zn > Cu > Cr > Cd > Mn > Pb, (b) kidney: Zn > Cr > Cd > Cu > Mn > Pb and (c) muscle: Zn > Cr > Mn > Cu > Cd > Pb.

Table 2. ANOVA results on the concentrations of heavy metal in *Helicophagus leptorhynchus* from Mun River.

Station x Season	Heavy metals (mg kg ⁻¹ DW ⁻¹)					
	Zn	Mn	Cu	Pb	Cd	Cr
Wy_summer	54.68±6.71	4.67±0.80 ^b	11.90±4.87 ^b	1.07±0.71 ^a	8.15±1.79 ^{abc}	12.69±2.72
Wy_rainy	62.38±5.13	4.51±1.02 ^b	9.94±3.62 ^b	2.20±1.01 ^a	8.22±1.82 ^{abc}	11.63±2.66
Wy_winter	64.94±6.04	4.83±1.01 ^{ab}	13.47±5.18 ^b	15.23±3.46 ^b	9.00±1.51 ^{abc}	15.41±3.03
Bt_summer	68.06±5.77	6.54±0.83 ^{ab}	11.05±4.88 ^b	4.39±1.89 ^a	4.47±1.38 ^c	13.56±2.50
Bt_rainy	70.69±6.63	6.73±0.98 ^{ab}	9.59±3.56 ^b	2.75±1.57 ^a	4.25±1.04 ^c	11.65±2.90
Bt_winter	81.30±8.52	5.33±1.06 ^{ab}	21.26±7.84 ^a	4.17±1.80 ^a	6.28±1.39 ^{bc}	8.38±1.98
Bd_summer	80.41±11.80	6.88±0.87 ^{ab}	20.87±8.32 ^a	2.45±1.53 ^a	12.89±3.48 ^{ab}	12.48±1.29
Bd_rainy	86.40±13.74	5.21±0.68 ^{ab}	23.83±9.69 ^a	0.93±0.92 ^a	14.07±3.75 ^{ab}	11.82±0.86
Bd_winter	81.48±18.32	7.90±1.31 ^a	28.70±13.48 ^a	1.21±1.21 ^a	15.45±3.48 ^a	15.38±1.57

Note: Different letters (a, b and c) in each column indicate statistical different at $\alpha = 0.05$.

(Wy= Wangyang; Bt= Buatha; Bd= Bandan)

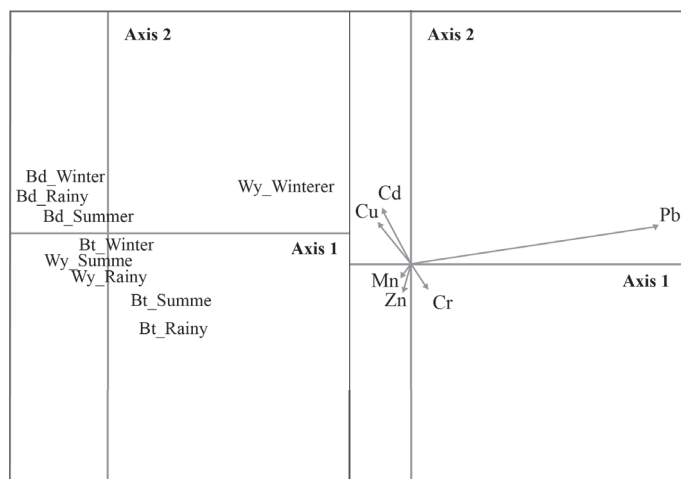


Figure 5. Ordination diagram of the correspondence analysis showing the relationship of combinations of stations x seasons and selected heavy metals.

Note: Wy = Wangyang; Bt= Buatha; Bd = Bandan

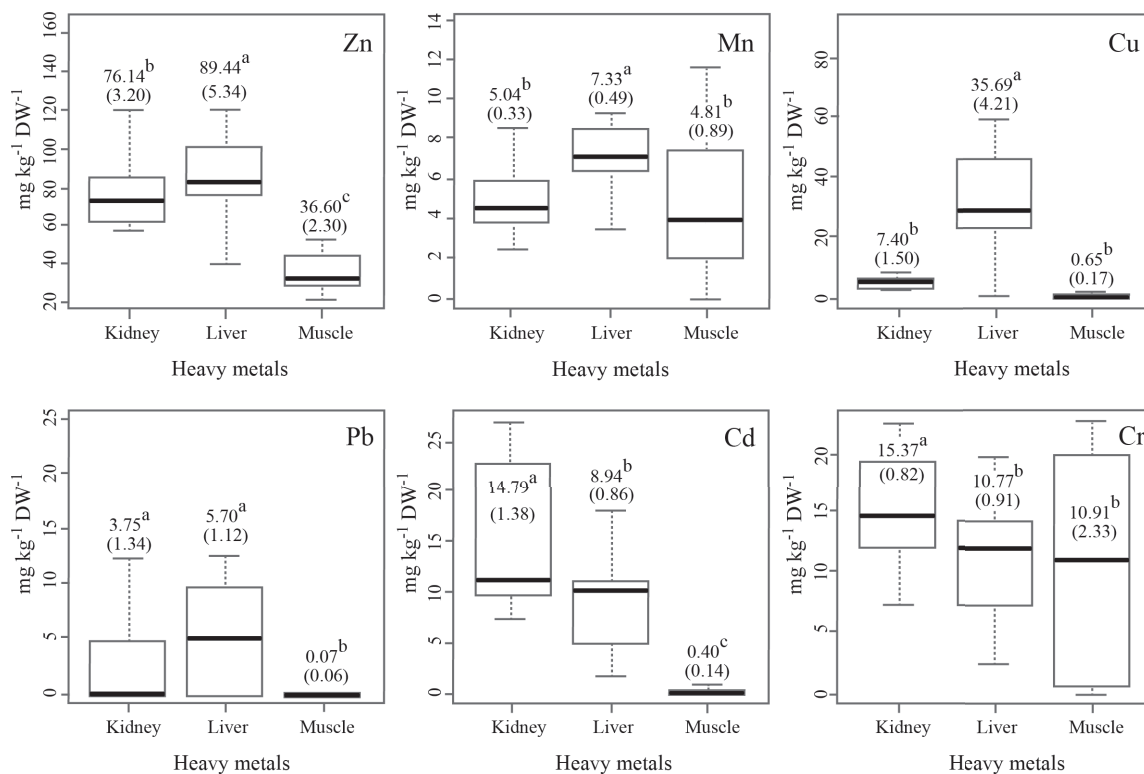


Figure 6. Boxplots presenting the distribution of concentration of each heavy metal in fish tissues, in *H. leptorhynchus* from the Mun River. The average associated with standard error, in parenthesis, of each heavy metal is presented above the box. The different letters, behind the averages, indicate statistical different at $\alpha = 0.05$.

DISCUSSION

The concentrations of all investigated heavy metals contaminating *H. leptorhynchus* varied among sites and seasons, in which Zn showed the highest concentration level. This heavy metal is, therefore, of serious concern because the Zn concentrations in all samples were either double or triple the acceptable value for Zn in edible fish (i.e. 30 mg kg⁻¹ DW⁻¹; FAO, 1983; FDA 2011). This heavy metal is likely coming from rubber plantations, which are now very popular in northeast of Thailand. Zinc oxide is commonly used as a white pigment for preparing the rubber latex (Fierro, 2006), and the residues could be washed away to the river. In addition, in addition to Zn, the findings revealed that the average concentration levels of all the other heavy metals investigated were more than the permissible concentrations for human consumption (FDA, 2011). This is, therefore, alarming as to the high intensity of heavy metal contamination in *H. leptorhynchus* in the Mun River as well as in the Mekong mainstream. The sources of these heavy metals include surface runoff from agricultural lands or urban wastewater. For example, Pb and Cr are used as pesticides, meanwhile Cu and Pb are from construction sites (Fierro, 2006). Similar results on high concentration of heavy metals in other fishes from nearby rivers, i.e. the Pong and Chi Rivers, were also reported (Priprem *et al.*, 2007), although not as high as the levels in *H. leptorhynchus* from the Mun River.

In terms of spatial approach, the impacts from terrestrial effluences were obvious. Wangyang site is surrounded by agricultural

areas (*personal observation*), therefore, there is a high chance of contamination by Pb which is used for pest control. Meanwhile, high concentrations of Cd and Cu in Bandan are attributed to the mining activities in Lao PDR (U.S. Geological Survey, 2012) as well as accumulation in the downstream site (Qadir and Malik, 2011). Seasonal differences in rainfall and river flow can also influence metal accumulation and bioavailability (Simkiss and Mason, 1983; Qadir and Malik, 2011). High concentrations of heavy metals tend to increase in the rainy season due to terrestrial runoffs, then accumulate in winter while the flow rate is relatively low (Agarwal *et al.*, 2007). Moreover, it has also been reported that Zn and Cu would accumulate more in fishes in the summer due to the increase in temperature (Ibrahim and Omar, 2013).

Variations in contamination of heavy metals were also different among fish organs. In general, fish organs show significant variations in metal accumulation, which is related to differences in uptake, absorption, storage, regulation and excretion abilities of fish species (Agarwal *et al.*, 2007; Qadir and Malik, 2011). The present results are in agreement with Velcheva (2006), who reported that heavy metals were significantly higher in tissues in fish liver and kidney than in the muscle. Four heavy metals *viz.*, Zn, Mn, Pb and Cu were accumulated in high concentrations in the liver of *H. leptorhynchus* because fish liver is a storage organ and thus it accumulates the highest level of heavy metals (Priprem *et al.*, 2007). This is due to the fact that the liver is a vital organ concerned with basic metabolism and acts like a filter that eliminates unwanted

substances, including heavy metals (Figueiredo *et al.*, 2006). The remaining heavy metals, Cr and Cd were high in the kidney. The reason for Cu to be highest in the kidney is because the fish kidney contains a cystine rich copper binding protein, which is thought to have either a detoxifying or storage function, meanwhile Cd is accumulated and form as metalloprotein complexes in kidney (Ashraf, 2005). The lowest heavy metal concentration is in the muscle. This was an expected result and similar as reported elsewhere. It has been pointed out that the muscle is not an active tissue in accumulating heavy metals in fishes (Legorburu *et al.*, 1988).

CONCLUSION

The high concentration of heavy metals in *H. leptorhynchus* is an alarming sign of environmental integrity and human consumption of this fish from the Mun River. Investigation on this matter should be conducted with other fish species as well. A number of studies reported on the variations in contamination levels of heavy metals among fish species (e.g. Legorburu *et al.*, 1988; Begüm *et al.*, 2005; Velcheva 2006; Priprem *et al.*, 2007). The variability of heavy metals in different species depends on feeding habitats, ecological needs, metabolism, age, size, length of the fish and their habitats (Priprem *et al.*, 2007). This is not only impacting the fishes, but this will also eventually impact the ecological integrity of aquatic resources and to the people around the Mun River. This will be harmful and they must be concerned of this.

Differences in metal concentrations in fish can be attributed to the presence of metal contaminants in surface water (Qadir and Malik, 2011) and hence people who use surface water for consumption must be aware of their possible toxic effect. Thus it is recommended that a monitoring program on heavy metals in freshwater fish in Thailand must be established and a mitigation program must be considered a priority concern to be addressed urgently.

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