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Bioaccumulation of heavy metals in fish from the Chi River, Maha Sarakham Province, Thailand

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Abstract

Heavy metals are the most significant pollutants in the problem of environmental pollution worldwide. Metals tend to accumulate in water and then move up through the food chain, therefore accumulating in the fish, the main protein source for locals. This research thus aims to study the accumulation of heavy metals in sediment, water, and fish species in the Chi River. The soil sediment and water samples were collected from six points along the Chi River in Muang District, Maha Sarakham Province. Analysis showed that concentrations of heavy metals, including zinc (Zn), copper (Cu), iron (Fe), and lead (Pb) in soil sediments were 5.377-15.669 mg/kg, 5.220-6.894 mg/kg, 1210.149-1324.430 mg/kg, and 7.547-47.560 mg/kg, respectively. The concentrations of Zn, Cu, and Fe in the water were 0.11-0.32 mg/L, 0.076-0.086 mg/L, and 1.272-2.644 mg/L, respectively. Pb was not detected in the water samples. Seven fish samples were collected, namely *Chitala ornata*, *Cyclocheilichthys enoplos*, *Kryptopterus apogon*, *Morulius chrysophekadion*, *Mystus gulio*, *My. ruckioides*, and *Notopterus notopterus*, for analysis of heavy metals accumulation in the different fish organs. The heavy metals detected in the fish, shown in diminishing order, were Fe > Pb > Zn > Cu. The distribution of heavy metals in selected organs analyzed appeared in the order of liver > stomach-intestine > gill > muscle. Fortunately, concentrations of Zn, Cu, and Fe in the muscle did not exceed standard level. However, it should be taken into consideration that concentration of Pb detected in fish were higher than the standard levels. This should be a concern, because lead potentially affects fish consumers.

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Introduction

Heavy metals refer to stable metals of density greater than around 5 to 6 g/cm^3, or refer to all high-density metallic elements which may have hazardous effects on plant or animal ecosystems when present in higher concentrations than found naturally (Keepax *et al.*, 2011).

Heavy metal pollution in rivers has become a matter of great concern over the last few decades, not only because of the threat to public water supplies, but also the hazards posed to human consumption of fishery resources. Heavy metals from many sources, e.g. natural, industrial or sewerage effluents, agricultural drainage, and domestic wastewater, are continually released into rivers, and they are serious threats because of their toxicity, long persistence, and bioaccumulation and biomagnifications in the food chain (Terra *et al.*, 2008). In freshwater systems, not only is fish one of the aquatic products humans consume, it also provides a good indicator of trace element pollution (Rashed, 2001). Heavy metal contamination in water with its uptake by fish species is directly transferred to human populations via fish consumption. Toxicity of heavy metals as a result of fish contamination has led to many studies on the heavy metal bioaccumulation in fishes in other parts of the world (Rashed, 2001; Chi *et al.*, 2007; Dural *et al.*, 2007; Nesto *et al.*, 2007; Terra *et al.*, 2008; Vinodhini and Narayanan, 2008; Abdel-Baki *et al.*, 2011; Ambedkar and Muniyan, 2011; Abah *et al.*, 2013).

Thailand's Chi River is the one major river playing an important role in water and fish supplements in northeastern Thailand. There are 88 fish species in the Chi River and most of them are consumed as food for humans (Dumrongtripob *et al.*, 2002). Our previous study showed that accumulation of heavy metals in fish along the Chi River was found in various fish species (data not shown). Reports using levels of heavy metals in fish to indicate and assess the contamination of heavy metals in water have been found to be very useful for environmental monitoring (Rashed, 2001). There is little or no information regarding heavy metal accumulation in fish habitats along the Chi River, particularly with respect to the distribution of heavy metals caused by run-off and flooding.

This research aims to determine the contents of heavy metals in different tissues, *viz*. muscle, gill, stomach-intestine, and liver, of frequently consumed fish species caught from the Chi River.

Materials and methods

Description of the study area

This study was conducted in the Chi River in Maha Sarakham Province, Thailand. The Chi River is the longest river in Thailand, running 765 km. The river originates in the Phetchabun Mountain Range; runs east through the central northeastern provinces of Chaiyaphum, Khon Kaen and Maha Sarakham; turns south in Roi Et, runs through Yasothon and joins the Mun River in Kanthararom district, Si Sa Ket. The river carries approximately 9,300 cubic kilometres of water per annum. In the rainy season, there are concerns about flash floods in the floodplain of the Chi River Basin.

Collection of fish samples

Fish species were caught by the local fishermen using gill nets of various sizes. After identification the fish samples were ice-packed, transported to the laboratory, and analyzed. Total fish length and weight were measured, and then gill, liver, muscle, and stomach-intestine tissues were dissected using clean equipment. After the dissection, the tissues were completely dried in an oven at 105°C.

Sampling

Six sampling stations were established along the Chi River in Muang District, Maha Sarakham Province, Thailand with the most upstream study site at station 1 and the most downstream site at station 6 (Table 1 and Fig. 1). The sampling stations were chosen based on ecological settings and human activities in the area and using geographic information system (GIS). At each of the stations, water samples were collected in triplicate at a depth of 50 cm below the water surface.

The water was preserved in plastic bottles by the addition of a few drops of nitric acid. Soil sediment samples were collected in triplicate from the same locations as water samples using an Eckman grab. The triplicates were unloaded from the grab into a plastic tub, mixed together, then put into a bottle. The water and sediment samples were transported on ice to the laboratory.

Table 1. List of sampling stations.

Station number	Location
1	16°11.747N′ 103° 9.366E′
2	16°11.727N′ 103° 13.454E′
3	16°13.030N′ 103° 16.745E′
4	16° 13.014N′ 103° 20.446E′
5	16° 14.041N′ 103° 25.864E′
6	16° 10.897N′ 103° 27.221E′

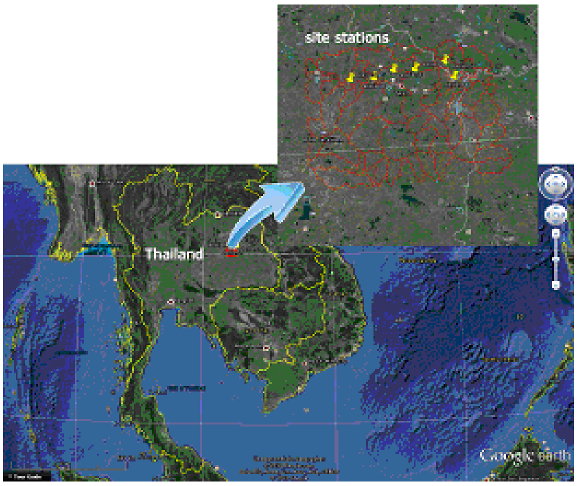

Fig. 1. Map of Thailand and locations of the six stations studied.

Heavy metals analysis

The water samples (50 mL) were digested with 12 mL of $HClO_4$: HNO_3 mixture (1:3) at 80°C for 2 h and were brought up to 50 mL in a volumetric flask with deionized-distilled water (Agunbiade *et al.*, 2009). The digestion was done in glassware previously soaked in nitric acid and washed with deionized-distilled water.

The sediment samples were dried in an oven at 105°C for 24 h. After drying, the samples were cooled and then crushed with a mortar and pestle. Approximately 1 g of each of the dried samples was digested in 12 mL of $HClO_4$: HNO_3 mixture (1:3) and boiled at 100°C for 30 min (Pyle *et al.*, 2005).

The dried fish tissues were finely ground and 1 g of each of the powdered tissues was digested with 12 mL of $HClO_4$: HNO_3 mixture (1:3) at about 80°C until all the tissues were dissolved (Agunbiade *et al.*, 2009). The resulting clear colored solutions were brought up to 50 mL in a volumetric flask with deionized-distilled water.

The digested samples of water, sediment, and fish tissues were analyzed for heavy metals concentrations (Fe, Pb, Zn, Cu) using an AA 6200 Atomic Absorption Spectrophotometer (Shimadzu, Japan). All the analyses were done in triplicate.

Data analysis

The mean and the standard error of the mean of the metals concentrations in water, sediment and fish tissues were calculated. The results were analyzed descriptively by means of a one way-ANOVA, followed by Tukey's post-hoc test ($p < 0.05$). All statistical analyses were performed with SPSS 19.0 for Windows.

Results

Heavy metals in water samples

The total heavy metals concentrations in water samples from the six different sampling sites are presented in Table 2. The concentrations were found to decrease in the sequence of Fe > Cu > Zn. Fe showed the highest concentration (2.644 mg/L), significantly higher than that of Cu and Zn. Pb was not detected.

Heavy metals in sediment samples

Table 3 shows concentrations of the heavy metals in the sediments, values for which decrease in the sequence of Fe > Pb > Cu > Zn. Fe shows the highest concentration (1324.430 mg/kg), significantly higher than the concentrations of Pb, Cu or Zn.

Table 2. The concentrations of Fe, Pb, Cu and Zn in the water samples from the six stations on the Chi River.

Sampling Station	Heavy metals concentration (Mean±SD, mg/L)			
	Iron (Fe)	Lead (Pb)	Copper (Cu)	Zinc (Zn)
1	2.626±0.369	ND	0.086±0.000	0.032±0.008
2	2.631±0.240	ND	0.086±0.003	0.015±0.007
3	2.644±0.286	ND	0.079±0.002	0.029±0.020
4	2.554±0.472	ND	0.077±0.001	0.011±0.012
5	1.272±0.714	ND	0.076±0.001	ND
6	2.116±0.079	ND	0.091±0.001	0.060±0.032

ND= Non Detected

Table 3. The concentrations of Fe, Pb, Cu and Zn in the sediment samples from the six stations on the Chi River.

Sampling Station	Heavy metals concentration (Mean±SD, mg/kg dry weight)			
	Iron (Fe)	Lead (Pb)	Copper (Cu)	Zinc (Zn)
1	1210.149±43.267	ND	5.220±0.274	5.377±0.084
2	1222.966±9.700	ND	5.532±0.298	6.053±0.527
3	1211.990±14.383	ND	5.702±0.723	6.673±2.830
4	1324.430±7.735	23.271±3.913	6.894±0.113	15.669±2.943
5	1314.565±20.325	47.560±2.106	7.617±0.371	13.297±1.107
6	1251.172±27.754	7.547±1.930	5.745±0.390	5.665±1.094

ND= Non Detected

Table 4. The concentrations of Fe, Pb, Cu and Zn in different the tissues of the seven fish species caught from the Chi River.

Heavy Metals	Fish species	Concentration (Mean±SD, mg/kg dry weight)			
		Muscle	Liver	Gill	Stomach-intestine
Fe	*Chitala ornata*	52.650±8.795	220.060±0.000	191.348±5.556	122.857±53.773
	Cyclocheilichthys enoplos	54.010±52.938	1363.012±306.888	344.666±104.029	1050.938±1071.480
	Kryptopterus apogon	1.901±5.593	2692.073±2368.939	79.308±33.740	211.695±54.821
	Morulius chrysophekadion	74.615±0.000	ND	447.132±0.000	734.420±0.000
	Mystus gulio	77.515±11.610	4031.352±1729.686	490.901±167.364	1049.994±671.800
	My. ruckioides	48.809±0.000	1425.530±0.000	494.426±0.000	722.250±0.000
	Notopterus notopterus	1.248±0.000	764.054±0.000	86.948±0.000	27.065±0.000
Pb	*Ch. ornata*	39.743±8.183	40.221±0.000	47.380±5.146	37.693±1.487
	Cy. enoplos	34.062±3.861	382.827±412.040	47.951±5.609	143.362±42.281
	K. apogon	36.529±0.093	711.597±87.432	94.333±27.926	165.649±9.954
	Mo. chrysophekadion	35.573±0.000	ND	71.085±0.000	48.695±0.000
	My. gulio	46.349±6.792	517.620±141.243	100.083±4.988	149.998±10.937
	My. ruckioides	39.403±0.000	153.953±0.000	47.906±0.000	40.299±0.000
	N. notopterus	33.840±0.000	84.978±0.000	60.539±0.000	70.319±0.000
Cu	*Ch. ornata*	3.046±0.470	31.150±0.000	4.121±0.929	4.111±1.154
	Cy. enoplos	3.741±0.028	174.185±11.136	5.339±1.261	31.939±1.882
	K. apogon	2.293±0.066	52.452±6.165	5.176±1.817	12.774±1.516
	Mo. chrysophekadion	3.444±0.000	ND	8.128±0.000	21.442±0.000
	My. gulio	4.247±0.452	91.218±47.167	8.284±1.000	16.655±2.502
	My. ruckioides	2.459±0.000	43.416±0.000	4.007±0.000	6.579±0.000
	N. notopterus	2.421±0.000	3.958±0.000	38.858±0.000	10.309±0.000
Zn	*Ch. ornata*	21.733±10.436	55.427±0.000	57.745±27.750	53.586±3.110
	Cy. enoplos	24.171±1.993	151.888±6.040	45.496±4.900	51.299±3.833
	K. apogon	31.583±1.011	110.255±4.460	66.926±30.351	62.677±13.965
	Mo. chrysophekadion	28.727±0.000	ND	101.233±0.000	76.546±0.000
	My. gulio	29.831±5.396	105.383±50.115	54.252±5.355	52.807±9.979
	My. ruckioides	14.596±0.000	155.915±0.000	42.252±0.000	52.803±0.000
	N. notopterus	14.613±0.000	36.329±0.000	38.422±0.000	36.310±0.000

Heavy metals in fish samples

Concentrations of the heavy metals in selected tissues, *viz*. liver, gill, muscle, and stomach-intestine, of the seven fish species (*Chitala ornata*, *Cyclocheilichthys enoplos*, *Kryptopterus apogon*, *Morulius chrysophekadion*, *Mystus gulio*, *My. ruckioides*, and *Notopterus notopterus*) are presented in Table 4. The mean concentrations of heavy metals were not significantly different between the fish species. The results showed that Fe had the highest concentration of the heavy metals contained in the sampled fish; the mean concentration of Fe in the fish samples was significantly higher than that of Pb, Cu or Zn. The mean concentrations of heavy metals in the fish species showed values decreasing in the sequence of Fe > Pb > Zn > Cu. Considering each tissue, values decreased in the sequences of Fe > Pb > Zn > Cu in the liver, Fe > Pb > Zn > Cu in the stomach-intestine, Fe > Pb > Zn > Cu in the gill, and Fe > Pb > Zn > Cu in the muscle. The liver showed significantly higher accumulations of the heavy metals than the other tissues. The distribution of heavy metals in each type of tissues was found in diminishing order of liver > stomach-intestine > gill > muscle.

Discussion

Good quality foods are very important for the wellbeing of humanity, and animal health has long been used as an environmental indicator of food source quality (Findik and Cicek, 2011). However, there are no current studies addressing potentially harmful conditions of foods or ecotoxicology in the Chi River. Concentrations of heavy metals (Fe, Pb, Zn, Cu) in water and sediment samples recorded in this study were compared with the Sediment and Water Quality Guidelines issued by the Thailand Pollution Control Department (Ministry of Public Health, 1986). Comparisons showed that these concentrations did not exceed the guidelines. Sediment accumulated more heavy metals than water, a trend which has been observed in other water researches by Davies *et al.* (2006), Aderinola *et al.* (2009), Öztürk *et al.* (2009), Vicente-Martorell *et al.* (2009), Tabari *et al.* (2010) and Chawpaknum *et al.* (2012). Suspension of sediments into the water body may increase metals concentrations in the water. River sediments are the major reservoirs and primary source of metals. When heavy metals are accumulated in river sediments at levels above the tolerable limits for aquatic organisms, they become a threat to the organisms, particularly to the fish species that derive a greater chunk of their food from sediments (Edet and Ubuo, 2013). Metals mainly tend to accumulate in fine grained sediments; therefore, larger particles have a dilutive effect on metals concentrations (Kruopiene, 2007). In addition, heavy rainfall leads to farm drainage. Large amounts of pesticides, herbicides and chemical fertilizers containing metal compounds are brought via surface runoff from farms to the river, contributing highly to pollution from agriculture (Modaihsh *et al.*, 2004; Alluri *et al.*, 2007). Moreover, the Chi River has received wastewater that is not pretreated from households and transportation. Heavy metals contamination in sediment can affect the water quality and bioaccumulation of metals in fish species, resulting in potential long-term implications on human health and the ecosystem (Fernandes *et al.*, 2007; Öztürk *et al.*, 2009). Fish are often at the top of the aquatic food chain and may contain concentrated, large amounts of heavy metals, such as Pb, Zn, Fe, Cu, cadmium (Cd), chromium (Cr) and mercury (Hg). These metals accumulate differentially in fish organs and cause serious health hazards to human life. For this reason, the problem of heavy metals contamination in fish has received a lot of attention (Mansour and Sidky, 2002; Vinodhini and Narayanan, 2008; Öztürk *et al.*, 2009; Yousafzai *et al.*, 2010; Abdel-Baki *et al.*, 2011; Ambedkar and Muniyan, 2011; Chawpaknum *et al.*, 2012). The concentrations of heavy metals in selected organs, *viz.* liver, gill, stomach-intestine and muscle, of the seven fish species (*Chitala ornata*, *Cyclocheilichthys enoplos*, *Kryptopterus apogon*, *Morulius chrysophekadion*, *Mystus gulio*, *My. ruckioides*, and *Notopterus notopterus*) are presented in Table 4. The distribution of heavy metals in all the fish organs analyzed were in the diminishing order of Fe > Pb > Zn > Cu. The average concentrations of the metals other than Pb found in the muscles of the fish species were within limits not considered to be cause for toxicology concerns by IAEA-407 (IAEA, 2003) or the Thailand Pollution Control Department Guidelines (Ministry of Public Health, 1986). Of all the metals examined, iron was found to be the most abundant metal in the fish organs that were studied. The results of our study concerning high Fe concentrations are in line with previous studies worldwide. According to Öztürk *et al.* (2009), Fe was found to be the most concentrated metal in all studied tissues of *Cyprinus carpio* from Avsar Dam Lake in Turkey. Chawpaknum *et al.* (2012) showed Fe as the most highly concentrated metal in *Oreochromis niloticus* in the Bangpakong River of Chachoengsao Province, Thailand. Mediterranean fish species demonstrated the same trend (Canli and Atli, 2003). Fe is a basic component of the haemoglobin protein, which is stored in the liver and reused in the genesis of red blood cells. High concentrations of Fe accumulated in the liver indicate its metabolic and physiological role in the fish body (Avenant-Oldewage and Marx, 2000). Fe, Zn and Cu are included in the group of essential trace elements required for maintaining cellular function and are integral components of numerous metal-containing enzymes. However, even essential metals, depending on their concentration, may exert beneficial or harmful effects on fish (Rajkowska and Protasowicki, 2013). Pb concentration in the organs of the seven fish species was found at higher than the standard level of 0.12-1 mg/kg, as issued by the Thailand Pollution Control Department Guidelines (Ministry of Public Health, 1986), Joint FAO/WHO Food Standards Programme Codex Committee on Contaminants in Foods (WHO, 2012), EU (Commission Regulation (EC) No 1881, 2006) and IAEA-407 (IAEA, 2003). The extremely high values of Pb in the samples indicate that the environment is highly stressed with respect to Pb. These findings are in accordance with other studies that have shown high Pb concentrations in fish (Öztürk *et al.*, 2009; Tabari *et al.*, 2010; Yousafzai, 2010). Pb is known to cause serious concerns to health as it inhibits the synthesis of hemoglobin. This, in combination with a shortening of the life span of erythrocytes, results in anemia, one of the chief manifestations of Pb poisoning. Moreover, Pb can adversely affect the central nervous system, causing psychopathological symptoms including restlessness, dullness, irritability, and memory loss (Manahan, 1992).

The results of the analysis of metals in different fish organs show that different fish species contained different metal levels in their tissues due to variations in feeding habits, habitats and behaviors (Dural *et al.*, 2007; Yousafzai *et al.*, 2010). The levels of Zn, Cu, Fe, and Pb in the muscle of each species were determined because of their importance for human consumption. Also, the liver, gill and stomach-intestine were analyzed since these organs tend to accumulate metals (Öztürk *et al.*, 2009). Our results show that generally metal accumulation is highest in the liver, stomach-intestine and gills, while it is lower in the muscle of all species. Roméo *et al.* (1999) reported that in pelagic and benthic fishes from the Mauritania coast, Cd, Cu, Zn, and Hg accumulations in the liver and gills were at the highest levels, while accumulations of these metals were lowest in the fish muscle. Dural *et al.* (2007) reported the highest levels of Cd, Pb, Cu, Zn, and Fe in the liver and gills of three fish species (*Sparus aurata*, *Dicentrarchus labrax* and *Mugil cephalus*). Chi *et al.* (2007) and Rauf *et al.* (2009) also reported that the fish liver exhibited the highest tendency to accumulate heavy metals. The liver plays an important role in accumulation and detoxification of heavy metals. Induction of metallothioneins (MTs) in the liver is the main metabolic process to act as a form of storage and detoxication of heavy metals in fish (Roméo *et al.*, 1999).

Knowledge of heavy metals concentrations in fish is important with respect to both management of nature and human health. The toxicity of metals most commonly involves the liver, brain, and kidney, affecting biochemical mechanisms, such as the inhibition of enzyme action. In addition, heavy metals are a cause of mutagenesis, carcinogenesis, and suppression of the immune system. This research upholds for consideration that the concentration of Pb detected in fish is higher than standard level. This should be a concern, because Pb potentially affects fish consumers. High levels of Pb in the body can result in two types of symptoms. Firstly, acute symptoms can include a headache, confusion, depression, insomnia, blurred vision, severe abdominal pain, kidney damage, vomiting or spasmodic breakdown of red blood cells. Secondly, symptoms of chronic Pb poisoning are anemia, anorexia, constipation, abdominal cramps, wrist drop and cerebral palsy or meningitis (Manahan, 1992).

Conclusions

Contents of Fe, Cu, and Zn in muscle of the fishes were not exceeding the Standard Level on Contaminants in Foods issued by Ministry of Public Health, Thailand (1986). Unfortunately, the contents of Pb were higher than the Standard Level. The total metals accumulation was the greatest in liver and was the lowest in muscle. The results of this research should be broadcasted to the public for proper consideration to be taken about consumption of river fishes. Guidance for farmers and others should be provided, especially concerning the instructions for use of pesticides, herbicides, or fertilizers. Other actions that are necessary include determination of the sedimentation process in the Chi River, control of household wastewaters that are currently dispersed into rivers and crops, establishment of reference laboratories equipped with analytical apparatuses, etc. The relevant agencies should find ways to achieve a solution and a plan for managing water resources.

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