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Arsenic, Cadmium and Lead Levels in Freshwater Fish Collected from Paddy Field Ponds in the Northeastern of Thailand

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Abstract: This study was to determine arsenic, cadmium and lead of five freshwater fish species collected from the paddy field ponds in 5 provinces of the Northeastern region, Thailand between July and December 2012. The average concentrations (on a wet weight basis) were ranges from 0.22±0.15 $\mu g\ g^{-1}$ in muscle of common silver barb (*Barbonymus gonionotus*) to 0.78±0.25 $\mu g\ g^{-1}$ in liver of striped snakehead (*Channa striata*) for arsenic; 0.02±0.01 $\mu g\ g^{-1}$ for muscle climbing perch (*Anabas testudineus*) and 0.10±0.02 $\mu g\ g^{-1}$ in kidney of striped snakehead (*Channa striata*) for cadmium and 0.30±0.09 $\mu g\ g^{-1}$ in muscle climbing perch (*Anabas testudineus*) and 0.72±0.22 $\mu g\ g^{-1}$ in liver of striped snakehead (*Channa striata*) for lead. Heavy metal levels found in freshwater fish samples in the present study were lower than the Thai regulatory standard.

Key words: Arsenic, cadmium, lead, freshwater fish, Thailand

INTRODUCTION

Arsenic, cadmium and lead are recognized as the most toxic elements to animals and humans and they are used in many industrial processes and anthropogenic activities. These heavy metals can cause a potential risk to human health both carcinogenic and non-carcinogenic diseases (FAO/WHO, 1989). Uptake of trace elements in animals may occur as a result of ingestion of contaminated plants and diffusion pass into fish organs. Fish has been reported contain the high levels of heavy metals in comparison with other food of animal origins. The concentration of lead ranged from not detectable to 0.52 $\mu g\ g^{-1}$ for all food categories (ATSDR, 2007a). Arsenic is also found in many foods at concentrations that usually range from 0.20-0.14 $\mu g\ g^{-1}$ (ATSDR, 2007b; WHO, 2001). The concentrations of cadmium ranged from not detectable to 0.30 $\mu g\ g^{-1}$ (ATSDR, 2012; WHO, 2002). Heavy metals levels in food can vary greatly depending on the type of food, agricultural and cultivating practices and amount atmospheric deposition and other anthropogenic contamination.

At this time, there is little published data available regarding on the actual concentrations of arsenic, cadmium and lead in freshwater fish from Thailand (Dummee *et al*., 2012; Jankong *et al*., 2007; Ruangwises *et al*., 2012; Saipan *et al*., 2012) and only a few studies have addressed the concentrations of heavy metals in freshwater fish collected from the Northeastern region, Thailand (Priprem *et al*., 2007). Thailand is divided into four administrative regions: Central, North, Northeast and South with an approximately 63,878,267 people. The Northeastern region was selected for this study since this region has the highest population of 21,573,318 inhabitants living in 20 provinces (NSO, 2012). Generally, five fish species consisted of common climbing perch (*Anabas testudineus*), Nile tilapia (*Oreochromis niloticus*), common silver barb (*Puntius gonionotus*), striped snakehead (*Channa striata*) and walking catfish (*Clarias batrachus*) are usually consumed by human in this region. Tilapia, walking catfish, silver barb, striped, snakehead and climbing perch have annual production of 268,000, 151,800, 82,900, 26,800 and 15,500 tons, respectively almost of which are consumed domestically. Approximately 15% of fish is exported as many forms of fish products (DOF, 2011).

Thailand has rice planted area approximately 79,754,000 rais and the Northeastern area has 39,367,962 rais (1 acre = 2.5 rais) (OAE, 2012). Many farmers raised five fish species in paddy field ponds (Little *et al*., 1996). In paddy field, almost of farmer used pesticides, insecticides, fungicides and fertilizers for control pests and grow rice in field. The increasing of chemicals from industries, agricultures and community wastes has been discharged into the environmental media. Fish and freshwater animals can accumulate of heavy metals by intake of contaminated environmental media and feed and then these fish tissues residues of heavy metals can be

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harmful to human health who consumed the fish. The northeast region of Thailand was selected for this study as this region has the highest population and may be the highest consumption of freshwater fish (NSO, 2012). Human in this region generally consumed spicy minced meat salad, grilled, fried or steamed and then gastrointestinal organs, liver, kidney and other tissues are used for Thai style soup. This study was assessed the current status concentrations of arsenic, cadmium and lead of the five freshwater fish species and also investigated whether the contamination levels are within the regulatory limit.

MATERIALS AND METHODS

A hundred of fish samples representing five types of freshwater fish (n = 20 each fish sp. and each province) were determined of arsenic, cadmium and lead concentrations. The five fish species consisted of common climbing perch (*Anabas testudineus*), Nile tilapia (*Oreochromis niloticus*), common silver barb (*Puntius gonionotus*), striped snakehead (*Channa striata*) and walking catfish (*Clarias batrachus*). All samples were purchased from farmers who raised fish in rice field ponds in Chaiyaphum, Khonkaen, Loei, Mahasarakham and Ubonratchathani Provinces, Northeast region of Thailand. Fish samples of the same size from each fish type were used in this study and were collected between July and December 2012. The samples were put in polyethylene bags, placed in ice boxes and transported to the laboratory. Sizes and weights of individual fishes were measured before muscle parts were separated from other parts. Approximately 10 g of the muscle excluding the skin was cut from each side of fish. Liver, kidney and gastrointestinal organs (including intestine and stomach) were separated; the gastrointestinal tissues did not include any of the stomach and intestine content and then were washed with double-distilled water, homogenized, weighed, freeze-dried and kept in glass bottles at -18°C until analysis.

Sample preparation for determination of heavy metal was performed by a modified procedure of Ruangwises and Ruangwises (1998). Briefly, 0.5 g wet sample of different tissues (muscle, liver, kidney and gastrointestinal) from each fish were digested overnight in 15 mL of a mixture of 12 mL ultrapure nitric acid (65%) and 3 mL perchloric acid (70%). The digest was performed on a hotplate at 80°C and then was ignited at 550°C in a muffle furnace for 3 h. The solution was left for 30 min; 10 mL of 1 N HCl was then added to the solution. The solution was filtered through a Whatman No. 1 filter paper into a 25 mL volumetric flask and adjusted to volume with 1 N HCl. Arsenic, cadmium and lead levels were determined under specified condition according to the manufacturer as graphite furnace atomic absorption spectrophotometer using Spectra AA640Z (Variance, Australia).

Standard stock solutions containing 1000 mg L^{-1} of each element were purchased from Fluka (UK) and were used to prepare calibration standards. HNO_3 and $HClO_4$ were purchased from Merck Chemicals (Darmstadt, Germany); other chemicals were obtained from Sigma-Aldrich (St. Louis, MO). Deionized water (18 MU cm) was used for preparation of standards, reagents and samples throughout the study. All glassware was treated with 10% (v/v) HNO_3 for 20-24 h and washed three times with deionized water. Calibration standards were prepared by a manual standard addition in the four ranges of each element and blanks was analyzed for each calibration run using the same procedure. Overall recoveries for arsenic, cadmium and lead were 96.5, 95.2 and 95.8%, respectively and percent relative standard deviation ranged from 2.0-7.5, 2.5-8.0 and 2.6-9.5 for arsenic, cadmium and lead, respectively. The analytical detection limits for arsenic, cadmium and lead were 0.05, 0.009, 0.02 μg g^{-1}, respectively. Each sample has been analyzed in duplicate. Standard reference material, oyster tissue (SRM 1566b, NIST, USA) was used to verify the accuracy of determination.

About one half of the values of the respective detection limits was substituted for those values below the limits of detection and used in statistical analysis. Analysis of variance and Turkey-Kramer multiple comparison test were used to test for differences levels of elements between fish species and from the different tissues using SPSS Statistics version 17.0 for Windows.

RESULTS AND DISCUSSION

Concentrations of arsenic, cadmium and lead found in SRM 1566b (oyster tissue) were 7.45±0.25 μg g^{-1} (n = 5; reference value 7.65±0.65 μg g^{-1}), 2.40±0.10 μg g^{-1} (n = 5; reference value 2.48±0.08 μg g^{-1}) and 0.29±0.04 μg g^{-1} (n = 5; reference value 0.308±009 μg g^{-1}), respectively. The concentrations of heavy metals were in agreement with by the standard reference material. Weight and length of climbing perch, Nile tilapia, common silver barb, striped snakehead and walking catfish samples used in this study are presented in Table 1. The highest of weight and length found in striped snakehead with ranges 496.0-602.5 g and 32.5-39.0 cm. Arsenic, cadmium and lead were found in muscle at ranges of not determined 0.78, 0.12 and 0.68 μg g^{-1}, respectively. Arsenic in kidney, liver and gastrointestinal samples were ranges following; 0.05-0.96; 0.30-1.72 and 0.12-1.29 μg g^{-1}, respectively. Cadmium and lead ranged as 0.01-0.15 and 0.10-0.90 μg g^{-1} in kidney; 0.01-0.16 and 0.09-0.98 μg g^{-1} in liver and not determined-0.12 and not determined 0.95 μg g^{-1} in gastrointestinal samples, respectively. Average and ranges concentrations of arsenic, cadmium and lead in all samples are shown in Table 1-3. There are differences among the fish samples for the levels of heavy metals. Arsenic, cadmium and lead are expected to vary in a wide concentration range causes they reflect the exposure to environmental levels and feeding behavior. Carnivorous fish such as striped snakehead, generally, showed the higher concentration of heavy metal more than the omnivorous fish (Zeng *et al*., 2012).

Internationally published reports on the levels of arsenic in freshwater animals from Thailand are limited. Jankong *et al*. (2007) determined concentrations of total arsenic in striped snakehead collected from the Suphan River, Thailand. The reported total arsenic level was 1.9±1.46 μg g^{-1} which was slightly greater than the values found in this study. Ruangwises *et al*. (2012) determined arsenic concentrations in four freshwater fish species collected from central region of Thailand; the arsenic in the tilapia, silver barb, striped catfish, striped snakehead fish samples were 0.140-0.286, 0.156-0.315, 0.135-0.234 and 0.214-0.516 μg g^{-1} wet wt., respectively similar to the results found by Saipan *et al*. (2012) study. They reported that arsenic in seven freshwater types collected from central part of Thailand ranged 0.183-0.711 μg g^{-1} wet wt. which were slightly lower than the values found in this

Table 1: Arsenic concentrations in organs of fish samples

Species (n = 20 each sp.)	Length (cm)	Weight (g)	Arsenic concentrations (μg g^{-1}, wet weight)* Muscle	Kidney	Liver	Gastrointestinal tissues
Climbing perch	13.4±1.15	65.6±3.25	$0.32±0.14^a$	$0.54±0.23^a$	$0.65±0.32^a$	$0.61±0.25^a$
(*Anabas testudineus*)	(10.5-15.2)	(60.5-68.4)	(ND-0.52)	(0.10-0.86)	(0.35-1.55)	(0.25-1.29)
Nile tilapia	26.0±1.68	368.0±8.10	$0.24±0.12^a$	$0.45±0.08^a$	$0.50±0.25^a$	$0.48±0.20^a$
(*Oreochromis niloticus*)	(22.0-27.5)	(345.0-370.0)	(ND-0.40)	(0.08-0.60)	(0.30-1.36)	(0.12-1.17)
Silver barb	18.5±1.40	145.2±5.82	$0.22±0.15^a$	$0.40±0.24^a$	$0.54±0.28^a$	$0.54±0.20^a$
(*Barbonymus gonionotus*)	(15.2-22.4)	(130.0-165.6)	(ND-0.42)	(0.05-0.74)	(0.50-1.42)	(0.20-1.20)
Striped snakehead	36.4±4.20	583.4±10.05	$0.44±0.18^a$	$0.60±0.15^a$	$0.78±0.25^a$	$0.60±0.15^a$
(*Channa striata*)	(32.5-39.0)	(496.0-602.5)	(0.14-0.78)	(0.22-0.96)	(0.60-1.72)	(0.15-0.95)
Walking catfish	25.2±3.85	220.5±9.60	$0.40±0.15^a$	$0.58±0.20^a$	$0.76±0.15^a$	$0.64±0.25^a$
(*Clarias batrachus*)	(21.0-30.5)	(205.6-252.5)	(0.10-0.65)	(0.09-0.86)	(0.42-1.05)	(0.15-1.02)

Table 2: Cadmium concentrations in organs of fish samples

Species (n = 20 each sp.)	Length (cm)	Weight (g)	Cadmium concentrations (μg g^{-1}, wet weight)* Muscle	Kidney	Liver	Gastrointestinal tissues
Climbing perch	13.4±1.15	65.6±3.25	$0.02±0.01^a$	$0.06±0.03^a$	$0.05±0.01^a$	$0.04±0.01^a$
(*Anabas testudineus*)	(10.5-15.2)	(60.5-68.4)	(ND-0.06)	(0.01-0.14)	(0.01-0.10)	(ND-0.08)
Nile tilapia	26.0±1.68	368.0±8.10	$0.04±0.01^a$	$0.08±0.04^{ab}$	$0.06±0.02^a$	$0.05±0.02^a$
(*Oreochromis niloticus*)	(22.0-27.5)	(345.0-370.0)	(ND-0.10)	(0.02-0.14)	(0.03-0.12)	(ND-0.07)
Silver barb	18.5±1.40	145.2±5.82	$0.03±0.02^a$	$0.05±0.02^a$	$0.07±0.03^{ab}$	$0.04±0.03^a$
(*Barbonymus gonionotus*)	(15.2-22.4)	(130.0-165.6)	(ND-0.09)	(0.01-0.10)	(0.02-0.12)	(ND-0.09)
Striped snakehead	36.4±4.20	583.4±10.05	$0.05±0.02^a$	$0.10±0.02^b$	$0.08±0.02^b$	$0.06±0.02^a$
(*Channa striata*)	(32.5-39.0)	(496.0-602.5)	(ND-0.12)	(0.02-0.15)	(0.03-0.16)	(ND-0.10)
Walking catfish	25.2±3.85	220.5±9.60	$0.04±0.03^a$	$0.08±0.03^{ab}$	$0.06±0.04^{ab}$	$0.06±0.03^a$
(*Clarias batrachus*)	(21.0-30.5)	(205.6-252.5)	(ND-0.08)	(0.03-0.14)	(0.02-0.12)	(ND-0.12)

Table 3: Lead concentrations in organs of fish samples

Species (n = 20 each sp.)	Length (cm)	Weight (g)	Lead concentrations (μg g^{-1}, wet weight)* Muscle	Kidney	Liver	Gastrointestinal tissues
Climbing perch	13.4±1.15	65.6±3.25	$0.30±0.09^a$	$0.38±0.10^a$	$0.40±0.15^a$	$0.36±0.15^a$
(*Anabas testudineus*)	(10.5-15.2)	(60.5-68.4)	(ND-0.52)	(0.10-0.60)	(0.09-0.82)	(ND-0.55)
Nile tilapia	26.0±1.68	368.0±8.10	$0.34±0.10^a$	$0.40±0.06^a$	$0.36±0.05^a$	$0.30±0.12^a$
(*Oreochromis niloticus*)	(22.0-27.5)	(345.0-370.0)	(ND-0.55)	(0.15-0.60)	(0.10-0.50)	(ND-0.60)
Common silver barb	18.5±1.40	145.2±5.82	$0.35±0.10^a$	$0.38±0.08^a$	$0.38±0.05^a$	$0.36±0.16^a$
(*Barbonymus gonionotus*)	(15.2-22.4)	(130.0-165.6)	(ND-0.52)	(0.12-0.62)	(0.15-0.55)	(0.08-0.60)
Striped snakehead	36.4±4.20	583.4±10.05	$0.40±0.14^a$	$0.65±0.15^b$	$0.72±0.22^b$	$0.54±0.18^a$
(*Channa striata*)	(32.5-39.0)	(496.0-602.5)	(0.18-0.68)	(0.28-0.90)	(0.20-0.98)	(0.10-0.95)
Walking catfish	25.2±3.85	220.5±9.60	$0.35±0.12^a$	$0.60±0.21^b$	$0.68±0.18^b$	$0.50±0.15^a$
(*Clarias batrachus*)	(21.0-30.5)	(205.6-252.5)	(0.10-0.63)	(0.32-0.90)	(0.20-0.95)	(0.16-0.80)

*Values are mean±standard deviations; numbers in parentheses are ranges; ND = Not Determined; value in the same column followed by different letters denote significant differences ($p<0.05$)

study. Mean arsenic level was found between 0.02 μg g^{-1} in common carp and 1.45 μg g^{-1} in pike samples (Al Sayegh Petkovsek *et al*., 2012). Yi *et al*. (2011) were found arsenic average ranges from not detectable to 0.039 μg g^{-1} wet wt. and Wang *et al*. (2007) reported that arsenic in nile tilapia was 0.357-1.047 μg g^{-1} wet wt. In this study, the mean values were generally comparable to the levels reported in the literature.

Several studies have showed the variability in levels of cadmium. Andreji *et al*. (2005, 2006) reported cadmium levels in fish collected from Nitra River, Slovakia were 0.06-2.76 μg g^{-1}. Burger and Gechfeld (2005), Copat *et al*. (2013), Has-Schon *et al*. (2006), Suhaimi *et al*. (2005) and Yi *et al*. (2011) presented cadmium concentrations ranged 0.0005-0.03, 0.004-0.09, 0.01-0.15, 0.005-0.055, not detectable 1.79 and 2.00 μg g^{-1}, wet wt., respectively. The present study, cadmium concentrations in all fish samples were not detectable to 0.45 μg g^{-1}. The values obtained in this study were generally comparable to the levels reported in the other studies. Lead concentrations were ranges not-detected to 0.64 μg g^{-1} (Burger and Gechfeld, 2005; Copat *et al*., 2013; Has-Schon *et al*., 2006; Noel *et al*., 2013; Suhaimi *et al*., 2005) which were slightly lower than the values found in this study. Yi *et al*. (2011) studied was higher level of lead, 0.009-10.1 μg g^{-1} than the resulted from the present the study, similar Ebrahimpour *et al*. (2011) report showed that cadmium and lead in muscle, kidney, liver and intestine were ranges 0.05-2.60 and 0.6-6.7 μg g^{-1} wet wt., respectively. However, it is difficult to directly compare heavy metal levels data because the residue magnitude can be influenced by the type and age of the animals, concentrations and rates of exposure to the element. Heavy metal levels are higher in closed water resources than open water resources. In this area, generally, water and soil in rice field pond is little transferred to outside the field and farmer use small community water resources for supplementary fish pond (Little *et al*., 1996). Thus, these elements may be accumulated in the high concentrations in paddy rice pond. The amount of heavy metal dissolved in waters is dependent on the pH and the dissolved salt content of the water. Heavy metals mobility in soil depends on several factors including the pH of the soil and the availability of organic matter. In present study, however, was not investigated sediment and water characteristics. In this study, statistical analysis results presented that cadmium and lead in liver and kidney were statically significant higher than those in muscle and gastrointestinal tissues ($p<0.05$) while arsenic concentrations in all tissues were not differences. In kidney and liver samples, cadmium concentrations in striped snakehead were significant ($p<0.5$) higher values than other species. Lead levels in kidney and liver of striped snakehead and walking catfish were significantly ($p<0.05$) higher than in silver barb, nile tilapia and climbing perch. Table 4 presents the difference levels of heavy metals in the same tissue of each fish. The present results were found that arsenic and lead difference ($p<0.01$) from cadmium in all of tissue samples. The results presented that heavy metal levels were in fishes in descending order of arsenic, lead>cadmium. The overall tissue concentrations followed the pattern liver, kidney>gastrointestinal>muscle and the elements accumulation in the fish species were following trend: Snakehead fish, walking catfish>climbing perch, Nile tilapia, silver barb.

Regulatory limits of arsenic, cadmium and lead in foods vary among countries. Thailand regulatory limits for general foods and seafood products are 2, 0.2 and 1 μg g^{-1} of arsenic, cadmium and lead, respectively (ACFS, 2005; Thailand Ministry of Public Health, 2003) whereas the EC (2006) had proposed cadmium and lead in muscle meat fish as 0.05 and 0.2 μg g^{-1}, Australia and New Zealand stated that levels of total arsenic, cadmium and lead in seafood or general foods were 1, 2 and 0.5 μg g^{-1}, respectively (ANZFA, 2009). China and several countries proposed the permitted levels of cadmium in fishery product as 2 μg g^{-1} (Suhaimi *et al*., 2005). Codex had proposed the maximum residue limits of cadmium and lead as 2 μg g^{-1} for seafood and 0.2 μg g^{-1} for fish muscle (CCFAC, 2001). At present, several countries including Thailand have not yet established specification regulatory limits of arsenic, cadmium and lead for freshwater animals and visceral organs of these animals. From Table 1-3, the levels of arsenic, cadmium and lead in all tissue samples were ranges not detectable

Table 4: Comparisons among heavy metals levels in fish organs (μg g^{-1} wet wt)

	Muscle			Kidney			Liver			Gastrointestinal		
Species*	As	Cd	Pb	As	Cd	Pb	As	Cd	Pb	As	Cd	Pb
Climbing perch	0.32 ± 0.14^{a}	0.02 ± 0.01^{b}	0.30 ± 0.09^{a}	0.54 ± 0.23^{a}	0.06 ± 0.03^{b}	0.38 ± 0.10^{a}	0.65 ± 0.32^{a}	0.05 ± 0.01^{b}	0.40 ± 0.15^{a}	0.61 ± 0.25^{a}	0.04 ± 0.01^{b}	0.36 ± 0.15^{ab}
Nile tilapia	0.24 ± 0.12^{a}	0.04 ± 0.01^{b}	0.34 ± 0.10^{a}	0.45 ± 0.08^{a}	0.08 ± 0.04^{b}	0.40 ± 0.06^{a}	0.50 ± 0.25^{a}	0.06 ± 0.02^{b}	0.36 ± 0.05^{a}	0.48 ± 0.20^{a}	0.05 ± 0.02^{b}	0.30 ± 0.12^{a}
Silver barb	0.22 ± 0.15^{a}	0.03 ± 0.02^{b}	0.35 ± 0.10^{a}	0.40 ± 0.24^{a}	0.05 ± 0.02^{b}	0.38 ± 0.08^{a}	0.54 ± 0.28^{a}	0.07 ± 0.03^{b}	0.38 ± 0.05^{a}	0.54 ± 0.20^{a}	0.04 ± 0.03^{b}	0.36 ± 0.16^{a}
Striped snakehead	0.44 ± 0.18^{a}	0.05 ± 0.02^{b}	0.40 ± 0.14^{a}	0.60 ± 0.15^{a}	0.10 ± 0.02^{b}	0.65 ± 0.15^{a}	0.78 ± 0.25^{a}	0.08 ± 0.02^{b}	0.72 ± 0.22^{a}	0.60 ± 0.15^{a}	0.06 ± 0.02^{b}	0.54 ± 0.18^{a}
Walking catfish	0.40 ± 0.15^{a}	0.04 ± 0.03^{b}	0.35 ± 0.12^{ab}	0.58 ± 0.20^{a}	0.08 ± 0.03^{b}	0.60 ± 0.21^{a}	0.76 ± 0.15^{a}	0.06 ± 0.04^{b}	0.68 ± 0.18^{a}	0.64 ± 0.25^{a}	0.06 ± 0.03^{b}	0.50 ± 0.15^{a}

*Values are mean±standard deviations; the different letters in the same row of each type of fish and each organ means the levels of arsenic, cadmium and lead are significantly different ($p<0.01$)

to 1.72, 0.16 and 0.98 μg g^{-1}, respectively. All of samples in this study were lower than the permitted level set by Thailand.

CONCLUSION

The results of the present study presented that higher concentrations of heavy metals were found in the visceral organ than in muscle. Consumption of visceral tissues of fish may be poses a potential risk to human health than meat tissue. From this study, it may be concluded that average concentration of total arsenic, cadmium and lead in freshwater fish samples were still within acceptable levels. Many people in Thailand consumed of a wide variety of freshwater animal species and tissues, e.g., freshwater snails, head, gill and other visceral organs of fishes thus further investigation of these organs is necessary including the inorganic arsenic species are the most toxic forms of arsenic. Knowledge of the toxic element levels in fish and visceral tissue is important for quantifying contaminant intake in human.

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