Journal of Food and Drug Analysis, Vol. 12, No. 4, 2004, Pages 358-366 藥物食品分析 第十二卷 第四期

Comparison of the Metal Concentrations in Muscle and Liver Tissues of Fishes from the Erren River, Southwestern Taiwan, after the Restoration in 2000

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(Received: August 11, 2003; Accepted: October 28, 2004)

ABSTRACT

Metal concentrations in the muscle and liver tissues of fishes from the Erren River in southwestern Taiwan were analyzed to evaluate the pollution status after the completion of the restoration plan in 2000. It was the first time the tissue concentrations of 4 important elements, i.e. Fe, Mn, As and Hg, were reported in fishes from this area. Samples were collected in January 2002 at the junction of the Erren River and the Sanyegong River, the most polluted section of the river. Five fish species, *Megalops cyprinoi*ds (Indo-Pacific tarpon), *Chanos chanos* (milkfish), *Liza macrolepis* (large-scaled mullet), *Mugil cephalus* (striped mullet) and *Orechromis* sp. (tilapia) were collected. A total of 91 fishes were dissected and the muscle and liver tissues taken for analysis of seven metals, Fe, Zn, Cu, Mn, As, Hg and Cd, on a wet weight basis (mg/kg). Among them, Fe and Zn were the highest, followed by Cu, Mn and As, while the Hg and Cd concentrations were the lowest. Except for As and Hg, which showed higher concentrations in the muscle tissues than in the livers, the remaining elements showed higher concentrations in livers. Significant species differences were found in all elements and tissues. The highest concentrations of Fe, Zn, Cu and Mn in muscles were found in tilapia (4.04), striped mullet (4.38), large-scaled mullet (0.20) and milkfish (0.534), respectively. The highest concentrations of As and Hg were found in striped mullet (1.59) and Indo-Pacific tarpon (0.081), respectively. The highest concentrations of Fe (3122), Hg (0.045) and Cd (0.123) were found in livers of large-scaled mullet, while striped mullet had the highest concentration of Zn (77.6), Cu (78.2) and As (2.39). The level of Mn in tilapia was 1.32 mg/kg.

The Zn, Cu and Cd concentrations of the muscles and livers were mostly 7-50% lower than the levels reported on the same species of the same tissue before the restoration indicating the improvement on the metal pollution status. The heavy metal concentrations in this area were similar to various uncontaminated areas around Taiwan and all were within the food standard levels of the FDA and NHMRC.

Key words: heavy metals, fishes, muscle, liver, baseline, Erren River (Ell-Ren River, Er-Jen River, Erhjin Chi), Sanyegong River

INTRODUCTION

The Erren River (Ell-Ren River, Er-Jen River, Erhjin Chi) is one of the major rivers in southwestern Taiwan. Since the 1970s, the development of a scrap-metal industry along the Sanyegong River, a tributary of the Erren River, has caused the sediment of the river to be severely polluted with metals(1,2). The "green oyster incident" outbreak in 1986, was caused by the discharge of large amount of wastewater from the acid-washing process. As a result, the sediment of the riverbed became polluted by heavy metals. At the junction of the Sanyegong River and the Erren River, the Cu concentrations in the top layer of the sediment ranged between 100 and 1,000 mg/kg dry weight, with the highest Cu concentrations actually as high as 86,000 mg/kg dry weight(3).

Since 1986, oyster cultivation has been forbidden at the estuary because of the green oyster incident that took place in the river mouth(4). Since 1993, the government has banned the import of waste-metals for the scrap-metal industry, and commenced a program for the restoration of the river(5). By 2001, all of illegal scrap-metal factories along the riverbank were demolished. The source of metal pollution of the river was thereby significantly decreased(2).

Most fishes found at the heaviest polluted tidal area of the Erren River and the Sanyegong River were estuarine

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Journal of Food and Drug Analysis, Vol. 12, No. 4, 2004

and secondary freshwater fishes, including Indo-Pacific tarpon (*Megalops cyprinoids*), milkfish (*Chanos chanos*), large-scaled mullet (*Liza macrolepis*), striped mullet (*Mugil cephalus*) and tilapia (*Orechromis* sp.). Among them, the tilapia is a true local resident that complete their life history in a water body[6], whereas the large-scaled mullet and striped mullet span most of their lifetime in an estuary and leave the estuary for off-shore spawning during the spawning season once a year[6,7]. On the other hand, the tarpon and milkfish stay in an estuary for a couple years before maturation[6]. They were highly pollution-tolerant species commonly found in an esturay[2,8]. Except for the tarpon, which is carnivorous, the remaining four species are benthic omnivorous. Their bottom-feeding habits brought them in constant and direct contact with the river's sediment. Along with their estuary-depending life style, these fish species could be used as a monitoring tool for the pollution status of the environment[9]. Moreover, except for the tarpon, the other four fishes were all edible and had a high economic value. Therefore, once humans consume the flesh of these fishes, the heavy metals accumulated are immediately transferred and might become a threat to the health of consumer[10].

Previous studies reported that the average Cu concentrations in the muscle of marine fishes caught in the Erren River were over 1 mg/kg wet weight prior to 2000[4,11-12]. This was 3-10 times higher than the Cu concentrations in most marine and freshwater fishes[13]. Furthermore, the liver concentrations of Cu and Zn recorded in this area in 1993-1994[4,12] were also 2-3 times higher than the concentrations found in normal fish liver in Taiwan[1,13]. Since the EPA (Environmental Protection Administration, Taiwan, R.O.C.) implemented the Erren River renovation plan in 2000, there have been no reports as to the extent of any improvement of the environment regarding metal concentrations in fish tissue. Apart from the fish muscle consumed by human beings, fish liver is a major detoxification organ[14]. Many poisonous materials absorbed from the environment are detoxified by the liver, leading the metal concentrations of the liver to indirectly reflect the pollution status of the environment. Therefore, in this study, we used the muscle and liver tissues of fishes to analyze their metal concentrations, to evaluate if they are safe for human consumption, as well as to measure the pollution status after the clean-up project.

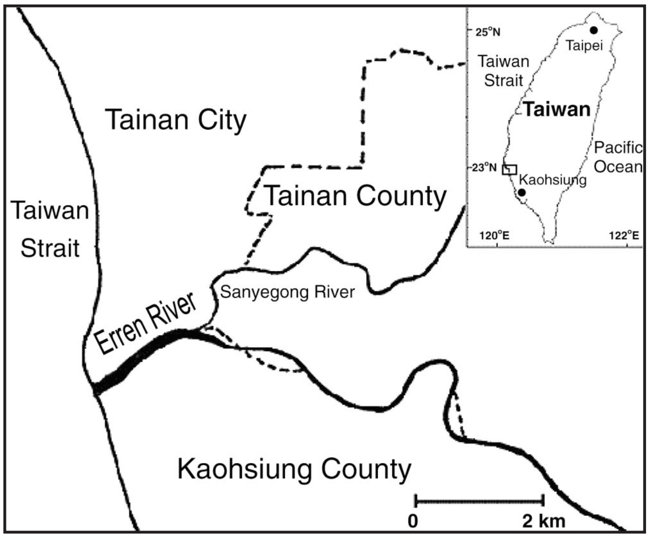

Figure 1. Sampling site.

MATERIALS AND METHODS

I. *Materials*

(I) *Collection of specimens*

In January 2002 (Figure 1) five fish species, including Indo-Pacific tarpon (*Megalops cyprinoids*), milkfish (*Chanos chanos*), large-scaled mullet (*Liza macrolepis*), striped mullet (*Mugil cephalus*) and tilapia (*Orechromis* sp.), were collected by gill-nets at the junction of the Sanyegong River and the Erren River, which was the heaviest polluted area in the drainage of the Erren River. A total of 260 fishes were caught. Among those a total of 91 fishes, including 31 randomly picked Indo-Pacific tarpon and 60 fishes of the other four species, were packed in ice and transported back to the laboratory for further treatment (Table1). In the pre-treatments of the samples, the fishes were first identified and measured for the weight and total length. Then, each fish was dissected to identify its gender. The muscle and liver tissues removed from each fish species having a similar total length were combined and

Table 1. Details of fish species used in this study

Species	Common name	Code	N[a]	n[b]		TL[c] (cm)	BW[d] (g)	FH[e]	LS[f]
				Muscle	Liver				
Megalops cyprinoides	Indo-Pacific tarpon	Mcy	31	8	8	32.9 ± 1.5	287.4 ± 35.8	c	J
Chanos chanos	Milkfish	Cc	27	7	7	32.0 ± 1.7	258.5 ± 39.9	bh	J
Liza macrolepis	Large-scaled mullet	Lm	6	3	3	25.2 ± 1.5	186.5 ± 32.9	bh	A
Mugil cephalus	Striped mullet	Mc	6	1	1	32.2 ± 1.8	273.6 ± 54.3	bh	J
Orechromis sp.	Tilapia	On	21	5	5	18.4 ± 1.7	120.5 ± 29.0	bh	A
Total			**91**	**24**	**24**				

[a]N: total number of samples.
[b]n: pooled sample number.
[c]TL: total length.
[d]BW: body weight.
[e]FH: feeding habit (h = herbivore, b = benthic, c = carnivore).
[f]LS: life stage (A: adult, J: juvenile).

homogenized as pooled samples[13,15]. Each pooled sample consisted of at least three individuals. All together 24-muscle and 24-liver pooled samples were obtained. The pooled samples were stored in acid-washed plastic bottles and frozen at -20˚C before metal analyses.

(II) *Reagents*

All the chemicals used in this study, including HNO_3, H_2SO_4, $SnCl_2$, $KMnO_4$ and the metal standard solutions (1000 mg/L), were of GR grade (Merck). Matrix matched standard reference materials (SRMs), e.g. DORM-2 (dogfish muscle) and DOLT-2 (dogfish liver), were purchased from the National Research Council of Canada.

II. Methods

(I) *Analysis of Fe, Zn, Cu, Mn, As and Cd*

Two grams of muscle or 0.5 g of liver tissue were digested with 10 mL of nitric acid in a 50-mL flask. Each sample was covered with a pear-shaped glass ball for acid reflux and heated to 120˚C at a rate of 10˚C/hr, and then held for at least 2 hr. The digests were concentrated to 1 mL, then made into 25 mL with 1 M nitric acid[16]. Fe, Zn and Cu were determined by flame atomic absorption spectrometry (AAS, Hitachi Zeeman-5000), whereas As, Cd and Mn were determined by inductively coupled plasma mass spectrometry (ICP-MS, Perkin-Elmer ELAN 5000).

(II) *Analysis of Hg*

Two grams of muscle or 1 g of liver tissue was digested with 4 mL of nitric acid and 6 mL of sulfuric acid in a graduated 75-mL tube. The tube was heated up to 70˚C for 2 hr. Ten milliliter of 5% $KMnO_4$ was added for further oxidation. Before made up to 25 mL with double distilled water, a few drops of 15% H_2O_2 were added to reduce the excessive $KMnO_4$. The digests were analyzed by cold vapor atomic absorption spectrometry (CVAAS, Hitachi Zeeman-8200) within 48 hr[17].

III. *Analytical Quality Control*

All glasswares used in this study were acid-washed. Duplicate standard reference materials and blanks were used simultaneously in each batch of analysis to verify the analytical quality. The recoveries of the two SRMs were controlled within 100 ± 15%, except for Hg in DORM-2 (Table 2). The detection limits were determined by the mean and three times standard deviation of blank absorbance[18-20]. Accordingly, the instrumental detection limits of muscle samples for Zn, Mn, As, Hg and Cd were 0.0125 mg/kg, and those of Fe and Cu were 0.125 mg/kg, respectively. On the other hand, the detection limits of liver samples for Zn, Mn, As and Cd were 0.05 mg/kg, 0.50 mg/kg for Fe and Cu, and 0.05 mg/kg for Hg.

IV. *Statistics Analysis*

All the results in this study were presented on a wet weight basis (mg/kg). The ratio used for transferring dry weight to wet weight was 1/4.5. The analysis of variance was performed using SAS software (one-way Analysis of Variance, ANOVA). The statistical significance level was set at $p < 0.05$ for all tests. Dietary safety was evaluated according to the level of the FDA (Food and Drug Administration, USA)[21] and NHMRC (National Health and Medical Research Council, Australia)[22-23].

RESULTS AND DISCUSSIONS

I. *Heavy Metals Concentrations in the Muscles of Fishes in 2002*

Among the four essential elements, Fe, Zn, Cu and Mn, in the muscles of the five fishes from the Erren River, the metal concentrations ranked as follows: Fe > Zn > Cu > Mn. The Fe concentration was generally the highest, except that in the tilapia (On). The Mn concentration was mostly the lowest, except that in the milkfish (Cc) (Table 3). Such ranking of metal concentrations was similar to that found in fish of the Annping estuary[16], but slightly different to that of Chigu (Chi-ku), which had Zn > Fe > Cu > Mn[13].

Significant differences of muscle tissues were found across species, depending on the type of metal (ANOVA, $p < 0.05$). Fe and Cu in large-scaled mullet, Zn in tilapia and Mn in milkfish were significantly higher than those of the

Table 2. Verification of standard reference materials and instrumental detection limits in this study

Element	DORM-2				DOLT-2				IDL[d]
	n[b]	Mean ± SD[a]	Certified value[a]	R[c] (%)	n[b]	Mean ± SD[a]	Certified value[a]	R[c] (%)	(mg/L)
Fe	4	153 ± 8	142 ± 10	108	4	1118 ± 70	1103 ± 47	101	0.010
Zn	4	25.9 ± 5.8	25.6 ± 2.3	101	4	88.2 ± 3.3	85.8 ± 2.5	103	0.001
Cu	4	1.93 ± 0.22	2.06 ± 0.01	88	4	27.0 ± 0.4	25.8 ± 1.1	105	0.010
Mn	4	3.44 ± 0.54	3.66 ± 0.34	94	4	5.59 ± 0.11	6.88 ± 0.56	81	0.001
As	4	18.3 ± 0.9	16.4 ± 1.1	102	4	16.5 ± 0.8	16.1 ± 1.1	102	0.001
Hg	8	3.68 ± 1.10	4.64 ± 0.26	79	8	1.92 ± 0.39	2.14 ± 0.1	90	0.001
Cd	8	0.039 ± 0.011	0.043 ± 0.008	91	6	17.6 ± 1.9	20.8 ± 0.5	85	0.001

[a]Unit: mg/kg dry wt. [b]n: sample size. [c]R: recovery rate. [d]IDL: instrumental detection limits.

Table 3. Metal concentrations (mg/kg wet wt) in muscle tissues of *Megalops cyprinoids* (Mcy), *Chanos chanos* (Cc), *Liza macrolepis* (Lm), *Mugil cephalus* (Mc), and *Orechromis* sp. (On) from Erren River, southwestern Taiwan in 2002

Species	Fe	Zn	Cu	Mn	As	Hg	Cd
Mcy	3.02 ± 0.39^{ab}	2.57 ± 0.35^{b}	0.132 ± 0.023^{b}	0.131 ± 0.048^{b}	0.683 ± 0.175^{a}	0.081 ± 0.026^{a}	<0.0125
Cc	3.36 ± 0.19^{ab}	3.30 ± 0.39^{b}	0.078 ± 0.036^{b}	0.534 ± 0.141^{a}	0.575 ± 0.039^{a}	0.021 ± 0.013^{b}	<0.0125
Lm	3.63 ± 1.06^{a}	2.58 ± 0.18^{b}	0.200 ± 0.048^{a}	0.037 ± 0.006^{b}	0.811 ± 0.243^{a}	0.011 ± 0.010^{b}	<0.0125
Mc	4.38	2.23	0.150	0.086	1.589	<0.0125	<0.0125
On	2.99 ± 0.21^{b}	4.04 ± 0.22^{a}	0.118 ± 0.004^{b}	0.106 ± 0.021^{b}	0.664 ± 0.085^{a}	0.013 ± 0.012^{b}	<0.0125

[a,b]The same letter across columns indicate no significant difference (Duncan's multiple range test, $p > 0.05$).

Table 4. Metal concentrations (mg/kg wet wt) in liver tissues of *Megalops cyprinoids* (Mcy), *Chanos chanos* (Cc), *Liza macrolepis* (Lm), *Mugil cephalus* (Mc), and *Orechromis* sp. (On) from Erren River, southwestern Taiwan in 2002

Species	Fe	Zn	Cu	Mn	As	Hg	Cd
Mcy	1187 ± 196^{b}	24.9 ± 2.5^{b}	10.1 ± 1.6^{b}	0.803 ± 0.365^{b}	0.252 ± 0.036^{b}	0.020 ± 0.008^{b}	0.012 ± 0.006^{b}
Cc	1420 ± 137^{b}	36.1 ± 6.9^{a}	44.1 ± 7.8^{a}	0.705 ± 0.068^{b}	0.535 ± 0.068^{b}	<0.025	0.042 ± 0.029^{b}
Lm	3122 ± 1758^{a}	22.6 ± 3.3^{b}	6.0 ± 1.4^{b}	0.880 ± 0.099^{b}	1.989 ± 0.784^{a}	0.045 ± 0.007^{a}	0.123 ± 0.112^{a}
Mc	1766	77.6	78.2	1.106	2.387	0.037	0.040
On	1135 ± 168^{b}	16.9 ± 10.8^{b}	38.7 ± 7.8^{b}	1.317 ± 0.298^{a}	0.369 ± 0.172^{b}	<0.025	0.022 ± 0.013^{b}

[a,b]The same letter across columns indicate no significant difference (Duncan's multiple range test, $p > 0.05$).

other species (Table 3). It was noted that higher Fe and Cu concentrations were found in the muscle tissues of mugillidae (i.e. striped mullet and large-scaled mullet) than other family of fishes[13].

The Zn and Cu concentrations in the muscles were within the levels recommended by NHMRC, Australia (Zn < 1000, Cu < 30 mg/kg)[22-23]. The RDNA (Recommended Daily Nutrient Allowance, Taiwan)[24] and USRDA (United States Recommended Dietary Allowance)[25] recommend daily Fe and Mn allowance for healthy life as 10-15 mg and 4 mg, respectively. Accordingly, under normal dietary habit (amount of daily fish intake 40-63 g)[26], the Fe and Mn in fishes would also be important nutritional trace elements for the general public.

Furthermore, among the three toxic elements, the concentrations of As in muscle tissue of the five fish species from the Erren River were higher than those of Hg and Cd. All Cd concentrations were below the detectable limit of 0.0125 mg/kg (Table 3). This ranking of metal concentrations, As > Hg ≥ Cd, was common in fish muscles[13].

Same as the essential elements, the Hg concentrations of muscle tissues showed significant differences across species (ANOVA, $p < 0.05$), whereas the As concentrations did not (Table 3). The highest Hg concentration found in the Indo-Pacific tarpon (Mcy) was 2.5 times higher than those of the other four species, which is the result of biomagnification-effect of Hg for the tarpon preying on them. No species difference of As concentrations was found in the muscle tissues, except striped mullet. The As concentration in the striped mullet were almost double than the other four species. The reason why striped mullet contained such high As concentrations in their muscles were unclear.

The metal concentrations of the three toxic elements in fish muscles were all within the levels recommended by the FDA (Hg < 1 and Cd < 2 mg/kg) and NHMRC (inorganic As < 1.5, Hg < 0.5 and Cd < 2 mg/kg)[21-23]. In the case of striped mullet, the muscle concentration of As (1.589 mg/kg) was higher than the NHMRC level. However, the major As species in fish flesh was in the low toxic organic As form[27], which was about 30% of the total As[27]. The concentration of inorganic As in the striped mullet was only 0.48 mg/kg which was lower than the NHMRC level[23]. Therefore, under normal dietary habits, eating fishes would not cause the excessive intake of the toxic metals.

II. *Heavy Metals Concentrations in the Livers of Fishes in 2002*

Metal concentrations in fish livers mostly differed from that of the fish muscles. Among the 5 fish species, only Indo-Pacific tarpon showed the same ranking of metal concentrations in the liver and muscle tissues. This finding corresponds to previous studies, such as the study of 21 fish species in Chigu Lagoon[13] and 9 species in Annping Estuary[16]. In the cases of milkfish and tilapia, a higher Cu and lower Hg concentrations found in their livers, the ranking changed to Fe > Cu > Zn > Mn > As > Cd > Hg. For the large-scaled mullet and the striped mullet, the orders became Fe > Zn > Cu > As ≥ Mn > Cd > Hg and Fe > Cu = Zn > As > Mn > Hg = Cd, respectively.

Significant species-differences of metal concentrations in the liver tissues were also observed (ANOVA, $p < 0.05$), which were similar to previous studies[13,16]. Fe, As, Hg and Cd concentrations in large-scaled mullet, Zn in milkfish, Cu in striped mullet and Mn in tilapia were significantly higher than those of the other species (Table 4). Comparing the metal concentrations of the livers to the various food standards stated earlier, most metal concentrations in the fish livers were within or below the allowable standards. Although the Cu concentrations in the livers of the milkfish, striped mullet and tilapia exceeded the NHMRC (Cu < 30 mg/kg) standards, general public did not normally consume the tissues. Even if some were consumed accidentally, the possibility of toxic effects would be very low due to the small amount of intake and the self-detoxification in the consumer's body. Furthermore, the higher total As concentrations in the livers of large-scaled mullet and striped mullet were total As, based on the conversion ratio of 1/0.3 for total to inorganic As[27]. The inorganic As concentrations in the livers of the two species were only 0.597 and 0.716 mg/kg, which did not exceed the NHMRC standard (inorganic As<1.5 mg/kg)[23].

Being a detoxification organ, metal concentrations in the livers were generally higher than those of the muscles, except As and Hg. Generally speaking, the liver concentrations of Fe, Cu, Zn, Mn and Cd were 150-900, 20-550, 4-35, 5-25 and 0-10 times higher than those of muscle tissues of fishes in a metal non-contaminated area[13]. However, As and Hg in tarpon and As in tilapia showed higher metal concentrations in the muscles.

III. *Comparison of the Present Results with Reported References*

The Fe and Mn concentrations of the muscle tissues in the five fish species ranged from 2.99 to 4.38 mg/kg and 0.037 to 0.534 mg/kg, respectively, which were similar to those recorded elsewhere (Tables 3 and 5). Although there were no historical levels of Fe and Mn concentrations in fish muscles for comparing the polluted status before and after the clean-up project of the Erren River (Table 5), Fe and Mn concentrations of the fish muscles reflected a normal range.

The Fe and Mn concentrations in the liver tissues of the five fish species ranged from 1135 to 3122 mg/kg and 0.705 to 1.317 mg/kg, respectively (Tables 4 and 5). No historical data of Fe and Mn concentrations of the liver tissues were available for comparison. However, Fe concentrations in the livers of large-scaled mullet and striped mullet found in this study were 2 to 5 times higher than those recorded in Chigu Lagoon[13] and Annping Coast[16]. In the case of Mn, the concentration in the liver of large-scaled mullet was only half of those in these two loactions[13,16]. On the other hand, the Mn concentration in the large-scaled mullet was same as striped mullet in Chigu Lagoon[13] (Table 5).

Mean Zn concentrations in the muscle tissues of the five fishes ranged from 2.23 to 4.04 mg/kg, representing normal Zn concentrations in fish-muscles (Tables 3 and 5). Compared to the same species of fish from different geographical areas, the Zn concentrations in Indo-pacific tarpon, milkfish and large-scaled mullet of the Erren River were all lower than those from Chigu Lagoon[13], a relatively unpolluted area in southwestern Taiwan, as well as the polluted areas, i.e. Annping Estuary[13], Yanshui River and Luermen River[28]. Furthermore, the Zn concentration of tilapia was only half of those from polluted Sindian harbour and Luremen River[28]. The data of striped mullet in Erren River were similar to those recorded in Chigu Lagoon[13] and Australia[29], but were much lower than those from the polluted lake in Tunisia[30]. Limited data were recorded before the restoration of Erren River. Of the one historical Zn data of milkfish from the aquaculture pond in Erren area in 1998[1] that was available, our data were about 2/3 lower (Table 5).

The mean Zn concentrations in liver tissues of the five fish species ranged from 22.6 to 77.6 mg/kg, representing normal Zn concentrations of fish livers (Tables 4 and 5). The Zn concentrations in the livers of large-scaled mullet and tilapia from Erren River were lower than those from Chigu Lagoon[13], Annping Estuary[16], Yanshui River and Luermen River[28]. It is worth noted that the Zn concentration of Indo-Pacific tarpon data obtained after the restoration were significantly reduced to half or one-third of the value before the restoration[1,31](Tables 4 and 5). However, the Zn concentrations in the livers of the large-scaled mullet and the tilapia did not show such marked decrease[1,32] (Table 5).

The mean Cu concentrations in the muscle tissues of the five fish species ranged from 0.078 to 0.200 mg/kg (Table 3), which were 4 to 10% lower than the data recorded in 1986 and 1990[11,12]. In comparision with the Cu concentrations of the same fish species from Chigu Lagoon[13], Annping Coast[16], Sindian harbour, Yanshui River and Luermen River[28] (Tables 3 and 5), we found the respective concentrations of Erren River were 50 to 20% lower. Furthermore, our Cu concentration of milkfish were only 17 to 20% of the historical data in a nearby aquaculture pond[1]. In addition, the Cu concentration in the muscle tissue of the striped mullet corresponded closely to that from unpolluted regions in Australia[29], but was 14% of the same species from a polluted region in Tunisia[30] (Table 5). As a result, we concluded that there was no sign of severe Cu pollution in the Erren River area.

Mean Cu concentrations in livers of the five fish species ranged from 6.0 to 78.2 mg/kg (Table 4), which were similar to the baseline data of Chigu Lagoon, except for the striped mullet (Table 5). The concentrations of the same fish species decreased significantly after the restoration, e.g. 1/2 to1/6 lower in the Indo-Pacific tarpon[1,31], 1/16 lower in the large-scaled mullet[32], and 1/2 lower in tilapia[1] (Tables 4 and 5). Comparing the Cu concentration with that from polluted areas of Taiwan, the concentration of the large-scaled mullet of Erren River was only 1/3 of the Yanshui's data[28] and 1/8 of the Annping's data[16] (Table 5). However, for some unexplained reason, the tilapia data reported from the polluted Luermen River[28] was one order of magnitude lower than the normal value that was considered unacceptable for comparison. From all of the above, we conclude that no sign of severe Cu pollution exists in this area after the restoration.

Mean As concentrations in the muscle tissue of the five fish species ranged from 0.58 to 1.59 mg/kg. This was the first time the As concentrations of muscle tissues of fish were reported in the area, therefore, no historical

Table 5. Comparisons of metal concentrations (mg/kg wet wt) in fish muscles and livers from various areas

Species/Location	Year	PS[a]	Fe	Zn	Cu	Mn	As	Hg	Cd	Ref
MUSCLE										
Megalops cyprinoids										
Chigu (Chi-ku) Lagoon, Taiwan	1997	U	2.51 ± 0.04	3.07 ± 0.23	0.34 ± 0.01	0.21 ± 0.02	1.91 ± 0.10	0.068 ± 0.005	<0.025	13
Sindian Harbor, Taiwan	2002	S	--[b]	2.51	0.25	--	--	0.0265	N.D	28
Chanos chanos										
Erren (Er-Jen) River, Taiwan	1998	P	--	5.57 ± 1.11[c]	0.45 ± 0.13[c]	--	--	--	0.016 ± 0.008[c]	1
		U	--	4.24[c]	0.38[c]	--	--	--	0.002[c]	1
Chigu Lagoon, Taiwan	1997	U	3.36 ± 1.15	4.72 ± 0.21	0.37 ± 0.06	0.89 ± 0.69	0.28 ± 0.02	<0.0125	<0.025	13
Liza macrolepis										
Chigu Lagoon, Taiwan	1997	U	3.71 ± 1.74	5.44 ± 0.82	0.38 ± 0.15	0.21 ± 0.26	0.95 ± 0.26	0.046 ± 0.005	<0.025	13
Annping Coast, Taiwan	1998	S	5.71 ± 2.47	4.01 ± 1.08	0.43 ± 0.06	0.13 ± 0.08	--	--	<0.025	16
Yanshui River	2002	P	--	6.03	0.42	--	--	0.003	N.D	28
Mugil cephalus										
Chigu Lagoon, Taiwan	1997	U	2.25 ± 0.58	2.31 ± 0.13	0.36 ± 0.04	0.20 ± 0.06	2.65 ± 2.22	<0.0125	<0.025	13
Australia	2001	U	--	2.16[c]	0.16[c]	--	--	--	--	30
Tunisia	2002	P	--	10.0[c]	1.07[c]	--	--	N.D	0.011[c]	31
***Orechromis* sp.**										
Sindian Harbor, Taiwan	2002	S	--	7.59	0.37	--	--	0.0295	N.D	28
Luermen River, Taiwan	2002	P	--	7.11	0.29	--	--	0.0278	N.D	28
LIVER										
Megalops cyprinoids										
Erren River, Taiwan	1994	P	--	53[c]	62[c]	--	--	--	--	"1"[d]
Erren coastal waters, Taiwan	1997	P	--	77.8	25.5	--	0.256	--	0.033	31
Liza macrolepis										
Erren River, Taiwan	1993	P	--	20[c]	95[c]	--	--	--	0.76[c]	32
Chigu Lagoon, Taiwan	1997	U	1452 ±660	32.5 ± 10.4	31.9 ± 24.8	1.63 ± 0.34	4.03 ± 1.66	0.124 ± 0.035	0.116 ± 0.034	13
Annping Coast, Taiwan	1998	S	646 ±431	34.2 ± 10.6	48.2 ± 66.5	1.62 ± 0.54	--	--	0.119 ± 0.078	16
Yanshui River, Taiwan	2002	P	--	49.5	22.7	--	--	0.0192	N.D	28
Mugil cephalus										
Chigu Lagoon, Taiwan	1997	U	442 ±136	46.8 ± 16.1	26.3 ± 23.7	1.19 ± 0.09	5.18 ± 3.44	0.044 ± 0.010	0.072 ± 0.049	13
***Orechromis* sp.**										
Erren River, Taiwan	1994	P	--	20[c]	89[c]	--	--	--	--	"1"[d]
Luermen River, Taiwan	2002	P	--	21.46	2.59	--	--	1.128	0.04	28

[a]PS: pollution status (U = unpolluted, S = slightly polluted, P = polluted).
[b]data unavailable.
[c]the data was transformed from dry weight to wet weight by a conversion factor of 1/4.5.
[d]"1": cited from reference 1.

data was available for comparison. Although the concentration of striped mullet was 2 to 3 times higher than the other four species in this study, it was still lower than that of the same species from Chigu lagoon[13] (Tables 3 and 5). Compared to the As concentration in the muscle tissue of the fishes from Chigu Lagoon[13], only milkfish contained a higher level of As than that from Chigu Lagoon. The other three species all contained lower As concentrations (Tables 3 and 5).

Mean As concentrations in the liver tissue from the five fish species ranged from 0.25 to 2.38 mg/kg. Although the concentrations of large-scaled mullet and the striped mullet were 4 to 8 times higher than those of the other three species, they were still 50% lower than those of the same species from Chigu Lagoon[13] (Tables 4 and 5). Comparing the As concentrations in the liver of the Indo-Pacific tarpon, we found that the data remained the same after the restoration project (Tables 4 and 5).

The mean concentrations of Hg in the muscle tissue of the five fish species ranged from <0.0125 to 0.081 mg/kg. Same as As, this was also the first time the Hg concentrationis was reported in the muscle tissue of fish in the Erren River. The Hg concentrations of the fish muscles were similar to those in Chigu Lagoon[13], Sindian Harbour and Luremen River[28], showing typical Hg concentrations in fishes in an uncontaminated aquatic environment[13] (Table 5).

The mean liver concentrations of Hg in the five species of fish, ranging from <0.025 to 0.048 mg/kg, were within the same range of baseline for Taiwan[13]. Furthermore, our tilapia data was 2 orders lower than that recorded from Luremen River[28] (Tables 4 and 5).

The mean Cd concentrations in the muscle tissue of the five fish species were all below 0.013 mg/kg and similar to the levels of Chigu Lagoon[13] and Annping Coast[16]. Compared to the historical data, the concentration of milkfish (0.016 ± 0.008 mg/kg) recorded in 1998 was slightly higher than that of this study. However, analytical bias might exist between laboratories, not to mention the variation of analysis even within the same laboratory. Therefore, the data could be considered without significant difference (Table 5).

Mean Cd concentrations in the livers of the five fish species ranged from 0.012 to 0.123 mg/kg that were 1/2 to 1/5 lower than those of Chigu Lagoon and Annping Estuary[13,16]. Compared to the historical data, the concentration of large-scaled mullet recorded in 1993 was estimated to be 0.76 mg/kg[32], which was 6 times higher than our data (Table 5). This once again suggested the improvement on environment of Erren River after the restoration.

The results showed that toxic elements As and Hg were not elevated in Erren River. The Zn, Cu and Cd concentrations in the muscle and liver tissues of the fishes reduced by 7% to 50% after the restoration. According to our data, the Cu pollution from the scrap metal smelters located at the Erren River in the 1980s and 1990s was no longer evident. These results agreed with the decreasing levels of metal pollution found in this area's water and sediment. From Tsai's report in 1998[33], the Zn and Cu concentrations in the sediment of Erren River were elevated to 358 and 151 mg/kg dry weight, respectively, at the time of the green oyster event. Today, the Zn and Cu concentrations in sediment of Sanyegong River decreased to 282 and 46 mg/kg[34]. Moreover, according to the database of the river water quality from EPA (Environmental Protection Administration), Taiwan, the metal concentrations in water were significantly decreased after cleaning up Erren River[35].

In conclusion, the metal concentrations in the muscle and liver tissues of the five fish species were all lower than the historical values, and similar to the baseline data established in Taiwan. As a result, we conclude that the clean up action in 2000 improved the pollution status of Erren River. Furthermore, metal concentrations in the muscle tissue were within the safety levels of FDA and NHMRC. According to the NAHSIT (Nutrition and Health Survey in Taiwan, 1993-1996) and based on the consumption of 40-63 g of fish flesh per day[26], the maximum intake of Fe, Zn, Cu, Mn, As and Hg from the fish of Erren River were 276, 255, 12.6, 34, 100 and 5.1 μg, respectively, which were only 1~10% of the ADI (Acceptable Daily Intake, based on a body weight of 70 kg, WHO)[36]. As a result, under a normal diet, there would be a low risk of excessive intake of heavy metals from eating fishes harvested from the Erren River after the restoration project.

ACKNOWLEDGMENTS

We thank Mr. Jung-Lu Su and MissYing-Nu Hung for their assistance with the collection of fish samples, Miss Pei-Fen Tsai for dissecting and pre-treatment of the samples and the ICP-MS laboratory, headed by Prof. Shie-Jen Cheng, Department of Chemistry, National Sun Yat-sen University, for the metal analysis. Financial support was partially from the National Science Council of Taiwan, R.O.C. (Grant Nos. NSC 90-2313-B-110-006, NSC 91-2313-B-110-007 and NSC 92-2313-B-110-004). Yi-Chun Chen appreciates the Ph.D. fund provided by Chung Hwa College of Medical Technology.

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