Hindawi Publishing Corporation
Journal of Marine Biology
Volume 2015, Article ID 132768, 5 pages
http://dx.doi.org/10.1155/2015/132768

Research Article

Heavy Metals in *Terapon puta* (Cuvier, 1829) from Karachi Coasts, Pakistan

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Received 21 July 2015; Revised 3 October 2015; Accepted 22 October 2015

Academic Editor: Horst Felbeck

This study was carried out to investigate the accumulation of Fe, Mn, Cd, Pb, and Ni in *Terapon puta* from Karachi Fish Harbor, Pakistan. There were no significant differences in the concentration of the metals except Mn in *T. puta*, which is obtained from the different seasons. The highest metal concentration is Fe followed by Mn. Cd, Pb, and Ni were measured relatively lower concentrations. The mean (± SD) Fe, Mn, Cd, Ni, and Pb levels in the muscles tissues of *T. puta* were 25.5 ± 13.71, 6.07 ± 3.58, 0.47 ± 0.29, 0.46 ± 0.27, and 0.43 ± 0.25 mg/kg dry wt., respectively, whereas the mean (± SD) Fe, Mn, Cd, Ni, and Pb levels in the liver tissues of *T. puta* were 562 ± 105, 18.62 ± 11.86, 1.06 ± 0.47, 1.20 ± 0.53, and 1.01 ± 0.50 mg/kg dry wt., respectively. Cd and Pb levels exceeded the Commission Regulation of European Union maximum limit of 0.05 mg/kg and 0.30 mg/kg, respectively. However, the results of Estimated Weekly Intake and Estimated Daily Intake indicate that the heavy metal levels in the samples are generally within respective recommended guidelines. Thus, it would appear from this study that the heavy metal levels in *T. puta* from Karachi coasts are low enough not to present a health hazard if the fish are consumed, although due attention should be paid to the maximum acceptable daily intake per kilogram body weight.

1. Introduction

Karachi is the biggest city in Pakistan. The major sources of pollution in eastern part of Karachi industries related to leather, paints, textile, pharmaceuticals, iron, steel, electrical appliances, refinery, oil, and electroplating and water from run-off and general untreated sewage. There are over 11,000 industrial units [1] in the city, distributed as >2,000 units in Federal B area zone, 2,571 units in Korangi zone, 2,000 units in North Karachi, 1,200 units in Landhi zone, and 4,000 units in Mangopir zone in Karachi. Moreover, Port Qasim is another industrial area but there is no detailed data available.

Karachi has five industrial estates and other areas are also dumping untreated toxic waste amounting to an estimated 200 million gallons per day [2]. The lack of regard for environmental standards also means that Pakistani companies may have trouble exporting their goods abroad [2]. Contamination of marine coastal areas includes a wide variety of organic and inorganic compounds. Among the myriad of these substances released into marine ecosystems, heavy metals have received considerable attention due to their different toxicity and potential bioaccumulation in aquatic organisms [3–5].

It is known that some trace metals are essential for biological processes and are absolutely required by biota to grow and complete their life cycle and yet can become toxic when their concentration levels exceed those required for correct nutritional response by factors varying between 40- and 200-fold [6]. Meanwhile, metals such as Pb, Hg, and Cd are toxic at quite low concentrations [7, 8]. Cd is considered one of the most toxic elements to human life. It causes "itai itai," a bone disease similar to rickets, and cardiac enlargement, anemia, gonadal atrophy, kidney failure, and pulmonary emphysema [9]. Pb is toxic as well and causes anemia, encephalopathy, weight and coordination loss, abdominal pain, vomiting, constipation, and insomnia [9]. Being nonbiodegradable like

Figure 1: Study area.

many organic pollutants, metals can be concentrated along the food chain, producing their toxic effects at points often far away from their original source as point of entry in the marine environment [10]. Accumulation of heavy metals in the food web can be either by accumulation from the surrounding medium, such as water or sediment, or by bioaccumulation from the diet [11]. Aquatic organisms have been widely used in biological monitoring and assessment of safe environmental levels of heavy metals. Fish have been used to monitor pollution load in coastal and marine environment [12]. Fish occupy the top of the food chain and may concentrate high metal levels from the surrounding waters and food and accumulate them in their tissues.

The present study was undertaken to investigate the levels of heavy metals (Fe, Mn, Cd, Pb, and Ni) in the fish *T. puta* collected from Karachi Fish Harbor, during January–December, 2012. It is also necessary to have data on the levels of heavy metals in fish species in order to assess whether there is a health hazard. Adults of this fish inhabit coastal waters and feed on other fishes and invertebrates. Although with no commercial value, *T. puta* is often consumed by locals, hence the necessity to assess its potential health hazard to humans.

2. Material and Methods

2.1. Study Area. Karachi Fish Harbor is located in Karachi, Sindh, Pakistan, close to the main business district and several industrial areas (Figure 1). The geographic position of the harbor places it in close proximity to major shipping routes. Moreover, due to heavy agricultural, domestic activities and urbanization in the region the harbor may receive large quantities of untreated agricultural and domestic sewage. Fisheries play an important role in the national economy of Pakistan. Over 90 percent of Pakistan's fish and seafood catch and exports pass through the harbor. Moreover, heavy metal contamination in the coast is an important issue regarding the health of the marine organisms and in turn health of the humans who feed on seafood.

2.2. Sampling. Thirty-six (36) *T. puta* specimens were obtained from Karachi Fish Harbor, Karachi coast, during premonsoon, monsoon, and postmonsoon season between January 2012 and December 2012. Fish samples in each season were transported to the laboratory in a thermos-flask with ice on the same day in each study period. In the laboratory, the fish samples were washed and then measured as fresh weights.

Table 1: Mean ± SD, minimum and maximum of length (cm) and weight (g) of *Terapon puta* during different seasons of the year 2012.

Seasons		Length (cm)	Weight (g)
Premonsoon	Mean	14.38	75.00
	Std. deviation	0.82	1.60
	Minimum	13.50	73.00
	Maximum	16.00	78.00
Monsoon	Mean	16.32	78.17
	Std. deviation	0.62	1.80
	Minimum	15.50	76.00
	Maximum	17.30	82.00
Postmonsoon	Mean	17.13	79.42
	Std. deviation	0.78	2.39
	Minimum	16.00	76.00
	Maximum	18.50	84.00

2.3. Preparation of Samples and Determination of Heavy Metals. The samples were rinsed with distilled water and approximately 5 g of muscle and entire liver of each sample were dissected with acid washed tools and rinsed to avoid cross-contamination and stored in a deep freezer at −21°C until being ready for analysis. The muscle and liver tissues of the fish were prepared for analysis according to the method described by Bernhard [13]. Samples were placed in a vented drying oven at 70°C and allowed to dry to constant weight. The samples were then calcined at 500°C for 3 hours and then ground. Each ash sample was weighed again and dissolved with 0.1 M HCl in beaker. The beakers then cooled to room temperature and one milliliter filtered solution was diluted with 25 mL distilled water. A stock solution of 1000 ppm of each metal was prepared by dissolving metals in distilled water. Standard solutions of each metal were prepared, three standards from 1000 ppm stock solution to 2 ppm, 4 ppm, and 6 ppm. A calibration curve was established using standard solutions to every analysis. The solutions were analyzed by Perkin-Elmer AAnalyst 700 Atomic Absorption Spectroscopy (AAS), with background correction and acetylene as carrier. The most widely used flame is the air/acetylene flame, which is transparent over a wide spectral range and displays low emission, making it ideal for the determination of many elements including Fe, Mn, Cd, Pb, and Ni.

2.4. Statistical Analysis. Data were expressed as mean ± standard deviation (SD). Data were analyzed by ANOVA at $\alpha = 0.05$. Comparison of means was performed by Tukey test and difference was considered significant at $P < 0.05$ [14]. Statistical analysis of data was carried out using Statistica version 7.0 software. All values were being expressed on a mg/kg dry wt. basis. Three replicates were made of each measurement.

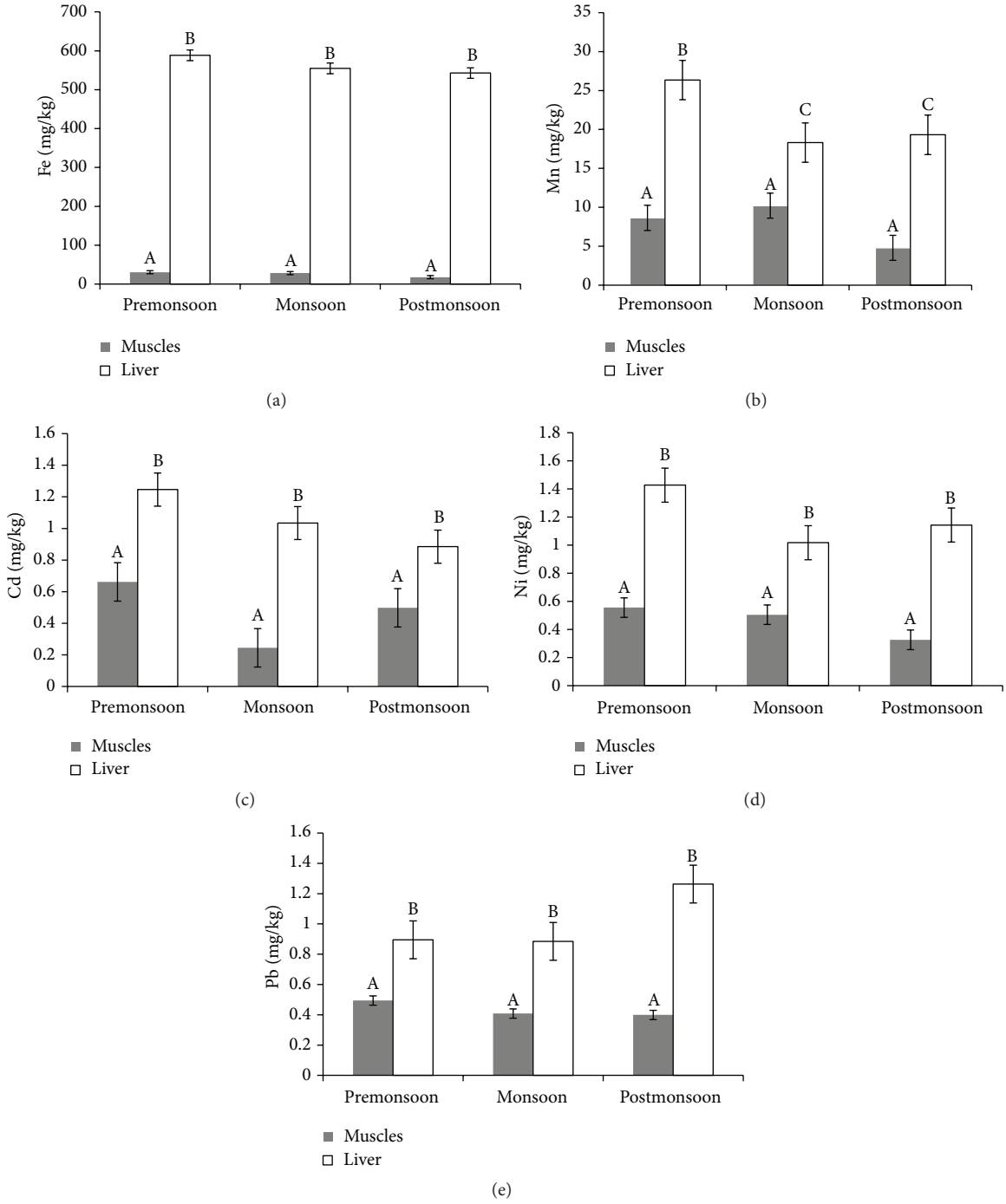

Figure 2: Metal concentration (mean ± SE) in muscles and liver *Terapon puta* from Karachi Fish Harbor for Fe (a), Mn (b), Cd (c), Ni (d), and Pb (e) during different seasons of January 2012 and December 2012. A, B, and C = the same letters beside the vertical bars in each graph indicate that the values are not significantly different ($P > 0.05$).

3. Results and Discussion

Thirty-six (36) fishes were obtained; their lengths and weights are given in Table 1. Lengths and weights of the fish samples were taken as equal as possible in the present study and there was no significant difference in the weights and lengths of the fish as a result of sampling period.

One of the purposes of the present survey was to determine current levels of selected heavy metals in fish in Karachi waters, in order to assess whether any changes are occurring on an annual basis. The data in Figure 2 on the distribution of the metals in the tissues of the fish samples indicate that there is a considerable greater accumulation of these metals in liver than those in muscle tissues. These results were similar

Table 2: Analysis of variance (ANOVA) of heavy metals in *Terapon puta* collected at Karachi Fish Harbor from January 2012 to December 2012.

Metals		Sum of squares	df	Mean square	F	P
Fe	Between groups	10419	2	5209	0.07	0.938
	Within groups	5565290	69	80656		
	Total	5575709	71			
Mn	Between groups	1202	2	601	5.92	0.004
	Within groups	7009	69	102		
	Total	8211	71			
Cd	Between groups	1.36	2	0.68	3.02	0.055
	Within groups	15.55	69	0.23		
	Total	16.91	71			
Pb	Between groups	0.19	2	0.09	0.44	0.648
	Within groups	14.87	69	0.22		
	Total	15.06	71			
Ni	Between groups	0.74	2	0.37	1.11	0.335
	Within groups	23.02	69	0.33		
	Total	23.76	71			

to those reported by other studies in Karachi coasts [15, 16]. These findings are totally normal and will also be found in noncontaminated areas since the liver is a detoxifying organ that contains all the metals accumulated along the life-history of the fish. Statistical test of significance using Tukey test and analysis of variance (ANOVA) showed significant differences in the concentration of Mn in *T. puta*, which is obtained from the different seasons (Table 2). Other metals did not show a significant difference. In *T. puta*, Fe is the most abundant of the metals examined. Fe contents in the fish in the seasons were not different. Fe is the main element of blood and is usually in excess in bodies, including liver which is where iron is also stored. However lower Mn, Cd, and Ni levels were found in the monsoon and postmonsoon seasons than those in premonsoon season. Pb contents in the liver of fish samples in the postmonsoon season were slightly higher than those for other seasons, but Pb contents in the muscles were not different. This high level of Pb could be due to heavy rainfall during the postmonsoon, which increases the metal content of water by washing down the agricultural wastes.

T. puta is distributed in tropical and subtropical waters. In Indo-West Pacific, it is found from the northern Indian Ocean and the Indo-Australian Archipelago. It is a lessepsian migrant, now prevalent in the Mediterranean [17]. The average consumption of this fish in this region is generally low. The current food standard regulations in the European Union [18] specify allowable maxima for the quantities cadmium and lead. These maxima are 0.05 mg/kg for Cd and 0.30 mg/kg for Pb, respectively. No maxima have been specified for the other metals. The results presented in Figures 2(a)–2(e) show that metal levels of the tissues for *T. puta* always exceeded the allowable levels, whereas MAFF [19] show that the maxima levels 2 mg/kg for Pb. Moreover, FAO evaluated that the normal tissue concentrations of heavy metals in marine organisms were between 0.001 and 1.5 mg/kg for Cd and between 0.05 and 5 mg/kg for Pb. The progress of agriculture and industrial development activities has led to the increased heavy metals emission into the coastal ecosystem.

The average daily fish consumption in Pakistan is 5 g per person [20]. This is also equivalent to 35 g/week. The Joint FAO/WHO has recommended a provisional tolerable weekly intake (PTWI) of 0.007 mg/kg body weight for Cd [21]. The mean (±SD) Cd levels in the muscles tissues of *T. puta* were 0.468 ± 0.286 mg/kg dry wt. This is equivalent to 0.49 mg/week/70 kg body weight. Estimated Weekly Intake (EWI) and Estimated Daily Intake (EDI) for a 70 kg body weight of an adult person on basis of the present study results were calculated as 0.016±0.010 and 0.002±0.001, respectively. Thus, considering normal diet from people around Karachi, both Cd and Pb are below the dose to induce harmful effects to an adult individual.

The Joint FAO/World Health Organization Expert Committee on Food Additives (JECFA) established the PTWI for Pb as 0.025 mg/kg body weight [21]. The mean (±SD) Pb level in the muscles tissues of *T. puta* was 0.4339 ± 0.25102 mg/kg dry wt. This is equivalent to 1.75 mg/week/70 kg body weight. EWI and EDI for a 70 kg body weight of an adult person on basis of the present study results were calculated as 0.015 ± 0.008 and 0.002 ± 0.001, respectively.

Nonessential metals such as Cd and Pb have been proved to be toxic to aquatic organism and marine environmental health as well as human. In conclusion, the heavy metal concentrations in *T. puta* of the Karachi Harbor do not present any danger to human health or human consumption. However it is recommended that the liver of fish should be completely removed and very well washed before consumption of the fish. Bear in mind that this fish should not be consumed very often because of critical Cd levels. The Marine Strategy Framework Directive (MSFD, 2008/56/EC) establishes a framework for the development of marine strategies designed to achieve Good Ecological Status (GES) in the

marine environment by the year 2020. Contaminants including heavy metals in fish for human consumption should not exceed levels established by Community legislation or other relevant standards [22]. In the cause of their toxicity and their possible bioaccumulation, these metals should be subject to mandatory monitoring. The present study provides a useful baseline against which to measure any future changes in metal pollution in Karachi coasts of Pakistan.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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