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Quratulan Ahmed
The Marine Reference Collection and Resources Centre, University of Karachi, Karachi 75270, Pakistan.

Levent Bat
Department of Hydrobiology, Sinop University, Fisheries Faculty, 57000 Sinop, Turkey.

Farzana Yousuf
Department of Zoology, University of Karachi, Karachi 75270, Pakistan.

Elif Arıcı
Department of Hydrobiology, Sinop University, Fisheries Faculty, 57000 Sinop, Turkey.

Correspondence
Quratulan Ahmed
The Marine Reference Collection and Resources Centre, University of Karachi, Karachi 75270, Pakistan.

Heavy metals in *Acanthopagrus arabicus* Iwatsuki, 2013 from Karachi Coasts, Pakistan and potential risk of human health

Quratulan Ahmed, Levent Bat, Farzana Yousuf, Elif Arıcı

Abstract
During the present concentrations of heavy metals (Fe, Mn, Zn, Pb and Cd) were estimated in muscle and liver tissues of commercially important marine fish Arabian yellowfin seabream (*Acanthopagrus arabicus* Iwatsuki, 2013) collected from Karachi Fish harbour during January-October, 2015. The results showed that muscle had the lowest metal concentrations as compared to liver tissues. The Fe concentration was the highest for both in muscle as well as for liver among the other heavy metals. The ranges of Fe concentration were found from 20.16 to 63.52 and 20.16 to 641.52 mg/kg in muscle and liver, respectively. The lowest metal concertation was Pb which was followed by Cd. Pb and Cd concentrations in the muscles of *A. arabicus* ranged from 0.01 to 0.52 and 0.23 to 0.88 mg/kg, respectively; whereas Pb and Cd concentrations in the liver of Arabian yellowfin seabream ranged from 0.01 to 2.58 and 0.23 to 2.64 mg/kg, respectively.

Keywords: Heavy metals, hazard index, estimated daily intakes, *Acanthopagrus arabicus*, Karachi Harbour, Pakistan

1. Introduction

Rapid industrialization and economic development in Pakistan has resulted in increased water pollution in the coastal areas [1-4]. Marine coastal environments are increasingly being contaminated with different kinds of chemicals [5, 6]. Pollutants are deposited into coastal water column and cause serious changes in the ecosystem, which in turn directly or indirectly affect the life and activities of marine organisms and accumulate a toxic concentration level [5]. Many of these dangerous pollutants, such as heavy metals, can readily accumulate within aquatic organism tissues at much higher concentrations than those in water column and sediment [5-9] and both essential and nonessential metals may be toxic at certain levels [5, 6, 10]. The heavy metals accumulated in fish not only damage to fish due to their high toxicity and accumulative behaviour but also affect the health of human beings through the food chain [6, 11]. Industrialization level and agricultural activities as well as uncontrolled urbanization along the coastal areas were indicated to be the leading potential source of the accumulation of chemicals including heavy metals in marine environment [1-4, 6, 12]. Research on metal pollution in Pakistan coast has been increased during the recent years [10, 13-19]. Most of them have dealt with metal concentration in fish species [10, 13-20]. In Pakistan fish are highly consumed by people and may accumulate large amounts of some metals from fish as food. Fishery plays an important role in the national economy of Pakistan [20]. Therefore it is important to determine the heavy metal levels in commercial fish in order to evaluate the possible risk of consumption for people health.

The present study was evaluated to determine the distribution of Fe, Mn, Zn, Pb and Cd levels in muscle and liver tissues of commercially important marine fish Arabian yellowfin seabream (*Acanthopagrus arabicus* Iwatsuki, 2013) which is called “Dandia” by local people, collected from Karachi Fish harbour during January-October, 2015. This species is pelagic-neritic and moderate vulnerability fish [21] and occurs in shallow coastal waters and enters river mouths and estuaries. They feed mainly on echinoderms, worms, crustaceans and molluscs [22] and were sold fresh in markets [23].

2. Materials and Methods

2.1. Study Area

Karachi Fish Harbour is located in Karachi, Pakistan, close to the main business district, major shipping routes and several industrial areas [20]. Thus, heavy metal levels in fish tissues are an important issue regarding the health of the human via food chain.

2.2. Sampling

Eighteen (18) *A. arabicus* specimens were obtained from Karachi Fish Harbour where is located in Karachi, Sindh on the Arabian Sea (Figure 1) during pre-monsoon, monsoon and post-monsoon season between January 2015 and October 2015. Six fish samples in each season were placed immediately in poly-ethylene bags, put into isolated container of thermos-flask with icebox and, then, brought to the laboratory at the same day in each study period. In the laboratory, the total length (cm) and the body wet weight (g) of each specimen were measured.

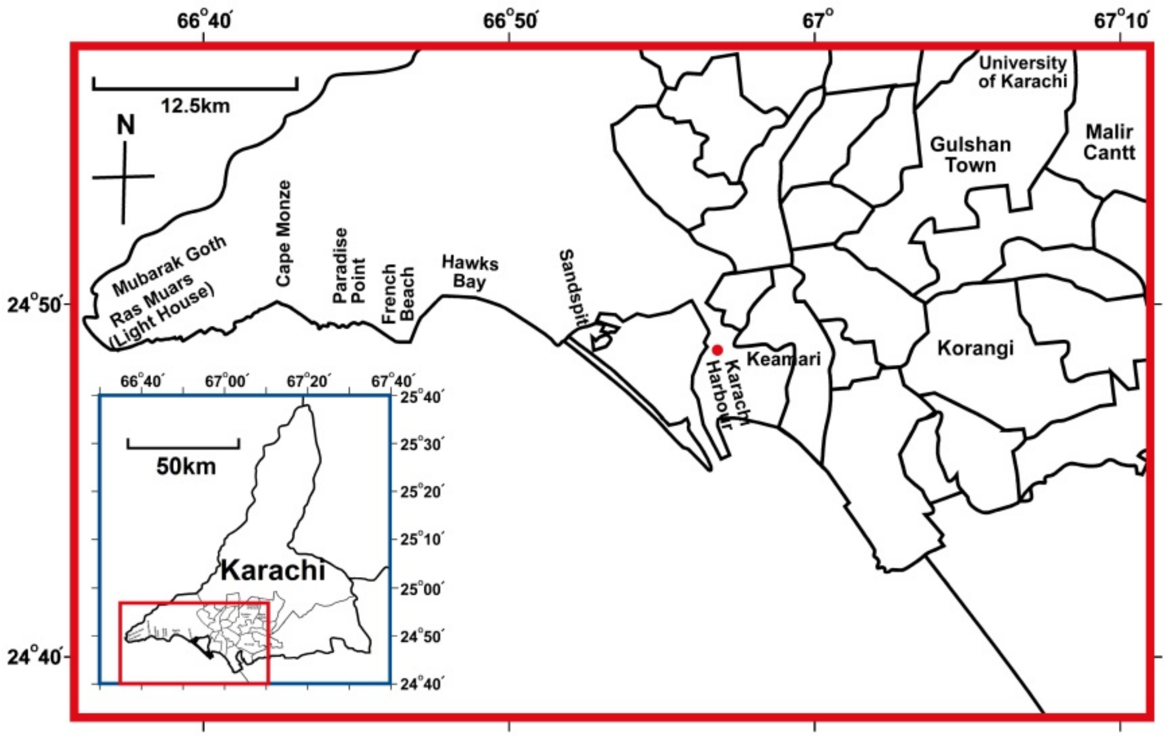

Fig 1: Fish Harbour of Karachi, Pakistan

2.3. Preparation 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 and stored in a deep freezer at -21 °C up to the analysis. The muscle and liver tissues of the fish were prepared for analysis according to the method described by Bernhard [24]. 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 until it turned to white or grey ash and ground. Each ash sample was weighed again and dissolved with 0.1 M HCl in beaker. The beakers then cooled to room temperature and residues were filtered using 0.45µm Whatman filter paper then transferred to 50 ml volumetric flask and were diluted to 25 ml deionised water for muscle and liver. Working standards were prepared from stock solutions. A calibration curve was established using standard solutions to every analysis. The solutions were analysed by AA-ANALYST 700 Atomic Absorption Spectrophotometer (AAS).

2.4. Reagents

De-ionized water was used to prepare all aqueous solutions. All plastic and glassware used were rinsed and soaked in 10% (v/v) HNO_3 overnight. They were rinsed with deionized water and dried prior to use. All acids were of highest quality from Merck, Germany. Double distilled water was used to prepare standard stock solutions of Fe, Mn, Zn, Pb and Cd in concentrations of 1,000 mg/L.

2.5. Assessments hazard index (HI) of heavy metals in fish

Risk from metals intake through ingestion may be characterized using a hazard index (HI) as the ratio of the estimated metal dose (EDI mg/kg of body weight per day) and the reference dose (Rf D mg/ kg. y). The HI was calculated by using the equations below [25-27].

$$HI = EDI/ RfD$$

If HI > 1.0, then the EDI of a particular metal exceeds the RfD, indicating that there is a potential risk associated with that metal. The estimated daily intake (EDI) depends on both the metal concentration level and the amount of consumption of fish. The EDI of metals was determined using the following equation.

$$EDI = C_{metal} \times W / bw$$

Where: C_{metal} is the concentration of metals in fish; W represents the daily average consumption of fish; bw is the body weight.

2.6. Statistical analysis
Statistical Analysis of data was carried out using Statistica version 7.0 software. A one- way analysis of variance (ANOVA) was performed, followed by Tukey post hoc comparisons for the source of statistically significant difference. The significance was set at 0.05 and P-values less of 0.05 were considered statistically significant [28]. All values were being expressed on mg/kg dry wt. basis.

3. Results and Discussion
3.1. Fish size
Metal concentrations in fish are highly dependent on fish size, so it is necessary to include it in the stats. The length and weight of the fish samples were taken as equal as possible in the present study and there was no significant difference in the length and weight of the fish as a result of sampling period (Figure 2).

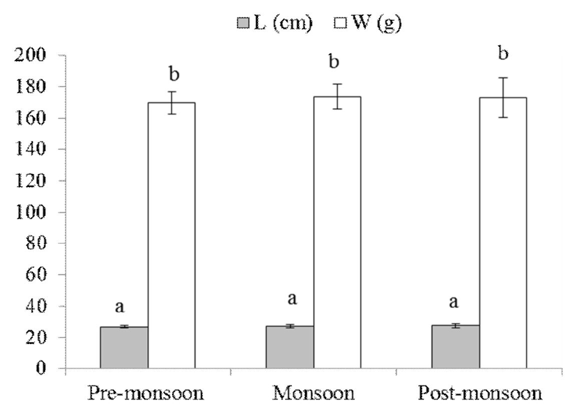

Fig 2: Length and weight of *A. arabicus* collected at Karachi Fish Harbour January 2015 and October 2015. The different letters beside the vertical bars indicate that the values are significantly different (p< 0.05).

3.2. Heavy metal levels in fish muscle and liver
The metal concentrations and the corresponding mean standard deviations (expressed as mg/kg dry wt.) were measured in muscle and liver tissues of *A. arabicus* sampled in Karachi harbour are given in Figures 3-7. The variance has revealed that the mean levels of Fe, Mn, Zn, Pb and Cd among the organs of *A. arabicus* were significantly different (P < 0.05). Findings of this study show that metal levels are higher in liver than those in muscular tissues (Figures 3-7). This is in agreement with previous studies by Ahmed and Bat [14, 17, 18], Ahmed *et al*. [19, 20]. Ahmed *et al*. [20] had stated that the liver is a detoxifying organ and contains all the metals accumulated along the life-history of the fish even in uncontaminated areas and hence the findings of this study are totally normal.
In this study Fe is the most abundant of the metals examined. However, it is an essential element. Fe contents in the fish in the seasons were different (Figure 3). Fe concentration in fish muscles from Karachi Harbour ranges between 20.16 mg/kg in Post-monsoon to 63.52 mg/kg in Monsoon and are as shown in Figure 3. Whereas statistical test of significance using Tukey test and analysis of variance (ANOVA) showed no significant differences between seasons in the concentration of Fe in liver tissues of *A. arabicus*. Zn levels in muscle and liver tissues did not show significant differences between seasons (Figure 5). The concentration of Mn in muscle of *A. arabicus* ranges from 3.22 mg/kg and 9.98 mg/kg and showed no significant differences between seasons (Figure 4), whereas the highest Mn in livers was 52.63 mg/kg with the mean 46±4.1 mg/kg in Post-monsoon and showed very significant differences between seasons. Mn is also an essential element for organisms and its deficiency results in severe skeletal and reproductive abnormalities.
Pb and Cd belong to non-essential and toxic metal group, implying no known function in biochemical processes. In general Pb levels in teleost liver are substantially higher than that in the corresponding muscle tissues, though the specific mechanism for the highly elevated Pb levels in liver remains unknown [29]. In this study the mean Pb levels in muscle and liver were 0.11±0.05 mg/kg and 1.36±0.28 mg/kg, respectively. The Pb concentrations ranged from 0.01 at Pre-monsoon to 0.46 at Post-monsoon mg/kg in muscle and 0.58 to 2.58 at Monsoon mg/kg in liver (Figure 6). Pb levels both in muscle and liver tissues showed significant differences between seasons. The mean Cd levels in muscle and liver were 0.56±0.19 mg/kg and 1.67±0.28 mg/kg, respectively. The Cd concentrations ranged from 0.23 at Pre-monsoon to 0.88 at Post-monsoon mg/kg in muscle and 1.18 at Pre-monsoon to 2.64 at Post-monsoon mg/kg in liver (Figure 7). Cd levels in muscle tissues were not different between seasons, but Cd levels in liver were statistically different. High accumulation of heavy metals in liver of fishes has been reported in many studies from Karachi Harbour [11, 13-20], which have suggested that the liver plays an important role in the metabolic processes of heavy metal in fishes. It is well known that liver as a tissue specialized in metal storage and detoxification [29].

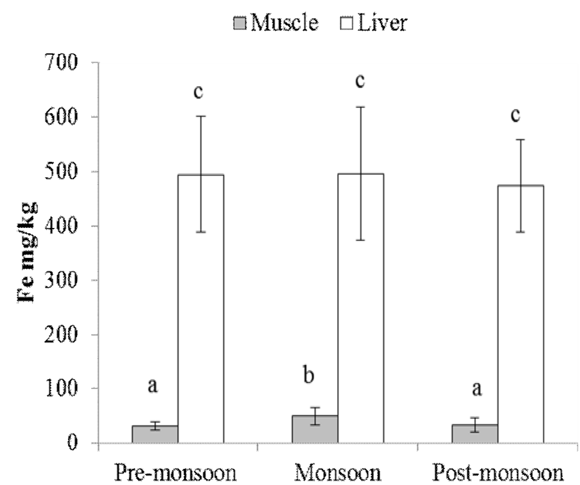

Fig 3: Differences of Fe concentrations in *A. arabicus* from Karachi Harbour among the seasons. The different letters beside the vertical bars indicate that the values are significantly different (p< 0.05).

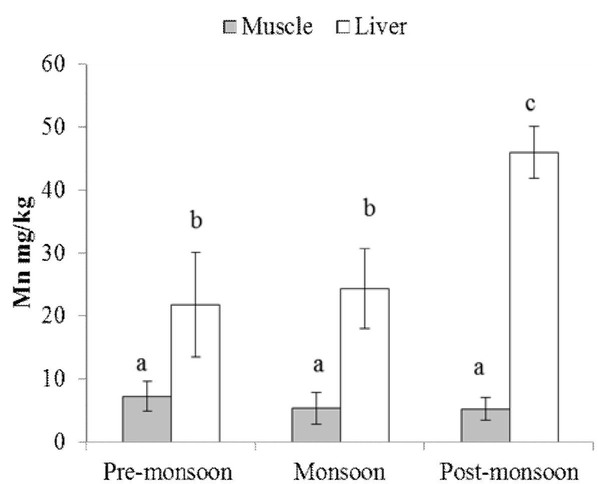

Fig 4: Differences of Mn concentrations in *A. arabicus* from Karachi Harbour among the seasons. The different letters beside the vertical bars indicate that the values are significantly different (p< 0.05).

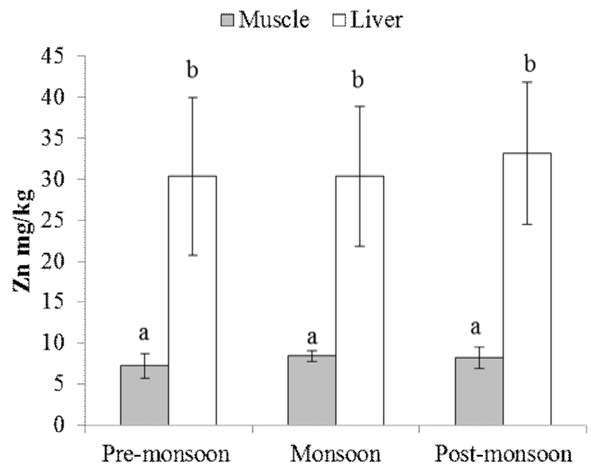

Fig 5: Differences of Zn concentrations in *A. arabicus* from Karachi Harbour among the seasons. The different letters beside the vertical bars indicate that the values are significantly different ($p< 0.05$).

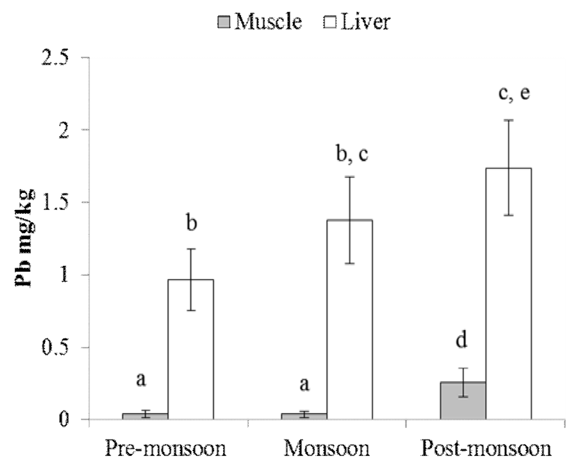

Fig 6: Differences of Pb concentrations in *A. arabicus* from Karachi Harbour among the seasons. The different letters beside the vertical bars indicate that the values are significantly different ($p< 0.05$).

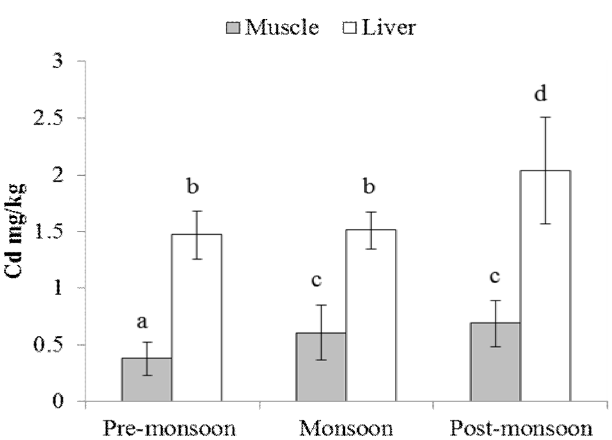

Fig 7: Differences of Cd concentrations in *A. arabicus* from Karachi Harbour among the seasons. The different letters beside the vertical bars indicate that the values are significantly different ($p< 0.05$).

3.3. Concentrations of heavy metals in *A. arabicus* and comparison with international guidelines

Legal thresholds are not available for essential elements in Europe Commission Regulation (EC) [30]. The mean concentration of Pb in the muscles tissues of *A. arabicus* was 0.11±0.05 mg/kg dry wt. This value is far away from the maximum acceptable limit of Europe Commission which limited the maximum concentration of Pb in fish muscles by 0.30 mg/kg wet wt.

Cd is also considered as non-essential metal for organisms. In the present study Cd in the muscles tissues of *A. arabicus* was 0.56±0.19 mg/kg dry wt. (approximately 0.140±0.048 mg/kg wet wt.). This value is nearly 28 times higher than the maximum acceptable limit of Cd in fish set by EC which detected it by 0.05 mg/kg wet wt., but the highest value of Cd in this study was 1.4 times lower than the maximum permissible value set by MAFF (The Ministry of Agriculture, Forestry and Fisheries) [31] which limited it up to 0.2 mg/kg wet wt. In the present the results are similar to those reported by Ahmed *et al*. [13, 19]. It is possible that short-term exposure to Cd may not cause immediate health threats to people [19].

3.4. Assessments hazard index (HI) of heavy metals in fish

According to the FAO (Food and Agriculture Organization) [32], the average quantity of fish consumed per person (assuming a 70-kg person) per day in Pakistan is 5 g. Multiplying this value by the average concentration of each metal (Fe, Mn, Zn, Pb and Cd) in analysed fish, the average daily intake of metals from fish can be estimated. Intake estimates were expressed as per unit body weight (mg/kg body wt. / daily). The estimated daily intakes (EDI) for Fe, Mn, Zn, Pb and Cd in the edible tissues of *A. arabicus* in this study were 0.195±0.055, 0.030±0.011, 0.040±0.006, 0.0006±0.00025 and 0.0028±0.00098, respectively.

Fe intake should not exceed a maximum of 15 mg/day [33]. National Academy of Sciences [33] recommended Zn allowance for adult men is set at 15 mg/day. The allowance for adult women, because of their lower body weight, is set at 12 mg/day. The required daily intake for adults is about 15 mg/day; however, the requirement also varies with age. A provisional daily dietary Mn intake for adults of 2.0 to 5.0 mg is recommended [33]. The tolerable weekly intake of heavy metals as PTWI (Provisional Tolerable Weekly Intake), are set by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA). The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for Fe, Mn, Zn, Pb and Cd as 5.6, 2-5, 7, 0.025 and 0.007 mg/kg body weight for a 70 kg adult person, respectively [33-36].

Estimated hazard index (HI) of Fe, Mn, Zn, Pb and Cd suggest that these metals in the edible tissues of *A. arabicus* do not pose any apparent threat to people, where the HIs of all the considered metals were below the value of 1 (Figure 8).

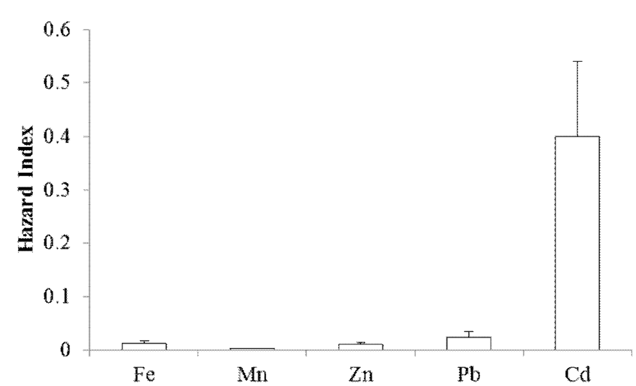

Fig 8: Total hazard index (HI) of Fe, Mn, Zn, Pb and Cd via consumption of *A. arabicus*

4. Conclusion

In the present study, essential (Fe, Mn, Zn) and non-essential toxic (Pb, Cd) metals in *A. arabicus* have been analysed. All metal levels are higher in liver than those in muscular tissues. The metal levels in Arabian yellowfin seabream from Karachi Harbour of Pakistan were not exceeded except Cd the permissible limits set by EU. On the other hand, Cd exceeds the maximum allowable standards proposed by international organizations, which makes their health at risk. Consequently, adverse human health effects may occur if contaminated fish is consumed too much. However, the daily intakes (EDI) of these metals were estimated taking into account the mean metal level in the muscle tissues of *A. arabicus* and the mean consumption of the Arabian yellow fin seabream per day for adults. Estimated HIs of all the considered metals were below the value of 1, therefore the metals in the edible tissues of fish do not pose any apparent threat to the population and indicated no adverse effects to the consumers.

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