ISSN: 2458-8989

Natural and Engineering Sciences

NESciences, 2017, 2 (3): 79-92

-RESEARCH ARTICLE-

Bioaccumulation of nine heavy metals in some tissues of *Anodontostoma chacunda* (Hamilton, 1822) in the Arabian Sea coasts of Pakistan

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Abstract
Concentrations of heavy metals Cd, Fe, Pb, Cu, Zn, Ni, Co, Cr and Mn in muscles, liver, kidney and gills of marine and native commercial fish of Chacunda gizzard shad *Anodontostoma chacunda* collected from Karachi fish harbour were determined to conduct a health risk assessment for people by the Atomic Absorption Spectrophotometer. The obtained results were expressed mg/kg dry weight. Results showed that Fe and Zn concentrations were the most abundant among all tissues whereas Cd and Pb levels were the lowest. The data obtained in this study were compared with the international maximum permissible values. The estimated values of all metals in muscles of fish in this study were below the permissible limits. Generally, risk values for the measured metals do not pose unacceptable risks at mean ingestion rate for edible tissues. It can be concluded that the heavy metal levels in edible parts of *A. chacunda* have no hazards for consumers.

Keywords:
Anodontostoma chacunda, heavy metals, Karachi fish harbour, Arabian Sea, Hazard Quotient

Article history:
Received 19 July 2017, Accepted 22 September 2017, Available online 30 October 2017

Introduction

Industrial, domestic, agricultural, fishing and tourisms activities continuously increase the quantity of heavy metals in the marine environment. It is well known that the heavy metals constitute the major pollutants in the marine environment. Since heavy metals cannot be bio- and thermo-

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degraded, and their biological half-lives are long, they ultimately accumulate in different tissues of aquatic organisms. Furthermore, heavy metals are the most important toxic pollutants for aquatic organisms (Govind & Madhuri, 2014).

The increasing request for food and food safety is getting important to the risks associated with consumption of fish contaminated with heavy metals. However, protection of fish from the environmental pollutants especially heavy metals is essential, as the fish are most often contaminated by these pollutants (Govind & Madhuri, 2014). Monitoring of toxic substances in the marine biota including fish is required in order to improve knowledge of the health state of the marine ecosystem under Descriptors 8 and 9 of EC Marine Strategy Framework Directive (MSFD, 2008). Fish are often used as bio-indicators of heavy metals pollution in marine ecosystem (Authman et al., 2015) since they are found at high trophic levels and are an important and relatively cheap food source (Bat, 2014). Fish represents a very valuable source of protein and essential nutrients for balanced nutrition and good health. However the consumption of contaminated fish constitutes an important route of human exposure.

In Karachi coasts of the Arabian Sea, increasing population growth and industrial activities in Karachi region are the main sources of heavy metal pollution. Jilani & Khan (2013) pointed out that more than 18 million population of Karachi and sixty percent of the country's industries have been releasing around 472 million gallons per day of industrial and municipal wastewater. The main industries in Karachi that are playing a part of relatively considerable pollution load include textile, pharmaceutical, organic and inorganic chemicals, food industries, ceramics, steel, oil mills and leather tanning (Jilani & Khan, 2013). Automobiles, ever-increasing industrialization and urbanization are the most well-known source of pollution. There is an increasing accumulation of poisonous substances and gases in atmosphere, watering and agricultural soils while the industrial estates of Karachi are discharging large amount of pollutants including heavy metals (Khattak et al., 2012). At the same time, the fisheries and tourism industry are the major sources of the anthropogenic input of heavy metals. The coastal zone of Karachi is about 167 km long and untreated domestic and industrial wastes are the main problem (Siddique et al., 2009). The Karachi Harbour area is now regarded to be the most heavily polluted marine ecosystem in Pakistan owing to obtain a continuous discharged of domestic and industrial wastes (Jilani, 2015).

Although people in Pakistan consume a low quantity of fish, Pakistan is rich in the marine fisheries resources (Nazir et al., 2015). FAO reported that fish consumption in Pakistan was recorded at 0.6 kg per capita per year (Needham & Funge-Smith, 2014). However, Karachi is the centre of the fishing industry which plays an important role in Pakistan's national income (Nazir et al., 2016). The main source of commercial fish in Pakistan is the wild caught fish which are usually captured along the Karachi coasts of the Arabian Sea.

Recently, more concerns were raised about the potential accumulation of heavy metals in fish captured from this region due to coastal heavy metal pollution (Yousuf et al., 2013; Ahmed et al., 2014, 2015a; Ahmed & Bat, 2015a, b, c).

One of the methods for estimating the consumer based health risk assessment is the Provisional Tolerable Weekly Intake (PTWI). Risk of intake of metal-contaminated fish to human health was characterized by Hazard Quotient (HQ). This is a ratio of determined dose to the reference dose (Rf. D.). The people will harm no risk if the ratio is less than 1 and if the ratio is equal or greater than 1 then people will have health risk.

Therefore, the main purpose of this study was to (1) investigate Cd, Fe, Pb, Cu, Zn, Ni, Co, Cr and Mn in muscles, liver, kidney and gills of chacunda gizzard shad from Karachi fish harbour during Post-monsoon (October to December 2015), Pre-monsoon (January to May 2016) and Monsoon (June to September 2016) and (2) to undertake a health risk assessment of the consumption of heavy metals via this fish. Chacunda gizzard shad was marketed fresh, frozen, dried, dried-salted and boiled.

Materials and Methods

Fish samples of *A. chacunda* were purchased from Karachi fish harbour in Post-monsoon (October to December 2015), Pre-monsoon (January to May 2016) and Monsoon (June to September 2016). Six individuals each sampling season were put in plastic bags and conserved at -21°C for heavy metals (Cd, Fe, Pb, Cu, Zn, Ni, Co, Cr, Mn) analysis in muscles, liver, kidney and gills of chacunda gizzard shad.

Preparation of subsamples and analysis were performed according to UNEP (1984 and 1985) and FAO Technical Paper (FAO/SIDA, 1983). For heavy metal analysis, frozen fish were partially thawed, and muscles, liver, kidney and gills of each fish individual were dissected using stainless steel instruments. They were then dried at 70°C to constant weight. Then they are finely ground using an agate mortar to avoid any external contamination by heavy metals. The composite samples of 2 to 5 g of dry weight of the biological material were used for subsequent analysis. The samples were digested with ultra-pure nitric acid (and per-chloric acid for gills 4:1) at 100°C until the solution become clear. The residue was transferred to 50 ml volumetric flasks and diluted to the level with deionized water and analysed for Cd, Fe, Pb, Cu, Zn, Ni, Co, Cr and Mn using the AAnalyst-700 model Atomic Absorption Spectrophotometer. All the analyses were carried out in triplicate. The obtained results were expressed mg/kg dry weight. The ratio of dry weight to wet weight is also calculated.

Hazard Quotient (HQ) value of 1 refers to the threshold reference value suggested by U.S. EPA (2002) as the acceptability for non-carcinogenic health effects (U.S. EPA, 2002). In cases in which the non-carcinogenic HQ does not exceed unity (HQ<1), no potential non-carcinogenic risks are assumed to occur in the study area. It was calculated as the ratio of the estimated metal dose (EDI mg/kg of body weight per day) and the reference dose (Rf. D. mg/kg) (RAIS, 2016). The average daily fish consumption in Pakistan is 1.64 g per person and 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.

Statistical Analysis

All data generated were analysed statistically by calculating the mean and standard deviation of the measured parameters. One way ANOVA was performed to test the differences between muscles, liver, kidney and gills and Duncan test was used to determine the difference among seasons (post-monsoon, pre-monsoon and monsoon). P value was considered statistically significant at $P<0.05$ (Zar, 1984). The statistical analysis was performed with Statistica version 7.0 software.

Results

The fish samples in this study were captured from different seasons. The length and weight of *A. chacunda* ranged from 15.5 to 21 cm and from 58 to 95 g, respectively (Figure 1). The metal concentrations and the corresponding mean standard deviations (expressed as mg/kg dry wt.) were measured in muscles, liver, kidney and gills of chacunda gizzard shad found in Karachi fish harbour and are given in Figures 2-10. The variance has revealed that the mean heavy metal levels among the organs of chacunda gizzard shad were significantly different (P < 0.05). Among all the heavy metals that were studied, Fe had the highest concentrations in the four organs of the analysed chacunda gizzard shad from different seasons followed by Zn.

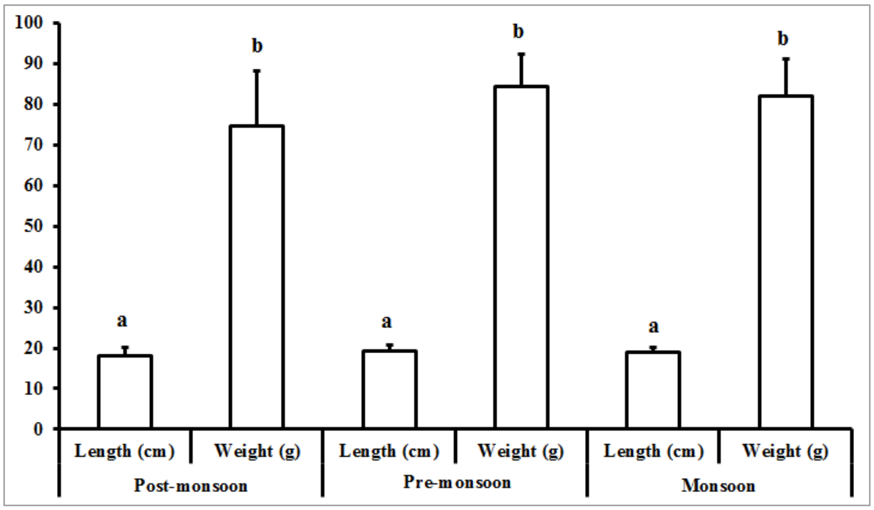

Figure 1. Mean length and weight of *A. chacunda* captured at Karachi Fish Harbour from October 2015 to September 2016.

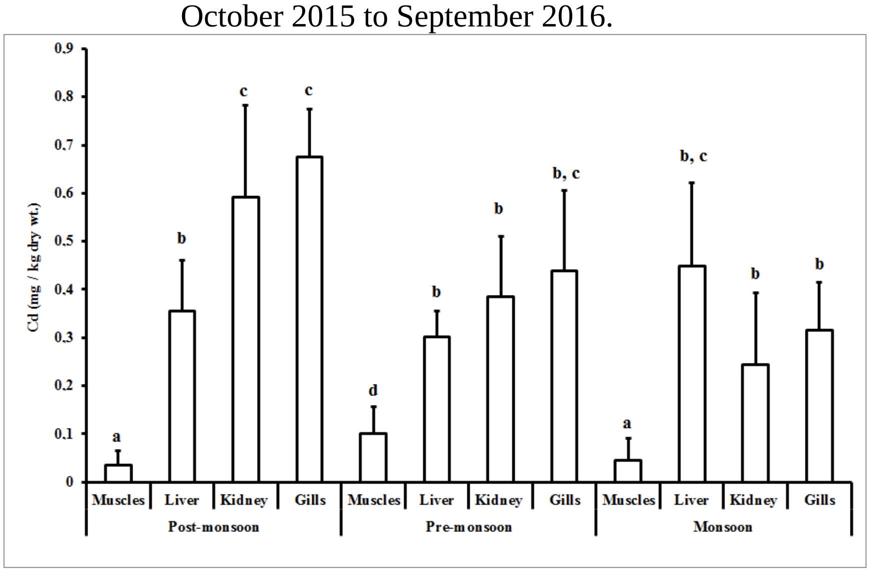

Figure 2. The means with standard deviations (vertical line) of Cd concentrations (mg/kg dry wt.) in the edible tissues of *A. chacunda* collected from Karachi fish harbour during Post-monsoon, Pre-monsoon and Monsoon in 2015 and 2016. The same letters above the vertical bars indicate the values are not significantly different (P>0.05).

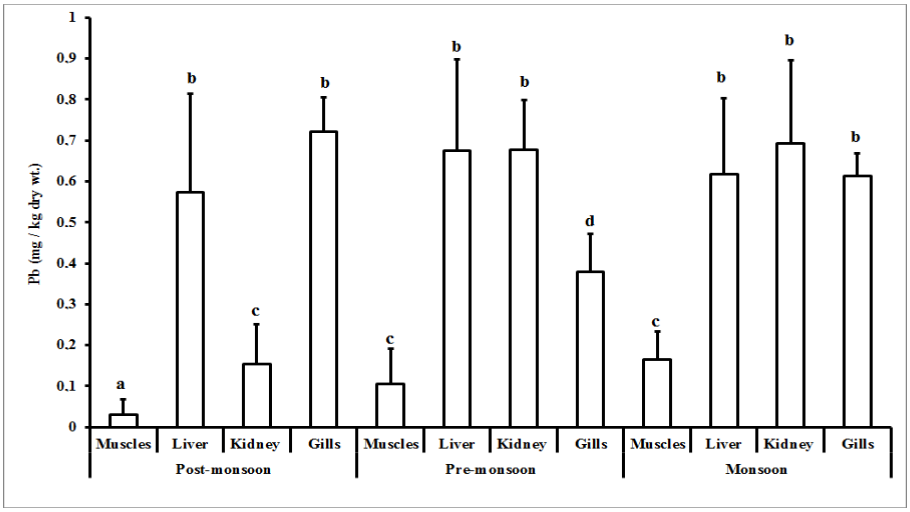

Figure 3. The means with standard deviations (vertical line) of Pb concentrations (mg/kg dry wt.) in the edible tissues of *A. chacunda* collected from Karachi fish harbour during Post-monsoon, Pre-monsoon and Monsoon in 2015 and 2016. The same letters above the vertical bars indicate the values are not significantly different (P>0.05).

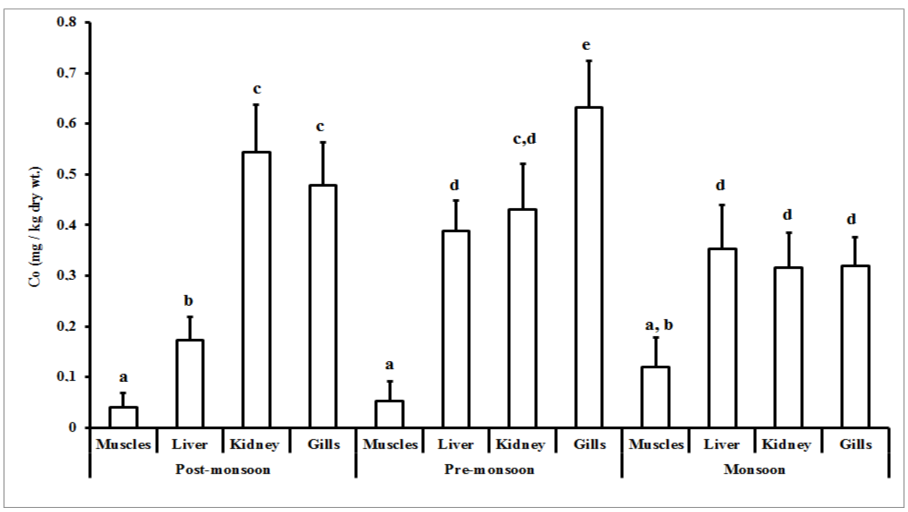

Figure 4. The means with standard deviations (vertical line) of Co concentrations (mg/kg dry wt.) in the edible tissues of *A. chacunda* collected from Karachi fish harbour during Post-monsoon, Pre-monsoon and Monsoon in 2015 and 2016. The same letters above the vertical bars indicate the values are not significantly different (P>0.05).

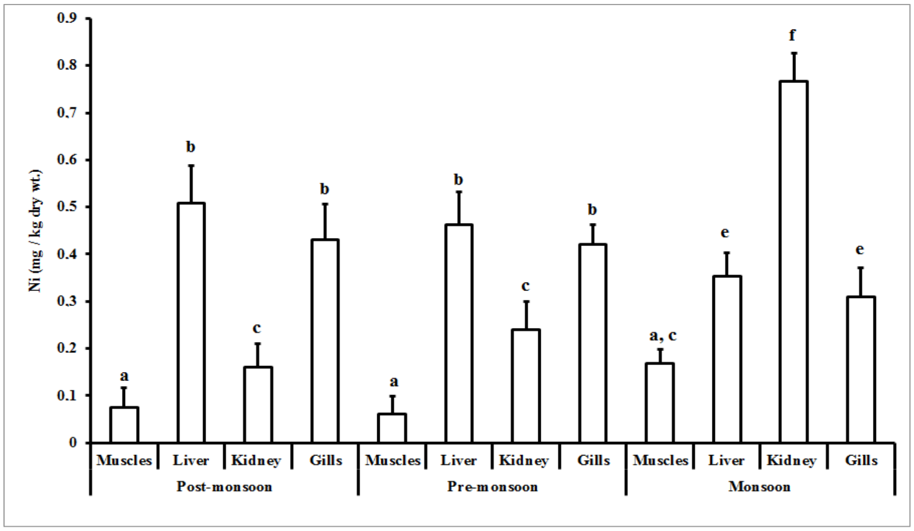

Figure 5. The means with standard deviations (vertical line) of Ni concentrations (mg/kg dry wt.) in the edible tissues of *A. chacunda* collected from Karachi fish harbour during Post-monsoon, Pre-monsoon and Monsoon in 2015 and 2016. The same letters above the vertical bars indicate the values are not significantly different (P>0.05).

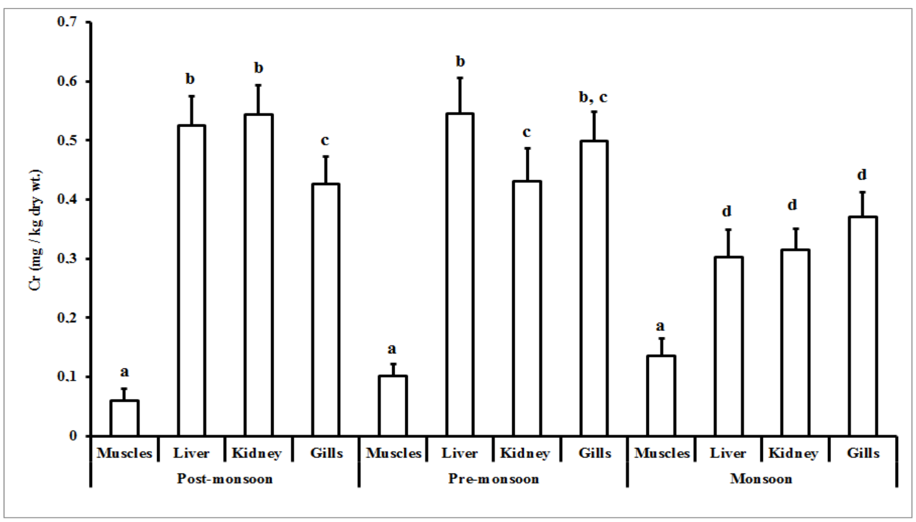

Figure 6. The means with standard deviations (vertical line) of Cr concentrations (mg/kg dry wt.) in the edible tissues of *A. chacunda* collected from Karachi fish harbour during Post-monsoon, Pre-monsoon and Monsoon in 2015 and 2016. The same letters above the vertical bars indicate the values are not significantly different (P>0.05).

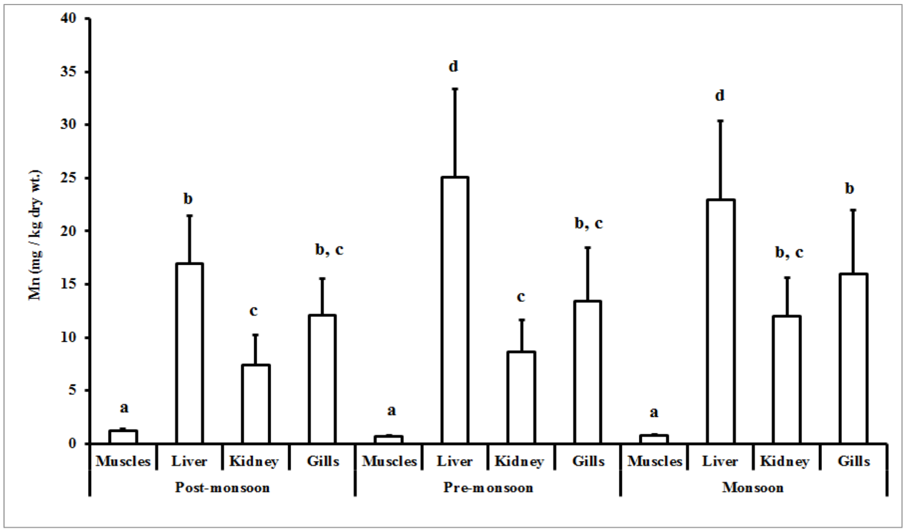

Figure 7. The means with standard deviations (vertical line) of Mn concentrations (mg/kg dry wt.) in the edible tissues of *A. chacunda* collected from Karachi fish harbour during Post-monsoon, Pre-monsoon and Monsoon in 2015 and 2016. The same letters above the vertical bars indicate the values are not significantly different (P>0.05).

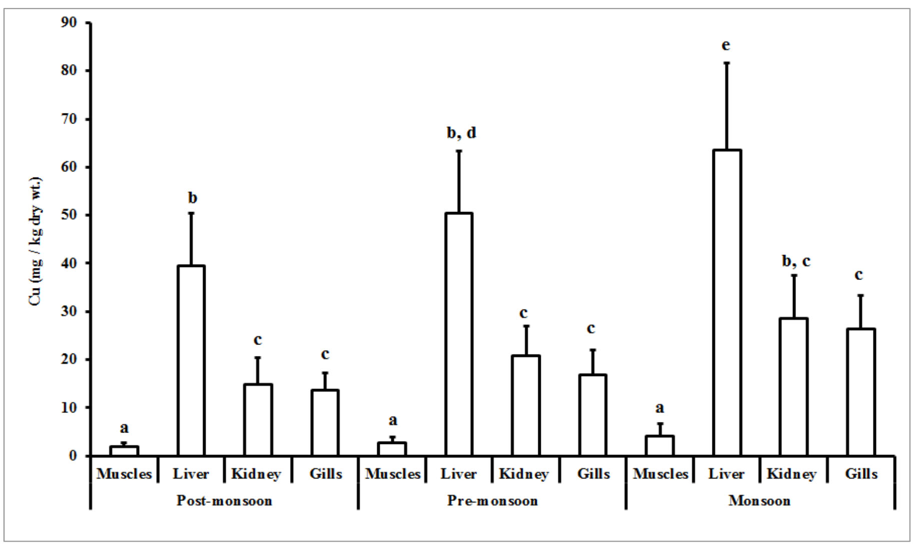

Figure 8. The means with standard deviations (vertical line) of Cu concentrations (mg/kg dry wt.) in the edible tissues of *A. chacunda* collected from Karachi fish harbour during Post-monsoon, Pre-monsoon and Monsoon in 2015 and 2016. The same letters above the vertical bars indicate the values are not significantly different (P>0.05).

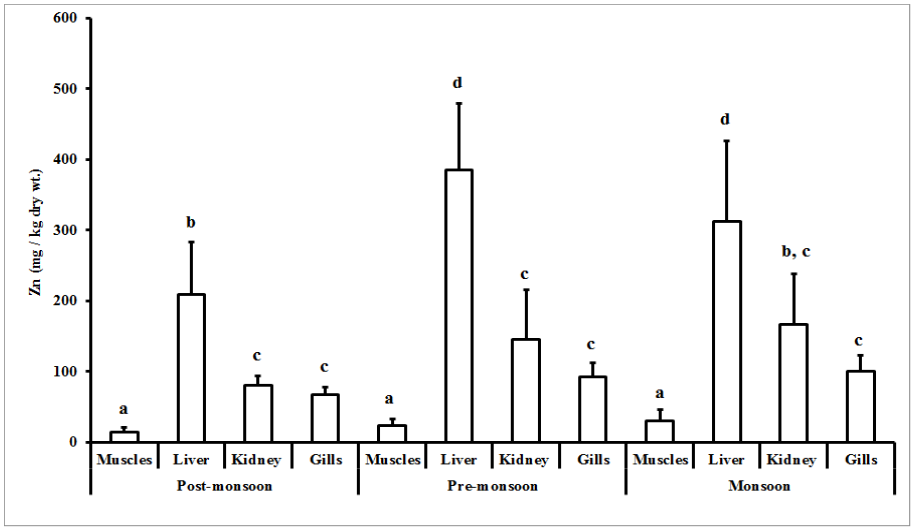

Figure 9. The means with standard deviations (vertical line) of Zn concentrations (mg/kg dry wt.) in the edible tissues of A. chacunda collected from Karachi fish harbour during Post-monsoon, Pre-monsoon and Monsoon in 2015 and 2016. The same letters above the vertical bars indicate the values are not significantly different (P>0.05).

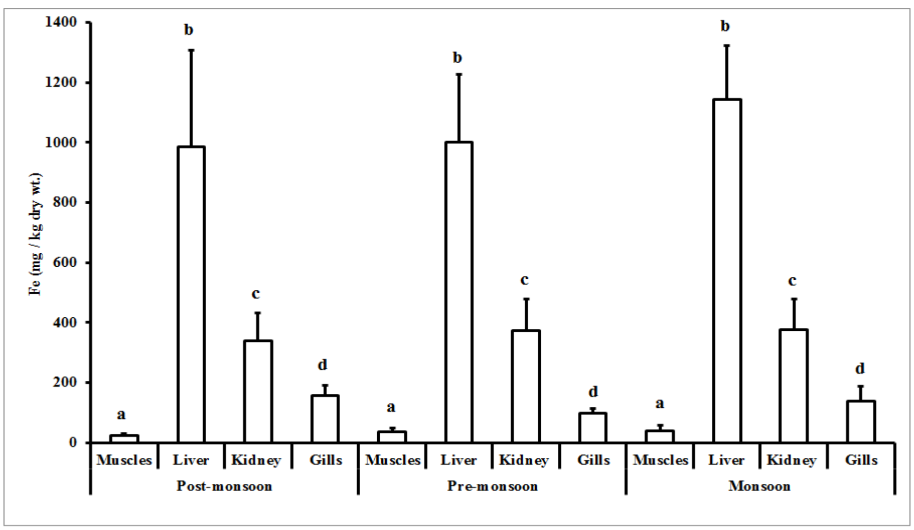

Figure 10. The means with standard deviations (vertical line) of Fe concentrations (mg/kg dry wt.) in the edible tissues of A. chacunda collected from Karachi fish harbour during Post-monsoon, Pre-monsoon and Monsoon in 2015 and 2016. The same letters above the vertical bars indicate the values are not significantly different (P>0.05).

Discussion

The heavy metal accumulations shown in Figures 2-10 are higher in liver, kidneys and gills compared to edible tissues. Higher levels of Mn, Cu, Zn and Fe were determined in liver at all seasons. High Ni levels were found in liver (post- and pre-monsoon) followed by kidney (monsoon). High Cr levels were detected in liver and kidney during post-monsoon, liver and gills during pre-monsoon and all organs except muscles during monsoon. High Co concentrations were found in kidney and gills (post-monsoon), gills (pre-monsoon) and liver (monsoon). Higher levels of Cd and Pb were also determined in gills, liver and kidney. The differences found in some heavy metal levels in different tissues between seasons may be due to heavy rainfall during monsoon which raise the metal contamination of water by washing down the agricultural waste (Kamaruzzaman et al., 2010). Seasonal changes subsistent circumstances like growth cycle and reproductive cycle and from changes in sea water temperature (Kamaruzzaman et al., 2010), this affects metal accumulation in fish.

This is in agreement with many previous studies from Pakistan coasts of the Arabian Sea by Yousuf et al. (2013), Ahmed et al. (2014, 2015a), Ahmed & Bat (2015a, b, c). Yousuf et al. (2013) found that Fe, Zn, Cu and Mn levels were higher in liver than those in muscles of *Trichiurus lepturus* from the coast of Karachi fish harbour between August 2011 and July 2012. The highest Fe concentration was recorded in liver (598±49.6 mg/kg dry wt.) and in muscles (42.6±4.07 mg/kg dry wt.) of *T. lepturus* (Yousuf et al., 2013). Similarly Ahmed et al. (2014) found that the highest mean concentration of Fe, Mn, Zn, Cd, Pb and Cu in *Sardinella albella* from Balochistan coast of Pakistan were 496.43±41.79, 9.42±0.81, 66.22±7.06, 2.15±0.19, 2.42±0.21 and 14.69±2.30 in liver, 45.21±5.03, 3.08±0.86, 16.64±1.39, 2.28±0.25, 2.15±0.19 and 3.25±0.37 in gills and 3.82±0.91, 1.41±0.62, 1.88±0.25, 0.64±0.16, 0.35±0.06 and 1.69±0.14 in muscles mg/kg dry wt., respectively. Again the highest mean concentration (mg/kg dry wt.) of Fe 36.43±11.41, Mn 1.29±1.08, Cu 23.35±11.47, Zn 3.89±2.23, Ni 0.35±0.12, Pb 0.27±0.12, Cd 0.71±0.13 and Cr 0.35±0.20 were recorded in the edible tissues of *Thunnus tonggol* from Karachi Fish Harbour (Ahmed et al. 2015a). Another study in Karachi Fish Harbour, the highest Fe and Mn concentrations were 47±12.5 and 10.4±3.2 mg/kg dry wt. in muscle and maximum concentrations of Fe (660±141 mg/kg dry wt. Mn (47.4±12.3 mg/kg dry wt.) and Ni (2.8±0.8 mg/kg dry wt.) in liver of *Euthynnus affinis* (Ahmed & Bat, 2015a). Ahmed and Bat (2015b) recorded the mean concentrations of Mn, Fe, Co, Cu, Zn and Pb in the muscles and liver of *Pampus chinensis* from Karachi Harbour were 0.95±0.08, 29.32±2.04, 0.13±0.02, 2.28±0.12, 4.00±0.20, 0.36±0.03 and 3.08±0.27, 414.30±18.07, 0.85±0.06, 32.75±1.67, 43.24±2.61 and 0.77±0.07 mg/kg dry wt., respectively. Alike Ahmed & Bat (2015c) determined that the concentrations of Pb and Cd in muscle, liver, gills and kidney tissues of *Alepes djedaba* ranged from 0.02 to 0.94, 0.19 to 3.10, 0.16 to 2.24 and 0.11 to 1.84 and ranged from 0.13 to 1.21, 0.19 to 2.93, 0.62 to 2.91 and 0.18 to 2.51 mg/kg dry wt., respectively (Ahmed & Bat, 2015c).

In the present study Fe levels in liver of *A. chacunda* ranged from 618.4 to 1564 mg/kg dry wt. and higher than those in *T. lepturus* (Yousuf et al., 2013), *Sardinella albella* (Ahmed et al., 2014), *Euthynnus affinis* (Ahmed & Bat, 2015a) and *Pampus chinensis* (Ahmed & Bat, 2015c), but Fe levels in muscles were similar (17.45- 66.98 mg/kg dry wt.) to those studies.

The highest Zn concentration was recorded in liver (75.0±7.61mg/kg dry wt.) and in muscles (16.8±3.22 mg/kg dry wt.) of *T. lepturus* (Yousuf et al., 2013). In the present study Zn levels in liver and muscles of *A. chacunda* ranged from 121 to 541and 8.24-56.23 mg/kg dry wt., respectively. These levels in livers are higher than those in *T. lepturus* (Yousuf et al., 2013), *Sardinella albella* (Ahmed et al., 2014), *Thunnus tonggol* (Ahmed et al. 2015a) and *Pampus chinensis* (Ahmed & Bat, 2015c).

The highest Mn concentration was recorded in liver (54.0±9.47 mg/kg dry wt.) and in muscles (3.31±0.53 mg/kg dry wt.) of *T. lepturus* (Yousuf et al., 2013). In the present study Mn levels in liver and muscles of *A. chacunda* ranged from 12.26 to 42.71 and 0.12-1.96 mg/kg dry wt., respectively. These levels in liver are much lower than those in *T. lepturus* (Yousuf et al., 2013), *Sardinella albella* (Ahmed et al., 2014), *Thunnus tonggol* (Ahmed et al. 2015a), *Euthynnus affinis* (Ahmed & Bat, 2015a), but similar levels in muscle to *Pampus chinensis* (Ahmed & Bat, 2015c).

The highest Cu concentration was recorded in liver (43.2±5.19 mg/kg dry wt.) and in muscles (6.56±0.92 mg/kg dry wt.) of *T. lepturus* (Yousuf et al., 2013). In the present study Cu levels in liver of *A. chacunda* ranged from 22.56 to 84.27 mg/kg dry wt. and higher than those in *T. lepturus*, but Cu levels in muscles were slightly low (0.22-7.96 mg/kg dry wt.).

When compare to non-essential heavy metals in edible tissues, in this study Pb accumulation in muscles of *A. chacunda* (0.1±0.084 mg/kg dry wt.) is much lower than those in *Trachinotus blochii* between 0.39 and 0.8 (Ahmed et al., 2009), *Acanthopagurus berda* between 0.25 and 0.5 (Zehra et al., 2003), *Alepes djedaba* with 0.335±0.213 mg/kg dry wt. (Ahmed & Bat, 2015c), *Euthynnus affinis* with 0.4958±0.13641 mg/kg dry wt. (Ahmed and Bat, 2015a), *Sardinella sindensis* with 0.87 mg/kg dry wt. (Ahmed et al., 2015b) and *Sardinella gibbosa* with 0.39±0.04 mg/kg dry wt. (Ahmed et al., 2015c). Cd values (0.01-0.18 mg/kg dry wt. in *A. chacunda*) are also much lower than those in the reported data from some studies by Ahmed et al. (2009), Zehra et al. (2003), Ahmed et al. (2015b), Ahmed & Benzer (2015), Ahmed & Bat (2015c) and Ahmed et al. (2015c).

Topping (1973) pointed out that mostly plankton feeding fish contain much higher heavy metal levels than those in bottom fish. *A. chacunda* is pelagic inshore fish and occurs Karachi coasts. Chacunda gizzard shad feeds on diatoms, radiolarians, molluscs, copepods and crustaceans (Whitehead, 1985; Carpenter et al., 1997). Bat et al. (2015) discussed that differences in heavy metal levels in pelagic and benthic fish species and concluded this depends of diet and feeding habits of fish species. Moreover accumulation of heavy metal levels in whether pelagic or benthic fish depend on ecological needs and size of individuals (Newman & Doubet, 1989; Zehra et al., 2003, Naeem et al., 2011). Jezierska & Witeska (2001) pointed out that smaller younger fish accumulate heavy metals except Hg than those in larger fish, which may develop out of the higher metabolic rate. Newman & Doubet (1989) found that smaller fish had generally higher equilibrium Hg concentrations than those in larger fish. It is also emphasized that size-dependent uptake rate was the dominant factor influencing size-dependent body burden for group II b metals (Newman & Doubet, 1989). It is by virtue of some heavy metals accumulate at higher whilst others at smaller rate compared with the rate of excretion as fish grows in size (Naeem et al., 2011). In this present study there was no significant difference in the lengths and weights of *A. chacunda* samples as a result of sampling period (Figure 1).

It is proven that muscles are not target tissues for heavy metal accumulation. On the other hand in polluted marine habitats the heavy metal levels in fish muscles may exceed the permissible limits for human consumption and refer to severe health risk (El-Moselhy et al., 2014). Legal thresholds are not available for essential elements in European Commission Regulation (EC). However, the maximum permissible limits for Pb and Cd in fish muscles are 0.30 and 0.05 mg/kg wet wt., respectively. Moreover, the Global Agricultural Information Network (GAIN) Report for China for Pb and Cd in edible fish tissues is 0.5 and 0.1 mg/kg wet wt. (GAIN Report, 2006). Since the results of this study have been given as dry wt., they have converted to wet wt. multiplying by 0.28 (the moisture is about 72% in the muscles of *A. chacunda*) as factor for comparison to the maximum permissible limits as recommended by the Turkish Food Codex and European Commission Regulation (TFC, 2002; EC, 2006). In this study the maximum Pb and Cd levels in edible tissues of *A. chacunda* were 0.067 and 0.054 mg/kg wet wt., respectively. Thus heavy metal levels in the muscles of chacunda gizzard shad from Karachi fish harbour did not exceeded the maximum permissible limits, which was no toxic risk to consumer.

In addition to that, the Joint FAO/WHO Expert Committee on Food Additives (FAO/WHO 2010) established Provisional Tolerable Weekly Intakes (PTWI) for Pb and Cd were 0.025 and 0.007 mg per kg body weight per week, which was equivalent to 1.75 and 0.49 mg/week for an adult, respectively. Permissible Tolerable Daily Intakes (PTDI) for Pb and Cd were 0.25 and 0.07 mg/day/70 kg body weight. Weekly intakes of Pb and Cd per kg of body values estimated as 0.003 and 0.002 mg/week/ kg body wt., respectively which were not above the limit of the PTWI. Estimated Daily Intake (EDI) of Pb and Cd per kg of body were 0.0004 and 0.0003 mg/day/70 kg body wt., respectively. Estimated hazard quotient (HQ) suggested that Cd, Fe, Pb, Cu, Zn, Ni, Co, Cr and Mn in the edible tissues of chacunda gizzard shad from Karachi fish harbour do not pose any apparent threat to consumer, which the total value (0.01327) was smaller than 1 (HQ<1), (Figure 11).

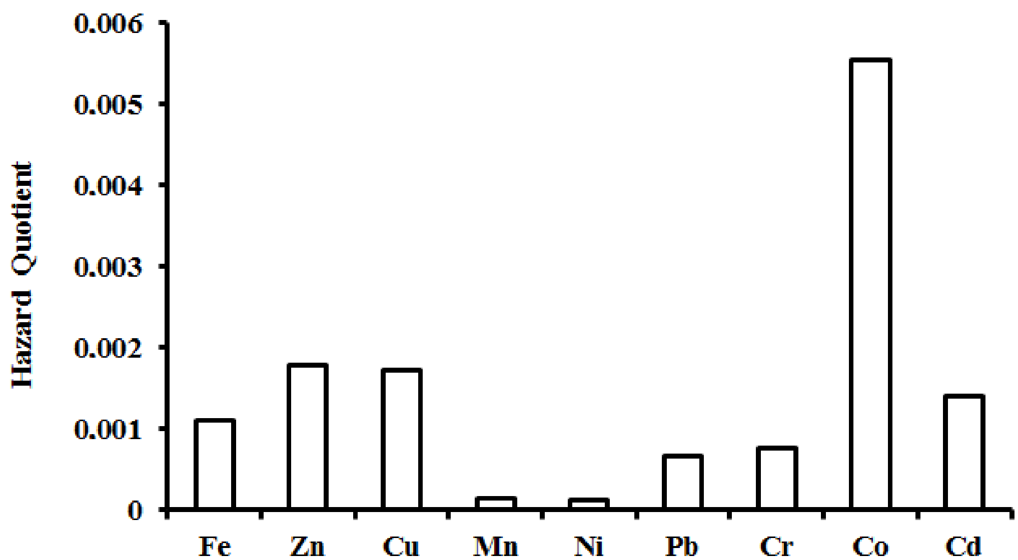

Figure 11. Total hazard quotient of Cd, Fe, Pb, Cu, Zn, Ni, Co, Cr and Mn via consumption of *A. chacunda* from Karachi fish harbour.

Conclusion

In this study, Cd, Fe, Pb, Cu, Zn, Ni, Co, Cr and Mn contents in muscles, liver, kidney and gills of chacunda gizzard shad from Karachi fish harbour have been analysed. The heavy metal accumulation is higher in liver, kidneys and gills of *A. chacunda* compared to edible tissues. According the results, mean concentrations of heavy metals in edible tissues were quite below the maximum recommended values by the EU. Moreover the total THQ were less than one, risk assessments suggested that chacunda gizzard shad in Karachi coasts of Pakistan did not pose any threat to consumer upon their consumption. Such assessment for the heavy metals is required for the human health.

Acknowledgements

The researchers wish to acknowledge the Marine Reference Collection and Resources Centre, University of Karachi for providing laboratory facilities.

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