

Concentration of trace metals and biochemical alterations in various species of fishes along the west coast of India

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Abstract

This study is to evaluate levels of seven trace metals (Cr, Mn, Fe, Cu, Zn, Pb and Hg) and biochemical (proteins, lipids and carbohydrates) alterations in muscle tissue of 27 commercially important fish species collected from six different estuaries along the west coast of India. The study was performed using ICP-OES, GF-AAS, FIMS and UV-Visible spectrophotometer. Estuaries along the central west coast of India (Maharashtra coast) receive wastewater from agricultural, domestic and industrial sources. The obtained results revealed that the abundance of trace metals and biochemical compounds in fish tissue followed the order: Fe(370.0±6.0µg/g) > Zn(92.0±4.2µg/g) > Mn(23.0±1.24µg/g) > Cu(27.9±1.8µg/g) > Cr(1.2±0.18µg/g) > Pb(0.28±0.06µg/g) > Hg(0.09±0.01µg/g) and proteins(43.5±1.6 mg/g) > lipids(23.6±1.6 mg/g) > carbohydrates(3.9±1.8 mg/g). The results indicate that various levels of trace metals exist in the studied fish species, but the concentrations are within permissible limits set by the USFDA, FAO/WHO, and EU for human consumption, except Mn (<1.0 µg/g) and Fe (<100.0 µg/g) in some of the species. Cu (r=-0.39) and Zn (r=-0.85) showed a negative correlation with total carbohydrates (n=120; p<0.05) indicating that accumulation of Cu and Zn can be impact the metabolic characteristics of the fish. Results of Principal Component Analysis (PCA) highlighted the same point source for these metals as influenced by the anthropogenic processes.

Keywords: Bioaccumulation, biochemical compounds, fish, food safety, India, trace metals

1.Introduction

Most of the population along coastline consumes fish, which are rich in protein. Besides protein, it is also a better choice of nutritional requirements such as essential fatty acids, vitamins and minerals (Hixson 2014). In recent years, contamination of the aquatic environment is a serious growing trouble throughout the globe. The industrialization, increased population, combustion of fossil fuels, mining operations and metallurgical discharge are the primary cause of trace metal contamination in the aquatic environment (Sankhla et al. 2016). Trace metals like Chromium (Cr), Manganese (Mn), Iron (Fe), Copper (Cu), and Zinc (Zn) are essential for the metabolic function in organisms, however, their increased concentration may lead to toxicity and can be harmful to survival (Pandey and Madhuri 2014). Heavy metals like Cadmium (Cd), Lead (Pb) and Mercury (Hg) are toxic even at trace levels and metabolic activity plays an important role in the bioaccumulation of trace metals in marine organisms (Asati et al. 2016; Velusamy et al. 2014). Trace metal's concentration beyond permissible limits is considered toxic to human beings and even aquatic biota (Thakur and Mhatre 2015). The metals can bioaccumulate in the organisms from ambient environment and may biomagnified in higher trophic levels through dietary exposure (Barwick and Maher 2003). Knowing changes in the biochemical profile of fish in relation to metal concentration will help to evaluate the sensitivity of the metabolism (Yacoub and Gad 2012).

Substantial number of studies has reported bioaccumulation of trace metals and biochemical compounds in different fish tissues (Mohamed and Gad 2008; Cao et al. 2010; Ebrahim and Taherianfard 2011; Yacoub and Gad 2012; Velusamy et al. 2014; El-Moselhy et al. 2014; Javed and Usmani 2015; Siva et al. 2017). However, scarce published information is available on the effect of trace metals on the biochemical compounds [Total Proteins (PRT), Total Lipids (LPD) and Total Carbohydrates (CHO)] in the fish. Since, seafood is important to study the bioaccumulation of trace metals, as it is the mainly favored food among the local public and tourists in the Maharashtra, India. In the present study, we have highlighted the bioaccumulation of trace metals and their alterations on biochemical compounds in different fish present in selected tropical estuaries. This coastal territory is one of the highly-urbanized and industrialized regions in India and thus most of the estuaries (Fig. 1) along the coast receive a significant amount of domestic as well as industrial effluents and transport into the Eastern Arabian Sea (CSIR-NIO technical report 2009; Rao et al. 2019). To study the accumulation of trace metals Cr, Mn, Fe, Cu, Zn, Pb and Hg, and biochemical compounds (PRT, LPD and CHO) alterations in muscle tissue of 27 commercially important fish species were the major objective of this study.

2. Materials and methods

Study area description

The selected estuaries make their way to the eastern Arabian Sea along the Maharashtra coast of India. Together these river- estuaries invade an area of $27 \times 10^3 \text{ km}^2$ with the net water flow of $53.3 \times 10^9 \text{ m}^3 \text{ yr}^{-1}$ which equals around 10% of total Maharashtra region and 20% of the total annual riverine flow into the Arabian Sea (Rao et al. 2019). When precipitation is taken into consideration the average rainfall rate is around 3322 mm yr^{-1} which is higher as compared to average precipitation in the state of Maharashtra 733 mm yr^{-1} (Sharad et al. 2007). As these estuaries are near to urban civilization and industries anthropogenic activities dominates the waste released in estuaries and their parent riverine system. The amount of Industrial Effluent (IE) and Domestic Waste (DW) per day received by the studied estuaries (Fig.1) varied i.e. Ulhas (IE; 60×10^3 & DW; $350 \times 10^3 \text{ m}^3$), Patalganga (both IE & DW; $15 \times 10^3 \text{ m}^3$), Kundalika (both IE & DW; $1.4 \times 10^3 \text{ m}^3$), Savitri (IE; 6×10^3 & DW; $7.5 \times 10^3 \text{ m}^3$) and Vashishti (IE; 4.5×10^3 & DW; $7.7 \times 10^3 \text{ m}^3$). The concentration of trace metals in the effluent disposal from these areas (Table 1) (CSIR-NIO technical report, 2018). These estuaries are governed by tides and coastal currents mostly semidiurnal with a diurnal inequality. The tidal range along these estuaries in the range of 1m to 4.3m (CSIR-NIO technical report, 2009; Rao et al. 2019).

Sample collection and analysis

27 different fish samples (commercially important) were collected during March-April, 2016 from different sites along Ulhas (S1), Patalganga (S2), Kundalika (S3), Savitri (S4), Vashishti (S5) and Shastri (S6) estuaries, which flow into the Arabian Sea, west coast of India (Fig. 1). Collection of fishes was done with the help of trawl net and the fishes caught were packed in aluminum foils and then preserved in the ice and brought to the laboratory. After identifying species of fish, its length and weight were measured (Table 2). Muscle tissue of fish samples were dissected using Teflon coated accessories, kept in polyethylene bags and frozen at -20°C until the analysis (Velusamy et al. 2014) and minimize the contamination while handling the fish sample (FAO, 1983). All dissection apparatus was pre-cleaned. Fish tissue was placed into glass beaker to dry at 50°C till the constant weight reached. Dried tissue was powdered in agate mortar and pestle. Hg was analyzed in the wet sample. A microwave accelerated digestion system (MARS-5, CEM) was used for digesting samples with addition of concentrated aqua regia HNO_3 : HCl (1:3). Cr, Mn, Fe, Cu, and Zn were analyzed on Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES, Optima 7300 DV, Perkin Elmer), whereas Pb was analyzed by using Graphite Furnace Atomic

Absorption Spectrometry (GF-AAS, Perkin Elmer, Analyst 600), Hg was analyzed with a Flow Injection Mercury System (FIMS-400, Perkin Elmer). Blanks in triplicate were processed and analyzed along with each set of the sample. Certified reference materials (TORT-2 and DOLT-4, NRC, Canada) were processed along with samples to authenticate the results and recovery was 95% to 105 % using various spiked metal concentrations (Table 3). The biochemical compounds were analyzed by using a spectrophotometrically (UV–Visible Spectrophotometer: SHIMADZU). The extraction of PRT, fish samples were digested in Sodium hydroxide (NaOH) by heating in a water bath. The slurry was centrifuged, and an aliquot of the clear supernatant was used for estimation of PRT using Folin phenol reagent (Lowry et al. 1951). PRT concentrations are given as Bovine Serum Albumin (BSA) equivalents. LPD was extracted from fish samples by elution with Chloroform and Methanol (Bligh and Dyer 1959), estimated by using sulphophosphovanillin method (Barnes and Blackstock 1973), and expressed as cholesterol equivalents. CHO was analyzed following the phenol-sulphuric acid method according to Dubois et al. (1956) and expressed as glucose equivalents. The precision of the method was ± 5 %. All the values were represented in an average of the triplicate along with standard deviation.

3. Results and discussion

Trace metals in fish tissue

The concentrations of trace metals in the muscle tissue samples of fishes in the study area are presented in Fig.2 as well as compared with metal concentrations reported in different areas around the globe (Table 5). The concentration of the trace metals analyzed in the present study varied according to the study area, species and feeding habit. However, in most of the species, variation was in order of Fe>Zn>Mn>Cu>Cr>Pb>Hg with Fe being maximum in all cases.

The concentration of Cr in the muscle tissue of fishes in this study varied from not detectable (nd) to 1.23 ± 0.18 $\mu\text{g/g}$ with the mean of 0.57 $\mu\text{g/g}$ (Table 4). The maximum concentration of Cr was recorded in *Pampus argenteus* (1.23 ± 0.18 $\mu\text{g/g}$) from the Ulhas Estuary followed by *Otolithoides biauritus* (1.22 ± 0.33 $\mu\text{g/g}$) from the Shastri Estuary. Cr was below detection limit in *Coilia dussumeiri*, *Pennahia anea*, *Thryssa malabarica* and *Liza parsia* from the Savitri Estuary. Cr is an essential trace element, which is play a vital function in the glucose metabolism (Mertz 1969). The mean concentration of Cr in this study is higher than reported in Masan Bay, Korea 0.18 – 0.25 $\mu\text{g/g}$; Kwon and Lee (1999) and lower than those reported in California waters 0.27 – 3.0 $\mu\text{g/g}$; Moeller et al.(2003). The concentration of Cr in fish tissues in this study was comparable to other Indian studies (i.e. 0.65 – 0.92 $\mu\text{g/g}$ from Raja et al (2009); 0.47 $\mu\text{g/g}$

from Velusamy et al (2014) . The highest permissible Cr concentration rank in fish is 12–13 µg/g dry wt USFDA (1993). Thus, Cr concentration in the fish species analyzed in this study area was under the acceptable level for human consumption.

The Mn concentration in fish samples ranged from 1.01 ± 0.19 – 22.59 ± 1.24 µg/g with the mean of 4.2 µg/g (Table 4). The maximum concentration of Mn was in *Cynoglossus arel* (22.59 ± 1.24 µg/g) from the Shastri Estuary, whereas, it was minimum (1.01 ± 0.19 µg/g) in *Gobiidae* sp from the Ulhas Estuary. Mn is a low toxic metal with a substantial biological significance and seems to accumulate in aquatic organisms Velusamy et al. (2014). The mean concentration of Mn in this study was higher than reported in the Mumbai harbor 3.47 µg/g; Velusamy et al (2014), the Parangipettai, India 0.31-1.2 µg/g; Raja et al (2009) and the Saricay stream, Turkey 0.11 - 24.23 µg/g, Yilmaz et al. (2007). The Mn concentration in this study was observed to be greater than the permissible limit 1.0 µg/g dry wt; FAO/WHO (1989) in all the species of all studied locations.

The Fe concentration was the most abundant trace element in tissue samples in this study. The concentration of Fe in muscle tissue varied from 29.39 ± 1.4 to 370.0 ± 6.0 µg/g with the mean of 100.8 µg/g (Table 4). The maximum concentration of Fe was in *Otolithoides biauritus* (370.0 ± 6.0 µg/g) from the Shastri Estuary while the minimum was in *Cynoglossus arel* (29.39 ± 1.4 µg/g) from the Vashishthi Estuary. Fe is one of the essential element required and deficiency of it may cause anemia (Hegazy et al. 2010). Fish is important sources for Fe in human (Pearson and Dutson 1994). The mean concentration of Fe in this study is almost similar to the Mumbai harbor 97.04 µg/g; Velusamy et al (2014). It was higher than the Parangipettai, India 24.1-50.3 µg/g; Raja et al. (2009) and in the Mediterranean Sea 59.6–73.4 µg/g; Kalay et al (1999). The high Fe concentration reported in *Otolithoides biauritus* from the Shastri Estuary might be because of the feeding behavior and environmental condition. The maximum concentration of Fe has been also reported in the aquatic region due to the increased contribution of organic matter as well as anthropogenic releases from industries and sewage waste Velusamy et al. (2014). However, it does not appear to be same in the present study, as the maximum concentration in *Otolithoides biauritus* is from the Shastri Estuary, which does not receive waste that can enhance the Fe concentration. However, the sediment of the region contains naturally occurring Fe in high concentration (CSIR-NIO technical report, 2009). The observed mean Fe concentration was higher than the acceptable limit 100 µg/g dry wt; FAO/WHO (1989) in the fish species *Otolithoides biauritus*, *Cynoglossus arel*, and *Megalaspis cordyla* from Shastri Estuary; *Loligo* from Vashishthi Estuary; *Johnius aneus* from Savitri Estuary and *Coilia dussumieri* from Patalganga, Savitri and Vashishthi Estuary (Table 4). Concentration of Fe in all other species was below the permissible limit.

The Cu concentration in all of the fish tissue samples varied between 0.34 ± 0.08 and 27.94 ± 1.8 $\mu\text{g/g}$ and mean value of 3.4 $\mu\text{g/g}$ for fishes along the study area (Table 4). The maximum concentration was recorded in *Gobiidae sp* (27.94 ± 1.8 $\mu\text{g/g}$) from the Ulhas Estuary and minimum in *Liza parsia* (0.34 ± 0.08 $\mu\text{g/g}$) in the Savitri Estuary. Cu is one of the essential element in fish and controlled in the muscle tissue with high molecular weight proteins (Kumar et al. 2011). It is well known that Cu plays a very important role in enzymatic processes and is crucial for the synthesis of hemoglobin in fish. Though, excessive consumption will cause severe health issues to the aquatic ecosystem and indirectly to the human being (Demirezen and Uruc 2006). The Ulhas Estuary receives effluent from industrial units and the sediment of it contains high concentration Cu (CSIR-NIO technical report 2009). Hence the observed values of Cu in present study were higher than found in the fishes in the Mumbai harbor (2.46 $\mu\text{g/g}$; Velusamy et al. (2014). It was lower than in the range (3.40 – 5.88 $\mu\text{g/g}$) of Mediterranean Sea Kalay et al. (1999). The mean Cu concentrations reported in fish species in this study were within the permissible limit (30 $\mu\text{g/g}$ dry wt; FAO/WHO (1989).

The Zn concentration in fish tissue samples from this study varied from 10.12 ± 0.58 – 91.95 ± 4.2 $\mu\text{g/g}$ and mean value was 39.4 $\mu\text{g/g}$ (Table 4). The accumulation of Zn varied significantly from species to species ($p < 0.05$). The maximum concentration of Zn was in *Arius maculatus* (91.95 ± 4.2 $\mu\text{g/g}$) from the Patalganga Estuary and lowest in *Liza parsia* (10.12 ± 0.58 $\mu\text{g/g}$) from the Savitri Estuary. Zn is also an essential element in food and also the second abundant metal followed by Fe (Kumar et al. 2011), though, high level of Zn in fish affects the survival, improvement and growth of fish. According to Chi et al. (2007) reported that the too much of Zn and Cu eating is damaging to human health as well as be able to cause fever, diarrhea, acute stomach pains, sickness and poisoning. The Patalganga Estuary receives wastes from chemical industries and sediment of it has a high concentration of Zn in the vicinity of effluent discharge (CSIR-NIO technical report 2011). The observed values of Zn in this study were higher than found in the Mediterranean Sea (14.1 – 33.5 $\mu\text{g/g}$; Kalay et al. (Kalay et al. 1999) and the Mumbai harbor (38.54 $\mu\text{g/g}$; Velusamy et al. (2014) in India. However, the average value of Zn in the study area was lower compared to the Black Sea (44.2 $\mu\text{g/g}$; Topcuoglu et al. (Topcuoğlu et al. 2002). The FAO/WHO allowable rank of Zn for human eating is 100 $\mu\text{g/g}$ dry wt. Thus the average concentration of Zn in the fish species in this study was found to be lower than the acceptable limit FAO/WHO (1989).

The concentration of Pb in the muscle tissue observed in this study varied from non-traceable limit to 0.28 ± 0.06 $\mu\text{g/g}$ with the mean of 0.15 $\mu\text{g/g}$ (Table 4). The maximum Pb value was detected in *Ilisha megaloptera* (0.28 ± 0.06 $\mu\text{g/g}$) and the lowest concentrations (0.1 ± 0.02 $\mu\text{g/g}$) were

in *Johnius belangerii* and *Stingray* from the Patalganga Estuary. Pb is a toxic element and causes carcinogenic effects in aquatic environment as well as humans (Velusamy et al. (2014). Pb concentrations were below the detection level in studied fish species except for the Ulhas and Patalganga Estuaries. However, the Pb concentrations in fish species in this study were found to be lower than the permissible limit recommended by the European Union (0.5-1.0 $\mu\text{g/g}$; EU, 2001; 2008).

In this study, the concentration of Hg in muscle tissue of fishes varied from non-traceable to 0.09 ± 0.01 $\mu\text{g/g}$ with the mean of 0.03 $\mu\text{g/g}$ (Table 4). The highest concentration was observed in *Pampus argenteus* (0.09 ± 0.01 $\mu\text{g/g}$) from the Ulhas Estuary, and lowest concentration (0.01 $\mu\text{g/g}$) was in *Liza parsia* from the Ulhas and Savitri estuaries. Its concentration in *Llisha megaloptera* from the Patalganga, *Loligo* from the Vashishthi and *Epinephelus sp*, *Harpadon nehereus* from the Shastri Estuary were lowest. Our result of Hg is in agreement with an earlier report on fish muscle from the Mumbai Harbor (0.06 $\mu\text{g/g}$; Velusamy et al. (2014). The level of Hg in fish during present study was low compared to other studies (Bordajandi et al. 2004; Burger et al. 2005; Cirillo et al. 2010). Although in low amounts, Hg is recognized as a highly toxic metal to biota, due to its affinity towards lipid, allowing movement across the cell membrane. Such movements of Hg may interfere with cell metabolism (Boening, 2000; Pinho et al. 2002). The Bioavailable Hg can be quickly accumulated in the aquatic organism, and biomagnified then after along the trophic chain (Pouilly et al. 2013). Once accumulated in the body, Hg reacts with methyl cobalamine and forms methyl mercury by replacing Cobalt (Co) (Hutchison and Atwood 2003). According to the Food Drug and Administration (FDA, 2003) set an acceptable range for fish species from $0.004 - 0.16$ $\mu\text{g/g}$. Whereas, the European Union (EU)'s permissible limit for Hg concentration in fish is 0.5 $\mu\text{g/g}$ wet wt EU (2008). In this study, Hg concentrations in fish species were reported to be lesser than the permissible limit recommended by the EU (2008). The Hg concentrations in the fish vary depending on feeding behavior, habitat, and seasonal changes Velusamy et al. (2014). The concentration of trace metals in the studied fish species might be because of the factors like pollution exposure, diet and behavior Henry et al. (2004). Dissimilar concentration of trace metals in different fish species might be a result of different metabolism, feeding patterns, ecological needs, and the contamination gradients of water, food and sediment, as well as ecological factors e.g size, age of the fish, salinity and temperature (Allen-Gil and Martynov 1995; Jezierska and Witeska 2006). In addition, species in the upper trophic levels in very impacted sites can accumulate higher metal, with the local playing a more critical function than the trophic web fish position (Terra et al. 2008). Overall, in this study, concentration of Mn in all the fish species and Fe in selected fish species of a particular estuary were

higher than the permissible levels, while concentration of other metals (Cr, Cu, Zn, Pb, and Hg) recorded in the fish muscle tissue from this study was lower than the acceptable ranges suggested by the USFDA (1993), FAO/WHO (1989) and EU (2001; 2008).

Generally metals can bioaccumulate through exposure to ambient environment and diet. The metal bioaccumulation factor (BAF) of 5000 times than ambient concentrations are designated as more bioaccumulative. The background concentrations of trace metals in the studied sampling sites are ranged between 0.1 µg/l to 5 mg/l (Table. 1) but their concentrations in the tissue samples were in 0.1 µg/g to 370 µg/g. This difference infers that they were accumulated into tissues from ambient exposure. The level of accumulation is differed among the sampling sites due to spatial difference in metal concentration. The variation of metal concentrations among the species is explained by difference in the uptake-assimilation-elimination efficiency and metabolic requirements (Jim et al. 2004).

Biochemical parameters in the fish tissue

The concentration of PRT in the muscle tissue of fishes in this study (Fig. 3) varied from 22.3 ± 1.6 – 43.5 ± 1.6 mg/g with the mean value 34.0 mg/g (Table 4). The maximum concentration of PRT was in *Coilia dussumeiri* (43.5 ± 1.6 mg/g) from the Vashishthi Estuary while the minimum was in *Liza Parsia* (22.3 ± 1.6 mg/g) from the Ulhas Estuary. One of the chief nitrogenous components of the body's tissue is PRT (Siva et al. 2017). Hence, acts as an important bioindicator for determining the physiological condition of the fish (Begum and Vijayaraghavan 1996). The fluctuations in the concentration of PRT in the studied muscle tissue of fishes were due to the release of discharged effluents from different sources, including hydrocarbons found in domestic wastes and trace metals in the industrial and agricultural drainage bring the change in the aquatic environment and ultimately results in reduction of protein content (Zaghloul 2000). The reduction in protein concentration might be due to the blocking of protein synthesis pathways or denaturation of proteins or negative affect on amino acid synthesis by metals (Jha 1991). The variation in PRT concentrations in the studied fish species was due to their seasonal variation, habitat preference, distribution and feeding habits. Ahmed et al. (1997) reported that the alterations in PRT and their metabolites in aquatic organisms are due to exposed to toxicants. Hence, the lowest concentration of PRT recorded in the *Liza Parsia* (22.3 ± 1.6 mg/g) from the Ulhas Estuary than the Savitri Estuary (*Liza parsia* = 37.5 ± 3.1 mg/g) may be due to the above mentioned reasons. The concentration of LPD in muscle tissue of fishes recorded during this study (Fig.3) varied from 7.3 ± 1.4 – 23.6 ± 1.6 mg/g with the mean of 14.1 mg/g (Table 4). The maximum concentration of LPD was in *Epinephelus diacanthus* (23.6 ± 1.6 mg /g) from the

Vashishthi Estuary while the minimum was in *Liza parsia* (7.3 ± 1.4 mg/g) from the Ulhas Estuary. Similar to glycogen, lipids also promotes storage of energy. They are organic compounds having negligible solubility in water but get easily dissolved in organic solvents as they have intense calorific value and acts as essential fatty acids (Siva et al. 2017). Sancho et al. (1998) reported that the fish uses energy-rich lipids for energy production during toxic stress, which reduces the concentration of LPD in the muscle of fish exposed to different pollutants and show decreased lipid content (Chandra et al. 2004; Blaner et al. 2005). The concentration of CHO in muscle tissue from this study (Fig.3) varied from 1.1 ± 0.2 – 3.9 ± 1.8 mg/g with the mean of 2.2 mg/g (Table 4). The maximum concentration of CHO was in *Liza parsia* (3.9 ± 1.8 mg/g) from the Savitri Estuary while the minimum was in *Panulirus polyphagus* (1.1 ± 0.2 mg/g) from the Patalganga Estuary. CHO consists of an important part of protoplasm as it participates in storage and release of energy. Some of the crucial carbohydrates persisting in the animal cells are glucose, fructose, mannose, sucrose, galactose, maltose, lactose, trihalose, all of these promote glycogen cellular functions thereby acting as energy generator (Siva et al. 2017). Glycogen in many marine animals does not add much to the reserves in the body might result in the lower level of CHO recorded in this study. Ramaiyan et al. (1976) reported similar findings in 11 species of Clupeids. The study thus supports the view that CHO plays an irrelevant role as an energy reserve in aquatic animals (Love, 1970), since it is in very low concentration in the muscle as well as that its role in the mobilization of energy during maturation and spawning (Shamsan and Ansari 2010). It is further supported by Vijaykumaran (1979) who stated that carbohydrates play a minor role in energy reserves of *Ambassis gymnocephalus* as well as its depletion during the spawning season is insignificant.

Relationship between trace metals and biochemical parameters

Overall, the biochemical concentrations analyzed in the fish species in this study decreased in the order of PRT>LPD>CHO. Such changes have also been attributed to various physiological and other factors like maturation, spawning, feeding etc (Shamsan and Ansari 2010; Siva et al. 2017). Also, Saha and Guha (1940) reported that the chemical composition is dependent on age, sex, habitat and seasons. The statistical analysis was carried out using Statistica 7.0 software. The correlation between species is performed and these results were observed, most of the fish species are mostly similar habitat and food habit (carnivores), moreover the species *Coilia dussumieri*, *Liza parsia* and *Johnius aneus* are found in more than two sites from polluted and unpolluted places hence it is worth to compare them for metal concentrations between sites. Furthermore, the correlation between absorption of trace metals, biochemical parameters, standard length, and total weight of fish species was observed. The metal concentrations were significantly varied in different fish species

however not correlated with fish length and weight ($p > 0.05$). A significant positive correlation was observed in tissue samples for the trace metal pairs of Cr and Mn ($p < 0.05$), Cr and Fe ($p < 0.05$) and Mn and Fe ($p < 0.05$) (Fig. 4). However, a significant variation between Zn and Pb ($p < 0.05$) were found in all analyses. These correlations revealed that the source of origin of the metal was anthropogenic. Hg showed no significant correlation with other studied metals, a similar result was reported by Velusamy et al. (2014) from Mumbai coast, India. Moreover, there was a negative correlation between Cu, Zn and CHO ($p < 0.05$) (Fig. 4). Similarly, James et al. (1991) reported toxic effects of Cu and Zn in *Oreochromis mossambicus*, and significant decrease in PRT, LPD and CHO were observed in muscle tissue and the whole body of *O. mossambicus* exposed to metals individually. While our results indicated reduced CHO in the fish tissue and there was no correlation between trace metals, PRT and LPD, but positive correlation between PRT and LPD ($p < 0.05$) (Fig. 4). It is concluded that the enhanced concentration of metals can impact the metabolic characteristics of the fish.

Additionally, the result of PCA analysis by using Primer 6 (Fig.5), two dominant factors, accounting for 40.3% of cumulative percentage, were identified as controlling factors for heavy metals with biochemical compounds. Factor 1 accounted for 21.0 % of total variance and indicated significant positive loading was observed for Zn and Cu and negative loadings were found for CHO, indicating that accumulation of Cu and Zn can be impact the metabolic characteristics of the fish. . Factor 2 accounted for 19.3 % of total variance. A positive loading was observed for Cr, Fe and Mn (Fig.5). Outcome of the PCA indicates same point source for these metals as influenced by the anthropogenic processes.

4. Conclusions

The concentrations of the trace metals and biomolecules analyzed in this study varied according to the study area, species and feeding habit. However, in most of the species variation was $Fe > Zn > Mn > Cu > Cr > Pb > Hg$ and $PRT > LPD > CHO$. The high concentration of trace metals in the studied fish species might be because of the factors like pollution exposure and diet of the fish. Moreover, different concentration of trace metals and biochemical alterations in different fish species might be a result of different ecological condition, metabolism, feeding patterns, the contamination gradients of water as well as factors such as size, age of the fish. Trace metal concentrations in the fish samples analyzed in this study are within the permissible limits (USFDA, FAO/WHO and EU). Therefore, the fish in this study area was found to be usually considered safe for human consumption and no risk to the health of human consumers except Mn in all the fish species and Fe in selected fish

species of a particular estuary. Based on result of PCA indicates same point source for these metals as influenced by the anthropogenic processes. Further, the negative correlation ($p < 0.05$) of Cu, Zn with CHO of the fish species indicated that accumulation of Cu and Zn affected the CHO in muscle tissue of the studied fish species or enhanced concentration of metals can impact the metabolic characteristics of the fish. The results of this study might be useful to find out the impacts and mode of action of trace metals on aquatic organisms and long-term monitoring in future to curtail the pollution load and restore ecological conditions. Moreover, continuous reclamation activities, especially for industrial effluent and domestic sewage, which poses even greater risks to public health and ecological degradation, has yet to gain policymakers' attention.

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References

- Ahmed R, Elumalai M, Ezhilarasi MP, Balasubramanian (1997) Individual and combined effect of copper and chromium on oxygen consumption and phosphatases of a marine edible crab, *Scylla serrata*. *Biomedical Letters* **55**: 147-179
- Allen-Gil SM, Martynov VG (1995) Heavy metal burden in nine species of freshwater and anadromous fish from the Pechora River, Northern Russia. *Sci Total Environ* **160**: 653-659
- Asati A, Pichhode M, Nikhil K (2016) Effect of heavy metals on plants: an overview. *Int J Innov Res Sci Eng Technol* **5**: 56-61
- Barnes H, Blackstock J (1973) Estimation of lipids in marine animal's tissues: Detailed investigation of the sulphophosphovanillin method for total lipids. *J Exp Mar Biol Ecol* **12**: 103-118
- Barwick M, Maher W (2003) Biotransference and biomagnification of selenium copper, cadmium, zinc, arsenic and lead in a temperate sea grass ecosystem from Lake Macquarie Estuary, NSW, Australia. *Mar Environ Res* **56**: 471-502
- Begum G, Vijayaraghavan S (1996) Alterations in protein metabolisms of muscle tissues in the fish *Clarias batrachus* by commercial grade Dimethioate. *Bull Environ Contam Toxicol* **57**: 223-228

- Blaner C, Curitis M, Chan H (2005) Growth, nutritional composition and hematology of Arctic char (*Salvelinus alpinus*) exposed to toxaphene and tapeworm (*Diphyllobothrium dendritium*). Arch Environ Contam Toxicol **48**: 397-404
- Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem **37**:911–917
- Boening DW (2000) Ecological effects, transport, and fate of mercury:a general Review. Chemosphere **40**: 1335-135
- Bordajandi L, Gomez G, Abad E, Rivera J, Fernandez-Baston M, Blasco J, Gonzalez MJ (2004) Survey of persistent organo-chlorine contaminants (PCBs, PCDD/Fs, and PAHs), and heavy metals (Cu, Cd, Zn, Pb and Hg), and arsenic in food samples from Huelva (Spain): levels and health implications. J Agr Food Chem **52**: 992–1001
- Burger J, Stern AH, Gochfeld M (2005) Mercury in commercial fish: optimizing individual choices to reduce risk. Environ Health Perspect **113**:1–6
- Cao L, Huang W, Liu J, Yin X, Dou S (2010) Accumulation and oxidative stress biomarkers in Japanese flounder larvae and juveniles under chronic cadmium exposure. Comp Biochem Physiol Toxicol Pharmacol **151**: 386-392
- Chandra S, Ram R, Singh I (2004) First ovarian maturity and recovery response in common carp *Cyprinus carpio* after exposure to carbofuran. J Environ Biol **25**: 239-249
- Chi QQ, Zhu GW, Alan L (2007) Bioaccumulation of heavy metals in fishes from Taihu Lake, China. J Environ Sci **19**: 1500–1504
- Cirillo T, Fasano E, Viscardi V, Arnese A, Cocchieri RA (2010) Survey of lead, cadmium, mercury and arsenic in seafood purchased in Campania, Italy. Food Addit Contam **3**: 30–38
- CSIR-NIO technical report (2009) Monitoring of Coastal marine and estuarine ecology of Maharashtra-Phase I. Part A (Main report)
- CSIR-NIO technical report (2011) Release of treated effluent from M/s. Lenzing Modi in Patalganga Estuary.
- CSIR-NIO technical report (2018) Assessment of impact of release of effluents on ecology of inshore and coastal areas of Maharashtra and their management.
- Demirezen D, Uruc K (2006) Comparative study of trace elements in certain fish, meat and meat products. Meat Sci **74**: 255–260
- Dubois M, Gilles KA, Hamilton JK, Reebers PA, Smith F (1956) Colorimetric method for the determination of sugars related compounds. Anal Chem **28**: 350-356
- Ebrahim M, Taherianfard M (2011) The effects of heavy metals on reproductive system of cyprinid fish from Kor River. Iran. J Fish Sci **10**: 13-24
- El-Moselhy KM, Othman AI, El-Azem HA, El-Metwally MEA (2014) Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. Egyptian. J Basic Appl Sci **1**: 97-105

- European Union, EU (2001) Commission Regulation as regards heavy metals. Directive 2001/22/EC, No: 466/2001
- European Union, EU (2008) Commission Regulation (EC) No. 629/2008. Setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, L, 173
- FAO (1983) Compilation of Legal Limits for Hazardous Substance in Fish and Fishery Products.
- FAO/WHO (1989) Evaluation of certain food additives and the contaminants mercury, lead and cadmium. WHO Technical Report, Series No. 505
- FDA (2003) FDA Consumer Advisory. Food and Drug Administration, Washington, DC. Available from: <<http://www.fda.gov/bbs/topics/ANSWERS/2000/advisory.html>>
- Hegazy AA, Zaher MM, Abd el-hafez MA, Morsy AA, Saleh RA (2010) Relation between anemia and blood levels of lead, copper, zinc and iron among children. *BMC research notes* **3(1)**: 133
- Henry F, Amara R, Courcot L, Lacouture D, Bertho ML (2004) Heavy metals in four fish species from the French coast of the Eastern English Channel and Southern Bight of the North Sea. *Environ Int* **30**: 675-683
- Hixson SM (2014) Fish Nutrition and Current Issues in Aquaculture: The Balance in Providing Safe and Nutritious Seafood, in an Environmentally Sustainable Manner. *J Aquac Res Dev* **5**: 234
- Hutchison AR, Atwood DA (2003) Mercury pollution and remediation: the chemist's response to a global crisis. *J Chem Crystallogr* **33(8)**: 631-645.
- James RV, Sampath K, Siva KV, Manthiramoorthy S (1991) Individual and combined effects of heavy metals on survival and biochemistry of *Oreochromis mossambicus*. *Indian J Fish* **38(1)**: 49-54
- Javed M, Usmani N (2015) Stress response of biomolecules (carbohydrate, protein and lipid profiles) in fish *Channa punctatus* inhabiting river polluted by Thermal Power Plant effluent. *Saudi J Biol Sci* **22(2)**: 237-242.
- Jeziarska B, Witeska M (2006) The metal uptake and accumulation in fish living in polluted waters. In *Soil and water pollution monitoring, protection and remediation* (pp.107-114). Springer, Dordrecht.
- Jha BS (1991) Alterations in the protein and lipid contents of intestine, Liver and gonads in the lead exposed fresh water murrel *Channa punctatus* (Bloch). *J Ecobiol* **12**: 9-34
- Jim M, Gerry H, Roman L, Nicholas F, Keith S, John D (2004) Issue paper on the bioavailability and bioaccumulation of metals. U.S. Environmental Protection Agency Risk Assessment Forum 1200 Pennsylvania Avenue, NW Washington, DC 20460
- Kalay M, Ay O, Canli M (1999) Heavy metal concentrations in fish tissues from the Northeast Mediterranean Sea. *Bull Environ Contam Toxicol* **63**: 673-681

- Kaya G, Turkoglu S (2017) Bioaccumulation of heavy metals in various tissues of some fish species and green tiger shrimp (*Penaeus semisulcatus*) from İskenderun Bay, Turkey, and risk assessment for human health. *Biol. Trace Elem Res* **180(2)**: 314-326
- Kojadinovic J, Potier M, Le Corre M, Cosson RP, Bustamante P (2007) Bioaccumulation of trace elements in pelagic fish from the Western Indian Ocean. *Environ Pollut* **146(2)**: 548-566
- Kumar B, Shah R, Mukherjee D (2011) Geochemical distribution of heavy metals in sediments from sewage fed fish ponds from Kolkata Wetlands, India. *Chem Spec Bioavailab* **23**: 24-32
- Kwon YT, Lee CW (1999) Application of multiple ecological risk indices for the evaluation of heavy metal contamination in a coastal dredging area. *Sci Total Environ* **214**: 203–210
- Love RM (1970) *The chemical biology of fishes*. London Academic Press 547
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin Phenol reagent. *J Biol Chem* **193**: 265-275
- Mertz W (1969) Chromium occurrence and function in biological system. *Physiol Rev* **49**: 163–239
- Moeller A, Mac Neil SD, Ambrose RF, Hee SSQ (2003) Elements in fish of Malibu Creek and Malibu Lagoon near Los Angeles, California. *Mar Pollut Bull* **46**: 424–429
- Mohamed FA, Gad NS (2008) Environmental pollution induced biochemical changes in tissues of *Tilapia zillii*, *Solea vulgaris* and *Mugilcapito* from Lake Qarun, Egypt. *Glob Vet* **2(6)**: 327-336
- Pandey G, Madhuri S (2014) Heavy Metals Causing Toxicity in Animals and Fishes. *Int j animal vet Fishery Sci* **2(2)**: 17-23
- Pearson AM, Dutson R (1994) *Quality Attributes and their Measurement in Meat, Poultry and Fish Products*. Oregon State of University.
- Pinho AP, Guimaraes JRD, Martins AS, Costa PAS, Olavo G, Valentin J (2002) Total Mercury in Muscle Tissue of Five Shark Species from Brazilian Offshore Waters: Effects of Feeding Habit, Sex, and Length. *Environ Res* **89**: 250-258
- Plotitsyna NF, Kireeva LI (1996) Contaminants in marine organisms from the Barents Sea. *Zagryaznyayuschiye veschestva v morskikh organizmah Barentseva morya*, Materials on PINRO Researches in 1995, PINRO, Murmansk, pp. 168–191 (in Russian)
- Pouilly M, Rejas D, Pérez T, Duprey JL, Molina CI, Hubas C, Guimaraes JRD (2013) Trophic structure and mercury biomagnification in tropical fish assemblages, Iténez River, Bolivia. *PLoS one* **8(5)**: 65054
- Prasad NV, Ch V (2017) Seasonal variations in the biochemical composition of muscle and liver of marine fishes, *Gazza achlamys* and *Ariomma indica* from Visakhapatnam coast, South India. *Int J Bioassays* **6(6)**: 5407-5414

- Raja P, Veerasingam S, Suresh G, Marichamy G, Venkatachalapathy R (2009) Heavy metals concentration in four commercially valuable marine edible fish species from Parangipettai Coast, South East Coast of India. *Int J Anim Vet Adv* **1(1)**: 10-14
- Ramaiyan V, Paul AL, Pandian TJ (1976) Biochemical studies on the fishes of the order clupeiformes. *J Mar Biol Ass India* **18(3)**: 516-524
- Rao NM, Ram A, Pradhan UK, Siddaiah V (2019) Factors controlling organic matter composition and trophic state in seven tropical estuaries along the west coast of India. *Environ Geochem Hlth* **41(2)**:545-562
- Saha KF, Guha BC (1940) Nutritional investigation of Bengal fish. *Indian J Med Res* **27**: 873- 876
- Sancho E, Ferrando MD, Fernandez C, Andreu E (1998) Liver energy metabolism of *Anguilla anguilla* after exposure to fenitrothion. *Ecotox Environ Safe* **41**: 168-175
- Sankhla MS, Kumari M, Nandan M, Kumar R, Agrawal P (2016) Heavy metals contamination in water and their hazardous effect on human health—a review. *Int J Curr Microbiol Appl Sci* **5(10)**: 759-766
- Shamsan EF, Ansari ZA (2010) Biochemical composition and caloric content in the sand whiting *Sillago sihama* (Forsskal), from Zuari Estuary, Goa. *Indian J Fish* **57 (1)**: 61-64
- Sharad KJ, Pushpendra K, Agarwal VPS (2007) *Hydrology and Water Resources of India*, Springer, 743
- Stange K, Maage A, Klungsoyr J (1995) Contamination in fish and sediment in the North Atlantic Ocean. *TemaNord* 1996:522. Nordic Council of Ministers, Copenhagen
- Terra BF, Araújo FG, Calza CF, Lopes RT, Teixeira TP (2008) Heavy metal in tissues of three fish species from different trophic levels in a tropical Brazilian river. *Water Air Soil Poll* **187**: 275-284
- Thakur J, Mhatre M (2015) Potential Health Risk due to the Bioaccumulation of Heavy Metals in the Fish found in Dharamtar Creek, India.
- Topcuoğlu S, Kırbaçoğlu Ç, Güngör N (2002) Heavy metals in organisms and sediments from Turkish Coast of the Black Sea, 1997–1998. *Environ Int* **27**: 521-526
- Tuzen M (2002) Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry. *Food Chem* **27**: 521–526
- USFDA (1993) Food and Drug Administration. Guidance Document for Chromium in Shellfish
- DHHS/PHS/FDA/CFSAN/Office of Seafood, *Washington, DC*
- Velusamy A, Kumar PS, Ram A, Chinnadurai S (2014) Bioaccumulation of heavy metals in commercially important marine fishes from Mumbai Harbor, India. *Mar Pollut Bull* **81(1)**: 218-224
- Vijayakumaran M (1979) Chemical composition and caloric content of *Ambassis gymnocephalus*. *J Mar Biol Ass India* **21**: 182-184

Yacoub AM, Gad NS (2012) Accumulation of some heavy metals and biochemical alterations in muscles of *Oreochromis niloticus* from the River Nile in Upper Egypt. *Int J Environ Sci Technol* **3**: 1-10

Yilmaz AB (2003) Levels of heavy metals (Fe, Cu, Ni, Cr, Pb, and Zn) in tissue of *Mugilcephalus* and *Trachurus mediterraneus* from Iskenderun Bay, Turkey. *Environ Res* **92**: 277-281

Yılmaz F, Ozdemir N, Demirak A, Tuna AL (2007) Heavy metal levels in two fish species *Leuciscus cephalus* and *Lepomis gibbosus*. *Food Chem* **100**: 830–835

Zaghloul, HK (2000) Effect of different water sources on some biological and biochemical aspect of the Nile tilapia *Oreochromis niloticus* and Nile cat fish *Clarias gariepinus*. *Egyptian J Zool* **34**: 353-377

Legend to Figure:

Fig. 1. Map showing study area and sampling locations.

Fig. 2. The concentration of trace metals in the muscle tissue samples of fishes in the study area.

Fig. 3. The concentration of PRT, LPD and CHO in the muscle tissue samples of fishes in the study area.

Fig. 4. Correlation between metals and biochemical compounds in the muscle tissue samples of fishes in the study area.

Fig. 5. Principal component analysis (PCA) representing loading pattern of total length, weight of the fish, various trace metals and biochemical compounds in the muscle tissue samples of fishes in the study area. Zn and Cu contributed to significant positive loading while CHO contributed negative loading in Factor 1 (21% variance). Additionally, positive loading was observed for Cr, Fe and Mn contributed positive loadings in Factor 2 (19.2% variance).

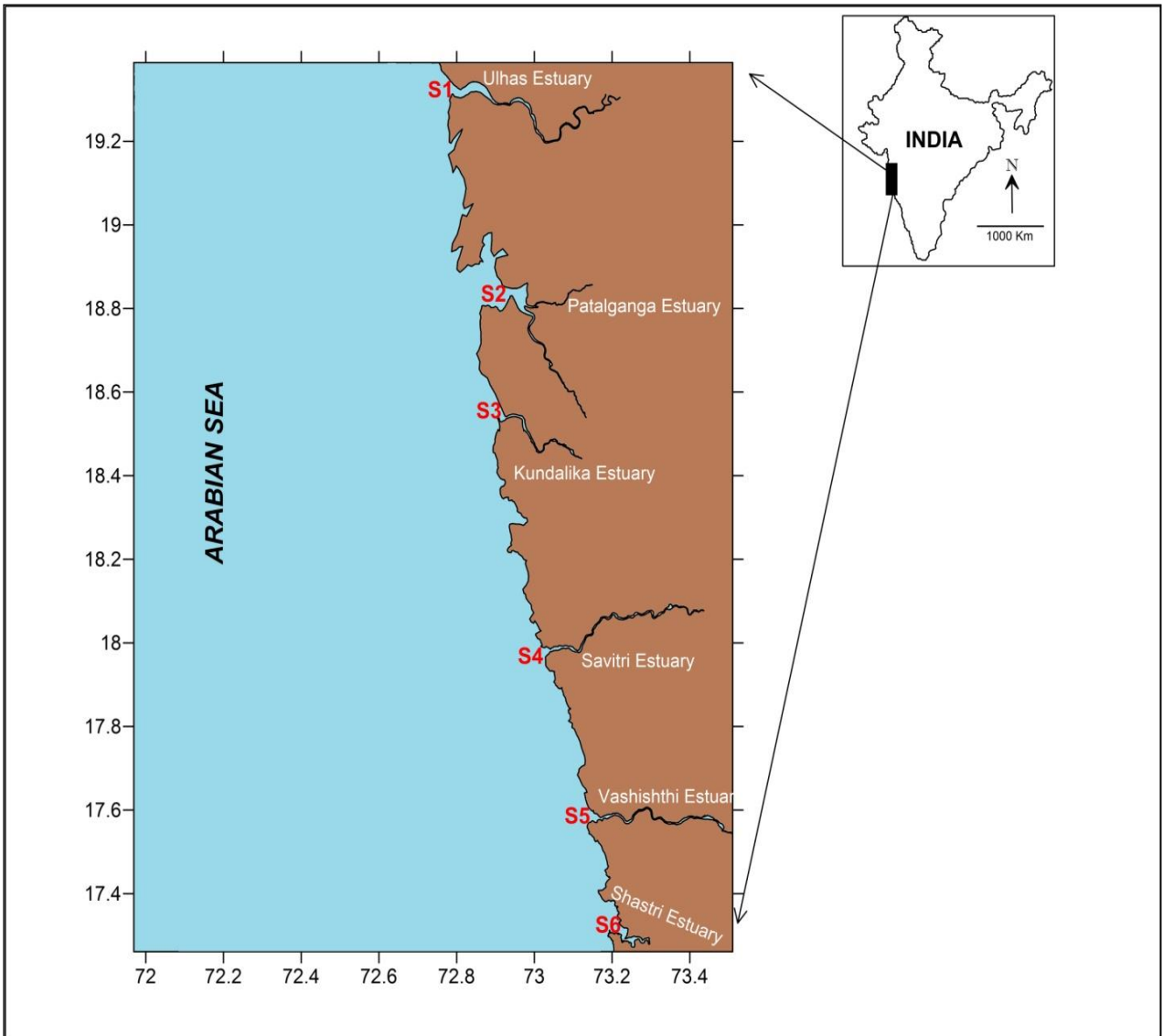


Fig.1

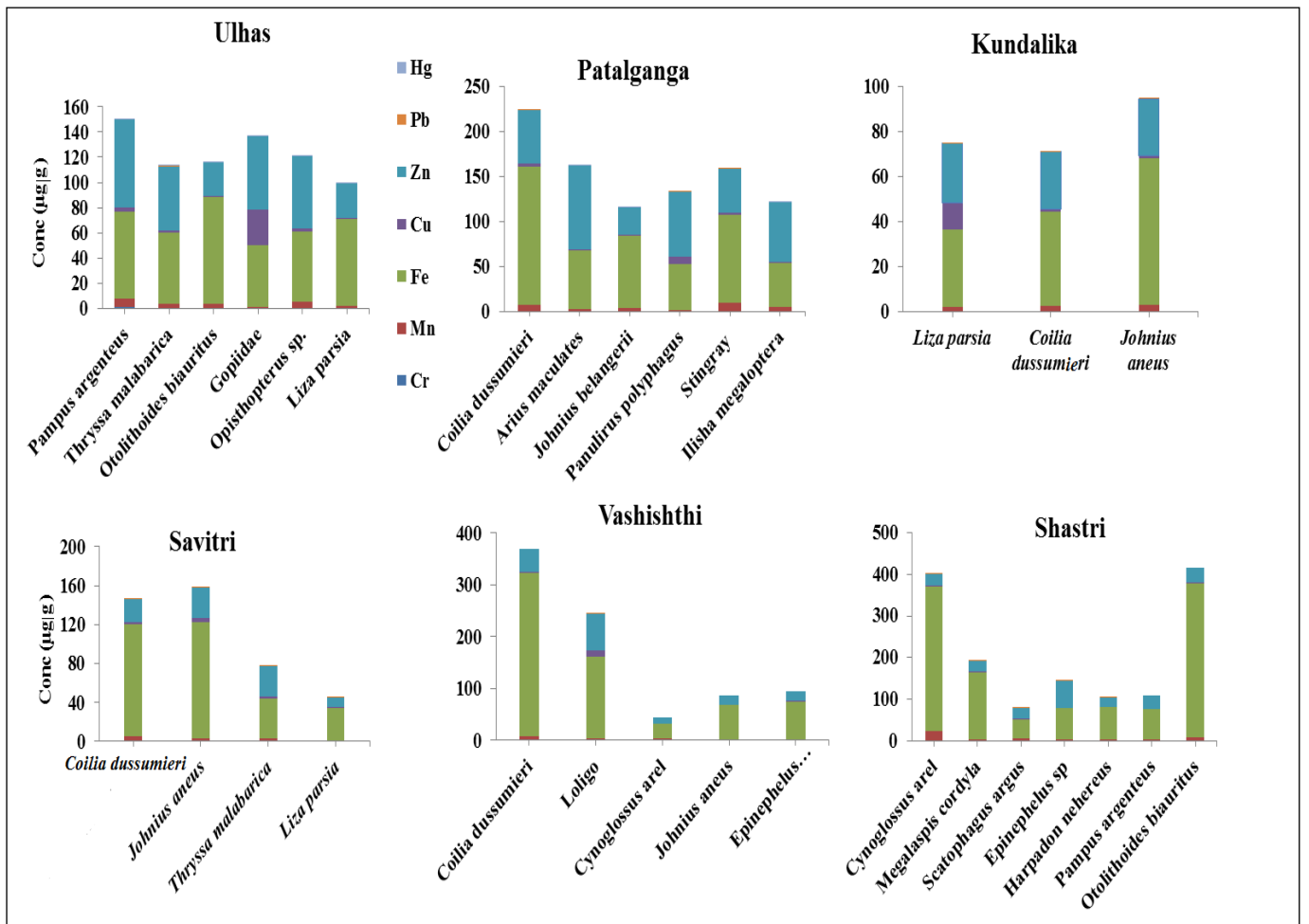


Fig. 2

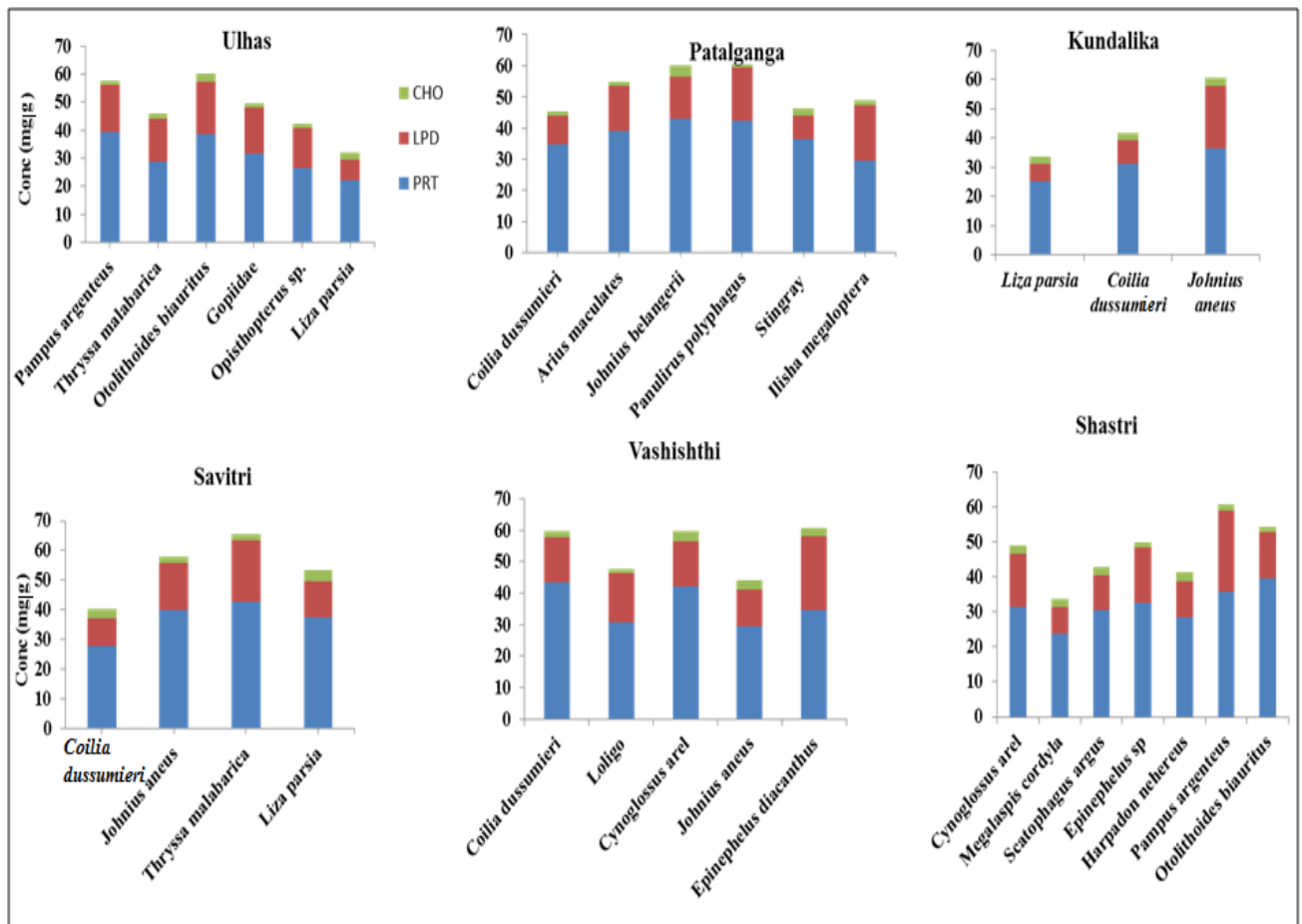


Fig.3

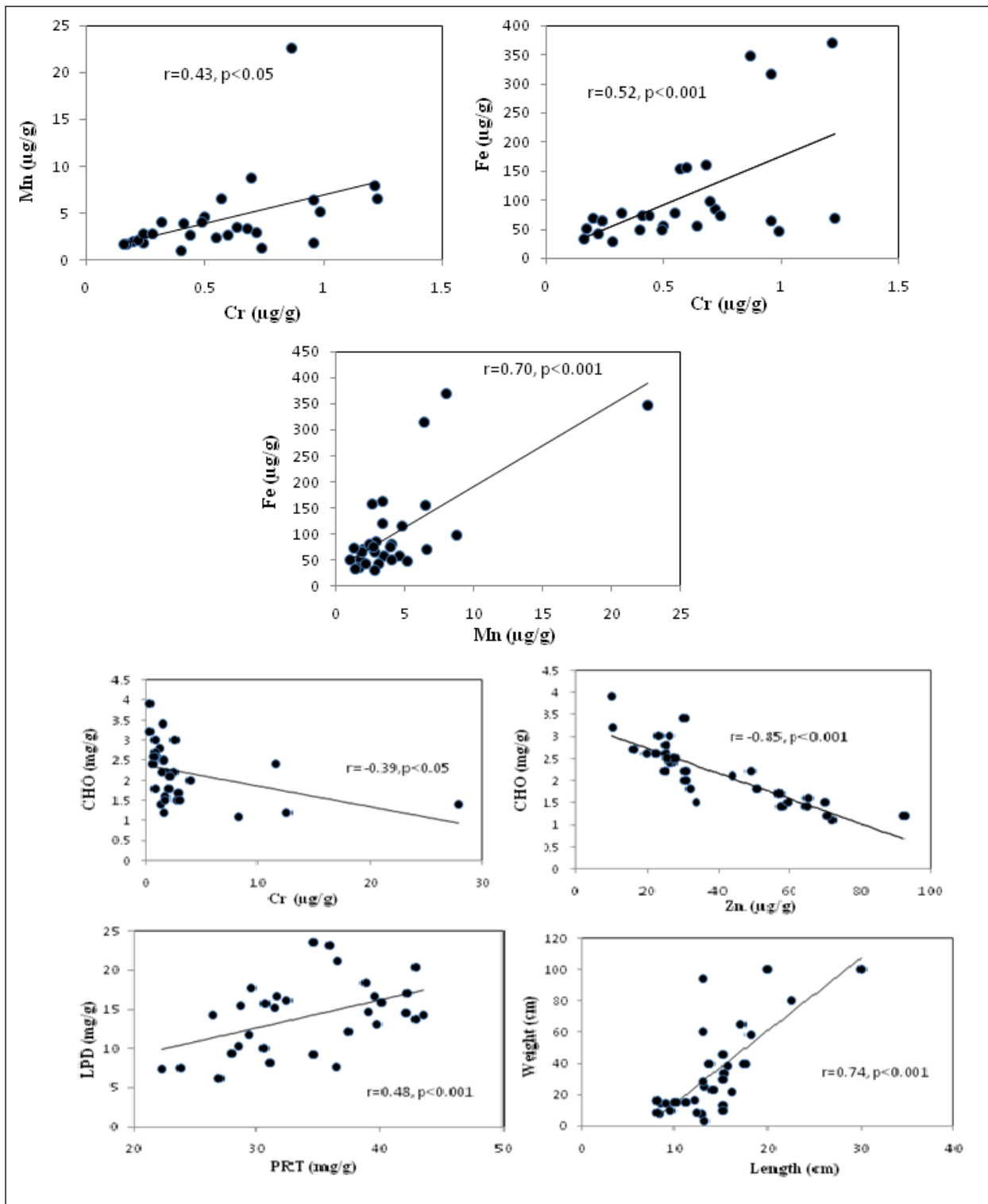


Fig.4

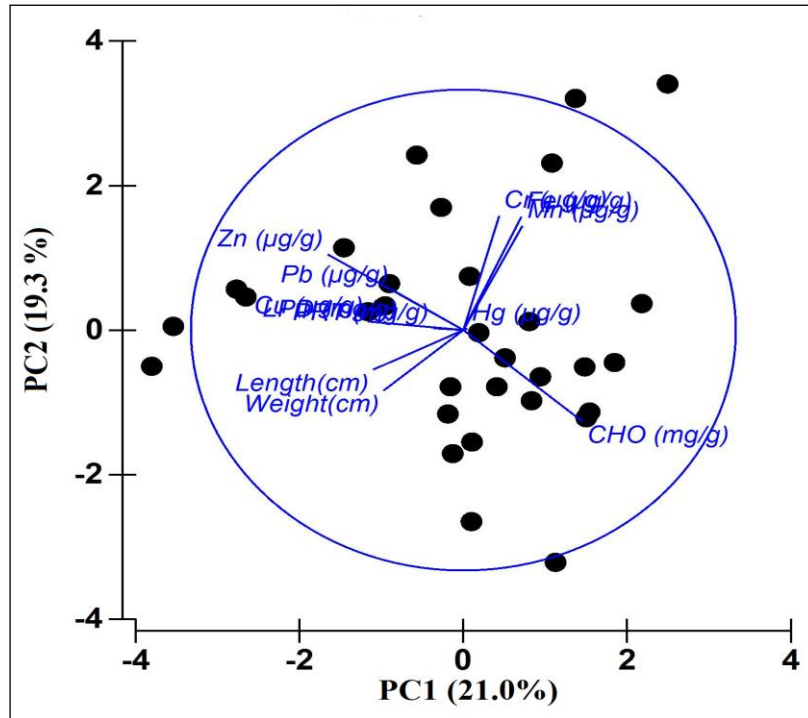


Fig.5

Table 1. Concentration of trace metals in the effluent disposal of the study area during post-monsoon and pre-monsoon (CSIR-NIO technical report 2018).

(µg/l)	Post-monsoon			Pre-monsoon		
	min	max	avg	min	max	avg
Cr	2.5	128	41.7	2.2	142.3	25.4
Mn	13	1787	352.4	93.1	543.3	244.3
Fe	8.9	5031	1041.9	91.2	3020	1105.9
Co	0.4	139	17.5	0.3	33.5	7.3
Ni	2.1	127.4	42.9	5.2	119.7	49.1
Cu	1.7	342.9	79.2	19.4	369.9	136.5
Zn	5.3	732.6	152.0	15.4	814	215.3
Hg	0.02	1.22	0.3	0.02	0.24	0.1
Pb	2.4	115.5	32.1	3.0	89.1	45.6

Table 2. Sampling location, average length, weight with \pm SD, and habitat and feeding of the fishes in the study area.

	Name of fish	n	Length (cm)	Weight (g)	Habitat	Feeding
Ulhas (S1) 19.31° N, 72.73° E - 19.32° N, 72.79° E	<i>Pampus argenteus</i>	3	9.5 \pm 1.3	9.9 \pm 1.5	Marine, Benthopelagic	Carnivorous
	<i>Thryssa malabarica</i>	4	15.6 \pm 1.5	38.2 \pm 2.5	Marine (Demersal)	Carnivorous
	<i>Otolithoides biauritus</i>	3	8.4 \pm 1.0	14.6 \pm 1.2	Marine, Brakish	Carnivorous
	<i>Gobiidae sp</i>	5	11 \pm 1.6	15 \pm 1.5	Marine, Flw, Brakish	Omnivorous
	<i>Opisthopterus tardoore</i>	4	14 \pm 1.1	23.5 \pm 1.8	Marine, Flw, Brakish	Carnivorous
	<i>Liza parsia</i>	4	10 \pm 2.1	15 \pm 2.8	Marine, Flw, Brakish	Omnivorous
	Patalganga (S2) 18.83°N, 72.91°E - 18.80°N, 72.98°E	<i>Coilia dussumeiri</i>	5	12.9 \pm 1.3	7.7 \pm 2.0	Marine, Flw, Brakish
<i>Arius maculatus</i>		4	17.4 \pm 1.6	40 \pm 3.3	Marine, Brakish	Carnivorous
<i>Johnius belangerii</i>		3	13.2 \pm 2.1	25 \pm 3.0	Marine, Brakish	Carnivorous
<i>Panulirus polyphagus</i>		4	20 \pm 1.8	100 \pm 5.6	Marine, Brakish	Omnivorous
<i>Stingray (himantura chaophraya)</i>		3	13 \pm 2.3	60 \pm 3.1	Marine, Brakish	Carnivorous
<i>Ilisha megaloptera</i>		3	12.3 \pm 1.2	9 \pm 1.6	Marine, Brakish	Carnivorous
Kundalika (S3) 18.55°N, 72.86°E - 18.54°N, 72.93°E	<i>Liza parsia</i>	4	15.2 \pm 2.4	34 \pm 3.4	Marine, Flw, Brakish	Omnivorous
	<i>Coilia dussumeiri</i>	5	8 \pm 1.8	16.3 \pm 2.2	Marine, Flw, Brakish	Carnivorous
	<i>Johnius aneus</i>	3	17 \pm 2.2	65 \pm 3.4	Marine, Brakish	Carnivorous

Savitri (S4) 17.97°,72.97°E - 17.98°N, 73.02°E	<i>Coilia dussumeiri</i>	6	15±2.0	13±1.2	Marine, Flw, Brakish	Carnivorous
	<i>Johnius aneus</i>	4	15±3.1	30±2.8	Marine, Brakish	Carnivorous
	<i>Thryssa malabarica</i>	3	15±2.3	10±1.6	Marine, Flw, Brakish	Carnivorous
	<i>Liza parsia</i>	4	18±2.6	58±3.8	Marine, Flw, Brakish	Omnivorous
Vashishthi (S5) 17.58°N- 73.08°E - 17.58°N- 73.15°E	<i>Coilia dussumieri</i>	5	13.2±2.8	3.4±1.5	Marine, Flw, Brakish	Carnivorous
	<i>Loligo</i>	3	30±3.4	100±4.6	Marine	Carnivorous
	<i>Cynoglossus arel</i>	3	22.5±2.5	80±3.4	Marine, Brakish (Demersal)	Carnivorous
	<i>Johnius aneus</i>	4	13±2.2	28.3±3.1	Marine, Brakish	Carnivorous
Shastri (S6) 17.31°N, 73.16°E - 17.30°N, 73.22°E	<i>Epinephelus diacanthus</i>	3	13.6±1.5	40±2.2	Marine (Demersal)	Carnivorous
	<i>Cynoglossus arel</i>	4	16±2.2	22±2.6	Marine, Brakish (Demersal)	Carnivorous
	<i>Megalaspis cordyla</i>	3	8.2±1.5	7.9±2.1	Marine, Brakish	Carnivorous
	<i>Scatophagus argus</i>	3	13±3.1	94±4.8	Marine, Flw, Brakish	Omnivorous
	<i>Epinephelus sp</i>	4	15±2.2	46±3.1	Marine (Demersal)	Carnivorous
	<i>Harpadon nehereus</i>	5	8±2.1	9±2.6	Marine, Benthopelagic	Carnivorous
	<i>Pampus argenteus</i>	5	9±2.5	14.5±2.8	Marine, Benthopelagic	Carnivorous
	<i>Otolithoides biauritus</i>	4	12±2.0	16.5±2.5	Marine (Demersal)	Carnivorous

Table 3. Trace metals concentration ($\mu\text{g/g}$) with $\pm\text{SD}$ in Certified Reference Materials (TORT-2 and DOLT-4) of the studied fish.

CRM→		TORT-2			DOLT-4		
Metal	Instrument	Certified	Observed	Recovery (%)	Certified	Observed	Recovery (%)
Cr	ICP-OES	0.77 \pm 0.15	0.81 \pm 0.18	105	-	-	-
Mn	ICP-OES	13.6 \pm 1.2	13 \pm 1.0	96	-	-	-
Fe	ICP-OES	105 \pm 13	101 \pm 11	96	1833 \pm 75	1788 \pm 80	98
Cu	ICP-OES	106 \pm 10	102 \pm 12	95	31.2 \pm 1.1	30.2 \pm 1.5	97
Zn	ICP-OES	180 \pm 6	175.1 \pm 8	97	116 \pm 6	112 \pm 8	97
Pb	GF-AAS	0.35 \pm 0.013	0.344 \pm 0.01	97	0.16 \pm 0.04	0.15 \pm 0.05	96
Hg	FIMS-400	0.27 \pm 0.06	0.265 \pm 0.08	98	2.58 \pm 0.22	2.55 \pm 0.31	99

Table 4. Mean with \pm SD values of concentration of trace metals (dry wt., except Hg, which is in wet wt. basis) and biochemical compounds (PRT, LPD and CHO) in muscle tissue of fishes along the study are

Name of fish	Cr (μ g/g)	Mn (μ g/g)	Fe (μ g/g)	Cu (μ g/g)	Zn (μ g/g)	Pb (μ g/g)	Hg (μ g/g; wet)	PRT (mg/g)	LPD (mg/g)	CHO (mg/g)
Ulhas (S1)										
<i>Pampus argenteus</i>	1.23 \pm 0.18 ^{ab}	6.56 \pm 0.97 ^a	69.09 \pm 2.1 ^a	2.86 \pm 0.44 ^a	69.81 \pm 1.88 ^{ab}	0.11 \pm 0.02 ^a	0.09 \pm 0.01 ^a	39.5 \pm 2.6 ^a	16.6 \pm 1.5 ^a	1.5 \pm 0.2 ^a
<i>Thryssa malabarica</i>	0.64 \pm 0.13 ^{ab}	3.45 \pm 0.69 ^a	55.86 \pm 1.7 ^a	2.13 \pm 0.63 ^a	50.7 \pm 1.55 ^{ab}	0.15 \pm 0.03 ^a	0.02 \pm 0.01 ^a	28.6 \pm 2.8 ^a	15.5 \pm 2.1 ^a	1.8 \pm 0.5 ^a
<i>Otolithoides biauritus</i>	0.72 \pm 0.04 ^{ab}	2.89 \pm 0.79 ^a	85.06 \pm 2.3 ^a	0.86 \pm 0.23 ^a	26.42 \pm 0.55 ^{ab}	0.2 \pm 0.04 ^a	0.04 \pm 0.01 ^a	38.8 \pm 3.1 ^a	18.4 \pm 1.2 ^a	3 \pm 1.0 ^a
<i>Gobiidae</i>	0.4 \pm 0.08 ^{ab}	1.01 \pm 0.19 ^a	49.12 \pm 1.8 ^a	27.94 \pm 1.8 ^a	57.72 \pm 1.45 ^{ab}	0.14 \pm 0.02 ^a	0.06 \pm 0.01 ^a	31.6 \pm 2.4 ^a	16.6 \pm 1.0 ^a	1.4 \pm 0.6 ^a
<i>Opisthopterus tardoore</i>	0.5 \pm 0.04 ^{ab}	4.55 \pm 0.33 ^a	55.84 \pm 1.7 ^a	2.97 \pm 0.48 ^a	57.02 \pm 1.8 ^{ab}	0.17 \pm 0.02 ^a	0.03 \pm 0.01 ^a	26.5 \pm 1.9 ^a	14.2 \pm 1.3 ^a	1.7 \pm 1.0 ^a
<i>Liza parsia</i>	0.2 \pm 0.05 ^{ab}	1.94 \pm 0.16 ^a	69.09 \pm 2.1 ^a	0.7 \pm 0.18 ^a	27.16 \pm 1.54 ^{ab}	0.17 \pm 0.04 ^a	0.01 \pm 0.0 ^a	22.3 \pm 1.6 ^a	7.3 \pm 1.4 ^a	2.4 \pm 1.3 ^a
Patalganga (S2)										
<i>Coilia dussumieri</i>	0.57 \pm 0.08 ^{ab}	6.52 \pm 0.5 ^a	154 \pm 4.1 ^a	3.04 \pm 0.23 ^a	59.51 \pm 3.8 ^a	0.13 \pm 0.02 ^a	ND	34.6 \pm 1.1 ^a	9.2 \pm 1.2 ^a	1.5 \pm 0.4 ^a
<i>Arius maculatus</i>	0.24 \pm 0.05 ^{ab}	1.87 \pm 0.2 ^a	65.58 \pm 2.1 ^a	1.7 \pm 0.15 ^a	91.95 \pm 4.2 ^a	0.11 \pm 0.01 ^a	0.02 \pm 0.01 ^a	39 \pm 2.5 ^a	14.6 \pm 1.8 ^a	1.2 \pm 0.3 ^a
<i>Johnius belangerii</i>	0.32 \pm 0.07 ^{ab}	4.01 \pm 0.8 ^a	79.08 \pm 2.3 ^a	1.58 \pm 0.28 ^a	30.18 \pm 1.74 ^a	0.1 \pm 0.02 ^a	0.03 \pm 0.01 ^a	42.9 \pm 3.3 ^a	13.7 \pm 2.1 ^a	3.4 \pm 1.2 ^a
<i>Panulirus polyphagus</i>	0.17 \pm 0.02 ^{ab}	1.73 \pm 0.08 ^a	50.55 \pm 1.5 ^a	8.3 \pm 0.6 ^a	72.07 \pm 2.4 ^a	0.13 \pm 0.02 ^a	ND	42.2 \pm 2.2 ^a	17.1 \pm 1.5 ^a	1.1 \pm 0.2 ^a
<i>Stingray (Himantura chaophraya)</i>	0.7 \pm 0.09 ^{ab}	8.71 \pm 0.5 ^a	97.34 \pm 4.2 ^a	2.5 \pm 0.38 ^a	49.17 \pm 1.68 ^a	0.1 \pm 0.02 ^a	ND	36.4 \pm 3.1 ^a	7.6 \pm 1.3 ^a	2.2 \pm 1.0 ^a
<i>Ilisha megaloptera</i>	0.49 \pm 0.05 ^{ab}	4.03 \pm 0.48 ^a	49.14 \pm 1.8 ^a	1.8 \pm 0.24 ^a	65.29 \pm 1.88 ^a	0.28 \pm 0.06 ^a	0.01 \pm 0.0 ^a	29.6 \pm 1.4 ^a	17.7 \pm 1.0 ^a	1.6 \pm 0.8 ^a
Kundalika (S3)										
<i>Liza parsia</i>	0.16 \pm 0.04 ^b	1.67 \pm 0.15 ^a	34.72 \pm 1.7 ^a	11.58 \pm 0.56 ^a	26.4 \pm 0.7 ^b	ND	0.04 \pm 0.01 ^a	26.9 \pm 2.5 ^a	6.2 \pm 1.6 ^a	2.4 \pm 1.4 ^a
<i>Coilia dussumieri</i>	0.22 \pm 0.06 ^b	2.17 \pm 0.18 ^a	42.12 \pm 1.2 ^a	1.0 \pm 0.21 ^a	25.41 \pm 0.7 ^b	ND	0.05 \pm 0.01 ^a	31 \pm 2.4 ^a	8.2 \pm 1.4 ^a	2.6 \pm 1.0 ^a
<i>Johnius aneus</i>	0.24 \pm 0.04 ^b	2.76 \pm 0.12 ^a	64.9 \pm 1.5 ^a	1.29 \pm 0.16 ^a	25.14 \pm 0.7 ^b	ND	0.06 \pm 0.01 ^a	36.5 \pm 1.8 ^a	21.2 \pm 1.2 ^a	2.8 \pm 1.1 ^a
Savitri (S4)										

<i>Coilia dussumieri</i>	ND	4.82±0.3 ^a	115±6.8 ^a	2.6±0.3 ^a	23.14±1.4 ^b	ND	0.04±0.01 _a	27.9±2.1 _a	9.4±1.5 ^a	3±1.3 ^a
<i>Johnius aneus</i>	ND	3.4±0.6 ^a	119±4.2 ^a	3.96±0.6 ^a	30.64±2.15 _b	ND	0.06±0.01 _a	40.1±2.4 _a	15.9±2.1 _a	2±1.4 ^a
<i>Thryssa malabarica</i>	ND	3.1±0.4 ^a	41.22±2.1 _a	1.95±0.18 ^a	30.52±1.79 _b	ND	0.03±0.01 _a	42.9±1.8 _a	20.4±1.6 _a	2.2±0.9 _a
<i>Liza parsia</i>	ND	1.36±0.2 ^a	33.1±1.8 ^a	0.34±0.1 ^a	10.12±0.58 _b	ND	0.01±0.0 ^a	37.5±3.1 _a	12.1±2.6 _a	3.9±1.8 _a
Vashishthi (S5)										
<i>Coilia dussumieri</i>	0.96±0.12 ^a	6.42±0.64 ^a	316±10.3 _a	2.26±0.21 ^a	44.09±1.3 ^b	ND	ND	43.5±1.6 _a	14.2±1.3 _a	2.1±1.0 _a
<i>Loligo</i>	0.6±0.1 ^a	2.61±0.21 ^a	157±5.8 ^a	12.64±0.54 _a	70.61±2.1 ^b	ND	0.01±0.0 ^a	30.6±3.2 _a	15.7±1.5 _a	1.2±0.6 _a
<i>Cynoglossus arel</i>	0.28±0.08 ^a	2.81±0.18 ^a	29.39±1.4 _a	0.34±0.08 ^a	10.56±0.5 ^b	ND	ND	42.1±2.6 _a	14.5±1.2 _a	3.2±1.0 _a
<i>Johnius aneus</i>	0.96±0.11 ^a	1.88±0.24 ^a	65.68±2.7 _a	0.86±0.21 ^a	16.5±0.4 ^b	ND	ND	29.4±1.6 _a	11.8±1.1 _a	2.7±0.8 _a
<i>Epinephelus diacanthus</i>	0.74±0.08 ^a	1.33±0.17 ^a	72.89±2.1 _a	0.84±0.23 ^a	19.9±0.7 ^b	ND	ND	34.6±2.1 _a	23.6±1.6 _a	2.6±1.1 _a
Shastri (S6)										
<i>Cynoglossus arel</i>	0.87±0.1 ^a	22.59±1.24 _a	347±10.4 _a	1.61±0.21 ^a	27.82±2.8 ^b	ND	0.04±0.01 _a	31.4±1.8 _a	15.2±1.2 _a	2.5±1.0 _a
<i>Megalaspis cordyla</i>	0.68±0.20 ^a	3.39±0.36 ^a	161±5.4 ^a	1.73±0.28 ^a	25.59±1.82 _b	ND	0.03±0.01 _a	23.8±1.6 _a	7.5±1.0 ^a	2.5±0.8 _a
<i>Scatophagus argus</i>	0.99±0.29 ^a	5.18±0.41 ^a	46.49±2.8 _a	1.44±0.19 ^a	24.8±1.54 ^b	ND	0.05±0.01 _a	30.5±3.3 _a	10±1.8 ^a	2.2±1.0 _a
<i>Epinephelus sp</i>	0.41±0.1 ^a	3.95±0.29 ^a	74.75±3.8 _a	1.34±0.16 ^a	64.97±3.65 _b	ND	0.01±0.0 ^a	32.4±2.1 _a	16.1±1.0 _a	1.4±0.6 _a
<i>Harpadon nehereus</i>	0.55±0.1 ^a	2.44±0.31 ^a	78.38±5.0 _a	0.76±0.10 ^a	22.52±1.88 _b	ND	0.01±0.0 ^a	28.5±1.6 _a	10.3±1.1 _a	2.6±0.9 _a
<i>Pampus argenteus</i>	0.44±0.14 ^a	2.69±0.18 ^a	73.91±5.7 _a	0.91±0.12 ^a	31.71±2.16 _b	ND	ND	35.8±2.3 _a	23.1±1.5 _a	1.8±0.5 _a
<i>Otolithoides biauritus</i>	1.22±0.33 ^a	7.94±0.59 ^a	370±6.0 ^a	1.8±0.35 ^a	34.02±2.88 _b	ND	ND	39.7±2.0 _a	13±1.6 ^a	1.5±0.7 _a

Trace metals and biochemical concentrations among the tissue from different fishes were compared statistically one-way ANOVA. Where: a, b and ab means for groups in homogenous subsets are displayed from Duncan multiple range test (DMRT) by using XLSTAT. All comparisons were statistically significant at $p < 0.05$.

ND; Not Detected.

Table 5. Trace metal concentrations ($\mu\text{g/g}$) in fish compared to others reported areas around the world.

Area	Cr	Mn	Fe	Cu	Zn	Pb	Hg	References
Karp Farvel, Greenland ^a	–	–	–	0.2–0.3	2.3–3.6	0.003–0.012	0.18–0.21	Stange et al. (1995)
Barents Sea ^a	–	–	–	0.6–0.6	5.6–7.8	<0.1	0.07	Plotitsyna and Kireeva (1996)
Mediterranean Sea ^b	1.28–1.6	–	59.6–73.4	3.40–5.88	16.1–31.4	–	–	Kalay et al. (1999)
Masan Bay, Korea ^b	0.18–0.25	–	–	–	6.33–12.9	–	–	Kwon and Lee (1999)
Middle Black Sea ^b	–	1.06–0.76	9.52–32.4	1.28–2.93	9.5–22.9	–	–	Tuzen (2002)
Black Sea ^b	<0.06–0.84	0.69–0.56	30–60	1.01–4.54	25.7–44.2	–	–	Topcuoglu et al. (2002)
Iskenderun Bay, Turkey ^b	1.03–1.79	–	0.9–3.6	0.66–1.98	8.99–2.18	–	–	Yilmaz (2003)
Mozambique Channel ^a	–	0.06	5.33	0.15	9.92	0.03	0.38	Kojadinovic et al. (2007)
Parangipettai, India ^b	0.65–0.92	0.31–1.2	24.1–50.3	0.12–0.31	14.1–33.5	–	–	Raja et al. (2009)
Kolkata ^a	–	4.24	58.66	7.54	47.8	–	0.41	Kumar et al. (2011)
Mumbai Harbor, India ^b	0.47	3.47	97.04	2.46	38.54	0.12	0.06	Velusamy et al. (2014)
İskenderun Bay, Turkey ^a	0.134–0.336	0.166–0.382	-	-	-	0.09–0.11	0.03–0.23	Kaya and Türkoğlu (2017)
West coast of India	0.5	4.2	100.8	3.4	39.4	0.1	0.03 ^a	In the present study

Where, a: Wet.Wt and b: Dry. Wt.