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# CONCENTRATION OF HEAVY METALS (FE, ZN, MN, CU, PB AND CD) IN *OTOLITHES RUBER* (BLOCH & SCHNEIDER, 1801) FROM DAMB FISH LANDING CENTRE AT SONMIANI, MAKRAN COAST, PAKISTAN

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**ABSTRACT:** Fish samples of *Otolithes ruber* (Bloch & Schneider, 1801) of various lengths (cm) and weight (g) were collected from Damb Fish Landing Centre at Sonmiani, Balochistan during 15$^{th}$ August 2009 to 15$^{th}$ April 2010. Total (56) fish samples were collected during the two major seasons Northeast Monsoon and Southwest Monsoon. The concentrations of Fe, Mn, Zn, Cu, Pb, and Cd were recorded in fish muscles. The mean concentration of Fe (4.23±1.38 µg/g), Mn (0.28±0.18 µg/g) and Cd (0.46±0.06 µg/g) were recorded in NE-monsoon. However Zn (3.34±1.19 µg/g), Cu (0.33±1.14 µg/g), and Pb (0.02±0.01 µg/g) showed highest concentration in SW-monsoon season. The results indicated that Fe concentrations were higher than other metals. Analysis of Variances (ANOVA) were performed in between seasons and metals, the significant difference was observed in Mn ($P$>0.01 at 0.05) and Pb ($P$>0.006 at 0.05) between seasons. The order of the metals in the fish samples based on concentrations in the tissues analyzed, as follows: Fe>Zn>Cu>Mn>Pb>Cd. Results concluded that the concentration of metals did not exceed the permissible limit of the food regulations of international standards. Hence there is no health hazards to humans consuming of *O. ruber*.

**KEYWORDS:** *Otolithes ruber*, Arabian Sea, heavy metals, Monsoon, target hazard quotients.

## INTRODUCTION

Toxicity heavy metals is highly persistent and widespread environmental problems owing to the stability and non-degradable properties of them. They can enter in marine coastal ecosystem through natural environmental constituents and human anthropogenic activities such as extensive fertilizers and pesticides application in agriculture, waste disposal, domestic wastes, fossil fuels burning, atmospheric deposition and discharge from manufacturing industries (Bat *et al*., 2018c). They can be accumulated in the coastal environment and biomagnified in biota and they may be toxic even at low levels (Bat and Arici, 2018).

Toxic metals such as Cd and Pb have no physiological functions in biota and are associated with organs dysfunctions, nervous system and bone diseases (EFSA, 2010 and 2012). However fundamental metals such as Fe, Zn, Cu and Mn are essential for biota at low levels to get normal development and maintenance of physiology, but the high levels exposure to these metals causes toxicity risks (WHO, 1996). Monitoring of heavy metal levels in seafood like fish by estimation of these contaminants potential risks is necessary. Risk assessment is a method required for human health hazard estimation which depends on the target hazard quotients (THQ), a ratio between the chemical estimated level and the oral reference dose (RfD) as there is no significant risk below if the THQ is <1. Thus, the present work investigated the Fe, Zn, Mn, Cu, Pb and Cd amounts in muscle of *Otolithes ruber* by using of the Atomic Absorption Spectrophotometer and assessed the human risk of these essential heavy metals.

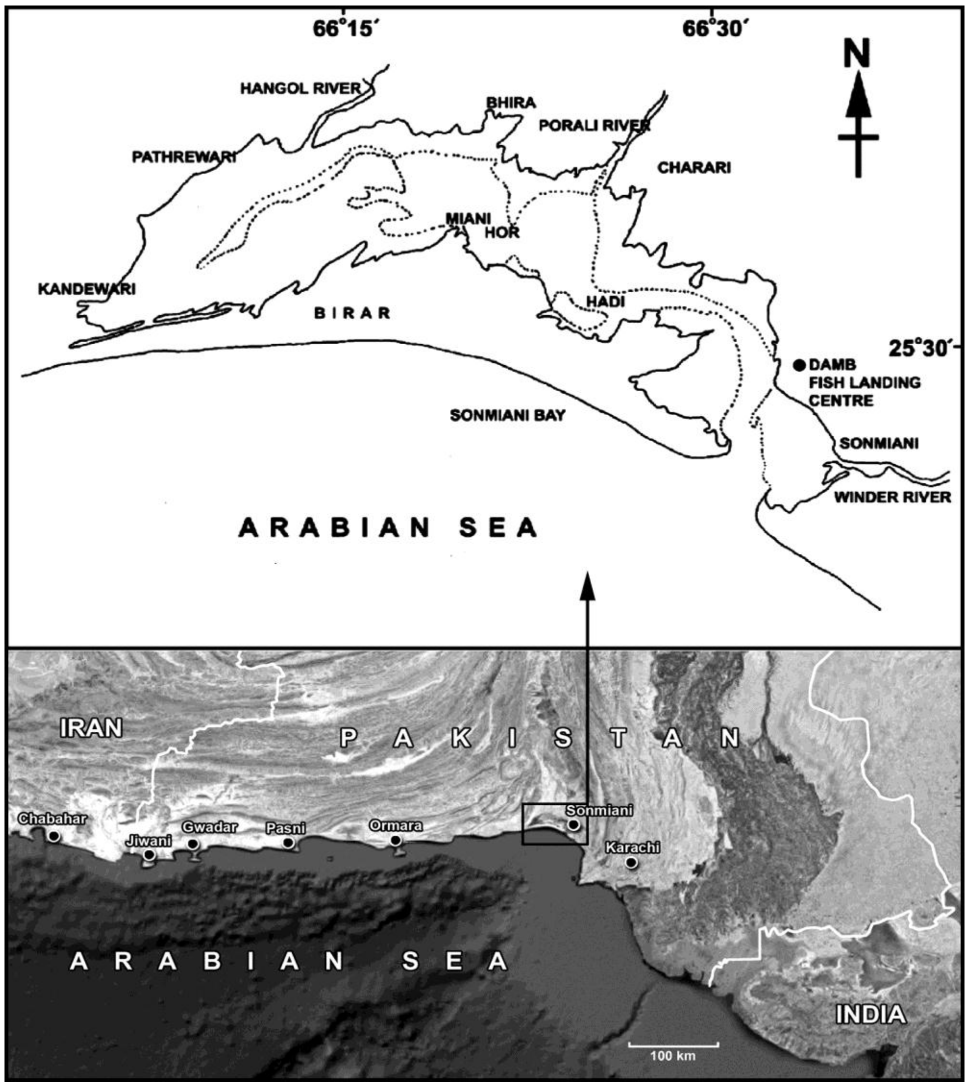


Fig. 1. Sampling area.

## MATERIALS AND METHOD

Fish **s**amples (*Otolithes ruber*) were collected in Damb Fish Landing Centre at Sonmiani, Balochistan during 15th August 2009 to 15th April 2010. Total (56) fish samples were collected during the two major seasons Northeast Monsoon and Southwest Monsoon. (Fig. 1).

Fishes were dissected using steel scissors and scalpels to remove approximately 5 g edible tissues (Bernhard, 1976). The fish samples were washed with distilled water and fresh weight of each sample were measured in (g). Samples were ground and calcinated at 500 $^{O}$C for 3 hrs till it turned to white or grey ash and reweighed. The ashes were dissolved with 0.1 M HCl according to the method of (Gutierrez *et al.*, 1978) and filtered with Whatman filter paper. One ml filtered solution was diluted with 25 ml distilled water for metal analyses. Blank solution was prepared from 2 ml of perchloric acid (70 %), 10 ml of nitric acid (65 %) and the standards were prepared (modified by UNEP 1984 and 1985). Heavy metals analysis was measured by atomic absorption spectrophotometer (Analyst 700). The results were expressed as µg per g dry weight.

The concentrations of heavy metals were expressed as µg/g dry weight. Metal concentrations in the fish samples were compared with the maximum permissible limits of metals set by European Commission for Standardization (European Commission Regulation, 2001 and 2006) and other international standards (MAFF, 1995; Georgian Food Safety Rules, 2001; TFC, 2002 and 2009; GAIN Report Russian Federation, 2002).

**Assessment of human health risk:** Risk assessment was estimated depend on the metal amounts in muscles by means of United States Environment Protection Agency (US-EPA, 2000) for human health risk assessment.

**Estimated daily intake (EDI):** The heavy metals estimated daily intake (EDI) was dependent on the heavy metal amounts in muscle tissues and the consumption rate. The EDIs were calculated using the following equation: EDI=(Cm x FIR)/BW, as Cm = concentration of the heavy metal in the edible tissues; FIR = fish ingestion rate (5 g /day) (FAO, 2010), BW is the adults body weight = 70 kg.

**Target Hazard Quotient (THQ):** THQ was used to evaluate health risk accompanied with the non-carcinogenic and carcinogenic effect of any heavy metals. Edible tissues of fish were evaluated according to the THQ. The THQ is a relation between the determined metal level and the level of reference dose (RfD). The RfD is the daily exposure of a chemical estimated which the population exposed along a lifetime continually with no major hazard (US-EPA, 2000). If this relation is <1, the population has improbable no noticeable bad effects. The method of estimating THQ was provided. The risk assessment was determined by using the following equation: THQ=EF x ED x FIR x CRFD x BW x ATx$10^{-3}$, as EF is the frequency of exposure (365 days/year), ED is the duration of exposure (70 years, mean duration), FIR is the ingestion rate of edible tissues of fish (g/day), C is the heavy metal level in fish muscles (mg/kg); BW is the adult mean body weight (70 kg), RfD is the oral reference dose (mg/kg/day) and AT is the exposure time average (365 days/ year × exposure years number, assume 70 years). The oral reference dose values for Fe, Zn, Mn, Cu, Pb and Cd are 0.7, 0.3, 0.14, 0.04, 0.004 and 0.001 (mg/kg bw/day), respectively (US-EPA, 2000).

**Hazard index (HI):** To assess the potential people health risk, the hazard index (HI) has been established by the summation of the THQ for the heavy metals in the subsequent equation. HI = Σ HQ = HQ Fe + HQ Zn + HQ Mn + HQ Cu + HQ Pb + HQ Cd. Where Σ HQ is the summation of hazard quotients of all metals. When HI higher than 1, it is risk for people health (National Academy of Science, 1989; FAO/WHO, 2010 and 2011, Bat, 2017).

**Statistical analysis:** The data was analyzed with SPSS version 12. The obtained results were expressed as the mean ± standard deviation (SD). All the statistical analyses were done at the significance level of 0.05.

## RESULTS AND DISCUSSION

Mean and standard deviation of heavy metals levels in the edible tissues of *Otolithes ruber* analyzed in the present study are shown in Figure 2. In general, edible tissues were relatively high in Fe followed by Zn, Mn and Cu which are essentials followed by levels of Pb and Cd which have no biochemical functions and are toxic even at low levels. Results of statistical analysis of heavy metals in the examined samples of fish were given in Table 1.

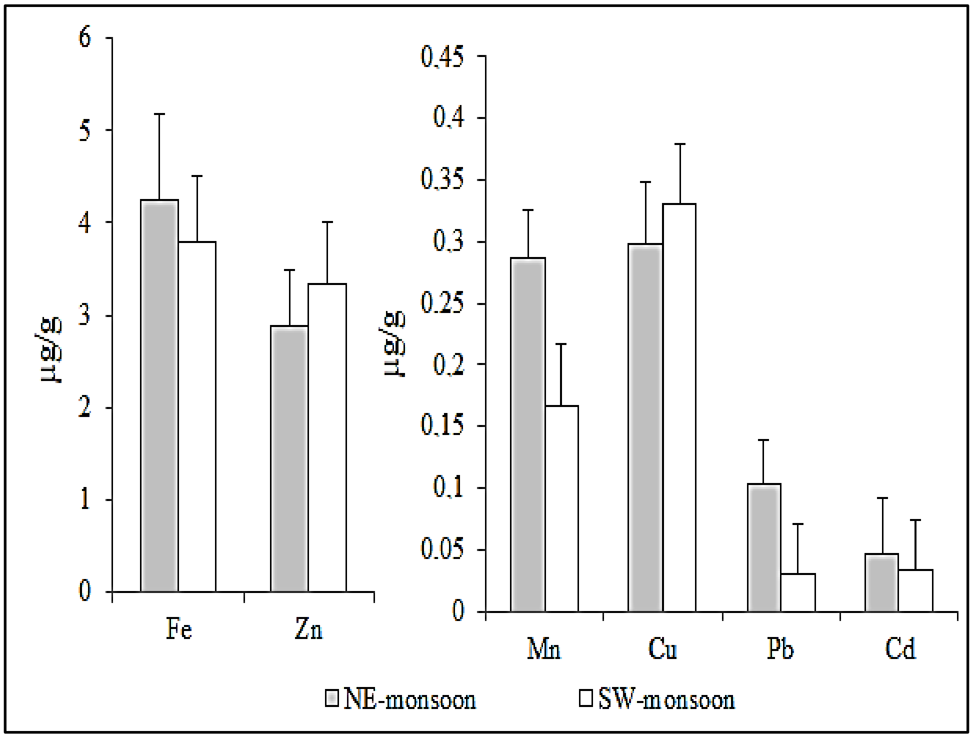


Fig. 2. The means with standard deviations (vertical line) of heavy metal concentrations (µg/g dry wt.) in the edible tissues of *Otolithes ruber* collected from Damb Fish Landing Centre at Sonmiani, Balochistan during Northeast Monsoon and Southwest Monsoon between 15th August 2009 and 15th April 2010.

**Table 1. Results of statistical analysis of Fe, Mn, Zn, Cu, Pb and Cd in the examined samples of *Otolithes ruber*.**

| | | | Sum of Squares | df. | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|---|
| Fe * Seasons | Between Groups | (Combined) | 02.763 | 01 | 2.763 | 1.571 | .215 |
| | Within Groups | | 94.963 | 54 | 1.759 | | |
| | Total | | 97.726 | 55 | | | |
| Mn * Seasons | Between Groups | (Combined) | 00.202 | 01 | 0.202 | 6.006 | .018 |
| | Within Groups | | 01.812 | 54 | 0.034 | | |
| | Total | | 02.014 | 55 | | | |
| Zn * Seasons | Between Groups | (Combined) | 02.885 | 01 | 2.885 | 2.323 | .133 |
| | Within Groups | | 67.056 | 54 | 1.242 | | |
| | Total | | 69.941 | 55 | | | |
| Cu * Seasons | Between Groups | (Combined) | 00.014 | 01 | 0.014 | 0.412 | .523 |
| | Within Groups | | 01.894 | 54 | 0.035 | | |
| | Total | | 1.908 | 55 | | | |
| Pb * Seasons | Between Groups | (Combined) | 0.077 | 01 | .077 | 8.222 | .006 |
| | Within Groups | | 0.506 | 54 | .009 | | |
| | Total | | 0.584 | 55 | | | |
| Cd * Seasons | Between Groups | (Combined) | 0.003 | 01 | .003 | 0.980 | .327 |
| | Within Groups | | 0.151 | 54 | .003 | | |
| | Total | | 0.154 | 55 | | | |

Relatively high concentrations of iron, manganese, lead, and cadmium were measured in NE monsoon whereas Zn and Cu are high compare to SW monsoon. However these metals in *Otolithes ruber* from both sampling times are lower than those in permitted levels (Table 2). However, the mean heavy metal levels in fish muscles were well below the maximum tolerance levels for human consumption established by compared with the international regulations (Table 2).

In the present study, heavy metal levels are given as dry wt., but regulation values are given as wet wt.. Thus, Fe, Mn, Zn, Cu, Cd and Pb in fish samples were estimated to be much below measurable amounts. Also it can be seen from Table 3 that the measured EDIs of the heavy metals in the present study are far below the suggested values and indicated no toxic effects to the people.

THQ for Fe, Mn, Zn, Cu, Pb and Cd ranged from 0.027 to 0.031, 0.048 to 0.056, 0.0059 to 0.010, 0.037 to 0.041, 0.037 to 0.13 and 0.16 to 0.23, respectively (Fig. 3).

**Table 2. The tolerable values of measured metals in the fish (mg/kg wet wt.)**

| Standards | Cd | Pb | Cu | Zn |
|---|---|---|---|---|
| MAFF (1995) The Food Safety (Fish Product) | <0.2 | 2.0 | 20 | 50 |
| EC (2001) The European Commission Regulation | 0.05 | 0.2 | -- | -- |
| Georgian Food Safety Rules (2001) | 0.20 | 1.0 | 10 | 40 |
| TFC (2002) Turkish Food Codex | 0.10 | 0.4 | 20 | 50 |
| GAIN Report (2002) Russian Federation | 0.20 | 1.0 | 10 | 40 |
| EC (2006) The European Commission Regulation | 0.05 | 0.3 | -- | -- |
| TFC (2009) Turkish Food Codex | 0.05 | 0.3 | -- | -- |

**Table 3. Mean amounts (mg/kg wet wt.), EDIs of heavy metals in *Otolithes ruber* due to ingestion of adults.**

| Toxic Metals | Rf. D mg/day/ kg body wt. | EDI mg/day/ kg body wt. | |
|---|---|---|---|
| | | NE-monsoon | SW-monsoon |
| Fe | 0.7 | 0.021191 | 0.018969 |
| Zn | 0.3 | 0.014456 | 0.016725 |
| Mn | 0.14 | 0.001432 | 0.000832 |
| Cu | 0.04 | 0.001491 | 0.001652 |
| Pb | 0.004 | 0.000518 | 0.000147 |
| Cd | 0.001 | 0.000234 | 0.000164 |

The results of health risk of Fe, Mn, Zn, Cu, Pb and Cd in *O. ruber* from Damb Fish Landing Centre at Sonmiani, Balochistan showed that the HI values were 0.485 for NE monsoon and 0.3259 for SW monsoon. HIs values were less than 1, it was concluded that there was no risk for people consumption from the Arabian Sea at both seasons.

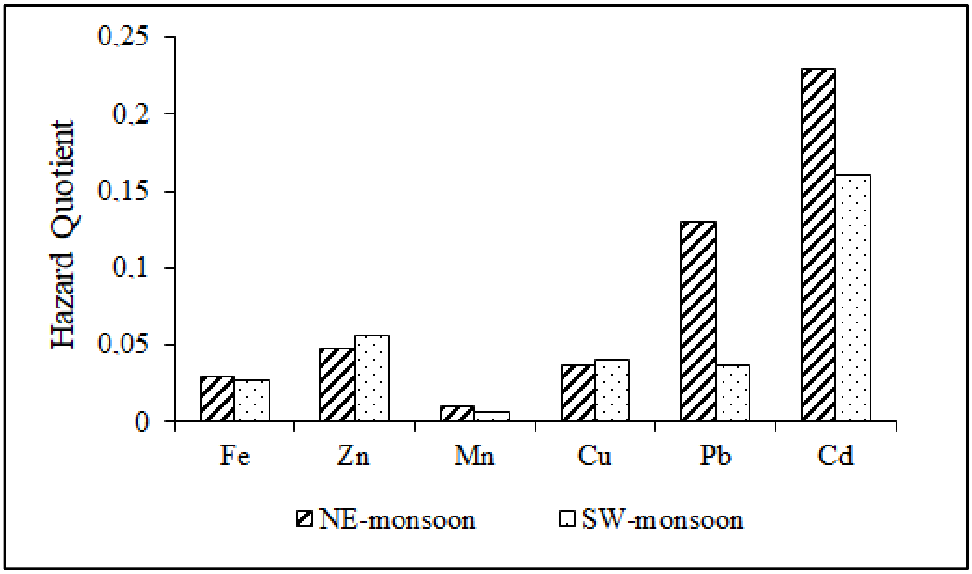


Fig. 3. Target hazard quotient (THQ) of heavy metals from consumption of *Otolithes ruber* from Damb Fish Landing Centre at Sonmiani, Balochistan.

These results were in agreement with Ahmed *et al.* (2016) and Ahmed *et al.* (2017) from the Arabian Sea, Bat *et al.* (2018a), Bat *et al.* (2018d) and Bat *et al.* (2018b) from the Black Sea, who determined THQ value below 1 for fish muscles.

## CONCLUSION

In the present study, the toxic and essential metals concentrations for *O. ruber* samples have been analyzed. The results of metals concentrations in *O. ruber* were not exceeded the allowable limits. The daily intakes (EDI) of the metals were estimated taking into account the mean of metal in *O. ruber* and the mean consumption of this fish species per day for adults. These results are much lower than the recommended values of international standarts. Calculated HIs of all the studied metals were below the value of 1, therefore the metals in *O. ruber* do not toxic to the population and this species is healthy for consumption.

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