Contamination of Cu, Zn, Fe and Mn in *Katsuwonus pelamis* (Linnaeus, 1758) from Karachi Fish Harbor and Potential Risks to Human Health

Quratulan Ahmed 1, Levent Bat 2, Farzana Yousuf 3

1 The Marine Reference Collection and Resources Centre, University of Karachi, Karachi, 75270, Pakistan
2 Sinop University Fisheries Faculty, Department of Hydrobiology, TR57000 Sinop, Turkey
3 Department of Zoology, University of Karachi, Karachi, 75270, Pakistan

International Journal of Marine Science, 2017; Vol.7, No. 9  doi: 10.5376/ijms.2017.07.0009

Abstract In the present study, skipjack tuna (*Katsuwonus pelamis*) from the Karachi coast between 2006 and 2011 were chosen and analyzed for the Fe, Mn, Cu, and Zn levels in the edible dorsal tissues, livers, kidney, gills and gonads. The liver tissues had the highest concentrations of metals (623±103 mg kg⁻¹ for Fe, 49±13 mg kg⁻¹ for Mn, 67±17 mg kg⁻¹ for Cu and 68±21 mg kg⁻¹ for Zn). The muscle maximum concentrations of Fe, Mn, Cu, and Zn were 46±17, 6±2, 7±2 and 7±2 mg kg⁻¹, respectively. The results revealed that Fe concentrations were higher than those of other metals. The values obtained were compared with the international regulation maximal allowable standards in seafood. The current work attested that calculated diurnal and hebdomadal intakes of Fe, Mn, Cu and Zn levels by way of consumption of skipjack tuna were not in excess of the Permissible Tolerable Daily Intake (PTDI) and Provisional Tolerable Weekly Intake (PTWI) values established by FAO/WHO. In conclusion, *K. pelamis* appears to be useful bioindicator due to their accumulation of the metals and continued sampling and pollution effects on food chain organisms comparatively are required for further investigations.

Keywords Heavy metals; *Katsuwonus pelamis*; Arabian Sea; Karachi; Estimated Daily Intakes

Introduction The family Scombridae incorporates many of the world’s most popular and commercial fishes. Tunas, mackerels and bonitos as a member of Scombrids are important sources of energy and protein and dietary associated with good health. In the Mediterranean countries, tunas have been caught locally for many years, yet intense commercial misuse of open-ocean tunas has led in some cases to decrease of tuna populations. Many of the tuna captured is used for canning (Anonymous, 2017). Pakistan has a coastline of well-nigh 990 km throughout with contiguous coastal zone of 240,000 km² in Northern Arabian Sea. Its’ coastal zone has rich living and non-living resources. The Karachi coastline of Pakistan accelerating pollution grade which is related to the extending of the shipping industry via port, is heavily contaminating the mangrove and aquatic life. Industries produce great amount of their waste (Anonymous, 1991a). Untreated contaminant and sewage are discharge into the marine coasts alongside oil spills from ships and fishing activities through the port. (Anonymous, 1991b). The Karachi coast is subject to pollution owing to anthropogenic activities such as domestic and industrial effluents, shipping and fishing activities, and consolidated chemicals from agricultural run-off from various hot spots (Rizvi et al., 1988; Saleem and Kazi, 1995; 1998; Saifullah et al., 2002). Most of the coastal pollution is condensed in the Karachi harbour where an approximate 90,000 tons of oil products from ships and seaport are drained off every year. Fish are the upper of the marine food chain and may bioconcentration of great quantities of some metals from surrounding water (Mansour and Sidky, 2002). In contaminated marine coastal environments, fish may be accumulated the metals in their edible tissue (Ahmed and Bat, 2015a; 2015b; 2015c; 2015d; 2015e; 2015f). Metal levels in the fishes determined as biomarkers can render as an index of the biologically existing metal burden. Hereby the metal levels in the edible tissues of fish can ensure a criterion for the extent of pollution in coastal waters. Muscle is a fish tissue mostly selected for assay because of edible tissue (Ahmed and Bat, 2015b). In addition to this, liver is a vital organ for metals storage (Ahmed and Bat, 2015a; 2015c; 2015e; Ahmed et al., 2015).
2015a; 2015b) and purge in fishes where the synthesis of metallothionein induced by metals occur (Boudou and Ribeyre, 1989). Other organ that may be regarded for assay is the gill, which is in steady contact with the surrounding water and the metals contained therein (Ahmed and Bat, 2015e; Ahmed et al., 2015a; 2015c). Fish is a major source of protein and gain for people living in the south-eastern Asia. However, detrimental substances such as metals released by anthropogenic activities have contaminated the marine coastal environment. For example, the Straits of Malacca, a momentous fishing place (Eng et al., 1989) is badly polluted by international shipping activity, intense industrialization and urbanization, and oil spills (Eng et al., 1989; Abdullah et al., 1999). The main aim of the current study to determine Fe, Mn, Cu and Zn concentrations in Katsuwonus pelamis collected from Karachi fish harbour (Figure 1).

![Map of Karachi coast](image)

**Figure 1** Map of Karachi coast

### 1 Materials and Methods

*K. pelamis* specimens of similar sizes were collected seasonally between August 2006 and December 2011. After biometric measurements, specimens were dissected using steel scissors and scalpels to take about 5 g edible muscles, entire organs (liver, kidney and gonads) and two rakers of gills. They were cleaned with deionized water and weighed (modified from UNEP, 1984; 1985; Tuzen, 2003; Karadede et al., 2004; Papagiannis et al., 2004). The specimens were crushed and calcinated at 500°C for three hours til they turned to white or grey. Then they were prepared with respect to the procedure of Gutierrez et al. (1978). The cinders were melted in 10 ml (HCl) in beakers and which the residues were filtered with Whatman filter paper and after were diluted to 25 ml distilled water with 1 N HNO₃. The equipment AAS Perkin Elmer, model AAnalyst 700, USA, with background adjustment and acetylene as fuel were initiated and presented programme win lab 32 software. The absorption wavelengths (nm) for Cu, Zn, Fe and Mn were 324.7, 213.9, 248.3 and 279.5, respectively. The detection limits of Cu, Zn, Fe and Mn were 0.23, 0.50, 0.45 and 0.58 ppb, respectively. At least three standards from 1000 ppm stock solution to 2, 4 and 6 ppm were made. The equipment together with the referred standards were calibrated. The specimens severally were aspirated to determine Cu, Zn, Fe and Mn levels. A one-way analysis of variance (ANOVA) in which the within sample variances and between the sample variances were employed to regard the initial step interactions between the organs, year and seasons (Snedecor and Cochran, 1967). The Bonferroni test was applied for correction to set against the problem of multiple comparisons. All values were expressed as µg metal g dry wt.
2 Results

The maximum mean length of *K. pelamis* (65±2 cm) was measured in summer (2010-2011) and minimum mean length (55±2 cm) was recorded in autumn (2008-2009). The maximum mean weight (3200±309 g) was obtained in summer (2010-2011) and minimum mean weight (1750±259 g) was recorded in autumn (2007-2008) (Table 1).

Table 1 Length and weight and seasonally distribution of *K. pelamis* samples

| Years     | Seasons | N  | Length (cm) (X±SD) | Weight (g) (X±SD) |
|-----------|---------|----|-------------------|-------------------|
| 2006-2007 | autumn  | 16 | 58±2              | 1800±239          |
|           | winter  | 14 | 59±2              | 1800±255          |
|           | spring  | 16 | 60±2              | 2100±239          |
|           | summer  | 20 | 59±3              | 2200±300          |
| 2007-2008 | autumn  | 14 | 56±3              | 1750±295          |
|           | winter  | 16 | 57±3              | 1900±290          |
|           | spring  | 12 | 60±3              | 2400±337          |
|           | summer  | 18 | 62±3              | 2750±342          |
| 2008-2009 | autumn  | 16 | 55±2              | 1800±198          |
|           | winter  | 18 | 58±2              | 2200±200          |
|           | spring  | 14 | 60±2              | 2350±291          |
|           | summer  | 23 | 61±2              | 2800±295          |
| 2009-2010 | autumn  | 12 | 57±1              | 1800±175          |
|           | winter  | 18 | 59±2              | 2200±252          |
|           | spring  | 16 | 59±2              | 2350±287          |
|           | summer  | 18 | 62±2              | 2700±281          |
| 2010-2011 | autumn  | 14 | 58±2              | 1950±289          |
|           | winter  | 18 | 61±3              | 2300±357          |
|           | spring  | 12 | 61±3              | 2700±483          |
|           | summer  | 20 | 65±2              | 3200±309          |

Note: X=mean, SD= standard deviation, N= Number of samples

Table 2 Mean Fe concentrations (μg/g dry wt.) in selected organs of *K. pelamis* from the Karachi coast

| Years     | Seasons | n   | Muscles (X±SD) | Liver (X±SD) | Kidney (X±SD) | Gills (X±SD) | Gonads (X±SD) |
|-----------|---------|-----|---------------|--------------|---------------|--------------|---------------|
| 2006-2007 | autumn  | 16  | 23±8          | 34±105       | 16±6          | 15±12        | 9±5           |
|           | winter  | 14  | 24±13         | 37±112       | 17±14         | 25±11        | 7±5           |
|           | spring  | 16  | 39±20         | 385±148      | 18±15         | 24±15        | 14±6          |
|           | summer  | 20  | 42±20         | 523±114      | 31±18         | 26±9         | 12±5          |
| 2007-2008 | autumn  | 14  | 35±16         | 430±112      | 27±9          | 26±16        | 9±5           |
|           | winter  | 16  | 38±27         | 475±145      | 39±25         | 43±16        | 13±7          |
|           | spring  | 12  | 30±8          | 623±103      | 34±10         | 27±9         | 15±9          |
|           | summer  | 18  | 27±13         | 523±122      | 23±8          | 32±11        | 29±9          |
| 2008-2009 | autumn  | 16  | 46±17         | 469±185      | 27±8          | 29±11        | 19±13         |
|           | winter  | 18  | 25±17         | 413±129      | 35±20         | 35±17        | 37±23         |
|           | spring  | 14  | 35±21         | 424±118      | 42±12         | 18±7         | 28±11         |
|           | summer  | 23  | 36±19         | 452±119      | 30±17         | 26±19        | 45±20         |
| 2009-2010 | autumn  | 12  | 32±20         | 441±106      | 24±12         | 30±18        | 40±19         |
|           | winter  | 18  | 25±15         | 531±123      | 22±12         | 32±12        | 34±21         |
|           | spring  | 16  | 17±8          | 502±114      | 30±23         | 32±23        | 36±18         |
|           | summer  | 18  | 26±14         | 476±128      | 47±16         | 43±24        | 53±20         |
| 2010-2011 | autumn  | 14  | 29±15         | 522±153      | 46±22         | 44±13        | 17±8          |
|           | winter  | 18  | 16±6          | 445±112      | 20±8          | 46±24        | 32±19         |
|           | spring  | 12  | 27±10         | 425±102      | 37±8          | 22±13        | 25±14         |
|           | summer  | 20  | 26±12         | 531±108      | 29±14         | 26±13        | 35±35         |
(i) Fe (Iron): The highest mean concentration of Fe (623±103 µg/g dry wt.) was determined in liver during spring (2007-2008) and the lowest mean concentration in liver (344±105 µg/g dry wt.) was determined in autumn (2006-2007). Highest mean level of muscles (46±17 µg/g dry wt.) was found in summer 2008-2009) and the lowest mean level of muscles (16±6 µg/g dry wt.) was detected in winter (2010-2011). The highest mean value of kidney (47±16 µg/g dry wt.) was found in autumn (2009-2010) and lowest mean concentration of kidney (16±6 µg/g dry wt.) was detected in autumn (2006-2007). In winter (2010-2011), the highest mean concentration of gills (46±24 µg/g dry wt.) was recorded; and the lowest mean concentration of gills (15±12 µg/g dry wt.) was recorded in autumn (2006-2007). Finally, the highest mean concentration of gonads (53±20 µg/g dry wt.) was determined in summer (2009-2010) and the lowest mean concentration of gonads (7±4 µg/g dry wt.) was recorded in winter (2006-2007) (Table 2).

(ii) Mn (Manganese): The highest mean concentration of Mn (49±13 µg/g dry wt.) was obtained in liver during autumn (2010-2011) and the lowest mean concentration in liver (28±12 µg/g dry wt.) was determined in winter (2010-2011). In spring, maximum mean value of muscles (6±2 µg/g dry wt.) was obtained (2007-2008) and the lowest mean concentration of muscles (2±1 µg/g dry wt.) was obtained in winter (2006-2007). Maximum mean concentration of kidney (9±2 µg/g dry wt.) was recorded in summer (2008-2009 and 2010-2011) and the lowest mean concentration of kidney (5±1 µg/g dry wt.) was recorded in winter, spring and summer (2006-2007). The highest mean concentration of gills (18±4 µg/g dry wt.) was detected in spring (2009-2010) and the lowest mean concentration of gills (11±4 µg/g dry wt.) was determined in summer (2006-2007). In spring, maximum mean level of gonads (7±2 µg/g dry wt.) was obtained (2007-2008) and the minimum mean level of gonads was 3±2 µg/g dry wt. (2006-2007) (Table 3).

Table 3 Mean Mn concentrations (µg/g dry wt.) in selected organs of K. pelamis from the Karachi coast

| Years      | Seasons | n   | Muscles (X±SD) | Liver (X±SD) | Kidney (X±SD) | Gills (X±SD) | Gonads (X±SD) |
|------------|---------|-----|---------------|--------------|---------------|--------------|---------------|
| 2006-2007  | autumn  | 16  | 4±2           | 29±10        | 6±2           | 14±4         | 5±3           |
|            | winter  | 14  | 2±1           | 32±10        | 5±1           | 15±3         | 4±1           |
|            | spring  | 16  | 3±2           | 36±8         | 5±1           | 13±3         | 3±2           |
|            | summer  | 20  | 3±2           | 42±7         | 5±1           | 11±4         | 4±2           |
| 2007-2008  | autumn  | 14  | 5±2           | 38±11        | 6±1           | 14±3         | 4±1           |
|            | winter  | 16  | 5±2           | 40±12        | 5±2           | 17±5         | 5±2           |
|            | spring  | 12  | 6±2           | 39±19        | 6±2           | 13±3         | 7±2           |
|            | summer  | 18  | 5±1           | 38±16        | 6±2           | 13±3         | 6±1           |
| 2008-2009  | autumn  | 16  | 3±2           | 35±18        | 5±2           | 14±2         | 5±2           |
|            | winter  | 18  | 5±2           | 47±15        | 5±2           | 13±3         | 5±1           |
|            | spring  | 14  | 4±1           | 38±15        | 5±2           | 15±2         | 5±2           |
|            | summer  | 23  | 4±1           | 38±12        | 9±2           | 13±2         | 6±1           |
| 2009-2010  | autumn  | 12  | 6±1           | 44±12        | 7±1           | 16±3         | 4±1           |
|            | winter  | 18  | 5±2           | 41±17        | 7±1           | 13±3         | 5±1           |
|            | spring  | 16  | 4±2           | 32±15        | 8±3           | 18±4         | 6±1           |
|            | summer  | 18  | 5±2           | 42±11        | 8±1           | 16±4         | 4±1           |
| 2010-2011  | autumn  | 14  | 5±2           | 49±13        | 7±2           | 17±5         | 4±1           |
|            | winter  | 18  | 5±2           | 28±12        | 8±1           | 16±4         | 6±2           |
|            | spring  | 12  | 6±1           | 29±7         | 7±1           | 15±5         | 5±2           |
|            | summer  | 20  | 5±1           | 42±14        | 9±2           | 14±3         | 6±1           |

(iii) Cu (Copper): The highest mean level of Cu (67±17 µg/g dry wt.) was obtained in liver during summer (2007-2008) and the lowest mean level in liver (36±13 µg/g dry wt.) was determined in summer (2008-2009). In winter, highest mean level of edible tissues (7±2 µg/g dry wt.) was recorded (2010-2011). The maximum mean level of kidney (6±2 µg/g dry wt.) was obtained in autumn (2007-2008) and second maximum mean level (6±1 µg/g dry wt.) was also found in summer (2007-2008). Minimum average concentration of kidney (2±1 µg/g dry wt.) was found in spring (2006-2007) and second minimum average value (3±1 µg/g dry wt.) was found in spring
(2007-2008). The maximum average level of gills (7±2 µg/g dry wt.) was detected in summer (2010-2011). In summer, highest mean concentration of gonads (4±2 µg/g dry wt.) was obtained (2007-2008) (Table 4).

Table 4 Mean Cu concentrations (µg/g dry wt.) in selected organs of *K. pelamis* from the Karachi coast

| Years   | Seasons | n  | Muscles (X±SD) | Liver (X±SD) | Kidney (X±SD) | Gills (X±SD) | Gonads (X±SD) |
|---------|---------|----|----------------|--------------|---------------|--------------|---------------|
| 2006-2007 | autumn | 16 | 4±2            | 48±21        | 4±2           | 3±1          | 3±1           |
|           | winter  | 14 | 3±1            | 36±20        | 5±2           | 5±1          | 2±1           |
|           | spring  | 16 | 4±1            | 41±14        | 2±1           | 4±1          | 2±1           |
|           | summer  | 20 | 4±1            | 39±22        | 5±2           | 3±1          | 3±1           |
| 2007-2008 | winter  | 16 | 4±2            | 49±25        | 6±2           | 5±2          | 3±1           |
|           | spring  | 12 | 3±1            | 56±21        | 3±1           | 5±2          | 3±1           |
|           | summer  | 18 | 4±1            | 67±17        | 6±1           | 4±2          | 4±2           |
| 2008-2009 | autumn  | 16 | 4±1            | 50±21        | 5±2           | 4±2          | 4±1           |
|           | winter  | 18 | 3±2            | 51±12        | 4±2           | 6±1          | 3±1           |
|           | spring  | 14 | 5±2            | 41±16        | 5±1           | 5±1          | 3±1           |
|           | summer  | 23 | 6±2            | 36±13        | 5±2           | 6±2          | 4±1           |
| 2009-2010 | autumn  | 12 | 5±1            | 50±17        | 3±2           | 5±1          | 2±1           |
|           | winter  | 18 | 3±2            | 44±18        | 5±1           | 5±1          | 3±1           |
|           | spring  | 16 | 5±2            | 50±22        | 5±2           | 5±2          | 3±1           |
|           | summer  | 18 | 6±2            | 53±20        | 5±2           | 6±1          | 4±1           |
| 2010-2011 | autumn  | 14 | 6±1            | 61±16        | 4±2           | 6±2          | 3±1           |
|           | winter  | 18 | 7±2            | 50±18        | 5±2           | 6±1          | 3±1           |
|           | spring  | 12 | 3±1            | 63±21        | 5±2           | 5±1          | 2±1           |
|           | summer  | 20 | 4±1            | 62±23        | 4±2           | 7±2          | 2±1           |

Table 5 Mean Zn concentrations (µg/g dry wt.) in selected organs of *K. pelamis* from the Karachi coast

| Years   | Seasons | n  | Muscles (X±SD) | Liver (X±SD) | Kidney (X±SD) | Gills (X±SD) | Gonads (X±SD) |
|---------|---------|----|----------------|--------------|---------------|--------------|---------------|
| 2006-2007 | autumn  | 16 | 2±2            | 35±18        | 3±2           | 2±1          | 2±1           |
|           | winter  | 14 | 6±3            | 24±22        | 4±2           | 2±1          | 2±1           |
|           | spring  | 16 | 2±1            | 22±18        | 4±1           | 3±1          | 2±1           |
|           | summer  | 20 | 4±2            | 51±19        | 5±2           | 4±1          | 3±2           |
| 2007-2008 | autumn  | 14 | 2±1            | 36±20        | 5±1           | 2±1          | 4±1           |
|           | winter  | 16 | 5±2            | 39±12        | 5±1           | 3±2          | 2±1           |
|           | spring  | 12 | 3±2            | 34±17        | 4±1           | 3±2          | 3±2           |
|           | summer  | 18 | 5±2            | 35±20        | 5±2           | 4±1          | 2±1           |
| 2008-2009 | autumn  | 16 | 4±2            | 45±18        | 4±1           | 4±2          | 3±1           |
|           | winter  | 18 | 4±2            | 38±19        | 3±2           | 4±1          | 3±2           |
|           | spring  | 14 | 6±2            | 54±22        | 4±2           | 5±1          | 4±2           |
|           | summer  | 23 | 4±2            | 56±21        | 5±1           | 5±1          | 4±1           |
| 2009-2010 | autumn  | 12 | 6±3            | 47±20        | 5±1           | 6±2          | 5±2           |
|           | winter  | 18 | 6±2            | 68±21        | 4±2           | 5±2          | 4±1           |
|           | spring  | 16 | 5±2            | 49±19        | 6±3           | 5±2          | 5±2           |
|           | summer  | 18 | 6±3            | 49±20        | 4±2           | 5±2          | 3±1           |
| 2010-2011 | autumn  | 14 | 4±2            | 50±18        | 5±2           | 4±3          | 4±2           |
|           | winter  | 18 | 7±2            | 44±13        | 5±1           | 5±2          | 3±1           |
|           | spring  | 12 | 6±2            | 37±17        | 8±1           | 5±1          | 2±1           |
|           | summer  | 20 | 6±2            | 35±11        | 6±1           | 6±2          | 4±1           |

(iv) Zn (Zinc): The maximum mean value of Zn (68±21 µg/g dry wt.) was found in liver during winter (2009-2010) and the minimum mean value in liver (22±18 µg/g dry wt.) was determined in spring (2006-2007). In winter, highest mean concentration of muscles (7±2 µg/g dry wt.) were obtained (2010-2011) and the lowest
Average value of muscles (2±1 µg/g dry wt.) was detected in spring (2006-2007). The maximum average level of kidney (8±1 µg/g dry wt.) was recorded in spring (2010-2011) and the minimum average level of kidney (3±2 µg/g dry wt.) was obtained in autumn (2006-2007) and in winter (2008-2009). The highest mean concentration of gills (6±2 µg/g dry wt.) was recorded in autumn (2009-2010) and in summer (2010-2011). The maximum mean concentration of gonads (5±2 µg/g dry wt.) was found in both autumn and spring (2009-2010) (Table 5).

Analyses for the obtained results of metals are shown in Table 6 and Table 7.

### Table 6: Analysis of variance (ANOVA) in *K. pelamis* from the coast of Karachi

| Metals | Effect                  | Sum of square | df | Mean square | F    | p    |
|--------|-------------------------|---------------|----|-------------|------|------|
| Fe     | Organs                  | 5             | 4  | 1           | 3363 | 0.000|
|        | Season                  | 85765         | 3  | 28588       | 81   | 0.000|
|        | Year                    | 128197        | 5  | 32049       | 9    | 0.000|
|        | Organs * Season         | 197096        | 12 | 16425       | 5    | 0.000|
|        | Organs * Year           | 297154        | 16 | 18572       | 5    | 0.000|
|        | Season * Year           | 107487        | 12 | 8957        | 3    | 0.003|
|        | Organs * Season * Year  | 462903        | 48 | 9644        | 3    | 0.000|
|        | Error                   | 5435104       | 1525| 3564       |      |      |
|        | Total                   | 8             | 1625|           |      |      |
|        | Corrected Total         | 7             | 1624|           |      |      |
| Mn     | Organs                  | 282224        | 4  | 7056       | 1761 | 0.000|
|        | Season                  | 86            | 3  | 29         | 1    | 0.544|
|        | Year                    | 1286          | 5  | 322        | 8    | 0.000|
|        | Organs * Season         | 549           | 12 | 46         | 1    | 0.322|
|        | Organs * Year           | 1799          | 16 | 112        | 3    | 0.000|
|        | Season * Year           | 705           | 12 | 59         | 1    | 0.130|
|        | Organs * Season * Year  | 4127          | 48 | 86         | 2    | 0.000|
|        | Error                   | 61113         | 1525| 40        |      |      |
|        | Total                   | 692102        | 1625|           |      |      |
|        | Corrected Total         | 361252        | 1624|           |      |      |
| Cu     | Organs                  | 548994        | 4  | 137248     | 1789 | 0.000|
|        | Season                  | 412           | 3  | 137        | 2    | 0.147|
|        | Year                    | 2190          | 5  | 548        | 7    | 0.000|
|        | Organs * Season         | 430           | 12 | 36         | 0.5  | 0.934|
|        | Organs * Year           | 7229          | 16 | 452        | 6    | 0.000|
|        | Season * Year           | 606           | 12 | 50         | 0.7  | 0.793|
|        | Organs * Season * Year  | 4264          | 48 | 89         | 1    | 0.216|
|        | Error                   | 117024        | 1525| 77        |      |      |
|        | Total                   | 998302        | 1625|           |      |      |
|        | Corrected Total         | 694212        | 1624|           |      |      |
| Zn     | Organs                  | 420277        | 4  | 105069     | 1472 | 0.000|
|        | Season                  | 121           | 3  | 40         | 0.6  | 0.637|
|        | Year                    | 3833          | 5  | 958        | 13   | 0.000|
|        | Organs * Season         | 521           | 12 | 43         | 0.6  | 0.837|
|        | Organs * Year           | 7099          | 16 | 444        | 6    | 0.000|
|        | Season * Year           | 1890          | 12 | 158        | 2    | 0.010|
|        | Organs * Season * Year  | 7100          | 48 | 148        | 2    | 0.000|
|        | Error                   | 108859        | 1525| 71        |      |      |
|        | Total                   | 817089        | 1625|           |      |      |
|        | Corrected Total         | 561078        | 1624|           |      |      |

Note: * = significant at P < 0.05
Table 7 Bonferroni test in *K. pelamis* collected from Karachi coast

| Metals | Seasons (I) | Seasons (J) | Mean Difference (I-J) | SE  | P     |
|--------|-------------|-------------|----------------------|-----|-------|
| Fe     | Autumn      | Spring      | -8.33                | 4.48| 0.379 |
|        |             | Summer      | -17.70               | 4.14| 0.000 |
|        |             | Winter      | -1.89                | 4.29| 1.000 |
|        | Spring      | Autumn      | 8.33                 | 4.48| 0.379 |
|        |             | Summer      | -9.40                | 4.17| 0.149 |
|        |             | Winter      | 6.45                 | 4.32| 0.815 |
|        | Summer      | Autumn      | 17.70                | 4.14| 0.000 |
|        |             | Spring      | 9.37                 | 4.17| 0.149 |
|        |             | Winter      | 15.81                | 3.96| 0.000 |
|        | Winter      | Autumn      | 1.89                 | 4.29| 1.000 |
|        |             | Spring      | -6.45                | 4.32| 0.185 |
|        |             | Summer      | -15.81               | 3.96| 0.000 |
| Mn     | Autumn      | Spring      | 0.379                | 0.48| 1.000 |
|        |             | Summer      | -0.30                | 0.44| 1.000 |
|        |             | Winter      | -0.10                | 0.45| 1.000 |
|        | Spring      | Autumn      | -0.38                | 0.48| 1.000 |
|        |             | Summer      | -0.68                | 0.44| 0.752 |
|        |             | Winter      | -0.47                | 0.46| 1.000 |
|        | Summer      | Autumn      | 0.298                | 0.44| 1.000 |
|        |             | Spring      | 0.678                | 0.44| 0.752 |
|        |             | Winter      | 0.209                | 0.42| 1.000 |
|        | Winter      | Autumn      | 0.09                 | 0.45| 1.000 |
|        |             | Spring      | 0.49                 | 0.46| 1.000 |
|        |             | Summer      | -0.21                | 0.42| 1.000 |
| Cu     | Autumn      | Spring      | 0.90                 | 0.66| 1.000 |
|        |             | Summer      | -0.49                | 0.61| 1.000 |
|        |             | Winter      | 0.11                 | 0.63| 1.000 |
|        | Spring      | Autumn      | 0.90                 | 0.66| 1.000 |
|        |             | Summer      | -1.39                | 0.61| 0.137 |
|        |             | Winter      | -0.80                | 0.63| 1.000 |
|        | Summer      | Autumn      | 0.49                 | 0.61| 1.000 |
|        |             | Spring      | 1.39                 | 0.61| 0.137 |
|        |             | Winter      | 0.60                 | 0.58| 1.000 |
|        | Winter      | Autumn      | -0.11                | 0.63| 1.000 |
|        |             | Spring      | 0.80                 | 0.63| 1.000 |
|        |             | Summer      | -0.60                | 0.58| 1.000 |
| Zn     | Autumn      | Spring      | -0.52                | 0.63| 1.000 |
|        |             | Summer      | -0.61                | 0.59| 1.000 |
|        |             | Winter      | -0.059               | 0.61| 1.000 |
|        | Spring      | Autumn      | 0.52                 | 0.63| 1.000 |
|        |             | Summer      | -0.09                | 0.59| 1.000 |
|        |             | Winter      | 0.46                 | 0.61| 1.000 |
|        | Summer      | Autumn      | 0.61                 | 0.59| 1.000 |
|        |             | Spring      | 0.09                 | 0.59| 1.000 |
|        |             | Winter      | 0.56                 | 0.56| 1.000 |
|        | Winter      | Autumn      | 0.06                 | 0.61| 1.000 |
|        |             | Spring      | -0.46                | 0.61| 1.000 |
|        |             | Summer      | -0.56                | 0.56| 1.000 |
3 Remarks

The heavy metal pollution of marine environment may be evaluated by analysing seawater, sediments and biota. Because of metal levels in marine organisms are generally notably higher than those in other items of marine ecosystem (Bryan, 1976; Phillips and Rainbow, 1994). Phillips (1980) represented that for an organism to be a good indicator of metal contamination, there should be a simple correlation between metal values in the surroundings and in the organism. Fish are broadly chosen as sentinels of pollution in marine coastal area (Bat, 2014; Ahmed and Bat, 2016a; 2016b; Ahmed et al., 2016) and may accumulate metals over background levels and so, verify their likely as biomonitors of heavy metal pollution (Storelli and Marcotrigiano, 2005). Most living being need minor quantities of many essential metals such as iron, manganese, copper and zinc for their vital processes (Bryan, 1976). Besides, these ones have toxic when they passed over definite limits (Rainbow, 1985). In the present study, the concentrations of Cu, Zn, Fe and Mn have been measured in skipjack tuna (K. pelamis) collected from Karachi fish harbour of Arabian Sea. Skipjack tuna is a pelagic fish and is the top of food chain. They feed on mainly fish, crustaceans, cephalopods and mollusc (Kojadinovic et al., 2007; Jakimska et al., 2011). It is also notified that metabolic rates (Korsmeyer and Dewar, 2001) and digestion and growth rates (Storelli et al., 2005) of the pelagic fish are three and two to five folds higher than those of many fish species of same length, respectively. Because of their high rates of metabolism and xenobiotic uptake (Kojadinovic et al., 2007; Jakimska et al., 2011), it may results in a rise in the concentrations of heavy metal taken up and hence accumulated. Bustamente et al. (2003) pointed out that difference in metal levels coupled with food and nutrition temperaments of benthic and pelagic fish species and found that benthic fish generally accumulated higher metal levels than those in pelagic fish. This finding is disagreeing with the results of Topping (1973) who put forwarded that primarily plankton nourishment fish comprise much higher metal levels than those benthic nourishment fish from Scottish waters. Jakimska et al. (2011) found that carnivorous species accumulated higher heavy metals than herbivores and omnivores. These results are similar to Bat et al. (2012a) which show high heavy metal levels in sprat which is pelagic and zooplanktonivorous fish and has high metabolic rate. This makes skipjack tuna ideal bioindicator for assessing heavy metal pollution in marine ecosystems (Kojadinovic et al., 2007). Heavy metal levels varied among the tissues and organs with seasonally. This may be attributed to factors such as water temperature and food. The data in Table 2, Table 3, Table 4 and Table 5 on the dispersion of the metal levels in the organs and tissues of the skipjack tuna represent that there is a significant concentration of these metals in liver than those in edible parts. These findings were similar to those reported by other studies in Karachi coasts (Yousuf et al., 2013; Ahmed et al., 2014). This is also agreed with the study of Bat et al. (2012b) that heavy metal levels of bottom fish Psetta maximus in liver were greater than those in edible tissues and gonad for both male and female, recommending that liver is an organ in which metals are accumulated. The results for these metals also demonstrated that there was disparity in metal levels seasonally. Fe is the maximum available in large quantities of the metals investigated. In skipjack tuna, gill was the second target tissue for Mn and Cu. Dallinger et al. (1987) emphasizes that one of the major route of heavy metal assimilation in fish are water, through the gills. It is interesting to note that Zn in muscle tissue is higher than those in in gills and gonads. Cu in muscle tissues is also greater than those in gonad. From the current study, gonads generally accumulated low heavy metals except Fe. Maximum Cu and Zn levels in dorsal muscles were 7±2 μg/g dry wt. Statutory thresholds are not existing for requisite elements in European countries. Nevertheless, in the edible tissues of skipjack tuna the mean metal levels were beneath the maximal allowance values for people consumption set by compared the international regulations (Table 8). The Cu and Zn levels in kidney, gills and gonads were always lower than the permissible maxima, but liver Cu and Zn levels exceeded the allowable levels. It should be noted that the concentrations of metals were given as dry wt. in the present study. Even so it is recommended that the liver of skipjack tuna should be ejected and decently cleaned before consumption.

In addition, the Provisional Tolerable Weekly Intake (PTWI) level is a calculate of the quantity of a metal that may be eaten by human over a vita without perceptible hazard. PTWI is builded according to the Joint Food and Agricultural Organization for the United Nations (FAO) / World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (Anonymous, 2010a). PTWI levels were accessed in the current investigation to give
as exemplary levels for confident levels of the metals studied. Table 9 displays that known foolproof levels of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Council of Europe for both Cu and Zn. PTWI levels seeing a 70 kg body weight of an grown person for Cu and Zn.

Table 8 The tolerable values of Cu and Zn in the fish (mg/kg wet wt.)

| Standards                          | Metals          |
|-----------------------------------|-----------------|
| Anonymous (1995a) Turkish Legislation | Cu | Zn |
| Anonymous (1995b) The Food Safety (Fish Product) | 20 | 50 |
| Anonymous (2001a) Georgian Food Safety | 10 | 40 |
| Anonymous (2002a) Turkish Food Codex | 20 | 50 |
| Anonymous (2002b) Russian Federation | 10 | 40 |

Table 9 Internationally accepted safe levels for Cu and Zn

| Metals | Standard                  | References            |
|--------|---------------------------|-----------------------|
| Cu     | PTWI of 3.5 mg per kg body wt. per week | Anonymous 1996, Anonymous 2001b |
| Zn     | PTWI of 7 mg per kg body wt. per week |                       |

Table 10 Estimated Weekly Intakes (EWI) and Daily Intakes (EDI) of Cu and Zn in dorsal tissue of K. pelamis from Karachi fish harbor of Arabian Sea

| Metals | PTWI (mg/week/70 kg body wt.) | PTDI (mg/day/70 kg body wt.) | EWI   | EDI  |
|--------|-------------------------------|-----------------------------|-------|------|
| Cu     | 245                           | 35                          | 0.222 | 0.032|
| Zn     | 490                           | 70                          | 0.240 | 0.034|

According to the current study results Estimated Weekly Intake (EWI) and Estimated Daily Intake (EDI) for a 70 kg person weight were submitted in Table 10 and were incorporated with standards in Table 9 for evaluation of these metals in K. pelamis. The Joint FAO/WHO Expert Committee on Food Additives set PTWIs for both Cu and Zn were had in Table 9, which was tantamount to 245 and 490 mg/week for a 70 kg person, respectively. The main per diem fish consumption in Pakistan is 5 g per individual (Anonymous, 2010b). It is withal equal to 35 g/week. By calculating the averages of weekly fish eaten in Pakistan and the most Cu and Zn values in skipjack tuna, weekly entrance counted per body for these metals in edible muscles of K. pelamis. As it can be deduced out of the Table 10, the evaluated EWIs of both Cu and Zn in the current work is rather under the approved safe levels.

4 Conclusion

The present study had for objective to measure levels of the metals namely Fe, Mn, Cu and Zn in skipjack tuna (K. pelamis) from the Karachi coast between 2006 and 2011. The means levels of these metals varied depending on seasons. The weekly intakes of Cu and Zn per kg of person amounts not passed over the Provisional Tolerable Weekly Intake (PTWI) established. The heavy metal concentrations of skipjack tuna in Karachi fish harbour of Arabian Sea do not present any danger to people consumption. These information ensure a beneficial reference line to gage essential these metals any oncoming alters in local pollution. It is also suggested that the commercial fish used as pollution bio-indicators for assessment of environmental health of coastal areas.

References

Ahmed Q., and Bat L., 2015a, Heavy metal levels in Euthynnus affinis (Cantor, 1849) Kawakawa fish marketed of Karachi fish harbour, Pakistan and potential risk of human health, J. Black Sea/Mediterranean Environment, 21(1): 35-44

Ahmed Q., and Bat L., 2015b, Accumulations of Zn, Ni, B, Al and Co in Megalaspis cordyla from fish marketed by Karachi fish harbor of Pakistan, International Journal of Fisheries and Aquatic Studies, 2(4): 204-207
Bryan G.W., 1976, Marine pollution. Heavy metal contamination in the sea. In: R. Johnston (Ed.), Academic Press Inc., London

Bustamante P., Bocher P., Chérel Y., Miramand P., and Courant A., 2003, Distribution of trace elements in the tissues of benthic and pelagic fish from the Kerguelen Islands, The Science of the Total Environment, 313: 25–39

https://doi.org/10.1016/S0048-9697(03)00265-1

Dallingr R., Prosi F., Segner H., and Back H., 1987, Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research, Oecologia, 73(1): 91–98

https://doi.org/10.1007/BF00376982

PMid:28311410

Eng C.T., Paw J.N., and Guarin F.Y., 1989, The environmental impact of aquaculture and the effects of pollution on coastal aquaculture development in Southeast Asia, Marine Pollution Bulletin, 20: 335–343

https://doi.org/10.1016/0025-326X(89)90157-4

Gutiérrez M., Stabbler R.E., and Arias A.M., 1978, Accumulation y efectos histopatologicos del Cd yel Hg en el pez sapo (Halobatrachus didactylus), Investigaciones Pesqueras, 42: 141-154

Jakimska A., Konieczka P., Skora K., and Namiesnk J., 2011, Bioaccumulation of metals in tissues of marine animals. Part I: The role and impact of heavy metals on organisms, Polish Journal of Environmental Studies, 20(5): 1117-1125

Karadede H., Oymak S.A., and Unlu E., 2004, Heavy metals in mullet, Liza abu, and catfish, Sillarus triostegus, from the Ataturk Dam Lake (Euphrates), Turkey Environment International, 30: 183-188

https://doi.org/10.1016/S0660-4120(03)01697-7

Kejadinovic J., Potier M., Corre M.L., Cosson R.P., and Bustamante P., 2007, Bioaccumulation of trace elements in pelagic fish from the Western Indian Ocean, Environmental Pollution, 146: 548-566

https://doi.org/10.1016/j.envpol.2006.07.015

PMid:17084003

Korsmeyer K.E., and Dewar H., 2001, Tuna metabolism and energetics. In: Tuna physiology, ecology, and evolution. B. A. Block and E. D. Stevens, Academic Press, San Diego, California

https://doi.org/10.1016/S1546-7296(01)00088-7

Kojadinovic J., Potier M., Corre M.L., Cosson R.P., and Bustamante P., 2007, Bioaccumulation of trace elements in pelagic fish from the Western Indian Ocean, Environmental Pollution, 146: 548-566

https://doi.org/10.1016/j.envpol.2006.07.015

PMid:17084003

Korsmeyer K.E., and Dewar H., 2001, Tuna metabolism and energetics. In: Tuna physiology, ecology, and evolution. B. A. Block and E. D. Stevens, Academic Press, San Diego, California

https://doi.org/10.1016/S1546-7296(01)00088-7

Mansour S.A., and Sidky M.M., 2002, Ecotoxicological studies 3. Heavy metals contaminating water and fish from Fayoum, Governorate, Egypt, Food Chemistry, 78: 15–22

https://doi.org/10.1016/S0308-8146(01)00197-2

Papagiannis I., Kagalou I., Leonardos J., Petridis D., and Kalfakakou V., 2004, Copper and zinc in four freshwater fish species from Lake Pamvotis (Greece), Environment International, 30: 357-362

https://doi.org/10.1016/j.envint.2003.08.002

PMid:14987866

Phillips D.J.H., 1980, Quantitative aquatic biological indicators their use to monitor trace metal and organochlorine pollution, Applied Science Publishers Ltd., London

Phillips D.J.H., and Rainbow P.S., 1994, Biomonitoring of trace aquatic contaminants. Environmental Management Series, Chapman & Hall, London

Rainbow P.S., 1985, The biology of heavy metals in the sea, International Journal of Environmental Studies, 25: 195-211

https://doi.org/10.1080/00207238508710225

Rizvi S.H.N., Saleem M., and Baquer J., 1988, Steel mill effluents: Influence on the Bakran Creek environment. In: M.F. Thompson and N.M. Tirmizi, Editors, Proceedings of Marine Science of the Arabian Sea, living marine resources and the environment

PMId:25618265

Saleem M., and Kazi G.H., 1995, Distribution of trace metals in the seawater and surficial sediments of the Karachi harbor. In: M.F. Thompson and N.M. Tirmizi, Editors, Proceedings of the arabian sea, living marine resources and the environment, Vanguard books (pvt) LTD, Lahore, Pakistan

PMId:PMC41656

Saleem M., and Kazi G.H., 1998, Concentration and distribution of heavy metals (lead, cadmium, chromium, copper, nickel, zinc) in Karachi shore and offshore sediments, Pakistan Journal of Marine Science, 7: 71-79

Snoeck W.G., and Cochran W.G., 1967, Statistical methods. New York Oxford and IBH Publishing Co.

PMid:6027619

Storelli M.M., and Marcotrigiano G.O., 2005, Bioindicator organisms: Heavy metal pollution evaluation in the Ionian Sea (Mediterranean Sea-Italy), Environmental Monitoring and Assessment, 102: 159-166

https://doi.org/10.1007/s10661-005-6018-2

PMid:1586988

Storelli M.M., Giacominelli R., Storelli A., and Marcotrigiano G.O., 2005, Accumulation of mercury, cadmium, lead and arsenic in swordfish and bluefin tuna from the Mediterranean Sea: a comparative study, Marine Pollution Bulletin, 44: 281-288

https://doi.org/10.1016/j.marpolbul.2005.06.041

Topping G., 1973, Heavy metals in fish from Scottish waters, Aquaculture, 11: 373-377
Tuzen M., 2003, Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry, Food chemistry, 80: 119-123
https://doi.org/10.1016/S0308-8146(02)00264-9

UNEP, 1984, Determination of total Cd, Zn, Pb and Cu in selected marine organisms by flameless AAS, Reference Methods for Marine Pollution Studies, 11, Rev.1.

UNEP, 1985, GESAMP: Cadmium, lead and tin the marine environment. UNEP Regional Seas Reports and Studies, No.56

Yousuf F., Ahmed Q., Türkmen M., and Tabussum S., 2013, Heavy metal contents in largehead hairtail (Trichiurus lepturus) from the coast of Karachi, Karadeniz Fen Bilimleri Dergisi / The Black Sea Journal of Sciences, 3(8): 105-111