CONCENTRATIONS OF HEAVY METALS (Fe, Mn, Zn, Cd, Pb, AND Cu) IN MUSCLES, LIVER AND GILLS OF ADULT SARDINELLA ALBELLA (VALENCIENNES, 1847) FROM GWADAR WATER OF BALOCHISTAN, PAKISTAN

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Abstract

Fish samples of *Sardinella albella* were collected seasonally from Balochistan coast during (Pre-monsoon, Monsoon, Post-monsoon) season in (January 2012 - December 2012). The maximum mean length (cm) and weight (g) of the fish were $(20.1 \pm 0.29 \text{ cm})$ and $(93.13 \pm 1.9g)$ in Monsoon season and the lowest mean length $(18.5 \pm 0.58 \text{ cm})$ and weight $(82 \pm 3.60 \text{ g})$ were measured in Pre-monsoon season. Determinations of heavy metals were made by Atomic Absorption Spectrophotometer (AA Analyst). The highest mean concentration of Fe $(496.43 \pm 41.79 \mu g/g)$, Mn $(9.42 \pm 0.81 \mu g/g)$, Zn $(66.22 \pm 7.06 \mu g/g)$, Pb $(2.42 \pm 0.21 \mu g/g)$, and Cu $(14.69 \pm 2.30 \mu g/g)$ were recorded in liver. The lowest mean concentration of Fe $(3.82 \pm 0.91 \mu g/g)$, Mn $(1.41 \pm 0.62 \mu g/g)$, Zn $(1.88 \pm 0.25 \mu g/g)$, Cd $(0.64 \pm 0.16 \mu g/g)$, Pb $(0.35 \pm 0.06 \mu g/g)$, and Cu $(1.69 \pm 0.14 \mu g/g)$ were recorded in fish muscles. Metals concentration didn't vary seasonally and following trends of metal concentrations were observed in various organs of *S. albella*.

 $\begin{array}{ll} Muscles: \ Fe > Zn > Mn > Cd > Pb\\ Liver: \ Fe > > Zn > Cu > Mn > Pb > Cd\\ Gills: \ Fe > Zn > Mn > Cu > Cd > Pb \end{array}$

Introduction

Fish, as human food, are considered as a good source of protein, polyunsaturated fatty acids (particularly omega-3 fatty acids), calcium, zinc, and iron (Chan et al., 1999; Daviglus et al., 2002). Metal residues problems in the fish flesh are, however, very serious owing to the high metal concentrations recorded in the water and sediments (Wong et al., 2001). The major sources of pollution of surface waters include effluent discharges by industries, atmospheric depositions of pollutants and occasional accidental spills of toxic chemicals (Ikem and Egiebor, 2005) and trace metals are regarded as serious pollutants of the aquatic environment because of their toxicity, their persistence, their difficult biodegradability and their tendency to concentrate in aquatic organisms (Ikem and Egiebor, 2005; Schuurmann and Markert, 1998). They enter the marine environment through atmospheric and land-based effluent sources (Islam and Tanaka, 2004). Industrial and agricultural activities also significantly contribute to the accumulation of pollutants in the aquatics including seawater (Jordao et al., 2002). It has been recognized for many years that the concentrations of metals found in coastal areas, whether they are in the dissolved or particulate phases are derived from a variety of anthropogenic and natural sources (Burridge et al., 1999). The major part of the anthropogenic metal load in the sea and sea bed sediments and organisms has a terrestrial source from mining and intensive aquaculture and municipal wastewaters, industrial untreated effluents, harbor activities, urban and agricultural runoff along major rivers and estuaries and bays (Tarra-Wahlberg et al., 2001; Akif et al., 2002).

The degree of heavy metal contamination in some Karachi Seawater fishes is reported to be as *Otolithes ruber* > *Liza vaigiensis* > *Sardinella albella* > *Scomberomorus guttatus* > *Pomadasys olivaecum* (Ali *et al*, 2013). Some of the metals found in the fish might be fundamental as they play vital role in biological system of the fish as well as in human beings but some of them may, however, be toxic and may cause serious damage to the human health if present in excess to the permitted limits. The common heavy metals that are found in fish may include Cu, Fe, Zn, Ni, Mn, Hg, Pb, Cd, etc. from Pakistan waters or elsewhere (Connell, 1984, Rizvi *et al.*, 1988; Tariq *et al.*, 1991, 1998; Nair *et al.*, 1997; Zehra *et al.*, 2003; Agusa *et al.*, 2005, 2007; Dalman *et al.*, 2006; Naidu *et al.*, 2008; Tabinda *et al.*, 2010; Kumar *et al.*, 2012; Shivakumar *et al.*, 2014; El-Moselhy *et al.*, 2014). Ambedkar and Muniyan (2011) found Cr in maximum concentration followed by Cd > Cu > Pb > Zn in the fishes of Vellar River, Tamil Nadu (India) for the samples caught during January to June, 2010. Heavy metals have the tendency to differentially accumulate in various organs of marine organisms, especially fish, which in turn may enter into the human metabolism through consumption causing serious health hazards (Puel *et al.*, 1987). Iron, copper, zinc and manganese are essential (physiological) metals while, mercury, lead and cadmium are xenobiotic toxic metals (Kennish, 1992). Fish has been considered good indicators for heavy metal contamination in aquatic systems because they occupy elevated trophic levels with different sizes and ages

(Burger *et al.*, 2002). The levels of toxic elements in fish are related to age, sex, season and habitat (Kagi and Schaffer, 1998).

The aim of this study was to determine heavy metal Fe, Mn, Zn, Cd, Pb, and Cu concentrations in Sardinella albella fish muscles, liver and gills collected from Balochistan coast during (January 2012-December 2012). It is a small pelagic fish distributed in Indo-West pacific from Red Sea, Persian Gulf, East African coasts, Madagascar, eastwards to Indonesia and the Arabian Sea, North to Taiwan and South to Papua Guinea. Western New Also in and Southern Taiwanese waters, and Panghu (www.discoverlife.org/20/?search+Sardinella+albella). It is a planktivore in Sea and estuaries. It May reach 18 cm in length and have black spot on the origin of dorsal fin (Randall, 1995). Being small in size, it is graded as trash fish but it is nutritionally rich and good if prepared fresh (Chattopadhay et al., 2004). It is eaten by humans in coastal communities and powdered for poultry feed. Sardinella albella is reported to contain protein c $20.2 \pm$ 0.72 % and lipid 1.9 ± 0.10 % (Jayasantha and Patterson (2014). Its feeding habit and length-weight relationship are described by K.V.Sekhran (ND) in his doctoral thesis of Madras University, India.

Materials and Methods

Fish sample of *Sardinella albella* (Valenciennes, 1847) were collected seasonally (Pre-monsoon, Monsoon, Post-monsoon) from Gwadar water of Balochistan coast, Pakistan (Fig. 1 and 1B) during January to December 2012. The length (L) of the fish was measured from the tip of the mouth to the caudal fin (cm). Fish weight was measured after drying with a piece of clean towel. Total length (TL) and body weight (W) were measured with fresh samples to the nearest 0.1 cm and 0.01 g, respectively. Samples were collected for the analysis of heavy metals. Fishes were dissected using steel Scissors and scalpels to remove approximately 5 g dorsal muscles, entire liver and 2 rakers of gills. They were washed with deionized water and weighed after blotting excess surface water. Samples were ground and calcinated at 500°C for 3 hours until 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). The ashes were dissolved in 10 ml (HCl) in beaker and after which the dissolved ash residue was filtered with Whatman filter paper. One ml filtered solution was diluted with 25 ml distilled water for elemental analyses. The standards were prepared from 1000 ppm stock solution to 2 ppm, 4 ppm, 6 ppm, etc. Elemental analysis was made with atomic absorption spectrophotometer (Analyst 700) in the Centralized laboratories of University of Karachi. The concentration of metals was expressed as µg per g dry weight. The data was analyzed with SPSS version 12.

The contents of selected heavy metals in Pakistan Coast and the adjoining waters are presented in Table 1.



Fig. 1A. Map of Pakistan coast showing the study area. Adopted from Khan (1987). The map was originally drawn by Pakistan Generalized Soil Map. III. Draft (1969). Soil Survey Project, Pakistan.



Fig. 1B. An individual of *Sardinella albella* from Pakistan Coast. Source: Hamid Badar Osmani (hamid61612002@yahoo.com). www.fishbase.us / photos

Fe

+

±

0.154

(N=13)

2 - 180

0.0004

0.531

(35.9

0.11**

27.9) ***

2 - 220

0.0034

y metals in Seawater of Arabian Sea / Indian Ocean (in ppm).										
Mn	Cd	Pb	Cr	Reference						
-	0.064 ± 0.16	0.77 ± 0.16	-							
				Ismaili <i>et al</i>						
-	0.057	0.04	-	(2014)						
$0.302 \pm$	0.0133 ± 0.003	0.159 ±	$0.885 \pm$							

0.472

(N =12)

14.13±1.44

0.0002

0.0046

(N = 13)

 4.93 ± 0.77

 4.94 ± 1.33

 4.39 ± 0.824

 6.941 ± 1.92

0.00003

Table 1. Contents of heavy met

(N = 11)

 14.55 ± 4.42

 0.087 ± 0.008

 0.086 ± 0.004

 0.066 ± 0.0018

0.00011

Seawater (ppm) *, mean of six sites; **, mean excluding Bin Qasim sample (312 ppm) and Giddani sample (148 ppm) - heavily Fe-polluted sites; ***, Mean including Port Qasim and Giddani sites; ****, mean for thirteen sites calculated from the data of Mumtaz (2002).

Results and Discussion

Location

Keti Bunder to Giddani

mangroves

unpolluted

Karachi

(ppm) * Miani Hor (ppm)

(ppm) ****

 $(\mu g/L)$

Station I

Station II

Station III

Standard

South coast, India

Indian Ocean (µg/L)

Persian Gulf (ppm)

Thirty six Sardinella albella fish samples were collected from Gwadar, Balochistan coast during (Premonsoon, Monsoon, Post-monsoon) season in (January 2012- December 2012). The fish samples studied for metallic contents varied little in size - from 3.16 to 9.64% in length and 4.49 to 15.68% in weight season. The maximum mean length (20.1 ± 0.29 cm cm) and weight ($93\pm1.19g$ g) of fish were recorded in Monsoon season and the lowest mean length (18.5 \pm 0.17cm) and weight (82 \pm 1.04 g) were measured in Pre-monsoon season (Table 2). There was direct relationship between length and weight (r = 0.90, p < 0.0001) (Table 2).

The heavy metal (Fe, Mn, Zn, Cd, Pb, and Cu) concentration were measured in Muscles, Liver, and Gills of the fish. The highest mean concentration of Fe (496.43 + 41.79 μ g/g), Mn (9.42 + 0.81 μ g/g), Zn (66.22 + 7.06 μ g/g), Pb (2.42 ± 0.23 μ g/g), and Cu (14.69 ± 2.30 μ g/g) were recorded in liver in any season. The lowest mean concentration of Fe $(3.82 + 0.54 \mu g/g)$, Mn $(1.41 + 0.18 \mu g/g)$, Zn $(1.88 + 0.18 \mu g/g)$, Cd $(0.64 + 0.16 \mu g/g)$, Pb $(0.35 + 0.06 \,\mu\text{g/g})$, Cu $(1.69 + 0.14 \,\mu\text{g/g})$ were recorded in fish muscles. The maximum mean concentrations of heavy metals were associated with liver in all seasons. Muscles showed lowest level of concentration throughout the study (Table3, 4 and 5). The metals contents on annual basis are presented in Table 6.

Seasons	Length (cm) (Mean ± SE)	CV (Length)	Weight (g) (Mean ± SE)	CV (Weight)				
		(%)		(%)				
Pre-monsoon	18.5 ± 0.17	3.16	82 ± 1.04	4.49				
Monsoon	20.1 ± 0.29	3.98	93 ± 1.19	4.44				
Post-monsoon	19.5 ± 0.48	9.64	87 ± 3.94	15.68				
Annual	19.6	6.59	88.50	9.45				
Linear relationship: $W = -39.391 + 6.584 L \pm 4.237$								
t = -3.66 $t = 12.01$								
p < 0.001 $p < 0.0001$								
r = 0.90	$r^2 = 0.809$, Adj. $r^2 = 0.809$	= 0.804, F = 1	144.28 (p < 0.0001))				

Table 2. Mean length (L) and weight (W) of S. albella fish collected from Gwadar water.

Comparing the concentrations of metals in various parts of the fish with that of the Seawater (Table 1) it is clear that multi-fold bioaccumulation of metals has taken place in fish at different rates in different organs. Following trends of metal concentrations approximated the metals concentration in various organs of S. albella:

> Muscles: Fe > Zn > Mn > Cd > PbLiver: Fe >> Zn > Cu > Mn > Pb > CdGills: Fe > Zn > Mn > Cu > Cd > Pb

Mumtaz (2002)

et

al.

Kumar

(2013)

(2011)

Gaid (2011)

Khoshnoud et al.

Turekian (1968)

More or less similar Fe-dominated heavy metal trends in different organs of *Megalaspis cordyla* have been reported from Karachi coast, Pakistan (Ahmed, *et al.*, 2014).

Muscles:
$$Fe > Mn > Cd > Pb > Cr$$

Liver: $Fe > > Zn > Cr \approx Cd \approx Pb$
Gills: $Fe > Mn > Cd > Pb > Cr$

The overall metals concentration (($\mu g/g$) sequence irrespective of any season or organ of *S. albella* was as follows:

Fe
$$(160.25) >> Zn (23.05) > Cu (6.562) > Mn (4.989) > Cd (1.515) > Pb (1.394)$$

An order of heavy metals concentrations (Fe > Zn > Cu > Mn > As > Hg > Cd) with Fe being the predominant metal in the fishes of Northeast coast of India has also been reported by Kumar *et al.* (2012). Shivakumar *et al.* (2014) have also given Fe-dominated metallic accumulation sequences in some Indian fishes as follows:

Table 3. Concentration of heavy metals in S. albella during Pre monsoon season.

Organs	Fe (μ g/g) (Mean ± SE)	$\frac{Mn (\mu g/g)}{(Mean \pm SE)}$	$\frac{Zn (\mu g/g)}{(Mean \pm SE)}$	$\begin{array}{c} Cd \ (\mu g/g) \\ (Mean \pm SE) \end{array}$	$\begin{array}{c} Pb \ (\mu g/g) \\ (Mean \pm SE) \end{array}$	$Cu (\mu g/g) (Mean \pm SE)$
Muscles Liver Gills	$\begin{array}{r} 6.93 \pm 0.51 \\ 496.43 \pm 41.79 \\ 37.48 \pm 4.52 \end{array}$	$ \begin{array}{r} 1.64 \pm 0.14 \\ 9.42 \pm 0.81 \\ 3.08 \pm 0.42 \end{array} $	$2.66 \pm 0.36 \\ 50.15 \pm 5.92 \\ 16.64 \pm 1.39$	$\begin{array}{c} 0.70 \pm 0.10 \\ 1.64 \pm 0.14 \\ 1.48 \pm 0.23 \end{array}$	$\begin{array}{c} 0.35 \pm 0.06 \\ 2.42 \pm 0.21 \\ 1.64 \pm 0.14 \end{array}$	$ \begin{array}{r} 1.69 \pm 0.14 \\ 12.85 \pm 1.18 \\ 3.25 \pm 0.37 \end{array} $

Table 4. Concentration of heavy metals in S. albella during monsoon season.

	Fe (µg/g)	Mn (µg/g)	$Zn (\mu g/g)$	Cd (µg/g)	Pb (µg/g)	Cu (µg/g)
Organs	(Mean \pm SE)	$(Mean \pm SE)$	(Mean \pm SE)	(Mean \pm SE)	(Mean \pm SE)	(Mean \pm SE)
Muscles	3.82 <u>+</u> 0.54	2.15 <u>+</u> 0.19	1.88 ± 0.25	1.22 ± 0.17	0.47 ± 0.07	1.79 <u>+</u> 0.22
Liver	305.17 <u>+</u> 34.19	8.63 ± 0.70	66.22 ± 7.06	2.15 ± 0.19	2.42 ± 0.23	11.2 <u>+</u> 2.39
Gills	39.96 ± 4.68	3.08 + 0.86	13.88+1.93	2.28 + 0.25	1.34 + 0.10	2.77 ± 0.24
						_

Table 5. Concentration of heavy metals in S. albella during post monsoon season.

Organa	Fe $(\mu g/g)$	$Mn (\mu g/g)$	$Zn (\mu g/g)$	Cd (μ g/g)	Pb $(\mu g/g)$	$Cu(\mu g/g)$
Organs	$(Mean \pm SE)$	$(Mean \pm SE)$	$(Mean \pm SE)$	$(Mean \pm SE)$	(Mean \pm SE)	$(Mean \pm SE)$
Muscles	7.76 <u>+</u> 0.91	1.41 <u>+</u> 0.18	2.36 <u>+</u> 0.25	0.64 ± 0.16	0.38 <u>+</u> 0.06	2.08 <u>+</u> 0.23
Liver	476.85 <u>+</u> 48.53	8.57 <u>+</u> 0.73	45.64 <u>+</u> 4.48	1.65 <u>+</u> 0.18	1.61 <u>+</u> 0.13	14.69 <u>+</u> 2.30
Gills	45.21 <u>+</u> 5.03	2.24 ± 0.23	12.94 <u>+</u> 1.48	1.59 <u>+</u> 0.16	2.15 <u>+</u> 0.19	2.30 <u>+</u> 0.34

Table 6. Mean concentration of heavy metals	s in various organs of S albella	(data of all seasons pooled)
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Organs	Fe (µg/g)	Mn (µg/g)	Zn (µg/g)	Cd (µg/g)	Pb (µg/g)	Cu (µg/g)
	(Mean \pm SE)	$(Mean \pm SE)$	(Mean \pm SE)	$(Mean \pm SE)$	(Mean \pm SE)	$(Mean \pm SE)$
Muscles	6.16 ± 0.47 a *	1.77 ± 0.10 a	2.50 ± 0.171 a	0.812 ± 0.103 a	0.479 ± 0.048 a	1.975 ± 0.119 a
Liver	433.11± 25.31 b	$9.77\pm0.42~b$	$51.78 \pm 3.51 \text{ b}$	$1.84\pm0.100\ b$	2.077 ± 0.132 b	$14.738 \pm 1.154 \ b$
Gills	41.48\ ± 2.78 c	$3.42 \pm 0.57 \text{ c}$	14.86 ± 0.86 c	1.90 ± 0.316 b	1.624 ± 0.099 c	2.971 ± 0.193 c

*, Figures provided with similar letter in a column are not significantly different.



Fig.2. Comparison of seasonal values of liver: muscle ratio of heavy metals in S. albella.

Table 7. ANOVA for heavy metals data in various organs of fish S. albella captured from Gwadar water.

Source	SS	df	MS	F	р
Seasons	5143.23	2	2571.62	2.114	0.1217 (NS)
organs	2134742.09	5	426948.42	350.91	0.00001
Metals	904471.57	2	452235.79	371.69	0.00001
Seasons x Metals	35433.73	10	3543.37	2.912	0.0014
Seasons x Organs	10066.21	4	2516.55	2.0683	0.0835 (NS)
Metals x Organs	3190816.46	10	319081	26.225	0.00001
Seasons x organs x metals	62618.76	20	3130.94	2.573	0.0002
Error	722717.57	594	1216.70	-	-
Total	7066009.64	647	-	-	-

Metals			Organs			Seasons		
Rank	Metals	Mean	Rank	Organs	Mean	Rank	Seasons	Mean
1	Fe	160.253a	1	Liver	85.553 a	1	Post-Monsoon	35.868 a
2	Zn	23.05 b	2	Gills	11.044 b	2	Monsoon	33.864 a
3	Cu	6.562 c	3	Muscles	2.284 c	3	Pre-Monsoon	29.147 a
4	Mn	4.989 c						
5	Cd	1.514 c		LSD _{0.05} : 6.5	592			
6	Pb	1.954 c					LSD _{0.05} : 6.592	
LSD _{0.05} : 9.322								

Three way ANOVA of the metal data for factors such as seasons, metal types and the organ types indicated no influence of seasons over metal concentration (F = 2.1, p < 0.1217, NS). However, metal concentration was significantly influenced by metal types (F = 371.69, p < 0.0001) and the organ types (F = 350.91, p < 0.0001). There was significant interaction amongst the factors except that there was no significant interaction of seasons with the organ types (Table 7). The predominant metal in S. albella was Fe followed by Zn i.e. Fe >> Zn > Cu> Mn > Cd > Pb. The metal content of liver was much higher than that in gills or the muscles. The metal concentration in the fish didn't vary in the three seasons. The concentration of metals in fish organs are determined primarily by the level of pollution in their environment, in water and food (Farkas et al., 2003). The concentration in muscles and gills reflect the concentrations of metals in the water (Fathi et al., 2013). The maximum mean concentrations of heavy metals were found in liver in all seasons. Liver has also been reported to be the target organ for Cu, Zn and Fe accumulation by El-Moselhy et al., (2014). Pb and Mn were found in higher concentration in the gills by El-Moselhy et al. (2014). Highly increased concentrations of metals in general and iron in particular in liver may represent storage of sequestrated products in this organ (Hamilton and Mehrle, 1986). Iron is physiologically (reversibly) stored in liver as ferritin and hemosiderin (Ahmad, J, < www.fda.gov.ph/...annex% 20J%20....>). Ferritin has the capacity of about 4500 iron (III) ions per protein molecule. This is the major form of iron storage (< http://library.med.utah.edu/ NetBiochem/hi.htm>). The metallic accumulation may depend on seasonal variations also (Deram et al., 2006).

The liver / muscles ratio of metals varied seasonally and were always higher than one – maximally around 63.64 - 90.15 in case of Fe, 4.83-6.06 for Mn, 3.22-5.22 for Pb and 2.33 - 2.61 in case of Cd. The ratios for Fe, Zn was comparatively higher in monsoon season (Fig. 2). In other metals the liver / muscles ratio was relatively much lower and less variant amongst the seasons. Multi-fold accumulation (up to 100 times) of heavy metal in liver is well known (Agusa *et al.*, 2007). Accumulation of Fe in liver by 10-12 folds over muscles is also reported by Ahmed *et al* (2014) in *Megalaspis cordyla* from Karachi waters. The magnitude of such a ratio

should obviously depend upon not only the metallic concentration in the Seawater but also on the intrinsic metabolic characteristics related to metallic bioaccumulation in various organs of a species.

The concentrations of various metals in *Sardinella* spp. from various Coasts of India and Pakistan are outlined in Table 8. Fe in muscles of *Sardinella longiceps* was much higher than that recorded in *S. albella* in

Species	Organ	Fe	Mn	Zn	Cd	Pb	Cu	Reference & Locality
Sardinella. Longiceps μg/g DW ± SD	Muscles Al. canal Gills	$\begin{array}{c} 148.5{\pm}32.0\\ 2255.40{\pm}609\\ 428.39{\pm}68 \end{array}$	ND 6.14±1.1 16.39 ±2.4	$\begin{array}{c} 20.52 \pm 1.8 \\ 63.42 \pm 11 \\ 110.8 \pm 12.0 \end{array}$	-	-	1.54±0.10 6.94±0.41 2.72 ±0.1	Nair <i>et al.</i> (1997) India
Sardinella. Longiceps (ppm)	Muscles Gills Kidneys	-	-	-	0.24 0.33 0.30	0.25 0.33 0.32	1.4 1.62 0.30	Anand and Kumaraswamy (2013), India
Sardinella sindensis μg/g DW ± SD	Muscles	0.104 ± 0.001	-	1.215 ± 0.136	0.031 ± 0.003	0.200 ± 0.002	0.007 ± 0.001	Tabinda <i>et al.</i> (2010) Keti Bunder, Pakistan
Sardinella albella (India) µg/g DW ± SD	Muscles	32.2	3.2	19.30	-	-	-	Jayasantha & Patterson (2014) Tuticorin, India
Sardinella fimbriata Goa (India) ppm	Muscles	2.21	2.59	14.94	-	-	0.59	Sing bal <i>et al.</i> (1982), India
S, albella μg/g DW ± SD	Muscles Liver Gills	$\begin{array}{c} 6.16 \pm 2.79 \\ 433.1 \pm 151.8 \\ 41.48 \pm 16.7 \end{array}$	1.77 ±0.60 9.77 ±2.53 3.42 ±2.14	2.5 ±1.02 51.78 ±21.08 14.86 ±5.16	$\begin{array}{c} 0.81 \pm \! 0.62 \\ 1.835 \pm \! 0.60 \\ 1.897 \pm \! 1.79 \end{array}$	$\begin{array}{c} 0.48 \pm 0.29 \\ 2.08 \pm 0.79 \\ 1.62 \pm 0.60 \end{array}$	1.97 ±0.70 14.74 ±4.9 2.97 ±1.58	Present study

Table 8. Comparison of heavy metals contents in Sardinella spp. reported from Indo-Pak sub-continent.

the present study. It was heavily accumulated in alimentary canal in *S. longiceps* (Nair *et al.*, 1997). As compared to the present studies, Fe in muscles was higher in *S. longiceps* (Nair *et al.*, 1997) and *S. albella* from Tuticorin, India (Jayasantha and Patterson, 2014). Mn and Zn were also higher in concentration in gills of *S. longiceps*. Cu was somewhat higher in liver of *S. albella* (present study) than that in *S. longiceps* (Nair *et al.*, 1997) and *S. fimbriata* (Sing bal *et al.*, 1982). Pb was substantially higher in *S. albella* of Karachi water than that in *S. sindensis* of Keti Bunder (Tabinda *et al.*, 2010) that may presumably be attributed to higher level of pollution of Karachi Seawater (cf. Table 1).

Muscles which are the edible part of the fish showed the lowest level of metals concentration in *S. albella* throughout this study. El-Moselhy *et al.* (2014) have also reported muscles of the fish to possess the lowest concentration of metals. Quite varying concentrations of various heavy metals in fishes as a function of the species or the pollution of their environment have been reported by various authors. The metallic concentration amongst fishes of Cochin (India) varied from species to species. Cu, Zn, Fe and Mn showed increased concentrations in various species were attributed to their varying feeding habit (Nair *et al.*, 1997). According to the studies of Nair *et al.* (1997) Fe was dominant element which was 362.32 ± 70 in muscles, 347.82 ± 88.4 in gills and maximally $833.33 \pm 178 \ \mu g/g$ dry weight in alimentary canal in *M. cordyla* of Cochin. ShivaKumar *et al.* (2014) also showed relatively higher accumulation of metals in intestine and gills. Tuzen (2003) reported Pb levels in the fish of Black Sea in the range of $0.22-0.85 \ mg/kg$ and Uluozlu *et al.* (2007) reported lead in the fish edible tissue in the range of $0.33 - 0.93 \ mg/kg$. *Dicentrarchus labrax*, a fish of Güllük Bay (Aegean Sea, Turkey) was found to contain Pb (< $0.0042 - 0.4 \ mg/kg$) and Cd ($0.01-0.04 \ mg/kg$) - to be within permissible limit (Dalman *et al.*, 2006). Zehra *et al.* (2003) reported Cd ($0.04-0.15 \ \mu g / g$) and Pb ($0.25-0.5 \ \mu g / g$) in *Acanthopagurus berda* from Balochistan coast.

Some heavy metals are health damaging elements. Pb is known to induce reduced cognitive development and intellectual retardation in children and increase blood pressure and cardiovascular disease in adults (Commission of the European; 2002). It may cause learning disabilities, impaired protein and hemoglobin synthesis and shorten the lifespan of red blood cells which leads to severe anemia (hypochromic microcytic anemia) in children (Sultana and Rao, 1998). The most common toxic effects of cadmium in human are renal failure, accumulation in the bone resulting in calcium loss and malfunctioning of peripheral and central nervous system (Schroeder, 1965; Castro-Gonzalez and Méndez-Armenta 2008). Cd proves to be a risk factor for lung disease, kidney dysfunction, skeletal damage and reproductive deficiency (Nordberg, 2003). Gutenmann *et al.* (1988) indicated that a frequently used food safety limit for Cd in food is 2 ppm. In 1993, Food and Agriculture Organization (FAO) reduced the limit for Cd is 0.5 ppm. WHO (1990, 1993) indicated that Cd permissible limit

is 2.0 ppm for seafood and 0.70 ppm for water. Opinions differ regard the residual level of heavy metals in the water and their relation to the residuals level in fish flesh. Kock and Hofer (1998) reported that even low concentrations of heavy metal in the water may result in high concentrations in fish flesh. However, others such as Wong et al. (2001) reported that despite high metal levels in the Seawater and sediments, concentrations of Cd and Pb in fish flesh did not exceed permissible levels. WHO (1990, 1993) indicated that Cu permissible limit is 20 ppm for fish and 2.00 ppm for water. Cu occurs in foods in many chemical forms and has an important role in the physiological activities of living bodies. Abou-Arab et al. (1996) reported Cu residues in sardines and mackerel of 0.086 and 0.077 ppm, respectively. Cu is considered as public health hazard if an abnormal high level of Cu is ingested. Cu may cause Mediterranean anemia, hemochromatosis, liver cirrhosis and Wilson's disease (Underwood, 1977). Abou-Arab et al. (1996) reported mean Pb residue in whole sardines and mackerel of 11.1 and 12.6 ppm, respectively. Hodson et al. (1984) indicated that the Canadian Pb limit of 10 ppm was discontinued, but that the British limit remains at 2 ppm for fish. Abou-Arab et al. (1996) indicated that the FAO limit (1983) is 2.0 ppm. WHO (1990, 1993) indicated that Pb permissible limit is 2.0 ppm for seafood, and 0.50 ppm for water. Industrial and agricultural discharges are the sources of Pb pollution in Iran. Pb is identified as a serious public health problem particularly for children. The adverse toxic effect caused by Pb on human was recognized (Subramanian, 1988). Neurological defects, renal tubular dysfunction, anemia are the most characterized of Pb poisoning (Forstner and Wittmann, 1983). Zinc is known to be involved in most metabolic pathways in humans and zinc deficiency can lead to loss of appetite, growth retardation, skin changes and immunological abnormalities. Zinc is widespread among living organisms, due to its biological significance. The maximum zinc level permitted for fish is 50 mg/kg according to Food Codex (Maff et. al., 1993). The recommended daily intakes of zinc are 15 mg for adult males and 12 mg for adult females. Zinc causes slow growth in children, reduced fertility, dry mouth, headache and nausea (Schroeder, 1965). The United States environmental protection agency and the European Commission (US-EPA and EC) have not considered any standards or limits for the zinc concentrations (Alimentarius, joint FAO / WHO; 1994, Ashraf et. al., 2006). It is clear from the above discussion that the metals such as Cd, Pb, Cu and Mn in edible part of S. albella were within permissible limits.

Per capita fish consumption per year in Pakistan is the lowest in the World (c 2 kg per capita per year or 5.48g per capita per day (Government of Pakistan, Agric. Statistics, MINFAL, Islamabad (seen in Waseem, 2007) which is much lower than that of global estimate of 17 kg per capita per year and very much lower than that in southeast Asia (170 g per capita per day in Malaysia (Agusa *et al.*, 2007). Toxicity due to heavy metals by eating *S. albella* is, therefore, quite unlikely in general terms, in Pakistan but there remains great likelihood of heavy metal toxicity in Pakistan's populations due to heavy pollution of all kinds in the country. There is a great need to investigate heavy metals load in all kinds of fishes or Sea food consumed locally or exported elsewhere.

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(Accepted for publication November, 2014)