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Accumulation of heavy metals (Fe, Zn, Cu, Mn, Cd and Pb) in mullets *Planiliza subviridis* (Valenciennes, 1836) and *Ellochelon vaigiensis* (Quoy & Gaimard, 1825) from Damb Harbor, Balochistan, Pakistan

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Abstract

In this study, muscle samples collected from *Planiliza subviridis* (Valenciennes, 1836) and *Ellochelon vaigiensis* (Quoy & Gaimard, 1825) caught on the Balochistan coast (Damb Harbor) between January and December 2015 (during the northeast monsoon, post-monsoon and pre-monsoon seasons, and the southwest monsoon) were analyzed to determine concentrations of heavy metals: iron, zinc, copper, manganese, cadmium and lead by an atomic absorption spectrophotometer, expressed per unit of dry weight of each sample. The average measured level of Fe, Zn, Cu, Mn, Cd and Pb for *P. subviridis* was 26.70 ± 11.49 μg g^{-1}, 13.82 ± 4.56 μg g^{-1}, 1.66 ± 0.84 μg g^{-1}, 0.24 ± 0.10 μg g^{-1}, 0.06 ± 0.07 μg g^{-1} and 0.17 ± 0.14 μg g^{-1}, respectively. The average level of the same metals for *E. vaigiensis* was 29.26 ± 10.18 μg g^{-1}, 18.85 ± 6.28 μg g^{-1}, 2.18 ± 1.01 μg g^{-1}, 0.32 ± 0.14 μg g^{-1}, 0.25 ± 0.11 μg g^{-1} and 0.30 ± 0.12 μg g^{-1}, respectively. The highest Pb accumulation (0.56 μg g^{-1} and 0.61 μg g^{-1}) detected in *P. subviridis* and *E. vaigiensis* is above the limit value (0.50 μg g^{-1}) reported by FAO, hence the accumulation of Pb in these two fish species should be monitored in the future.

Key words: heavy metals, fish, *Planiliza subviridis*, *Ellochelon vaigiensis*, Balochistan Coast, Damb Harbor

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Introduction

Due to the increasing levels of pollution and its effects on human health, heavy metal pollution has become a serious problem worldwide. Various contaminants are highly toxic and can accumulate in seafood. Thus, the fact that people can be easily exposed to these contaminants means that ultimately human health can also be at risk. When toxic elements are absorbed for a long period of time, they can be highly harmful even at low concentrations (Biswas et al. 2012). Industrial effluents, agricultural runoff, transport, burning of fossil fuels, animal and human excretion, geological weathering, and domestic waste cause pollution of water bodies with heavy metals (Olowu et al. 2010).

Various marine pollution monitoring programs have been initiated due to the fact that marine organisms accumulate contaminants such as metals from the environment (Linde et al. 1998; de Mora et al. 2004). Fish accumulate metals contained in water, their food, seabed sediments and some other particulate material. In many countries, industrial waste, geochemical structure and mining of metals are known to cause heavy metal pollution in the aquatic environment owing to their toxicity and accumulation behavior. Under certain environmental conditions, these heavy metals can accumulate up to toxic concentration levels and cause ecological damage (Sivaperumal et al. 2007).

It should be noted that the level and variety of heavy metals among different fish species depend on their feeding, habits, age, size (including length and weight) and habitats (Amudsen et al. 1997). Contamination caused by heavy metals in animal-based food is a severe threat to human health due to their toxicity, persistence, bioaccumulation, and biomagnification (Kannan et al. 2007; Chary et al. 2008).

Fish can accumulate high concentrations of metals in their tissues, especially in muscles, which makes them a major source of these metals in the human diet (Rose et al. 1999). In general, residents of our coastal areas are very poor and do not have a good sense of food security. It is therefore the responsibility of relevant authorities to focus on this aspect and ensure that food security objectives are incorporated into national poverty reduction strategies. This would have an impact at national, sub-national, household and individual levels, with a particular emphasis on reducing hunger and extreme poverty (FAO 1983).

A large number of commercial fisheries are located in the coastal waters of Balochistan. The fishing pressure on pelagic and demersal fisheries resources has been gradually increasing. However, information on fishing pressure and sustainable stock status is limited and little information on the population dynamics and the status of exploitation in the coastal waters of Pakistan is available. The mullet *P. subviridis* is the cheapest popular food among the population of the coastal area of Balochistan since ancient times. *P. subviridis* is an inhabitant of coastal waters in estuaries and bays (Das 1992).

The objective of this study was to determine concentrations of heavy metals (Fe, Zn, Cu, Mn, Cd and Pb) in the mullets *P. subviridis* and *E. vaigiensis* caught between January and December 2015.

The carnivorous mullets *P. subviridis* and *E. vaigiensis* are important in research on heavy metal accumulation as they are abundantly harvested and are one of the most exported species. In addition, they are commercially important and most often consumed. Therefore, the health risk associated with heavy metals in fish has been assessed using the provisional tolerable weekly intake (PTWI) for both fishes.

Materials and methods

P. subviridis and *E. vaigiensis* samples were collected from the Damb Fish Landing Centre in Sonmiani (Miani Hor, Balochistan) between January and December 2015, during the northeast monsoon (December to February), the post-monsoon season (October and November), the pre-monsoon season (March and April) and the southwest monsoon (May to September), using a Thukri net (length 180–190 m, width 1.5–2.0 m). Fishing was banned on the scheduled days in June and July due to rough weather conditions and in the closed season by the Directorate of Fisheries, the Government of Balochistan. Sonmiani (Miani Hor) is a lagoon located about 10 km north-west of Karachi and mostly on the eastern part of the Balochistan coast between 25°35′N and 66°20′E. It is a 50 km long and 7 km wide contorted body of water, which is connected with the sea through a 4 km wide mouth. Two seasonal rivers enter the lagoon: the Porali River empties into the central part from the northern side and the Winder River enters near the mouth of the lagoon from the eastern side. Sonmiani is one of the important fish landing centers and comprises three villages (Sonmiani, Damb, Bhira and Balochi Goth). Fish samples were transported to a freezer in the laboratory immediately after collection, and then thawed and rinsed in distilled water to remove any foreign particles. The length (cm) and weight (g) of the collected fish were measured. Fish were labelled for identification and then frozen until analyzed.

Following the biometric measurements, approximately 2 g of the epaxial muscle from the dorsal surface of each sample were dissected, washed with distilled water, dried on filter paper, weighed, packed in polyethylene bags and kept at –20°C until analysis. An AAnalyst 700 Atomic Absorption Spectrophotometer was used to perform the analysis in the Centralized Science Laboratory of the University of Karachi. Absorption wavelengths (λ) used for the determination of the analyzed metals were as follows: Fe – 248.30 nm, Zn – 213.90 nm, Cu – 324.70 nm, Mn – 279.50 nm, Cd – 228.80 nm and Pb – 217.00 nm. Due to the absence of a standard reference material, the accuracy of the analysis and the effect of matrices in the media were controlled with the standard addition method using three randomly selected samples for each analyzed element, cut into the smallest possible pieces. The typical limits of detection were as follows: 0.1, 0.018, 0.035, 0.01, 0.025 and 0.012 μg ml^{-1} for iron, zinc, copper, manganese, cadmium and lead, respectively, calculated by regression analysis as suggested by the U.S. Environmental Protection Agency (EPA 2004).

A 1–2 g aliquot of each dry sample was placed in a cylindrical Teflon vessel and digested with 3 ml of a 1:2 v/v mixture of H_2O_2 and HNO_3 at 250°C. The organic part was discarded and the remaining part was diluted with demineralized water to 50 ml in a graduated flask (Bernhard 1976).

Concentrations of metals in *P. subviridis* and *E. vaigiensis* in the muscle tissues in different seasons were determined by analysis of variance (ANOVA) using Tukey's HDS post-hoc comparison method. The results were evaluated on the basis of homogenous groups with a significance level of $p < 0.05$. The common elements in the muscle tissue of *P. subviridis* and *E. vaigiensis* were assessed using Pearson's correlation coefficients. Data collection and statistical calculations were performed using the SPSS software (Ver 22).

Results and discussion

The length and weight (min.–max) of *P. subviridis* and *E. vaigiensis* determined in our samples were in the range of 11.00–23.00 cm and 24.00–82.00 g, and 13.50–26.50 cm and 31.00–106.00 g, respectively (Table 1). Seasonal and average distributions of metal (Fe, Zn, Cu, Mn, Cd and Pb) concentrations are presented in Table 2.

The accumulation of the metals in the muscles of *P. subviridis* and *E. vaigiensis* followed the order: Fe > Zn

Table 1

Seasonal metrics of *P. subviridis* and *E. vaigiensis*

Seasons		*P. subviridis*			*E. vaigiensis*		
		N	Length (cm)	Weight (g)	N	Length (cm)	Weight (g)
northeast monsoon	mean	10	13	30	10	17	46
	SD		23	82		20	66
	min.		16.60	46.40		18.60	56.60
	max		3.64	19.85		1.36	8.72
post-monsoon	mean	12	14	34	12	13.50	31
	SD		19	58		18	42
	min.		16.79	42.25		14.92	35.58
	max		2.03	7.47		1.79	3.90
pre-monsoon	mean	10	11	24	10	16	39
	SD		23	82		24	86
	min.		15.80	41.45		18.15	52.60
	max		3.28	16.33		2.03	12.61
southwest monsoon	mean	12	16	33	12	14	34
	SD		21	74		26.50	106
	min.		17.71	46.67		18.58	57.42
	max		1.97	14.78		5.63	31.58
average	mean	44	11	24	44	13.50	31
	SD		23	82		26.50	106
	min.		16.59	43.09		17.39	49.27
	max		2.73	13.89		3.62	20.13

Std. Deviation – SD

Table 2

Seasonal concentrations in *P. subviridis* and *E. vaigiensis* (µg g^{-1} d.w.)

		P. subviridis						*E. vaigiensis*					
		Fe	Zn	Cu	Mn	Cd	Pb	Fe	Zn	Cu	Mn	Cd	Pb
northeast monsoon	mean	19.44	12.04	1.91	0.26	0.05	0.20	31.31	15.28	2.20	0.32	0.25	0.30
	SD	7.92	3.54	0.91	0.07	0.05	0.07	10.24	2.88	0.92	0.12	0.11	0.13
	min.	12.36	7.08	1.23	0.16	0.00	0.05	15.56	11.98	1.33	0.16	0.03	0.16
	max	42.41	17.65	4.56	0.41	0.14	0.34	46.35	20.49	3.91	0.52	0.42	0.61
post-monsoon	mean	26.07	13.68	1.91	0.22	0.03	0.11	31.27	16.89	2.41	0.38	0.28	0.29
	SD	8.47	4.29	0.79	0.13	0.03	0.07	8.63	5.37	0.71	0.22	0.13	0.12
	min.	17.46	7.08	0.36	0.08	0.00	0.01	21.56	10.39	1.46	0.24	0.04	0.16
	max	38.75	19.32	3.14	0.45	0.08	0.23	48.41	26.16	3.68	0.93	0.51	0.56
pre-monsoon	mean	39.59	12.75	1.81	0.26	0.14	0.35	30.39	24.28	2.37	0.32	0.29	0.31
	SD	9.72	5.63	0.96	0.06	0.10	0.15	13.29	6.038	1.42	0.08	0.10	0.13
	min.	26.03	3.62	1.06	0.16	0.02	0.09	10.72	13.59	0.88	0.22	0.18	0.14
	max	51.68	21.16	3.98	0.34	0.34	0.56	58.66	32.66	5.85	0.51	0.42	0.51
southwest monsoon	mean	23.75	16.61	1.09	0.24	0.03	0.04	24.58	19.55	1.82	0.30	0.21	0.30
	SD	9.93	3.87	0.44	0.13	0.03	0.04	7.85	6.90	0.89	0.10	0.11	0.10
	min.	14.67	10.84	0.19	0.05	0.00	0.01	10.58	8.12	0.36	0.18	0.02	0.12
	max	45.79	24.16	1.63	0.46	0.08	0.16	38.08	28.76	3.10	0.46	0.41	0.48
average	mean	26.70	13.82	1.66	0.24	0.06	0.17	29.26	18.85	2.18	0.32	0.25	0.30
	SD	11.49	4.56	0.84	0.10	0.07	0.14	10.18	6.278	1.01	0.14	0.11	0.12
	min.	12.36	3.62	0.19	0.05	0.00	0.01	10.58	8.12	0.36	0.16	0.02	0.12
	max	51.68	24.16	4.56	0.46	0.34	0.56	58.66	32.66	5.85	0.93	0.51	0.61

Std. Deviation – SD

> Cu > Mn > Pb > Cd. Accordingly, the accumulation of the metals in the muscles of these two species during the northeast monsoon and the post-monsoon season followed the same order of Fe > Zn > Cu > Mn > Pb > Cd (Table 2).

The highest accumulation of Fe and Pb in *P. subviridis* was recorded in the pre-monsoon season. Fe and Zn were found to accumulate in the smallest amounts in the northeast monsoon season. Cu, Cd and Pb were recorded in the smallest amounts in the southwest monsoon season. The highest accumulation of Zn, Cd and Pb in *E. vaigiensis* was determined in the pre-monsoon season. The smallest amounts of Fe, Cu, Mn and Cd were detected in the southwest monsoon season, while the smallest accumulation of Zn was found in the northeast monsoon season.

In the northeast, post-monsoon and southwest monsoon seasons, the concentration of all elements was higher in *E. vaigiensis* than in *P. subviridis*. Only the accumulation of Fe in *P. subviridis* in the pre-monsoon season was higher compared to *E. vaigiensis*, while the accumulated amounts of other elements in the same season were higher in *E. vaigiensis* than in *P. subviridis* (Table 2).

The accumulation of Fe found in the muscles is lower than that reported by Al-Najare (2012) for *P. subviridis* and *E. vaigiensis*. However, it is higher compared to that reported by Al-Khafajy et al. (1997) for *P. subviridis* and *E. vaigiensis*. On the one hand, the Zn values are lower compared to some of the reported data (Mitra & Ghosh 2014; Chakraborty et al. 2016), but on the other, they are higher than those reported by other authors (Al-Khafajy et al. 1997; Ali et al. 2013) for *P. subviridis* and *E. vaigiensis*. The Cu values (Table 3) are lower than those reported in the literature (Al-Khafajy et al. 1997; Al-Najare 2012; Mitra & Ghosh 2014; Chakraborty et al. 2016). However, they are higher than those reported by Ali et al. 2013 for *P. subviridis* and *E. vaigiensis*. The Mn values are lower compared to the published data (Al-Khafajy et al. 1997; Al-Najare 2012) for *P. subviridis* and *E. vaigiensis*. The Cd values are lower than those reported in the literature for *P. subviridis* and *E. vaigiensis* (Al-Khafajy et al. 1997; Al-Najare, 2012; Norouzi et al. 2012; Ali et al. 2013). However, they are higher than those reported by Sai Su et al. (2009) for *P. subviridis* and *E. vaigiensis*. On the one hand, the Pb values (Table 3) are lower compared to some of the reported data (Al-Khafajy et al. 1997; Mitra & Ghosh 2014; Chakraborty et al. 2016), but higher than those reported by Sai Su et al. (2009), Norouzi et al. (2012) and Ali et al. (2013).

There is no difference ($p > 0.05$) between seasonal accumulations of Fe, Cu, Cd and Pb in *P. subviridis*. There is also no difference ($p > 0.05$) between seasonal accumulations of Zn in *E. vaigiensis*. Zn and Mn accumulations were found to be different ($p > 0.05$)

Table 3

Comparison of concentrations in fish tissues reported in the literature

Location	Fish	Metal concentration (μg g^{-1} d.w.)						Reference
		Fe	Zn	Cu	Mn	Cd	Pb	
Arabian Gulf	*L. subviridis*	10.7	7.0	3.7	3.55	0.09	1.36	Al-Khafajy et al. 1997
Manila Bay	*L. subviridis*	-	-	-	-	0.0170	0.0382	Sai Su et al. 2009
Oeshm Island	*L. vaigiensis*	-	-	-	-	0.16	0.11	Norouzi et al. 2012
Iraqi Marine	*L. subviridis*	57		9.5	6.72	5.9	-	Al-Najare 2012
Coastal regions of Karachi	*L. vaigiensis*	-	0.160	0.180	-	0.160	0.007	Ali et al. 2013
Indian Sunderbans S1	*Liza parsia*	-	124.12	75.91	-	4.01	19.89	Mitra & Ghosh 2014
Indian Sunderbans S2		-	94.63	54.39	-	BDL	15.16	
Indian Sunderbans S3		-	61.21	28.12	-	BDL	14.85	
Indian Sunderbans S4		-	27.67	21.01	-	BDL	13.92	
Indian Sunderbans S1	*Liza tade*	-	103.45	43.89	-	1.95	15.77	Mitra & Ghosh 2014
Indian Sunderbans S2		-	91.25	50.38	-	BDL	13.51	
Indian Sunderbans S3		-	53.98	27.91	-	BDL	15.79	
Indian Sunderbans S4		-	25.45	18.65	-	BDL	11.65	
Gangetic Delta Reg. S1	*Liza parsia*	-	102.78	70.11	-	-	13	Chakraborty et al. 2016
Gangetic Delta Reg. S2		-	80.16	49.99	-	-	11.64	
Gangetic Delta Reg. S3		-	56.12	28.46	-	-	9.21	
Gangetic Delta Reg. S4		-	34.66	19.50	-	-	8.43	
Gangetic Delta Reg. S1	*Liza tade*	-	96.41	52,60	-	-	11.23	Chakraborty et al. 2016
Gangetic Delta Reg. S2		-	72.33	42	-	-	10.44	
Gangetic Delta Reg. S3		-	49.87	25.26	-	-	7.4	
Gangetic Delta Reg. S4		-	24.89	15.9	-	-	6	
Balochistan	*L. subviridis*	26.70	13.82	1.66	0.24	0.06	017	This study
	E. vaigiensis	29.26	18.85	2.18	0.32	0.25	0.30	
International limits		100	50	-	1.00	1.00	2.00	WHO (1989)
		-	40	10–100	-	0.50	0.50	FAO (1983)

for *P. subviridis* in all seasons. Fe, Cu, Mn, Cd and Pb accumulations were found to be different ($p > 0.05$) for *E. vaigiensis* in all seasons.

Table 4 demonstrates that there is no high correlation between the metals for *P. subviridis* and *E. vaigiensis*. Cd showed a low correlation with Fe, whereas Pb showed a low correlation with Zn.

Concentrations of Fe, Zn, Cu, Mn, Cd and Pb found in daily fish consumption per capita are calculated to assess a potential health risk to Pakistanis. The average

Table 4

Pearson correlation coefficients between metal concentrations in the muscle tissue of *P. subviridis* and *E. vaigiensis*

Metal	Fe	Zn	Cu	Mn	Cd	Pb
			P. subviridis			
Fe	1.000					
Zn	−0.058	1.000				
Cu	−0.027	−0.122	1.000			
Mn	0.105	0.003	0.094	1.000		
Cd	0.380*	−0.004*	−0.021	−0.184	1.000	
Pb	0.385**	−0.315*	0.106	0.191	0.499**	1.000
			E. vaigiensis			
Fe	1.000					
Zn	−0.025	1.000				
Cu	−0.088	−0.034	1.000			
Mn	−0.144	−0.108	−0.178	1.000		
Cd	0.133	0.031	−0.034	0.229	1.000	
Pb	0.079	−0.022	0.09	0.011	−0.180	1.000

* indicates a significance level of $p < 0.05$. ** indicates a significance level of $p < 0.01$.

daily fish consumption by Pakistanis is 33 g per capita (Chughtai & Mahmood 2012). The Provisional Permissible Tolerable Weekly Intake (PTWI) of Fe, Zn, Cu, Mn, Cd and Pb (for a 60 kg adult person; g/week/60 kg body weight) was 5600, 7000, 3500, 980, 7 and 25, respectively, expressed in g/week/60 kg body weight (FAO/WHO 2004). The heavy metal accumulation in the muscles of *P. subviridis* and *E. vaigiensis* was found to be below nationally and internationally stipulated values and does not pose a serious health risk (Table 5).

Table 5

Estimated daily and weekly intakes for the economically significant fish species consumed by adults in Pakistan

Metal	PTWI*	PTWI[a]	PTDI[b]	*P. subviridis* EWI[c] (EDI)[d]	*E. vaigiensis* EWI[c] (EDI)[d]
Fe	5600	336000	48000.00	881.10 (125.87)	965.58 (137.94)
Zn	7000	420000	60000.00	456.06 (65.15)	622.05 (88.86)
Cu	3500	210000	30000.00	54.78 (7.82)	71.94 (10.28)
Mn	980	58800	8400.00	7.92 (1.13)	10.56 (1.51)
Cd	7	420	60.00	1.98 (0.28)	8.25 (1.18)
Pb	25	1500	214.29	5.61 (0.80)	9.90 (1.41)

* Provisional Permissible Tolerable Weekly Intake (PTWI) in g/week/kg body weight
[a] PTWI – permissible tolerable weekly intake (g/day/60 kg body weight)
[b] PTDI – permissible tolerable daily intake (g/day/60 kg body weight)
[c] EWI – estimated weekly intake in g/week/60 kg body weight
[d] EDI – estimated daily intake in g/day/60 kg body weight

The accumulation of Fe, Zn, Cu, Mn, Cd and Pd in the muscles of *P. subviridis* and *E. vaigiensis* (Table 3) is lower than the international limits (FAO 1983; WHO 1989).

The results of this study show that the accumulation of Fe, Zn, Cu, Mn, Cd and Pd in *P. subviridis* and *E. vaigiensis* caught at the Balochistan coast was generally below the international limits. The present study shows that the largest Pb accumulation (0.56 μg g^{-1} and 0.61 μg g^{-1}) detected in *P. subviridis* and *E. vaigiensis* is higher than the limit value (0.50 μg g^{-1}) reported by FAO (1983). The accumulation of Pb in these two fishes should be monitored in the future. Otherwise, the pollutants may be harmful to the health of the fish populations and humans who consume them.

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