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Assessment of heavy metals in *Oratosquillina interrupta* and *Miyakella nepa* (Crustacea: Stomatopoda: Squillidae) from Korangi Fish Harbor, Karachi, Pakistan

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Abstract: Scanty information is available on the accumulation of heavy metals in mantis shrimps (stomatopods). Present study is a pioneer attempt from Pakistan to determine heavy metal contents in two species (*Oratosquillina interrupta* and *Miyakella nepa*). This study focused on a comparative analysis of heavy metals concentrations (Fe, Mn, Pb, Hg, Cu, Zn and Cd) in these stomatopods from the Korangi Fish Harbor (Karachi, Pakistan) from February 2019 to December 2019. All samples were analyzed by using the AAnalyst 700 Flame Atomic Absorption Spectrophotometer. It was observed that the average concentrations (µg.g^{-1}) of Fe, Mn, Pb, Hg, Cu, Zn and Cd in muscle were, respectively: 4.308 ± 1.078, 0.352 ± 0.155, 0.022 ± 0.021, 0.001 ± 0.002, 1.373 ± 0.625, 1.943 ± 0.700 and 0.035 ± 0.031 µg g^{-1} for *O. interrupta*. For the same metals sequence in *M. nepa* these values in muscle were 4.342 ± 2.051, 0.507 ± 0.239, 0.036 ± 0.033, 0.004 ± 0.006, 0.407 ± 0.228, 1.852 ± 0.672 and 0.032 ± 0.031 µg g^{-1}, respectively. Metal accumulations in muscle tissues of these species from Korangi Fish Harbour did not exceed limit values. However, with the increase in daily seafood consumption, these results are moving towards the point of danger.

Résumé: *Évaluation des métaux lourds dans* Oratosquillina interrupta *et* Miyakella nepa *(Crustacea : Stomatopoda : Squillidae) de Korangi Fish Harbour, Karachi, Pakistan.* Peu d'informations sont disponibles sur l'accumulation de métaux lourds dans les crevettes mantes (stomatopodes). La présente étude est une tentative pionnière du Pakistan pour déterminer les teneurs en métaux lourds chez deux espèces (*Oratosquillina interrupta* et *Miyakella nepa*). Cette étude a porté sur une analyse comparative des concentrations de métaux lourds (Fe, Mn, Pb, Hg, Cu, Zn et Cd) dans ces stomatopodes du port de pêche de Korangi (Karachi, Pakistan) de février 2019 à décembre 2019. Tous les échantillons ont été analysés en utilisant le spectrophotomètre d'absorption atomique de flamme AAnalyst 700. Il a été observé que les concentrations moyennes (µg.g^{-1}) de Fe, Mn, Pb, Hg, Cu, Zn et Cd dans le muscle étaient respectivement : 4,308 ± 1,078, 0,352 ± 0,155, 0,022 ± 0,021, 0,001 ± 0,002, 1,373 ± 0,625, 1,943 ± 0,700 et 0,035 ± 0,031 µg g^{-1} pour *O. interrupta*. Pour la même séquence de métaux dans *M. nepa*, ces valeurs dans le muscle étaient de 4,342 ± 2,051, 0,507 ± 0,239, 0,036 ± 0,033, 0,004 ± 0,006, 0,407 ± 0,228, 1,852 ± 0,672 et 0,032 ± 0,031 µg.g^{-1}, respectivement. Les accumulations de métaux dans les tissus musculaires de ces espèces du port de pêche de Korangi n'ont pas dépassé les valeurs limites. Cependant, avec l'augmentation de la consommation quotidienne de fruits de mer, ces résultats se dirigent vers le point de danger.

Keywords: Heavy metal ● Stomatopods ● Korangi Fish Harbor ● *Oratosquillina interrupta* ● *Miyakella nepa* ● Pakistan

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Introduction

Heavy metal contamination in aquatic environment is an ever growing major threat globally. Untreated industrial and agriculture discharges containing toxic and hazardous substances are the main source of heavy metal contamination in aquatic ecosystems. These substances, particularly trace metals both essential and non-essential, can have carcinogenic, cytotoxic and mutagenic effects on aquatic organisms. These trace metals are taken up by invertebrates and accumulate in their bodies as they are not biodegradable. Heavy metals must be controlled in food sources in order to assure public safety (WHO, 1995). Etiologically, numerous diseases, especially cardiovascular, renal, neurological, and bone diseases, are associated with excessive concentrations of heavy metals in food (Chailapakul et al., 2008).

The mantis shrimps are hard shelled and economically important marine organisms, which belong to the order Stomatopoda within the subphylum Crustacea. In terms of fisheries, stomatopods are important resources, especially in Asia (Lui et al., 2007). Being a good source of trace minerals, these shrimps are commonly used as raw materials for production of fishmeal, poultry feed and pet food, although trace and heavy metals accumulate in their different organs (Murthy et al., 2015). There are many sources of metals and different routes of metal uptake in aquatic invertebrates including a direct contact with water and sediment, branchial hemostasis or food absorption (Luoma & Rainbow, 2008; Duarte et al., 2017). Seafood is widely used as one of the marine pollution bioindicators by metals (Abdel-Salam, 2013; Evans et al., 1993). It has been documented that marine organisms can be used as bioindicators of metal pollution in aquatic environments and for the definition of toxicological threshold with regard to human consumption (Yılmaz & Yılmaz, 2007; Yılmaz et al., 2010; Abdel-Salam & Hamdi, 2011; Mitra et al., 2012; Viswanathan et al., 2013).

In Pakistan, the stomatopods are represented by 7 families and 20 species. According to Ghory (2013), *Miyakella nepa* (Latreille in Latreille, Le Peletier, Serville & Guérin, 1828) is the only known Pakistani species of the genus *Miyakella.* Few studies on heavy metals were reported on stomatopods (Blasco et al., 2002; Wardiatno et al., 2012; Murthy et al., 2015; Anandkumar et al., 2017; Candra et al., 2019). There has been no study conducted to investigate heavy metal accumulation on *Oratosquillina interrupta* (Kemp, 1911) and *M. nepa* from Karachi coast in Pakistan. Therefore, this is the first attempt to report heavy metal accumulation in two commercially important species of mantis shrimp in Pakistan. Although, mantis shrimp is commercially exploited and consumed in many countries, it is not used for human consumption in Pakistan at the moment and it is only utilized as fish meal.

The aim of the present study was to determine heavy metal (Fe, Mn, Pb, Hg, Cu, Zn and Cd) concentration in muscles of *O. interrupta* and *M. nepa* collected seasonally from Korangi Fish Harbor, Karachi Pakistan.

Materials and Methods

Mantis shrimp are abundantly observed in fish harbours as a by-catch and thrown away. Sampling was done randomly at the Korang Fish Harbour, Karachi. Twenty-four specimens of *O. interrupta* (Total length (TL) = 5.0 to 10.5 cm; wet weight (WW) = 6.00 to 21.00 g) and 24 specimens of *M. nepa* (TL = 5.6 to 11.0 cm; WW = 6.32 to 20.79 g) were collected at three different seasons: Pre-monsoon (February-March), Monsoon (August-September) and Post-Monsoon (November-December) in 2019. The collected samples were stored in crushed ice and immediately brought to the laboratory, where they were washed with tap water and sorted for further study.

AAnalyst 700 Atomic Absorption Spectrophotometer was used to perform the analysis in the Centralized Science Laboratory of the University of Karachi. The absorption wavelengths (λ, in nm) which were used for the determination of the analyzed metals were as follows: Fe: 248.30; Mn: 279.50; Pb: 217.00; Hg: 253.65; Cu: 324.70; Zn: 213.90; and Cd: 228.80 nm. Due to the lack of reference standard material, accuracy of the analysis and the effect of the matrices in the media were controlled with the standard addition method. All studied elements were tested with standard addition method for 3 randomly selected samples. Approximately 20 mL filtered water and fish sample solution was taken for heavy metal analysis. Typical detection limit was 0.1, 0.01, 0.012, 0.01, 0.035, 0.018 and 0.025 $\mu g.mL^{-1}$ for Fe, Mn, Pb, Hg, Cu, Zn and Cd respectively, and were calculated by regression analysis as suggested by the US Environmental Protection Agency. An aliquot of 1-2 g of each dry sample was placed in 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 was diluted with demineralized water to 50 mL in a graduated flask (Bernhard, 1976).

Seasonal variations in heavy metal concentrations in *O. interrupta* and *M. nepa* were assessed by carrying out a one-way analysis of variance (ANOVA). Prior to ANOVA, normality (Shapiro-Wilk test) and homoscedasticity (Levene test) were tested. These tests demonstrate that the hypothesis of normality and homogeneity of variances can be accepted. For metals presenting a significant difference among seasons, the Tukey's HDS post-hoc comparison method was used to identify which seasons were significantly different from the others at a level of 5%. Relationships in metal concentrations in the muscle tissues of both mantis shrimps were assessed using the Pearson's correlation coefficients. Statistical calculations were performed using SPSS software (Ver. 24).

Fe, Mn, Pb, Hg, Cu, Zn and Cd concentrations per capita as daily seafood consumption were calculated to assess potential health risks for Pakistanis. Estimated Weekly Intake (EWI) was calculated by multiplying the average mass of weekly consumed fish (g) by the average metal concentrations (μg g^{-1}). The Estimated Daily Intake (EDI) was equal to one seventh of the EWI. One-seventh of the tolerable weekly intake is Permissible Tolerable Daily Intake (PTDI).

Results and Discussion

Total length, wet weight and heavy metal concentrations in *O. interrupta* and *M. nepa* were given in Tables 1 and 2 respectively.

Metal accumulation in the muscles was observed to follow the order Fe > Cu > Zn > Mn > Cd > Pb > Hg in pre-monsoon, Fe > Zn > Cu > Mn > Cd > Pb > Hg in monsoon and Fe > Zn > Cu > Mn > Cd > Pb > Hg in post monsoon for *O. interrupta*. Metal accumulation in the muscles was observed to follow the order Fe > Zn > Cu > Mn > Pb > Cd > Hg in pre-monsoon, Fe > Zn > Mn > Cu > Cd > Pb > Hg in monsoon and Fe > Zn > Mn > Cu > Pb > Cd > Hg in post monsoon for *M. nepa*. It has been determined that Mn accumulated more in *M. nepa* than in *O. interrupta* (Table 2). In general, Hg accumulation in the muscle tissues of both mantis shrimps occurred with the lowest concentrations, whereas the highest concentrations were recorded for Fe.

Abdel-Salam & Hamdi (2014) revealed that mantis shrimps are more vulnerable to metal pollution in comparison to other marine invertebrates (shrimps, crabs and cephalopods) in which higher heavy metal concentrations were recorded. Due to the lack of data in the literature about *O. interrupta* and *M. nepa*, comparisons were made with data which have been published for others crustacean species (Table 3).

The amount of Cu value found in the muscle of *O. interrupta* in the present study was higher than those reported by Castañeda-Chavez et al. (2014) in *Litopenaeus setiferus* (Linnaeus, 1767) in the Gulf of Mexico. However, all metal accumulation values of *O. interrupta* were lower than the data provided by other studies (Blasco et al., 2002; Raissy et al., 2011; Bat et al., 2013; Anandkumar et al., 2017) (Table 3). For *M. nepa*, all metal concentrations were found to be lower than those already observed for other crustaceans (Table 3). However, the metal accumulation in this species should be monitored, since *M. nepa* is used as a fishmeal (Bijoy et al., 2016) and consumed either directly or indirectly.

The one-way ANOVA analysis was applied to examine the variations in heavy metal concentration, length and weight among different seasons in *O. interrupta* and *M. nepa*. For *O. interrupta*, significant differences among seasons were reported only for Fe, Mn, length and weight ($p < 0.05$). Tukey HSD tests

Table 1. Biometric analysis (TL, total length and WW, wet weight) of the two mantis shrimps (*Oratosquillina interrupta* and *Miyakella nepa*) collected at Korangi Fish Harbor (Karachi, Pakistan).

Species	Season	TL (cm)		WW (g)	
		Mean ± SD	Min-Max	Mean ± SD	Min-Max
O. interrupta	Pre-Monsoon	7.262 ± 1.767	5.00-9.50	1.482 ± 5.658	6.00-19.86
	Monson	8.312 ± 0.883	7.00-9.00	14.466 ± 3.989	8.00-18.02
	Post-Monsoon	8.362 ± 0.934	7.50-10.50	15.785 ± 5.008	9.00-21.00
	All Seasons	7.979 ± 1.312	5.00-10.50	13.911 ± 5.059	6.00-21.00
M. nepa	Pre-Monsoon	7.350 ± 0.922	6.00-8.30	10.300 ± 2.748	7.00-14.60
	Monson	6.862 ± 1.114	5.60-9.00	9.407 ± 3.926	6.32-18.59
	Post-Monsoon	9.175 ± 1.293	7.50-11.00	15.630 ± 4.751	9.60-20.79
	All Seasons	7.795 ±1.476	5.60-11.00	11.779 ± 4.662	6.32-20.79

Table 2. Metal concentrations (mean ± standard deviation and range) of the two mantis shrimps (*Oratosquillina interrupta* and *Miyakella nepa*) in distinct seasons (I, pre-monsoon season; II, monsoon season; III, post-monsoon season; and IV, all seasons) collected at Korangi Fish Harbor (Karachi, Pakistan).

Species	Seasons	Fe	Mn	Pb	Hg	Cu	Zn	Cd
O. interrupta	I	**4.201 ± 0.821**	**0.280 ± 0.103**	**0.011 ± 0.009**	**0.000 ± 0.000**	**1.740 ± 0.737**	**1.447 ± 0.509**	**0.252 ± 0.218**
		(3.250-5.630)	(0.110-0.450)	(0.000-0030)	(0.000-0.002)	(0.330-2.630)	(0.520-2.100)	(0.000-0.070)
	II	**4.762 ± 1.523**	**0.456 ± 0.106**	**0.027 ± 0.023**	**0.002 ± 0.002**	**0.921 ± 0.380**	**2.145 ± 0.534**	**0.045 ± 0.044**
		(2.720-6.830)	(0.320-0.650)	(0.010-0.080)	(0.000-0.008)	(0.410-1.360)	(1.410-2.860)	(0.001-0.140)
	III	**3.961 ± 0.659**	**0.321 ± 0.193**	**0.029 ± 0.025**	**0.001 ± 0.001**	**1.460 ± 0.447**	**2.237 ± 0.800**	**0.037 ± 0.025**
		(3.160-5.220)	(0.180-0.680)	(0.013-0.090)	(0.000-0.004)	(0.880-2.290)	(1.250-3.660)	(0.010-0.080)
	IV	**4.308 ± 1.078**	**0.352 ± 0.155**	**0.022 ± 0.021**	**0.001 ± 0.002**	**1.373 ± 0.625**	**1.943 ± 0.700**	**0.035 ± 0.031**
		(2.720-6.830)	(0.110-0.680)	(0.000-0.090)	(0.000-0.008)	(0.330-2.630)	(0.520-3.660)	(0.000-0.140)
M. nepa	I	**2.499 ± 1.279**	**0.299 ± 0.119**	**0.043 ± 0.045**	**0.005 ± 0.010**	**0.374 ± 0.240**	**1.885 ± 0.515**	**0.024 ± 0.025**
		(1.160-4.670)	(0.110-0.480)	(0.010-0.140)	(0.000-0.030)	(0.130-0.870)	(1.110-2.670)	(0.001-0.060)
	II	**5.728 ± 1.735**	**0.633 ± 0.234**	**0.026 ± 0.023**	**0.003 ± 0.002**	**0.360 ± 0.205**	**1.696 ± 0.984**	**0.046 ± 0.041**
		(3.890-9.430)	(0.260-0.970)	(0.010-0.080)	(0.001-0.008)	(0.130-0.580)	(0.560-3.560)	(0.006-0.100)
	III	**4.799 ± 1.693**	**0.589 ± 0.209**	**0.038 ± 0.031**	**0.004 ± 0.003**	**0.488 ± 0.246**	**1.976 ± 0.454**	**0.025 ± 0.021**
		(2.360-7.200)	(0.230-0.870)	(0.010-0.090)	(0.000-0.010)	(0.160-0.920)	(1.270-2.590)	(0.001-0.061)
	IV	**4.342 ± 2.051**	**0.507 ± 0.239**	**0.036 ± 0.033**	**0.004 ± 0.006**	**0.407 ± 0.228**	**1.852 ± 0.672**	**0.032 ± 0.031**
		(1.160-9.430)	(0.110-0.970)	(0.010-0.140)	(0.000-0.030)	(0.130-0.920)	(0.560-3.560)	(0.001-0.100)

Table 3. Comparison of mean concentrations of metals (Fe, Mn, Pb, Hg, Cu, Zn and Cd) in crustacean species.

Area	Species	Metal concentration (μg g^{-1})							Reference
		Fe	Mn	Pb	Hg	Cu	Zn	Cd	
Persian Gulf	*Panulirus homarus*	-	-	0.63	0.05	-	-	0.25	Raissy et al. (2011)
Samsun Coast	*Crangon crangon*	30-58	6-15	0.29-0.49		5.85-14.77	18-36	0.23-0.48	Bat et al. (2013)
Gulf of Mexico	*Litopenaeus setiferus*	-	-	0.059	-	0.516	-	N.D.	Castañeda-Chavez et al. (2014)
Miri coast	*Parapenaeopsis sculptilis*	-	51.3	1.6	-	72.8	130.8	0.1	Anandkumar et al. (2017)
	Litopenaeus vannamei	-	31.7	3.0	-	51.3	72.4	0.1	
	Penaeus merguiensis	-	47.0	2.9	-	80.8	104.2	0.1	
	Harpiosquilla harpax	-	7.3	2.5	-	37.4	203	2.1	
	Argulus indicus	-	7.4	7.2	-	32.4	62.4	1.2	
Gulf of Cádiz	*Squilla mantis*	77.89	4.24	0.19	-	135.47	134.97	1.66	Blasco et al. (2002)
This study	***O. interrupta***	**4.308**	**0.353**	**0.023**	**0.001**	**1.374**	**1.943**	**0.036**	**Present study**
	M. nepa	**4.342**	**0.507**	**0.036**	**0.004**	**0.407**	**1.852**	**0.032**	
International Limits	**General**	**100**	**1**	**2**	**1.5**	**30**	**100**	**1**	**WHO (1989)**
		-	**-**	**0.5**		**10-100**	**30-100**	**0.5**	**FAO (1983)**
		-	**-**	**0.3**	**1**	**-**	**-**	**0.50**	**EC (2008)**

showed that that there were seasonal variations between Pre Monsoon - Monsoon and Pre Monsoon - Post Monsoon in Fe and Mn concentrations. On the other hand, seasonal differences were also detected between Pre Monsoon – Post Monsoon and Monsoon – Post Monsoon for length and weight. For *M. nepa,* seasonal differences were observed for Cu and Zn ($p < 0.05$). Tukey HSD tests showed that Cu concentrations varied significantly only between between Pre Monsoon and Monsoon. These tests were not able to detect significant differences between pairs of season for Zn although marginal differences were observed between Post Monsoon and Pre Monsoon ($p = 0.051$). No significant seasonal change in length and weight was reported for this later species.

No significant correlation was found between metal concentrations in muscle tissues of *O. interrupta* and *M. nepa* (Table 4). However, there was a significant correlation between the Cd concentration and the individual length and weight for *O. interrupta* at a level of 5% (Table 4).

The increase of metal concentrations in fish is reported to be related to species location and distribution, the local environmental characteristics (e.g. temperature), the biological characteristics of the fish species (e.g. feeding habits, age, size), the duration of exposure to metals, and the homeostatic regulation activity (Sankar et al., 2006). Fe, Mn, Pb, Hg, Cu, Zn and Cd concentrations per capita as daily seafood consumption were calculated to assess the potential health risks for Pakistanis.

Average daily fish and seafood consumption in Pakistan is 1,720 g per capita (Data, 2020). Provisional Permissible Tolerable Weekly Intake (PTWI) for a 60 kg adult person, expressed in $\mu g.week^{-1}$, was calculated as 5,600, 980, 25, 4, 3,500, 7,000 and 7 for Fe, Mn, Pb, Hg, Cu, Zn and Cd respectively (FAO/WHO, 2004). The heavy metal concentrations in muscles of *O. interrupta* and *M. nepa* was found to be below international thresolds (Table 5). However, estimated daily intakes of Cd for *O. interrupta* and Hg for *M. nepa* appeared to be close to the hazardous limits.This study was conducted with focus on the accumulation of some heavy metals in mantis shrimps. The results of this study showed that Fe, Mn, Pb, Hg, Cu, Zn and Cd concentrations in *O. interrupta* and *M. nepa* caught from Karachi Fish Harbor were generally below the international limits.

Table 4. Pearson correlation coefficients for the relationships between the concentrations of different metals in *O. interrupta* and *M. nepa* in the muscle tissues, obtained from Korangi Fish Harbor (Karachi, Pakistan). * correlation is significant at the 0.05 level; ** correlation is significant at the 0.01 level; a = cannot be computed (variables is constant), L = Length, W = Weight.

	Fe	Mn	Pb	Hg	Cu	Zn	Cd	L	W
					O. interrupta				
Fe	1.000								
Mn	0.368	1.000							
Pb	-0.018	-0.015	1.000						
Hg	a	a	a	1.000					
Cu	0.035	-0.217	-0.288	a	1.000				
Zn	-0.002	-0.052	0.305	a	-0.040	1.000			
Cd	0.176	-0.171	0.138	a	-0.193	0.221	1.000		
L	-0.089	-0.094	0.157	a	-0.073	0.375	0.496*	1.000	
W	-0.201	-0.290	0.267	a	-0.247	0.367	0.587**	0.889**	1.000
					M. nepa				
Fe	1.000								
Mn	0. 287	1.000							
Pb	0.166	-0.044	1.000						
Hg	a	a	a	1.000					
Cu	-0.242	0.209	-0.070	a	1.000				
Zn	-0.155	-0.19	0.089	a	0.170	1.000	-0.344		
Cd	0.199	0.133	-0.103	a	-0.264	-0.344	1.000		
L	-0.164	0.289	-0.026	a	0.239	0.006	0.110	1.000	
W	-0.165	0.299	-0.031	a	0.248	0.115	0.110	0.961**	1.000

Table 5. Estimated daily and weekly intakes for the economically significant mantis shrimp species consumed by adults in Pakistan. Where: PTWI*, provisional permissible tolerable weekly intake (in µg/week, according FAO/WHO, 2004); PTDI[a], permissible tolerable daily intake (in µg.day^{-1} for a 60 kg adult); EWI[b], estimated weekly intake (in g.week^{-1} for a 60 kg adult); and EDI[c], estimated daily intake (in g.day^{-1} for a 60 kg adult).

			O. interrupta	*M. nepa*
	PTWI*	**PTDI[a]**	**EWI[b] (EDI)[c]**	**EWI[b] (EDI)[c]**
Fe	5.600[a]	336.000	7.410 (1.059)	7.468 (1.067)
Mn	980[a]	58.800	607 (87)	872 (125)
Pb	25[a]	1.500	40 (6)	62 (8.85)
Hg	4	28	2 (0.25)	7 (1)
Cu	3.500[a]	210.000	2.363 (338)	700 (100)
Zn	7.000[a]	420.000	1.722 (246)	3.185 (455)
Cd	7[a]	420	62 (8.85)	55 (8)

However, with the increase in daily seafood consumption, these results are moving toward the point of danger. It is estimated that intake values may trigger problems in case of excessive consumption. Obviously, due to the subsequent industrialization, the entry of toxic effluents is increasing the risk of metal contamination in the environment which results in further entry into humans through seafood consumption. This study showed that effective measures should be taken to prevent metal pollutants in the Karachi Coast in the future. Corrective measures and prompt actions are inevitable from all the stakeholders for check/control the heavy metal contamination in the coastal areas.

The authors declare that they have no conflict of interest. This paper was written during the COVID-19 pandemic while quarantined at home.

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