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HEAVY METAL ACCUMULATION IN SEA CUCUMBER ACTINOPYGA MAURITIANA (ECHINODERMATA-HOLOTHUROIDEA) FROM CAPE MONZE BEACH KARACHI, PAKISTAN

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ABSTRACT: In the present study sea cucumber *Actinopyga mauritiana*, water and sediment samples collected during September to December 2018. Six heavy metals (Fe, Cu, Mn, Zn, Pb, and Cd) were analyzed in tissues (muscles, tentacles and guts), water and sediment by atomic absorption spectrophotometer (AAS). The average measured levels of heavy metals Fe, Cu, Mn, Zn, Pb and Cd in *A. mauritiana* muscle, tentacle and gut was 42.20 ± 9.47, 3.14 ± 0.48, 0.38 ± 0.22, 11.42 ± 4.34, 0.03 ± 0.00 and 0.02 ± 0.00; 36.90 ± 8.14, 3.22 ± 0.60, 0.40 ± 0.27, 10.50 ± 1.70, 0.04 ± 0.05 and 0.03 ± 0.02; 21.54 ± 5.06, 2.32 ± 0.66, 0.53 ± 0.16, 13.13 ± 2.90, 0.02 ± 0.02 and 0.02 ± 0.01 μg g^{-1} respectively. It is useful to monitor all metal accumulation in the future.

KEYWORDS: *Heavy metals, sea cucumber, A. mauritiana, Cape Monze Beach, Karachi*

INTRODUCTION

Heavy metals are known to be stable and persistent environmental contaminants, as they cannot be degraded or destroyed and they tend to accumulate in soil and sediments. Excessive levels of heavy metals in sediments affect marine biota and pose a risk to human health due to consumption of seafood (Mucha *et al.*, 2003; Zhang *et al.*, 2007). Heavy metals such as zinc, mercury, copper, cadmium and lead accumulate in the body of many marine animals (Riley and Segar, 1970). Commercially important mollusks, teleosts and crustaceans are being studied for the bioaccumulation of heavy metals (Xing and Chia, 1997).

Sea cucumbers belong to class Holoturoidea phylum Echinodermata appeared in the oceans during the evolution of the ocean 540 million years ago. Today, 1975 seafood species have been identified and reported in the world's seas (FDA, 2020). *A. mauritiana* is commonly found in the high-wave energy habitats of intertidal and tidal reefs in the Indian Pacific (Hopper *et al.*, 1998). The habitats of this species found approximately 1–3 m deep and are subject to strong waves and currents (Conand 1990, 1993; Hopper *et al.* 1998; Graham and Battaglene, 2004).

Haque (1969) reported *A. mauritiana* (surf redfish) for the first time from Cape Monze near Karachi, Pakistan. Clark and Rowe (1971) and Munir and Almas (2005) also reported this species from Pakistan’s coast but with no mention of a specific location.

Tahera (1996) and Tahera and Kazmi (2005), reported it from intertidal areas, where it was found hiding under small rocks exposed to surge and currents.

Sea cucumbers are considered a traditional medicine, delicacies and aphrodisiacs. Sea cucumbers offer high nutritional value due to their high protein, low fat content, and amino acid profile and are a rich source of trace elements (Wen *et al.*, 2010). Some studies have been conducted on metal accumulation of sea cucumbers, sediments and water (Siddique *et al.*, 2009; Trianni and Tenorio, 2011; Givianrad *et al.*, 2014; Jinadasa *et al.*, 2014; Hashmi *et al.*, 2014; Jiang *et al.*, 2015; Li *et al.*, 2016; Mohammadizadeh *et al.*, 2016; Ahmed *et al.*, 2017; Mohsen *et al.*, 2019), bioactive metabolites (Gao *et al.* 2015; Marques *et al.* 2016; Cuong *et al.* 2017) and elemental composition (Wen and Hu, 2010). Haider *et al.* (2015) evaluated the mineral contents of chromium, nickel, manganese, copper, lead, cadmium, zinc, sodium, potassium, calcium and magnesium in *H. arenicola* and *A. mauritiana* from Karachi coastal waters of Pakistan.

The aim of this study was to determine the heavy metal concentration (Fe, Cu, Mn, Zn, Pb and Cd) in commercially important sea cucumber species *A. mauritiana* muscle, tentacle, water and sediments from Cape Monze beach Karachi Pakistan. The present study was designed to assess this possibility by examining the heavy metals in the muscles, tentacles and guts.

MATERIALS AND METHODS

Study site and sample collection: The rocky ledge at Cape Monze is located at 24° 49' 35.0" N 66° 39' 53.0" E. The intertidal area at rocky ledge measures approximately 1000 m long and 120 m wide at 0.0 m low tide. The Cape Monze a high cliff projecting into the Arabian Sea also referred as Ras Mauri and is an example of raised beaches along the coast of Karachi (Fig. 1). The area is a continuation of ridges of hills composed of alternating beds of limestone, clay and sandstone. Deposits are derived from Gaj /Manchar formations of lower Miocene to Middle Miocene / Upper Miocene to Pliocene epochs. The ledge comprises of hard conglomerate and shale cliffs with unconsolidated sandy clays exposed to coastal weathering and erosion.

Sea cucumber samples (*A. mauritiana*) were collected from the intertidal region by hand-picking through forceps at low tide in September to December 2018. The samples obtained within the scope of the study were taken into containers filled with water and kept alive. Samples of sea cucumber were taken to the laboratory and transferred to well aerated aquaria.

Sample preparation and Chemical analysis: Length (mm) and weight (g) data were collected for each sea cucumber after allowing the sea cucumber to relax in water for 5 min. Total length from mouth to anus was measured to the flexible ruler. Wet weight was measured to the nearest 0.01 g immediately after removing the animal from the water to avoid evisceration. Sea cucumbers dissected and remove muscles, tentacles and gut for analysis of heavy metals. Tissues weighted (g) and chopped into small pieces and then ground and calcinated at 450 °C for 4 h. Sea cucumber Ash samples of each specimen were weighed (g), dissolved in HCl (0.1 mol/L) and further treated with H_2O_2 (30%) till lucid solutions were formed, and then diluted by water. The 0.45 μm Whatman filter papers were chosen for the filtration purposes. Analysis was performed in the laboratory using the AAnalyst 700 Atomic Absorption Spectrophotometer. The specimens were preserved in 5% neutralized formaldehyde and later transferred in to 70% ethyl alcohol and deposited in the repository of Marine Reference Collection and Resource Centre, University of Karachi.

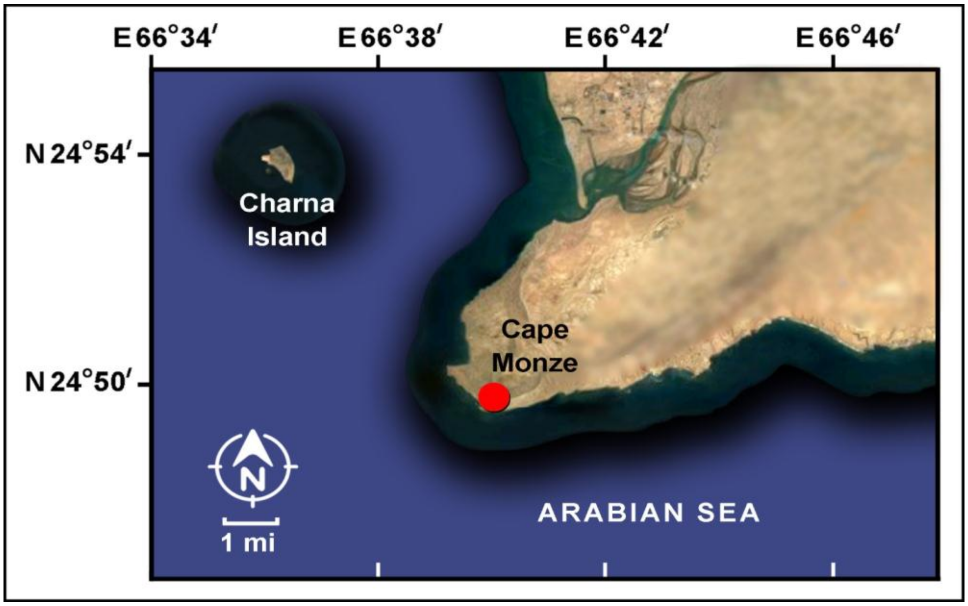

Fig. 1. Study area.

The absorption wavelengths (λ) used in the atomic absorption device are as follows: Fe (248.30 nm), Cu (324.70 nm), Mn (279.50 nm), Zn (213.90 nm), Pb (217.00 nm) and Cd (228.80 nm). The accuracy of the analysis and the effect of the matrices in the environment were cut into the smallest possible pieces and controlled by the standard collection method using three randomly selected samples for each analyzed item. The typical limits of detection were as follows: 0.1, 0.035, 0.01, 0.018, 0.012 and 0.025 µg ml−1 for iron, copper, manganese, zinc, lead and cadmium respectively, calculated by regression analysis (EPA, 2004).

The water samples were filtered through a 0.45 µm membrane as indicated by standard method by American Public Health Association (APHA, 1998) and preserved in refrigerator for laboratory analysis. The sediment samples were then dried at 105°C for 24 h, ground and sieved with a 63µm sieve, and exactly 1 g of the sample was digested with a mixture of concentrations H2O2, HCl and HNO3. The final solution was diluted to 40 ml with distilled water and filtered with Whatman No.1 filter paper into pre- cleaned 50 mL volumetric flasks as the method set by USEPA (1999) and kept in a fridge till metal analysis.

Statistical Analysis: Descriptive statistics (mean, standard deviation, standard error, minimum and maximum range) were conducted using statistical software's IBM SPSS (Mac ver 23) for statistical analysis.

RESULTS AND DISCUSSION

Total (30) specimens of *A. mauritiana* length and weight ranges 16.00–21.00 cm and 49.00-61.24 g (min and max) were collected from Cape Monze Beach during September to December, 2018 (Table 1). Six heavy metals (Fe, Cu, Mn, Zn, Pb, and Cd) were analyzed in tissues of *A. mauritiana* (muscles, tentacles and guts), water and sediment by atomic absorption spectrophotometer (AAS).

The mean values of heavy metal concentrations in water, sediments and sea cucumber were presented in (Table 2). The accumulation of the metals in the muscles, tentacles and guts of *A. mauritiana* followed the order: Fe > Zn > Cu > Mn > Pb > Cd (Table 2). The highest and lowest accumulation of Fe and Cd were reported in *A. mauritiana* muscles. Metal concentrations in Cape Monze Beach water and sediment decreased in the sequence of Fe > Zn > Cu = Mn > Pb > Cd; Fe > Zn > Cu > Mn > Cd > Pb, respectively (Table 2). Table 3 shows that high correlation between Cd and Fe for *A. mauritiana* in muscle. Zn showed a low correlation with Cu and Mn, whereas Pb showed a low correlation with Fe, Cu and Mn (Table 3).

Table 4 shows the metals with the correlation coefficients between the concentrations in tissues (muscle, tentacle and gut) of sea cucumber, water and sediment. The results show that there are metals with high correlation coefficients between tissues, water, and sediment. In addition, it was seen that it has high correlation coefficients in Mn, Zn and Pb metals for muscle in sediment compared to tentacles and gut samples.

The results showed that the iron concentrations in sea cucumber were lower than those found at Haider *et al.* (2015), Ahmed *et al.* (2017) (*H. aranicola*, *H. verrucosa*, *H. atra*, *O. ehrenbergii*, *S. buccalis*), de Fretes *et al.* (2020) (*S. herrmanni*- intestine) but were higher than those discovered at Wen and Hu (2010), Ahmed *et al.* (2017) (*H. cinerascens*, *H. leucospilota*), de Fretes *et al.* (2020) (*S. herrmanni*- muscle). As it can be understood from here, the results reported in the literature reveal that the metal content in sea cucumber varies greatly depending on the species and habitat. In current study Zn and Pb concentration in *A. mauritiana* was also greater the determined values by Haider *et al.* (2015) ve Jiang *et al.* (2015) but below the determined values by İsmail *et al.* (2004), Wen and Hu (2010), Givianrad *et al.* (2014), Mohammadizadeh *et al.* (2016), Ahmed *et al.* (2017), Mohsen *et al.* (2019), de Fretes *et al.* (2020). According to the review in the literature, while Cu and Cd accumulation results are similar, it was found that it is lower than other studies in terms of other metals (Table 5).

Table 6 shows the mean concentrations of metals in water and sediment in the Cape Monze Beach, and results from other studies. In the study area, all accumulation values in water and sediment samples were determined lower than the literature (Table 6). As can be seen from these results, the water and residue values were very good. The fact that the working area is far from people and opens to pollution also supports these results.

However, it has been noted that holothurians gradually remove elements and can act as integrators of metal and radionuclide contamination in the environment (Michel *et al.*, 2006). Therefore, their extensive distribution properties and easy collection properties make them the most important issues for the bioaccumulation of chemicals.

In addition to its use in meals, seafood is also used in the pharmaceutical and cosmetic industry (Sicuro and Levine, 2011). Bêche-de-mer is also called trepang, the boiled, dried and smoked meat of sea cucumber used to make soup. The concentrations of Fe, Cu, Mn, Zn, Pb and Cd found in daily seafood consumption per capita was calculated to assess a potential health risk to Pakistanis.

Table 1. Length and weight metric of *A. mauritiana*.

Actinopyga mauritiana		
	Length (cm)	**Weight (g)**
Mean	18.4375	55.6750
SD	2.04306	4.32002
Min	16.00	49.00
Max	21.00	61.24

Table 2. Heavy metal accumulation values in samples

***Actinopyga mauritiana* / Cape Monze Beach**							
Sample		**Fe**	**Cu**	**Mn**	**Zn**	**Pb**	**Cd**
Muscles	Mean	42.1963	3.1425	0.3800	11.4238	0.0263	0.0150
	SD	9.46763	0.48323	0.21633	4.34443	0.02925	0.00756
	Min	31.78	2.32	0.12	8.26	0.00	0.01
	Max	56.08	3.68	0.71	19.22	0.08	0.03
Tentacles	Mean	36.9025	3.2171	0.4000	10.5013	0.0375	0.0250
	SD	8.14363	0.60139	0.27045	1.69954	0.05175	0.02138
	Min	23.84	2.33	0.14	8.81	0.00	0.01
	Max	45.32	4.02	0.94	13.84	0.16	0.06
Guts	Mean	21.5475	2.3163	0.5325	13.1325	0.0212	0.0187
	SD	5.04578	0.66393	0.15536	2.90450	0.02532	0.01126
	Min	16.03	1.48	0.34	9.34	0.00	0.01
	Max	28.66	3.56	0.81	17.16	0.08	0.04
Sea water	Mean	0.6567	0.0567	0.0567	0.6133	0.0006	0.0003
	SD	0.1124	0.0252	0.0351	0.3329	0.0004	0.0003
	Min	0.5600	0.0300	0.0200	0.2300	0.0002	0.0001
	Max	0.7800	0.0800	0.0900	0.8300	0.0009	0.0006
Sediment	Mean	7.38333	1.10000	0.17000	5.13000	0.00433	0.00500
	SD	2.01053	0.21166	0.04583	1.32842	0.00208	0.00265
	Min	5.40000	0.86000	0.12000	3.86000	0.00200	0.00300
	Max	9.42000	1.26000	0.21000	6.51000	0.00600	0.00800

It was counts of seafood consumption in Pakistan remarked that the adult person eats the mean daily seafood consumption in Pakistan is 1720 g per person (Our World In Data, 2020). The Provisional Permissible Tolerable Weekly Intake (PTWI) of Fe, Cu, Mn, Zn, Pb and Cd was 5600, 3500, 980, 7000, 25 and 7, respectively, expressed in g/week/60 kg body weight (FAO/WHO, 2004). It has been determined that the heavy metal accumulation in A. mauritiana muscles is below the national and international predicted values and does not pose a serious health risk (Table 7).

Table 3. Correlation coefficients between metal concentrations in the muscle of *A. mauritiana*

Metal	Fe	Cu	Mn	Zn	Pb	Cd
Fe	1.000					
Cu	0.529	1.000				
Mn	0.592	0.770*	1.000			
Zn	0.370	-0.093	0.110	1.000		
Pb	-0.135	-0.139	0.023	0.317	1.000	
Cd	0.818*	0.465	0.446	0.428	0.291	1.000

Table 4. Correlation coefficients of metal concentrations in tissue compared with tentacle, gut, water and sediment

		Muscle	Tentacle	Gut	Water	Sediment
Muscle	**Fe**	1.000	-0.332	-0.414	-0.746	-0.853
	Cu	1.000	-0.052	-0.149	0.960	0.206
	Mn	1.000	-0.055	-0.186	0.300	-0.850
	Zn	1.000	-0.022	-0.361	0.775	0.987
	Pb	1.000	-0.337	-0.031	-0.335	0.999*
	Cd	1.000	0.595	0.755*	0.918	-0.655
Tentacle	**Fe**	-0.332	1.000	0.203	-0.994	0.185
	Cu	-0.052	1.000	-0.472	0.007	0.998*
	Mn	-0.055	1.000	-0.227	-0.296	-0.999*
	Zn	-0.022	1.000	0.081	-0.962	-0.700
	Pb	-0.337	1.000	-0.008	0.904	-0.693
	Cd	0.595	1.000	0.023	0.986	-0.817
Gut	**Fe**	-0.746	0.203	1.000	-0.287	0.834
	Cu	0.960	-0.472	1.000	0.176	0.995
	Mn	0.300	-0.227	1.000	-0.875	0.251
	Zn	0.775	0.081	1.000	-0.284	-0.723
	Pb	-0.335	-0.008	1.000	-0.904	0.693
	Cd	0.918	0.023	1.000	-0.737	-0.371

* $p < 0.05$; indicate significance levels.

The results of the present study need to be followed as sea cucumber species can accumulate significant levels of heavy metals and can serve as a good bio-indicator. These findings suggest that sea cucumbers from Cape Monze Beach in future can be contaminated with these heavy metals or that these heavy metals are capable of deposition.

Table 5. Comparison of concentration found in tropical sea cucumber tissues from literatures.

Location	Cucumber	Metal concentration (μg g-1)						References
		Fe	Cu	Mn	Zn	Pb	Cd	
Pulau Pangkor	*S. herrmanni*	-	-	-	43.54	9.87	10.48	İsmail *et al.*, 2004
	H. atra	-	-	-	30.38	10.32	8.00	
Pulau Kapas	*S. hermanni*	-	-	-	59.13	15.77	12.09	
	H. atra	-	-	-	51.67	15.67	33.03	
Guangzhou, China	*A. mauritiana*	660	14	9.2	57	0.11	0.05	Wen and Hu, 2010
Qeshm, Persian Gulf	*H. parva* (muscles)	-	-	-	-	14.98	1.06	Givianrad *et al.*, 2014
	H. parva (guts)	-	-	-	-	8.21	0.25	
Bohai and Yellow Sea	*A. japonicus*		0.179		2.634	0.065	0.161	Jiang et al., 2015
Buleji	*H. arenicola*	10.7	7.0	3.7	3.55	0.09	1.36	Haider *et al.*, 2015
	A. mauritiana	-	-	-	-	0.017	0.0382	
Qeshm Island	*H. leucospilota*	-	66.35	-	39.64	11.42	0.17	Mohammad izadeh *et al.*, 2016
		-	74.17	-	31.16	14.28	0.18	
		-	82.28	-	12.30	11.98	0.29	
	H. scabra	-	44.48	-	19.30	2.55	0.16	
		-	47.91	-	29.12	1.69	0.17	
		-	81.16	-	21.46	1.52	0.13	
Buleji and Sunehri coasts (max value)	*H. arenicola*	47	2.23	5.32	11-28	2.33	1.42	Ahmed *et al.*, 2017
	H.verrucosa	29	3.76	2.47	12-30	1.03	1.76	
	H. atra	26	3.89	2.49	18-24	1.23	1.11	
	O. ehrenbergii	34	3.98	3.91	24-32	3.02	0.52	
	H. cinerascens	52	8.93	4.64	37	2.12	2.67	
	S. buccalis	14	2.46	3.02	19	0.82	0.11	
	H. leucospilota	73	8.64	7.12	46	2.19	1.02	
Qianshan Island	*A. japonicus*	-	7.79	17.25	29.98	1.56	0.31	Mohsen *et al.*, 2019
Kayeli Bay	*S. herrmanni* (intestine)	0.427	-	-	-	-	-	Fretes *et al.*, 2020
	S. herrmanni (muscle)	1502.9	-	-	-	-	-	
Cape Monze	*A. mauritiana* (muscles)	42.20	3.14	0.38	11.42	0.03	0.02	This study
	A. mauritiana (tentacles)	36.90	3.22	0.40	10.50	0.04	0.03	
	A. mauritiana (guts)	21.55	2.32	0.53	13.13	0.02	0.02	
International limits		-	10-10	-	40	0.50	0.50	FAO, 1983
		100	-	1.00	50	2.00	1.00	WHO, 1989

Table 6. Comparison of sea water and sediment from literatures.

Location	Sample	Sediment (µg g-^{1}), Water (µg L-^{1})						References
		Fe	**Cu**	**Mn**	**Zn**	**Pb**	**Cd**	
Karachi Region	Sediment Bin Qasim	3.94	27.19	273.67	104.56	14.28	2.03	Siddique *et al*. 2009
Qeshm, Persian Gulf	Sediment St1	-	-	-	-	23.85	4.00	Givianrad *et al.*, 2014
	Sediment St2	-	-	-	-	43.26	2.96	
	Sediment St3	-	-	-	-	57.88	2.86	
Qeshm Island	Sediment St 1	-	47.11	-	55.78	28.97	0.6	Mohammadi-zadeh *et al.*, 2016
	Sediment St 2	-	18.31	-	44.28	11.18	0.91	
	Sediment St 3	-	51.89	-	28.26	15.78	1.15	
Buleji coasts	Sediment PreM	220	5.52	137	14	37	2.42	Ahmed *et al.*, 2017
	Sediment M	218	4.92	133	26	33	1.91	
	Sediment PosM	218	5.27	132	39	37	1.88	
Sunehri coasts	Sediment PreM	241	5.99	134	19	56	1.18	
	Sediment M	222	7.83	140	15	51	3.07	
	Sediment PosM	222	9.04	143	22	47	2.60	
Buleji coasts	Water PreM	206	33	273	4.23	73	0.17	
	Water M	478	37	323	4.21	88	0.86	
	Water PosM	264	19	308	3.38	67	1.22	
Sunehri coasts	Water PreM	308	57	229	3.41	66	1.83	
	Water M	222	44	199	2.03	52	3.11	
	Water PosM	372	63	294	1.55	47	5.57	
Farms in China	Sediment Pans han	-	27.09	182.83	-	11.88	0.17	Mohsen *et al.*, 2019
	Sediment Lvs hunkou	-	30.85	409.17	-	11.81	0.15	
Cape Monze	Sediment	7.383	1.100	0.170	5.130	0.004	0.005	This study
	Sea water	0.657	0.057	0.057	0.613	0.0006	0.0003	

The present study highlights and provides new information of heavy metal accumulation of commercially important sea cucumber *A. mauritiana* tissues, water and sediments from Cape monze beach. There are no reports/data available on heavy metals in sea cucumber, water and sediments of this area and this is a first study providing information and accurate data for future research. Therefore it is concluded that there is a dire need to monitor these coastal areas and to devise an alarming system on heavy metal levels/contamination.

Table 7. Estimated daily and weekly intakes values.

Metal	PTWI*	PTWI[a]	PTDI[b]	*Actinopyga mauritiana* EWI[c] (EDI)[d]
Fe	5600	336000	48000.00	72577.63 (10368.23)
Cu	3500	210000	30000.00	5405.10 (772.16)
Mn	980	58800	8400.00	653.60 (93.37)
Zn	7000	420000	60000.00	19648.93 (2806.99)
Pb	25	1500	214.29	40.60 (5.80)
Cd	7	420	60.00	25.80 (3.69)

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

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