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**DETERMINATION OF MERCURY (Hg) IN TWO SEA  
CUCUMBER SPECIES *OHSHIMELLA EHRENBORGII*  
(SELENKA, 1868) AND *STOLUS BUCCALIS* (STIMPSON, 1855)  
FROM THE KARACHI COAST**

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**ABSTRACT:** In this study the amounts of mercury (Hg) were determined in tentacles and muscle tissues of *O. ehrenbergii* and *S. buccalis* from two coastal sites of Karachi; Buleji and Sunehri during Southwest monsoon (August and September) and Northeast monsoon (December and January) seasons of the year 2018. The mean amounts of Hg in edible tissues of sea cucumbers were as follows: *O. ehrenbergii* (0.0176 mg/kg dry wt.) and *S. buccalis* (0.0155 mg/kg dry wt.). Hg amounts in muscles of both species are much lower than the maximum permissible limits (0.5 mg/kg wet wt.). Estimated Daily Intakes for adults consuming *O. ehrenbergii* and *S. buccalis* are lower than published RfD values. Total Target Hazard Quotient (TTHQ) values (0.00787) are also lower than 1, it may be concluded that the consumption of these sea cucumbers from Karachi, do not pose any health hazards to human as Hg amounts were concerned.

**KEYWORDS:** *Ohshimella ehrenbergii*, *Stolus buccalis*, Mercury, Buleji, Sunehri, Karachi, Estimated Daily Intakes, Target Hazard Quotient

### INTRODUCTION

In marine coastal environment, toxic heavy metals are accumulated by biota either directly from surrounding water or through their food. Bio-accumulation of heavy metals depends on the bioavailability of the element in the surrounding waters, storage, ingestion rate and excretion mechanisms of aquatic organisms. Toxic heavy metals especially mercury (Hg) in edible organisms can counteract their beneficial effects leading to adverse effects of heavy metals on human health including serious threats such as renal failure, liver damage, cardiovascular diseases, carcinogenic effect and even death.

Not only toxic metals pose hazardous effects to humans but also may become toxic to natural biota when entering the marine coastal environment. Hg is now widely studied due to causing deleterious effect in marine ecosystem. Hg is an element that has no benefit for living organisms that are not easily degraded in nature. It has toxic effects on marine organisms even in very trace amounts. The use of this toxic metals in industry is leading to widespread marine coastal contamination. During the last few years' heavy metal amounts in holothuroids is increasing (Haider *et al.*, 2015; Ahmed *et al.*, 2017 and 2018).

The main objective of this study is to determine the concentrations of Hg in the tentacles and body muscle tissues of *Ohshimella ehrenbergii* (Selenka, 1868) and

*Stolus buccalis* (Stimpson, 1855) collected from two coastal sites Buleji and Sunehri along the Karachi coast, Pakistan during Southwest monsoon (August and September) and Northeast monsoon (December and January) of 2018.

From Pakistan Ahmed *et al.*, (2018) reported mercury (Hg) in four holothurians species from Karachi coast, this was the first document from Pakistan on Hg accumulation in sea cucumber. *Ohshimella ehrenbergii* belongs to family: Sclerodactylidae has been rediscovered in Pakistan after 43 years which was first reported by Clark and Rowe in 1971. *O. ehrenbergii* reported from Sunehri in April to May 2014 and this species was recorded for the first time from Buleji coast in 2017. *Stolus buccalis* belongs to family-Phyllophoridae, the taxonomic studies on this species was done by Clark and Rowe in 1971; Tahera, 1992; Ahmed and Ali, 2014. Both the species do not found in abundance on Pakistan coast as compare to other species of sea cucumbers. Both species are not commercially important as food mentioned by Purcell *et al.*, 2012 but we can use these species for monitoring of heavy metals accumulation levels in marine environment. Also we can conclude on the basis of results it is safe for human consumption if local community will consume it or if they will export to other countries.

## MATERIALS AND METHODS

### Sampling sites:

The sea cucumber species were collected seasonally from Buleji and Sunehri coasts of Karachi, Pakistan (Fig.1).

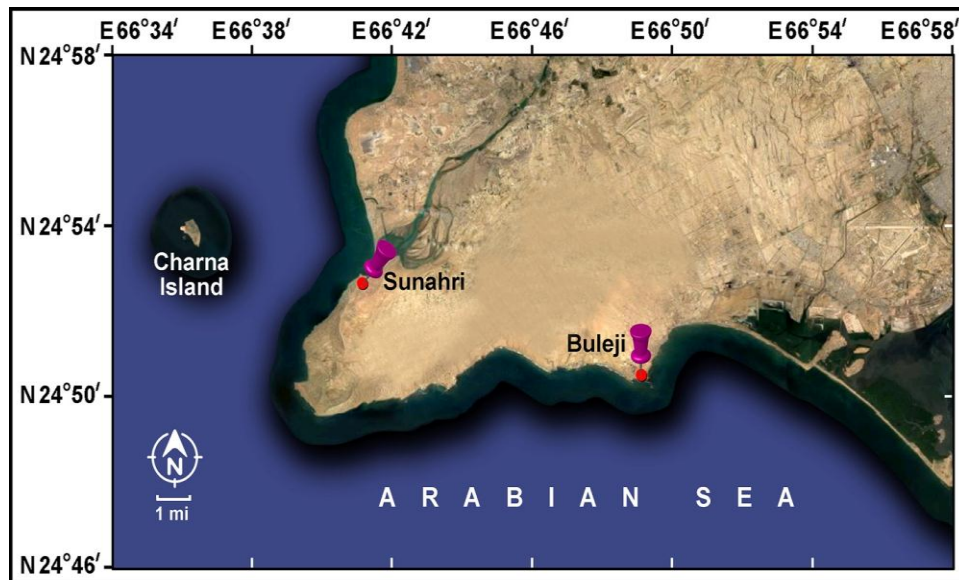


Fig. 1. Sampling area map.

**Sample collection:**

*O. ehrenbergii* and *S. buccalis* were caught during Southwest monsoon (August and September) and Northeast monsoon (December and January) of 2018. Collected sea cucumber samples were put in clean seawater and transferred to the laboratory. Total length and weights of the specimen were recorded.

**Determination of heavy metals:**

Total 28 specimen were dissected, removing tentacles and muscle tissues of sea cucumbers and were placed in separate containers. About 3 grams of each tissue samples were placed into the crucibles and were dried in furnace at 600°C for three hours and then dry ash were weighted on digital balance. These ashes were digested in the flasks containing 10 ml of concentrated HCl. When the digestions were completed, the solutions were cooled to ambient temperature and the digest was filtered and made up to 100 ml in a volumetric flask using ultrapure water as the diluent. The analysis for total Hg was performed on Mercury Hydride system (cold vapor Technique) Atomic Absorption Spectrometer (AAS). Analysis followed the methods described by Ahmed *et al.* (2018). The AA Analyst 700 AAS with 253.7 wavelengths was used to determine Hg concentrations. Detection limit was 2 ng Hg (0.00004 mg/L in a 50 mL sample).

**Intake Levels Calculation:**

The daily intake amounts were calculated using the mean Hg concentration in sea cucumber species EDI (Estimated Daily Intakes) = the mean concentration of Hg (mg/kg) multiplied by holothuroids consumption (kg/70 kg body wt. /week). The health hazards from consumptions of these sea cucumber species by human were calculated based on the target hazard quotient (THQ). The estimating risk using THQ was supplied in the US Environment Protection Agency (US EPA, 2018) region risk based amount table and it is described by the following equation:

$$THQ = \frac{EF \times ED \times FI \times MC}{RfD \times BW \times AT}$$

where, THQ refers to target hazard quotient; EF is the exposure frequency (365 days/year); ED is the exposure duration (70 years old); FI is the mean ingestion rate, 5 g/person/day in Pakistan (FAO, 2010); MC is the metal concentration in fish muscles (g/kg, on wet wt.); RfD is the oral reference dose for Hg 0.0003 mg/kg/day; BW is the average body weight (70 kg); and AT is the averaging time.

**Statistical analysis:**

ANOVA with post hoc test analyses based on Tukey was used to compare the differences between the seasons, tissues and localities. A P-value of <0.05 or less was considered statistically significant. All analysis was carried out using the SPSS, version 21 and Excel 2010 to analyses the influence of pre-monsoon, monsoon and post-monsoon (or Southwest and Northeast monsoon). All values were being expressed on mg/kg dry wt. basis.

**RESULTS AND DISCUSSION**

The mean lengths (cm) and weights (g) with standard deviations and ranges of *O. ehrenbergii* and *S. buccalis* from Buleji and Sunehri coasts of Karachi are given in Figs. 2 and 3.

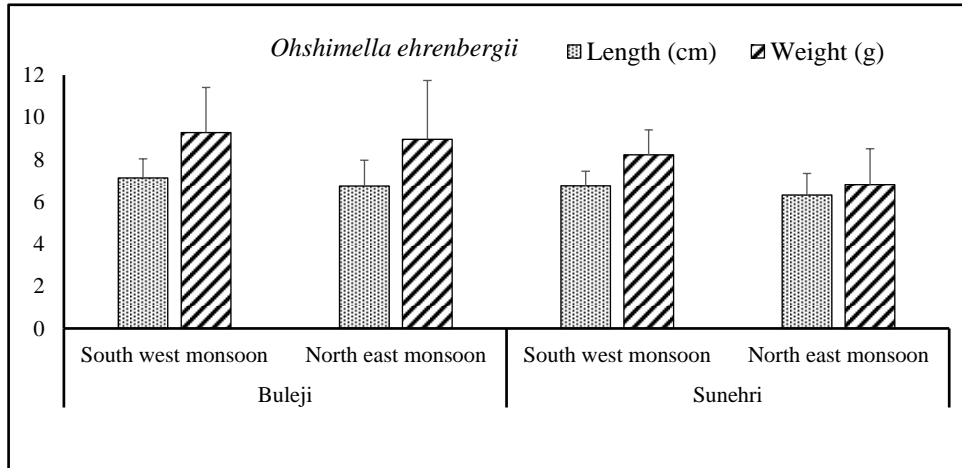


Fig. 2. Mean  $\pm$  SD of length (cm) and weight (g) of *O. ehrenbergii* from Buleji and Sunehri coasts of Karachi during Southwest monsoon (August and September) and Northeast monsoon (December and January) seasons of the year 2018.

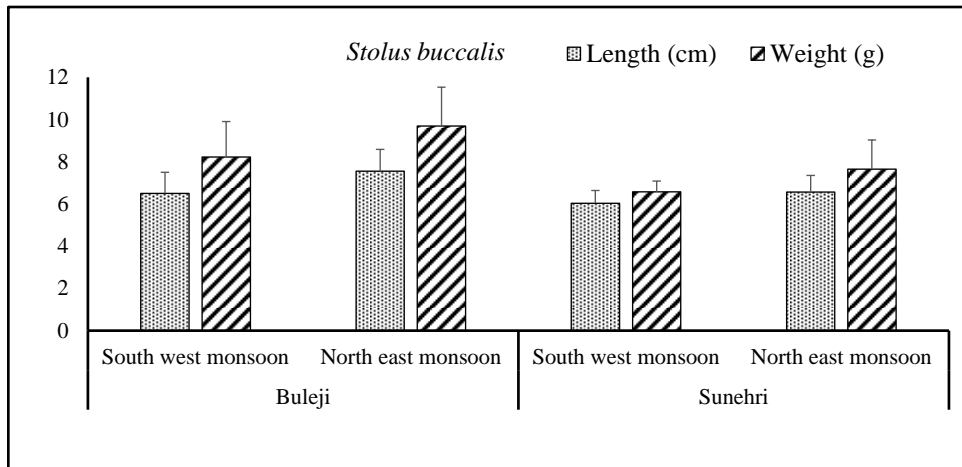


Fig. 3. Mean  $\pm$  SD of length (cm) and weight (g) of *S. buccalis* from Buleji and Sunehri coasts of Karachi during Southwest monsoon (August and September) and Northeast monsoon (December and January) seasons of the year 2018.

The concentrations of Hg in tentacles and muscle tissues of *O. ehrenbergii* and *S. buccalis* from Buleji and Sunehri coasts of Karachi are presented in Figs. 4-5.

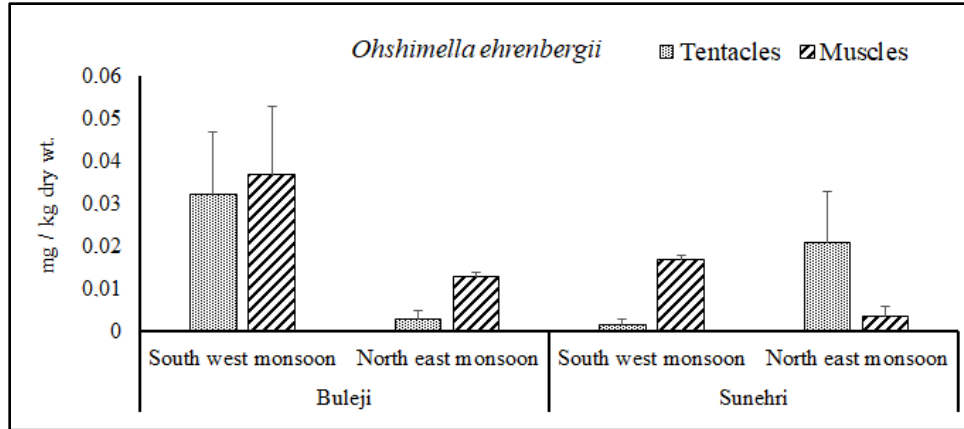


Fig. 4. The means with standard deviations (vertical lines) of Hg concentrations (mg/kg dry wt.) in tentacles and muscle tissues of *O. ehrenbergii* from Buleji and Sunehri coasts of Karachi during Southwest monsoon (August and September) and Northeast monsoon (December and January) seasons of the year 2018.

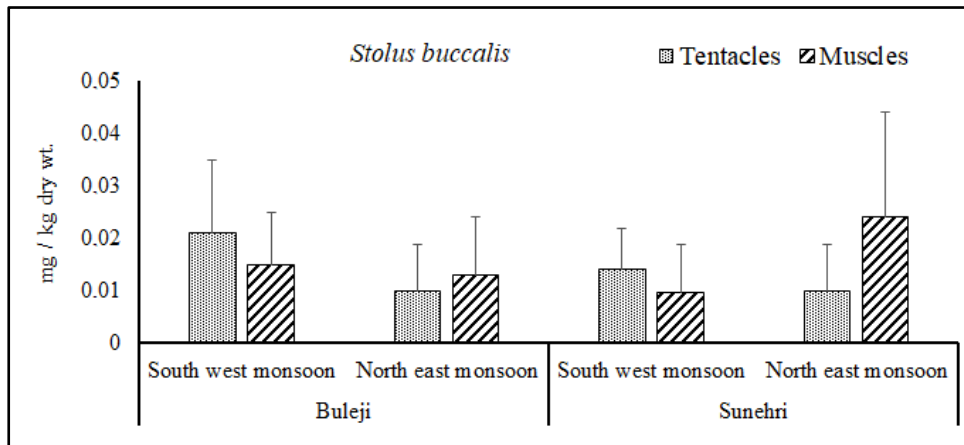


Fig. 5. The means with standard deviations (vertical lines) of Hg concentrations (mg/kg dry wt.) in tentacles and muscle tissues of *S. buccalis* from Buleji and Sunehri coasts of Karachi during Southwest monsoon (August and September) and Northeast monsoon (December and January) seasons of the year 2018.

The results of analysis of variance were presented in Tables 1 and 2, that there were significance differences in the amounts of Hg in tentacles and muscle tissues of *O. ehrenbergii* from Buleji and Sunehri coasts of Karachi during Southwest monsoon (August and September) and Northeast monsoon (December and January) seasons. However, there was no statistically significant difference between the concentrations of Hg in tentacles and muscle tissues of *S. buccalis* through seasons and localities ( $P > 0.05$ ).

In the present study, statistical analysis of the results displayed significant differences ( $P < 0.05$ ) between *O. ehrenbergii* and *S. buccalis*. The mean Hg levels varied amongst these species. The mean amounts of Hg in edible tissues of sea cucumbers are as follows: *O. ehrenbergii* (0.0176 mg/kg dry wt.) and *S. buccalis* (0.0155 mg/kg dry wt.). It is clear that Hg amounts in muscles of both species are much lower than the maximum permissible limit (0.5 mg/kg wet wt.) set by the European Union and Turkish Food Codex (EU Commission Regulations, 2006; TFC, 2008). Environmental Quality Standards (EQS) for Hg in biota is given in European Union Legislative acts as 0.02 mg/kg wet wt. (Official Journal of the European Union, 2013). The results of the present study were given in dry wt., mean Hg levels converted to wet wt. dividing by 8.0 as factor (Ahmed *et al.*, 2018). It is seen that overall average of Hg amounts in muscle tissues were between 9.09 and 10.3 times lower than EQS value (0.02 mg/kg wet wt.).

**Table 1. Analysis of variance (ANOVA) in tentacles and muscle tissues of *O. ehrenbergii* from from Buleji and Sunehri coasts of Karachi during Southwest monsoon and Northeast monsoon seasons.**

			Sum of Squares	df	Mean Square	F	Sig.
Hg * Seasons	Between Groups	(Combined)	.000	1	.000	.035	.853
	Within Groups		.007	54	.000		
	Total		.007	55			
Hg * Tissues	Between Groups	(Combined)	.001	1	.001	7.848	.007
	Within Groups		.006	54	.000		
	Total		.007	55			
Hg * Locality	Between Groups	(Combined)	.000	1	.000	3.633	.062
	Within Groups		.007	54	.000		
	Total		.007	55			

Hg amounts in the present study were generally in low ranges with the literature, where it has been reported as 0.018 – 0.036 mg/kg dry wt., for different sea cucumber species (*Holothuria (Thymiosycia) arenicola* Semper, 1868, *Holothuria (Lessonothuria) pardalis* Selenka, 1867, *Holothuria (Lessonothuria) verrucosa* Selenka, 1867 and *Holothuria (Halodeima) atra* Jaeger, 1833) collected during different seasons (pre-monsoon, monsoon, and post-monsoon) in 2015 at Buleji and Sunehri coasts of Karachi (Ahmed *et al.*, 2018).

Food and Agriculture Organization (FAO) counts of seafood consumption in Pakistan remarked that the adult person eats the mean daily seafood consumption in Pakistan is 5 g per person (FAO, 2010). The Estimated Daily Intake (EDI) and Target

Hazard Quotient (THQ) of Hg for people in Buleji and Sunehri coasts of Karachi via consumptions of sea cucumbers are presented in Table 3.

**Table 2. Analysis of variance (ANOVA) in tentacles and muscle tissues of *S. buccalis* from from Buleji and Sunehri coasts of Karachi during Southwest monsoon and Northeast monsoon seasons.**

			Sum of Squares	Df	Mean Square	F	Sig.
Hg * Seasons	Between Groups	(Combined)	.000	1	.000	.026	.873
	Within Groups		.012	54	.000		
	Total		.012	55			
Hg * Tissues	Between Groups	(Combined)	.000	1	.000	1.002	.321
	Within Groups		.012	54	.000		
	Total		.012	55			
Hg * Locality	Between Groups	(Combined)	.000	1	.000	.039	.844
	Within Groups		.012	54	.000		
	Total		.012	55			

**Table 3. EDI and THQ of Hg via consumptions of sea cucumber muscles in Buleji and Sunehri coasts of Karachi.**

Species	EDI (µg/kg/day)	THQ
<i>O. ehrenbergii</i>	0.001259	0.004196
<i>S. buccalis</i>	0.001104	0.003678

EDI values for adults consuming *O. ehrenbergii* and *S. buccalis* from Buleji and Sunehri coasts of Karachi lower than published RfD values. As all THQ and Total Target Hazard Quotient (TTHQ) values (0.00787) were lower than 1, it may be concluded that

the consumption of these sea cucumbers from Karachi, do not pose health hazards to humans.

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