KSÜ Tarım ve Doğa Derg 23 (6): 1561-1568, 2020
KSU J. Agric Nat 23 (6): 1561-1568, 2020
DOI:10.18016/ksutarimdoga.vi.715683

Assessment of Hg in *Holothuria (Mertensiothuria) leucospilota* (Brandt, 1835) from Karachi coasts, Pakistan

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ABSTRACT
The aim of the present study was to determine mercury levels in sediments, surface sea water and *Holothuria leucospilota* muscles and skin in two selected sites from Karachi coast. Samples were collected during two seasons south-west monsoon (August-September) and north-east monsoon (December-January). The concentrations of Hg in sediment ranged from 0.0012 to 0.0023 mg kg^{-1}, and in surface water from 0.00018 to 0.00034 (µg l^{-1}), while in muscle and skin of black sea cucumber ranged from LOD to 0.0034 and LOD to 0.0046 mg kg^{-1} dry wt., respectively. Moreover, Hg showed the highest accumulation rate in the skin tissue with BSAF values ranging between 2 in Buleji and 2.69 in Sunehri at north-east monsoon. The BSAF values in the muscles of the black sea cucumber ranged from 1.44 at south-west monsoon in Buleji to 1.63 at north-east monsoon in Sunehri, respectively. Since BSAF value is higher than 2 on the skin of sea cucumber, it is evaluated as macro-concentrator. However, the BSAF value varies between 1 and 2 in muscle, it has been determined as micro-concentrator. Hg in the black sea cucumbers from Buleji and Sunehri sites of the Karachi coasts is "very bio-accumulative" (BCF > 5000) during both sampling seasons.

Research Article

Article History
Received : 07.04.2020
Accepted :

Keywords
Mercury
Holothuria leucospilota
Karachi
Pakistan
Arabian Sea

Pakistan Karachi sahillerinden *Holothuria (Mertensiothuria) leucospilota* türünde (Brandt, 1835) Hg değerlendirmesi

ÖZET
Bu çalışmanın amacı, Karachi kıyısında seçilen iki bölgede sediment, yüzey deniz suyu ve *Holothuria leucospilota* kasları ve derisindeki cıva seviyelerini belirlemektir. Örnekler iki mevsim güney-batı musonu (Ağustos-Eylül) ve kuzey-doğu musonu (Aralık-Ocak) boyunca toplanmıştır. Sediment içindeki Hg konsantrasyonları kuru ağırlık olarak 0,0012 ila 0,0023 mg kg^{-1} ve yüzey suyunda 0,00018 ila 0,00034 (µg l^{-1}) arasında değişirken, kara deniz hıyarının kas ve derisinde ise LOD değerleri 0,0034 - 0,0046 mg kg^{-1} arasında değişmektedir. Üstelik, Hg deri dokusunda en yüksek birikim oranını, kuzeydoğu musonunda Buleji'de 2 ile Sunehri'de 2,69 arasında değişen BSAF değerleri ile gösterilmiştir. Kara deniz hıyarı kaslarındaki BSAF değerleri, Buleji'de güneybatı musonda 1,44 ile Sunehri'nde kuzeydoğu musonda 1,63 arasında değişmektedir. Kara deniz hıyarının derisinde BSAF değeri 2'den yüksek olduğu için makro-yoğunlaştırıcı olarak değerlendirilmiştir. Bununla birlikte, BSAF değeri kasta 1 ile 2 arasında değiştiğinden, mikro-yoğunlaştırıcı olarak belirlenmiştir. Karachi kıyılarının Buleji ve Sunehri bölgelerindeki kara deniz hıyarlarında Hg, her iki örnekleme mevsimi boyunca biyo-birikime sahip olduğu görülmüştür (BCF> 5000).

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 07.04.2020
Kabul Tarihi :

Anahtar Kelimeler
Cıva
Holothuria leucospilota
Karachi
Pakistan
Arap Denizi

To Cite :. Ahmed Q, Bat L 2020. Assessment of Hg in *Holothuria (Mertensiothuria) leucospilota* (Brandt, 1835) from Karachi coasts, Pakistan. KSU J. Agric Nat 23 (6): 1561-1568. DOI: 10.18016/ksutarimdoga.vi.715683.

INTRODUCTION

Industrialization and urbanization of marine coastal areas are very prevalent in countries such Pakistan and cause serious damage to marine ecosystems. However, environmental changes due to human activities are known to have potential effects of marine coastal areas, especially from hot spots. The release of pollutants into marine ecosystems is an important issue worldwide. The main sources of heavy metal contamination, such as Hg are the run-off from urban areas and agricultural, from industrial sites, discharges from factories, municipal sewer systems and mining and atmospheric depositions (Bat 2017; Bat et al. 2018; Bat and Özkan 2019). Mercury (Hg) is considered a very important contaminant of marine ecosystems owing to its capability to enter and accumulate in the food web. Hg is also acquainted with likely accumulate in sediments which attend as a repository of contaminants. Sediment-bound metals could be let to the surrounding water, occuring possible hazard effects to marine biota. Hg is a potentially very toxic trace metal. Hg accumulation in aquatic biota is a big ecological concern, especially because of its capability to accumulate rapidly. Moreover, Hg is known to bioaccumulate in edible tissues of seafood thus, representing a health risk to people.

The holothurians or sea cucumbers, can be found in great numbers in the marine environment throughout the worldwide, with an important role on transferring energy in the ecosystem. *Holothuria leucospilota*, commonly known as the black sea cucumber, belongs to the family Holothuriidae. It has been included in the subgenus *Mertensiothuria* doing its full scientific name *Holothuria (Mertensiothuria) leucospilota*. The black sea cucumbers are found in the coastal areas of warm-temperate zones (Purcell et al. 2012; Yu et al. 2013) and is generally found at the edge of reef shores in the intertidal zone, where it lives in the tidal pool with the depths a few centimetres to a half meter or being entirely exposed (Sloan 1979). This species is relatively tolerant salinity and temperature changes and is usually found in between the hard rocks and tolerant to air exposure over a long period of time and might be found in the area closest to the coast (Iliyas 2010). It lives shallow water at the bottom of the seabed. Sea cucumber ingested bacteria, dissolved organic matters, meiofauna, inorganic components and decaying organic debris that are accessible on surface sediments as their primary source of food (Roberts et al. 2001; Gao et al. 2014).

Contaminations of heavy metals have been broadly studied in Karachi coasts, However, the continuity of such studies is extremely important. The European Union Marine Strategy Framework Directive (MSFD) strongly recommends this for the achievement of Good Environmental Status (GES) in the seas (Official Journal of the European Union 2008). With this respect, it is significant to evaluate the sediment and water contamination of this toxic contaminant for preferable management and conservation of these valuable coastal ecosystems on the beaches of Buleji and Sunehri along the Karachi coasts.

Few studies on heavy metal accumulation on sea cucumbers from Karachi coast were reported by (Ahmed and Ali 2014; Ahmed et al. 2017). Previously (Ahmed et al. 2018; Ahmed et al. 2019) analyzed six holothurians species of mercury (Hg) from Karachi coast. However, this is the first attempt to determine of Hg in skin and muscles tissues of *H. leucospilota* from Karachi shores of the Arabian Sea. The aim of this study to determine Hg concentration in sea water, sediment and *H. leucospilota* tissues from two selected sites namely Bulji and Sunehri of Karachi coasts, Pakistan in August 2018 and January-2019.

MATERIAL and METHOD

Sample collection for Hg analysis in sea water, sediment and black sea cucumbers

The present study was carried out Buleji 24°50'20.41" N, 66°49'24.15" E and Sunehri 24°52'33.49" N, 66°40'40.20"E of Karachi coasts (Figure 1) between August -2018 and January-2019. A total of 24 *Holothuria* (*Mertensiothuria*) *leucospilota* individuals were collected seasonally in south-west monsoon (August-September) and north-east monsoon (December-January) during low tide from intertidal zone. After collection, individuals from the sampling stations were kept in seawater and transferred to the laboratory. They were placed in well aerated aquarium for length and weight measurement. Length (cm) and weight (g) data were taken for each individual after allowing the black sea cucumber to put in water for 5 min. Total length from mouth to anus was measured by the flexible ruler. Wet weight was measured to the nearest 0.01 g immediately after removing the animal from the water prior to evisceration (Ahmed et al. 2017). The sediments were sampled by pushing a plastic core into sediment to a depth of roughly five cm. The sediments were then placed in plastic zipper bags and taken to the laboratory. The water samples were taken into 500 ml white polyethylene bottles and added few drops of HNO_3 and keep in the refrigerator until analysis.

Sample preparation for (Hg) analysis in sea water, sediment and black sea cucumbers

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 H_2O_2, HCl and HNO_3. 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 Keeney and Nelson (1982) and kept in a fridge till Hg analysis.

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.

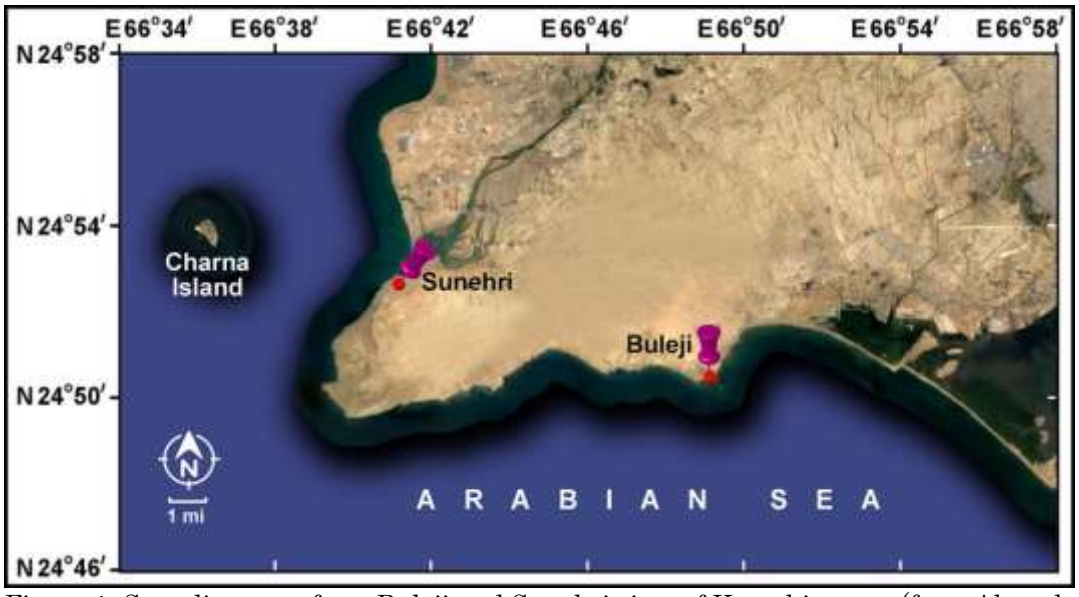

Figure 1. Sampling area from Buleji and Sunehri sites of Karachi coasts (from Ahmed and Ali, 2020)
Şekil 1. Karaçi kıyılarının Buleji ve Sunehri bölgelerindeki örnekleme alanı (Ahmed ve Ali'den, 2020)

For Hg analysis in skin and muscles tissues of the black sea cucumbers were then separated carefully according to Bernhard (1976) to remove all the internal organs of the specimen and the muscles and skins were used for analysis after cleaning. The tissues were weighted (g) and chopped into small pieces and then ground and calcinated at 650°C for 3 h. Ash samples of each individuals were weighed (g), dissolved in HCl (0.1 mol/l) and further treated with H_2O_2 (30%) until lucid solutions were formed, and then diluted by deionized water (Hashmi et al. 2014). When the digestions were completed, the solutions were cooled to ambient temperature and the digest was filtered the 0.45 µm Whatman filter papersand made up to 100 ml in a volumetric flask using ultrapure water as the diluents.

Total mercury analysis was performed on Mercury Hydride system (cold vapor Technique) Atomic Absorption Spectrometer. The AA Analyst 700 atomic absorption spectrometer with 253.7 wavelengths was used to determine Hg concentration. Detection limit was 2 ng Hg (0.00004 mg/l in a 50 ml sample). For each run, three blanks were analysed using the equal procedure for the purpose of control the purity of reagents and if any possible contamination. The quality of the analytical data was ensured through careful standardization, blank measurements and triplicate samples.

Statistical analysis

The concentrations were expressed as mg/kg dry weight. All values were expressed as mean± SE. Statistical analysis of data was performed by using a one-way analysis of variance (ANOVA) and Duncan multiple range test. The significance levels of 5% were used for Hg to examination for significant differences between sites and seasons. All statistical analyses were performed by using the IBM® SPSS® Statistics for Windows, Version 21.0 (SPSS 2012) and Office Excel 2010 software package (Microsoft Corporation 2018).

Moreover bio-sediment accumulation factor (BSAF) and bio-concentration factor (BCF) were also calculated. Hg levels in tissues of the black sea cucumbers from the sediments are defined as the BSAF.

The BSAF is a parameter that assigns the bio-accumulation of sediment-bound Hg in tissues of the black sea cucumber and is calculated using the below equation:

$$\boldsymbol{BSAF = \frac{C_{bsc}}{C_{sed}}}$$

where, C_{bsc} is the Hg concentration in tissues of the black sea cucumber (mass of Hg per kg of black sea cucumber/dry wt.), while C_{sed} is the concentration in the related sediment (mass of Hg per kg of sediment/dry wt.).

The BCF is the ratio of the level of Hg in tissues of the black sea cucumber to the level in the medium (mass of Hg/l).

BCF was calculated by the below formula:

$$\boldsymbol{BCF = \frac{C_{bsc}}{C_{wat}}}$$

where, C_{bsc} is the Hg concentration in tissues of the black sea cucumber, C_{wat} is the

Hg concentration in the ambient water.

RESULTS and DISCUSSION

In the sediment samples, the concentration of Hg was higher in north-east monsoon (Figure 2). While there was changeability between Buleji and Sunehri, the overall amount range for Hg was relatively close, with no values that come out to be abnormal. Buleji site demonstrated relatively the higher levels of Hg contamination measured during this study. This may be clarified by the increasing fishing and touristic activities at this site.

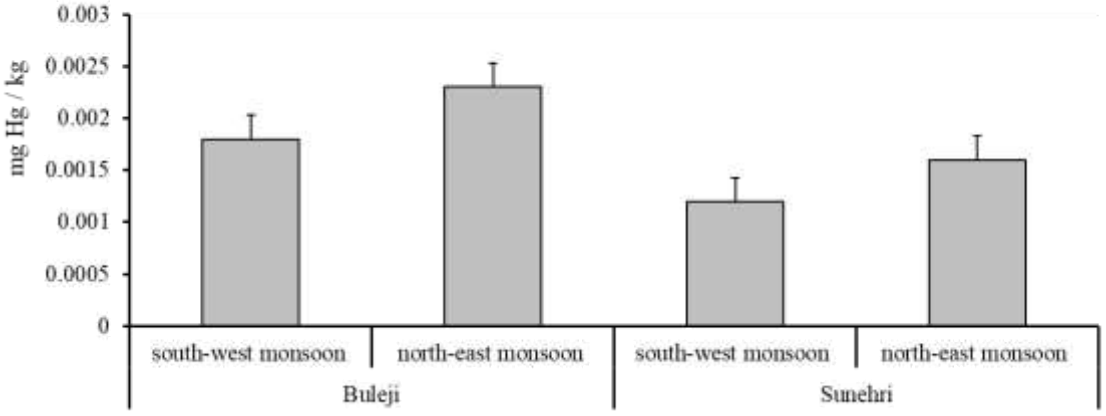

Figure 2. Mean concentrations of Hg (mg/kg) (±SE) in sediments collected from Buleji and Sunehri sites of the Karachi coasts during south-west monsoon and north-east monsoon.

Şekil 2. Güney-batı musonu ve kuzey-doğu musonu sırasında Karachi kıyılarının Buleji ve Sunehri yerlerinden toplanan sedimentlerdeki ortalama Hg (mg / kg) (±SH) konsantrasyonları.

In the surface water samples collected during the present study, the concentrations of Hg were higher in north-east monsoon (Figure 3). Similar to the sediment samples, there was variability between Buleji and Sunehri sites, the overall concentration range for Hg was relatively small, with no values that appeared to be unusual. Water contamination was also found to be higher in north-east monsoon than in south-west monsoon along the Buleji and Sunehri sites on the Karachi coasts.

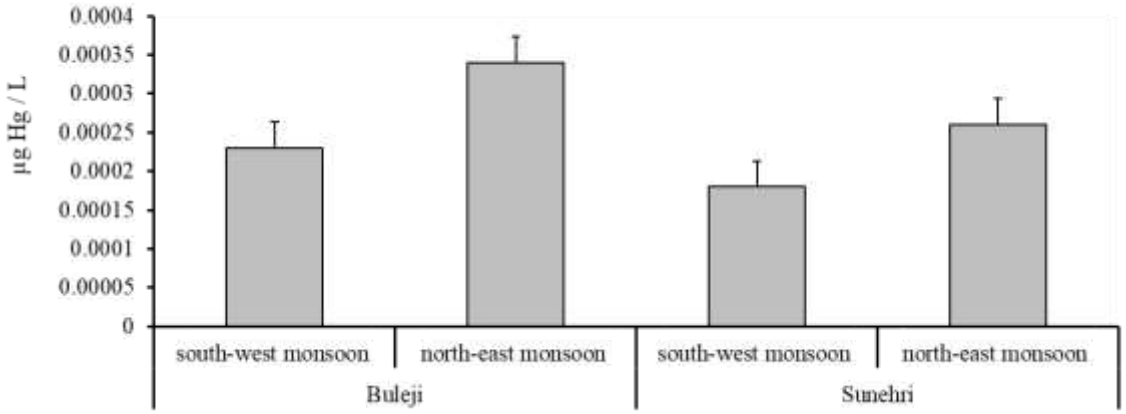

Figure 3. Mean concentrations of Hg (µg/l) (±SE) in surface waters collected from Buleji and Sunehri sites of the Karachi coasts during south-west monsoon and north-east monsoon.

Şekil 3. Güney-batı musonu ve kuzey-doğu musonu sırasında Karachi kıyılarının Buleji ve Sunehri bölgelerinden toplanan yüzey sularındaki ortalama Hg (µg / l) (±SH) konsantrasyonları.

The concentrations of Hg (mg kg^{-1}) in the muscle and skin of the black sea cucumbers are shown in Figure 4. The concentration of Hg in the black sea cucumber samples was also higher in the north-east monsoon than in the south-west monsoon at both Buleji and Sunehri sites. Hg levels in the black sea cucumbers from Sunehri site are relatively lower than Buleji site. However, in general the amounts of Hg in the muscle and skin of the black sea cucumbers from both sites were quite low. Similar findings were given by Ahmed

et al. (2017), Ahmed et al. (2018) and Ahmed et al. (2019) in different sea cucumber species at same sites. In this study the amounts of Hg in the black sea cucumber differed within an unimportant range in both the north-east monsoon and the south-west monsoon and across the sampling Buleji and Sunehri sites due to alike environment circumstances. *H. leucospilota* is a scavenger and is fed by using its tentacles to shovel organic matter lying on the bottom into its mouth. In the process it swallows an important amount of sediment, which passes through the gut (Roberts et al. 2001; Gao et al. 2014).

The highest Hg concentration in the black sea cucumber is less than the maximum permissible levels given by European Union. Environmental Quality Standards (EQS) for Hg in biota are given in European Union legislative acts as 0.02 mg/kg wet wt. (European Union 2014). Mean Hg in muscle and skin were between 7.7 and 5.2 times lower than EQS value, respectively.

Holothuria (Mertensiothuria) leucospilotais locally not consumed. Moazzam and Moazzam (2020) pointed out that sea cucumbers are not consumed in Pakistan and there was also no organized fishery for harvesting or processing. Only one species *Holothuria (Thymiosycia) arenicola* have been made since 1990 to process them into beche-de-mer, but none of them were successful until 2012 when one of the seafood processors started pro¬ducing as beche-de-mer from Pakistan coast. Worldwide utilization of sea cucumbers to supply consumer request is getting a rising conservation concern (Eriksson and Clarke 2015; Meloni and Esposito 2018). However, the black sea cucumber is recorded as one of the consumable species with functional ingredients in medical sector, it has little toxic effect (Pangestuti and Arifin 2018). Usually, the reconstituted body wall (bêche-de-mer) is eaten by Asians. The whole organism or its intestine and/or gonads may be consumed as a delicacy or as protein in traditional diets or in times of shortage (Purcell et al. 2012).

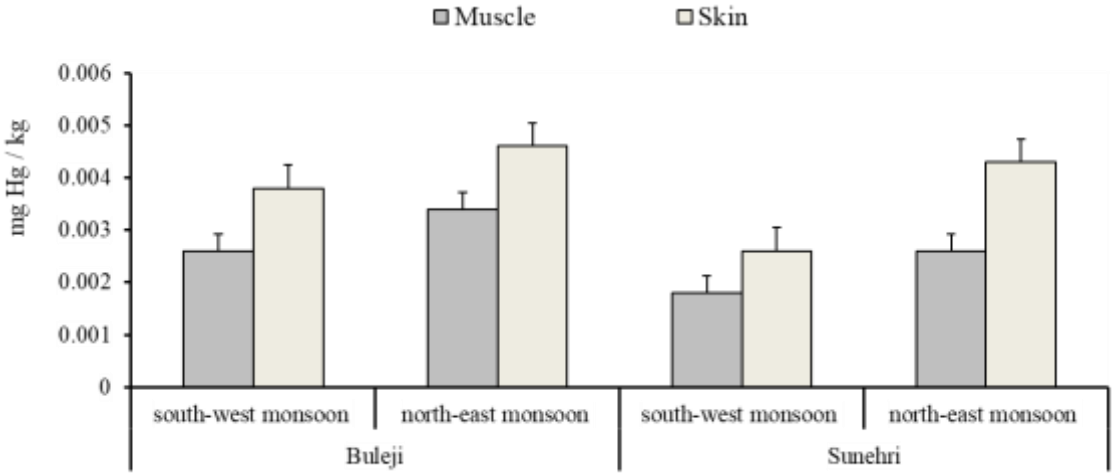

Figure 4. Mean concentrations of Hg (mg/kg) (±SE) in tissues of the black sea cucumber collected from Buleji and Sunehri sites of the Karachi coasts during south-west monsoon and north-east monsoon.

Şekil 4. Güney-batı musonu ve kuzey-doğu musonu sırasında Karachi kıyılarının Buleji ve Sunehri bölgelerinden toplanan kara deniz hıyarı dokularında ortalama Hg (mg / kg) (±SH) konsantrasyonları.

The BSAF and BCF of the heavy metals are noted to be a bio-indicator of these contaminants in the tissues of marine biota when indicated in relative to their amount in the sediment and surrounding water (Geyer et al. 2000; Dimitrov et al. 2003; Kleinow et al. 2008). The BSAF and BCF values for Hg in the muscle and skin of the black sea cucumbers are given in Figures 5 and 6.

Hg showed the highest accumulation rate in the skin tissue with BSAF values ranging between 2 in Buleji and 2.69 in Sunehri at north-east monsoon. The BSAF values in the muscles of the black sea cucumber ranged from 1.44 at south-west monsoon in Buleji to 1.63 at north-east monsoon in Sunehri, respectively. BSAF was used to classify the black sea cucumber species as a macro-concentrator (BSAF > 2), micro-concentrator (1 < BSAF < 2) or de-concentrator (BSAF < 1). Since the BSAF value is higher than 2 on the skin of sea cucumber, it is considered as a macro-concentrator. However, the BSAF value varies between 1 and 2 in muscle, it has been determined as micro-concentrator.

According to Regulation (EC) No. 1907/2006 (REACH), the heavy metals are classified as "bio-accumulative" if BCF varies between 1000 and 5000 and "very bio-accumulative" if the BCF was higher than 5000 (Regulation 1999). Hg in the black sea cucumbers from Buleji and Sunehri sites of the Karachi coasts is "very bio-accumulative" (BCF > 5000) during both sampling seasons.

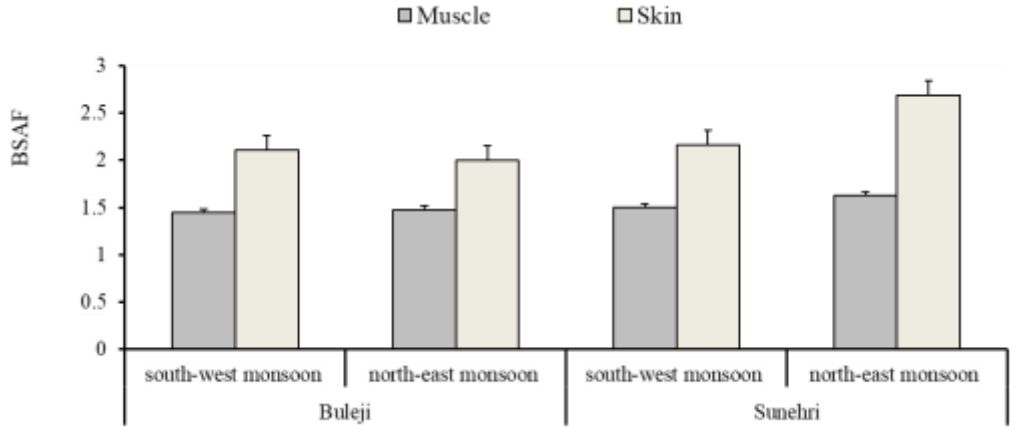

Figure 5. BSAF values of Hg (±SE) in the tissues of the black sea cucumber and sediments.
Şekil 5. Sedimentlerde ve kara deniz hıyarı dokularında Hg'nin (±SH) BSAF değerleri.

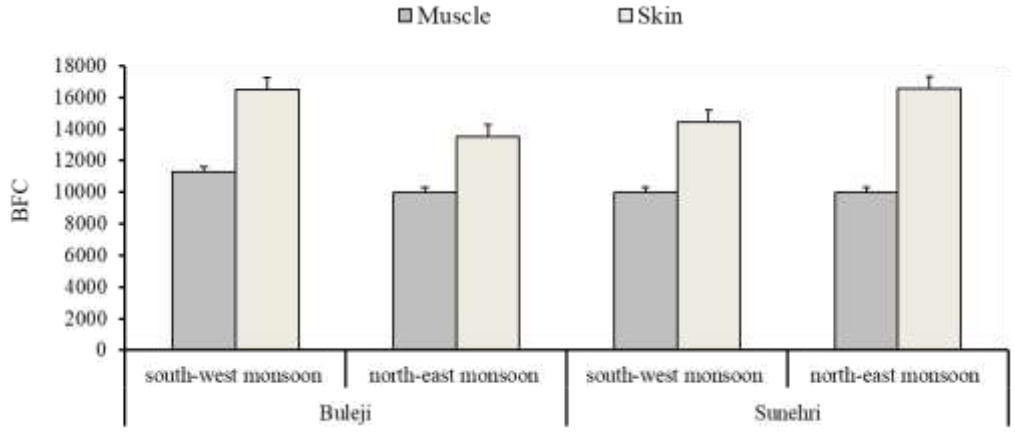

Figure 6. BFC values of Hg (±SE) in the tissues of the black sea cucumber and surface waters.
Şekil 6. Yüzey sularında ve kara deniz hıyarı dokularında Hg'nin (±SH) BFC değerleri.

There were differences in Hg levels in Buleji and Sunehri sites, these differences in Hg concentration were statistically insignificant ($p > 0.05$). On the other hand, there is no Hg extra source in both these sites sampled for the current study. These results indicate that the black sea cucumber has a bioavailability to accumulate Hg from the sediment and ambient water and can be suggested that the black sea cucumber is a well bio-indicator for the existence of Hg.

CONCLUSION

The present study was achieved to supply data on the amounts of Hg in sediment, surface water and the black sea cucumber from the Buleji and Sunehri sites of the Karachi coasts Pakistan. This study revealed that the Hg levels in the muscle and skin of the black sea cucumbers were below the maximum permissible concentrations for people consumption. Although, Hg is low in sediment, surface water and the black sea cucumber a likely threat may rise in the later depending on the local waste, fishing and farming activities in these sites. Such studies will support the improving of a successful coastal administration programme to keep safe the environmental quality of these beneficial marine ecosystems and the well-being of people connected with it. In this sense, heavy metal monitoring studies in in bio-indicator organisms such as the black sea cucumber should be continued in these regions.

The mean Hg concentrations in the black sea cucumber were high in north-east monsoon, and these differences in amounts were statistically significant ($p< 0.05$), showing that environmental conditions change seasonally. It may be concluded that Buleji and Sunehri sites of the Karachi coast are in general not considered Hg contamination.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

REFERENCES

Ahmed Q, Ali QM 2014. Abundance and distribution of Holothuroidea (Echinodermata) with emphasis on heavy metals accumulation in organism and its

habitat, Project: Higher Education Commission of Pakistan, (Grant No. IPFP/HRD/HEC/1688).

Ahmed Q, Ali QM, Bat L 2017. Assessment of heavy metals concentration in Holothurians, sediments and water samples from coastal areas of Pakistan (northern Arabian Sea). Journal of Coastal Life Medicine 5(5): 191-201.

Ahmed Q, Bat L, Ali QM 2018) Analysis of mercury (Hg) in four Holothurians species (Phylum-Echinodermata) from Karachi coast-northern Arabian Sea. Aquatic Research 1(2): 55-63.

Ahmed Q, Bat L, Ali QM 2019. Determination of mercury (hg) in two sea cucumber species *Ohshimella ehrenbergii* (Selenka, 1868) and *Stolus buccalis* (Stimpson, 1855) from the Karachi coast. Pakistan Journal of Marine Sciences 28(1): 55-62.

Ahmed Q, Ali QM 2020. Holothurians from Pakistan: New addition of *Holothuria (Theelothuria) notabilis* (Ludwig, 1875) and rediscovery of *Actinocucumis typica* (Ludwig, 1875) from the Karachi coast, northern Arabian Sea. Beche-De-Mer information bulletin 40: 40-42.

American Public Health Association 1998. Water Environment Federation, Standard methods for the examination of water and wastewater. Stand Methods, 541.

Bat L 2017. The contamination status of heavy metals in fish from the Black Sea, Turkey and potential risks to human health. In: Sezgin, M., Bat, L., Ürkmez, D., Arıcı, E., Öztürk, B. (Eds.) Black Sea Marine Environment: The Turkish Shelf. Turkish Marine Research Foundation (TUDAV), Publication No: 46, Istanbul, TURKEY, 322-418.

Bat L, Öztekin A, Şahin F, Arıcı E, Özsandıkçı U 2018. An overview of the Black Sea pollution in Turkey. MedFAR 1(2): 67-86.

Bat L, Özkan EY 2019. Heavy Metal Levels in Sediment of the Turkish Black Sea Coast. In I. Management Association (Ed.), Oceanography and Coastal Informatics: Breakthroughs in Research and Practice Hershey, PA: IGI Global 86-107.

Bernhard M 1976. Manual of Methods in the Aquatic Environment Research. FAO Fisheries Technical Paper FIRI/T no.158, Food and Agriculture Organization, Rome.

Dimitrov SD, Dimitrova NC, Walker JD, Veith GD, Mekenyan OG 2003. Bioconcentration potential predictions based on molecular attributes–an early warning approach for chemicals found in humans, birds, fish and wildlife. QSAR & Combinatorial Science 22(1): 58-68.

Eriksson H, Clarke S 2015. Chinese market responses to overexploitation of sharks and sea cucumbers. Biological Conservation 184: 163-173.

European Union 2014. Common implementation strategy for the water framework directive (2000/60/EC) Guidance, Document No. 32 on biota monitoring (the implementation of EQS biota) under the water framework directive. EU Technical Report - 2014 – 083.

Gao F, Li F, Tan J, Yan J, Sun H 2014. Bacterial community composition in the gut content and ambient sediment of sea cucumber *Apostichopus japonicus* revealed by 16S rRNA gene pyrosequencing. PloS one 9(6).

Geyer HJ, Rimkus GG, Scheunert I, Kaune A, Schramm KW, Kettrup A, Zeeman M, Muir DCG, Hansen LG, Mackay D 2000. Bioaccumulation and occurrence of endocrine-disrupting chemicals (EDCs), persistent organic pollutants (POPs), and other organic compounds in fish and other organisms including humans. In Bioaccumulation–New Aspects and Developments. Springer, Berlin, Heidelberg, 1-166.

Hashmi MI, Thilakar R, bin Syed Hussein MA, Hoque Z 2014. Determination of seven heavy metals in eight species of Sea Cucumbers. Sci Int 26 (1).

Ilias Z 2010. Gamat perairan Malaysia: kepelbagaian spesies dan kegunaannya. Kuala Lumpur: Dewan Bahasa dan Pustaka.

Keeney DR, Nelson DW 1983. Nitrogen-inorganic forms. Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties 9: 643-698.

Kleinow KM, Nichols JW, Hayton WL, McKim JM, Barron MG 2008. Toxicokinetics in fishes. The toxicology of fishes 55-152.

MeloniD, Esposito G 2018. Hygienic and commercial issues related to the illegal fishing and processing of sea cucumbers in the Mediterranean: A case study on over-exploitation in Italy between 2015 and 2017. Regional Studies in Marine Science 19: 43-46.

Microsoft Corporation 2018. Microsoft Excel. Retrieved from https://office.microsoft.com/excel

Moazzam M, Moazzam N 2020. Annotated checklist of sea cucumbers from Pakistan with new records of *Holothuria (Theelothuria) hamata* (Pearson, 1913) and Stichopus herrmanni (Semper, 1868). Beche-De-Mer information bulletin 40: 32-39.

Official Journal of the European Union 2008. Directives Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). L 164: 19-40.

Pangestuti R, Arifin Z 2018. Medicinal and health benefit effects of functional sea cucumbers. Journal of traditional and complementary medicine 8 (3): 341-351.

Purcell SW, Samyn Y, Conand C 2012. Commercially important sea cucumbers of the world. FAO Species Catalogue for Fishery Purposes No. 6. Rome, FAO.

Regulation EC 1999. No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration. Evaluation, Authorisation and Restriction of Chemicals

(REACH), establishing a European Chemicals Agency, amending Directive 45: 1-849.

Roberts D, Moore HM, Berges J, Patching JW, Carton MW, Eardly DF 2001. Sediment distribution, hydrolytic enzyme profiles and bacterial activities in the guts of *Oneirophanta mutabilis*, *Psychropotes longicauda* and *Pseudostichopus villosus*: what do they tell us about digestive strategies of abyssal holothurians? Progress in Oceanography 50(1-4): 443-458.

Sloan NA 1979. Microhabitat and resource utilization in cryptic rocky intertidal echinoderms at Aldabra Atoll, Seychelles. Marine Biology 54(3): 269-279.

SPSS 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.

USEPA 1999. United State Environmental Protection Agency. SW – Method 3050B. Standard Operating Procedure for the digestion of soil sediment samples using a hotplate/beaker digestion technique, Chicago.

Yu Z, Hu C, Zhou Y, Li H, Peng P 2012. Survival and growth of the sea cucumber *Holothuria leucospilota* Brandt: a comparison between suspended and bottom cultures in a subtropical fish farm during summer. Aquaculture Research 44 (1): 114-124.