Journal of Coastal Life Medicine

journal homepage: www.jclmm.com



Original article

https://doi.org/10.12980/jclm.5.2017J7-56

©2017 by the Journal of Coastal Life Medicine. All rights reserved.

Assessment of heavy metals concentration in holothurians, sediments and water samples from coastal areas of Pakistan (Northern Arabian Sea)

Quratulan Ahmed^{1*}, Qadeer Mohammad Ali¹, Levent Bat²

¹The Marine Reference Collection and Resource Centre, University of Karachi, Karachi, 75270 Pakistan

²Department of Hydrobiology, Fisheries Faculty, Sinop University, TR57000 Sinop, Turkey

ARTICLE INFO

Article history:
Received 23 Mar 2017
Received in revised form 2 May 2017
Accepted 15 May 2017
Available online 20 May 2017

Keywords: Heavy metal Sea cucumber Karachi coast Northern Arabian Sea

ABSTRACT

Objective: To determine heavy metal concentrations in holothurians (*Holothuria arenicola*, *Holothuria pardalis*, *Holothuria verrucosa*, *Holothuria atra*, *Ohshimella ehrenbergii*, *Holothuria cinerascens*, *Stolus buccalis* and *Holothuria leucospilota*), sediments and sea water samples from both Buleji and Sunehri coasts bordering Northern Arabian Sea during January to December 2014 and to assess the level of bioaccumulation using bioaccumulation factors.

Methods: Fe, Mn, Zn, Cu, Cd and Pb levels were determined by Perkin Elmer AAnalyst 700 Atomic Absorption Spectrophotometer. Biota concentration factor (BCF) and biota sediment accumulation factor (BSAF) were also calculated.

Results: The mean concentrations of heavy metals in body wall of sea cucumber ranged from 0.11 to 2.67, 0.43 to 8.93, 14 to 73, 0.76 to 7.12, 0.52 to 3.02 and 11 to 46 μg/g dry wright for Cd, Cu, Fe, Mn, Pb and Zn, respectively. The greatest biota-sediment bioaccumulation factor (BSAF) value for Zn (3.29) was observed in *H. leucospilota* at Buleji during pre-monsoon, indicating the species as microconcentrator. The BSAF values for Cd in sea cucumber ranged from 0.042 to 1.492.

Conclusions: The results suggested that the studied sea cucumber species ranged from being microconcentrators to deconcentrators. The BSAF values of Cu, Fe, Mn and Pb were low, indicating the species as deconcentrators. Zn in all species from Sunehri coast is "very bioaccumulative" (BCF > 5000) during all sampling periods. Cu, Fe, Mn and Pb can be considered "bioaccumulative" (BCF < 5000).

1. Introduction

During the last few decades, the state of marine coastal pollution has become a matter of growing global concern. Heavy metals are regarded as a main anthropogenic pollutant in coastal and marine environments globally[1]. Heavy metals pose a major danger to people health, living organisms and natural ecosystems by virtue of their toxicity, constancy and bio-accumulation and such characteristics[2]. Heavy metals can conduce to disruption of marine environments by diminishing species diversity and abundance and by dint of accumulation of metals in biota and food web[3]. Heavy metals can enter to marine coastal area through the agency of a kind of sources, including industries and domestic wastes[4].

Metals are a natural part of the earth's crust and are permanent and

sediments and biota[5-7]. The heavy metal amounts in biota are oft notably higher than those in other sections of marine ecosystem[8,9]. Some of these are compulsory elements necessary for biological activities, whereas they can also be toxic to biota when exposure to high levels. This threshold level counts on the metals, the biota, but also on the ecologic factors, which detect the availability[10,11]. The exposing of biota to toxic amounts of metals can lead to injury to tissue, incapability to regenerate injuried tissue, growth restriction, and harm to DNA. Because of aquatic organisms' ability to concentrate heavy metals from their habitat, it is essential to know the changes in the metal levels that should be considered within a normal range, and how much their levels may be increased above threshold levels[12]. Ideally, all components of a coastal ecosystem would be analyzed several times a year. In this manner, spatial and temporal distributions of pollutants and their dynamics in coastal ecosystems could be measured directly or inferred[13]. Heavy metals in the marine environment are primarily concentrated in coasts,

close to densely populated areas and industrialized regions. They are

cannot be disintegrated, which make it easy for them to accumulate

in the marine ecosystem. The contaminant amounts of marine coastal area by heavy metals can be determined by analyzing water,

Tel: +92 345 2983586

E-mail: quratulanahmed_ku@yahoo.com

Foundation Project: Supported by Higher Education Commission of Pakistan (Grant No. IPFP/HRD/HEC/1688).

The journal implements double-blind peer review practiced by specially invited international editorial board members.

^{*}Corresponding author: Quratulan Ahmed, The Marine Reference Collection and Resource Centre, University of Karachi, Karachi, 75270 Pakistan.

mostly connected with particles, and these particles are frequently very tiny, and can hence remain in solution for a long time. Eventually they will fall down in the sediments; hence amounts of heavy metals in the sediments are often 10 to 100 times higher than those in surrounding water. In the sediments, these particles may be a serious derivative source of pollution, even after the main resource disappeared[14,15]. The effects of heavy metals on marine organisms can be divided into direct effects and indirect effects. The direct influences are viewed in physiology, reproduction, metabolism, behavior, migration, development and growth of organisms. The latter can be effects on food web and environment stress. Due to their capacity to accumulate metals from the environment, Echinodermata species have been chosen as qualitative biological indicators of metal contamination. Of the Echinodermata, sea cucumbers now receive considerable attention as subjects of heavy metal pollution studies. Sea cucumbers have been proposed as suitable bio-indicators for monitoring heavy metal pollution in the marine ecosystem. They possess maximum of the obligatory characteristics of a bio-indicator such as worldwide distribution, long survival, plausible size, simple collection, abundance and the capability to accumulate heavy metals. They are mostly soft-bodied echinoderms consisting of a variety of pliable, prolonged, worm-like organisms, with a leathery skin and gelatinous body, looking like a cucumber. Usually, they tend to live on the sea bed in deep seas[16]. They are generally scavengers, feeding on debris in the benthic zone of the ocean and playing a major ecological role on account of their contribution to sediment recycling or bioturbation. Holothurians are important components of the marine ecosystem comprising 90% of the total mass of the macrofauna[17]. They are distributed in all oceans of the world, at depth deeper than 8.9 km (5.5 miles). They have a mean life span of 5-10 years, and the organisms achieve sexual maturity by 2 to 6 years with many species being broadcast spawners, and some reproduce by fission. It is shown that as deposit feeders, holothurians ingest carbonate sand and rubble through their digestive tract and dissolve the sediment CaCO₃ as part of their digestive processes. Such activity allows natural CaCO₃ turnover and increases alkalinity, as the sea cucumber secretes ammonia (NH₃, which becomes NH₄⁺ in the aqueous medium) as a by-product of their digestive process. They contribute to the nutrient cycling process and buffer the seawater pH from ocean acidification. Thus, it is postulated that holothurians can reduce the impact of ocean acidification[18].

Hashmi *et al.*[19] reported the concentrations of heavy metals in *Holothuria scraba*, seawater and sediments from sea ranching in Sabah Malaysian Borneo. Hashmi *et al.*[20] also determined the concentrations of As, Pb, Cd, Zn, Cr, Cu, and Mn in *Holothuria leucospilota*, *Holothuria edulis*, *Thelenota ananas*, *Thelenota anax*, *Actinopyga lecan*, *Bohadachio vitiens*, *Stichopus vastus*, *Phyllophogius spiculata* from Kota Kinabalu, Sabah Malaysia. Jinadasa *et al.*[21] recorded concentrations of Cu, Fe, Zn, Pb, Cd, Co, Cr and Hg in ten sea cucumber (holothurians) species in the northwestern sea of Kalpitiya and Dutch Bay area, Sri Lanka. Haifeng *et al.*[22] studied the Cu, Zn, Cr, Pb, Cd, As and Hg levels in *Apostichopus japonicus* juveniles off the coasts of Bohai and Yellow seas in Northern China. Haider *et al.*[23] studied mineral contents (Cr, Ni, Mn, Cu, Pb, Cd, Zn, Na, K, Ca and Mg) in *Holothuria arenicola* and *Actinopyga mauritiana* from coastal waters of Pakistan.

Karachi is a megacity and the commercial hub of Pakistan which is continuously pouring untreated effluents (domestic and industrial) in the coastal areas. High heavy metal levels in many fish of Southeast Arabian Sea were found[24]. Saif et al.[25] pointed out that wastewaters of the urban area and several industries of Korangi area are dumped to Malir River and then finally transferred to Arabian Sea. It is the reason for rise in the level of marine toxic pollutants including transition metals in the coastal areas. The transition metals are required sometimes in small amounts for metabolic activities, but they have toxic effects in higher concentration[26]. The bioaccumulation of intractable toxic pollutants is reported to be capable of decreasing immune system, interfering and damaging respiratory, reproductive and nervous system of various organisms which are transferred to the top predators[27]. Matranga et al.[28] pointed out that echinoderms are considered qua liable animals to detect permanent marine ecological stress and a long-term biological impact. They are proved in many studies[29-33] as ideal subjects for ecotoxicological studies because they are directly exposed to emerging anthropogenic pollutants in both their planktonic and benthic lives.

In the current study, heavy metal contents were investigated in the body wall of sea cucumbers (*Holothuria arenicola*, *Holothuria pardalis*, *Holothuria verrucosa*, *Holothuria atra*, *Ohshimella ehrenbergii*, *Holothuria cinerascens*, *Stolus buccalis* and *Holothuria leucospilota*) from Karachi coast bordering Northern Arabian Sea during January to December 2014. Cd, Cu, Fe, Mn, Pb and Zn were chosen for this study because their toxicities to aquatic organisms were well documented[34-44]. Heavy metals like Cu, Fe, Mn and Zn are essential for the growth of organisms, while Cd and Pb are not only biologically non-essential heavy metals, but also may be hazardous and toxic[45]. It is well known that the Pb cations have high affinity for calcic skeletons and have been indicated to be heavily concentrated in the body walls of various echinoderms[46].

There is no available data on heavy metal analysis on these sea cucumber species except *H. arenicola* in Pakistan coasts^[23]. The basic aim of the current study was to conduct the analysis of heavy metal concentrations in sea water, sediments and sea cucumber species, and to generate a base line data regarding heavy metal pollution level in sea cucumbers.

2. Material and methods

2.1. Study area

Two rocky shores Buleji and Sunahri coasts were selected in this study (Figure 1). The geographical descriptions of both sites are given below.

Buleji (rocky shore) is located at 24°50′20.41" N, 66°49′24.15" E on the northeastern border of the Arabian Sea. This coast is found near the fishing village of Buleji. The rocks of Buleji were laid in the post-tertiary period. These rocks are more or less like mounds. They are rough and hard. The area under investigation is two hundred fifty meters long and one hundred fifty meters wide; the rocky bulge is progressively curved which protrudes out in the Arabian Sea. The site may be classified as exposed wave-beaten rocky coast, with slightly uneven profile consisting of slightly elevated and depressed areas. Therefore part of the shores, which is exposed to direct wave action, is somewhat elevated compared to the hinder part where small and large pools of water are created at low tides. Boulders of Different dimensions can be viewed scattered on the rocky ledge but mostly high water mark. Buleji has muddy sandy pebbly beach lying at relatively low level compared to the main rocky ledge.

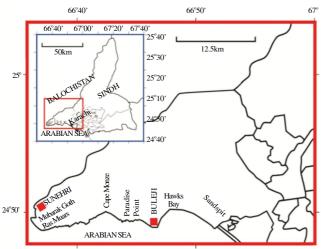


Figure 1. Sampling region.

Sunehri (rocky shore) is located at 24°52'33.49" N, 66°40'40.20" E on the northeastern border of the Arabian Sea. This coast is found near a fishing village Goth Manjhar. The area is a continuation of lime stone ridges of Jhill hills. Jhill hills submerge into the Arabian Sea at Cape Monze forming peninsular area between the Hub estuary and the open sea coast. Soil texture varies from coarse to clay. The sampling area is rocky ledge about three hundred meters wide and twelve hundred meters long which is gradually sloping and more protruding out in the Arabian Sea. The shore has relatively rich diversity of flora and fauna. The habitat is complex, with the existence of rockpools, gullies, cracks and boulders augmenting the variety of habitats and accordingly the quantity of species. Inasmuch as the substrate is non-deployable, it makes a reliable surface for a range of aquatic organisms such as barnacles, mussels, limpets and seaweeds.

While there is no specific source of chemical pollution in Buleji and Sunhehri beaches, heavy metals pollution such as Cd and Pb pollution exist in these regions due to fishing activities, oil spill and domestic wastes. The littoral zone of Pakistan coasts is facing many environmental issues due to increasing pollution and environmental changes caused by human. Over 18000 small fishing vessels are recorded in the Marine Fisheries Department in Karachi; a minimum of 12000 of them are operational throughout the 999 km sea coasts. These trawlers have been dredging the territorial waters of Pakistan for a long time, and polluting the sea[47]. Laxen[48] pointed out that high Pb levels in the coastal area of sea is attributed to boats exhausts, oil spill, and other petroleum compounds from mechanized vessels employed for fishing and sewage wastes discharged into water and such. A major source of Cd into the marine ecosytem is primarily electroplating, agricultural and industrial activities[49,50]. The industrial and municipal wastes input into the sea has bben sharply increased through the river Indus, Layari, Malir, Hub, and Windler[51].

2.2. Samples collection

2.2.1. Water samples

The water samples were collected into plastic bottle and acidified immediately with 2 mL of HNO₃ per liter of water and preserved in refrigerator for laboratory analysis[52].

2.2.2. Sediment samples

Sediment samples were collected and put in sanitized plastic sack and froze in ice box for transfer to the laboratory for the detection of metal concentrations. The sediment samples were then dried at 105 °C, ground and sieved (through a 63 µm sieve), and about 1 g of the sample was digested with a mixture of concentrations of H₂O₂, HCl and HNO₃. The digested samples were then diluted to 40 mL with deionized water and filtered through Whatman No.1 filter paper into pre-cleaned 50 mL volumetric flasks as the method described[53,54] and kept in a fridge until analysis.

2.2.3. Sea cucumber samples

Sea cucumber samples were collected by hand-picking through forceps at low tide from intertidal zone during pre-monsoon (January to May), monsoon (June to August) and post-monsoon (September to December) in 2014. Collected samples were kept alive in containers filled with water. Samples of sea cucumber were taken to the laboratory and transferred to well aerated aquaria.

2.3. Length and weight measurement of specimens

Length (mm) and weight (g) data were captured for each sea cucumber after allowing the sea cucumber to relax 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.

2.4. Identification

For taxonomic studies and identification, morphological features were examined and microscopic examinations were conducted. Ossicles were taken from three positions (dorsal and ventral body walls and tentacles), and wet mounts were made by laying a little part of skin tissue on slide and adding few drops of 3.5% bleach. The slides were then rinsed with drops of distilled water. The slides were examined under a microscope at 10×10 magnifications (Nikon LABOPHOT-2). Microphotography was also performed through digital camera (Fujifilm 16 MP).

2.5. Sample preparation for metal analysis

The body wall of sea cucumber was separated to remove all the internal organs before analysis and only the body wall was used for analysis after cleaning. The body walls were weighted (g) and chopped into small pieces and then ground and calcinated at 450 °C for 4 h. Ash samples of each specimen were weighed (g), dissolved in HCl (0.1 mol/L) and further treated with H₂O₂ (30%) till lucid solutions were formed, and then diluted by water[20]. The 0.45 µm Whatman filter papers were chosen for the filtration purposes. Fe, Mn, Zn, Cu, Cd and Pb levels were determined by Perkin Elmer AAnalyst 700 Atomic Absorption Spectrophotometer. The calibration and standards from 1000 ppm stock solution to 0.5, 1, 2, 4 and 6 ppm were prepared for metal analysis. The calibration graphs acquired for standard solution of each metal indicated a straight-line relation between levels and absorption values. To avoid contaminations the beakers and sample vessels were pre-cleaned

by soaking in a 10% HNO₃ solution more than overnight, and then rinsed at least 3 times with deionized water prior to use. The detection limits (μ g/L) were 0.45 for Fe, 0.58 for Mn, 0.50 for Zn, 0.22 for Cu, 0.38 for Cd and 0.28 for Pb.

2.6. Statistical analysis

One-way ANOVA was applied following Tukey *post hoc* comparisons for the results of statistically significant difference[55]. The significance was adjusted at 0.05 and statistical programme was realized using Statistica and Excel 2010 to analyse the influence of seasons (pre-monsoon, monsoon and post-monsoon) and the metal levels in sea cucumber species. The concentrations were expressed as µg/g dry weight. Biota concentration factor (BCF) and biota sediment accumulation factor (BSAF) were also calculated. Bioconcentration is the uptake of the metals by species only from media and the result of such a calculation is called the BCF. The bioconcentration factor (BCF) is the ratio of the concentration of a metal in an organism to the concentration in the medium (mass of metal/L)[56]. BCF was calculted by the following formula:

BCF = CB/CW

where, CB is concentration of the metal in the organism, CW is the concentration in the medium.

Heavy metals accumulation in tissues of sea cucumbers from the sediments is expressed by the BSAF. BSAF is a parameter defining bioaccumulation of sediment-associated organic compounds or metals in tissues of the organism[57]. The BSAF is calculated using the following equation:

BSAF = CB/CS

where, CB is the chemical concentration in the biota (mass of chemical per kg of biota/dry weight), while CS is the concentration in the related sediment (mass of chemical per kg of sediment/dry weight).

3. Results

3.1. Organisms size

One hundred specimens were collected from Buleji coast and a total of 196 specimens were from Sunehri coast. Sea cucumber species were indentified as Holothuria (Lessonothuria) pardalis Selenka, 1867 (collected from Buleji and Sunehri during all seasons), Holothuria (Holothuria) atra Jaeger, 1833 (collected from Buleji and Sunehri during all seasons), Holothuria (Thymiosycia) arenicola Semper, 1868 (collected from Buleji and Sunehri during all seasons), Holothuria (Lessonothuria) verrucosa Selenka, 1867 (collected from Buleji and Sunehri during all seasons), Holothuria (Mertensiothuria) leucospilota (Brandt, 1835) (collected from Buleji during just monsoon season), Stolus buccalis (Stimpson, 1855) (collected from Sunehri during just post-monsoon season), Ohshimella ehrenbergii (Selenka, 1868) (collected from Sunehri during pre- and postmonsoon season) and Holothuria (Semperothuria) cinerascens (Brandt, 1835) (collected from Sunehri during post-monsoon season). It is well known that metal concentrations in an organism are highly dependent on organism's size and species. The length and weight of the sea cucumber species were given in Table 1.

3.2. Heavy metals in surface sediments and in sea water

The levels of Cd, Cu, Fe, Mn, Pb and Zn in surface sediments from Blueji and Sunehri coasts during January to December 2014 are

presented in Table 2. One-way ANOVA analysis showed that there were not significant (P > 0.05) temporal differences of the metal levels in the sediments for Cu, Fe, Mn and Pb. However there were significant (P < 0.05) spatial differences of metal levels in the sediments for Cd, Cu, Pb and Zn. Spatial variations of the metal levels were found over the whole sampling period at both Blueji and Sunehri coasts. All highest metal concentrations except Zn were found at Sunehri coasts.

Table 1
Length and weight of sea cucumber species collected from Blueji and Sunehri coasts during January to December 2014.

Holothuria Buleji Pre-monsoon 23 ± 6	79 ± 21
arenicola 7 Monsoon 36 ± 12	105 ± 30
Post-monsoon 18 ± 5	34 ± 18
Sunehri Pre-monsoon 16 ± 3	22 ± 6
27 Monsoon 20 ± 5	50 ± 27
Post-monsoon 25 ± 5	81 ± 16
Holothuria Buleji Pre-monsoon 7 ± 3	10 ± 4
pardalis 47 Monsoon 9 ± 4	13 ± 6
Post-monsoon 9 ± 2	15 ± 3
Sunehri Pre-monsoon 9 ± 3	13 ± 4
110 Monsoon 6 ± 2	10 ± 7
Post-monsoon 9 ± 3	12 ± 4
Holothuria Buleji Pre-monsoon 14 ± 2	20 ± 5
<i>verrucosa</i> 34 Monsoon 10 ± 3	14 ± 4
Post-monsoon 11 ± 3	15 ± 3
Sunehri Pre-monsoon 14 ± 2	18 ± 4
33 Monsoon 11 ± 2	16 ± 2
Post-monsoon 9 ± 1	13 ± 2
Holothuria Buleji Pre-monsoon 20 ± 8	49 ± 37
atra 11 Monsoon 21 ± 12	34 ± 18
Post-monsoon 14 ± 3	19 ± 3
Sunehri Pre-monsoon 18 ± 4	34 ± 10
19 Monsoon 22 ± 3	46 ± 9
Post-monsoon 19 ± 5	40 ± 15
Ohshimella Buleji Pre-monsoon	
ehrenbergii Monsoon	
Post-monsoon	
Sunehri Pre-monsoon 6 ± 1	8 ± 1
5 Monsoon	
Post-monsoon 5 ± 2	9 ± 4
Holothuria Buleji Pre-monsoon	
cinerascens Monsoon	
Post-monsoon	
Sunehri Pre-monsoon	
1 Monsoon	
Post-monsoon 28	248
Stolus Buleji Pre-monsoon	
buccalis Monsoon	
Post-monsoon	
Sunehri Pre-monsoon	
1 Monsoon	
Post-monsoon 5	7
Holothuria Buleji Pre-monsoon 51	180
leucospilota 1 Monsoon	
Post-monsoon	
Sunehri Pre-monsoon	
Monsoon	
Post-monsoon	

Data are expressed as mean ± SD.

The heavy metal levels in seawater are shown in Table 3. In seawater the level of heavy metals (μ g/L) was in the order of Fe > Mn > Pb > Cu > Zn > Cd.

3.3. Heavy metals in the soft tissues of sea cucumbers

The heavy metal levels in body wall of sea cucumber species from

Table 2
Mean concentration of heavy metals (μg/g dry weight) in sediments collected from Blueji and Sunehri coasts during January to December 2014.

Region	Periods	Cd	Cu	Fe	Mn	Pb	Zn
Buleji	Pre-monsoon	2.42 ± 0.13^{a}	5.52 ± 0.31^{a}	220 ± 25^{a}	137 ± 13 ^a	37 ± 4^{a}	14 ± 2^{a}
	Monsoon	1.91 ± 0.08^{b}	4.92 ± 0.18^{a}	218 ± 22^{a}	133 ± 11^{a}	33 ± 3^{a}	26 ± 3^{b}
	Post-monsoon	1.88 ± 0.07^{b}	5.27 ± 0.28^{a}	218 ± 21^{a}	132 ± 10^{a}	37 ± 4^{a}	$39 \pm 5^{\circ}$
Sunehri	Pre-monsoon	$1.18 \pm 0.04^{\circ}$	5.99 ± 0.35^{a}	241 ± 26^{a}	134 ± 12^{a}	56 ± 8^{b}	19 ± 3^{a}
	Monsoon	3.07 ± 0.16^{d}	$7.83 \pm 0.57^{\text{b}}$	222 ± 26^{a}	140 ± 17^{a}	51 ± 8^{b}	15 ± 2^{a}
	Post-monsoon	2.60 ± 0.14^{a}	9.04 ± 0.64^{b}	222 ± 28^{a}	143 ± 19^{a}	47 ± 7^{b}	$22 \pm 4^{a,b}$

The same letters in each column indicate that the values are not significantly different (P > 0.05).

Table 3
Mean concentration of heavy metals (μg/L) in sea water collected from Blueji and Sunehri coasts during January to December 2014.

Region	Periods	Cd	Cu	Fe	Mn	Pb	Zn
Buleji	Pre-monsoon	0.17 ± 0.02^{a}	33 ± 4^{a}	206 ± 28^{a}	273 ± 28^{a}	73 ± 7^{a}	4.23 ± 1.10^{a}
	Monsoon	0.86 ± 0.04^{b}	37 ± 5^{a}	478 ± 54^{b}	323 ± 39^{b}	88 ± 9^{b}	4.21 ± 1.00^{a}
	Post-monsoon	$1.22 \pm 0.10^{\circ}$	19 ± 3^{b}	264 ± 32^{a}	308 ± 35^{b}	67 ± 7^{a}	3.38 ± 0.60^{b}
Sunehri	Pre-monsoon	1.83 ± 0.15^{b}	$57 \pm 8^{\circ}$	$308 \pm 41^{\circ}$	$229 \pm 27^{\circ}$	66 ± 6^{a}	3.41 ± 0.70^{b}
	Monsoon	3.11 ± 0.76^{d}	44 ± 5^{a}	222 ± 26^{a}	$199 \pm 25^{\circ}$	$52 \pm 5^{\circ}$	$2.03 \pm 0.50^{\circ}$
	Post-monsoon	5.57 ± 0.87^{e}	63 ± 9^{c}	$372 \pm 48^{\circ}$	294 ± 30^{a}	$47 \pm 4^{\circ}$	$1.55 \pm 0.40^{\circ}$

The same letters in each column indicate that the values are not significantly different (P > 0.05).

Blueji and Sunehri coasts during January to December 2014 are shown in Table 4. The mean concentrations of heavy metals in body wall of all sea cucumbers ranged from 0.11 to 2.67, 0.43 to 8.93, 14 to 73, 0.76 to 7.12, 0.52 to 3.02 and 11 to 46 μ g/g dry weight for Cd, Cu, Fe, Mn, Pb and Zn, respectively. The spatial and seasonal variations of concentrations in tissue were statistically significant (P < 0.05) as concluded by one-way ANOVA analysis. The highest concentrations of Cd (2.76 μ g/g dry weight) and Cu (8.93 μ g/g dry weight) in *H. cinerascens* occurred at Sunehri coasts during post-monsoon, while the highest concentrations of Fe (73 μ g/g dry weight), Mn (7.12 μ g/g dry weight) and Zn (46 μ g/g dry weight) in *H. leucospilota* occurred at Buleji coasts during pre-monsoon. The

highest Pb level (3.02 μg/g dry weight) was found in *O. ehrenbergii* at Sunehri coasts during pre-monsoon.

3.4. The bioconcentration factors (BSAF and BCF)

The concentrations of heavy metals in water may vary considerably depending on seasonal fluctuations. BCF and BSAF are used to estimate contaminant loads in organisms. BCF is used to evaluate the amount of metal found in the organism and in the surrounding water. BSAF is used to calculate the ratio of metal found in the organism to that in the sediments. BSAF was used to classify the sea cucumber species as a macroconcentrator (BSAF > 2), microconcentrator (1 <

Table 4
Mean concentration of heavy metals (μg/g dry weight) in sea cucumbers collected from Blueji and Sunehri coasts during January to December 2014.

Species	Region	Periods	Cd	Cu	Fe	Mn	Pb	Zn
H. arenicola	Buleji	Pre-monsoon	0.14 ± 0.01^{a}	2.23 ± 0.07^{a}	33 ± 4^{a}	5.32 ± 0.34^{a}	2.33 ± 0.08^{a}	17 ± 2 ^a
		Monsoon	0.87 ± 0.04^{b}	1.09 ± 0.05^{b}	44 ± 5^{b}	4.11 ± 0.44^{b}	1.38 ± 0.04^{b}	11 ± 1^{a}
		Post-monsoon	0.12 ± 0.01^{a}	0.95 ± 0.03^{b}	47 ± 5^{b}	5.16 ± 0.51^{a}	1.19 ± 0.03^{b}	13 ± 2^{a}
	Sunehri	Pre-monsoon	1.10 ± 0.01^{b}	1.13 ± 0.02^{b}	39 ± 5^{b}	2.45 ± 0.03^{b}	1.10 ± 0.02^{b}	28 ± 5^{b}
		Monsoon	$0.65 \pm 0.01^{\circ}$	1.98 ± 0.06^{a}	41 ± 6^{b}	2.89 ± 0.07^{b}	1.43 ± 0.03^{b}	22 ± 5^{c}
		Post-monsoon	1.42 ± 0.04^{d}	0.43 ± 0.02^{c}	35 ± 4^{a}	3.98 ± 0.22^{b}	0.92 ± 0.01^{b}	22 ± 4^{c}
H. pardalis	Buleji	Pre-monsoon	0.21 ± 0.01^{e}	1.13 ± 0.02^{b}	29 ± 6^{a}	3.76 ± 0.24^{b}	$0.76 \pm 0.02^{\circ}$	17 ± 3^{a}
		Monsoon	$0.67 \pm 0.03^{\circ}$	$1.98 \pm 0.03^{a,d}$	32 ± 8^{a}	3.91 ± 0.30^{b}	1.01 ± 0.03^{b}	19 ± 3^{a}
		Post-monsoon	0.88 ± 0.04^{b}	1.65 ± 0.02^{b}	$37 \pm 9^{a,b}$	$2.18 \pm 0.08^{\circ}$	$0.88 \pm 0.03^{\circ}$	19 ± 4^{a}
	Sunehri	Pre-monsoon	0.99 ± 0.05^{b}	2.34 ± 0.05^{a}	29 ± 7^{a}	1.56 ± 0.05^{d}	1.22 ± 0.05^{b}	$25 \pm 5^{b,c}$
		Monsoon	$0.43 \pm 0.02^{\rm f}$	1.42 ± 0.03^{d}	33 ± 6^{a}	1.23 ± 0.04^{d}	1.18 ± 0.04^{b}	$25 \pm 5^{b,c}$
		Post-monsoon	0.88 ± 0.03^{b}	1.73 ± 0.04^{d}	32 ± 6^{a}	$2.02 \pm 0.06^{\circ}$	1.56 ± 0.06^{b}	23 ± 4^{c}
H. verrucosa	Buleji	Pre-monsoon	1.12 ± 0.03^{a}	2.24 ± 0.04^{a}	$24 \pm 6^{\circ}$	$2.23 \pm 0.04^{\circ}$	$0.89 \pm 0.03^{\circ}$	15 ± 3^{a}
		Monsoon	0.87 ± 0.04^{b}	2.39 ± 0.05^{a}	29 ± 7^{a}	$2.47 \pm 0.05^{\circ}$	1.03 ± 0.04^{b}	12 ± 3^{a}
		Post-monsoon	0.76 ± 0.04^{b}	$1.98 \pm 0.03^{a,d}$	$27 \pm 7^{a,c}$	1.11 ± 0.03^{d}	$0.67 \pm 0.03^{\circ}$	14 ± 4^{a}
	Sunehri	Pre-monsoon	1.76 ± 0.04^{g}	3.76 ± 0.06^{e}	$27 \pm 8^{a,c}$	0.76 ± 0.04^{e}	0.54 ± 0.02^{d}	$30 \pm 7^{\rm b}$
		Monsoon	1.45 ± 0.02^{d}	2.23 ± 0.04^{a}	23 ± 4^{c}	0.94 ± 0.03^{e}	$0.86 \pm 0.03^{\circ}$	23 ± 4^{c}
		Post-monsoon	1.09 ± 0.01^{b}	$2.89 \pm 0.05^{\rm f}$	25 ± 5^{c}	$1.08 \pm 0.02^{d,e}$	0.52 ± 0.02^{d}	27 ± 6^{b}
H. atra	Buleji	Pre-monsoon	$0.63 \pm 0.03^{\circ}$	3.89 ± 0.07^{e}	$26 \pm 5^{\circ}$	1.33 ± 0.04^{d}	1.23 ± 0.03^{b}	18 ± 4^{a}
		Monsoon	$0.41 \pm 0.02^{\rm f}$	$3.11 \pm 0.05^{e,f}$	23 ± 4^{c}	$2.49 \pm 0.06^{\circ}$	$0.81 \pm 0.03^{\circ}$	$23 \pm 5^{\circ}$
		Post-monsoon	$0.52 \pm 0.02^{c,f}$	2.26 ± 0.04^{a}	$25 \pm 6^{\circ}$	$2.18 \pm 0.04^{\circ}$	$0.87 \pm 0.02^{\circ}$	$24 \pm 5^{b,c}$
	Sunehri	Pre-monsoon	1.11 ± 0.01^{b}	$2.78 \pm 0.05^{a,f}$	$23 \pm 5^{\circ}$	$1.09 \pm 0.02^{d,e}$	1.00 ± 0.01^{b}	$24 \pm 6^{b,c}$
		Monsoon	0.88 ± 0.03^{b}	2.03 ± 0.04^{a}	19 ± 3^{c}	$1.18 \pm 0.03^{d,e}$	$0.80 \pm 0.02^{\circ}$	19 ± 3^{a}
		Post-monsoon	0.74 ± 0.02^{b}	$3.21 \pm 0.07^{e,f}$	22 ± 4^{c}	1.67 ± 0.05^{d}	$0.69 \pm 0.03^{\circ}$	21 ± 4^{a}
							continued	on next page

Table 4 (continued)

Species	Region	Periods	Cd	Cu	Fe	Mn	Pb	Zn
O.	Buleji	Pre-monsoon						
ehrenbergii		Monsoon						
		Post-monsoon						
	Sunehri	Pre-monsoon	$0.52 \pm 0.02^{c,f}$	3.98 ± 0.08^{e}	32 ± 7^{c}	$2.11 \pm 0.06^{\circ}$	3.02 ± 0.07^{e}	$32 \pm 7^{b,d}$
		Monsoon						
		Post-monsoon	0.31 ± 0.02^{e}	2.23 ± 0.05^{a}	$34 \pm 8^{\circ}$	3.91 ± 0.08^{b}	2.54 ± 0.06^{a}	$24 \pm 6^{b,c}$
H.	Buleji	Pre-monsoon						
cinerascens		Monsoon						
		Post-monsoon						
	Sunehri	Pre-monsoon						
		Monsoon						
		Post-monsoon	2.67 ± 0.06^{h}	8.93 ± 0.19^{g}	52 ± 11^{d}	$4.64 \pm 0.09^{a,b}$	2.12 ± 0.05^{a}	37 ± 8^{d}
S. buccalis	Buleji	Pre-monsoon						
		Monsoon						
		Post-monsoon						
	Sunehri	Pre-monsoon						
		Monsoon						
		Post-monsoon	0.11 ± 0.01^{a}	2.46 ± 0.07^{a}	14 ± 3^{e}	3.02 ± 0.08^{b}	$0.82 \pm 0.07^{\circ}$	19 ± 4^{a}
H.	Buleji	Pre-monsoon	1.02 ± 0.05^{b}	8.64 ± 0.17^{g}	$73 \pm 13^{\rm f}$	$7.12 \pm 0.72^{\rm f}$	2.19 ± 0.04^{a}	46 ± 8^{e}
leucospilota		Monsoon						
		Post-monsoon						
	Sunehri	Pre-monsoon						
		Monsoon						
		Post-monsoon						

The same letters in each column indicate that the values are not significantly different (P > 0.05).

BSAF < 2) or deconcentrator (BSAF < 1). According to Regulation (EC) No. 1907/2006 (REACH) and US EPA Toxic Substances Control Act, the heavy metals were classified as "bioaccumulative" if BCF ranged between 1000 and 5000 and "very bioaccumulative" if the BCF was greater than 5000. The bioconcentration factors (BSAF and BCF) are presented in Tables 5 and 6.

Table 5BSAF of metals in the tissues of sea cucumber species and sediments.

Species	Region	Periods	Cd	Cu	Fe	Mn	Pb	Zn
H. arenicola	Buleji	Pre-monsoon	0.058	0.404	0.150	0.039	0.063	1.21
		Monsoon	0.456	0.223	0.200	0.031	0.042	0.42
		Post-monsoon	0.064	0.180	0.220	0.039	0.032	0.33
	Sunehri	Pre-monsoon	0.932	0.189	0.162	0.018	0.019	1.47
		Monsoon	0.212	0.253	0.185	0.021	0.028	1.47
		Post-monsoon	0.546	0.048	0.158	0.028	0.020	1.00
H. pardalis	Buleji	Pre-monsoon	0.087	0.205	0.132	0.027	0.021	1.21
		Monsoon	0.351	0.402	0.147	0.029	0.031	0.73
		Post-monsoon	0.468	0.313	0.170	0.017	0.024	0.49
	Sunehri	Pre-monsoon	0.839	0.391	0.120	0.012	0.022	1.32
		Monsoon	0.140	0.181	0.149	0.009	0.023	1.67
		Post-monsoon	0.338	0.191	0.144	0.014	0.033	1.05
H.	Buleji	Pre-monsoon	0.463	0.406	0.109	0.016	0.024	1.07
verrucosa		Monsoon	0.455	0.486	0.133	0.019	0.031	0.46
		Post-monsoon	0.404	0.376	0.124	0.008	0.018	0.36
	Sunehri	Pre-monsoon	1.492	0.628	0.112	0.0057	0.010	1.58
		Monsoon	0.472	0.285	0.104	0.007	0.017	1.53
		Post-monsoon	0.419	0.320	0.113	0.008	0.011	1.23
H. atra	Buleji	Pre-monsoon	0.260	0.704	0.119	0.010	0.033	1.27
		Monsoon	0.215	0.635	0.106	0.019	0.025	0.89
		Post-monsoon	0.277	0.429	0.115	0.017	0.023	0.62
	Sunehri	Pre-monsoon	0.941	0.464	0.096	0.008	0.018	1.26
		Monsoon	0.287	0.260	0.086	0.008	0.016	1.27
		Post-monsoon	0.285	0.355	0.099	0.012	0.015	0.96
O.	Buleji	Pre-monsoon						
ehrenbergii		Monsoon						
		Post-monsoon						
	Sunehri	Pre-monsoon	0.441	0.664	0.133	0.016	0.054	1.68
		Monsoon						
		Post-monsoon	0.119	0.247	0.153	0.027	0.054	1.09
				(ontinue	ed on the	right co	lumn

 Table 5 (continued)

Species	Region	Periods	Cd	Cu	Fe	Mn	Pb	Zn
Н.	Buleji	Pre-monsoon						
cinerascens		Monsoon						
		Post-monsoon						
	Sunehri	Pre-monsoon						
		Monsoon						
		Post-monsoon	1.027	0.988	0.234	0.032	0.045	1.68
S. buccalis	Buleji	Pre-monsoon						
		Monsoon						
		Post-monsoon						
	Sunehri	Pre-monsoon						
		Monsoon						
		Post-monsoon	0.042	0.272	0.063	0.021	0.017	0.86
Н.	Buleji	Pre-monsoon	0.421	1.570	0.332	0.052	0.059	3.29
leucospilota		Monsoon						
		Post-monsoon						
	Sunehri	Pre-monsoon						
		Monsoon						
		Post-monsoon						

Table 6BCF of metals in the tissues of sea cucumber species and sea water.

Species	Region	Periods	Cd	Cu	Fe	Mn	Pb	Zn
H. arenicola	Buleji	Pre-monsoon	824	68	160	19	32	4019
		Monsoon	1012	29	92	13	16	2613
		Post-monsoon	98	50	178	17	18	3846
	Sunehri	Pre-monsoon	601	20	127	11	17	8211
		Monsoon	209	45	185	15	28	10837
		Post-monsoon	255	7	94	14	20	14194
H. pardalis	Buleji	Pre-monsoon	1235	34	141	14	10	4019
		Monsoon	779	54	67	12	11	4513
		Post-monsoon	721	87	140	7	13	5621
	Sunehri	Pre-monsoon	541	41	94	7	18	7331
		Monsoon	138	32	149	6	23	12315
		Post-monsoon	158	27	86	7	33	14839
H.	Buleji	Pre-monsoon	6588	68	117	8	12	3546
verrucosa		Monsoon	1012	65	61	8	12	2850
		Post-monsoon	623	104	102	4	10	4142
					con	tinuea	on ne	ext page

Table 6 (continued)

Species	Region	Periods	Cd	Cu	Fe	Mn	Pb	Zn
	Sunehri	Pre-monsoon	962	66	88	3	8	8798
		Monsoon	466	51	104	5	17	11330
		Post-monsoon	196	46	67	4	11	17419
H. atra	Buleji	Pre-monsoon	3706	118	126	5	17	4255
		Monsoon	477	84	48	8	9	5463
		Post-monsoon	426	119	95	7	13	7101
	Sunehri	Pre-monsoon	607	49	75	5	15	7038
		Monsoon	283	46	86	6	15	9360
		Post-monsoon	133	51	59	7	15	13548
O.	Buleji	Pre-monsoon						
ehrenbergii		Monsoon						
		Post-monsoon						
	Sunehri	Pre-monsoon	284	70	104	9	46	9384
		Monsoon						
		Post-monsoon	56	35	91	13	54	15484
Н.	Buleji	Pre-monsoon						
cinerascens		Monsoon						
		Post-monsoon						
	Sunehri	Pre-monsoon						
		Monsoon						
		Post-monsoon	479	142	140	16	45	23871
S. buccalis	Buleji	Pre-monsoon						
		Monsoon						
		Post-monsoon						
	Sunehri	Pre-monsoon						
		Monsoon						
		Post-monsoon	20	39	38	10	17	12258
Н.	Buleji	Pre-monsoon	6000	262	354	26	30	10875
leucospilota		Monsoon						
		Post-monsoon						
	Sunehri	Pre-monsoon						
		Monsoon						
		Post-monsoon						

It can be seen in Table 7 that heavy metal levels in sea cucumber species in the coasts of different countries are compared with the present study.

4. Discussion

4.1. Organisms size

The difference in heavy metal concentrations observed in the Buleji and Sunehri coasts is obviously due to an increase and/ or decrease in the input of chemicals from different sources such as fishing activities, marine culture, domestic effluents, wastes disposal, agriculture, salt making and unplanned tourism[58]. Moreover, the fluctuations observed during the sampling periods resulted both from exogenous and endogenous factors. It was found that in sea cucumber species of different sizes, the lengths and weights varied with season and the concentrations of heavy metals were inversely proportional to these variations. Seasonal length and weight variations are involved in the yearly reproductive period of the organisms and food conditions. This may be a reason for the high concentrations of Cd and Cu in tissue of H. cinerascens at Sunehri coasts during post-monsoon, the high concentrations of Pb in O. ehrenbergii at Sunehri coasts during pre-monsoon and the high concentrations of Fe, Mn and Zn in H. leucospilota at Buleji coasts during pre-monsoon. It is well known that alteration in land drainage results in changes of metal entries into coastal region which may cause fluctuations in the metal levels in biota. It may be suggested that a constant input of heavy metals from industrial and urban sources occurs in these areas during pre- and post-monsoon of the year. Relatively low concentrations in monsoon may be due to high rainfall. Such fluctuations may also be related to productivity and availability of food and to the reproductive cycle of sea cucumbers. Unfortunately, detailed studies neither of productivity along the

Table 7

Comparison of heavy metal levels (µg/g dry wt.) in cucumbers species from various countries and maximum permitted limits.

Species	Cd	Cu	Fe	Mn	Pb	Zn	References
Holothuria arenicola	0.12-1.42	0.43-2.23	47–33	2.45-5.32	0.92-2.33	11-28	Present study
Holothuria verrucosa	0.76-1.76	1.98-3.76	23-29	0.76 - 2.47	0.52-1.03	12-30	Present study
Holothuria atra	0.52 - 1.11	2.03-3.89	19-26	1.09-2.49	0.69 - 1.23	18-24	Present study
Ohsimella ehrenbergii	0.31-0.52	2.23-3.98	32-34	2.11-3.91	2.54-3.02	24-32	Present study
Holothuria cinerascens	2.67	8.93	52	4.64	2.12	37	Present study
Stolus buccalis	0.11	2.46	14	3.02	0.82	19	Present study
Holothuria leucospilota	1.02	8.64	73	7.12	2.19	46	Present study
Holothuria leucospilota	0.16-0.45	64.81-97.69	-	-	19.09-23.24	40.00-46.18	[72]
Holothuria scabra	0.13-0.17	44.48-81.16	-	-	1.52-2.55	19.30-29.12	[72]
Holothuria atra	0.072 ± 0.008	3.18 ± 1.02	-	-	0.097 ± 0.054	24.38 ± 3.96	[21]
Holothuria fuscogilva	-	-	250 ± 36	9.4 ± 0.3	-	-	[68]
Holothuria fuscopunctata	-	-	100 ± 20	12 ± 0.6	-	-	[68]
Holothuria scabra	-	-	130 ±19	1.4 ± 0.1	-	-	[68]
Holothuria mexicana	-	-	190 ± 29	1.6 ± 0.2	-	-	[68]
Holothuria tunulosa	1.27	1.48	52.07	-	4.71	15.37	[71]
Isostichopus badionotus	0.795	1.01	-	-	4.311		[70]
Holothuria floridana	2.731	1.047	-	-	2.047		[70]
US	1.5	60	-	-	3	200	[67]
Australia	0.8	-	_	-	6	-	[69]

Northern Arabian Sea coasts nor of the reproductive cycle of sea cucumber species in these regions yet exists.

4.2. Heavy metals in surface sediments and sea water

The high Cd, Cu and Pb levels in the Sunehri coasts could be attributed to fishing activities from Goth Manjhar, sewage and waste discharges; whereas the high concentration of Zn was found in the Blueji coasts. There are about 200 million gallons sewage per day (909.05 m³/day) and waste water from industrial and municipal sources in Karachi city[59-61]. Both Mn and Fe levels were not significant in the Blueji and Sunehri coasts. The present study showed that sediment heavy metal levels were mainly fluctuated seasonally. The higher Cd and Cu levels in the surface sediment were found in pre-monsoon at Blueji coasts. On the contrary, Zn reached the highest level in post-monsoon at Blueji coasts. Higher Cu and Zn levels were found in post-monsoon in Sunehri coasts, but the highest Cd level occurred in monsoon. The seasonal changes of metal levels were related to the hydrological parameters such as temperature and salinity, or maybe because different levels of metal are entered via rivers. Cu and Zn concentrations in sediments of the Blueji and Sunehri coasts are similar to those reported for Sinop coast of the Southern Black Sea[62]. Cd and Pb levels in the Blueji and Sunehri coasts were higher than those in Sinop coast of the Southern Black Sea; whereas Fe and Mn levels were low. Yücesoy and Ergin[63] mentioned that the high metal levels can be associated simply with the geological construction and mineralogy of the region. Anthropogenic activities such as industrialization, urbanization, deposition of industrial wastes can also impact on the metal levels in these coastal sea bed[7,62]. However, it seems that high concentrations of non-essential heavy metal in surface sediments from the Blueji and Sunehri coasts may indicate a moderate pollution.

Cd and Cu levels in sea water from Sunehri coasts were higher than those in Blueji coasts; whereas levels of rest heavy metals were high in Blueji coasts. In terms of seasons, higher Cd and Cu concentrations were found in post-monsoon. The highest Fe, Mn and Pb concentrations were detected in monsoon and the highest Zn level was found in pre-monsoon.

4.3. Heavy metals in the soft tissues of sea cucumbers

Of the six metals studied, Fe had the highest level in the body wall of sea cucumbers during sampling periods, followed by Zn. Cd had the lowest level in the sea cucumber species, followed by Pb and Cu. Seasonal fluctuations of the metal concentrations were observed in sea cucumbers from Buleji and Sunehri coasts. The concentrations of Cd, Cu and Zn increased in the Sunehri coasts; whereas Mn level was higher in Buleji coasts. The highest Cd and Pb levels were obtained in *H. cinerascens* from Sunehri coasts during post-monsoon and in *O. ehrenbergii* from Sunehri coasts during pre-monsoon, respectively. The highest Cu level was also found in *H. cinerascens* from Sunehri coasts during post-monsoon, followed by *H. leucospilota* in Buleji

coasts during pre-monsoon. The highest Fe, Mn and Zn values were also detected in *H. leucospilota* from Buleji coasts during pre-monsoon. This may be because circumstances in the marine ecosytem could modify suddenly from pre-monsoon to post-monsoon. Thus it might affect the physiology of the sea cucumbers. There is agriculture, industrial and urban water pollution and the fisheries activity[64-66], and the higher metal levels probably resulted from the drainage of the enterprises. It is likely, therefore, that the very high concentrations of heavy metals obtained in sea cucumbers originated from industrial and/ or domestic effluents which were discharged deliberately or carried by torrents of the ground water to the marine coast.

4.4. The bioconcentration factors (BSAF and BCF)

The highest BSAF value for Zn was observed in *H. leucospilota* (3.29) at Buleji during pre-monsoon, indicating it as macroconcentrator. The BSAF values for Zn in *H. arenicola*, *H. pardalis*, *H. verrucosa*, *O. ehrenbergii* and *H. cinerascens* from Sunehri coasts ranged from 1 to 1.68. This suggests that they are microconcentrator. The BSAF values for Cd in sea cucumber species ranged from 0.042 to 1.492. This suggests that the sea cucumber species studied ranged from being microconcentrators to deconcentrators. The BSAF values of Cu, Fe, Mn and Pb were low indicating they as deconcentrators.

According to EU regulation, Zn in all species from Sunehri coasts is "very bioaccumulative" (BCF > 5000) during all sampling periods. Zn in *H. pardalis* in Buleji coats during post-monsoon, *H. atra* during both monsoon and post-monsoon and *H. leucospilota* during pre-monsoon is also "very bioaccumulative" (BCF > 5000). Cu, Fe, Mn and Pb can be considered "bioaccumulative" (BCF < 5000).

4.5. Comparison with data in the literature

When compared with the other studies, the results in the present study for Cd and Zn showed similarity to those of the other researches; whereas levels of the rest metals were lower than those in other studies. The current study showed that the levels of essential metals Cu, Fe, Mn and Zn in sea cucumber were under the excusable values for man consumption proposed by U.S. FDA[67] and Wen and Hu[68]. The nonessential metal Pb concentration was below recommended limits by Otway[69], Medina et al.[70], Michel et al.[71] and Mohammadizadeha et al.[72]. In current study Cd concentration in all sea cucumber species was also below the allowable levels reported by Medina et al.[70] but greater than recommended values by Jinadasa et al.[21], U.S. FDA[67], Otway[69], Michel et al.[71] and Mohammadizadeha et al.[72]. H. cinerascens and O. ehrenbergii showed higher concentration of Cd (2.67 µg/g dry weight) and Pb (3.02 µg/g dry weight). The current study ensures important notice on heavy metal levels in H. arenicola, H. pardalis, H. verrucosa, H. atra, O. ehrenbergii, H. cinerascens, S. buccalis, H. leucospilota from Pakistan coast, Northern Arabian Sea. Cu and Zn levels were beneath allowable limits for man consumption, and at the meantime Cd and Pb levels in O. ehrenbergii and H. cinerascens were high. Consequently, there could be a major health hazard to man eating these organisms. Turk Çulha[73] recently reviewed

the heavy metal concentrations in sea cucumbers in the world's several seas and concluded that metal concentrations attained from various regions of the world have similarities and differences counting on contamination aspects and geographical properties of the regions, environmental pollution and pollutants. Heavy metals can be uptaken by sea cucumber through different sources. They can accumulate through food consumption or due to pollution of the seas. Michel et al.[71] pointed out that the sampling location and depth of the water mostly crucially affected metal levels in the holothurians. Moreover sea cucumber has long life span (4-8 years) and they are deposit feeder, so they can accumulate heavy metals from sediments where they live in[71,74]. Highest Pb level was quite high in sea cucumber species. It is known that Pb has affinity for calcic skeletons and mainly accumulates in the body walls of many echinoderms[46]. Michel et al.[71] showed that holothurians do quite efficiently concentrate heavy metals in laboratory experiments. However, holothurians depurate elements stepwise (halflives from through 51 to 130 days considering the metal), advising that they could act as integrators of metal and radionuclide pollution in the medium[71].

The current study gives data on the heavy metal levels present in holothurians, sediments and sea waters samples from both Buleji and Sunehri coasts bordering Northern Arabian Sea during January to December 2014. Many chemicals have been discharged into aquatic environment in excess for many years. In these chemicals, heavy metals have long been known as main contaminants of the marine ecosystems. They are progressively loaded to the coastal regions from rivers and nonpoint sources, particularly in growing countries. Sea cucumbers could uptake heavy metals at pretty higher concentrations than those established in the surrounding water and sediments. Therefore their characteristics of extensive distributions and easy collection make them as the foremost subjects for working bioaccumulation of chemicals. Newly, with the progress of industry and economy, great quantities of waste effluent have been discharged to the Northern Arabian Sea, especially Karachi coasts have undergone significant destruction and pose a peril to living organisms[75]. The objective of the current study is to evaluate the degree of metal contamination of the Northern Arabian Sea using sea cucumber species as bioindicators.

Results of the present study indicate that sea cucumber species is able to accumulate most heavy metals at considerable levels comparable to molluscs and crustaceans, and may serve as a good bioindicator. These findings also imply that sea cucumbers in both Blueji and Sunehri coasts are contaminated with these heavy metals or have mighty capability for accumulation of these heavy metals.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The researchers wish to acknowledge the Higher Education Commission of Pakistan, (Grant No. IPFP/HRD/HEC/1688) which supported by financially for sample collection of holothurians and heavy metal analysis test in sea cucumber, sea water and sea sediments and also to acknowledge the Marine Reference Collection and Resources Centre, University of Karachi for providing laboratory facilities during the study.

References

- [1] Yu R, Yuan X, Zhao Y, Hu G, Tu X. Heavy metal pollution in intertidal sediments from Quanzhou Bay, China. J Environ Sci (China) 2008; 20: 664-9.
- [2] Deforest D, Brix K, Adams W. Assessing metal bioaccumulation in aquatic environments: the inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration. *Aquat Toxicol* 2007; 84: 236-46.
- [3] Hosono T, Su CC, Delinom R, Umezawa Y, Toyota T, Kaneko S, et al. Decline in heavy metal contamination in marine sediments in Jakarta Bay, Indonesia due to increasing environmental regulations. *Estuar Coast Shelf Sci* 2011; 92: 297-306.
- [4] Fu F, Wang Q. Removal of heavy metal ions from wastewaters: a review. J Environ Manage 2011; 92: 407-18.
- [5] Ozkan EY, Buyukisik HB, Kontas A, Turkdogan M. A survey of metal concentrations in marine sediment cores in the vicinity of an old mercurymining area in Karaburun, Aegean Sea. *Environ Sci Pollut Res* 2017; doi: 10.1007/s11356-017-8792-0.
- [6] Bat L, Raffaelli D. Effects of gut sediment contents on heavy metal levels in the amphipod *Corophium volutator* (Pallas). *Tr J Zool* 1999; **23**: 67-71.
- [7] Bat L, Özkan EY. Heavy metal levels in sediment of the Turkish Black Sea coast. In: Bikarska I, Raykov V, Nikolov N, editors. *Progressive* engineering practices in marine resource management. USA: IGI Global; 2015.
- [8] Bat L. Heavy metal pollution in the Black Sea. In: Düzgüne E, Öztürk B, Zengin M, editors. *Turkish fisheries in the Black Sea*. Turkey: Turkish Marine Research Foundation; 2014.
- [9] Simpson SL, Batley GE. Sediment quality assessment: a practical guide.2nd ed. Australia: CSIRO Publishing; 2016.
- [10] Authman MMN, Zaki MS, Khallaf EA, Abbas HH. Use of fish as bioindicator of the effects of heavy metals pollution. *J Aquac Res Develop* 2015; 6(4): 328.
- [11] Santana LMBM, Blasco J, Abessa DMS, Campana O. Bioaccumulation kinetics of copper in *Ruditapes philippinarum* exposed to increasing, continuous and pulsed exposure: implications for growth. *Sci Total Environ* 2017; 595: 920-7.
- [12] Ober GA, Gonzalez M, Maria IS. Heavy metals in molluscan, crustacean and other commercially important Chilean marine coastal waters species. *Bull Environ Contam Toxicol* 1987; 38: 534-9.
- [13] Farrington JW, Davis AC, Tripp BW, Phelps DK, Galloway WB. "Mussel watch" – measurements of chemical pollutants in bivalves as one indicator of coastal environmental quality. In: Boyle TP, editor. New approaches to monitoring aquatic ecosystems. Philadelphia: American Society for Testing and Materials; 1987.
- [14] Temara A, Skei JM, Gillan D, Warnau M, Jangoux M, Dubois P. Validation of the asteroid Asterias rubens Echinodermata as a bioindicator of spatial and temporal trends of Pb, Cd, and Zn contamination in the field. Mar Environ Res 1998; 45: 341-56.
- [15] Elliott M, Hemingway KL, editors. Fishes in estuaries. Oxford: Blackwell

- Science; 2002, p. 636.
- [16] Conand C. The fishery resources of Pacific island countries. Part 2: holothurians. Rome: Food and Agriculture Organization of the United Nations; 1990. [Online] Available from: http://www.fao.org/docrep/008/t0293e/t0293e00.htm [Accessed on 16th March, 2017]
- [17] Fish JD, Fish S. A student's guide to the seashore. 2nd ed. Cambridge: Cambridge University Press; 1996, p. 564.
- [18] Lovatelli A, Conand C, Purcell S, Uthicke S, Hamel JF, Mercier A. Advances in sea cucumber aquaculture and management. Rome: Food and Agriculture Organization of the United Nations; 2004. [Online] Available from: http://www.fao.org/docrep/007/y5501e/y5501e00.htm [Accessed on 16th March, 2017]
- [19] Hashmi MI, Mustafa S, Nitisewojo P, Tariq SA. Distribution of heavy metals in seawater, sea sediments and sea cucumber (*Holothuria* scraba), and its implications for sea ranching in Sabah Malaysian Borneo. Sci Int 2002; 14(1): 29-32.
- [20] Hashmi MI, Thilakar R, Hussein MABS, Hoque Z. Determination of seven heavy metals in eight species of sea cucumbers. Sci Int (Lahore) 2014; 26(1): 261-2.
- [21] Jinadasa BKKK, Samanthi RI, Wicramsinghe I. Trace metal accumulation in tissue of sea cucumber species; North-Western Sea of Sri Lanka. Am J Public Health Res 2014; 2(5A): 1-5.
- [22] Haifeng J, Tang S, Qin D, Chen Z, Wang J, Bai S, et al. Heavy metals in sea cucumber juveniles from coastal areas of Bohai and Yellow Seas, North China. *Bull Environ Contam Toxicol* 2015; **94**: 577-82.
- [23] Haider MS, Sultana R, Jamil K, Lakht-e-Zehra, Tarar OM, Shirin K, et al. A study on proximate composition, amino acid profile, fatty acid profile and some mineral contents in two species of sea cucumber. *J Anim Plant Sci* 2015; 25(1): 168-75.
- [24] Jaffer M, Ashraf M, Tariq J. Assessment of current trace metal pollution status of the South East Arabian Sea Coast of Pakistan through fish analysis. J Chem Soc Pak 1995; 17 (4): 204-7.
- [25] Saif MS, Hao MU, Memon KS. Heavy metals contamination through industrial effluent to irrigation water and soil in Korangi area of Karachi (Pakistan). *Int J Agric Biol* 2005; 7(4): 646-8.
- [26] Zhang Y, Lu X, Wang N, Xin M, Geng S, Jia J, et al. Heavy metals in aquatic organisms of different trophic levels and their potential human health risk in Bohai Bay. *China Environ Sci Pollut Res* 2016; doi: 10.1007/s11356-016-6948-y.
- [27] Govind P, Madhuri S. Heavy metals causing toxicity in animals and fishes. *Res J Anim Vet Fish Sci* 2014; **2**(2): 17-23.
- [28] Matranga V, Pinsino A, Randazzo D, Giallongo A, Dubois P. Longterm environmental exposure to metals (Cu, Cd, Pb, Zn) activates the immune cell stress response in the common European sea star (*Asterias rubens*). *Mar Environ Res* 2012; **76**: 122-7.
- [29] Coteur G, Gosselin P, Wantier P, Chambost-Manciet Y, Danis B, Pernet PH, et al. Echinoderms as bioindicators, bioassays and impact assessment tools of sediment-associated metals and PCBs in the North Sea. Arch Environ Contamin Toxicol 2003; 45: 190-202.
- [30] Schröder HC, Di Bella G, Janipour N, Bonaventura R, Russo R, Müller WEG, et al. DNA damage and developmental defects after exposure to UV and heavy metals in sea urchin cells and embryos compared to

- other invertebrates. Prog Mol Subcell Biol 2005; 39: 111-37.
- [31] Canty MN, Hutchinson TH, Brown RJ, Jones MB, Jha AN. Linking genotoxic responses with cytotoxic and behavioural or physiological consequences: differential sensitivity of echinoderms (*Asterias rubens*) and marine molluscs (*Mytilus edulis*). Aquat Toxicol 2009; 94: 68-76.
- [32] Sugni M, Tremolada P, Porte C, Barbaglio A, Bonasoro F, Carnevali MD. Chemical fate and biological effects of several endocrine disrupters compounds in two echinoderm species. *Ecotoxicology* 2010; 19: 538-54.
- [33] Falugi C, Aluigi MG, Chiantore MC, Privitera D, Ramoino P, Gatti MA, et al. Toxicity of metal oxide nanoparticles in immune cells of the sea urchin. *Mar Environ Res* 2012; 76: 114-21.
- [34] Strode E, Jansons M, Purina I, Balode M, Berezina NA. Sediment quality assessment using survival and embryo malformation tests in amphipod crustaceans: the Gulf of Riga, Baltic Sea AS case study. J Mar Syst 2017; 172: 93-103.
- [35] Bat L, Raffaelli D. Sediment toxicity testing: a bioassay approach using the amphipod *Corophium volutator* and the polychaete *Arenicola marina*. J Exp Mar Biol Ecol 1998; 226: 217-39.
- [36] Ahmed Q, Bat L, Yousuf F. Contamination of Cu, Zn, Fe and Mn in *Katsuwonus pelamis* (Linnaeus, 1758) from Karachi fish harbor and potential risks to human health. *Int J Mar Sci* 2017; **7**(9): 76-87.
- [37] Bat L, Üstün F, Öztekin HC. Heavy metal concentrations in zooplankton of Sinop coasts of the Black Sea, Turkey. *Mar Biol J* 2016; 1(1): 5-13.
- [38] Bat L, Arici E. Health risk assessment of heavy metals in *Sarda sarda* Bloch, 1793 for people through consumptionfrom the Turkish Black Sea coasts. *Int J Zool Res* 2016; **1**(1): 1-7.
- [39] Çulha ST, Çulha M, Karayücel I, Çelik MY, Isler Y. Heavy metals in Mytilus galloprovincialis, suspended particulate matter and sediment from offshore submerged longline system, Black Sea. Int J Environ Sci Technol 2017; 14: 385-96.
- [40] Bat L. A review of sediment toxicity bioassays using the amphipods and polychaetes. *Tr J Fish Aquat Sci* 2005; **5**: 119-39.
- [41] Bat L, Oztekin HC. Heavy metals in Mytilus galloprovincialis, Rapana venosa and Eriphia verrucosa from the Black Sea coasts of Turkey as bioindicators of pollution. Walailak J Sci Technol 2016; 13(9): 715-28.
- [42] Bat L, Arici E. Heavy metal concentrations in macroalgae species from Sinop coasts of the Southern Black Sea. J Coast Life Med 2016; 4(11): 841-5.
- [43] Bat L, Öztekin HC, Arıcı E, Visne A. A preliminary study on the heavy metal levels of dwarf eelgrass *Zostera noltii* Homermann in the Black Sea. *J Aquat Mar Biol* 2016; doi: 10.15406/jamb.2016.04.00072.
- [44] Bat L, Arici E. Heavy metal levels in tissues of *Merlangius merlangus* (Linnaeus, 1758) from the Black Sea coast of Turkey and potential risks to human health. *Int J Mar Sci* 2016; **6**(10): 1-8.
- [45] Chatta AM, Khan MK, Mirza ZS, Ali A. Heavy metal (cadmium, lead, and chromium) contamination in farmed fish: a potential risk for consumers' health. *Turk J Zool* 2016; **40**: 1-9.
- [46] Beeby A. Toxic metal uptake and essential metal regulation in terrestrial invertebrates: a review. In: Newman MC, McIntosh AW, editors. *Metal* ecotoxicology: concepts and applications. USA: Lewis Publ; 1991, p.

- 65-89
- [47] Baloch L. Overfishing, pollution continue to threaten marine resources. DAWN; 2008. [Online] Available from: https://www.dawn.com/news/418705/overfishing-pollution-continue-to-threaten-marine-resources [Accessed on 16th March, 2017]
- [48] Laxen DPH. The chemistry of metal pollution in water. In: Harrison RM, editor. *Pollution-causes, effects and control*. London: The Royal Society of Chemistry; 1983, p. 104.
- [49] Sireli UT, Göncüoglu M, Yıldırım Y, Gücükoglu A, Çakmak O. Assessments of heavy metals (cadmium and lead) in vaccum packaged smoked fish species (mackerel, Salmo salar and Oncorhynhus mykiss) marketed in Ankara (Turkey). Ege Univ J Fish Aquat Sci 2006; 23: 353-6
- [50] Kamal T, Tanoli MAK, Mumtaz M, Ali N, Ayub S. Bioconcentration potential studies of heavy metals in *Fenneropenaeus penicillatus* (Jaira or Red Tail Shrimp) along the littoral states of Karachi city. *J Basic Appl Sci* 2015; 11: 611-8.
- [51] Qari R, Siddiqui SA. Heavy metal pollution in coastal seawater of Nathia Gali, Karachi (Pakistan). J Environ Res Develop 2008; 3(1): 9-19.
- [52] Ahmad MK, Islam S, Rahman MS, Haque MR, Islam MM. Heavy metals in water, sediment and some fishes of Buriganga River, Bangladesh. *Int J Environ Res* 2010; **4**(2): 321-32.
- [53] U.S. NRC. United State Nuclear Regulatory Commission Method 3050B. Acid digestion of sediments, sludges, and soils. Revision 2. Washington, DC: U.S. NRC; 1996. [Online] Available from: https://www.nrc.gov/docs/ML1105/ML110590640.pdf [Accessed on 16th March, 2017]
- [54] Keeney DR, Nelson DW. Nitrogen in organic forms. In: Page AL, Miller RH, Keeney DR, editors. *Methods of soil analysis. Part 2. Agronomy No. 9.* Madison: American Society of Agronomy; 1982, p. 643-98.
- [55] Zar JH. Biostatistical analysis. 2nd ed. New Jersey: Prentice Hall Int.; 1984.
- [56] Geyer HJ, Rimkus GG, Scheunert I, Kaune A, Kettrup A, Zeeman M, et al. Bioaccumulation and occurrence of endocrine-disrupting chemicals (EDCs), persistent organic pollutants (POPs), and other organic compounds in fish and other organisms including humans, in bio-accumulation. New aspects and developments. In: Beek B, editor. The handbook of environmental chemistry. New York: Springer-Verlag Berlin Heidelberg; 2000, p. 167.
- [57] Kleinov KN, Nichols JW, Hayton WL, Mc Kim JM, Barron MG. Toxicokinetics in fish. In: Di Giulio RT, Hinton DE, editors. *The toxicology of fishes*. Boca Raton: Taylor and Francis Group LLC; 2008, p. 55-152.
- [58] Iqbal M, Kazmi MA. Coastal zone management in Pakistan with special reference to marine pollution impact and ICAM plan. In: Proceedings of the UNESCO workshop on coastal aquaculture; 1996, p. 47-52.
- [59] Ahmed M. The present status of marine pollution in Pakistan. In: Beg MAA, editor. Proceedings National and Regional Seminar for Protection of Marine Environment and Related Eco-system. Karachi; 1979, p. 1-6.
- [60] Ashraf M, Tariq J, Jaffer M. Pollution assessment through trace metal analysis of sediments of four tributary rivers of Pakistan draining in to the Arabian Sea. *Indian J Mar Sci* 1992; 21: 292-5.
- [61] Qasim R, Siddiqui PJA, Qari R. A survey of pollution in Korangi Creek water. In: Majid A, Khan MY, Moazzam M, Ahmed J, editors. In: Proc.

- Nat. Sem. Fish Policy and Plan, Karachi, Marine Fisheries Department; 1994, p. 591-601.
- [62] Bat L, Ozkan EY, Oztekin HC. The contamination status of trace metals in Sinop coast of the Black Sea, Turkey. Caspian J Environ Sci 2015; 13(1): 1-10
- [63] Yücesoy F, Ergin M. Heavy metal geochemistry of surface sediments from the southern Black Sea shelf and upper slope. *Chem Geol* 1992; 99: 265-87.
- [64] Waqas U, Hasnain SA, Ahmad E, Abbasi M, Pandrani A. Conservation of green turtle (*Chelonia mydas*) at Daran beach, Jiwani, Balochistan. *Pak J Zool* 2011; 43(1): 85-90.
- [65] Khattak MI, Khattak MI, Mohibullah M. Study of heavy metal pollution in mangrove sediments reference to marine environment along the coastal areas of Pakistan. *Pak J Bot* 2012; 44(1): 373-8.
- [66] Elahi N, Ahmed Q, Bat L, Yousuf F. Physicochemical parameters and seasonal variation of coastal water from Balochistan coast, Pakistan. J Coast Life Med 2015; 3(3): 199-203.
- [67] Food and Drug Administration. National Shellfish Sanitation Program. Guide for the control of molluscan shellfish. USA: Food and Drug Administration; 2003. [Online] Available from: https://www.fda.gov/downloads/Food/GuidanceRegulation/FederalStateFoodPrograms/ UCM505093.pdf [Accessed on 25th February, 2017]
- [68] Wen J, Hu C. Elemental composition of commercial sea cucumbers (holothurians). Food Addit Contam Part B Surveill 2010; 3: 246-52.
- [69] Otway NM. Bioaccumulation studies on fish: choice of species, sampling, designs, problems and implications for environmental management. In: Miskiewicz AG, editor. Assessment of the distribution, impacts and bioaccumulation of contaminants in aquatic environments. Proceedings of a Bioaccumulation Workshop. Sydney: Water Board and Australian Marine Sciences Association Inc.; 1992, p. 103-13.
- [70] Medina RG, Carlos ZM, Manuel CB, Roberto PC. [Concentration of Cd, Cr, Cu and Pb in sediments and in three species of sea cucumber (Holothuroidea class) of the coasts of State of Yucatan, Mexico]. *Ingeniería* 2004; 8: 7-19. Spanish.
- [71] Michel W, Sandrine D, Gilles L, Alessia M, Rodrigues Y, Philippe D. Heavy metals in the sea cucumber *Holothuria tubulosa* (Echinodermata) from the Mediterranean Posidonia oceanica ecosystem: body compartment, seasonal, geographical and bathymetric variations. *Environ Bio-indicators* 2006; 1: 268-85.
- [72] Mohammadizadeha M, Bastami KD, Ehsanpour M, Afkhami M, Mohammadizadeh F, Esmaeilzadeh M. Heavy metal accumulation in tissues of two sea cucumbers, *Holothuria leucospilota* and *Holothuria* scabra in the northern part of Qeshm Island, Persian Gulf. Mar Pollut Bull 2015: 103: 354-9.
- [73] Turk Culha S, Dereli H, Karaduman FR, Culha M. Assessment of trace metal contamination in the sea cucumber (*Holothuria tubulosa*) and sediments from the Dardanelles Strait (Turkey). *Environ Sci Pollut Res* 2016; 23: 11584-97.
- [74] Sicuro B, Piccinno M, Gai F, Abete MC, Danieli A, Dapra F, et al. Food quality and safety of Mediterranean sea cucumbers *Holothuria* tubulosa and *Holothuri apolli* in Southern Adriatic Sea. Asian J Anim Vet Adv 2012; 7(9): 851-9.
- [75] Ahmed Q, Bat L, Yousuf F, Ali QM, Nazim K. Accumulation of heavy metals (Fe, Mn, Cu, Zn, Ni, Pb, Cd and Cr) in tissues of Narrow-barred Spanish mackerel (Family-Scombridae) fish marketed by Karachi Fish Harbor. Open Biol Sci J 2015; 1: 20-8.