Sea cucumber as bioindicator of trace metal pollution in coastal sediments

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Abstract

Sea cucumbers are fished worldwide for export to Asia, but few studies have evaluated metal pollution and risk assessment. This study assessed concentration of trace metals and the potential ecological risk in sea cucumber (*Holothuria floridana*) and sediments at the Cispatá Bay, in the Caribbean coast of Colombia. The trace metal concentrations in biota and sediments showed a similar decreasing trend as follows: Cu > Zn > Hg > Pb > Cd. The highest bioconcentration factor was found for Hg, and according sediment quality guidelines, Cu levels indicate adverse biological effects in the ecosystem. In this regard, Cu levels were higher than effects range low and the threshold effect levels (TEL) and lowest effect level in all the stations, whereas Hg levels were higher than TEL in most of them. Results for health risk assessment based on the maximum allowable daily consumption rate (CR_{lim}) suggest that there is no risk to adults, however children should limit or avoid its consumption. Results from multivariate statistical analysis suggest that agricultural activities (i.e. application of fertilizers and agrochemicals) were identified as the main anthropogenic sources of metal pollution. This research suggest that sea cucumber could be used as a bioindicator species in studies of monitoring metal contamination, with special attention to the highly significant correlation between Zn in tissue and sediments. This study also reveals that anthropic activities may have negative effects in the quality of the sediments of the bay and contributing to the knowledge of metal accumulation in sea cucumber.

Keywords: *Holothuria floridana*; metals, sea cucumber; sediments; Cispatá Bay; Colombia.

1. Introduction

Different anthropogenic stressors, such as climate change, marine transportation and land based sources of pollution, have the potential to adversely impact marine ecosystems, so environmental assessment of pollutants is a critical issue for ecosystems management [1]. Globally, pollution by metals is one of the most critical problems for marine aquatic ecosystems because some toxic elements tend to bioaccumulate in organisms (echinoderms, bivalves, crustaceans, etc.) and sediments, exerting harmful effects on organisms and human health by biomagnification in the food chain [2, 3]. In aquatic ecosystems, sediments are the main sink and source of metals, serving as the largest pool for the storage of toxic metals [4]. Due to the variabilities of metals in the water column, the sediments usually provide more information to understand different dynamics of metals in the aquatic environment.

Sea cucumbers (*Holothuroidea*) are invertebrates that move slowly and live in contact or buried in the sediment; they have an essential role in the marine ecosystem by recycling nutrients, and therefore they can directly accumulate metals from the sediment because it is their food source [5, 6]. Consequently, a scientific interest focused on the use and conservation of sea cucumber has increased in recent years [7–9]. These organisms have been considered appropriate to be used as biomonitors because they have certain characteristics such as type of feeding, easy identification, low mobility, and small size, although with enough tissue to perform analysis of contaminants [10, 11].

On the other hand, sea cucumbers are highly prized in Asia and sold as trepang or bêche-de-mer, a dried product that is exported worldwide to Asian seafood markets [12], where is eaten for its health and medicinal benefits [13, 14]. Given the high demand for this resource, mainly by Asian countries such as China, Korea or Japan, overexploitation of sea cucumber fishing has led to the closure of some fisheries in the Pacific Island countries, and has expanded in the catch in other coasts of the world, including Latin

American: Peru, Ecuador, Chile, Cuba, Costa Rica and Colombia [6, 7, 15]. Thus, in these countries sea cucumber is not consumed locally but has awakened export interests due to the high prices in the international market. One of the sea cucumber species, *Holothuria floridana* is found in the Gulf of Mexico, Cuba, Florida, Bahamas, Puerto Rico, Aruba, Jamaica, The Lesser Antilles, Honduras, Venezuela, Panamá, and Colombia. In the last two decades, fisheries have begun in Latin America and the Caribbean, which, according to reports and studies, are mostly unsustainable [7].

The Cispatá bay is a protected mangrove swamp and a highly productive ecosystem located in the Caribbean Sea of Colombia. It is delimited on the west by the Sinú River, on the south by the Sicará channel and the Soledad swamp, and on the north by the Caribbean Sea [16, 17]. Currently, the bay is surrounded by areas with intensive agriculture, livestock and a great variety of commercial fisheries (including shrimp and tilapia), and also with touristic activities that have experienced a fast growth. This area has been reported to be impacted by indiscriminate discharges of untreated sewage, industrial and domestic solid wastes and wastewaters from agricultural activities and livestock [18]. Recent studies indicated that sediments, fish and seabirds were polluted by indiscriminate discharges of untreated wastewater, industrial and domestic solid waste, wastewater from agricultural and livestock activities, and spills of harmful substances such as hydrocarbons [19–21]. Additionally, high concentrations of metals have been reported in the middle and lower basin of the Sinú River, which flows into the study area [21–23]. These evidences place in the foreground the problem of metal pollution in the Cispatá bay, where *H. floridana* could be found. Despite the ecological and economic importance of sea cucumbers, there is scarce information on metals levels in sea cucumbers worldwide, and no information in the Caribbean Coast.

The main aims of this study were (i) to determine the concentrations of metals in sea cucumber and sediments, (ii), to calculate the ecological risk of metals on sea cucumber by means of pollution indices, and (iii) to evaluate if sea cucumbers are good bioindicators.

2. Materials and Methods

2.1. Study area

The Cispatá Bay is a protected mangrove swamp of 11000 ha, located in the Colombian Caribbean Coast (Department of Córdoba), between 09°25'12"–09°20'8"N and 75°47'37"–75°55'30"W (Figure 1). In addition, the study site area might be impacted by the offshore oil terminal activity situated in Coveñas.

2.2. Collection and analysis of samples

The locations of the selected sampling stations are shown in Figure 1. Sampling of the Florida sea cucumber (*H. floridana*) was carried out by diving and manual collection, considering similar sizes [24]. From each station, eight specimens of *H. floridana* were taken (mean (SD) length: 16.5 (2.9) cm; weight: 115.4 (28.9) g). At the same sampling site, one composite sediment sample was collected from at least 4 subsamples using a Van Veen grab sampler. Sediments and organisms were placed in plastic bags, labeled, packed in ice and transported to the laboratory. Once in the lab, the sediments were dried in an oven at 40 °C for 48 h, and the organisms were dissected using stainless steel scissors and a lancet after filtering. The samples tissues were kept in sterile polythene bags in a freezer at –20°C until analysis. The analytical method used to determine Hg concentration in the sediments and tissue samples (30 mg, dry weight, dw) was based on thermal decomposition with detection by atomic absorption spectrometry using a milestone DMA-80 (Direct Mercury Analyzer) [25]. For Cu, Zn, Cd and Pb analysis, sediments and tissues were digested with HNO₃/HCl (1:3 v/v) using Method 3051 A [26], and according to

the procedure described by Karadede and Ünlü (2007) [27], respectively. Analyses were performed using a Thermo Elemental Solaar S4 coupled flame spectrometer for Cu and Zn and graphite furnace for Cd and Pb. The method was validated with IAEA-405 and DORM-2, and the recovery average percentages (n=3) for metals were 94.6% (sediment) and 96.2% (tissue).

2.3. Bioconcentration factor

The bioconcentration factor (BCF) biota-sediment, expressed as the ratio of the metal concentration in the tissue to the metal concentration in the sediment [28], was estimated using the obtained concentrations of metals. The BCF of metals by organism occurs when $BCF > 1$ [29].

2.4. Pollution and ecological risk assessment

The contamination degree was calculated in function of the pollution load index (PLI), the potential ecological risk assessment (RI), the sediment quality guidelines (SQG), and the mean ERM Quotient (M-ERM-Q) (Table 1) as previously described elsewhere [30]. Background levels for metals in sediment were obtained by taking a core depth of 45 cm collected at station 5, subdivided into 1 cm layers and subsequent analysis of metals were performed for each fraction. The baseline or background values were: Hg 0.042 µg/g, Cu 9.77 µg/g, Pb 0.032 µg/g, Cd 0.015 µg/g and Zn 21.14 µg/g.

2.5. Human health risk assessment

Due to lack of available data on the estimated daily intake of sea cucumber from both exporting and importing countries, quantifying risks is more challenging. To this purpose, a good approach is possible by calculating the maximum allowable daily sea cucumber consumption rate (CR_{lim}), according to the USEPA [31] equation $CR_{lim} = (RfD \times BW) \times C^{-1}$;

where reference dose (RfD) is the daily oral exposure to the human population that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime, BW is consumer body weight, and C is the mean metal concentration of sea cucumber.

Values of CR_{lim} for *H. florida* based on a contaminant's noncarcinogenic health effects, was expressed in kilograms per day. RfDs values (Table 2) were taken from USEPA [32] and WHO [33], values for BW of 15 kg for children and 80 kg for adults, were assumed according USEPA recommendation [34]. Because no oral RfDs value for Pb exists, a reference value for Pb (Table 2) was taken from the European Food Safety Authority [35].

2.6. Statistical analysis

The results for each sample were expressed as the mean $\pm$ the standard deviation (SD) of three replicates. A Student's t-test was used to evaluate if there were significant differences between the average concentrations from different sampling stations. The criterion for significance was set at $p < 0.05$. Principal component analysis (PCA) with Varimax rotation was employed with the aim of identifying associations and possible origin among metals. The results from the PCA were interpreted according to the hypothetical sources of trace metals. Statistical analysis was performed with SPSS v23.0.0.0.

3. Results and discussion

3.1. Metal concentrations in tissue and sediment samples

Concentrations of metals in tissue and sediment samples in each sampling site are shown in Figure 2. Concentrations of metals (in $\mu\text{g/g}$) in tissues ranged from 7.72-9.67 (Cu), 5.39-8.03 (Zn), 0.074-0.090 (Hg), 0.030-0.038 (Pb) and 0.016-0.026 (Cd); while in sediments 53.85-62.87 (Cu), 46.53-58.21 (Zn), 0.102-0.148 (Hg), 0.088-0.122 (Pb) and 0.053-0.078 (Cd). The descending order as a function of the average concentrations of metals in tissue and sediments was $\text{Cu} > \text{Zn} > \text{Hg} > \text{Pb} > \text{Cd}$. The concentrations of metals in tissue and

sediments do not present statistically significant differences for the different sampling stations ($p>0.05$). This indicates that, although organisms and sediments could have been exposed to different degrees of metal pollution levels, there are no spatial variations.

The concentrations of Cu and Zn were lower in tissues than in sediments, probably because they are essential elements of great importance in many functional roles and they are co-factor for numerous enzymes that were regulated by sea cucumber tissues [29, 36, 37]; however, the accumulation of metals in organs depends on intrinsic and extrinsic factors to the organism [38, 39]. The accumulation of metals in sediments is consequence of the geochemical processes that occur *in situ* and in flooded soils, the discharges of sewage from population centers and primary, agro-industrial, tourist and industrial activities that take place around the Cispatá bay [17, 18, 21]. Additionally, the influence of the Sinú River should be considered since high concentrations of metals have been reported in sediments and soils of its middle-low basin that can reach the study site by runoff, dragging and subsequent deposition [19, 20].

Despite Hg, Pb, and Cd were the least accumulated elements in the tissue, their relatively low concentrations in the tissue do not rule out their potential risk, because these metals are precursors of toxic effects of great impact over time. In addition, since sediments are the main sink of metals from the water column, and therefore the food source of metals for *H. floridana*, those can be assimilated and bioaccumulated in their tissues for further biomagnification in the food chain [36, 40, 41]. Moreover, there are numerous factors that influence the accumulation of elements in living organisms: characteristics of the accumulated elements (essential or non-essential, solubility in water, form, etc.), concentration, exposure time, presence of other elements or compounds in the environment, physicochemical parameters of the environment, habitat, diet, sex, and metabolic rate of the organism in some cases [39, 42].

The efficiency of bioaccumulation of metals in *H. floridana* was evaluated by calculating BCF values. The bioaccumulation of metals by organism occurs if $BCF > 1$ [29]. Results showed that Hg (0.612) has much higher average values of BCF compared to Cu (0.152), Zn (0.122), Pb (0.328) and Cd (0.334), but all of them are lower than 1 for all trace metals ($BCF < 1$), indicating that accumulation was not found in the sea cucumber *H. floridana*. Results highlighted the fact that regardless these organisms acquired their food from sediments, these organisms have excretion mechanisms that prevent a bioaccumulation greater than their metabolic and physiological needs require, at least in the conditions in which this study was carried out. The above indicates a high accumulation of Hg, possibly due to the high bioavailability of Hg in sediments near the study area as reported by Paternina-Urbe [21]. For this reason, *H. floridana* could be considered as a biomonitor to determine the influence of Hg contamination on the ecosystem.

As was pointed out above, metals such as Cu and Zn have important physiological functions unlike to Hg, Cd and Pb. These nutrients are absorbed and eliminated frequently, and maintaining the levels through homeostatic mechanisms, as excess can be toxic and scarcity can generate physiological deficiencies. Hence, despite presenting the highest concentrations in tissue and sediments, BCF values for Cu and Zn are very low. Sea cucumbers are classified commercially important according to the different type of species, organoleptic characteristics (color, appearance, smell, body thickness), abundance and demand in the global market [15]. Although sea cucumbers are consumed as part of the Asian tradition, for their medicinal benefits and as part of culinary delicacy [6, 9], the *H. floridana* species still does not have great value as food, although the constant pressure and overexploitation of other sea cucumbers could increase their future demand.

3.2. Ecological risk potential assessment

To evaluate the ecological risk of metals in sediments, different indices were used such as the estimated potential ecological risk factor (E_{ri}) with individual elements in conjunction with the full potential ecological risk index (PRI), sediment quality guidelines (SQGs), as well as the mean effect coefficient values (M-ERM-Q) (Table 1). As it can be seen, the content of Pb, Zn and Cd were lower than the corresponding values of effects range low (ERL), effects range median (ERM), probable effect level (PEL), lowest effect level (LEL) and severe effect level (SEL). Levels of Cu were greater than ERL, TEL, and LEL, whereas Hg shows higher values than TEL in all the stations, except S1 and S2. This indicated that Cu and Hg levels in sediments can cause adverse biological effects on biota [43, 44]. Moreover, Cu had the highest concentration in tissue and Hg was the metal with the highest BCF, validating the biological effects that may occur in *H. floridana*. Moreover, stations S3, S4 and S7 presented a 21% probability of sediment toxicity ($0.11 = M-ERM-Q < 0.5$). As a function of E_{ri} , sediments were classified with a potential ecological risk factor from low ($E_{ri} < 40$) to considerable ($E_{ri} = 80-160$). Afterward, Hg and Cd exhibited considerable ecological risk, unlike Cu, Zn and Pb displayed low ecological risk ($E_{ri} < 40$). Likewise, surface sediments presented moderate ecological risk indices (RI, 150-300) in more than half of the samples (57%), with higher values in S3. Finally, according to the PLI values, the surface sediments in all the sampling sites indicated an environmental deterioration ($PLI > 1$), with greater impact for the different stations depending on the concentrations of metals in the following decreasing order: $Cu > Zn > Hg > Pb > Cd$.

3.3. Human health risk assessment

Table 2 shows maximum allowable daily sea cucumber consumption rate (CR_{lim}). As can be seen, the amount of daily consumption allowed ranged from 0.07 to 3.81 kg/day regardless of the metal considered. According to the calculated CR_{lim} values and considering that *H. floridana* in Colombia has a weight between 0.07 and 0.2 kg, its

consumption is not recommended for children, due to the levels of Cu, Hg and Pb reported. There is no expected risk to adults, although an increase of the maximum daily intake can lead to an increased risk. Indeed, excess of sea cucumber consumption (e.g. more than 3 to 5 per day, every single day, and depending on their size) can pose a threat to human health, due to Cu and Hg exposure.

3.4. Multivariate statistical analysis

In order to evaluate the relationship between trace metals and groups, some of the multivariate analysis techniques were carried out, including Pearson correlation and PCA with Varimax rotation to maximize the sum of the variance of the factor coefficients. Table 3 shows the values of the Pearson correlation coefficients in tissues (t) and sediments (sed). In sediments, Pb(sed) present a statistically significant correlation with Cu(sed) and Cd(sed) ($r = 0.790$ and $r = 0.767$, $p < 0.05$, respectively), indicating that these metals could have similar origin. Likewise, Zn(sed) presented a strong positive correlation with Zn(t) and Hg(t) in tissues ($r = 0.958$; $p < 0.01$ and $r = 0.776$, $p < 0.05$, respectively) indicating the metals absorption influence of Zn and Hg by *H. floridana*. Finally, Cd(t) in tissue also showed a strong statistical correlation with Hg(sed) in sediments ($r = 0.829$, $p < 0.05$).

The PCA method led to a reduction of the initial size of the data set to two components that explained 73.1% of the data variation (Figure 3). Therefore, these two factors played an important role in explaining metal contamination in the study area. According to the matrix of rotated components, trace metals Cu, Hg, Pb and Cd in sediments had higher loads in the first component (PC1), while trace metals in tissues Cu, Zn and Hg had a higher load in the second component (PC2). Hence, Pb(sed) showed a high contribution in PC1 (0.984) together with Cu(sed) (0.828) and Cd(sed) (0.705), suggesting that these metals may have same anthropogenic sources, which is also supported by the high correlations obtained between Pb and Cu, and Pb and Cd (Table 3). These three trace

metals are well-known markers of agricultural activities, specifically related to the application of phosphate-based fertilizers [45, 46]. The application of Cu-based pesticides and fungicides on long-term soils contributes to the increased accumulation of this metal potentially accumulated in sediments [47]. Results for Pb can also be attributed to petrogenic sources due to the transport of crude oil in a nearby oil port and tourist activities with gasoline engines. However, Burgos-Núñez et al. [20] reported that there is no relationship between the concentration of metals and hydrocarbon contamination in sediments near the study area. Hg sed also has a contribution in PC1 (0.721), however, the anthropogenic source of Hg sed should be different because there are no significant correlations with other metals except with Cd t ($p < 0.05$) (Table 3). The use of fungicides containing phenylmercury was very common in this area, as well as the massive aerial fumigation of rice fields with mercurial agrochemicals in previous years, which were banned by the ICA (Colombian Institute of Agriculture) according to the resolution 2189 of 1974, more than 50 years ago. Previous studies have demonstrated the presence of metals in sediments [22], in soils of the middle and lower basin of the Sinú River [23], and in fish species in the upper basin of the Sinú River [18], where soil flooding possibly accelerated the washing process and the metal extraction [22]. Therefore, the Sinú River could be considered a potential source of metals through water network interconnected with the study area that can increase the content of metals in sediments [17, 20, 21].

Contribution of Zn sed (0.911), Zn t (0.912) and Hg t (0.841) are shown in PC2 in Figure 3, additional to a strong Pearson's correlation between the metals (Table 3). These significant correlations indicate that they may be derived from similar sources, originated from anthropogenic activities, and possibly the presence of Zn in sediments and tissues, influences the absorption of Hg by *H. floridana*. Intensive livestock activities [21] near the study area can contribute between 37-40% of Zn to the soil through manure, which can eventually be deposited in sediments [48]. Hence, Zn is probably associated to traditional

agricultural practices developed in the area, such as fertilization, irrigation and the application of pesticides that are considered probable anthropogenic sources of trace metal accumulation [49, 50].

4. Conclusions

Results suggested that *H. floridana* could be used as a bioindicator species in studies of monitoring metal contamination in the Caribbean Sea, with special attention to the highly statistically significant correlation between Zn in tissue and Zn in sediments. Metal pollution in sediments came mainly from anthropogenic sources through intense agricultural activities by the application of agrochemicals and fertilizers. Moreover, metal levels in sediments and biota showed a similar decreasing trend as follows: Cu>Zn>Hg>Pb>Cd.

The contamination degree calculated in function of the pollution load index and the potential ecological risk assessment revealed a potential moderate ecological risk towards *H. floridana* together with a great environmental deterioration in surface sediments for the different sampling stations.

Results for health risk assessment based on the maximum allowable daily consumption rate (CR_{lim}) suggest that children should limit or avoid its consumption. It seems there is no risk to adults, however an increase of the maximum daily intake can lead to an increased risk.

This is the first study of accumulation of metals in *H. floridana* in tropical estuaries, and the results provided in this study is expected to serve as a useful guide for future management of the bay concerning potentially pollution activities that impact in marine aquatic ecosystems of the Colombian Caribbean coast.

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Table 1. Sediment Quality Guidelines (SQG; µg/g dw), PLI and RI classification

	Stations									NOAA				CMEE	
	1	2	3	4	5	6	7	Mean	E _r (media)	ERL	ERM	TEL	PEL	LEL	SEL
Hg	0.102	0.121	0.143	0.148	0.132	0.136	0.142	0.132	125.7	0.15	0.71	0.13	0.70	0.2	2.0
Cu	57.36	53.85	55.76	61.42	58.23	54.78	62.87	55.70	29.6	34	270	18.7	108.2	16	110
Zn	46.53	55.61	58.21	48.98	52.71	50.98	56.35	52.80	0.4	150	410	124	271	120	820
Cd	0.054	0.065	0.058	0.078	0.067	0.053	0.059	0.062	124.0	1.2	9.6	0.68	4.21	0.6	10
Pb	0.088	0.091	0.094	0.122	0.097	0.092	0.107	0.099	15.4	46.7	218	30.24	112.18	31	250
RI	248.4	287.8	295.8	347.9	305.1	278.4	302.5	295.1							
M-ERM-Q	0.095	0.102	0.111	0.113	0.108	0.105	0.115	0.107							
PLI	3.1	3.5	3.6	4.0	3.6	3.4	3.7	3.5							

NOAA: National Oceanic and Atmospheric Administration, USA; CMEE: Canadian sediment quality guidelines for the protection of aquatic life

Table 2. Maximum allowable daily consumption rate (CR_{lim}) of *H. floridana* expressed in kg per day by different age groups.

Metal	Concentration (mg/kg)	RfD (mg/kg bw/day) ^a	CR_{lim} (kg/day)	
			Children ^b	Adults ^c
Cu	8.75	0.04	0.07	0.37
Zn	6.44	0.3	0.70	3.73
Hg	0.081	5.7×10^{-4}	0.11	0.56
Pb	0.032	^d 6.3×10^{-4} / ^e 1.5×10^{-3} / ^f 5×10^{-4}	0.23	^d 1.58 – ^e 3.75
Cd	0.021	0.001	0.71	3.81

^a Values of oral reference dose (RfD) from [32, 33]

^b BW= 15 kg and ^c BW=80 kg [34]

Values for Pb: ^d BMDL₁₀ for nephrotoxicity (adults), ^e BMDL₀₁ for cardiovascular effects (adults), and ^f BMDL₀₁ for developmental neurotoxicity (children) [35]

Table 3. Correlation of Pearson among metals in sediments and tissues

	Cu t _a	Cu sed _b	Zn t	Zn sed	Hg t	Hg sed	Pb t	Pb sed	Cd t	Cd sed
Cu t	1									
Cu sed	-0.058	1								
Zn t	0.566	0.070	1							
Zn sed	0.455	-0.114	0.958**	1						
Hg t	0.504	0.140	0.724	0.776*	1					
Hg sed	-0.073	0.407	0.453	0.457	0.363	1				
Pb t	0.687	-0.598	0.238	0.276	0.136	-0.095	1			
Pb sed	-0.244	0.790*	0.001	-0.090	0.141	0.688	-0.572	1		
Cd t	0.208	0.628	0.377	0.330	0.536	0.829*	-0.006	0.693	1	
Cd sed	-0.132	0.406	0.018	-0.085	-0.046	0.423	-0.430	0.767*	0.233	1

t_a: tissue; sed_b: sediments; *Significance at the 0.05 probability level; ** Significance at the 0.01 probability level

Figure 1. Study area and sampling stations from the Cispatá Bay, Colombia.

Figure 2. Trace metal concentrations in (a) tissues, and (b) sediments

Figure 3. Principal Component Analysis (PCA)