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Mytilus Galloprovincialis and Metal Contaminants: Health Risk Assessment from Sinop Coasts

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Abstract

The goal of this study was to quantify the content of heavy metals (Zn, Cu, Cd, Pb, Hg) in the bivalve *Mytilus galloprovincialis*, which was obtained from the Black Sea's Sinop peninsula, as well as estimate the health risks. Concentrations of heavy metals in mussels' soft tissues ranged between 16.4-21.8, 0.52-0.93, 0.13-0.45; 0.015-0.019 and 0.006-0.011mg kg^{-1} wet weight for Zn, Cu, Pb, Hg and Cd, respectively. In general, metal concentrations in mussels were quite low. In mussels, Zn was the most prevalent element, followed by Cu. Regarding the monthly variation, although relatively higher tissue metal concentrations were observed in August compared to July, no statistical difference was found. The estimated daily intakes did not exceed the tolerable intakes. There was no health risk since the total hazard index was far below critical value 1. This demonstrates that eating mussels from the Sinop coasts in 2019 does not cause any health risks in infants, children, or adult. Mussels have a risk index of less than 10^{-6}, which is considered insignificant. In conclusion, the results of this study show that there is no heavy metal pollution in the mussels collected from Sinop coasts.

Keywords: Black Sea, Sinop, *Mytilus galloprovincialis*, heavy metals, hazard index

Major classifications: Food Science (Restaurant Marketing, Healthy Food), Health Science (Environmental Safety and Engineering)

1. Introduction

Increased industrialization, expanding human population, oil exploitation, agricultural and domestic waste run-off all contribute to the ongoing introduction of contaminants into the marine coastal environment. Because contaminants can harm living beings, bioaccumulation is an important part of the process. Heavy metals have long been established as important contaminant of the marine environment among these contaminants. The bioaccumulation of heavy metals in marine creatures is extremely difficult to comprehend. This is because various elements that influence bioaccumulation, such as the physico-chemical qualities of the waters, the chemical properties of the contaminant, and the biologic components of the organism, are intertwined.

Over the previous few decades, large volumes of contaminants have been dumped into marine and estuarine environments. Heavy metals are still being dumped into the coastal zone from rivers and nonpoint sources, particularly in riparian countries. The Black Sea waters feed major cities and receive waste waters from numerous industrial units and home garbage without treatment (Bat, Öztekin, Şahin, Arıcı, & Özsandıkçı, 2018a). As a result, the study of coastal water pollution and its consequences on the environment has been a key focus of various studies, with a greater emphasis on biological material examination (Bat & Arici, 2018). Because of their persistent nature, toxicity, tendency to accumulate in organisms and undergo food chain amplification, and the fact that they are non-degradable, heavy metals are one of the most harmful classes of contaminants.

Heavy metal contamination in the marine environment could be assessed using a variety of organisms, such as fish, molluscs, and crustacea (Bat & Arıcı, 2018), polychaetes (Bat & Kurt, 2020), sea cucumbers (Bat, Ahmed, Öztekin, & Arıcı, 2020), macro-algae and seagrass (Bat, Arici, & Öztekin, 2021). As they feed by filtering particles from water, including metals from saltwater, food, and sediment, bivalves collect metals from their surroundings. Bivalves are reflective of an area's contamination since they can absorb metals at considerably higher quantities than those observed in the water column. Furthermore, because of their extensive distribution, sessile, and ease of collection, bivalves are an ideal option for studying metal bioaccumulation (Phillips, 1977; Phillips, 1980; Phillips & Rainbow, 1994). Mussels meet most of these criteria. Therefore, it is among the most preferred species for metal pollution (Bat, Gundogdu, Ozturk, & Ozturk, 1999; Bat, Üstün, & Gökkurt-Baki, 2012; Bat & Öztekin, 2016; Bat, Arıcı, Sezgin, & Şahin, 2016; Bat, Arıcı, Öztekin, Yardım, & Üstün, 2018b, c).

With the growth of industry and the economy, enormous amounts of waste effluent have been released into the Black Sea's semi-enclosed aquatic system (Bat et al., 2018a). The environment in this area has deteriorated significantly, posing a threat to the biota, particularly mussels. It is required to investigate the causal linkages between contaminant exposure and detectable biological impacts in marine animals to assess the contamination trend in the Black Sea. Sinop is in the middle of the southern Black Sea. It is one of the smallest cities in Turkey with a population of approximately 58 thousand. However, during the summer months, especially in July and August, the number of tourists visiting Sinop has exceeded 1 million. Hundreds of thousands of tourists visit this place because it is the happiest city in Turkey, its natural beauties and the clean sea compared to other places in the Black Sea of Turkey. Most of these people who come to Sinop also consume seafood in restaurants. One of the most consumed of these seafood products is mussels.

In order to assess possible harm to human health, it is critical to always determine the bioaccumulation capacity of heavy metals in species, particularly edible creatures. In fact, *M. galloprovincialis* has been found to be a good heavy metal bioindicator species. It has a higher capacity for accumulating heavy metals than many other creatures. It has a wide geographic distribution, as well as a large enough size and tolerance range for a variety of salinities and temperatures.

The main purpose of this study is to determine the metal levels in mussels collected especially in the most consumed months and to make possible risk analyzes that may give to hundreds of thousands of people who eat them.

2. Methods and Materials

2.1. Sampling sites

There are dead mussel shells on the coasts of Sinop peninsula. That is why mussels are not dense everywhere. Larger and abundant mussels are found at the very point of Sinop. It is usually collected from here to restaurants or fish markets. In this study, mussel samples were collected by scuba divers from the rocks in the snout of Sinop (Figure 1) in July and August in 2019. After captured mussels were placed in plastic flasks filled with seawater of sampling area, then immediately transported to the laboratory. Each month, twenty specimens were chosen at random based on their shell length and wet weight data. The lengths of the shells did not differ significantly between samplings ($p>0.05$, One-way ANOVA).

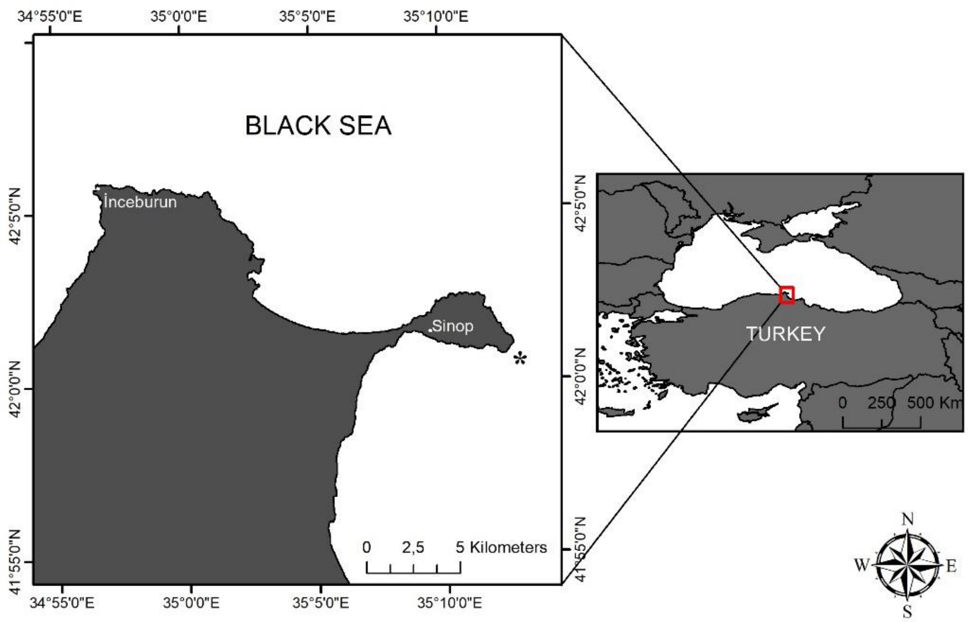

Figure 1: Mussels' sampling area

2.2. Experimental protocol

Mussels were measured (Figure 2) and were rinsed and put in constantly aerated clean seawater for 48h to allow depuration then subjected to one of the following treatments:

Method 1) Half of the mussels were then frozen (-21℃) for metal analysis.

Method 2) Rest of the mussels immediately were then killed by placing them in bi-distilled boiling water.

Figure 2: Measurement of mussels

2.3. Sample preparation

The complete tissues of mussel samples were prepared for metal analysis according to Bernhard's procedure (1976). The shells were carefully opened so as not to damage tissues. The samples were conserved in sterilized petri dishes and stored at a temperature of -21℃.

2.4. Analytic procedure

The mussels' entire tissues were digested with Suprapur® HNO_3 (nitric acid) using a Milestone Systems Start D 260 microwave digestion device for metal analysis. Using the certified reference material NRCC-TORT-2 lobster hepatopancreas, blanks and certified reference material samples were made in parallel to validate the accuracy and precision of the procedure. The percentages of these reference resources that were retrieved ranged from 98 to 104 percent. Limits of determination and quantification were considered the equipment's instrumental reaction. Tissues were evaluated in triplicate using an Agilent Technologies 7700x ICP-MS, with the results represented in mg kg^{-1} wet weight (Bat et al., 2020).

2.5. Risk assessment for health

Risk evaluations for adults were conducted in order to determine the potential hazards that may arise as a result of consuming metals found in mussels from the Sinop cape in the Black Sea. This was determined by calculating the probability of a health hazard using likely exposure. The average daily intake of heavy metals (mg/kg day) must be taken into account when calculating risk exposure. The estimated daily intake (EDI) is calculated based on element levels and the amounts of mussels consumed. The following equation was used to calculate the EDI of heavy metals:

$$\mathrm{EDI} = \frac{\mathrm{C_{metal}\ x\ W_{mussel}}}{\mathrm{BW}}$$

Where C_{metal} is the amounts of metals in whole tissues; W_{mussel} is the daily mean consumption of seafood, which is reported as 0.013, 0.027, and 0.041 kg/day for infants, children, and adults, respectively. BW is the body weight of 10 kg for infants, 30 kg for children, and 70 kg for adults (UNSCEAR, 2010).

A hazard index (HI) can be determined as the ratio of the calculated metal dosage (EDI mg/kg of body weight per day) to the reference dose (Rf. D. mg/kg day^{-1}). The HI was discovered using the formula below:

$$\mathrm{HI} = \frac{\mathrm{EDI}}{\mathrm{Rf.\,D.}}$$

If HI > 1.0, the EDI of a particular metal exceeds the Rf. D., indicating that the metal is potentially hazardous. It is dependent on both metal levels and the amounts of mussel consumed. For lead and compounds, there is no Rf. D. value. Oral slope factor (SF), on the other hand, is only indicated for lead and compounds as 0.0085 mg/kg-day (RAIS, 2019). The following formula was used to compute the risk index (RI):

$$\mathrm{RI} = \mathrm{EDI\ x\ SF}$$

The RI is taken account of insignificant if the $RI<10^{-6}$, allowable or tolerable if RI is $10^{-6}<RI<10^{-4}$ and likewise considered significant if the $RI > 10^{-4}$.

The Hazard Index (THI) is defined as the sum of the HIs, as shown in the following equation:

$$\mathrm{THI} = \mathrm{HI\ (Zn) + HI\ (Cu) + HI\ (Pb) + HI\ (Hg) + HI\ (Cd)}.$$

3. Results and Discussion

The average length of the collected mussels was 65±4 mm, their weight was 33±3 g, their width was 3.6±0.2 cm and their thickness were 2.8±0.1 cm.

The heavy metal values found in mussels collected from the farthest point of Sinop are shown in Figure 3.

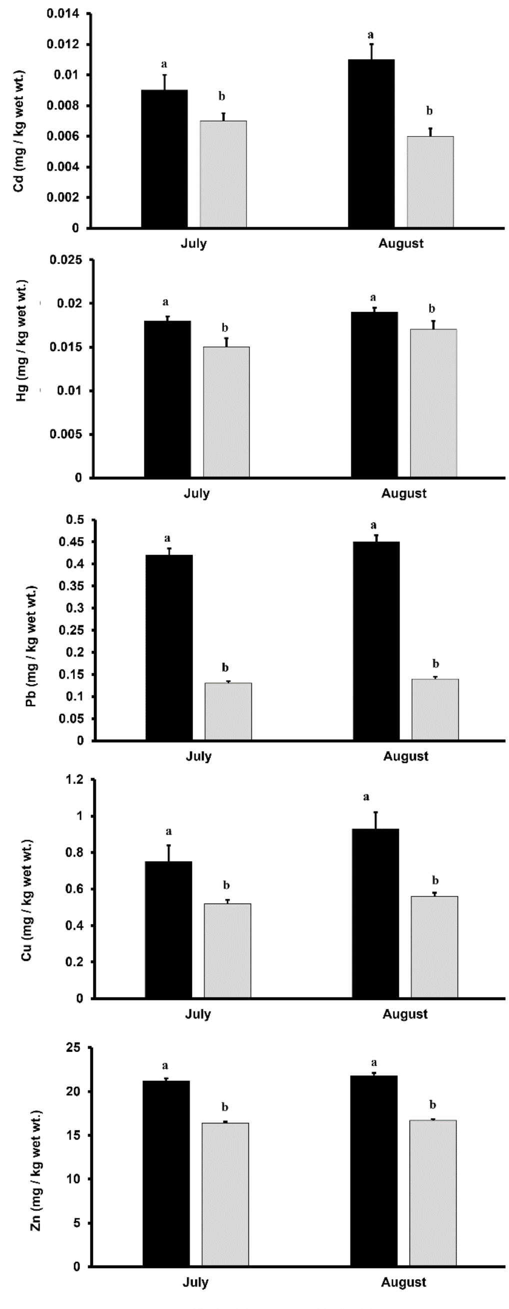

Figure 3: Mean heavy metals (mg/kg wet wt.) in mussels by months and two different methods in the mussels. Vertically, letters a and b show statistically significant differences ($p<0.05$).

Concentrations of heavy metals in mussels’ soft tissues ranged between 16.4-21.8, 0.52-0.93, 0.13-0.45; 0.015-0.019 and 0.006-0.011mg kg^{-1} wet weight for Zn, Cu, Pb, Hg and Cd, respectively. In general, the concentrations of trace elements found in mussels were minimal. Zn was the most abundant element in mussels, followed by Cu. The detected heavy metals had a low tendency to accumulate in the mussels in general. Regarding the monthly variation, although relatively higher tissue metal concentrations were observed in August compared to July, no statistical difference was found. The hydrological characteristics of the marine environment, such as temperature and salinity, as well as the physiological mechanisms that drive reproductive activity, were found to be associated to monthly fluctuations in heavy metal concentrations in mussels. The temperature and salinity in Sinop snout water between July and August were similar. This indicates that the ambient environment was identical over these two months, and hence the mussels' physiology was not affected.

However, statistical differences were found in the amounts of raw and cooked mussels. All heavy metal amounts in mussels measured by boiling were found to be less than those measured raw. The quantities of heavy metals in mussel tissues were compared to Turkish Food Codexis (Official Gazette of Republic of Turkey, 2002 and 2009) and the EU Commission Regulations (Council of Europe, 2001; Commission Regulation, 2006) standard values in this study. Heavy metal levels in the edible parts of the mussels from Sinop snout in the Black Sea are below the proposed human consumption limit values. Acceptable values of Cd, Pb, Hg, Cu, and Zn are indicated as 1.0, 1.5, 0.5 (Council of Europe, 2001; Commission Regulation, 2006; Official Gazette of Republic of Turkey, 2009), 20 and 50 (Official Gazette of Republic of Turkey, 2002), mg/kg wet wt., respectively. The results in the analyzed site show that there is no health danger for mussel users in Sinop, according to acceptable limits.

The daily consumption of mussels in Turkey is stated as 1.0 g by the Food and Agriculture Organization of the United Nations (FAO, 2010). In Turkey, seafood consumption is low, while it is higher in locations where people are better educated and have higher earnings (Food and Agriculture Organization of the UN, 2013) and inhabit coastal cities including Sinop province (Bat, 2017). This value does not reflect the reality especially for the province of Sinop. For this reason, the values specified in the United Nations Scientific Committee on the Effects of Atomic Radiation report (UNSCEAR, 2010) were taken as basis in this study.

The amounts of heavy metals in marine coastal ecosystems are often done by measuring their concentrations in water, sediments, and biota (Bat, 2017; Bat & Arıcı, 2018; Bat & Özkan, 2019; Bat & Kurt, 2020: Bat et al., 2021). The accumulating of a chemical to a dangerous level in the body of an organism is known as bioaccumulation. Heavy metals can be accumulated by marine species, such as fish and mussels, by direct absorption or through their food chain, and then passed on to humans through eating, causing chronic or acute illness.

Mussels feed by filtering the water they live in. During filtration, they accumulate the contaminants in the water and particles in their body. Studies have shown that metals accumulate more in mussels than in fish (Bat & Arıcı, 2018). Therefore, it is harmful to consume mussels collected in metal-contaminated areas. In other words, it would be inconvenient to consume mussels from contaminated areas or from unknown origin. However, mussels are a good source of vital amino acids and abundant in omega-3 polyunsaturated fatty acids (Carboni, Kaur, Pryce, McKee, Desbois, Dick, Galloway, & Hamilton, 2019). It has been suggested that replacing the protein component of lunch meals with mussels three times a week for two weeks is sufficient to moderately improve omega-3 status (Carboni et al., 2019).

Mussel consumption in Sinop is quite high when compared to many regions of Turkey. Especially in July and August, this rate increases with the opening of the sea season. The increase in mussel consumption almost broke a record, as the number of people coming to Sinop as tourists in the summer months of 2019 exceeded 1 million. It was mostly consumed in the form of mussel skewers, mussel pans and stuffed mussels. Therefore, in our current study, risk analyses were performed by taking the metal levels in cooked mussels.

The EDI, the calculated THQ and HI values and risk index of mussels from Sinop snout in the Black Sea in 2019 for infants, children and adults are given in Tables 1.

The EDI levels of the heavy metals examined in mussels were extremely low. These numbers were found to be lower than their Rf. D. counterparts. Similarly, the HI values of these metals were determined to be extremely low. Infants' HI values were found to be greater than children's and adults', indicating that infants are more likely to be at risk than children and adults at a relatively high level of exposure. It's also worth noting that infants weigh significantly less than children and adults. The THI, which is the sum of the HI values for all of the heavy metals tested, was, nevertheless, less than the threshold value of 1. This demonstrates that eating mussels from the Sinop snout in 2019 does not cause any health risks in infants, children, or adults.

The SP value is only supplied for Pb and its derivatives among the metals covered here in the Risk Assessment Information System. A person's lifetime (LT) is estimated to be 70 years on average, with an exposure duration (ED) of 26 years (RAIS, 2019). Mussels have a risk index of less than 10^{-6}, which is considered insignificant.

Table 1: Estimated Daily Intakes (EDI), Hazard Index (HI), Total Hazard Index (THI) and Risk Index (RI) of metals via consumption of mussels from Sinop snout in the Black Sea in 2019. Rf. D. and EDI values are given as mg/kg body wt. /day. Other parameters are unitless.

Metal	Rf. D.	SF	EDI			HI			RI
			(infants)	(children)	(adults)	(infants)	(children)	(adults)	
Hg	3E-04		2.08E-05	1.44E-05	9.37E-06	6.93E-02	4.80E-02	3.12E-02	
Cd	1E-03		8.45E-06	5.85E-06	3.80E-06	8.45E-03	5.85E-03	3.81E-03	
Cu	4E-02		7.02E-04	4.86E-04	3.16E-04	1.75E-02	1.22E-02	7.91E-03	
Zn	3E-01		2.15E-02	1.49E-02	9.69E-03	7.17E-02	4.96E-02	3.23E-02	
Pb		8.5E-03							6.72E-07
					THI	1.28E-01	8.86E-02	5.76E-02	

When we look at the previous studies, it is seen that the amount of metal in mussels is variable. These changes are evident in mussels collected from different regions and times. Bat et al. (2018c) found that mussels are generally safe to consume, although there are some high-value metals in mussels in their risk analysis calculations on mussels collected from the Black Sea coast.

Mussels are frequently used in metal pollution monitoring studies (Schulz-Baldes, 1974; Davies & Pirie, 1978; Ünlü & Fowler, 1979) because they are ingested by humans (Bat & Arıcı, 2018) and have a wide geographic range (Fish & Fish, 1996). The importance of mussels has long been recognized by the global Mussel Watch program, which was founded by Goldberg (1975) in the United States in the mid-1960s, and mussels are regarded as an ideal bio-monitor due to their propensity to bioaccumulate heavy metals. In the 1970s, several European countries (including France, Italy, and Spain) initiated national Mussel Watch programs (Baena & Thébault, 2007). In 1996, the "Réseau Intégrateur Biologique (RINBIO)" was established to track contaminant concentrations in mussels imprisoned for several weeks prior to biomonitoring (Andral, Stanisiere, Sauzade, Damier, Thebault, Galgani, & Boissery, 2004). Despite the existence of multiple ongoing state projects, according to Baena & Thébault (2007), no large-scale Mussel Watch network was coordinated at the Mediterranean level until 2002. Using the mussel *M. galloprovincialis* as a bio-monitor species, CIESM established a regional "Mediterranean Mussel Watch (MMW)" (y Baena & Thébault, 2007). Metal accumulations in *M. galloprovincialis* have been made in the seas of Turkey for the last fifty years and these studies are increasing gradually (Bat & Arıcı, 2018). Studies on *M. galloprovincialis* are also working on the coasts of the Sinop peninsula. These studies are presented in Table 2 for comparison with the current study.

Table 2: Comparison of heavy metal concentrations in *M. galloprovincialis* from Sinop coasts in the Black Sea.

Year	Wet/dry wt.	Zn	Cu	Cd	Pb	Hg	References
1992-1993	Wet wt.	1.58-7.28	0.1-1.89	0.03-0.24	0.11-1.18	--	Bat et al., 1999
2010	Dry wt.	79-163	2.41-4.82	0.27-0.98	2.10-4.10	--	Bat et al., 2012
2013	Dry wt.	35.4-47	0.8-1	0.4-0.77	0.15-0.7	<0.05	Bat & Öztekin, 2016
2015	Wet wt.	8-27	0.5-1.8	0.04-0.10	0.06-0.31	0.03-0.07	Bat et al., 2018c
2016-2018	Wet wt.	--	--	0.05-0.08	0.15-0.23	0.007-0.10	Bat, 2019
2019	Wet wt.	16.4-21.8	0.52-0.93	0.006-0.011	0.13-0.45	0.015-0.019	This study

Considering the other studies conducted on the coasts of Sinop (Bat et al., 1999; Bat et al., 2012; Bat and Öztekin, 2016; Bat et al., 2018b,c, Bat, 2019), the results seem to be similar to this study. Looking at the years, there is a decrease in the metal levels measured in mussels. This is a pleasing result. In fact, in this study, it was found that the amount of mussels collected from the snout of Sinop was partially lower. It has emerged as a result of the fact that this region is far from the settlements and that there are flowing and clean waters.

4. Conclusion

In the current study the contents of Zn, Cu, Cd, Pb, Hg in *M. galloprovincialis,* which was collected from Sinop snout in the Black Sea, as well as estimate the health risks. Heavy metal in soft tissues ranged between 16.4-21.8, 0.52-0.93, 0.13-0.45; 0.015-0.019 and 0.006-0.011mg kg^{-1} wet weight for Zn, Cu, Pb, Hg and Cd, respectively. Zn was the most prevalent metal, followed by Cu. Regarding the monthly variation, although relatively higher tissue metal concentrations were observed in August compared to July, no statistical difference was found. The estimated daily intakes did not exceed the tolerable intakes. There was no health risk since the total hazard index was far below critical value 1. This demonstrates that eating mussels from the Sinop coasts in 2019 does not cause any health risks in infants, children, or adult.

Consumption of mussels (one or two 100 g servings per week) appears to appropriate, but risks may be lowered by any level of mussel's consumption evaluated (up to two or three 100 g servings per week) unless high contaminants levels are present. Via the food chain, toxic substances are ingested and biomagnified. Therefore, it is very important where the consumed sea food is collected or caught.

Heavy metal contamination is one of the most serious environmental issues in locations with high levels of anthropogenic activity, and it has the potential to harm people health. Considering metal contamination in marine ecosystem can be hazardous to people health, it's critical to monitor and manage the contamination levels in seafood.

In conclusion, the results of this study show that there is no heavy metal pollution in the mussels collected from Sinop coasts. Sinop is the least populated coastal city in Turkey. There is no industrial activity in this region. Domestic waste is the first source of pollution that draws attention. However, in the summer months, the population increases excessively due to the fact that tourists come both for historical tours and for the sea. Due to insufficient lower capacity and touristic activities, contamination is not to be underestimated. It is important to design and implement regulations for the management of evacuations, along with remediation pilot projects, in order to limit the contamination of the Sinop coasts.

5. Acknowledgements

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6. Conflict of interest statement

There are no conflicts of interest declared by the authors.

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