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Assessment of Some Heavy Metals Using Sediments and Bivalvia (*Mytilus galloprovincialis*) Samples Collected from Tobruk Coast

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ABSTRACT

This study is designed to assess the concentrations of lead, copper, zinc, iron, cadmium and manganese in sediments and bivalvia (*Mytilus galloprovincialis*) tissues of Tobruk coast. The samples of sediments and bivalvia were collected from four sites around Tobruk city namely as Main port, Ledo, Andolus and Rasbayad (control site), during summer, 2021. The heavy metals of sediments and bivalvia tissues were determined using atomic absorption spectrometry. Also water samples were taken from four sites and physical and chemical properties of water were measured. The results of sediments showed that the Ledo site significantly recorded the higher concentrations of lead (0.624 mg/kg), zinc (73.77 mg/kg), copper (0.450mg/kg) and iron (94.38mg/kg) than those of the other sites, however, the metals of sediments collected from Rasbayad recorded the lowest concentrations. The results of bivalvia tissues revealed that Main port site recorded the greater concentration of lead (0.420 mg/l), zinc (2.185mg/l), cadmium (0.385 mg/l) and copper (3.010 mg/l) than that of other sites. Moreover, Andolus site recorded significantly the higher concentration of iron (14.135 mg/l) than that of other three sites. On the other hand, the Rasbayad recorded the lowest concentration of lead (0.070 mg/l), zinc (0.115 mg/l), cadmium (0.020 mg/l) and copper (0.030 mg/l) in bivalvia tissues. In general, the results indicated a high degree of contamination by heavy metals in sediments and bivalvia of three sites when compared with the control site (Rasbayad) and it is reflect the environmental pressure surrounding the coast of Tobruk, an urgently action is needed to reduce the causes of pollution and contamination by heavy metals.

1 Introduction

Heavy metals pollution in the marine ecosystems is a result of anthropogenic activities such as mining, shipping, agriculture and domestic waste (Haynes and Johnson, 2000; Islam and Tanaka, 2004). Humans as well as aquatic organisms are under the threat of a rise in heavy metals concentration in the marine environment (Uluturhan et al., 2007; Naji et al., 2010). In general,

heavy metals enter the aquatic environment through atmospheric deposition, erosion of the geological matrix, or due to anthropogenic activities caused by industrial effluents, domestic waste, sewage, shipping, agriculture and mining wastes (Tervainen et al., 1997; Stephen et al., 2000, Haynes and Johnson, 2000). The contamination chain in the aquatic environment has been always in the following cycle: industry, atmosphere, soil, water, phytoplankton, zooplankton, fish and human

beings or: soil, plant, animal and human beings in the terrestrial environment. Heavy metals contamination of coastal water and sediment has been identified as a serious pollution resulting from industrialization.

The bivalve molluscs are the second class of molluscs by number of species (about 30.000). This class includes mussels (*Mytilus galloprovincialis*), clams (*Venus gallina*), oysters (*Ostrea edulis*) and other edible species. Bivalve molluscs are very much appreciated by consumers for their nutritional properties, and their consumption has increased worldwide (Oliveira et al., 2011). Bivalve molluscs are suspension feeders that selectively filter small particles, phytoplankton zooplankton and inorganic matter from the surrounding water (mussels filter about 36 l/day of water and oysters filter up to 350 l/day). This aspect, together with their persistence in the same place, makes them excellent bio-indicators of the pollution level of the marine ecosystem (Eister, 1981; Rainbow, 1995). The mollusks are sensitive to environmental impact.

Intensive anthropogenic activities (Environmental pollution/pollutants originating in human activity) cause the accumulation of various xenobiotics whose final destination is predominantly the aquatic environment. Many environmental contaminants can accumulate in the tissues of aquatic organisms and exert toxic effects that are related to oxidative stress. Several studies of the stress response associated with oxidative stress in aquatic organisms are used as tools that aid in examining an environment's quality (Valavanidis et al., 2006).

The chemicals like polycyclic aromatic hydrocarbons and metal elements elevate the production of reactive oxygen species (ROS) in cells by different mechanisms and may lead to a state of oxidative stress. Cellular antioxidant enzymes respond to increased ROS levels with different levels of magnitude (Zhu et al., 2009) and their activities change under the influence of seasonal rhythmicity, adaptation to low environmental temperatures, and site-specific environmental influences (Vidal et al., 2010). According to Torres et al. (2002), taking into consideration that oxidative stress responses are directly associated with cellular function, oxidative stress parameters may give a good indication of the environment's local pollution status. Coastline of Libya is longest of any African country bordering the Mediterranean, there are a neglected studies were done to assess various contamination on Libyan coasts, (especial the eastern coast). The aim of this study is to assess of the trace elements in sediments and bivalvia tissues during the summer season and to compare the concentrations of the trace elements between different collection sites from Tobruk coast, east of Libya. Materials and Methods

2 Study area and sampling

Tobruk bay basin (Map1) is located in the south east of the Tobruk city, which is positioned at longitude

23.59 and 13.06 E and latitude 32.04 and 09.46 N, northeast of Libya. Some economic activities are concentrated on the two sides of the bay, such as Turkish port (for fishing boats), commercial port is used to receive merchant ships, fishing and rescue boats, and the Cornice resort which is used for entertainment. Bay basin suffers from direct sewage discharge (Fitori et al., 2020).

The samples of sediments and bivalvia (*Mytilus galloprovincialis*) were collected during the summer, 2021 from three sites and points located around Tobruk bay basin as (Main port, Ledo, Andolus), and Rasbayad as (control site). Some physicochemical parameters of water bodies at all studied sites were measured: including water temperature by thermometer, dissolved oxygen, salinity and pH by a multi-portable device (HACH, USA).

2.1 Analysis of bivalvia samples

The sampling of bivalvia (*Mytilus galloprovincialis*) was performed with benthic hand nets (kick and sweep multi-habitat semi-quantitative technique) (David et al., 1998). After sampling, the animals transferred to the laboratory. The soft tissues immediately were separated from the shells, frozen, and preserved at -80°C . For heavy metals analysis, the frozen tissues were washed by bi-distilled water, then oven-dried to constant weight at 105°C . The dried tissues were crushed and powdered in an agate mortar, then, they were kept in polyethylene bottles for analysis. One gram portions of tissues were digested by means of a microwave after addition of nitric acid and hydrogen peroxide. The elements of concern lead (Pb), copper (Cu), manganese (Mn), zinc (Zn), iron (Fe), cadmium (Cd) in the samples were determined by Atomic Absorption Spectrometer (AAS).

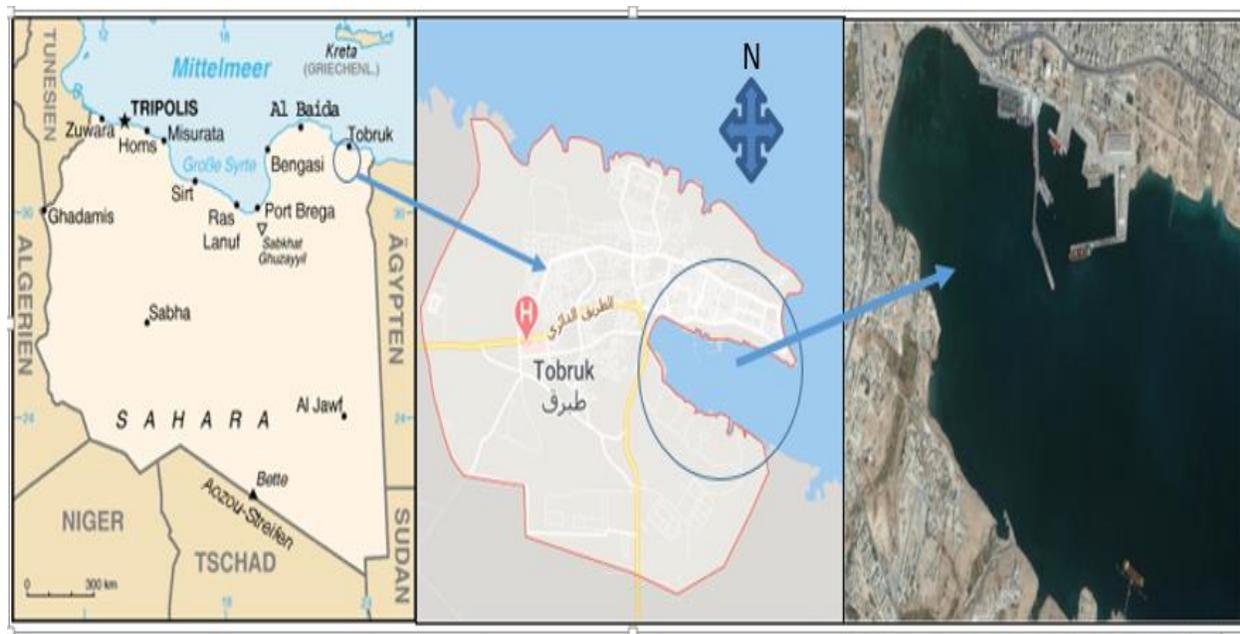
2.2 Sediment analysis

Sediment samples from the selected sites were collected by using Ekman dredge and kept frozen until analyzed. For total heavy metals, sediment samples were allowed to defrost, then air-dried in a circulating oven at 30°C and sieved mechanically using a 2 mm sieve. For the digestion of samples, 1gram sieved sediment was digested with repeated addition of nitric acid and hydrogen peroxide according Ackwerth and Würfels (Ackwerth and Würfels, 1994) method to complete dissolve all elements present in the sediments. The resultant digested was reduced in volume while heating and then diluted to a final volume of 100 ml. The elements of concern Pb, Cu, Mn, Zn, Fe, and Cd in the samples were determined by Atomic absorption spectrophotometer (AAS).

Statistical analysis

The collected data were analyzed using Statistical Package for Social Science (SPSS, version, 23). The analysis of variance (One-way Anova) was run to

investigate the effect of site on detecting heavy metals of sediment and bivalvia tissues, while the means were separated by Duncan Multiple Range Test (DRMRT). The results were presented as mean \pm SD



Map1. Tobruk bay basin (Fitori, et al 2021)

Table 1. Physical and chemical Parameters of the studied area

Site	Parameters			
	Temperature (°C)	pH	Salinity (%)	Dissolved oxygen (mg/l)
Main port	22.6	7.9	36.2	9.4
Andolus	20.3	6.8	34.7	8.9
Ledo	19.3	8.1	38.4	9.7
Rasbayad	20.3	7.2	37.4	12.9

3 Results

3.1 Physical and chemical parameter of water in studying area

The values of physical and chemical parameters of sea water *views*: Temperature (°C), Salinity (%), pH and Dissolved oxygen were given in Table (1). The water temperature (°C) values were measured in the studied locations exhibited small variations, where the minimum and maximum values of 19.3 and 22.6 °C was recorded in Ledo and Main port site respectively. The pH-values were ranged between a minimum value of 6.8 in Andolus site and a maximum value of 8.1 in Ledo. The values of salinity in the studied sites were fluctuating between 34.7% (Andolus) and 38.4% (Ledo). The

distribution pattern of dissolved Oxygen in the different areas varied from a minimum of 8.9 mg/l at Andolus site to a maximum of 12.9 mg/l at Rasbayad site.

3.2 Concentration of heavy metals in sediments

The heavy metal concentrations in the sediments of the four sites were found in Table 2. The results showed that the site of sediment collection significantly ($P < 0.01$) affected the concentration of heavy metals with respect to: lead, cadmium, zinc, copper, manganese and iron. The results of the means separation showed that the Ledo site recorded the highest concentration of lead (0.624 mg/kg), zinc (73.77 mg/kg), copper (0.450 mg/kg) and iron (94.38 mg/kg), followed by the main port site, then the Andolus site, while Rasbayad site

recorded the lowest concentration of heavy metals mentioned previously. On the other hand, the main port site showed that had the greatest concentration of cadmium (0.962 mg/kg) and manganese (42.99 mg/kg), followed by Ledo and Andolus sites, while Rasbayad site recorded the lowest concentration of cadmium (0.002 mg/kg) and manganese (0.01 mg/kg) in the sediment.

3.3 Concentration of heavy metals in tissues of *Mytilus galloprovincialis* collected from the studied sites

Concentration of heavy elements in tissues of bivalvia (*Mytilus galloprovincialis*) which collected from the studied sites was presented in Table 3. The results showed that the site of bivalvia collection significantly ($P < 0.05$) affected concentration of some heavy elements (lead, zinc and iron) in their tissues,

while the site insignificantly ($P > 0.05$) influenced the concentration of cadmium, copper and manganese. The mean separation results revealed that Main port site recorded significantly ($P < 0.05$) greatest concentration of lead (0.420 mg/kg) and zinc (2.185 mg/kg) in tissues of bivalvia, followed by Andolus site then lido, while the Rasbayad recorded the lowest concentration of lead (0.070 mg/kg) and zinc (0.115 mg/kg). On the other hand, Andolus site was showed had significantly ($P < 0.05$) highest concentration of iron (14.13 mg/kg) in bivalvia tissues, followed by main port (2.490 mg/kg), then Ledo site (2.470 mg/kg), while Rasbayad site significantly ($P < 0.05$) recorded the lowest concentration of iron (0.004 mg/kg) in tissues of bivalvia. Also the results revealed that the insignificantly ($P > 0.05$) greatest concentration of cadmium, copper and manganese in tissues bivalvia of main port site, while the lowest concentrations of the above three metals were found in tissues of bivalvia collected from Rasbayad site.

Table 2. Concentration of heavy metals (mg/kg) in sediments

Site	Lead	Cadmium	Zinc	Copper	Manganese	Iron
Main port	0.511 ^b ±0.013	0.962 ^a ±0.0117	11.38 ^b ±0.00	0.104 ^b ±0.001	42.99 ^a ±0.01	34.60^b±0.73
Andolus	0.316 ^c ±0.007	0.844 ^c ±0.0082	8.77 ^c ±1.14	0.060 ^b ±0.004	30.09 ^b ±0.18	026.13^c±0.30
Ledo	0.624 ^a ±0.004	0.937 ^b ±0.0091	73.77 ^a ±0.02	0.450 ^a ±0.034	42.09 ^a ±0.68	94.38^a±1.05
Rasbayad	0.005 ^d ±0.003	0.002 ^d ±0.0014	0.01 ^d ±0.00	0.003 ^c ±0.001	0.01 ^c ±0.01	0.003^d±0.001
Sig. Level	*	*	*	*	*	*

*: Significant level at $P < 0.01$,

a,b,c: Means with the same letter are insignificantly ($P > 0.05$) different

Table3. Concentration of heavy metals (mg/kg) in the tissues of bivalvia (*Mytilus galloprovincialis*) collected from the studied sites

Site	Lead	Cadmium	Zinc	Copper	Manganese	Iron
Main port	0.420 ^a ±0.042	0.385 ^a ±0.035	2.185 ^a ±0.078	3.010 ^a ±0.141	0.460 ^a ±0.325	2.490 ^b ±0.099
Andolus	0.220 ^b ±0.071	0.380 ^a ±0.467	0.485 ^b ±0.177	0.120 ^a ±0.014	0.795 ^a ±0.629	14.135 ^a ±7.029
Ledo	0.150 ^b ±0.085	0.180 ^a ±0.028	0.115 ^b ±0.148	1.665 ^a ±2.185	0.145 ^a ±0.162	2.470 ^b ±0.382
Rasbayad	0.070 ^b ±0.014	0.020 ^a ±0.014	0.115 ^b ±0.134	0.030 ^a ±0.028	0.016 ^a ±0.020	0.004 ^b ±0.004
Sig. Level	*	NS	*	NS	NS	*

*: Significant level at $P < 0.01$, NS insignificant at level $P > 0.05$

a,b,c: Means with the same letter are insignificantly ($P > 0.05$) different

4 Discussion

In this study the sea water temperature values were ranging between 19.3 and 22.6 °C. The temperature is one of the most important environmental factors, which directly affects the aquatic ecosystem, by direct effect on the metabolic activities of most aquatic organisms. The water temperature values depend up on different reasons such as the season of samples collection, the effect of out

let discharges and some of human activities as desalination stations and / or the presence of some industries (El-Khair, 1993). The values of salinity in the present study were fluctuating between 34.7 - 38.4%. Salinity, as temperature, is one of the most important limiting factors of biological distribution in aquatic environment (Langston, 1990). The pH-values were ranged between a minimum value of 6.8 and a maximum value of 8.1, the pH-values tended to increase at some locations. The main factors controlling the distribution

of pH in the marine environment are; dissolved oxygen, water temperature, sewage discharge, decomposition of organic matter, photosynthetic activity of aquatic plants, respiration of aquatic organisms, as well as some physicochemical processes, such as precipitation and oxidation reduction processes taking place in the environment so, it is not surprising to find obvious seasonal, monthly and diurnal variations. Hydrogen ion concentration plays an important role in many life processes. Living organisms are very dependent on/and sensitive to pH value (Masoud et al., 2003). In the present study, most of seawater was well oxygenated at all sites, Dissolved Oxygen is an important and useful parameter for identification of different water masses. It has been used as basic water criteria in assessing the degree of pollution in any aquatic environment (Masoud et al., 2003).

Heavy metals contamination of water bodies is one of the major quality issues in fast growing cities because maintenance of water quality and sanitation infrastructure do not increase along with population and urbanization growth especially in developing countries (Akoto et al., 2008; Ahmad et al., 2010). Marine sediments act as metal integrators and concentrators (García et al., 2004). The site of sediment collection significantly influenced the concentration of heavy elements *vis*: lead, cadmium, zinc, copper, manganese and iron. The Ledo site recorded greatest concentration of lead, zinc, copper and iron, while the Rasbayad as control site recorded the lowest concentration of the previous mentioned heavy metals. Moreover, the concentrations of metals such as cadmium and manganese were found greater in Main port site when compared with its contractions in the other sites. Generally the result obtained from the analysis of heavy metals in the Tobruk bay basin clearly showed that Mn, Zn and Fe are the most abundant elements, and indicated the present and an impact of neighboring industries on concentrations of minerals. Atypical or similar finding of the abundance of the above element was reported by Mora et al. (2013). Regarding to copper element, the levels can be considered as low compared to the rest of the elements in our current study. Previous studies reported up to 23 µg/g of Cu in the surface sediments of Baja California, Mexico with an increasing trend compared to regions, they are considered contamination-free (Villaescusa et al., 2000). The high copper concentrations have been related to wastewater discharges and hydrocarbons (González et al., 2006). Especially since the Tobruk bay basin dock receives many ships loaded with petroleum oils, in addition to the sewage that the city suffers from as a result of human activities (Fitori et al., 2021). Regarding to cadmium, it recorded an increase in the three study sites (Main port, Ledo and Andolus). Cadmium is considered as one of the most toxic elements to the environment. Recent studies indicated that cadmium poses a serious ecological threat and contributes greatly to the toxicity response rates, as it is even more toxic than arsenic and lead (Min et al., 2013). The concentrations of Pb in the

sediments were exceeded the permissible limits (FEPA, 1991).

Concentration of heavy elements in *Mytilus galloprovincialis* collected from four studied sites was investigated. The concentrations of heavy elements such as lead, zinc and iron in *Mytilus galloprovincialis* tissues were significantly affected by site of collection. While the concentrations of cadmium, copper and manganese were not affected by site of collection. The main port site recorded significantly greater concentration of lead and zinc in tissues of *Mytilus galloprovincialis* than that of the other sites. While the Rasbayad (control site) recorded the least concentrations of lead and zinc. The Andolus site was showed had significantly highest concentration of iron, while Rasbayad site significantly recorded the least concentration of iron in tissues of *Mytilus galloprovincialis*. Bivalves are some of the most suitable bio-indicators for bio-monitoring studies and appropriate for transplantation along coastlines due to their wide geographical distribution, sedentary filter-feeding organisms and ability to accumulate high levels of contaminants including metals in proportion to ambient concentrations in seawater (Goldberg 1975; Andral et al., 2004). The levels of Zn, Pb, Cd, Zn, Cu, Fe and Mn are similar or higher than their concentrations found in *Mugilcephalus* fishes collected from main port of Tobruk (Fitori et al., 2020) and samples of fishes collected from Khomes (Metwally and Fouad 2008). The results of the present study are in broad agreement with that the results obtained as part of the MYTIOR project in 2009. Heavy metals and organic compounds pollution assessed on mussels grown in 16 different stations along the coast of Libya. These stations were located miles away industrial/urban marine resources but in the open sea provision original results related to background contamination instead from its association with a particular coastal source of pollutants (Francois et al., 2014). Consequences indicated lead (Pb, 0.44 - 0.71 mg/kg dry weight) in the same range with present findings of bivalvia tissues (Main port site) and greater than that collected from Rasbayad as control region. On the other hands, Copper (Cu, 3.56-4.21 mg/kg dry weight) of mussels (Francois et al., 2014) is in slightly similar to the present finding of bivalvia collected from Main port and Ledo sites and greater that of Rasbayad site.

Generally, from the previous results of heavy metals concentrations in the sediments and bivalvia tissues, the control site (Rasbayad) recorded the least concentrations of the assessed metals, that mean is less polluted and contaminated in comparing with the other sites. Because, the Rasbayad located outside the Tobruk bay basin, while the other three sites located inside and around the bay basin, where drainages of rain and sewage opened on it, and receiving all merchant ships for longshore and loading. In addition, these sites are rest and recreation areas and entertainment for the majority of people.

5 Conclusion

Through the results we obtained in this study, we found that the levels of contamination with heavy metals in sediments and mussels (*Mytilus galloprovincialis*) were higher in all study sites except for Rasbayad and reflected environmental pressure on Tobruk coast. Reduction of human activities should be recommended regarding to decrease the levels of heavy metals pollutants in the region.

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Conflict of interest: The authors declare that there are no conflicts of interest.

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