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Temporal and Spatial Effects on Some Physiological Parameters of the Bivalve *Lithophaga lithophaga* (Linnaeus, 1758) from Coastal Regions of Alexandria, Egypt

Eman Hashem Radwan[1*], Sherifa Shaker Hamed[2,3], Gaber Ahmed Saad[3,4]

[1]Department of Zoology, Faculty of Science, Damnhour University, El Behara, Egypt
[2]Department of Zoology, College of Science, King Saud University, Saudi Arabia, KSA
[3]Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt
[4]Department of Biology, College of Medicine, Dammam University, Saudi Arabia, KSA
Email: [*]Dr_eman_hashem@yahoo.com

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Abstract

The date clam, *Lithophaga lithophaga* is a popular edible bivalve. It was collected from three different locations (Abo-Quir, Miami and El-Mex) along the coast of Alexandria-Egypt to determine the effect of two heavy metals as copper (Cu) and cadmium (Cd). The three organs were selected: gill, mantle and digestive gland. The enzymatic change is correlated with the concentration of metals. The present results suggest that Miami water is less polluted with Cu and Cd as compared to the two other locations. The physicochemical parameters are not significant among the three locations. Water of Abu-Quir and El-Max may be hazardous to bivalves. The order of levels of Cu and Cd was: gill > digestive gland > mantle. The present study illustrated that, in Abu Quir, there was a high positive correlation between Cd and Succinate dehydrogenase (SDH) as $r = 0.969$ whereas Cu and SDH as $r = -0.91$.

Keywords

***Lithophaga lithophaga*, Heavy Metals, Cu, Enzyme Activities**

1. Introduction

Pollution of the aquatic environment by heavy metals has received considerable attention in recent years. Ele-

[*]Corresponding author.

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vate concentrations of metals in aquatic ecosystems can be caused by the release of sewage and industrial effluents, non-point source runoff from agricultural and urban areas, and atmospheric fallout. When heavy metals enter the aquatic ecosystems, their mechanisms can cause stress effects due to their ability to accumulate [1]. [2] reported that cadmium sources in the anthropogenic sites came from ship traffic and tourist boats. The major environmental problem in the coastal area is directly related to the impact of domestic effluents [3]. The origin of trace elements in the sampled water of the Egyptian Mediterranean is mainly the waste water discharge [4]. Element concentrations in mollusks at the same location differ between different individuals [5] and different animals in the same community at the same trophic level could accumulate pollutants differently [6]. [7] said that the excess metal accumulation had the potential to exert toxic effects including cellular toxicity in bivalves.

The aim of the present study relates the concentration of heavy metal pollution in the coastal water on the physiology of a bivalve (*Lithophaga lithophaga*). Three localities from the Mediterranean Sea, Alexandria and Egypt were chosen for this study (Abo Quir, Miami and El-Mex). Specimens were collected seasonally throughout the year 2011(January 2011-December 2011). The present study aims also to: assess the level of copper and cadmium in seawater collected from three different locations; assess the level of these metals in different organs (gills, mantle and digestive gland); and investigate the impact of these metals on the enzymatic status of these organs.

2. Materials and Methods

2.1. Study Area

Localized pollution problems are mainly due to increasing coastal development activities including large urbanization and industrial development. The three locations were chosen: Abo Quir, Miami and El Mex. Water and mollusk samples were collected from these stations seasonally during the year, 2011, no.250/location/season.

2.2. Sampling

2.2.1. Water Samples

Coastal water samples were collected from the three selected locations on the coast of the Alexandria at 3 - 5 m depth for the determination of copper, and cadmium. Seawater samples were filtered through 0.45 µm millipore filters to remove any debris particles then stored at −20˚C until analysis. All concentrations are reported as µg/L for seawater. All the precautions recommended by [8] to minimize risks of sample contamination were followed during collection and treatment of samples.

2.2.2. Mollusk Samples

The Bivalve *Lithophaga lithophaga* (20 - 25 gm, 9 - 10 cm shell length, antero-posterior axis), were collected in sterile plastic bag (no.250/location/season, replicant, 5 times) and were cleaned from attached organisms and then rinsed with seawater from their sampling locations and transported to the laboratory within 4 - 6 hrs.

2.3. Analytical Methods

2.3.1. Heavy Metals in Seawater

The concentration of Cu, and Cd were determined in the collected seawater samples using Graphite Furnace Atomic Absorption Spectroscopy (Perkin-Elmer model 2380) under the recommended conditions and the detection limits in the manual for each metal [9].

2.3.2. Physicochemical Analysis of Seawater

Surface water samples were collected five times; bi-week from three sites representing the coastal area in front of Alexandria city starting from Abo-Quir, at the east, Miami, and El-Mex at the west. At each location water samples were collected using polyethylene bottles (2-liters capacity). The polyethylene bottles were previously cleaned with detergent rinsed several times with distilled water, soaked in 1 N HCl for several days and finally rinsed with re-distilled water. At each site a 150 mL dissolved oxygen bottle was firstly filled and immediately fixed, using manganoussulphate and alkaline potassium iodide solution [10]. Some parameters were totally or partially measured in the field *i.e.* as soon as the sample was collected. These steps of the methods would be explained by the term "*in situ*" in the text. Temperature measurements: *In situ* at each station, air and water temperatures were measured at the time of water sampling using an ordinary thermometer. Salinity (S‰): Salinity was determined by measuring the electrical conductivity using an inductive Salinometer (Beckman; model RS. 10). Hydrogen-ion concentration [pH]: The pH-value of water sample was measured in the laboratory immediately after collection using Bench type (JENWAY, 3410 Electrochemistry Analyzer pH-meter). Dissolved oxygen (DO): It was determined by a modified Winkler's method [10].

2.4. Analysis of Physiological Parameters

1) Superoxide dismutase (SOD): Antioxidative enzyme activities determination: Tissue cell suspension was separated on an ice-cold surface. Tissue cell suspension homogenates were prepared. Superoxide dismutase is measured based on [11].

2) Glutathione-S-transferase (GST): Activities of the following enzyme in the extracts were determined spectrophotometricaly using a Spectra MAX 340 microplate reader. GST activity was determined using a modified method of [12].

3) GPX activity: (nmol/min/mg protein) was measured using an adaptation of the method of [13].

4) Succinic dehydrogenase (SDH) is measured by [14].

2.5. Heavy Metals in Tissue

The preparation of samples to determine concentration of heavy metals was carried out animals were separated from the shells; weighed and digested using conc. HNO_3 in Taflon digestion vessels. Wet digested samples were diluted with deionized distilled water and analyzed by Ion-selective electrod AVL. The obtained data were expressed as µg/g wet weight [6]. The analytical method was checked by (5 replicate) measurements for the studied metals in a sample of marine.

2.6. Statistical Analysis

Statistical analysis was performed using two-way ANOVA using SPSS computer program (version 14.0) to check for significant difference between metal concentrations in different localities.

3. Results and Discussion

The results of the present study showed that there was incraese in Cu in seawater in Abo Quir as 2.45 > El Mex as 1.56 > Miami as 1.4 whereas for Cd, the highest results was represented also in Abo Quir as 1.82 > El Mex as 1.0 > Miami as 0.4, (**Table 1**).

In the current study, the physicochemical parameters for temperature, the highest mean reading was, in Miami as 29.7 > El Mex as 28.8 > Abo Quir 28.5. For the salinity, the highest value was reported at Miami as 38.8 comparing to El Mex as 38.2 > Abo Quir as 38.7, (**Table 2**). pH recorded the highest value in both El Max and Miami as 8 > Abo Quir as 7.44. The dissolved oxygen was recording the highest mean level in Miami as 5.74 followed by both El Mex and Abo Quir as 5.1, (**Table 2**).

For physicochemical parameters of seawater the highest mean of salinity was reported as Abo Quir > El Mex > Miami, whereas, for the mean of temperature, DO, pH, the highest mean level was as Miami > El Mex > Abo Quir (**Table 2**). For Cu in gills, the highest mean reading was found in Miami as 6.0 followed by El Mex as 4.4 then Abo Quir as 3.4 and the Cu in mantle reported the highest mean reading in Miami as 2.6 followed by both Abo Quir and El Mex as 0.8. The mean Cu in the digestive gland, the highest mean reading was reported as 4.3 in Miami followed by Abo Quir as 2.9 then El Mex as 2.5 (**Table 3**). The mean of Cd in gills was reported as 3.4 in Miami > 0.97 in El Mex followed by Abo Quir as 0.8 whereas for Cd in mantle, the highest reading was reported in Miami as 1.7 > El Mex as 1.4 then Abo Quir as 0.5. For Cd in the digestive gland, the highest mean reading was reported in Miami as 2.4 > El Mex as1.9 then > Abo Quir as 1.1 (**Table 3**).

In the current study, the mean level of SDH concentration in gills highly significant increase in El Mex > Miami > Abo Quir whereas, for mean concentration level of GST in gills reported highest level in Abo Quir > El Mex > Miami. The highest level of concentration of GPx in gills reported in Abo Quir > El Mex > Miami whereas, the highest mean concentration level of SOD in gills, was reported as Abo Quir > Miami > El Mex. In the present study, the highest concentration level of SDH in mantle was as followed, El Mex > Miami > Abo Quir, whereas GST concentration level in the mantle reported as El Mex > Abo Quir > Miami, (**Figures 1-3**).

The high mean concentration level of GPx has no significant difference between the three locations, whereas

Table 1. Heavy metal concentration (μg/L) in the three study areas along Alexandria coast.

Heavymetals/locations	Abo Quir	Miami	El Max	Admissible levels of heavy metals
Cu (μg/L)	2.45 ± 0.54	1.40 ± 0.44	1.56 ± 0.26	3.1 (μg/L) USEPA(2005)
Cd (μg/L)	1.82 ± 0.23	0.42 ± 0.28	1.03 ± 0.20	40 (μg/L) USEPA (2005)

Table 2. Physicochemical parameters of seawater collected from the three study areas along Alexandria coast.

Physicochemical parameters/location	Abo Quir	Miami	El Mex
Temperature	28.52 ± 0.49	29.7 ± 1.33	28.76 ± 0.58
Salinity (mg/L)	38.72 ± 0.15	38.16 ± 0.21	38.24 ± 0.15
pH	7.44 ± 0.36	8.14 ± 0.19	8.0 ± 0.06
Dissolvedoxygen (DO) (mg/L)	5.16 ± 0.18	5.74 ± 0.13	5.10 ± 0.14

Table 3. Heavy metal concentration (μg/g) in some organs of *Lithophaga lithophaga* (in wet weight) collected from the three study areas along Alexandria coast.

Heavy metals/location	Abo Quir	Miami	El Mex
Cu			
Gills	3.35 ± 0.18	6.03 ± 0.03	4.36 ± 0.26
Mantle	0.76 ± 0.03	2.62 ± 0.37	0.81 ± 0.07
Digestive glands	2.86 ± 0.05	4.24 ± 0.33	2.54 ± 0.33
Cd			
Gills	0.79 ± 0.15	3.55 ± 0.28	0.97 ± 0.13
Mantle	0.47 ± 0.08	1.65 ± 0.12	1.42 ± 0.22
Digestive glands	1.12 ± 0.23	2.44 ± 0.30	1.88 ± 0.13

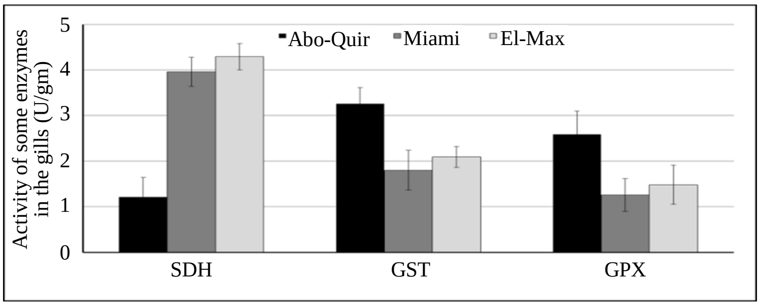

Figure 1. Activity of SDH, GST and GPx in gills of *Lithophaga lithophaga*.

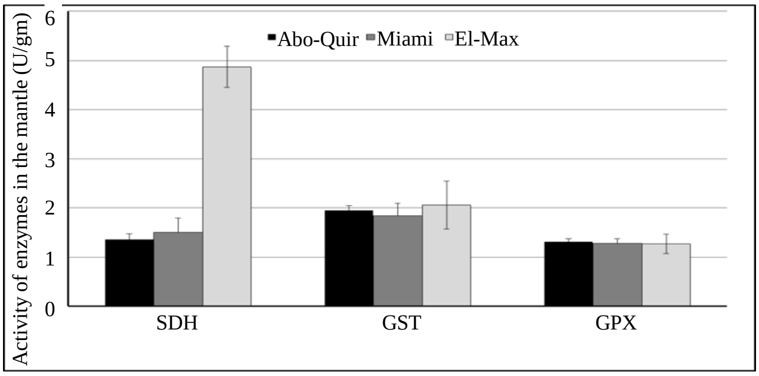

Figure 2. Activity of SDH, GST and GPx in mantle of *Lithophaga lithophaga*.

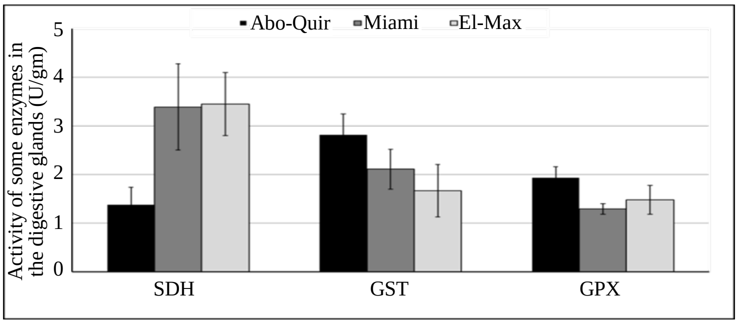

Figure 3. Activity of SDH, GST and GPx in digestive gland of *Lithophaga lithophaga*.

SOD highest mean concentration level in mantle was reported in Miami > El Mex > Abo Quir. The activity of some enzymes in the digestive gland was reported in the present study, the highest mean concentration level of SDH in the digestive glands was as El Mex > Miami > Abo Quir, whereas for GST in digestive gland was reported as Abo Quir > Miami > El Mex. The high concentration level of GPx in the digestive gland was reported as, Abo Quir > El Mex > Miami, whereas, for SOD it was reported as Abo Quir > Miami > El Mex, (**Figures 1-3**).

In the present study, the highest mean concentration level for GST, SOD, GPx in gills was reported in Abo Quir, whereas the highest concentration level of SDH was in El Mex. In the current study, for GST, SOD, GPx in the digestive glands, the highest reading was in Abo Quir whereas for SDH, the highest mean reading was reported in El Mex. For GPx in the mantle, the highest mean concentration level was reported in Abo Quir whereas for GST and SDH, the highest reading was in El Mex but SOD reported the highest concentration level in Miami. **Table 4** illustrated that, in Abo Quir, there is high positive correlation between Cd and SDH as $r = 0.969^{**}$ whereas in Miami, there was high positive correlation between SOD and GST as $r = 0.937^{**}$ but in El Mex, there was high negative correlation between Cu and SDH as $r = -0.91^{**}$ and also high negative correlation between Cd and GPx as $r = -0.92^{**}$.

Temperature, salinity, diet and individual variation are among other factors affecting accumulation of heavy metals [15]. [16] reported that all physiological mechanisms of response to cadmium differed at high and low cadmium levels. It is concluded here that there is no risk to eat date clam collected from Alexandria coast from the chosen locations as the levels of different heavy metal concentrations in tissues are below the safety levels reported by [17]. [6] reported that different animals in the same community at the same trophic levels could accumulate pollutants differently due to differences in habitat physical and chemical properties.

Variations in heavy metals concentration may be caused by factors such as a difference in seawater temperature, and food supply for the population of *Mytilus galloprovincialis* [18]. [16] and [19] agreed on that the temperature of the ambient water influences the metabolic rate of the organisms and hence the uptake and release of contaminants. Temperature may affect the growth, distribution of aquatic organisms. [19] and [20] reported that in temperature climates, oysters start to accumulate glycogen before winter and that, metal accumulation in the oyster soft tissues has little toxic effect on overall metabolism. [21] recorded pH-values of water at Abu-Quir site as (7.57 - 8.26). They added that the decrease in pH-value is coincided with the drop in oxygen content due to the effect of discharge of brackish water whereas, [22] reported that the values of hydrogen-ion concentration pH showed high concentration at the Eastern section of coast Mediterranean sea. The increase and decrease of pH values may be due to the mixed drainage water at the represented sites [23]. [21] reported that pH-value decreases with the increasing distance from the points of discharge. He recorded the pH-value in the El-Mex Bay as 8.06, whereas Abu-Quir Bay recorded pH-value as 7.76. pH concentration may be highly significant factor in determining or limiting the threshold concentration. [22] reported that dissolved oxygen is considered as one of the most important and useful parameters in identification of different water masses and in assessing the degree of pollution. The maximum value of DO can be attributed to lower water temperature as well as strong winds [22]. The relationship between temperature and DO is reversed [23]. Salinity reflects the degree of contamination in aquatic environment [22]. El-Mex Bay area exhibited wide fluctuation in salinity affected by discharge of huge amounts of agricultural, sewage and industrial waste waters [24]. At S‰ was equal 30 ppm, clams may be releasing a protein due to the stress, they under that may cause cloudiness [25].

Table 4. Correlation coefficient of Cu, Cd and enzyme activity of *Lithophaga lithophaga* in the three locations.

Location			Cd	SDH	SOD	GST	GPx
Abu Quir	Cu	r	−0.304	−0.309	−0.237	−0.769	0.79
	Cd	r		0.969*	0.317	−0.153	0.004
	SDH	r			0.504	−0.211	−0.116
	SOD	r				−0.361	−0.288
	GPx	r					−0.712
Miami	Cu	r	0.04	0.09	−0.619	−0.633	0.717
	Cd	r		0.169	0.240	0.112	0.03
	SDH	r			0.416	0.161	0.501
	SOD	r				0.937*	0.579
	GST	r					−0.75
El Mex	Cu	r	0.34	−0.906*	−0.615	−0.695	−0.038
	Cd	r		−0.48	0.321	−0.308	−0.917*
	SDH	r			0.339	0.640	0.46
	SOD	r				0.695	−0.326
	GST	r					0.181

[25] stated that variation in the physiology of marine animal might have affected the intake of metals by the organisms and that the effectiveness of metal intake may differ in relation to ecological needs and metabolism of animals and concentrations of the heavy metals in water and food as well as other factors such as salinity and temperature. [26] and [27] reported that a single biomarker cannot provide the basic answers as to what is the actual state of health in the natural populations and that, environmental stress may cause changes in cellular function that alter the physiology of organ system in organisms, suggesting that pollutants might influence the metabolism of the whole organism. [28] and [29] discovered a relationship between xenobiotic chemicals accumulated in oyster (*Crassostrea virginica*) tissue and various measures of physiological condition and that the possibility of variations of metals concentration due to physiological parameters have been taken into consideration such as stress. In the present study, variations of metal concentrations are to be attributed to variation of metal concentrations in the surrounding water.

In the present study the mean level of copper concentration in seawater of Abo-Quir was reported as, the highest mean level (2.45 µg/L) as comparing to the other locations, this might be due to leaching of copper from ships antifouling paints that could be a possible source for copper in Abo Quir. Abo Quir Harbor is affected by three continental discharges; they are; boughaz El-Maadia opening, the tapia pumping station and the opening of Rashid Nile branch [30]. In the present study Cu level for the three locations is lower than the Critirion Maximum Concentration (CMC) level for copper which is 4.8 µg/L and the Critirion Continous Concentration (CCC) level which is 3.1 µg/L stated by United State Enviromental Protection Agency [31]. [32] reported that the sources for Cd exposure are; air, water, other sources for Cd arise from the recycling and incineration of mincipal solid waste and hazardous wastes and pipe galvanization which are also abundant in the vicinity of polluted sites. [33] detected (40 µg/L) to be the criteria maximum concentration CMC for Cd. Sing of Cd in Abo-Quir in the present study is may be due to the biological activities, the industrial and sewage effluents.

In the present study the mean concentration of Cd is considered less than that reported by [34]. It is concluded that the coastal area in Mediterranean sea of Egypt might be considered relatively unpolluted with heavy metals this conclusion is in agreement with that of [35] who reported that; the coastal area in Mediterranean sea of Egypt might be considered relatively unpolluted with heavy metals. [2] [36] [37] reported that the uncontaminated sites recorded higher values compared to the human impact sites as well as it is suggested that, the deposition of contaminants into a marine area was due to transport of contaminants by coastal currents from upstream coastal industrial activities which is in agreement with the present study. [4] stated that the surface east water current and South west winds blowing on the Mediterranean coast of Egypt contribute mostly in spreading the trace elements to wide areas of the coast. The highest concentration of dissolved Cu at Alexandria coast may be due to the high decomposition rate of organic matter and the release of Cu from decaying organisms by the action of bacteria [25].

[27] found out that: the degree of environmental contamination is only one among several factors that influ-

ence metal concentrations in animals. Bioavailablity or specific sources may be responsible for higher concentrations in apparently less impacted environments. [36] suggested that, the deposition of contaminants into a marine protected area was due to transport of contaminants by coastal currents from upstream coastal industrial activities. [36] noticed that gills were the only organ to exhibit a continued increase in the gills of clam tissue Cu concentration with the corresponding increase of Cu in the seawater. Cu concentrations were significantly high in the gills confirming that it is the main organ for metal bioaccumulation in *Bathymodiolus azoricus* (mussels) is the gills [3]. [37] and [38] reported that, Cu in the gills of *Bathymodiolus azoricus* and Cu in the gills of mussels from reference sites was higher when compared with similar tissues of coastal mussels, *Mytilus edulis* and *Mytilus galloprovincialis* from heavily polluted environments. The uptake of metals may take place at the gills of bivalve and that the gills function as a site of metal uptake [5]. [39] stated that, in Quebec, gills of fresh water bivalve (*Pyganodon grandis*) respond to metals Cu contamination gradient. [40] reported that, only gills of bivalves can be considered as an adequate target tissue for Cu. The present study is in agreement with that conclusion as the gills were the target organ for Cu accumulation. Gills are frequent targets of environmental pollutants because they are the main interface between the organisms and their environment [41].

It had been suggested that the gill in Mussel *Mytilus edulis* appears to be more suitable organ for biomonitoring heavy metals [42] and that bivalves are frequently used in marine eco-toxicology for the purpose of assessing seawater quality because they are very sensitive to pollutants [43]. [37] reported that, the hepatopancreas of bivalve in the uncontaminated conditions demonstrates the highest values of toxic metals as a result of its major role in the uptake of metals from food. [44] reported that, gills are suggested as a possible route for accumulation of Cd as a possible route for Cd excretion. [45] reported Cd was present in the effluent and had accumulated significantly in mussels' gills. Metals could pose a health risk to heavy seafood consumers [46]. The average concentration of heavy metals found in Mussels were 0.64 µg/g for Cd [47]. [45] reported Cd accumulated significantly in mussels' gills. Uptake of metal in bivalves may take place at the gills and their relative importance is a function of the speciation of the metals in the environment [5]. The surveys of contaminants in shellfish conducted by Agency for Toxic Substances and Disease Registry [17] which reported the mean of Cd level for shellfish as 360 µg/g dry weight. [48] reported that there were different factors affecting the accumulation of metals in the bivalve species such as ambient concentrations of metals, growth rate, uptake and excretion rates of metals and biological development of animals. [4] reported that, the average concentrations of trace elements in Egyptian Mediterranean coast are far from the hazardous concentrations.

[44] suggested that, the gill appears to be a more suitable organ for bio monitoring than that the hepatopancreas. [37] [49] and [41] reported that gill represents the quick answer of mussels to water concentrations of metals. Every increase in dissolved Cd concentration in water resulted in a significant increase in its concentration in (Mussels) *Dreissena polymorpha* [31]. Any decrease in salinity makes a proportionately greater increase in the activity of the free cadmium ion [50]. The highest concentration of Cd was found in digestive gland in the clam *Ruditapes philippinarium* for Cd [40]. [38] reported the level of Cd level in the digestive gland of mussels collected from France as 0.05 + 0.01 µg/g wet weight. [51] found that Cd could be trapped in the digestive gland of the bivalves *Crassostrea gigas* (oyster). [42] found that when *Ruditapes philippinarum* (Bivalvia) were exposed to different concentrations of Cd (200 - 600 µg/g); the highest concentrations for Cd were found in the digestive gland. [38] reported that in uncontaminated conditions, the hepatopancreas demonstrates the high toxic metals as a result of its major role in the uptake of metals from food and that the time of exposure is more important than the concentration of toxic metals. [27] reported that variation in the environmental conditions might have affected the intake metals by the organisms. Many metals were found in agricultural products, those present in fertilizers include Cd [5]. Cd accumulation can be considered a typically low concentration in many aquatic fauna relative to environmental levels [52]. This report is in agreement with the present data as levels of Cd in the gill, digestive gland and mantle tissue considered low according to [17].

The digestive gland is the main copper accumulation organ when oysters were exposing to low concentrations (0.05 µM) [52]. [53] reported that the digestive gland appears as the center of accumulation of Cu, as Cu is strongly accumulated in the digestive gland and that digestive gland is the main site of Cu accumulation in mollusk. The present study did not agree with that observation as gills represented the main site of Cu accumulation in *Lithophaga lithophaga*, whereas, [54] reported that the digestive gland of *Mytilus edulis* is the preferential organ for the accumulation of heavy metals. The uptake of metals may take place on the surface of the mantle of bivalve [5]. [33] reported that accumulation of Cu in mussel—soft tissue—from seawater of the Mediterranean sea—industerial and urban areas—showed a range of (3.89 - 16.56 µg/g dry weight). [55] reported that Cu tissue levels increased with exposure time reaching maximum levels after 24 days. [56] reported that metal concentrations recorded in the soft tissues of mussels *Mytilus galloprovincialis* increased without a source of extra metals in water. The average concentrations of Cu in all dissected organs, mantle, gills and digestive gland of *Lithophaga lithophaga*, in the present study, were lower than that stated by [17] 7800 µg/g for soft tissues of shellfish.

Very high Cd concentration may result from food chain bioaccumulation of elevated Cd levels brought into the productive surface water by upwelling into the region [57]. [58] reported Cd in *Mytilus edulis* which had been collected from Barents sea (Russia) are largely within a worldwide reported range (1 - 2 µg/g wet weight in *Mytilus edulis*. They hypothesized that a potential copper deficiency might be related to an increased uptake of Cd due to an insufficient selectivity of the uptake process for the essential element Cu. [59] reported the mean of heavy metals in *Pinctadaradiata* collected from Akkuyu Bay (Icel Turkey—not polluted area) as 0.0058 µg Cd/g wet weight. They concluded that there was no heavy metal pollution in Akkuyu Bay Icel, Turkey. [60] stated that toxicity of Cd decreased with increasing the exposure time. [48] concluded that different factors can affect the accumulation of metals and its relationships in bivalve species such as food supply for the species population, growth rate and uptake and excretion rates of the metals. [33] reported that the effectiveness of metal uptake from food may differ in relation to ecological needs and concentration of the heavy metals in food. The irregular discharge of drainage water leads to variation in heavy metals concentrations [61].

Pollution in marine bivalve mollusks can induce effects on the immune system and thus diseases susceptibility can be enhanced [62]. [63] studied the expression of Glutathione peroxidase (GPx) that is known to be directly involved in oxidative metabolism and reported that, oxygen is at the basis of oxidative metabolism, genes encoding enzymes involved in the cellular regulation of oxidative stress such as antioxidants are consequently expected to be regulated by hypoxia. [64] found out that, aerobic organisms are protected against oxidative stress by antioxidant systems which mobiles enzymes such as the (Cu/Zn—superoxide dismutase) which transfers O_2 to H_2O_2. The prooxidant effects of transition metals such as Cu on marine organisms have been assessed showing that these metals can induce oxyradical production leading to lipid peroxidation [65]. [41] reported that, there were some evidence for oxidative stress was noted in the gills of fresh water bivalve (*Pyganodon grandis*) in Quebec but not in the digestive gland. [63] studied the expression of GPx that is known to be directly involved in oxidative metabolism. [38] emphasized that in the same time frame bio-accumulated toxic metals modified the general metabolism in the hepatopancreas of mussels and suggested the possibility of Cd binding with glutathione.

[64] reported that the activity levels of glutathione-s-transferase, glutathione peroxidase and supperoxide dismutase in the gills and digestive glands of blue mussels (*Mytilus edulis*) collected in North-West coast of France were used as biomarkers. [64] and [25] found out that, the significant decrease in the activity of tissue enzymes, suggesting some kind of functional damage in the cells lead to the leakage of these cellular enzymes into the extracellular fluid. These findings are in full agreement with those of the present study suggesting that there was a strong relationship between inhibition of tissue enzymes activities and tissue damage. Enzymatic defenses involved in protection from oxygen radical damage were determined in gills and mantle in *Bathymodiolus azoricus*. SOD level was higher in gills whereas GPx level was higher in the mantle [65]. Variations in the physiology of animal might have been affected by the intake of metals by the organisms. Metal intake may differ in relation to ecological needs, metabolism of animals. In the present study, the average concentrations of Cu in all dissected organs—mantle, gills and digestive gland—of *Lithophaga lithophaga* were lower than that stated by [17] for soft tissues. The present study reported that in terms of geographical locations the highest values appeared in the reference site is may be due to the discharge of untreated domestic wastes and other coastal and human activities which give rise to high metal concentration.

Cu in the gills of *Bathymodiolus azoricus* from reference sites was higher when compared with similar tissues of coastal mussels, *Mytilus edulis* and *Mytilus galloprovincialis* from heavily polluted environments [37]. It has been reported that in the uncontaminated conditions the hepatopancreas demonstrates the highest values of toxic metals as a result of its major role in the uptake of metals from food [36]. The major environmental problem in the coastal area is directly related to the impact of domestic effluents [3]. The average concentrations of Cu and Cd in all dissected organs—mantle, gills and digestive gland—of *Lithophaga lithophaga* in the present study were lower than that reported by [17] 7800 µg/g for soft tissues of shellfish. The surveys of contaminants in shellfish conducted by Agency for Toxic Substances and Disease Registry [17] which reported the mean of Cd level for shellfish as 360 µg/g dry weight.

In the present study the antioxidant enzymes measured in the gill, mantle and the digestive glands collected from the clams showed fluctuations at the different study areas as shown. Variations in the physiology of animal might have been affected by the intake of metals by the organisms [25]. The present study is in agreement with that of the reference [25], as, metal intake may differ in relation to ecological needs, metabolism of animals. Environmental stress may cause changes in cellular function that alter the physiology in organisms. The present study used a set of biomarkers to assess the state of health of *Lithophaga lithophaga* in the inspected locations. It is strongly recommended that the discharges of waste products of many industrial factories and the wastes of summer season and facilities is to be prevented from pouring directly or indirectly into Alexandria coast before subjected to several treatments and refinement procedures to reduce their harmful effects on the marine biota and the ecosystem as a whole. It is may be suggested disturbances in the functioning of the internal organs as a consequence of protein synthesis.

4. Conclusion

It is concluded from the present study that the toxic effects of heavy metals are the result of interaction between the metal, the physical environment and the biota. It is concluded here that there is no risk to eat clam collected from Alexandria coast from the chosen locations as the levels of different heavy metal concentrations in tissues are below the safety levels reported by [17].

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