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Physiological responses (Hsp70, Mt), Oxidative stress, toxicity impacts, and risk assessment of the biomarker (*Enochrus tenuicosta*) to heavy metals contamination along the Red Sea coasts- Egypt.

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Abstract

This study aimed to investigate the effect of heavy metals accumulation in midgut tissues of *Enochrus tenuicosta* beetles in the Red Sea also in water and sediments of the selected locations. Also to analyze the biochemical response and HSP70 expression. Our results demonstrated that the heavy metals and oxidative stress (MDA) concentrations in the polluted location were higher than the reference one. The response of the antioxidant defense system is significantly higher in the beetles of the reference ones. MT expression and HSP70 were much higher in the polluted beetles than in the reference ones.

Keywords: Toxicity, Heavy metals, Biomarker, Pollution, Antioxidants

Introduction

The Red Sea is a semi-enclosed body of water about 2000 km long, the basin is lengthy from Suez to the Bab El-Mandeb Strait (1). The Red Sea does not have recurrent water flows and only it has torrential downpours and waves, which typically originate from the northwest (2). However, at least three sources of emissions have been present: (i) petroleum emissions from tanker operations, oil fields, and refineries (3).; (ii) eutrophication from waste, sewage, phosphate extraction, and loading operations, and (iii) heavy metal pollution as a result of anthropogenic industrial pollution and the mining of deep hot brine muds in the central Red Sea (4). The most ecologically vulnerable locations are typically along the coast; hence it is crucial to investigate the causes of this deterioration (5).

Environmental studies have concentrated on the deterioration of coastal ecosystems in the Red Sea and Gulf of Suez (6). This semi-enclosed region in the Red Sea is relatively important due to its location that is next to a vital international trade route of the Suez Canal, also the Gulf of Suez is economically valuable for its tourist and fishing resources (7). The Gulf of Suez shoreline has become more industrialized; there are several sources of water pollution, including organic pollutants, sewage, heavy metals, and rubbish (8).

Heavy metals contaminations are currently becoming more common in coastal ecosystems and are thought to be one of the most urgent global challenges (9). Heavy metals are the principal pollutants that have an immediate impact on the marine environment. Although heavy metals like

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Cu, Ni, and Zn are essential for aquatic life, they can be hazardous in certain quantities and endanger the biological variety of the aquatic environment (10). Aquatic wildlife and plants are toxically affected by heavy metals-contaminated sediment and water (11). Increasing heavy metals levels in the aquatic environment have deleterious effects not only on aquatic living species but also on humans because aquatic living organisms are a component of the food chain. Heavy metals exposure in humans happens through the gastrointestinal tract (drinking water and eating food), inhalation, and skin contact (12). Significant levels of heavy metals are added to the sediments in oil-contaminated areas by the spilled oil. The terrigenous materials, which are the cause of metals present in the subsurface sediments in the oil spill-affected areas, are overprinted on the heavy metals from oil spills at these sites (13). Although the presence of particular ratios of trace elements is necessary for marine species to survive, if it surpasses certain limitations, it may be lethal. Due to their significant effect on the ecosystem and human safety, several heavy metals, such as Hg, Zn, Pb, and Cd are public health concerns (14).

Insects are growing commonplace worldwide and have been recognized as possible bioindicators of water quality and ecosystems**.** Due to their accurate sensory system, which enables them to qualify environmental conditions in specific situations and also quantify environmental damages caused to the environment, they refer to changes in the environment because they are very sensitive to pollutants and other types of environmental stress (15,16). To assess the water quality, physico-chemical characteristics, and insects as indicators may be used. Changes in the physical-chemical parameters of water have an impact on the distribution patterns of aquatic insects in the water (12). Additionally, an insect's gut is recognized as a crucial organ in ecotoxicological studies since the epithelium midgut is the first organ that an insect is exposed to at high toxin concentrations (17). Concerning harmful compounds absorbed while feeding, the midgut also provides a physical and chemical barrier.

The largest subgroup of the superfamily Hydrophiloidea, are the water scavenger beetles (Family: Hydrophilidae), this family is well-known for its aquatic members, as well as frequently found in rivers, streams, and seepage habitats (18). An incredibly effective and protective system known as the "antioxidant defensive system" has evolved in living things. To protect the body from the harmful effects of ROS (Reactive Oxygen Species), a variety of antioxidants must be present in copulative amounts. The enzyme system is the primary intracellular endogenous antioxidant defense (19).

Heavy metals would encourage the development of ROS (20). Under normal conditions, the equilibrium between the creation and removal of ROS is maintained due to antioxidant enzyme activities (21). Insects may have evolved special physiological and biochemical detoxification functions to maintain the homeostasis of the internal environment and prevent harm caused by excessive heavy metals (13).

Low molecular weight metallothionein (MT) proteins are involved in metal homeostasis and detoxification, in some contaminated situations, it has been observed that MTs from insects and other invertebrates act as biomarkers, so MT proteins bind metals that interact with the superoxide dismutase (SOD) system, ROS generation under stressful conditions causes the production of MT proteins (22).

Intracellularly localized Hsp70s (Heat shock proteins) are essential parts of the cell's machinery for folding proteins and helping to protect cells from the harmful effects of physiological stresses, members of the Hsp70 family are markedly increased by stress and hazardous substances, especially heavy metals (23).

The current search aims to study the accumulation of heavy metals in aquatic coleopteran

insects from Hurghada and the Gulf of Suez and the ability to use insects (*Enochrus tenuicosta)* as sensitive indicators of environmental contamination by heavy metals in the aquatic environment.

Methodology

The selected study areas

The Red Sea is a semi-enclosed basin that stretches for about 1936 km between the southern end of Bab El-Mandeb Strait (13 N) and the northern end of the Suez Gulf (30 N) (24). The study areas are Hurghada (Latitude: 27° 15' 26.57" N Longitude: 33° 48' 46.48" E) which is considered the less polluted site (site (A) and Gulf of Suez (Latitude: 28° 09' 60.00" N Longitude: 33° 26' 59.99" E) which considered the heavily polluted site (site (B). Fig (1).

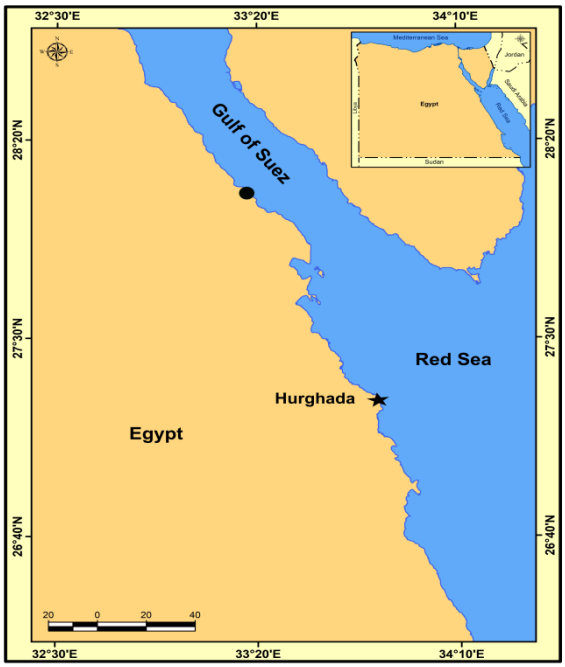


Fig. (1). Map showing the two selected studied locations

Samples collection and identification

The samples (beetles and sediments) were gathered from two places: Hurghada (site A) and the Gulf of Suez (site B). Both locations are spread out along the Egyptian Red Sea coast. The Gulf of Suez region is known for human activities such as industrial, oil pollution, and sewage (25). A Van Veen grab sampler (0.025 m^2) was used to gather sediment samples and beetles at a depth of between 3cm and 5 cm. Samples were kept and moved to a cooler. The cooler's contents were homogenized with a Teflon spoon until no color, or textural distinctions could be seen after enough sediment had been collected from a given station. The coolers were then brought to the lab after being refrigerated to -4 °C. Three copies of each sample were collected from each sampling site. In labeled plastic bags, samples were delivered to the lab and kept there at -20 °C until analysis. Sediment samples were air-dried and then put in an oven and dried at 105 °C. After being homogenized, each sample was sieved through a 0.75 mm plastic sieve and ground into a powder in an agate mortar (26). The identification of the beetles was done in the Entomology department, Faculty of Agriculture, Alexandria University as *Enochrus tenuicosta*, five samples of the aquatic beetle, *E. tenuicosta*, consisting of 20 units, were taken from each of the study locations.

This resulted in a total of 200 identically sized individuals being sampled. Insects were collected in a metal strainer (20 cm in diameter) with an aluminum handle before being placed into glass containers filled with lake water. Insects were moved to the lab and were sorted, cleaned of any debris with running water, and then stored at 20 °C until processing (24).

Analysis of water

To evaluate the chemical composition, water temperature, pH, salinity, and total dissolved solids (TDS), the water at each site was analyzed, water samples were haphazardly taken from each sampling station, 30 cm below the water's surface to minimize floating debris. On-site measurements of the water's pH were made using an Orion Research Model PTI20 digital pH meter, and measurements of dissolved oxygen (DO), water temperature (T), and electric conductivity (EC) were made using a multi-parameter analyzer (model YK-22DO). The turbidity of the water was assessed using a Cyber-bcan WL Turbidimeter TB1000 by Eutech Instruments.

Heavy metals analysis in water samples

The dosage of heavy metals (Pb, Cr, Cu, Cd, Fe, Cr, As, Al, Ni, and Zn) (µg/L) is made by an atomic absorption spectrophotometer (Varian AA 240 Z) equipped with a graphite furnace (GTA 120). Iron and Zinc are analyzed by flame atomic absorption spectrophotometer (Varian AA 240 FS). The pH, total dissolved salts, air temperature, water temperature, salinity, DO, TDS, alkalinity, and nutrient salts such as phosphate, ammonia, nitrate, and nitrite (PO4-P, NH4-N, NO2-N) were measured by standard methods for the analysis were described by the American Public Health Association (USEPA, 2015).

Determination of heavy metals in the midgut tissues of insects

According to Venter et al. 2017, the levels of the most prevalent metals, including Pb, Cu, Cr, Cd, Cr, Fe, As, Ni, Al, and Zn, were measured in homogenate tissues of the midgut (27).

Biomarker of oxidative stress

To assess the functioning of biochemical elements, such as enzymes involved in metabolism and antioxidants, in the midgut tissues of the insect under study. The midgut of the investigated insect was dissected, and the tissues were then washed three times with sterilized saline solution before being blotted with filter paper. The tissues were then weighed and thoroughly homogenized for 2 minutes in 400 L of potassium phosphate buffer (pH 7.0, 50 mM) at a ratio of 1:5. The obtained supernatants from the centrifugation (15,000 g: 4 °C, 60 min) of the mixtures were then used for the biochemical analyses.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured as previously published by Huang et al. (2006), and Bradford's technique was used to calculate the protein content (28). Using the kits purchased from Cayman Chemical Company (Michigan, USA), the amount of malondialdehyde (MDA), the activity of glutathione S-transferase (GST), and the catalase activity (CAT) were assessed. Additionally, reduced glutathione (GSH) was assessed using the methodology described in Beutler et al. 1963(29). The method was implemented using a spectrophotometer to measure the yellow chemical produced by the reduction of 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) with GSH at 412 nm. According to Misra and Fridovich (1972) (30), SOD activity was assessed. The method is based on monitoring the rate of adrenaline autoxidation. The supernatant, sodium carbonate buffer (200 mM; pH 10.0), EDTA (10 mM), and freshly produced epinephrine (15 mM) were all included in the reaction mixture, and the absorbance of the mixture was measured at 480 nm. SOD activity is displayed in the chart as mU/mg protein. The amount of enzyme that blocked 50% of the control reaction of

adrenaline autooxidation per minute was used to determine the activity unit. Nitric oxide (NO) was measured using a nitric oxide assay kit (ab65328, Abcam Co., Berlin, Germany) following the manufacturer's instructions to determine the index of inflammation in the midgut homogenates.

Evaluation of Hsp70 gene & MT expression using Real-time PCR

Expression of the Hsp70 gene and Mt were assessed as follows; three midgut tissues of the studied insect were randomly selected for total RNA isolation utilizing TRIzol reagent (Thermo Fisher Scientific, USA) following the manufacturer's instructions. The integrity and purity of the isolated RNA were evaluated using agarose gel electrophoresis and a spectrophotometer at 260/280 nm, respectively. After removing any genomic DNA contamination from total RNA (1.0 µg per sample) using DNase I (Fermentas, USA), The reverse transcription reaction of the total RNA was performed in a final volume of 20 µl using a high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific, USA). The thermal cycler was set up following the manufacturer's guidelines as follows: 10 min at 25 ◦C, 120 min at 37 ◦C, followed by incubation for 5 min at 85 ◦C. Following this, the cDNA was maintained at 80 ◦C. The quantities of RNA in the samples were determined by using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) set to 260 nm. Following this, the cDNA was maintained at 80 ◦C for further use in Real-time polymerase chain reactions (RT-PCR). RT-qPCR was used to assess the expression of the Hsp70 gene using cDNA in the presence of a forward primer.

5′-ATGGC(GAT)AA(GA)GC(AC)GC(AC)GT(GAC)GG -3′ and a reverse primer 5′-TTAGTCGACCTCTTCGATAGTTGG -3′, while the 18S rRNA gene was used as a control gene and amplified using a forward primer

5′- ATGCAAACAGAGTCCCGACCAGA -3′ and a reverse primer

5′ GCGCAGAACCTACCATCGACAG -3′ (31). In a 25 µL mixture containing 1 µL of cDNA, 12.5 µL of SYBR Green, 2.5 µL of each primer, and 9 µL of H2O, qRT-PCR reactions were performed using the Qiagen Rotor-Gene SYBR Green PCR Kit. An initial step of 95°C for 5 minutes was followed by 40 cycles of 95°C for 15 seconds and 60°C for 10 seconds in the qRT-PCR program. The assays were performed using Rotor-Gene Q-Pure Detection version 2.1.0 in the Rotor-Gene Q. (Qiagen, USA). The comparative $2^{-\Delta\Delta}$CT method was used to quantify the transcript level of Hsp70 mRNA (32).

Statistical analysis

The differences between the parameters in the two sites were statistically tested for significance using the student's t-test, One Way ANOVA, and data were tested at $p \leq 0.05$.

Results

Water physical and chemical characteristics:

The outcomes obtained showed the physical and chemical properties of the water gathered from both the reference location and the polluted site. The polluted site's pH values were essentially neutral, albeit slightly more alkaline. Additionally, data from chemical analysis of water samples taken from the contaminated location revealed a substantial rise in the concentrations of nutritional salts (ammonia and nitrite) in comparison to those from the reference site. As indicated in Table 1, water samples taken from a contaminated location showed significantly (p0.05) higher concentrations of TDS and PO_4, while the DO and alkalinity were marginally higher at the reference site.

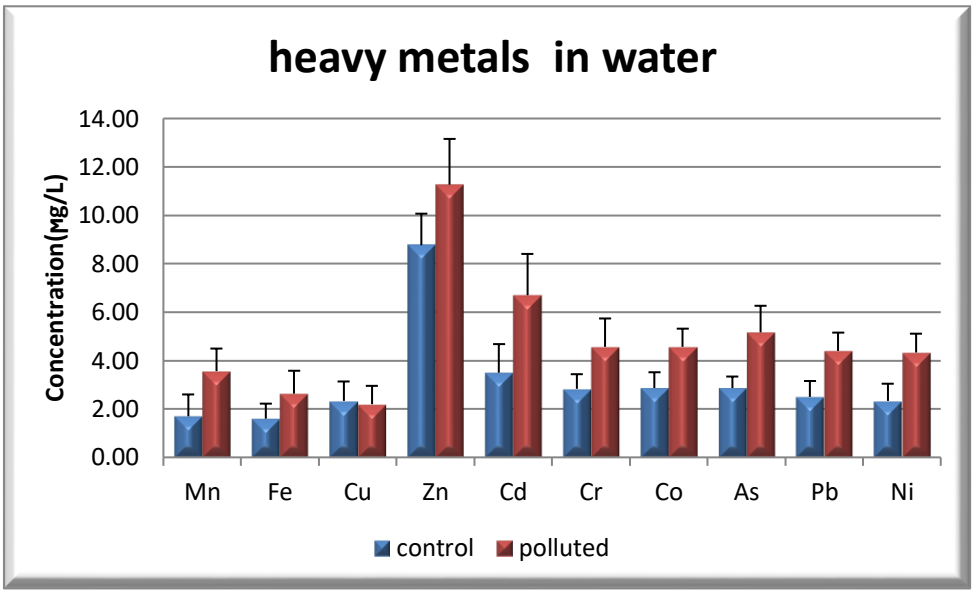
Table (1): The mean concentration of water physicochemical parameters in the two locations

The concentration of water icochemical parameters	**Site A(n=3)**	**site B (n = 3)**	**T-test**	**P**
Air temperature	15.0 ± 1.8	13.4 ± 2.0	0.604	0.578
Water temperature	11.2 ± 1.8	9.7 ± 1.3	0.691	0.527
DO (mg/L)	1.9 ± 0.3	2.9 ± 0.9	1.015	0.367
BOD (mg/L)	2.0 ± 0.3	2.3 ± 0.6	0.507	0.639
Alkalinity	126.7 ± 7.3	175.0 ± 23.6	1.955	0.122
Total hardness	95.0 ± 8.7	198.3 ± 21.3	4.498*	0.011*
pH	7.0 ± 0.2	7.4 ± 0.3	0.921	0.409
NH_4 (mg/L)	0 ± 0	0.1 ± 0.0	1.765	0.152
NO_2 (mg/L)	0 ± 0	0.11 ± 0	2.219	0.091
Transparency	11.0 ± 3.2	2.3 ± 1.5	2.457	0.070
Turbidity	15.7 ± 7.4	86.7 ± 22.0	3.051*	0.038*
Electrical conductivity (µS/cm)	0.3 ± 0.0	0.2 ± 0.1	0.331	0.757
S‰	0.4 ± 0.2	0.2 ± 0.0	0.821	0.496
PO_4	0.0 ± 0.0	0.1 ± 0.0	2.977*	0.041*
Total dissolved solids (TDS)	106.6 ± 6.3	192.3 ± 11.6	6.476*	0.003*

Data was expressed by using mean ± SE. t: Student t-test. p: p-value for comparing between the two studied groups. *: Statistically significant at $p \leq 0.05$.

Concentration and assessment of heavy metals in water:

The coordinates of the selected water samples and the concentrations of HMs (µg/L) were presented in Figure (2 a & b). It was clear that the mean concentration of (Pb, Cd, Co, Zn, Cr, Ni, Mn, As, and Fe) in the polluted site was significantly higher than those of the reference site, except the mean concentration of Cu in the polluted site was lower than those of the reference site.



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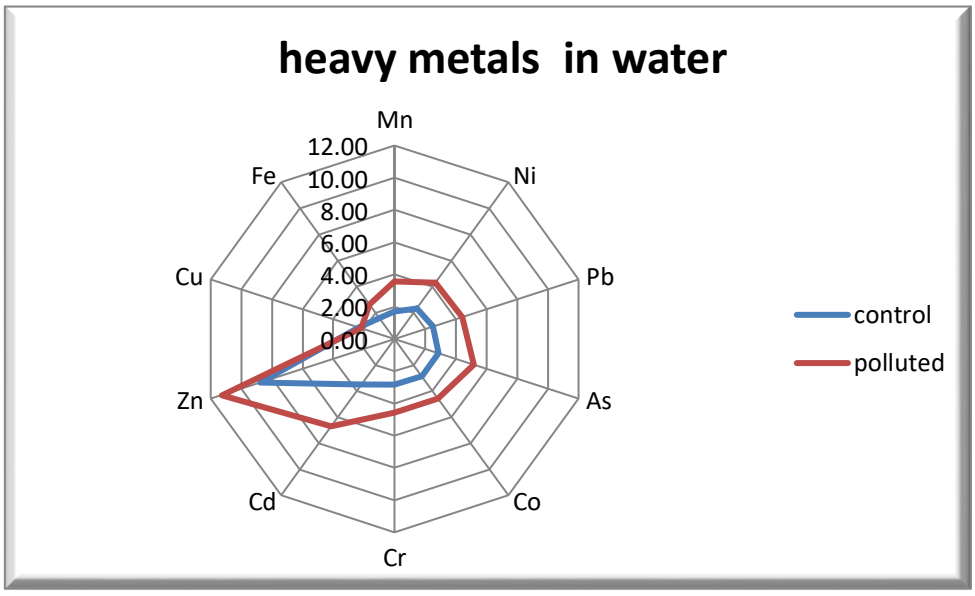

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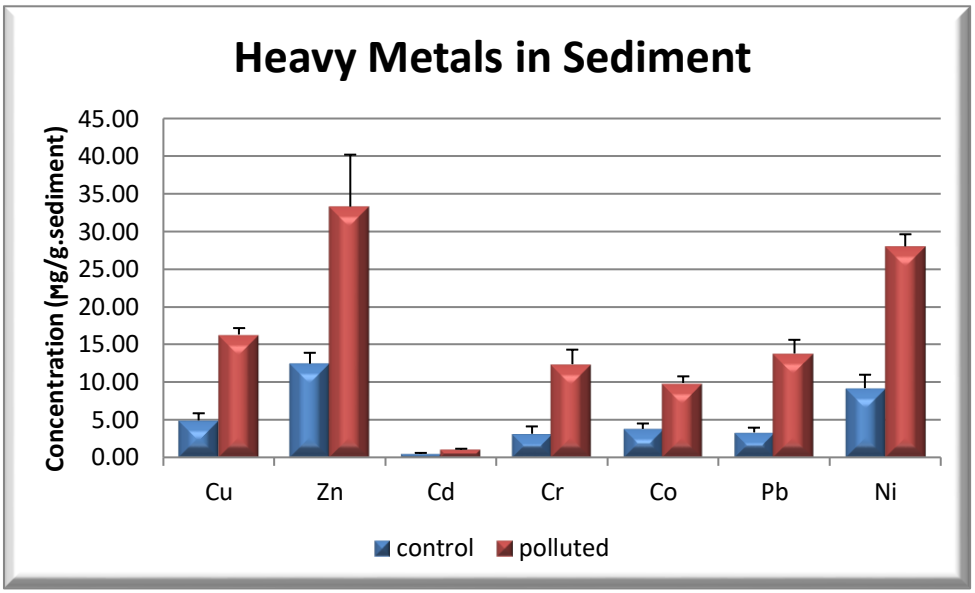
Fig. (2 a & b): the mean concentration of heavy metals (µg/L) in water samples

Concentration and assessment of heavy metals in sediments:

The coordinates of the selected coastal sediments and the concentrations of HMs (m/g sediment) were presented in Figure (3 a & b& c&d). It was clear that the mean concentration of (Pb, Cd, Co, Cu, Zn, Cr, Ni, Mn, As, and Fe) in the polluted site was significantly higher than that of the reference site.



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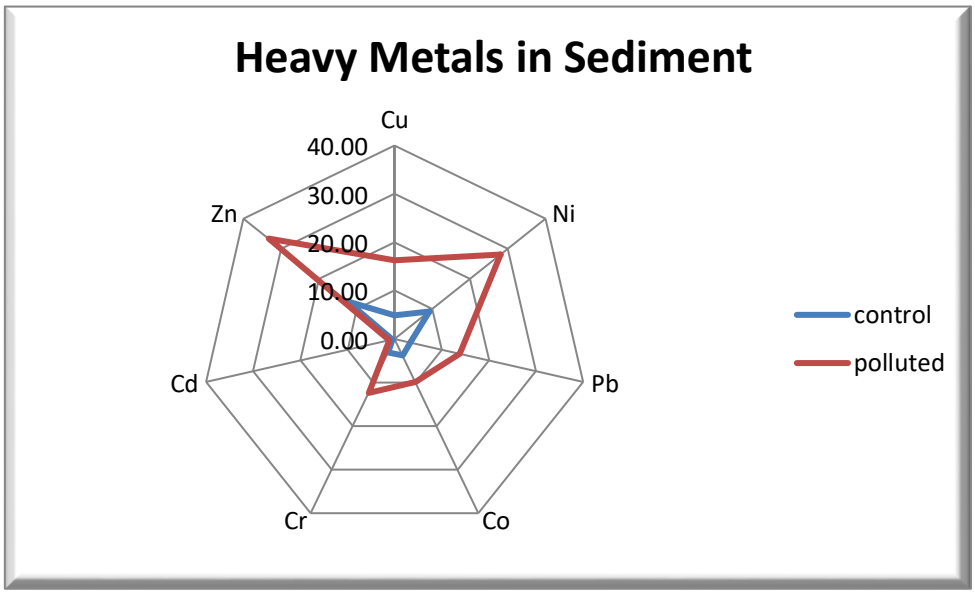

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Fig. (3 a & b): the mean concentration of heavy metals (Pb, Cd, Co, Cu, Zn, Cr, Ni) (µg/g. sediment) in sediments

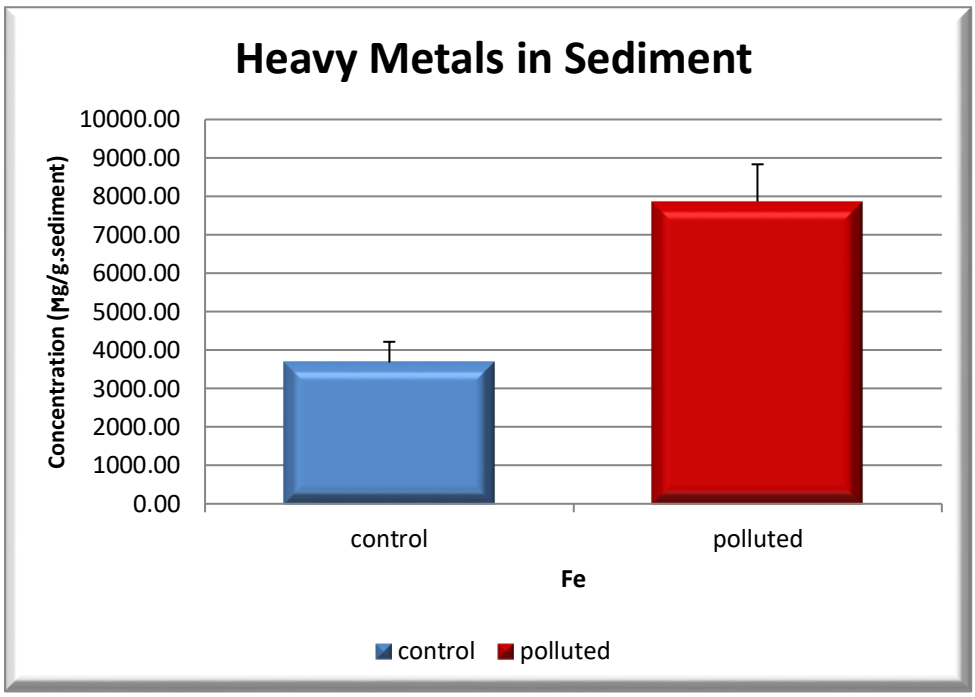

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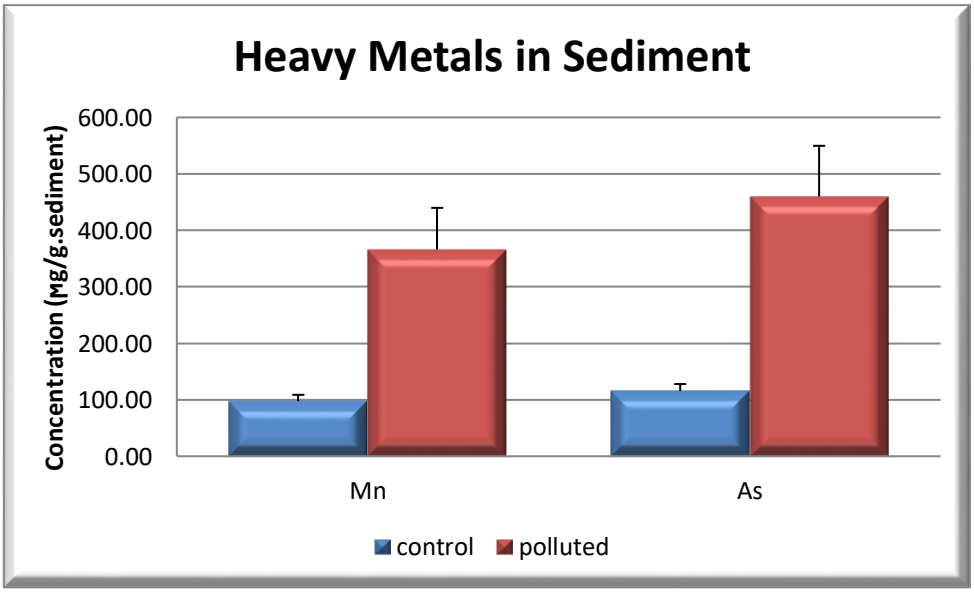
Fig. (3 c): the mean concentration of heavy metal (Fe) (µg/g. sediment) in sediments

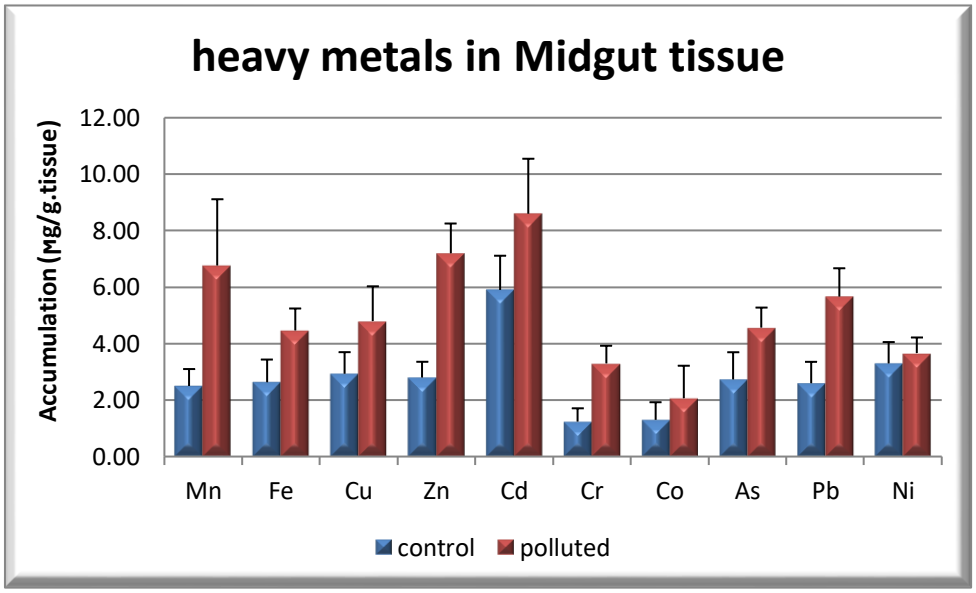


d

Fig. (3 d): the mean concentration of heavy metals (Mn, As) (µg/g. sediment) in sediments

Concentration and assessment of heavy metals in the midgut tissues of E. *tenuicosta*:

The coordinates of the midgut tissues of *E. tenuicosta* and the concentrations of HMs (µg/g.tissue) were presented in Figure (4 a & b). It was clear that the mean concentration of (Pb, Cd, Co, Cu, Zn, Cr, Ni, Mn, As, and Fe) in the polluted site was significantly higher than that of the reference site.



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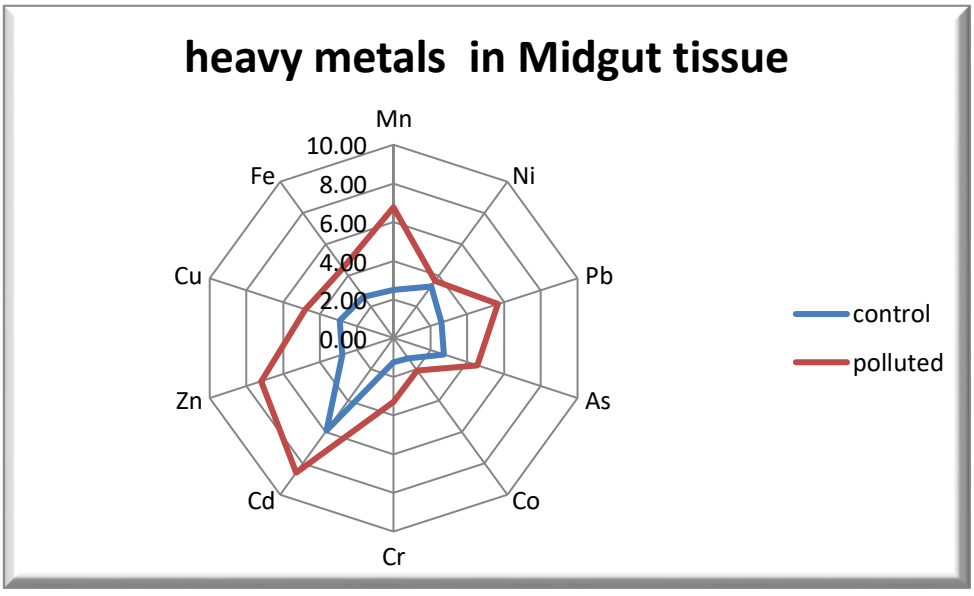


b

Fig. (4 a & b): the mean concentration of heavy metals (µg/g. tissue) in midgut tissues of *E. tenuicosta*

Biochemical measurements:

Figure (5a, b) revealed the concentrations of (ALT and AST) which show a significant increase in their concentrations, while (T. protein) revealed a decrease in the selected insects of the polluted site than those of the reference site. SOD, CAT, and GST concentrations were shown in Figure (6 a,b) which reveals a significant variation in the value. Figure (7a, b) reveals the mean concentration of (MDA, GSH, and NO) in the two locations. MDA and NO were significantly higher in the midgut tissues of the polluted site compared with the reference site. Our results also demonstrated that Mt expression was higher in tissues of the polluted samples. It is also evident from the data in (Fig 8) that the gene expression of Hsp70 was significantly upregulated ($P \leq 0.05$) about threefold in the midgut tissues of the insect from the polluted site compared to the reference site. These findings imply that exposure to heavy metals accumulation incited oxidative stress in the midgut tissues, which further resulted in the upregulation of Hsp70 to protect cells.

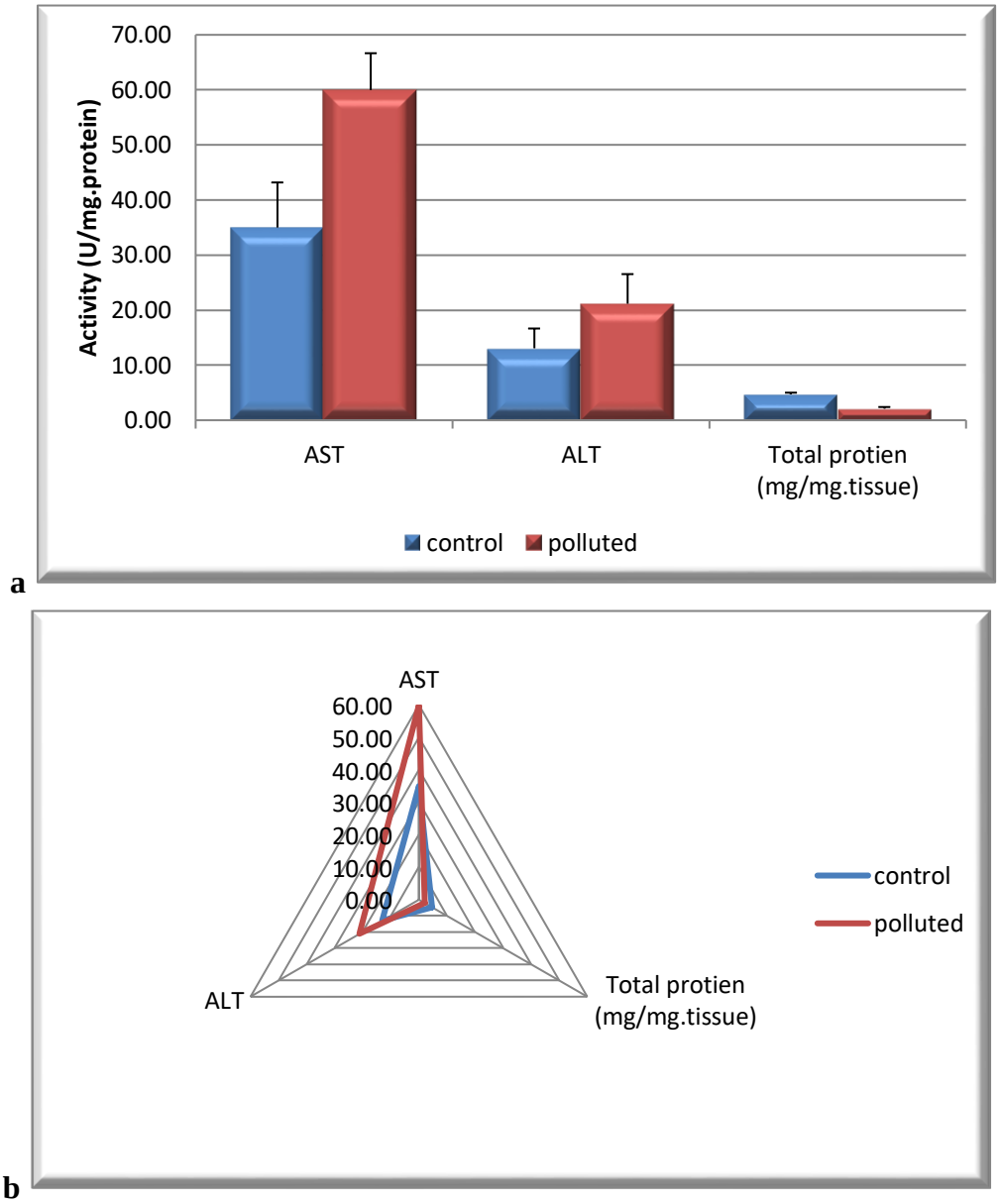
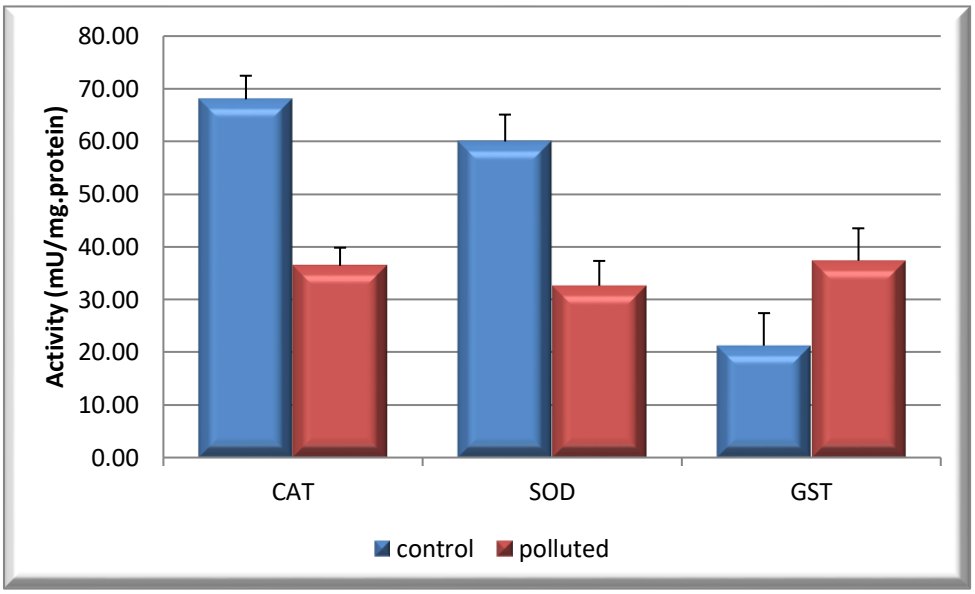


Fig 5 (a&b): the mean concentration of ALT, AST, and T. protein in midgut tissues of *E. tenuicosta*



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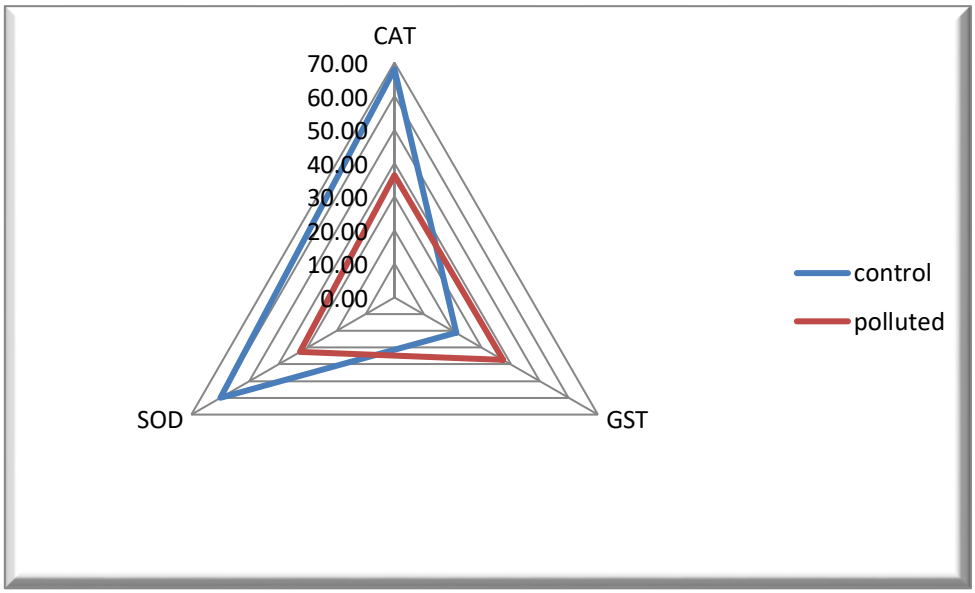

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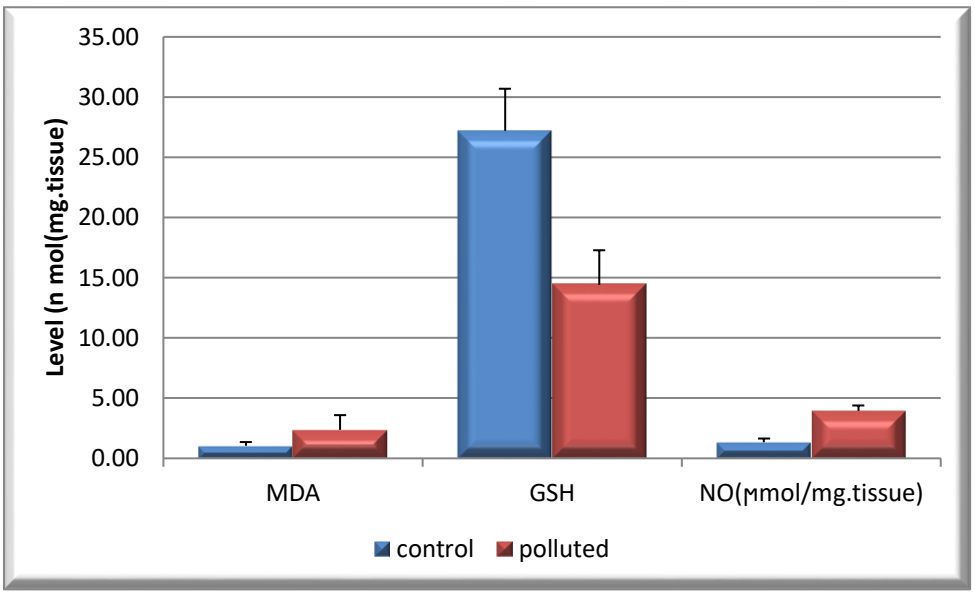
Fig 6 (a & b): the mean concentration of SOD, CAT and GST in midgut tissues of *E. tenuicosta*



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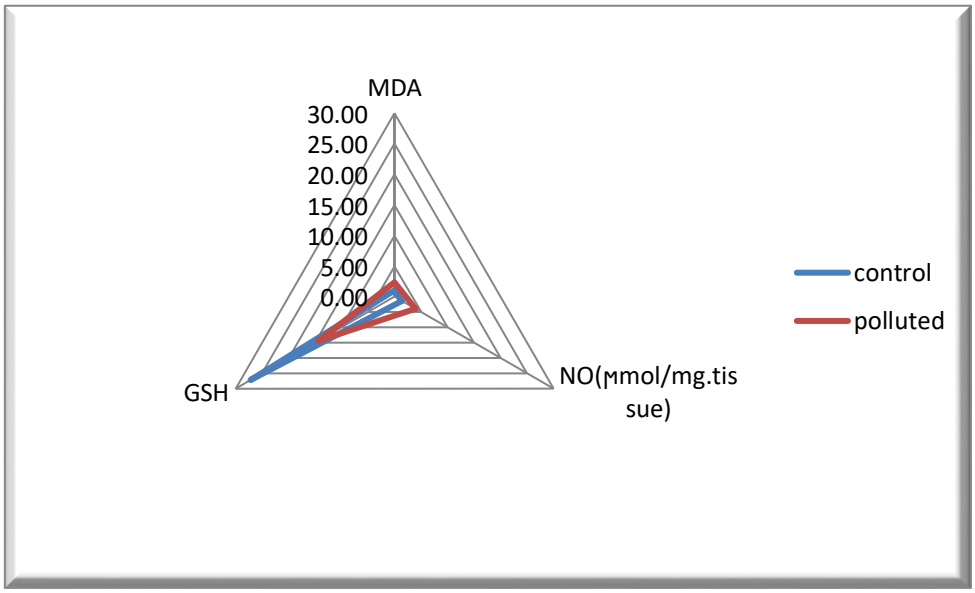

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Fig 7 (a & b): the mean concentration of MDA, GSH, and NO in midgut tissues of *E. tenuicosta*

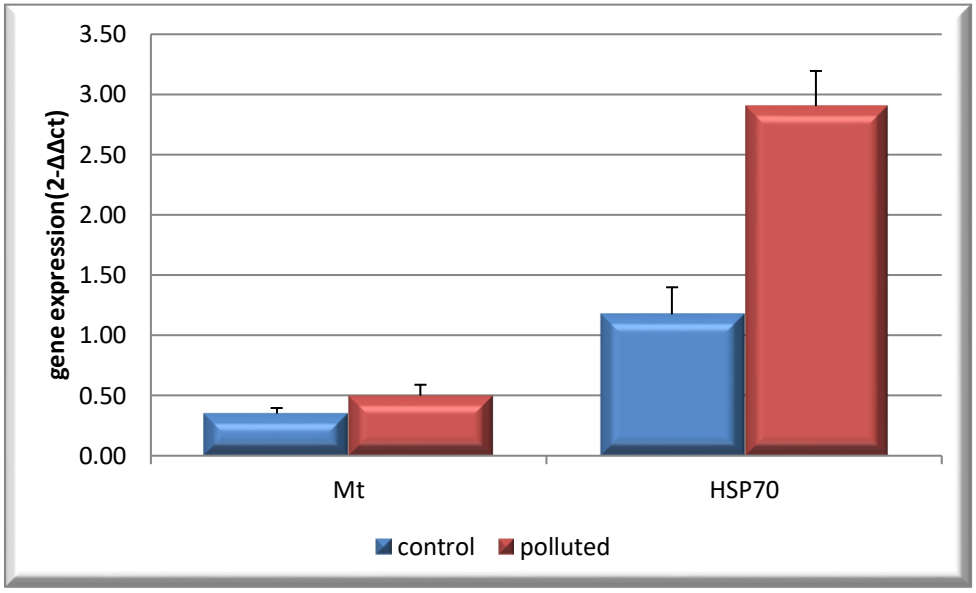

Fig 8: showing the expression of MT & HSP70 in midgut tissues of *E. tenuicosta.*

Discussion

This study investigated the biochemical responses associated with heavy metals in the Gulf of Suez ecosystems on the Red Sea coast, of Egypt. Specifically, we assessed the biochemical response to heavy metals in *E. tenuicosta* midgut tissues, the physico-chemical properties of water samples, and heavy metal accumulation in sediments. Due to its high salt content, the Gulf of Suez location had the highest salinity value, which had a substantial impact on the biodegradation of hydrocarbons (33). Environmental studies show that the high concentrations of heavy metals in the sediments of the Gulf of Suez area present a contamination risk. Notably, the levels of Cr, Hg, and Pb were higher than those allowed by the WHO. There is a substantial chance that these heavy metals' high concentrations will enter the human food chain and have cytotoxic, mutagenic, and cancerous impacts on people's health (34). The varied pollution sources in this area may be the cause of the relatively higher heavy metal values in sediments from the Gulf of Suez location than in sediments from Hurghada sites.

The current study's findings on the physico-chemical characteristics of water samples indicated that the water from the two places was on the alkaline site. Due to oil spills, increasing maritime activity, the volume of sewage released into hot spot locations, and the presence of acidic chemicals at the polluted site, the pH of the area may have decreased (7).

The available data demonstrated that the values of the eutrophication parameters varied in more notable ways. The presence of NO2 and NH4 in the water in the current study can be attributed to the fact that nitrite content in the Gulf of Suez location increased due to a shift in the load of wastes from household and industrial activities discharged into the water (35). In agreement with Saad et al. (2017) research that greater reactive phosphate concentrations are mostly caused by the action of drainage water that has been enhanced with phosphorous compounds, PO4 may thus be present in water samples from the Gulf of Suez (36).

One of the most crucial water quality indicators for marine life is the concentration of dissolved oxygen (37). Surface water's dissolved oxygen is mostly produced by algae and aquatic plants through photosynthetic activity in the atmosphere. The oxygen partial pressure of the atmosphere, water temperature, salinity, and the availability of nutrients are just a few of the variables that affect the concentration of dissolved oxygen on a daily and seasonal basis, according to Aljahdali and Alhassan, (2020b) (38). Due to the huge surface area and increased exposure, site B had higher average dissolved oxygen readings as well as higher turbidity measurements.

Ammonia may be present naturally in surface or waters and usually, its concentration is quite low due to its easy adsorption by soil particles or oxidation to nitrite and nitrate. However, the occurrence of high concentrations may be due to sources of the reduction of nitrate by bacteria or by ferrous ions. The presence of ammonia has a significant effect on the chlorine disinfection of water by the formation of chloramines, which have low bactericidal activity (39).

When compared to those of the reference site, the results of the current study demonstrated a highly significant increase in the mean concentrations (Pb, Cd, Cu, Co, Zn, Cr, and Ni) in the water samples, sediments, and midgut tissues from the contaminated site. According to Men et al. (2021), many mechanisms impacted by anthropogenic activity, including run-off from urban and rural regions, factory discharges, and industrial site leaching, may have contributed to the elevated amounts of heavy metals in natural streams (40).

The activity of shipping mineral products, particularly phosphate, has an impact on the highest Zn value in the water and sediments in the polluted region according to Wang et al. (2020a), The highest concentration of Cu can reflect seafaring activity (41). The highest Ni content can be found in contaminated areas. However, nickel can enter surface waters from natural sources including particulate matter in rains, through the dissolving of bedrock minerals, and soil phases. This greatest value may be associated with product shipping activities. The polluted location's highest Pb concentrations are noted. They most likely have anything to do with the delivery of mineral goods (38).

The results of the current investigation revealed that tissue MDA concentration levels of *E. tenuicosta* recovered from site B were significantly higher than those from site A. Our results corroborated those of Abu El-Saad (2017), who found that the midgut MDA levels of *Apis mellifera* from the polluted areas were high (42). The higher levels of MDA may be caused by the mitochondrial electron transport system's inhibitory action, which stimulates the generation of intracellular ROS (40). In the current investigation, *E. tenuicosta* tissues collected from site B had significantly higher levels of AST and ALT activity than those from site A. According to Shonouda et al. (2016), *A. globulus* tissues from heavy metal-polluted locations had higher levels of AST and ALT activity (43).

According to Sharifi-Rad et al. (2020), the rise in AST and ALT activity levels may be caused by an increase in the concentration of free amino acids because of protein degradation through proteolysis and the suppression of protein synthesis after exposure to pollution (44). The current study's highly significant decrease in the protein content of *E. tenuicosta* may be explained by the enhanced protein breakdown into free amino acids after exposure to pollution.

The GSH concentration of *E. tenuicosta* taken from site B was significantly lower than that of site A, according to the findings of the current study. According to El-Samad et al. (2015), the decline in GSH levels may be caused by their consumption in the process of scavenging free radicals (45). Asaeda and Barnuevo, (2019) reported that the decrease in total protein may reflect the decrease in the enzymatic activities of GST, GPx, and SOD (46). The results of the current study showed a significant

decrease in tissue GPx activity levels of *E. tenuicosta* sampled from site B when compared with those of site A. This may be because of the decrease in total protein. The current results are in agreement with Aljahdali and Alhassan, (2020a) who reported that the activity of GSH-dependent enzymes decreased in insects that were exposed to heavy metals (47).

Antioxidant enzymes such as catalase (CAT), glutathione-S-transferase (GST), and mitochondrial and cytosolic SOD are all affected differently by metal exposure. Aluminium (Al) toxicity in D. melanogaster is dependent on interactions with Fe, is mediated by ROS production, results in increased expression of mitochondrial SOD but not cytosolic SOD, and is suppressed by overexpression of CAT (48). This study once again emphasizes the possibility of indirect interactions and the complexity of genetic responses.

To delve into the expression of important stress factors, we investigated the impacts of heavy metals on the expression of HSP 70 and MT in the midgut tissues of *E. tenuicosta*, as depicted in Figure (6). The results reveal a significant upregulation in HSP 70 genes and an increase in MT expression in the beetles of polluted site (B) compared to site (A) beetles. These results indicate that heavy metals could modulate the stress response, which supports the previous biochemical assays.

Exposure to heavy metals induces the expression of metallothionein (MT). Of the two isoforms of MT present, Tunçsoy et al., (2021) found that MT2 is localized to intracellular compartments that are involved in detoxification and respond to Cd on both a transcriptional and a translational level (49). Metals have been shown to increase the expression of the MT gene in a wide range of taxa, and this gene expression has been used to establish a biomarker for metal contamination in insects and other animals (50).

According to El-Gendy et al., (2020), heat shock proteins (HSPs) are widely known to be essential for insects and humans in maintaining cellular homeostasis (23). As a defense mechanism to control oxidative stress, we observed a discernible overexpression of some protective proteins in our earlier research, such as HSPs, (16). According to earlier research, *A. domesticus* had higher levels of HSP70 after being exposed to heavy metals (51).

Conclusion

The Red Sea coast is subjected to natural and anthropogenic sources of trace elements. The natural sources include *Enochrus tenuicosta* eathering of rocks, thermal springs, and vegetation. Inputs from anthropogenic sources include tourist activity, smelting, oil spills, industrial and mining operations, waste disposal, agricultural activities, and domestic sewage.

In summary, this research explores the harmful influences of heavy metals in the midgut tissues of water scavenger beetles (*E. tenuicosta*). The exposure of beetles to heavy metals engendered substantial aberrations in physiological characteristics. Precisely, considerable disorders were perceived in the oxidative stress factors and antioxidant enzymes linked with remarkable changes in HSP 70 and MT gene expression for the polluted site beetles compared with the reference site beetles.

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