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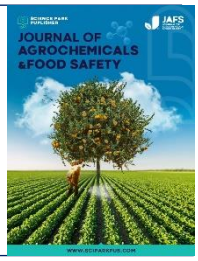

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Bioaccumulation of potentially toxic metals in four bivalve species from Ismailia, Egypt ecosystem in association with oxidative stress and biochemical defects

Khaled Y. Abdel-Halim[*,1], Yousry M. Ahmed[2], Amira A. A. Frahat[2], Usama M. Abu El-Ghiet[3]

[1] Mammalian & Aquatic Toxicology Department, Central Agricultural Pesticides Laboratory (CAPL), Agricultural Research Center (ARC), 12618-Dokki, Giza, Egypt

[2] Department of Plant Protection, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt

[3] Biology Department, Faculty of Science, Jazan University, Saudi Arabia

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Abstract

The study's purpose is to look into the bioaccumulation pattern of heavy metals (HMs) in bivalve species taken from Temsah Lake in Egypt's Ismailia region, as well as the relationship between oxidative stress and metabolic changes. Six heavy metals (HMs): cadmium (Cd), lead (Pb), manganese (Mn), chromium (Cr), tin (Sn), and mercury (Hg) were evaluated. The bivalve species were collected from five locations of Temsah Lake during the winter and summer of 2020/2021. The measured heavy metal concentration was higher in bivalve tissues than in sediments. The bioconcentration factor (BCF) mean values of Cr for the following species: comb circe, surrclam, grooved carpet shell, and golden venus, respectively, showed great values of 208.28, 224.15, 224.91, and 142.55. These were followed by Pb (68.46, 49.66, 53.84, and 43.86) and Sn (24.59, 32.51, 23.13, and 64.86) for the aforementioned species. Increases in carbonyl protein (CP) and malondialdehyde (MDA) were seen in the tested metals.

Keywords: Heavy metals, bioaccumulation, oxidative stress, bivalves, Temsah Lake, Egypt

1. Introduction

Bivalves are among the seafood that are mostly well-suited to nutrition, but they are also high in heavy metals (HMs), which can be harmful to one's health. A multitude of metabolic variables influence bivalves' HM intake, putting consumers at risk. Bivalves can absorb the majority of metals found in industrial waste and urban home pollutants. Molluscs are a diverse category of invertebrates found around the world. Temsah Lake is home to a variety of clam species, including grooved carper shell (*Tapes decussatus*), comb circe (*Gafrarium pectinatum*), golden venus (*Venerupis aurea*), and surrclam (*Paphia undulata*). Nevertheless, the bivalve clam *P. undulata* Born, 1778 (Bivalvia: Veneridae) is economically and ecologically significant as a source of food and biomass, while also having an impact on communities. It accounts for a major portion of the shellfish market in various coastal countries across the world [1]. Therefore, their seafood products and commercial value are stressed. However, consuming them as seafood may be risky because they are believed to be hyper-accumulators of HMs and other chemicals [2]. As mentioned earlier, aquatic invertebrates are inevitably impacted by metal contaminants in water and sediments, and they can act as biomarkers for deterioration in water quality. Bioaccumulation in mussels and possible harmful metals in the environment could pose health risks to the general people [3]. According to the European Water

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Framework Directive, some heavy metals have been categorized as urgent pollutants because of their high rate of accumulation in living things. Many investigations have examined the consumption of heavy metals (HMs) and their effects on the majority of edible marine species [4].

Also, several studies have shown that the sediments from a highly polluted area (HCA) are significantly impacted by trace element (TE) contamination. Published studies show that parameters including ingestion rates, gut fluid quality, and detoxication methods frequently affect how quickly TE builds up in the digestive gland of *Ruditapes decussates* [5, 6]. Furthermore, several studies have demonstrated that trace element (TE) contamination has a major effect on the sediments from a highly contaminated area (HCA). The pace at which TE accumulates in the digestive gland of *R. decussates* is often influenced by factors such as ingestion rates, gut fluid quality, and detoxication techniques, according to published research [7].

In food chains, poisonous substances known as heavy metals (HMs) accumulate at an accelerated rate. They are considered the most important environmental contaminants due to their toxicity and tendency to accumulate in marine species [8]. Numerous human activities, such as traffic, smelting, burning fossil fuels, industrial processes, and some agricultural runoff, contribute to their creation [9]. Non-essential HMs become potent poisons, when they bioaccumulate in living things and produce intoxication [10-12]. Although critical metals can bioaccumulate to a dangerous amount, they nonetheless serve typical physiological regulating purposes. Oxidative damage and metal cytotoxicity have been widely linked [13]. More precise attention needs to be paid to the biological effects on tissues and the cellular and/or molecular causes of its toxicity [14]. Numerous metals are known to induce oxidative stress, but transition metals including iron (Fe) and copper (Cu), O_2,¯, and H_2O_2 are especially known to do so because they can create ˙OH through the Fenton reaction [15]. Other non-transition metal ions may also be connected to the production of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) in mitochondria. Cadmium (Cd), for example, can generate ROS, which can impair the mitochondrial electron transport chain [16]. Therefore, one possible effect of ROS is oxidative stress, which is the breakdown of biological components and tissues. When the generation of free radicals exceeds the breakdown of those radicals, it manifests in living organisms [17]. Antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) quickly break down ROS. However, the production of ROS can initiate lipid peroxidation (LPO), which causes the cell to produce too much malondialdehyde (MDA) [18]. This synthesis has a rapid boundary with the biomolecules, leading to different toxicities. Irreversible protein oxidative modification brought on by negative stress circumstances can result in the development of carbonyl protein (CP), which is thought to be the best class of oxidized/or carbonyl proteins of oxyradicals, with a formation rate higher than their breakdown [19].

Due to its reversible role in converting pyruvate to lactate, the enzyme lactate dehydrogenase (LDH) is believed to be a biomarker for detecting stress conditions [20]. It was found to be a reliable indicator of tissue injury in invertebrates [21]. Oxidative stress and other metabolic alterations are critical for evaluating the effects of contaminants on animals in varied environmental situations [22]. Furthermore, the interactions between xenobiotics and the components of the antioxidant system provide a pattern of performance for ecotoxicological disorders in the organism in relation to its environment [23]. The novel component of this work is that it investigates the impact of HMs on organisms using a linear regression model for biological reactions. There are few comparable discoveries in Egypt. Thus, the current study seeks to examine the bioaccumulation pattern of HMs in bivalve species taken from Temsah Lake in relation to oxidative stress and specific metabolic changes.

2. Materials and methods

2.1. Chemicals and reagents

The following materials were provided by BDH Chemical Ltd., Poole, England: solvents (ethanol and ethyl acetate), folin reagent, ethylene diamine tetra-acetic acid, disodium salt (EDTA), and trichloroacetic acid (TCA). Chemical suppliers included LOBA Chemie-Jehanjer villa, Mumbai, 400004, India; other suppliers provided sodium azide, sulfosalicylic acid, thiobarbituric acid (TBA), and magnesium sulphate ($MgSO_4$, H_2O_2). Other chemicals supplied by J.T. Baker Chemical Co., Phillipsburg, N.J. 08865, included sodium chloride (NaCl), sodium citrate, sodium hydrogen citrate, phosphate buffer, sodium phosphate mono and dibasic, sodium carbonate (Na_2CO_3), hydroxide (NaOH), sodium-potassium tertrate, copper sulphate ($CuSO_4$), and acids, hydrochloric (HCl),

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Research Article

sulphuric (H_2SO_4), glacial acetic and nitric (HNO_3). Mumbai, India-based Oxford Lab Chem provided the guanidine chloride. Bovine serum albumin (BSA), sodium pyruvate, and several standards of HMs were acquired from Sigma Chemical.

2.2. Description of the studied region

The examination was carried out at five separate Temsah Lake locations. This lake is located 80 km south of Port Said in the middle of the Suez Canal and has an area of roughly 15 square kilometres between 32° 17' 30"; 32° 18' 30" E latitude and 30° 32' 30"; 30° 40' 30" N longitude. Figure 1 depicts a selection of sampling locations, with the site (5) chosen as a reference due to its distance from the source of contamination, and the other four sites (1-4) labelled as polluted regions. Furthermore, inhabitants are employed in the tourism and fishing businesses, which constitute a large amount of the region's income [24]. The lake receives high salinity water from the Suez Canal [25]. It receives freshwater from the Ismailia Canal, El-Forsan Drain, Al-Mahasama Drain, and Abu Jamous Drain through the western lagoon [26]. The dredging activities of the Suez Canal led to the demolition of fisheries [27]. The dramatic increase of human activities in recent years near the lake, such as shipping and servicing, municipal wastewater damping off, and agricultural drainage loading into the lake, has greatly accelerated the lake's depletion and pollution state [28].

2.3. Sample collection and preparation

Bivalve samples were obtained from the investigation sites during the winter and summer of 2020 and 2021. Every type of sample was placed in a plastic bag, labeled, and transported to the laboratory in an icebox for analysis. Sediment samples were collected at a 5 cm distance from the ground surface using auger equipment. The water was then removed from the equipment, placed in a plastic bag with a label, and delivered to the laboratory for analysis. Before usage, the samples were air-dried in a dark room for 72 hours.

2.3.1. Soft tissues

The following freshwater bivalve species were gathered from five sites during the above-described periods: grooved carper shell (*T. decussatus*), comb circe (*G. pectinatum*), golden venus (*V. aurea*), and surrclam (*P. undulata*). After being dissected to harvest soft tissue, they were packaged, labeled, and kept at -20 ˚C until needed.

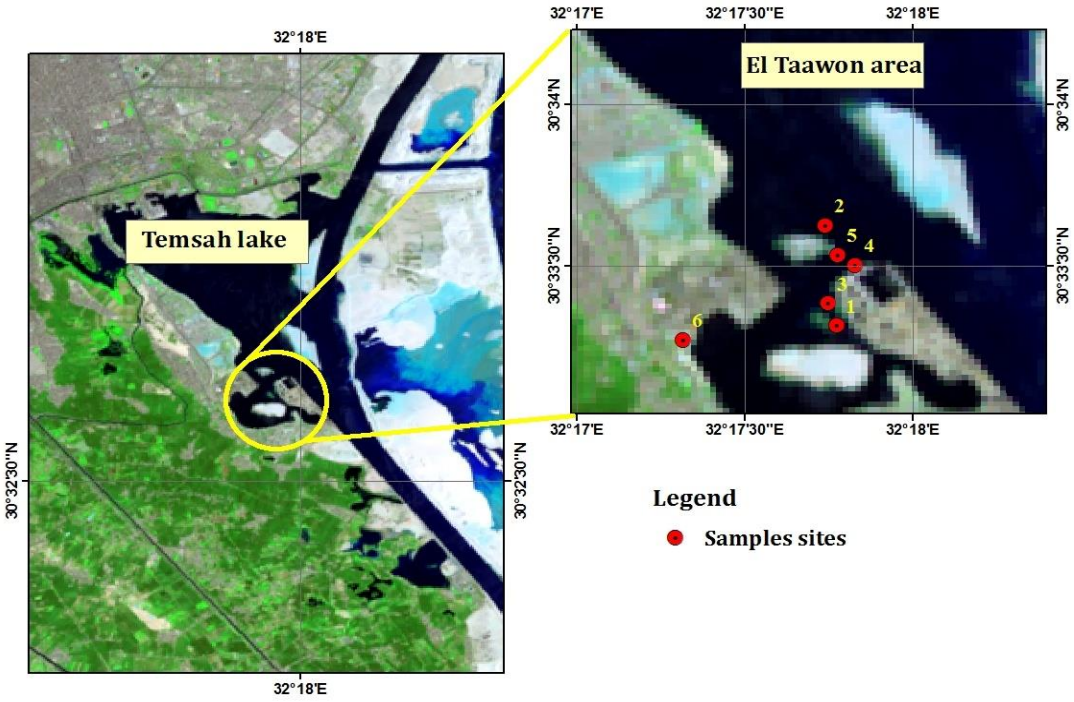

Figure 1. Google map demonstrating sampling sites of Temsah Lake.

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2.4. Determination of heavy metals (HMs)

2.4.1. In sediment

The Olowu et al. technique [29] was used to measure the amounts of HMs. An orbital shaker was used to agitate an aliquot of dried material (5 g, each) for 30 min after it was dispersed with 50 ml of 0.1M HCl into a 150 ml conical flask. The extract was then filtered, added 0.1M HCl to make it up to a volume of 50 ml, and used to test HMs.

2.4.2. In soft tissues

A mixture of 1 g of dried material and 10 ml of HNO_3: H_2SO_4 (at a ratio of 4:1 v/v) was prepared. Using a magnetic agitator and reflux condenser, the mixture was brought to a boil on a hot plate for one hour. The sample was chilled, and then heated once more after adding 10 milliliters of HCl. To determine HMs, it was diluted to an impressive level with deionized water and then reduced to a constant volume of 5 ml. This was done using an instrument, Inductive Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) [30].

2.5. ICP-OES

ICP-OES was used to measure a variety of metals, including chromium (Cr), manganese (Mn), lead (Pb), and cadmium. All measurements were done with the Agilent 4200 MP-AES microwave plasma model. An auto-sampler was used to deliver the sample (3 replicates) to the double-pass cyclonic Agilent SPS 3 instrument's cyclonic spray chamber, which was filled with mass flow-controlled nebulizer part (0.60 L/min). The system supported cooled CCD detection and operated in a fast-sequential mode with a solution time of 30 s and an equilibrium time of 15 s. Background and spectrum interference might be precisely and easily managed using Agilent's MP Expert Software.

The amounts of tin (Sn) and mercury (Hg) were measured using ICP-MS/MS (8900 Triple Quadrupole ICP-MS method). The apparatus was run in three modes: SQ-KED, SQ-CP-NH_3 (cold plasma with NH_3 reaction gas), and TQ using pure oxygen (TQ-O_2). The high sensitive PFA double-pass spray chamber was employed, with the nebulizer part set to 10 µl/min. The apparatus was set up for triple quad mode, with a forward power of 1550 W, nebulizer part of 0.999 ml/min, and CRC gas of 0.3 ml/min, with dwell periods ranging from 100 to 300 ms per metal.

For blank response, the limits of detection (LODs) for these metals were determined as two times the standard deviation over a range of solution quantities [31]. To ensure the reliability of the findings, working standards, quality assurance processes, and safety precautions were implemented. To avoid contamination, deionized water was used, and samples were handled with considerable caution. The techniques were followed as previously described, and a recovery experiment was authorized by spiking the blank with 50 and 100 ppm of the metal's standard.

2.6. Bioconcentration factor (BCF)

Bioconcentration factor (BCF) was estimated as a ratio between metal concentration in the tissue (C_t), with respect to its concentration in the surrounding media (C_{med}) as shown in equation (1) [32]:

$$BCF = \frac{Ct}{Cmed} \quad (1)$$

2.7. Biochemical quantifications

2.7.1. Preparation of the tissues

Each tissue sample weighed half g and the samples were mechanically homogenized for 15 s with an ice-cold saline solution (ratio 1:10 w/v). The extract was centrifuged for 20 min at 5000 rpm. The supernatant was utilized as a source for the measurement of other enzymes, the homogenate was utilized as a source for LPO and LDH.

2.7.2. Lipid peroxidation (LPO)

The spectrophotometric determination of MDA level was approved with the guidance of thiobarbituric acid reactive compounds (TBARS) [33]. In summary, 0.25 mL of tissue homogenate was added to a test tube containing two mL of 0.37% (w/v) thiobarbituric acid (TBA) and one mL of 15% (w/v) trichloroacetic acid (TCA) in 25 mM of HCl. After being heated to 100 ˚C for ten minutes, it was quickly cooled and centrifuged at 5000 rpm for five minutes. At 535 nm, a spectrophotometric determination was made. The MDA level was approximated as mM/g of tissue using an extinction value of 156 mM^{-1}.

2.7.3. Catalase (CAT)

Following the decrease in absorbance at 240 nm, which was attributed to the depletion of hydrogen peroxide (H_2O_2), the activity of CAT was measured [34]. An aliquot of the enzyme source was introduced to the cuvette along with 1 ml of 12.5 mM H_2O_2 (substrate) and 2 ml of 66.7 mM phosphate buffer (pH 7.0). In U/mg protein, the enzyme activity was measured. The quantity of enzyme that releases half of the peroxide oxygen

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from the H_2O_2 solution in 100 µl at 25 degrees Celsius, regardless of concentration, is the unit of CAT.

2.7.4. Glutathione peroxidase (GPx)

Using a cuvette containing phosphate buffer solution (100 mM), EDTA (50 mM), sodium azide (250 mM), H_2O_2 (10 mM), and enzyme, the activity of GPx was measured using the Flohe and Gunzler [35] technique. For 40 s, the absorbance at 340 nm was recorded every 3 s. The activity was calculated as mU GPx/mg protein. The quantity of enzyme required to oxidize 1 µM of β-nicotinamide adenine dinucleotide-reduced form (β-NADPH) per minute is known as one unit of GPx.

2.7.5. Carbonyl protein (CP)

Tissue samples that had been frozen were weighed, ground in ice-cold sulfosalicylic acid (5%) at a ratio of 1:20 w/v, and centrifuged for 15 min at 13,000 rpm. The sample pellets were combined with 0.5 ml of a solution of 2, 4-dinitrophenylhydrazine (10 mM) and vigorously vortexed for one hour at room temperature. After thoroughly mixing the same amount of TCA (20% w/v), the samples were centrifuged as previously mentioned. The excess 2, 4-dinitrophenylhydrazine was eluted three times using ethanol: ethyl acetate (1:1 v/v) (1 ml each time), which was recorded using a strong vortex and centrifuging again as previously mentioned. After being suspended in 6 M guanidine chloride, the final pellets were incubated for 15 min at 37 ˚C [36].

2.7.6. LDH

The enzyme activity in tissue homogenate was quantified according to method of McComb [37]. Also, sodium pyruvate was used as a substrate and the activity was estimated as U/L.

2.7.7. AST/ALT

Aliquots of the supernatant were used to assess AST and ALT activities by using specific kits. The enzyme activity was estimated as U/L [38].

2.7.8. Total Protein content

Protein content was quantified according to method of Lowry et al. [39] and bovine serum albumin (BSA) was used as a standard.

2.8. Statistical analysis

ANOVA, or analysis of variance, was employed to compare treatment means. The Student-Newman-Keuls test [40] was used to compare the least square means in order to identify any significant changes between treatments. In order to perform principal component analysis (PCA), multi-linear regression between HMs levels and physiological variables was accomplished [41]. The appropriate analysis was carried out using the Costat programme [42].

3. Results

3.1. Bioaccumulation of HMs

Table 1 displays the modified quantified levels of HMs at 100% recovery. Sn (2.50 mg/kg) and Mn (1.00 mg/kg) were the mean values, while Hg contents in sediment samples ranged [below the detection limit (BDL)-41.18 mg/kg] (mean, 8.99 mg/kg) during the summer. The metal Pb had the lowest average value (0.79 mg/kg). Throughout the winter, all tested HMs had greater values than in the summer. Table 2 shows the mean values for Mn, Cr, Cd, Pb, Hg, and Sn, which were calculated to be 15.20, 16.52, 17.79, 18.35, 16.41, and 16.85 mg/kg, respectively. Nonetheless, the measured HMs met the aforementioned regional standards of 8.10, 8.72, 9.30, 9.57, 12.70, and 9.68 mg/kg for HMs.

All HMs measured in bivalve tissues had greater regional mean values than those found in sediments (Table 3). Pb concentrations in the collected species of Comb circle, Surrclam, Grooved carpet shell, and Golden venus were exceptional, with mean values of 180.66, 223.09, 201.11, and 201.02 mg/kg. These were followed by Sn (164.42, 179.64, 228.49, and 286.22 mg/kg) and Cr (194.84, 208.91, 209.29, and 135.03 mg/kg) for the aforementioned species. The levels of Hg were 130.55, 53.19, 96.04, and 41.89 mg/kg, whereas the Mn bioaccumulation patterns for the aforementioned species were 53.31, 54.09, 50.80, and 51.98 mg/kg. They had low mean Cd values of 3.48, 3.64, 3.15, and 4.22 mg/kg, respectively. Table (3) shows the estimated bioconcentration factor (BCF) of the observed HMs as a ratio of tissue concentration to media concentration (sediment). For the species Comb circe, Surrclam, Grooved carpet shell, and Golden venus, the BCF of Cr was 208.28, 224.15, 224.91, and 142.55, respectively. Pb (68.46, 49.66, 53.84, and 43.86) and Sn (24.59, 32.51, 23.13, and 64.86) had high levels for the same species. In the Comb Circle, Hg had the greatest BCF (10.28), followed by Grooved Carpet Shell (7.55) and Surrclam (4.19), in that order. Mn's BCF pattern was 11.81, 7.98, 7.52, and 6.96 for the following species: Golden Venus, Surrclam, Comb Circe, and Grooved Carpet Shell, in that order.

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Research Article

Table 1. Recovery percentages and limits of detection (LODs) of HMs in bivalves and sediments collected from Temsah Lake.

Metal	Recovery (%)		LOD (ppb)
	Tissue	Sediments	
Pb	95.6	100.0	10
Cr	94.4	87.7	300
Cd	95.4	90.2	700
Mn	95.0	89.7	650
Sn	100.0	100.0	0.01
Hg	98.0	93.0	0.01

Table 2. Concentrations of HMs (mg/kg) in sediment samples collected from Temsah Lake.

Metal	Range	Mean	C.V (%)	*R. Mean*
-Summer season				
Mn	(*BDL-4.24)	1.00	52.56	
Cr	(BDL-4.08)	0.92	52.47	
Cd	(BDL-3.40)	0.80	52.45	
Pb	(BDL-3.60)	0.79	52.39	
Hg	(BDL-41.18)	8.99	52.51	
Sn	(BDL-8.36)	2.50	37.45	
-Winter season				
Mn	(BDL-53.33)	15.20	52.58	**8.10**
Cr	(BDL-61.22)	16.52	51.71	**8.72**
Cd	(BDL-61.78)	17.79	39.49	**9.30**
Pb	(BDL-62.12)	18.35	52.67	**9.57**
Hg	(BDL-63.31)	16.41	48.79	**12.70**
Sn	(BDL-60.35)	16.85	48.83	**9.68**

-*BDL= Below Detection Limits, R. mean= regional mean, and C.V.%= Coefficient of variation percent.

Table 3. Bioconcentration of HMs (mg/kg) in bivalve's tissues collected from Temsah Lake.

Species	HMs (mg/kg)												
	Mn		Cr		Cd		Pb		Hg		Sn		LSD (5%)
	S. Mean	BCF	*S. Mean*	BCF	*S. Mean*	BCF	*S. Mean*	BCF	*S. Mean*	BCF	*S. Mean*	BCF	
Comb circe													
S1	17.78 ±0.02	17.78	382.88 ±0.00	416.17	2.76 ±0.16	3.45	95.16 ±0.01	120.46	105.95 ±0.004	11.79	87.06 ±0.00	34.82	0.37
S2	88.65 ±0.00	5.83	6.37 ±0.07	0.39	4.55 ±0.09	0.26	301.87 ±0.001	16.45	155.51 ±0.003	9.48	242.04 ±0.002	14.36	0.48
R. Mean	**53.22**	**11.81**	**194.63**	**208.28**	**3.66**	**1.86**	**198.52**	**68.46**	**130.73**	**10.64**	**164.55**	**24.59**	-
Surrclam													
S1	9.51 ±0.04	9.51	412.11 ±0.00	447.95	1.68 ±0.22	2.10	61.92 ±0.01	78.38	68.52 ±0.01	7.62	128.28 ±0.003	51.31	0.67
S2	97.99 ±0.01	6.45	5.80 ±0.09	0.35	5.50 ±0.10	0.31	384.23 ±0.00	20.94	37.88 ±0.02	2.31	230.96 ±0.00	13.71	0.96
R. Mean	**53.75**	**7.98**	**208.96**	**224.15**	**3.59**	**1.21**	**223.08**	**49.66**	**53.20**	**4.97**	**179.62**	**32.51**	-
Grooved carpet shell													
S1	9.02 ±0.02	9.02	413.52 ±0.00	449.48	1.89 ±0.21	2.36	70.92 ±0.01	89.77	154.95 ±0.003	17.24	55.95 ±0.004	22.38	0.71
S2	92.11 ±0.02	6.06	5.69 ±0.40	0.34	4.68 ±0.49	0.26	328.67 ±0.01	17.91	7.53 ±0.30	0.46	400.67 ±0.01	23.78	4.14
R. Mean	**50.57**	**7.52**	**209.61**	**224.91**	**3.29**	**1.31**	**199.80**	**53.84**	**81.24**	**8.85**	**228.31**	**23.13**	-
Golden venus													
S1	7.57 ±0.03	7.57	261.81 ±0.00	284.58	2.41 ±0.09	3.02	54.68 ±0.00	69.22	51.69 ±0.003	5.75	279.39 ±0.001	111.76	0.40
S2	96.39 ±0.00	6.34	8.65 ±0.05	0.52	6.05 ±0.07	0.34	347.57 ±0.00	18.49	32.70 ±0.01	1.99	302.57 ±0.001	17.96	0.72
R. Mean	**51.98**	**6.96**	**135.23**	**142.55**	**4.23**	**1.68**	**201.13**	**43.86**	**42.20**	**3.87**	**290.98**	**64.86**	-

- Each value is the mean of 4 replicates ± SE during (S1) summer and (S2) winter season, BCF= bioconcentration factor and R. Mean= regional mean during four seasons, and S. Mean= seasonal mean.

Journal of Agrochemicals and Food Safety, 2025, Vol. X, Iss. X, 10-28
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Research Article

3.2. Biochemical responses

The enzyme activity of each species of trapped bivalve was assessed. It was revealed that bivalves' tissue homogenate contained exceptionally low amounts of MDA. Table 4 reveals that the seasonal average values did not exceed 0.02 mM/g tissue. Throughout the research periods, no obvious variation was seen among the bivalve species (Table 5).

The sequence of events below represented the CAT activity in tissue homogenate: The Grooved Carpet Shell, Golden Venus, and Comb Circe had mean protein concentrations of 60.35, 13.74, 10.99, and 4.76 U/mg (Table 5). In contrast, Table 6 reveals that the activity in samples taken from the sites was 42.41, 24.86, 19.01, 15.24, and 10.78 U/mg protein for sites 4, 1, 3, 2, and 5. Winter enzyme activity was higher than summer activity (see Table 4). The GPx enzyme was active in the homogenate of bivalve tissue in the order shown below. The grooved carpet shell, comb circle, golden venus, and surrclam had mean protein concentrations of 0.04, 0.03, and 0.02 mU/mg, respectively.

The CP levels in the bivalve tissue homogenate were determined in the following order: Mean values of 0.22, 0.20, 0.14, and 0.14 mM/g tissue were found in the olden venus, grooved carpet shell, surrclam, and comb circles, respectively. Protein levels in samples obtained from locations 2, 4, 1, 3, and 5 were 0.21, 0.19, 0.18, 0.16, and 0.14 mM/g tissue, respectively. Wintertime saw the highest amount (0.35 mM/g tissue).

The sequence of events below describes the LDH enzyme activity in tissue homogenate: The average values for Comb Circle, Grooved Carpet Shell, Surrclam, and Golden Venus are 1301.43, 114.47, 50.31, and 49.84 U/L, respectively. The mean enzyme activity values in the samples collected from the locations were 1524.50, 152.17, 121.90, 51.68, and 44.81 U/Lin for sites 3, 1, 2, 4, and 5. The summertime mean value (576.69 U/L) was higher than the wintertime mean value (181.33 U/L).

The following order could be applied to the ALT activity in bivalve tissue homogenate: The mean values of Comb Circle, Surrclam, Grooved Carpet Shell, and Golden Venus were 36.39, 35.07, 34.45, and 33.88 U/L, in that order. On the other hand, mean values of 37.50, 35.34, 35.27, 33.43, and 33.21 U/L were detected in the activities in samples that were taken from sites 1, 3, 4, 2, and 5, respectively. However, the AST activity showed the following order: 14.22, 18.21, 17.59, and 21.60 U/L for the mean values of Golden Venus, Surrclam, Grooved Carpet Shell, and Comb Circle, in that order. But in sites 4, 3, 1, 2, and 5, the activity in samples taken from the locations was discovered to have mean values of 23.43, 17.03, 16.81, 16.44, and 15.82 U/L, respectively.

3.3. Correlation analysis

A noteworthy (P<0.05) inverse relationship was discovered between the concentrations of HMs and ALT activity, except for Mn and Hg, which showed positive correlations with Pearson's correlation coefficients of 0.73 and 0.81, respectively. The enzyme AST was shown to have positive relationships (r=0.87 and 0.82) with the Cd and Sn concentrations. Significant positive correlations were seen between LDH activity and Mn and Hg concentrations (r=0.96* and 0.97*), whereas negative correlations were observed for other HMs. The CP level and the amounts of Cd, Pb, and Sn showed a marginally positive connection (r=0.26, 0.05, and 0.44, respectively). The concentrations of HMs were negatively correlated with the level of MDA, the activities of CAT, and GPx, with the exception of Pb, which showed positive correlations with the concentrations, imposing r values of 0.86, 0.82, and 0.85, respectively (Figure 2).

Table 4. Seasonal mean values of enzyme activities in four bivalve species collected from five sites of Temsah Lake.

Seasons	ALT (U/L)	AST (U/L)	LDH (U/L)	CP (mM/g tissue)	MDA (mM/g tissue)	CAT (U/mg protein)	GPx (mU/mg protein)
Summer	57.53 ±3.87	28.06 ±4.21	576.69 ±14.28	0.003 ±0.001	0.010 ±0.001	4.50 ±1.07	0.030 ±0.003
Winter	12.37 ±0.78	7.75 ±1.65	181.33 ±107.17	0.350 ±0.053	0.020 ±0.003	40.42 ±31.88	0.020 ±0.015
LSD (5%)	17.79	8.00	155.71	0.14	0.00	14.15	0.00

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Research Article

Table 5. The enzyme activities in four species of bivalves collected from Temsah Lake.

Species	ALT (U/L)	AST (U/L)	LDH (U/L)	CP (mM/g tissue)	MDA (mM/g tissue)	CAT (mM/g tissue)	GPx (mU/mg protein)
Comb circe	36.39* ±8.23	14.22 ±4.15	1301.43 ±202.79	0.14 ±0.06	0.01 ±0.003	10.99 ±2.64	0.03 ±0.005
Surrclam	33.88 ±8.06	18.21 ±3.84	50.31 ±15.43	0.14 ±0.06	0.01 ±0.003	4.76 ±0.80	0.02 ±0.005
Grooved carpet shell	34.45 ±7.00	17.59 ±2.87	114.47 ±95.96	0.20 ±0.07	0.01 ±0.003	60.35 ±44.31	0.04 ±0.019
Golden venus	35.07 ±8.41	21.60 ±6.75	49.84 ±14.93	0.22 ±0.07	0.01 ±0.002	13.74 ±4.76	0.02 ±0.005
$LSD_{5\%}$	**0.60**	**1.68**	**342.94**	**0.02**	**0.00**	**14.22**	**0.01**

-* Each value represents the mean of three replicates±SE.

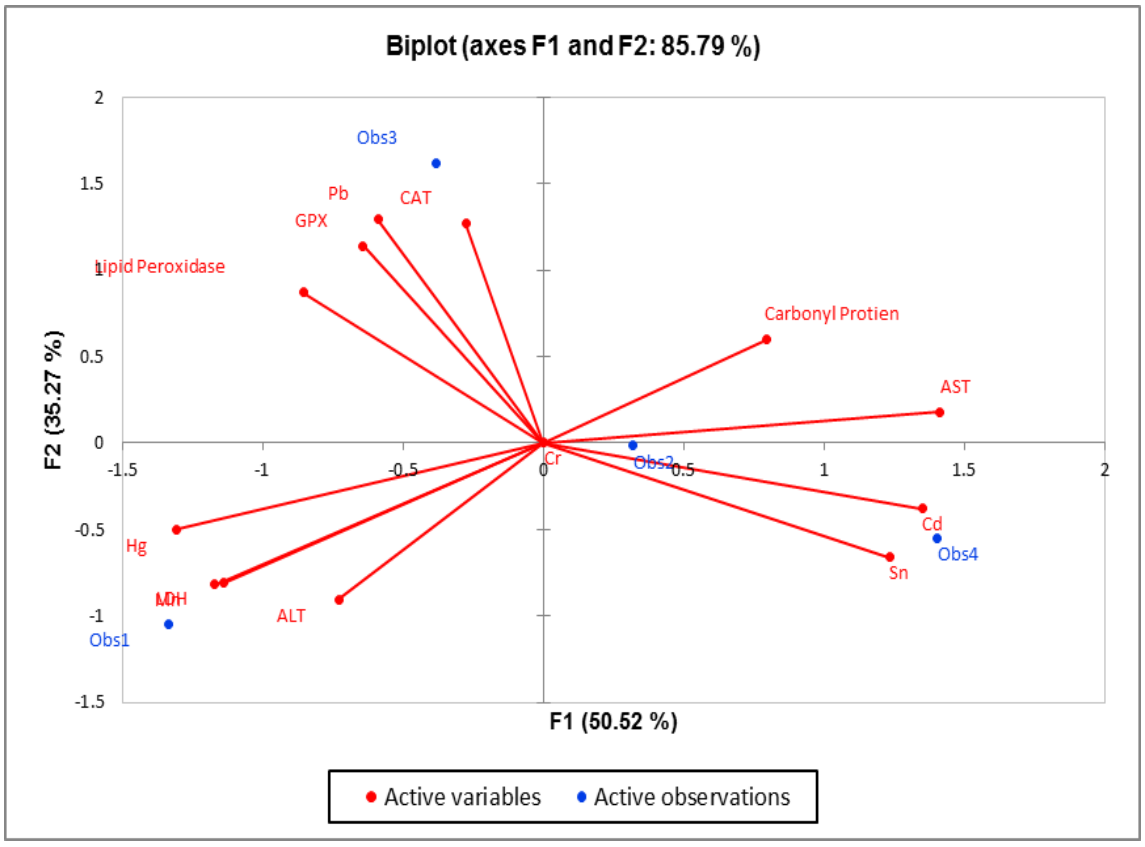

Figure 2. Correlation pattern of HMs levels in bivalves and some biochemical variables. Linear correlation between the variables and HMs obtained after Pearson's test (P<0.05).

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4. Discussion

The present work focused on HMs impact on four species of bivalves commonly distributed in Ismailia ecosystem in association with oxidative stress and other biochemical alterations.

4.1. Accumulation

A significant (P<0.05) negative connection was discovered. Polycyclic aromatic hydrocarbons (PAHs) and some potentially harmful metals are the main causes of pollution in maritime coastal habitats because of shipping and oil exploitation activities. Because of their ability to filter water, beavers are regarded as hyper-accumulators of contaminants and are seen as bio-pointers of contamination. As is known, for the past few decades, marine invertebrates such as bivalve mollusks have frequently been evaluated as sentinel models for ecosystem contaminants linked to their creation of ROS [43-45].

The current study investigated the accumulation of HMs in a large ecosystem model and discovered negative outcomes related to HM levels. Several risk factors, including rapid population growth, increased development, increased manufacturing, consideration and corruption of natural resources, factors such as irrigation expansion, the spread of other modern agricultural practices, and a lack of environmental regulations, may be to blame for the elevated rate of heavy metal pollution. Furthermore, owing to the industrial zone in Ismailia City and shipping traffic in the Suez Canal, HMs may be released into the Lake in large quantities. According to Abd El-Azim et al. [46] and Nasr et al. [47], the western lagoon on Temsah Lake is the principal source of pollution, discharging large amounts of sewage, agricultural effluents, and industrial waste.

Pollution's impact on aquatic systems has received global attention [48]. Likewise, some parameters, including pH and temperature, may alter HM ingestion, dispersion, and potential detrimental effects in ecosystems [49]. As a result, high concentrations of these metals can have unanticipated ecological consequences on the biota, potentially reducing fertility and/or interfering with reproduction [50]. Furthermore, HMs can affect biochemical and physiological processes in aquatic species' blood and tissues [51]. Pollutant processes and pathways from one trophic level to the next are explained by HM bioaccumulation and/or bio-magnification in organisms. HMs can infiltrate the food chain when ingested directly in water.

Figure 2 shows that the bioaccumulation patterns of HMs in mollusc tissues are significantly higher than those in sediments. According to Lau et al.'s study [53], the HMs As, Cu, Fe, Se, and Zn accumulated in the tissues of *Brotiacostula*, *Melanoides tuberculata*, and *Clithonsp* at higher levels than in the sediments, which is consistent with the profile presented here. Furthermore, the current findings are consistent with those obtained by Abd El-Azim et al. [54], who found that high levels of bioaccumulation factor (BAF) of Mn, Cd, and Cr are suitable bio-indicators for monitoring pollution in Bitter Lakes for fish species. Mn, Cd, Cr, Cu, and Pb were shown to accumulate in the soft tissues of *Pomadasys*. Numerous characteristics, including feeding habits, development rate, age, and metal bioavailability, have been associated with HM bioaccumulation in aquatic species [55, 56]. Certain molluscs living in sediments can acquire more HMs than those dwelling on the surface of rocks or algal diatoms. Water-dwelling organisms with a rapid growth rate accumulate HMs in their tissues at a lesser rate. This pattern may be connected with a rise in the weight of the tissue and shell, which occurs quicker than the accumulation process. El-Sawy et al. [57] documented this idea, with total quantities of arsenic (As), sulphur (Sn), mercury (Hg), and selenium (Se) in the sediment compartment of Bitter Lakes in 2018 being 22400.0, 605.85, 40.91, and 446.55 ng/g, respectively. Despite efforts to reduce their discharge, potentially hazardous metals continue to pose a significant threat to ecosystems. Numerous potential toxic metals are currently on the list of urgent elements contaminating surface waters, while alarms are triggered by the rising usage of technology-critical metals such as metallic nanoparticles (NPs), rare-earth, and platinum group metals. [58]

The recent findings of HM accumulation levels in bivalves' tissues were found to be higher than the limitations set by international authorities. For example, the established Mexican and international regulations (in wet weight) are as follows: As (80 mg/kg) [61], Hg (1.0 mg/kg) [61], Pb (1.0 mg/kg) [61], Cd (0.5 mg/kg) [61], Cu (35.5 mg/kg) [59], Cr (13.0 mg/kg) [60], and nickel (Ni) (80 mg/kg) [60] are listed in that order. In contrast, the bivalve Cerastoder maglaucum from Temsah Lake shows accumulation levels of Mn, Ni, Fe, and Cr that exceed the WHO's recommended limits [62]. The Malaysian Food Regulations (1985) [63] are arranged in the following order (in dry weight): Cd (1.0 mg/kg), Cu (30 mg/kg), Pb (2.0 mg/kg).

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It has been reported that HMs and metalloids cause cellular disruption, particularly by inducing oxidative stress independent of ROS initiation, damaging DNA and impairing its repair mechanism, delaying normal cell membrane function and nutrient adaptation, and inducing protein breakdown [64–65]. Furthermore, it is widely acknowledged that the majority of metals generate covalent bonds that disturb the normal functions of several organ systems [66]. For example, Pb has been associated with cancer and non-cancer hazards that exceed one EPA unit. As described in the literature, Pb poisoning in humans can result in arthritis, hypertension, brain and kidney damage, and muscle weakness. Furthermore, it may cause birth defects and mental retardation, especially in susceptible populations such as children and expectant mothers [67].

Based on this present research, Mn may be released via fertilization procedures, metal mining, agricultural runoff, and a range of household wastes produced in the Ismailia governorate. Although excessive exposure can result in tumors, hypotension, central nervous system disruption, and changes in fetal development, it is nevertheless recognized as a vital enzyme activator [68]. Such findings found that Mn levels in all studied bivalves were above EPA regulatory guidelines, implying that taking Mn often over an extended tolerance may pose health risks. Similarly, as previously stated, consuming bivalve species such as Pacific oysters and marsh clams may raise your chance of developing metal poisoning from As, Cu, Pb, Zn, and Hg, as evidenced by different Total Hazard Index (THI) values [69].

Low levels of Hg can harm to adult or fetal neurological system. Furthermore, exposed persons have effects on their immune, cardiovascular, reproductive, and renal systems [70]. According to recent results, the potential detrimental effects of low Pb doses and environmental contamination should be explored. Only a few countries run awareness programs, and they are occasionally successful. Administrators of relevant nations should witness the absence of Hg from food and the environment by creating rigorous norms for industrial sectors that pollute, validating correct handling of Hg waste, and promising not to undertake any Hg-related acts. Due to current findings about the adverse impact of such pollution in this study, the lake's water quality has an impact on the critical role of the Suez Canal as a key waterway for species movement [71]. In this regard, clams are consumed locally in Egypt, and their population has declined due to overexploitation, pollution, and parasites [72–74].

4.2. Biochemical responses

The findings obtained indicate that a potential hazardous technique to evaluate the biological impact and danger of HMs may be feasible. Even in the lack of local species, employing marine bivalves as antioxidant enzymes could be a reliable and straightforward solution to improve existing monitoring methods. The biological relevance of the findings is especially significant, given the HMs that have accumulated and the toxicological reactions to them.

When compared with another reference site (site 5), the results revealed that the observed HMs discharged into Temsah Lake could produce oxidative stress in samples collected from polluted locations. Most studies on aquatic species focused on oxyradicals (H_2O_2, the element $O^{\bullet -}$, and OH^-) as the key ROS sources. The presence of a wide range of xenobiotics, both natural and synthetic, can boost ROS production. Persistent organic pollutants (POPs) and HMs are two potential causes of elevated ROS and other pro-oxidant free radicals [75, 76]. In the current study, ROS were found to cause an intracellular excess of MDA in molluscs collected from all sites but the reference site. Formation of MDA in cellular membranes disrupts normal metabolism, triggering an adaptive response that eventually leads to cell death [77]. According to studies, various stresses resulted in higher MDA levels in mussel species [78–81]. El-Saidy et al. [62] found that the accumulation of Cu, Cr, and Fe in C. glaucum tissues in Temsah Lake correlated positively with MDA levels and negatively with CAT activities. Hossain et al. [3] found that Pb exposure had a significant impact on the physical health of the freshwater pearl mussel, *Lamellidens marginalis*. Furthermore, the persistent influence resulted in moderate to severe modifications in internal cellular structure.

However, during H_2O_2 detoxication, CAT and GPx have complementary functions. When tissue homogenates from all sites were compared to the reference site, the animals in the present investigation showed increased CAT activity. As previously stated by Hermes-Lima [82], these increases are produced by ROS regulation. The majority of seasons saw a decline in GPx activity in animals collected from polluted locations of the Lake. Previous studies discovered that various pollutants in coastal areas, marine ecosystems, and freshwater influenced the oxidative stress of many mollusc species. This was found with mussels, notably *Mytilus galloprovincialis* [83],

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Perna perna [84], *Ruditapes philippinarum* [85], and freshwater mussels [86].

The cytotoxic effects of HMs were determined by measuring LDH in the tissue homogenate of animals collected during the study period. Positive LDH activity was detected in all infected tissues. Elevated LDH release can also be used to detect cellular and membrane damage [87].

Furthermore, all mollusk species had noticeably larger excesses of MDA and LDH in the chosen snail. This profile reveals a probable relationship between HM toxicity, accumulation, and oxidative damage. Previous studies in contaminated locations found higher MDA levels and CAT activity in the digestive gland when compared to reference organs such as mussels *Mytella guyanensis* [88], snails *Theba pisana* [89], and *Helix aspersa* [90].

The quantification of CP generation in this work provides a metric of protein oxidation in molluscs collected to investigate HM bioaccumulation. The collected data revealed that the majority of the animals obtained from the sites had CP. Additionally, the study found that extended exposure to HMs could cause considerable amounts of irreversible change, such as protein carbonylation. McDonagh et al. [91] have already stated this concept. Furthermore, when compared to other seasons, winter was the most contaminated due to HMs, which increased CP induction. In contrast, CP levels in bivalve samples from contaminated sites were greater than in the reference zone. This finding is consistent with previous studies that used proteomics to assess tissue-specific CP generation caused by environmental pollutants such as POPs and HMs [92, 93]. Prior studies have shown that exposure to oil [94] or PAHs [95, 96] raises levels of CP. Significant quantities of CP were identified in the digestive gland tissues of PAH-exposed gastropods [97]. According to Abdel-Halim et al. [98], the land snail, *H. aspersa*, in Egypt's Kafr El-Zayat region was affected by inhaling contaminated air containing industrial emissions, which resulted in a significant induction of CP in the digestive gland, notably in groups near brick and pesticide businesses. The results gathered are consistent with the findings of Gupta et al. [99], who found a link between the development of CP content and ROS generation and the up-regulation of the letter in organisms exposed to environmental toxicants, implying that ROS plays a role in induction protein modification.

Table 6. Regional mean values of enzyme activities in four bivalve species collected from Temsah Lake.

Location	ALT (U/L)	AST (U/L)	LDH (U/L)	CP (mM/g tissue)	MDA (mM/g tissue)	CAT (U/mg protein)	GPx (mU/mg protein)
Site 1	37.50 ±8.78*	16.81 ±3.71	152.17 ±107.95	0.16 ±0.06	0.01 ±0.003	24.86 ±17.21	0.02 ±0.005
Site 2	33.21 ±8.11	16.44 ±3.33	121.90 ±107.50	0.21 ±0.07	0.01 ±0.003	10.78 ±3.10	0.02 ±0.006
Site 3	35.34 ±7.84	17.03 ±3.34	1524.50 ±225.74	0.14 ±0.05	0.01 ±0.003	19.01 ±11.32	0.02 ±0.004
Site 4	33.43 ±8.13	23.43 ±7.70	44.81 ±13.50	0.18 ±0.07	0.01 ±0.002	42.41 ±47.54	0.04 ±0.021
Site 5**	35.27 ±8.01	15.82 ±3.54	51.68 ±19.22	0.19 ±0.07	0.01 ±0.003	15.24 ± 05.65	0.03 ±0.007
LSD5%	0.97	1.74	357.58	0.02	0.00	6.85	0.01

*Each value represents the mean of three replicates±SE.

**Site 5 was considered as a reference zone

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Research Article

5. Conclusion

The current study found that aquatic HM pollution caused silt from Temsah Lake (Ismailia, Egypt) to bioaccumulate in the bivalves' tissues. This buildup induced oxidative stress and metabolic alterations in the organisms, affecting the dangers to local consumers (Ismailia residents). Given this, precautions must be taken to avoid further HM contamination. Furthermore, it improves baseline data and health risk assessments for these contaminants in commonly consumed bivalves in the Ismailia region. These findings provide significant information about the safety of routinely ingested bivalves.

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Compliance with ethical criteria

The tests have been approved in agreement with the European Ethical Procedures [100].

Declaration of conflicting of interest

The authors state that no possible conflicts of interest with deference to the investigation, invention, and/or publication of this manuscript.

Authors' contributions

Khaled Y. Abdel-Halim: investigation, writing-original draft, formal analysis, supervision [**K.A.**], **Yousry M. Ahmed**: investigation, formal analysis, writing-original draft, supervision **[Y.A.], Amira A. A. Frahat**: methodology, visualization, writing formal analysis [**A.F**.], **Usama M. Abu El-Ghiet**: reviewing manuscript and editing [**U.A**]. All authors reviewed the manuscript.

Supplementary data

No extra data are delivered.

Author information

Corresponding author: Khaled Y. Abdel-Halim*****

E-mail: Khaled_yassen68@yahoo.com

ORCID iD: 0000-0003-3389-2693

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