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Biochemical Changes, Antioxidants, and Protein Profile as Indicators of Heavy Metal Impact on *Solea aegyptiaca* in Lake Qarun: A Comparative Study

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ABSTRACT

Heavy metals pose a significant environmental threat to aquatic ecosystems, particularly in hypersaline lakes like Lake Qarun. These metals accumulate in aquatic organisms, including fish, disrupting biochemical and physiological functions. *Solea aegyptiaca*, an economically significant species, serves as a sentinel organism for assessing pollution impacts due to its ecological relevance. This study investigated the biochemical responses, antioxidant activity, and protein profile alterations in *Solea aegyptiaca* exposed to heavy metal contamination in Lake Qarun. Metal concentrations (Fe, Cu, Zn, Cd, Pb, and Ni) were analyzed in gills, liver, kidney, muscles, and skin of fish collected from various locations across the lake. Results showed significantly higher metal levels in fish from the eastern and southern regions compared to the northern and western regions ($P< 0.05$). Essential metals such as Zn, Fe, and Ni exceeded permissible thresholds for human consumption in muscle tissues, especially in the eastern and southern zones, while Cd and Pb remained below the acceptable limits. Biochemical assays revealed elevated glucose, AST, ALT, ALP, creatinine, and uric acid levels in fish from polluted regions, whereas unpolluted areas exhibited higher total protein, albumin, and hemoglobin levels. Antioxidant activity varied, with decreased SOD, GST, and GSH levels but increased CAT activity in polluted regions. Protein profiling showed the emergence and absence of specific molecular weight bands in gills, liver, and muscles, reflecting heavy metal stress. These findings demonstrate the significant physiological and biochemical impacts of heavy metals and underscore the potential of these biomarkers for environmental monitoring in aquatic ecosystems.

INTRODUCTION

Water contamination poses a significant challenge as a result of the escalating human activities in proximity to various aquatic environments **(El Agawany *et al.*, 2021)**. A multitude of ecological transformations in aquatic ecosystems have resulted from the escalation of industrial, agricultural, and commercial chemical effluents driven into water bodies. Despite the latest enactment of laws aimed at protecting the environment, unregulated discharge of wastewater coupled with inadequate water resource

management has led to the deterioration of numerous lakes in Egypt **(Abdel-Khalek *et al.*, 2020)**. Qarun Lake is positioned approximately 80km southwest of Cairo, within the Fayoum depression along the western desert boundary of Egypt. This lake, a confined basin, spans 5.7km in width, 45km in length, with an average depth of 4.2 meters and a surface area of 243km^2 **(Redwan & Elhaddad, 2017)**. The primary inflow sources for the lake are the Batts and Wadi drains, which channel a significant portion of agricultural runoff, alongside smaller drains that contribute minor volumes of drainage water into the lake **(Elwasify *et al.*, 2021)**. Consequently, Lake Qarun faces a multitude of environmental challenges that detrimentally impact its biodiversity, stemming from the influx of substantial quantities of untreated water laden with various aquatic pollutants **(El-Agri *et al.*, 2021, 2022)**.

Heavy metals are recognized as significant anthropogenic pollutants in marine ecosystems, posing a great danger to marine organisms due to their toxicity, persistence, and propensity for bioaccumulation **(Abdel-Khalek *et al.*, 2020)**. While essential metals like copper (Cu), iron (Fe), and zinc (Zn) are trace elements necessary for many biological processes, they can turn toxic if their levels exceed a certain threshold. Toxic metals like lead (Pb), cadmium (Cd), and nickel (Ni) can cause toxicity even at low concentrations **(Elwasify *et al.*, 2021; El-Agri *et al.*, 2022)**. Fish can accumulate metals by consuming food particles contaminated with metals or by coming into direct touch with the environment through their skin and gills **(Tunçsoy *et al.*, 2017)**. The toxicological effects of metals on various tissues are anticipated to increase when their rates of absorption and accumulation surpass their rates of excretion and detoxification **(Xu *et al.*, 2021)**.

Currently, biochemical parameters of fish are primarily utilized to evaluate their health condition **(Al-Hasawi & Hassanine, 2022)**. These parameters serve as sensitive and valuable indicators for water contamination, with their levels considerably altered in fish inhabiting metal-polluted habitats **(Hossain *et al.*, 2021; Ishaq *et al.*, 2023; Gaafar & Mohamed, 2024)**, resulting in severe health complications.

Metals have the potential to trigger oxidative stress in fish by facilitating the production of reactive oxygen species (ROS) such as oxygen radicals **(Flora *et al.*, 2008)**. The evaluation of alterations in antioxidants is recognized as a validated approach for evaluating biomarkers of oxidative stress **(Morshdy *et al.*, 2021)**. In this context, examining the defensive reactions in the liver, gills, and muscle of a native species of fish becomes crucial in biomonitoring investigations. The liver plays a pivotal role in numerous essential functions such as the accumulation, transformation, and elimination of pollutants **(Monteiro *et al.*, 2009; Tsurkan *et al.*, 2020)**, thus enhancing its capacity to tolerate high concentrations of pollutants, metabolites, and ROS. Fish gills are an essential organ since they are the main site for gas exchange, ion control, and the removal of waste products from metabolism **(Monteiro *et al.*, 2009; Rudyk-Leuska *et al.*, 2021)**.

Compared to other muscle proteins, fish muscle stands as a significant reservoir of animal protein with superior biological value **(Avigliano, 2019; Rudyk-Leuska *et al.*, 2021)**.

The electrophoresis of proteins is a proficient technique for obtaining systematic data from macromolecules as proteins exhibit species-specific characteristics, and electrophoretic separations can be conveniently performed **(Corzo *et al.*, 1984; Jesslin *et al.*, 2013)**. Protein profiling has been extensively utilized in detecting intra and inter-specific variations among species, which may mirror the organism's metabolic status and its adaptations to environmental fluctuations **(Muhammad *et al.*, 2018)**.

In this perspective, there have been limited studies conducted on *Solea aegyptiaca* inhabiting Lake Qarun. Therefore, this research was undertaken to assess the concentrations of various heavy metals in *Solea aegyptiaca*'s vital organs (such as gills, liver, kidney, muscles, and skin) that were collected from diverse locations along Lake Qarun, as well as examining the implications of such contaminants on biochemical responses, antioxidants, and protein profile.

MATERIALS AND METHODS

1. Sampling sites and fish collection

120 *Solea aegyptiaca* fish (30 fish per site) were gathered from four distinct locations along Lake Qarun: the first location (site 1) is in the lake's eastern sector, close to the mouth of the El-Bats drainage channel; the second location (site 2) is in the middle of the southern sector, close to the mouth of the El-Wadi drainage channel; the third location (site 3) is in the lake's northern sector, far from drainage water; and the fourth location (site 4) is the western sector of the lake, which is a relatively unpolluted area where no source of drainage water is detected.

1.1. Fish sampling

Using fishermen's nets, *Solea aegyptiaca* specimens were collected alive from the previously chosen places along Lake Qarun. They were then brought to the lab in aerated, cooled containers filled with seawater from the sampling sites. Blood samples were extracted from the arteria caudles prior to tissue biopsy. In a dorso-cranial direction, the needle (a heparinized glass pipette) was inserted fairly deeply into the middle line, directly behind the anal fin. Additionally, serum was separated from blood samples by centrifuging them at 4°C for 15 minutes at 3000rpm. The serum was then stored at -80°C to evaluate its biochemical properties.

To remove any extra blood, the gills, livers, kidney, skin, and muscles were sampled, rinsed in a phosphate-bufered saline (PBS) solution (pH7.4), and then mixed.

To ascertain the metal content, samples were grounded into a fine powder after being dried in an oven set at 105°C for 48 hours. For the purpose of measuring antioxidants, gill, liver, and muscle samples were homogenized using a Glas-Col motor-driven homogenizer (USA) in 5 milliliters of cold buffer (50mM potassium phosphate, pH 7.4) per gram of tissue. After centrifuging at 100,000xg for 15 minutes at 4°C, the homogenates were stored at -80°C. Furthermore, the muscle, liver, and gill samples from each group were immediately taken out, preserved in normal saline, and stored at -80°C for SDS gel electrophoresis.

The fish sampling was conducted with approval from the NIOF committee for ethical care and use of animals/aquatic animals (NIOF-IACUC) according to the certificate number NIOF–FW3–F-22-R-018.

2. Heavy metal analysis

Following the process described by **Ghazally (1988)**, the dried samples (gills, liver, kidney, skin, and muscles) were digested, and the amounts of Fe, Zn, Cu, cd, Pb, and Ni were measured using the Inductively Coupled Plasma Emission Spectrometer (ICP) (ICAP-6300 Duo) **(APHA, 2012)**. The results were expressed as mg/kg dry weight.

3. Blood biochemical parameters

Blood hemoglobin was evaluated using **Betke and Savelsberg**'s **(1950)** chemical method; blood glucose was measured using **Trinder**'s **(1969)** method; albumin was determined using **Doumas *et al.***'s **(1971)** method; uric acid was evaluated using **Fossati *et al.***'s **(1980)** method; total protein was measured using **Doumas et *al.,***'s **(1981)** method; AST and ALT were quantitatively determined using **Gella *et al.***'s **(1985)** method; creatinine was measured using **Weber and Van Zanten**'s **(1991)** method; and ALP was quantified using **Rosalki *et al.***'s **(1993)** method.

4. Tissue antioxidants

Tests for SOD, CAT, GST, and GSH were performed on the homogenized tissues (muscles, liver, and gills) in accordance with **Beutler *et al.* (1963)**, **Nishikimi *et al.* (1972)**, **Habig and Jakoby (1974)** and **Aebi (1984)**.

5. SDS-PAGE

SDS gel electrophoresis of gill, liver, and muscle proteins was performed according to **Laemmli (1970)**.

Statistical analysis

Version 23 of the SPSS Statistical Package Program (SPSS, 2015) was used to analyze the data using a general linear model. Post hoc testing was performed by the ĺeast significant difference (LSD) to compare means. For every test, the statistical significance was $P< 0.05$. The means ± standard error (SE) were utilized to express the results.

RESULTS

1. Residual heavy metals

The concentrations of the investigated heavy metals (Fe, Cu, Zn, Cd, Pb, and Ni) in the gills, liver, kidney, skin, and muscles of *Solea aegyptiaca* fish from various sites around the lake exhibited significantly higher levels ($P< 0.05$) in specimens collected from the eastern and southern regions compared to the less contaminated northern and western sectors, as illustrated in Tables (1, 2, 3, 4, and 5) for the gills, liver, kidney, skin, and muscles, respectively. Moreover, Fe, Zn, Pb, and Ni were predominantly accumulated in the kidney, while Cu and Cd displayed the highest concentrations in the liver. The muscle tissues showed the lowest metal concentrations. In the muscle tissue, essential elements such as Zn, Fe, and Ni exceeded the established threshold for human consumption in all four surveyed locations, whereas Cu surpassed the permissible limit only in fish gathered from the eastern and southern areas. Conversely, Cd and Pb levels were below the acceptable threshold at all four studied sites.

Table 1. Heavy metal concentrations in gills

Heavy metal	Eastern sector	Southern sector	Northern sector	Western sector	*P value*
Fe (mg/kg dry weight)	426.82±3.17 a	346.81±4.31 b	278.62±3.89 C	247.38±5.36 d	0.000
Cu (mg/kg dry weight)	12.75±0.19 a	15.61±0.93 a	4.53±0.75 B	3.60±0.18 b	0.000
Zn (mg/kg dry weight)	143.97±0.85 a	137.03±0.66 a	112.05±0.21 B	101.05±1.91 c	0.000
Cd (mg/kg dry weight)	0.48±0.04 a	0.44±0.03 a	0.13±0.005 B	0.05±0.001 b	0.001
Pb (mg/kg dry weight)	3.00±0.20 a	2.38±0.02 a	0.82±0.07 b	0.41±0.03 b	0.000
Ni (mg/kg dry weight)	6.71±0.48 a	6.62±0.25 a	3.95±0.04 b	3.45±0.005 b	0.002

In each row, statistically significant differences ($P< 0.05$) exist between mean values with different letters although means with the same letter do not differ significantly ($P> 0.05$).

Table 2. Heavy metal concentrations in liver

Heavy metal	Eastern sector	Southern sector	Northern sector	Western sector	*P value*
Fe (mg/kg dry weight)	675.27±10.07 a	499.10±4.25 b	361.58±5.68 C	351.32±3.52 c	0.000
Cu (mg/kg dry weight)	70.47±1.05 a	83.33±1.42 b	57.24±0.34 C	53.15±0.49 c	0.000
Zn (mg/kg dry weight)	106.75±1.17 a	87.15±1.38 b	65.02±1.34 C	59.98±2.46 c	0.000
Cd (mg/kg dry weight)	0.61±0.005 a	0.43±0.03 ab	0.25±0.04 bc	0.18±0.01 c	0.001
Pb (mg/kg dry weight)	1.64±0.28 a	1.39±0.14 ab	0.70±0.10 ab	0.38±0.03 b	0.017
Ni (mg/kg dry weight)	6.17±0.27 a	5.52±0.14 a	3.57±0.31 b	3.19±0.27 b	0.003

In each row, statistically significant differences ($P< 0.05$) exist between mean values with different letters although means with the same letter do not differ significantly ($P> 0.05$).

Table 3. Heavy metal concentrations in kidney

Heavy metal	Eastern sector	Southern sector	Northern sector	Western sector	*P value*
Fe (mg/kg dry weight)	731.96±11.36 a	593.26±8.06 b	331.46±5.74 c	311.16±5.73 c	0.000
Cu (mg/kg dry weight)	19.34±0.57 a	32.65±0.76 b	7.23±0.88 c	5.72±0.78 c	0.000
Zn (mg/kg dry weight)	259.53±2.56 a	246.54±1.75 a	219.52±2.22 b	216.58±1.98 b	0.000
Cd (mg/kg dry weight)	0.42±0.05 a	0.33±0.05 ab	0.14±0.003 b	0.10±0.001 b	0.01
Pb (mg/kg dry weight)	10.79±0.31 a	7.31±0.57 b	1.67±0.32 c	1.09±0.07 c	0.000
Ni (mg/kg dry weight)	7.25±0.13 a	6.98±0.18 a	4.13±0.29 b	3.71±0.21 b	0.001

In each row, statistically significant differences ($P< 0.05$) exist between mean values with different letters although means with the same letter do not differ significantly ($P> 0.05$).

Table 4. Heavy metal concentrations in skin

Heavy metals	Eastern sector	Southern sector	Northern sector	Western sector	*P value*
Fe (mg/kg dry weight)	201.49±3.31 a	195.60±4.60 a	105.81±2.31 b	94.99±4.60 b	0.000
Cu (mg/kg dry weight)	10.96±0.53 a	12.19±0.57 a	3.37±0.72 b	2.66±0.52 b	0.001
Zn (mg/kg dry weight)	138.43±1.67 a	137.57±1.13 a	119.95±1.48 b	102.90±1.40 c	0.000
Cd (mg/kg dry weight)	0.41±0.01 a	0.34±0.02 ab	0.23±0.02 b	0.15±0.002 bc	0.003
Pb (mg/kg dry weight)	1.90±0.25 a	1.66±0.24 a	0.26±0.01 b	0.07±0.03 b	0.004
Ni (mg/kg dry weight)	4.43±0.23 a	3.69±0.20 a	2.22±0.21 b	1.71±0.20 b	0.002

In each row, statistically significant differences ($P< 0.05$) exist between mean values with different letters although means with the same letter do not differ significantly ($P> 0.05$).

Table 5. Heavy metal concentrations in muscles

Heavy metal	Eastern sector	Southern sector	Northern sector	Western sector	*P value*	P.L. WHO
Fe (mg/kg dry weight)	153.90±5.22 a	122.38±4.91 a	57.85±4.42 b	49.31±5.54 b	0.000	5.00
Cu (mg/kg dry weight)	9.34±0.42 a	12.47±0.50 b	3.89±0.12 c	3.82±0.14 c	0.000	5.00
Zn (mg/kg dry weight)	101.78±2.99 a	69.44±2.88 b	51.17±2.02 c	45.92±2.05 c	0.000	40.00
Cd (mg/kg dry weight)	0.31±0.005 a	0.22±0.005 b	0.13±0.01 c	0.10±0.01 c	0.000	0.5
Pb (mg/kg dry weight)	1.22±0.23 a	1.06±0.13 a	0.34±0.02 ab	0.07±0.005 b	0.010	2.00
Ni (mg/kg dry weight)	3.65±0.10 a	3.18±0.08 a	1.58±0.13 b	1.46±0.13 b	0.000	0.5-0.6

In each row, statistically significant differences ($P< 0.05$) exist between mean values with different letters although means with the same letter do not differ significantly ($P> 0.05$). P.L. permissible level according to **WHO (1993)** for (Fe, Cu, Zn, Pb, and Cd) and **WHO (1985)** for (Ni).

2. Biochemical parameters

According to the current findings, fish exposed to El-Bats and El-Wadi drains in the lake's eastern and southern parts had significantly higher levels of blood glucose, AST, ALT, ALP, creatinine, and uric acid ($P< 0.05$). In contrast, the fish gathered from the northern and western sectors that were less contaminated showed the greatest mean amounts of hemoglobin, albumin, and total protein (Table 6).

Table 6. Biochemical parameters of *Solea aegyptiaca* from the studied locations

Parameter	Eastern sector	Southern sector	Northern sector	Western sector	*P value*
Glucose (mg/dl)	127.94±1.47 a	112.00±1.49 B	87.01±1.23 c	81.56±1.11 d	0.000
Total protein (g/dl)	2.52±0.14 b	2.99±0.14 B	3.80±0.16 a	4.42±0.21 a	0.000
Albumin (g/dl)	0.32±0.01 b	0.46±0.01 B	2.04±0.16 a	2.47±0.15 a	0.000
Hb (g/dl)	3.72±0.13 b	4.24±0.22 B	6.58±0.22 a	7.35±0.20 a	0.000
AST (U/l)	97.55±3.18 a	61.80±2.25 B	35.22±2.15 c	23.50±1.67 d	0.000
ALT (U/l)	31.50±2.10 a	23.44±1.34 B	12.61±1.13 c	7.22±1.21 c	0.000
ALP (U/l)	108.00±2.59 a	72.44±2.16 B	57.16±2.10 c	41.0±1.50 d	0.000
Creatinine (mg/dl)	0.80±0.04 a	0.62±0.03 B	0.36±0.02 c	0.25±0.01 c	0.000
Uric acid (mg/dl)	2.89±0.13 a	2.70±0.10 A	0.90±0.07 b	0.75±0.01 b	0.000

In each row, statistically significant differences ($P< 0.05$) exist between mean values with different letters although means with the same letter do not differ significantly ($P> 0.05$).

3. Antioxidants

As depicted in Table (7), the antioxidant enzymes' activities (SOD and GST) and the non-enzymatic antioxidant (GSH) levels exhibited a notable decrease ($P< 0.05$) in the gill, liver, and muscle tissues of *Solea aegyptiaca* subjected to elevated concentrations of metals in the eastern and southern regions, as opposed to the relatively unpolluted northern and western areas of the lake. Interestingly, the activity of the CAT enzyme showed a significant rise ($P< 0.05$) under the same conditions.

Table 7. Antioxidants in organs of *Solea aegyptiaca* from the studied locations

Antioxidant	Organs	Eastern sector	Southern sector	Northern sector	Western sector	*P value*
SOD (U/ g tissue)	Gills	211.73±9.50 B	217.10±9.86 b	256.57±5.69 a	252.08±7.72 A	0.001
	Liver	258.33±11.05 B	263.15±11.86b	322.36±11.86 a	363.15±8.70 a	0.000
	Muscles	328.94±4.32 b	333.86±3.05 b	342.10±3.28 ab	348.68±3.28 a	0.002
CAT (U/ g tissue)	Gills	0.78±0.02 a	0.76±0.02 ab	0.65±0.01 b	0.62±0.04 bc	0.001
	Liver	0.87±0.02 a	0.80±0.03 ab	0.73±0.04 b	0.60±0.02 c	0.000
	Muscles	0.62±0.045 a	0.50±0.04 ab	0.34±0.05 b	0.32±0.02 bc	0.000
GST (U/ g tissue)	Gills	0.03±0.009 b	0.04±0.01 b	0.09±0.005 ab	0.19±0.04 a	0.001
	Liver	0.04±0.006 b	0.05±0.007 b	0.07±0.003 ab	0.10±0.01 a	0.000
	Muscles	0.01±0.0009 bc	0.02±0.006 b	0.03±0.004 ab	0.04±0.002 a	0.000
GSH (mg/ g tissue)	Gills	56.10±2.81 b	65.86±2.69 b	108.83±5.64 a	126.63±18.28a	0.000
	Liver	81.83±8.98 bc	93.30±11.83 b	135.53±15.33ab	167.73±13.66a	0.000
	Muscles	48.86±2.94 b	53.96±2.90 b	88.83±9.63 a	97.73±12.25 a	0.000

In each row, statistically significant differences ($P< 0.05$) exist between mean values with different letters although means with the same letter do not differ significantly ($P> 0.05$).

4. SDS gel electrophoresis

Protein pattern of gills, liver, and muscles of *Solea aegyptiaca* obtained from different locations along Lake Qarun is depicted in Fig. (1). Gill samples exhibited the presence of an additional band at a molecular weight of 59 KDa in the eastern sector and at 64KDa in the southern sector, in contrast to the relatively unpolluted northern and

western sites. Conversely, liver samples from the eastern, southern, and northern sectors displayed the absence of a band between molecular weights of 26 and 59kDa compared to the western site. Muscles from the contaminated eastern sector revealed the emergence of two additional bands, one at a molecular weight of 59kDa and the other between molecular weights of 59 and 64 kDa in comparison to the other collection sites.

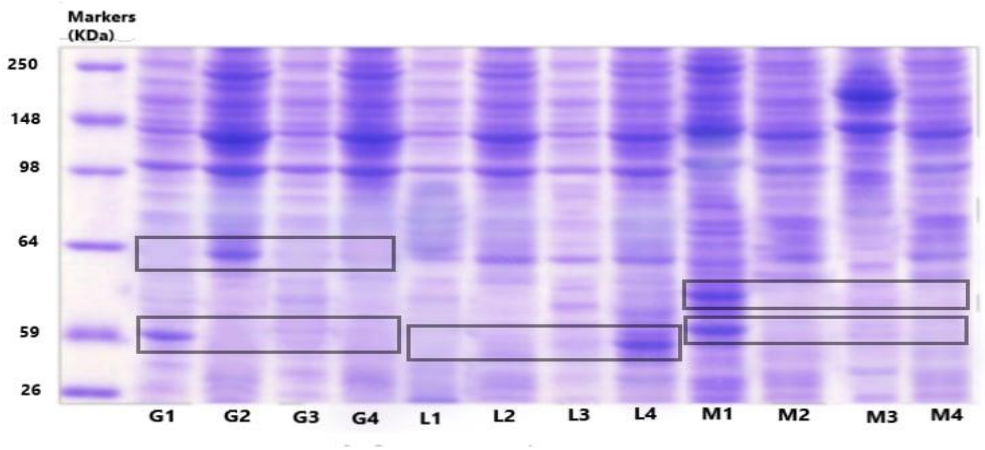

Fig. 1. Electrophoretic protein pattern for gills (G), liver (L), and muscles (M) of *Solea aegyptiaca* collected from the eastern (1), southern (2), northern (3), and western (4) sectors along Lake Qarun

DISCUSSION

Human health may be threatened by the presence of heavy metals that enter the food chain and accumulate in fish tissue within aquatic environments. These metals, which encompass Cu, Zn, Pb, and Cd, among others **(Alprol *et al.*, 2022)**, have exhibited a notable escalation in concentrations across various organs (such as gills, liver, kidney, muscles, and skin) of *Solea aegyptiaca* specimens obtained from the eastern and southern sectors. This trend aligns with previous studies examining the chemical composition of Lake Qarun water **(El-Agri *et al.*, 2021; Hamad *et al.*, 2024)**, reinforcing the assertion that the eastern and southern regions of the lake stand as the most contaminated zones. The substantial influx of agricultural and industrial effluents, laden with heavy metals, directly discharged into the lake in these specific locations from El-Bats and El-Wadi drains, contributes significantly to this pollution. Our findings find additional support from the works of **Ali and Fishar (2005)** and **Hussein *et al.* (2008)**.

Metabolically active tissues, including the liver, kidneys, and gills, are target organs that exhibit a tendency to accumulate heavy metals at increased levels **(Qiao-qiao *et al.*, 2007)**. The heightened metal concentration in these tissues may be attributed to their role in the absorption and depuration of ingested heavy metals, facilitating the

elimination of metal ions from the organism **(Jiaojiao *et al.*, 2020)**. The notable increase in residual heavy metals found in the viscera of *Solea aegyptiaca*, encompassing the liver and kidneys, compared to the edible muscle tissues, aligns with previous findings by **Zaghloul *et al.* (2011)** and **Hamad *et al.* (2024)**. Conversely, the minimal bioaccumulation of heavy metals in the muscles could be associated with the lipid content in muscle tissues, their limited affinity for heavy metal binding, and/or their lower metabolic activity **(Uluturhan & Kucuksezgin, 2007)**.

Toxicants in aquatic ecosystems affect cellular and molecular levels, resulting in notable changes in the biochemical compositions of aquatic biota **(Chowdhury *et al.*, 2004)**. Blood glucose has been utilized as a precise and reliable indicator of fish environmental stress **(Chowdhury *et al.*, 2004; Gagnon *et al.*, 2006)**. According to the current study, the heavy metal contamination from the effluents released directly from the El-Bats and El-Wadi drainage channels may be the cause of the observed hyperglycemia in fish collected from the lake's eastern and southern sectors, where it was demonstrated that metals bioaccumulation alters carbohydrates metabolism, inducing hyperglycemia in fish **(Kumar *et al.*, 2018)**. The observed outcome is consistent with those of **Abdel-Khalek *et al.* (2015)** and **Jagadeshwarlu and Sunitha (2018)**, who noted an elevation in blood glucose level in fish under heavy metal stress conditions.

An essential role of blood proteins is to uphold the osmotic equilibrium between blood and tissue spaces, while also being highly susceptible to metal toxicity **(Sakr & Al Lail, 2005)**. The levels of serum total protein and albumin in fish serve as crucial indicators of liver health and overall well-being **(Nguyen, 1999; Nordlie *et al.*, 1999; Sandre *et al.*, 2017)**. In this study, a decreased total protein and albumin levels was observed in the contaminated *Solea aegyptiaca* from the eastern and southern regions in contrast to the less contaminated specimens from other sites. A significant reduction in total protein content signifies that stress induced by effluent exposure triggers proteolysis to cope with heightened demands **(Kumar & Banerjee, 2016)**. Similar observations regarding protein content were noted by **Zaghloul *et al.* (2011)** and **Abdel-Khalek *et al.*, (2015)**, who documented a decrease in plasma proteins following exposure to heavy metals. Instances of protein loss, including albumin, occur during hemorrhage or external injuries. The diminishing levels of total blood protein and albumin, particularly in response to polluted effluents, reflect the immunosuppressive and hepatic impairing effects of the effluents **(Srivastava & Reddy, 2020)**. Consistent findings were also observed at various sampling intervals in *Labeo rohita* exposed to effluents from thermal power stations **(Anant & Suresh, 2015)**.

Regarding Hb, **Svobodova *et al.* (1997)** asserted that exposure to heavy metals results in diminished Hb content due to hemopoietic disturbances and rapid erythrocyte

cell membrane breakdown. Consequently, the significant decline in Hb content in fish collected from the lake's southern and eastern sections, which were subjected to heavy metal contamination from El-Bats and El-Wadi effluents, can be attributed to the decrease in red blood corpuscles production in the hematopoietic organs under the influence of elevated metal concentrations **(Zaghloul *et al.,* 2016)** or the oxidative harm of RBCs **(Zaghloul *et al.,* 2023)**.

Toxicant-induced hepatotoxicity or liver function is often indicated by enzymes like AST, ALT, and ALP **(Roy and Bhattacharya, 2005; Datta *et al.,* 2007)**. **Boyd (1983)** proposed that the liver harbors abundant AST, ALT, and ALP, hence any injury could lead to the release of substantial amounts of these enzymes into the bloodstream. The notable escalation in AST, ALT, and ALP levels in *Solea aegyptiaca* specimens retrieved from the lake's eastern and southern sections that were subjected to heavy metal contamination from El-Bats and El-Wadi drainage canals compared to the less contaminated northern and western sections, is consistent with the findings of **Zaghloul *et al.* (2011)** and **Omar *et al.* (2013)**. These studies elucidated that the surge in liver enzyme levels could be ascribed to hepatic tissue damage caused by bioaccumulated heavy metals, predominantly metabolized by hepatic parenchymal cells as a crucial detoxification defense mechanism against toxic substances. The elevation in enzyme concentrations in fish due to heavy metal pollution has been documented in numerous other investigations **(Soleimany *et al.,* 2016; Ugbomeh *et al.,* 2019; Al-Hasawi & Hassanine, 2022)**.

Plasma levels of creatinine and uric acid offer a broad indication of glomerular filtration rate and renal impairment **(Ismail & Mahboub, 2016)**. Diminished concentrations of creatinine and uric acid may lack significance, yet an escalation in their levels signifies the existence of various dysfunctions within the kidney **(Maxine & Benjamine, 1985)**. The rise of renal parameters (uric acid and creatinine) could stem from oxidative harm **(Prusty *et al.,* 2011)**. *Solea aegyptiaca* obtained from the heavy metal contaminated eastern and southern sectors of the lake exhibited a notable increase in plasma creatinine and uric acid, elucidated by the impact of heavy metals on the glomerular filtration rate, inducing renal cell impairment and malfunction **(Omar *et al.,* 2013; ALzhrani, 2020)**. **Osman *et al.* (2018)** asserted that harm to muscle tissue, disturbances in nitrogen metabolism, and renal dysfunction lead to diminished excretion of these compounds and a rise in their blood stream levels.

Various categories of environmental pollutants or their metabolites, including heavy metals, can enhance the intracellular generation of ROS, resulting in oxidative damage in fish **(Vinodhini & Narayanan, 2009; Alak *et al.,* 2013)**. According to **Lushchak (2011)**, ROS is an essential marker of oxidative stress in fish, which causes a variety of molecular or cellular changes, including changes in proteins, lipids, carbohydrates, and antioxidants. ROS causes cellular membranes to break down,

becoming permeable and altering fish physiology. Necrosis and apoptosis are the final results of this process **(Ullah *et al.*, 2019)**. Cells have defense systems that include both enzymatic and non-enzymatic antioxidants to protect against oxidative damage **(Padmini & Rani, 2009)**. The utilization of antioxidant profiles, particularly in response to heavy metal exposure, holds significant toxicological implications **(Kumari *et al.*, 2014)**.

Among antioxidant enzymes, SOD stands out as the primary line of defense against oxygen toxicity, attributed to its suppressive impact on oxyradical generation **(Li *et al.*, 2010)**. According to **Peixoto *et al.* (2013)**, SOD helps the superoxide anion radical dismutase into hydrogen peroxide and water, which are then neutralized by CAT. The notable decline in SOD activity observed in *Solea aegyptiaca* tissues retrieved from the eastern and southern regions of Lake Qarun, where heavy metal pollution from drainage channels prevails, aligns with the findings of **Liebler and Reed (1997)**, **Hamed *et al.* (2003)** and **Carvalho *et al.* (2012)**. These studies suggest that the pronounced reduction in SOD activity may stem from the direct binding of metals to the enzyme, triggering oxidative stress and promoting lipid peroxidation.

Together with SOD, CAT constitutes the primary line of defense toward ROS formation **(Pandey *et al.*, 2003)**. It effectively eliminates hydrogen peroxide, a non-radical ROS that has the capability to permeate all biological membranes and directly deactivate specific enzymes. CAT stands as a crucial and delicate biomarker of oxidative stress, shedding light on the impact of biological processes on the redox equilibrium of marine organisms **(Sanchez *et al.*, 2005)**. In the current investigation, the notable escalation in CAT activity within the tissues (gills, liver, and muscle) of *Solea aegyptiaca* collected from the heavy metal contaminated eastern and southern regions of Lake Qarun, compared to the relatively unpolluted northern and western sites, implies the presence of elevated peroxide levels. A comparable trend in CAT activity has been documented in numerous prior studies examining diverse fish species residing in metal-contaminated aquatic environments **(Khalil *et al.*, 2017; Ullah *et al.*, 2021; Ishaq *et al.*, 2023; Gaafar & Mohamed, 2024)**. The increase in CAT levels may be attributed to the existence of a robust antioxidant defense mechanism designed to combat the oxidative stress triggered by metal exposure. Moreover, it could potentially offset the decline observed in other enzymatic antioxidants, such as SOD and GST.

The GST is a collection of widely distributed enzymes that catalyze the conjugation of GSH with substances that contain reactive electrophilic groups, especially xenobiotics like metals and pesticides. These enzymes help create less toxic and more water-soluble particles **(Olsen *et al.*, 2001)** and are essential for preventing oxidative damage **(Barata *et al.*, 2005; Fernandes *et al.*, 2008)**. Nucleophilic groups in macromolecules such as proteins and nucleic acids are protected by this metabolic

process. The phase II biotransformation pathway includes the induction of GST by organic pollutants **(Banni *et al.,* 2011)**, but GST inhibition has been recognized as an unusual reaction to chemical exposure **(Greco *et al.,* 2010)**. The diminished activity of GST observed in all organs of *Solea aegyptiaca* fish from the impacted regions of Lake Qarun may result from inactivation by ROS produced by pollutants **(Martinez-Lara *et al.,* 1996)** or could be linked to reduced levels of GSH available for conjugation **(Carvalho *et al.,* 2012)**. Furthermore, consistent with our findings, **Beyer *et al.* (1997)**, **Vigano *et al.* (2001)**, **Oliva *et al.* (2012)** and **Jebali *et al.* (2013)** stated no alterations or a decline in GST activity in various fish organs under stressful conditions and field exposure to toxic substances, indicating a limited detoxification process taking place.

GSH is the most extensively researched antioxidant molecule in fish **(Viarengo & Nott, 1993)**. It interacts with various environmental contaminants **(Rajeshkumar & Li, 2018)** and is acknowledged as a primary cellular defense against metals through chelation and detoxification processes, as well as the scavenging of oxyradicals and involvement in detoxification reactions catalyzed by glutathione peroxidase **(Sies, 1999)**. Prior investigations have demonstrated that glutathione reductase, the enzyme that converts glutathione from its oxidized form (GSSG) to its reduced form (GSH), is rendered inactive by heavy metals, leading to diminished GSH levels **(Sandhir *et al.,* 1994)**. The depletion of cellular GSH content below a critical threshold can induce oxidative stress, hindering the binding of metals to GSH and allowing them to bind covalently with cellular proteins **(Yamano & Morita, 1995)**. The substantial reduction in GSH concentration in the organs (gills, liver, and muscle) of polluted *Solea aegyptiaca*, in comparison to less contaminated fish, aligns with the findings of **Yildirim *et al.* (2011)** in the liver and gills of *Capoeta trutta* fish, as well as **Ahmed (2013)** in the gills and muscles of *Tillapia niloticus* and *Siluriformes* fish. The decline in activity of GSH could be attributed to an increased GSH use, leading to the conversion of GSH into its oxidized form, and ineffective GSH synthesis **(Yildirim *et al.,* 2011)**. In addition to its antioxidant function, GSH acts as a substrate for GST activity and plays a crucial role in scavenging hydrogen peroxide within cells and promoting detoxification processes **(Ruas *et al.,* 2008)**. Hence, the diminished GST activity in the organs of polluted *Solea aegyptiaca* fish, as observed in this study, may be linked to the reduced GSH levels.

In the current investigation, electrophoresis analysis of gills, liver, and muscle proteins was employed to distinguish between the various study sites, revealing a pronounced disparity between samples from polluted and less polluted environments. It is postulated that the emergence of novel protein bands in the gill and muscle tissues of *Solea aegyptiaca* in heavy metal contaminated areas may signify the presence of stress proteins that serve to counteract the harmful consequences of heavy metal exposure, as documented by **Muthukumaravel *et al.* (2007)**. Conversely, the reduction in protein bands in the liver of contaminated *Solea aegyptiaca* could be attributed to either the disruption of the protein synthesis pathway **(Díaz-Villanueva *et al.,* 2015)** or the

depletion of reserve proteins aimed at mitigating the stress induced by heavy metal exposure, given the liver's susceptibility to environmental stressors. Our findings align with prior research indicating that the disappearance of protein bands is a consequence of pollution exposure **(Osman *et al.*, 2010; Hamdy *et al.*, 2016)**. Overall, electrophoresis revealed unique protein bands in fish from the polluted zone, suggesting stress-induced protein expression or degradation.

CONCLUSION

This study emphasizes how seriously heavy metal pollution affects Lake Qarun's *Solea aegyptiaca*, as evidenced by alterations in metal accumulation, biochemical responses, antioxidant activities, and protein profile. Fish from the lake's eastern and southern portions that were exposed to drainage water from El-Bats and El-Wadi drains, exhibited significantly higher levels of heavy metals in vital organs, with Zn, Fe, and Ni concentrations in muscle tissue exceeding permissible thresholds for human consumption. Biochemical markers such as elevated glucose, liver enzymes (AST, ALT, ALP), and kidney functions indicate physiological stress caused by metal exposure. The observed variations in antioxidant enzyme activities, with reductions in SOD, GST, and GSH levels and increased CAT activity, reflect adaptive responses to oxidative damage. Protein profile changes, including the emergence and disappearance of specific bands, further confirm the detrimental effects of heavy metal exposure on cellular function. These findings demonstrate the utility of biochemical, antioxidant, and molecular markers as effective tools for assessing environmental pollution and its biological consequences. They also emphasize the urgent need for implementing stricter pollution control measures and continuous biomonitoring to safeguard aquatic environments and public health.

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