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Research Article

Ecotoxicological Consequences of Heavy Metals on Emperor Fish *(Lethrinus)* Species in the Red Sea: Histopathology and Biochemistry

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Abstract

Heavy metal (HMs) contamination in marine environments is a global concern, affecting fish health and seafood safety. This study examined the effects of HMs on 90 emperor fish from the Red Sea, focusing on metal levels in water and fish liver, blood parameters, and liver histopathology. Using inductively coupled plasma mass spectrometry, different levels of heavy metals were detected. While some metals, such as Pb, Cu, and Zn, were undetectable in seawater from both northern and southern regions, Ni, Fe, and Mn were higher in the north. As a result, compared to the South area, fish from the North showed a significant increase in serum hepatic enzymes, with ALT, AST, ALP, and GGT elevated by 27.3%, 36.9%, 11.6%, and 35.4%, respectively. Bilirubin levels were 20.5% higher in the North, indicating liver dysfunction. This matches liver histology findings, where the North area had a mean liver damage score of 2.0, indicating moderate hepatocellular degeneration, congestion, and prominent melanomacrophage centers. Conversely, the South area had a lower mean liver damage score of 0.5, reflecting mostly normal or mild changes and relatively intact liver structure. The study concludes that HMs poisoning significantly harms fish in the north, emphasizing the importance of biochemical and histopathological markers in assessing marine pollution and its risks to both environmental health and food safety.

Keywords: Biomarker's analysis, Fish, Food safety, Heavy metals, Environmental pollution, Histological techniques, Liver

Introduction

Food security, defined in numerous ways, is commonly understood as ensuring access to sufficient, quality food for all, through availability, accessibility, and sustainable practices and prevent hunger [1]. A strong food security system ensures access to safe, nutritious food for a country's population [2]. Aquatic animal production from fisheries and aquaculture worldwide reached 177.8 million tons in 2020 [3-5]. For many people, especially in coastal cities, fish is an important source of food because they are high in vitamins and minerals, low in fat, and have a considerable source of protein. For the prevention of chronic diseases linked to diet, public health guidelines often include 1-2 weekly servings of fish [6]. The internationally recognized definition of marine pollution encompasses the introduction of chemicals into the marine environment, either directly or indirectly through human activities, resulting in detrimental effects on biota and water quality [7]. Marine ecosystems face significant risk from industrial and domestic pollutant discharge [8,9]. The residence time of pollutants in estuaries results in a significantly greater impact on the coastal zone compared to inland river systems [10]. The presence of heavy metals and organic pollutants within marine environments constitutes a significant ecological concern. Heavy metal deposition in fish organs is influenced by metal concentration, exposure time, absorption, environmental factors (temperature, hardness, pH salinity, water and sediment metal levels, ecological needs), and fishing period and the water's physical and chemical properties, and biological factors (age, feeding, size, gender). The primary organs of metal bioaccumulation are the gills, kidneys, and liver [11-13].

Heavy metal toxicity represents a major environmental and health concern, as exposure to these elements is associated with a wide range of adverse health effects. Although heavy metals have no essential biological role in the human body, their accumulation can impair physiological processes and disrupt normal biological functions. Prolonged retention of these toxic elements may result in chronic health conditions. The severity of metal toxicity depends on the absorbed dose, the route of exposure, and the duration of contact [14]. Heavy metals exhibit several detrimental characteristics that make them particularly concerning from both environmental and biological perspectives. They possess long biological half-lives, allowing them to remain in organisms for extended periods without efficient metabolic elimination. Additionally, they have a high capacity for bioaccumulation and biomagnification, enabling them to concentrate in tissues and increase in abundance through the food chain. Their chemical stability contributes to strong environmental persistence, meaning they resist natural degradation processes and remain in ecosystems for decades or longer, posing sustained ecological and health risks [15].

The Red Sea represents a highly diverse and ecologically rich marine ecosystem, with more than 1.200 documented species of fish [16]. Sewage represents a significant environmental threat across the region. Coastal zones also host power and desalination plants, oil refineries, fertilizer production facilities, and chemical industries [17].

Fish and other seafood are frequently among the primary sources of metal exposure in the general population. When toxic metal concentrations in foods exceed permissible limits, they pose risks to human health and are prohibited from trade under many national and international regulations [18]. The experimental sites in Jeddah are subject to multiple contamination sources that significantly impact coastal water quality and marine ecosystems. The northern site (Dahaban) is near to Distillation Plant Dahaban which is a Water treatment plant. The southern site (Sarum) is close to the SAWACO seawater desalination plant and the Jeddah 2nd industrial city. These anthropogenic inputs may create distinct pollution gradients, making these locations ideal for comparative studies on heavy metal contamination and its effects on marine biota in the Red Sea ecosystem. This study investigates the effects of heavy metals on fish from the Red Sea, Jeddah. The research involves measuring the concentrations of selected heavy metals in seawater and fish liver, evaluating their impact on specific blood parameters, and examining histopathological alterations in fish liver tissue.

Materials and Methods

Ethical Approval

The animal study has been reviewed and approved by ZU-IACUC committee. was performed in accordance with the guidelines of the Egyptian Research Ethics Committee and the guidelines specified in the Guide for the Care and Use of Laboratory Animals (2025). Ethical code number ZU-IACUC/3/F/284/2025.

Study Sites and Fish Sampling

This study was conducted from 2023 to 2024 in one of the major urban cities of the Kingdom of Saudi Arabia. Two sampling sites were selected, situated in the north and south of Jeddah. The northern site (Dahaban) is located about 50 km north of Jeddah city, at GPS coordinates 22°03'11.5"N and 38°55'16.5"E. It includes fishing areas and is close to the Distillation Plant Dahaban, which is a Water treatment plant. The southern site (Sarum) is located on the southern corniche of Jeddah, at GPS coordinates 21°07'32.5"N and 39°10'48.7"E, close to SAWACO seawater desalination plant and the Jeddah 2nd industrial city *(Fig. 1)*.

Fig 1. Detailed map of Jeddah showing sampling locations

Eighteen water samples were collected from each site to ensure the collection of representative data. A map showing the geographic locations of these sampling stations in the northern and southern regions of Jeddah. The sites were selected based on their contrasting environmental conditions and pollution levels.

A total of 90 specimens of the emperor fish species, carset *(Lethrinus lentjan,* 21), Abu Bose *(Lethrinus microdon,* 12), Mohaysni *(Lethrinus mahsena,* 15), Abu Sirin *(Lethrinus obsoletus,* 9), khorrami *(Lethrinus nebulosus,* 8), Abu Nuqtah *(Lethrinus harak,* 7), saqa *(Lethrinus borbonicus,* 9), and abu Zahu - Kharmiya *(Lethrinus xanthochilus,* 9) were collected in a fish trap (cage) by local fishermen at each site. The species of fish were identified according to external features by Abu Shusha et al.[19]. Fish samples were kept alive in clean tanks containing aerated seawater and taken to the laboratory. For each fish, morphological measurements and blood drawn were taken. Then, all fish samples were dissected on the same day. The most abundant species of emperor fish were *Lethrinus lentjan* (Carset), *Lethrinus mahsena* (Mohaysni) and *Lethrinus microdon* (Abu Bose).

Water Analysis

The surface water samples from the north and south areas were collected in clean 1-L plastic bottles. All sample bottles were immediately transferred to a cool box to the National Water Company laboratory in Jeddah, where analyses were conducted on the same day of collection to avoid potential temporal effects on water quality parameters and heavy metal speciation.

Physicochemical Parameters

Each water sample was analyzed for water temperature (°C), pH, total dissolved solids (mg/L), and conductivity (µS/cm) using the multi meter (HACH, HQ40d, Loveland, CO, USA). Turbidity (NTU) was measured with the portable turbidimeter (HACH, 2100Q). Total hardness (mg/L), total alkalinity (mg/L), and chloride (mg/L) were determined using the AUTO Titration (Metrohm, 905 Titrando, potentiometric titration, Switzerland). Ammonia (mg/L), nitrate (mg/L), sulfate (mg/L), and iron (mg/L) were measured with the Laboratory Spectrophotometer (HACH, DR 5000™ UV-Vis).

Heavy Metal Analysis

The seawater samples were acidified to pH <2 with ultrapure nitric acid immediately upon arrival at the laboratory and then subjected to membrane filtration (0.45 µm) to remove particulates. Acidification and digestion were performed in the laboratory on the same day as collection, rather than in the field, to ensure sample integrity while allowing precise control of reagent purity and digestion conditions. Seawater samples were then diluted and analyzed for copper (Cu), zinc (Zn), lead (Pb), manganese (Mn), cadmium (Cd), and nickel (Ni) using a Perkin Elmer NexION 300X Inductively Coupled Plasma–Mass Spectrometer (ICP-MS). The ICP-MS was selected for seawater analysis due to its superior multi-element capability, very low detection limits (sub-µg/L), and high throughput, which are critical for trace-level determinations in complex saline matrices. The metals concentration was analyzed according to the American Public Health Association (APHA 3125) standard method. Metal concentrations in water samples are expressed as mg/L.

Morphological Parameters of Fish

In the laboratory, morphological measurements as conducted for all collected specimens. For each fish, the total body length was measured to the nearest centimeter using a tape measure on a flat surface, and the total body weight was recorded to the nearest gram using digital balance (OHAUS, Scout pro balance). The condition factors of fish samples were calculated using the following equation [17]:

Condition Factor (K) = [body weight (g)/body length^3 (cm^3)] *100.

In addition, other parameters (standard length, total length, fork length, head length, eye diameter, and body depth) of each fish were measured in (cm).

Biochemical and Hematological Parameters

The fish were anesthetized by adding 3-5 drops of clove oil to the water tank. Blood samples were withdrawn from the caudal vein using a heparinized syringe. Each blood sample was analyzed for the total cell count of red blood cells (RBCs), white blood cells (WBCs), neutrophils, lymphocytes, monocytes, eosinophils, basophils, hemoglobin (HGB), and platelets (PLT). A commercial kit for a complete blood count (CBC) from Bio-Lab Diagnostics Ltd. (Mumbai, India) was used to assess the blood cell count, following the manufacturer's recommendations. A hemocytometer was used in blood cell count, in which the blood diluting fluid was prepared as described by Svobodova et al.[20]. The blood cells were counted on the counting chamber of a hemocytometer with the aid of a compound microscope.

In addition, blood samples were centrifuged at 2000 rpm for 20 min to obtain blood serum which was kept frozen at –20°C until being processed for various biochemical analyses: total protein [TP, Cat no 04810716, Roche Diagnostics (Switzerland)], albumin [ALB, OSR6102, Beckman Coulter (USA)], total bilirubin [BL, DF30, Siemens Healthineers (Germany)], aspartate aminotransferase [AST, 3L82, Abbott Diagnostics (USA)], alanine aminotransferase [ALT, 3L52, Abbott Diagnostics (USA)], gamma-glutamyl transferase [GGT, 3L72, Abbott Diagnostics (USA)], and alkaline phosphatase [ALP, 3L62, Abbott Diagnostics (USA)]. The biochemical analyses of blood samples were analyzed by Dynex Best 2000 automated microplate Immuno analyzer (DSX Automated Elisa System, Germany, Listing# 835485).

Collection of Liver Tissue for Heavy Metal Analysis

The dissected liver was removed and weighed to calculate the liver weight to body weight ratio then it was divided into liver tissue which will be fixed in 10% buffered formalin for histopathological examination. The other liver lobe will be frozen and kept at -20°C until biomarker analysis. Also, the concentrations of heavy metals iron (Fe, mg/kg), zinc (Zn, mg/kg), copper (Cu, mg/kg), manganese (Mn, mg/kg), nickel (Ni, mg/kg), lead (Pb, mg/kg), and cadmium (Cd, mg/kg) were measured in liver tissue using atomic absorption spectrophotometer (BIO RAD, SmartSpec Plus spectrophotometer). Liver digests were analyzed by

Atomic Absorption Spectrophotometry (AAS) because tissue matrices often require matrix-matched calibration, and AAS provides robust, cost-effective quantification for individual metals at higher concentration ranges. All metal determinations followed the APHA Method 3125 standard protocol, and results are reported as mg/L for water and mg/kg wet weight for liver samples. The liver tissues were transferred to an oven set at 80°C for 8 h until completely dried. Dry tissues were then digested in a mixture of concentrated nitric (HNO_3) and perchloric acids ($HClO_4$) according to the method described by Neugebauer et al.[21]. The mixture in a flask was gently shaken and placed on a hot plate until the tissues were completely digested to clear solutions. Then, the concentration (mg/L) of heavy metals in the solution is measured using an atomic absorption spectrophotometer.

Collection of Liver Tissue for Histological Examination

Fixed liver in phosphate-buffered formal saline was dehydrated and embedded in paraffin as blocks. Then they were sectioned (8 µm thickness), spread on glass slides, and stained with haematoxylin and eosin (H & E) [22].

Statistical Analysis

The statistical analyses for the data were conducted using the Statistical Package for the Social Sciences (SPSS). Significant differences between the north and south areas were tested using two-sample t-tests at a probability level of P<*0.05*.

Results

Physicochemical Parameters of Seawater

The physicochemical parameters of seawater at the north and south areas are presented in in *Table 1*. Surface water temperature and total hardness were relatively consistent between the two sites. Electrical conductivity and total dissolved solids (TDS) were slightly higher in the northern area compared to the southern area. A notable difference in pH was observed, with the southern area showing a significantly higher pH than the northern area (P<0.01). Additionally, turbidity was significantly higher in the north than in the south (P<0.01). Total alkalinity was significantly greater in the southern site compared to the northern site (P<0.05). The sulfate concentration was higher in the north than in the south (P<0.05). No noticeable differences were observed in chloride and nitrate levels between the two locations, and ammonia concentrations remained below detectable levels (<0.1 mg/L) across all samples.

Heavy Metals in Water

Table 2 displays the amounts of heavy metals in water samples taken from the north and south locations. All samples had cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn) levels below the limit of detection, which is less than 0.002 mg/L. The north had greater quantities of nickel (Ni) than the south. The northern samples had slightly higher concentrations of iron (Fe) than the southern ones. The north area had a higher concentration of Mn in water samples compared to the south area.

Morphological Parameters of Fish

Table 3 and *Table 4* display the data for each fish species from the north and south areas with respect to their total body weight, liver weight, standard length, fork length, body depth, head length, eye diameter, condition factor (K), and hepatosomatic index, which is the ratio of liver weight to body weight. The total body weight of *L. lentjan* was highest in the northern region, whereas that of *L. borbonicus* was lowest. When compared to other species in the south, *L. xanthochilus* had the highest total body

Table 1. *Mean ± SE of physicochemical parameters in seawater from two areas*

Parameters	North	South	P Value
Temperature (°C)	22.143±0.15	22±0	0.9 NS
Electrical Conductivity (mS/cm)	62.09±0.93	61.84±0.05	0.89 NS
Total dissolved solids (mg/L)	41942.86±1046.96	39820±20	0.75 NS
pH	7.45±0.14	8.082±0.03**	<0.01
Turbidity (NTU)	9.14±1.08**	5.38±0.20	<0.01
Total Hardness(mg/L)	7862.43±55.64	7898.4±22.77	0.99 NS
Total Alkalinity(mg/L)	180±10.19	210.4±6.14*	<0.05
Ammonia(mg/L)	<0.1	<0.1	-
Chloride(mg/L)	34423.86±2408.54	29236.2±713.84	0.13 NS
Nitrate(mg/L)	1.02±0.31	1.19±0.08	0.89 NS
Sulfate(mg/L)	861.86±316.12*	349.4±48.55	<0.05

*Two- sample t-test, * P<0.05; ** P<0.01, NS: Not significant*

Table 2. Mean ± SE of some heavy metal concentrations in water from two areas

Heavy Metals	North	South	WHO (2011)	P Value
Cd(mg/L)	<0.002	<0.002	0.003	0.99 NS
Pb(mg/L)	<0.002	<0.002	0.01	0.95 NS
Cu(mg/L)	<0.002	<0.002	2.00	0.99 NS
Zn (mg/L)	<0.002	<0.002	3.00	0.99 NS
Ni (mg/L)	1.68±1.32*	0.18±0.05	0.05	<0.05
Fe (mg/L)	0.077±0.006	0.070±0.006	0.30	0.98 NS
Mn (mg/L)	1.04±0.22	0.67±0.19	0.50	0.045

*Two- sample t-test; * P<0.05; ** P<0.01; NS: Not significant*

weight while *L. obsoletus* had the lowest. The liver weights of the northern *L. lentjan* and *L. nebulosus* were higher than those of the other species, whereas the liver weights of the *L. microdon* were much lower. Liver weight was highest in *L. mahsena* and lowest in *L. obsoletus* in the southern region.

In the northern region, *L. lentjan* had the longest total body length, whilst *L. borbonicus* had the shortest. The total body length of *L. xanthochilus* was the longest in the southern region, whilst that of *L. obsoletus* was the shortest.

Similarly, in the northern region, *L. lentjan* had the longest forks and *L. borbonicus* the shortest. In the southern region, *L. xanthochilus* had the longest forks and *L. obsoletus* the

Table 3. Mean ± SE of morphological parameters of fish from two areas

Area	Species	Total Body Weight (g)	Liver Weight (g)	Total Body Length (cm)	Condition Factor (K)	Hepatosomatic Index
North	*L. lentjan*	397.24±26.01	3.90±0.35	30.11±0.79	1.42±0.03	1.01±0.07
	L. obsoletus	240.25±0.25	2.60±0.2	25.7±0.10	1.46±0.025	1.05±0.05
	L. nebulosus	226.5±36.5	3.45±0.95	24.75±1.05	1.48±0.05	1.49±0.18
	L. mahsena	193.58±20.28	2.60±0.37	22.49±0.76	1.63±0.03	1.30±0.09
	L. microdon	165.29±37.80	1.68±0.44	22.60±1.55	1.30±0.05	1.02±0.11
	L. borbonicus	165.15±22.85	1.80±0	22.30±0.40	1.48±0.13	1.11±0.15
South	*L. xanthochilus*	221.25±68.75	1.85±0.55	25.40±2.10	1.30±0.09	0.84±0.01
	L. mahsena	218.67±45.20c	3.13±0.84	23.23±1.25	1.70±0.09	1.40±0.08
	L. harak	183.08±11.79	2.34±0.23	23.18±0.54	1.41±0.02	1.22±0.09
	L. obsoletus	126.4±0.2	1.15±0.15	21.25±0.5	1.31±0.12	0.80±0.01

Table 4. Mean ± SE of morphological parameters of fish from two areas

Area	Species	Fork Length (cm)	Standard Length (cm)	Body Depth (cm)	Head Length (cm)	Eye Diameter (cm)
North	*L. lentjan*	28.23±0.71	25.70±0.65	10.08±0.28	8.44±0.25	1.65±0.04
	L. obsoletus	24.55±0.05	22.45±0.25	8.50±0.1	7.70±0.2	1.75±0.15
	L. nebulosus	23.30±0.70	20.95±0.55	8.35±0.45	6.95±0.25	1.60±0.1
	L. mahsena	21.12±0.70	19.71±0.85	8.67±0.25	6.52±0.21	1.65±0.07
	L. microdon	21.03±1.39	19.38±1.29	7.45±0.29	6.55±0.53	1.54±0.04
	L. borbonicus	20.70±0.20	19.65±0.15	8.10±0.80	6.20±0	1.45±0.15
South	*L. xanthochilus*	23.70±2.20	22.0±1.90	7.25±0.15	7.75±0.85	1.90±0.10
	L. mahsena	21.77±1.16	20.27±1.16	8.97±0.52	6.63±0.33	1.60±0
	L. harak	21.70±0.51	19.77±0.49	7.70±0.19	6.37±0.18	1.60±0.04
	L. obsoletus	19.75±0.5	18.1±0.1	7.15±0.05	6.05±0.15	1.45±0.05

shortest. Similar patterns emerged when looking at the standard length, with *L. lentjan* being the longest in the northern region and *L. borbonicus* and *L. microdon* were the shortest. Standard length was greatest for *L. xanthochilus* and lowest for *L. obsoletus* in the southern region. Among the northern species, the body depth in north area was the highest in *L. lentjan* and whereas was the lowest in *L. microdon*. The body depth in south area of *L. mahsena* was the highest, whereas *L. obsoletus* was the lowest among other fish species.

Head length was greatest in *L. lentjan* in the northern region and lowest in *L. borbonicus*. The southern region showed the greatest head length in *L. xanthochilus* and the shortest in *L. obsoletus*. In terms of eye diameter, the northern species of *L. obsoletus* had the largest measurement, while the southern species of *L. borbonicus* had the smallest. In the southern region, *L. obsoletus* had the smallest eyes and *L. xanthochilus* the biggest.

In the northern region, *L. mahsena* had the greatest condition factor (K) value, whilst *L. microdon* had the lowest. While *L. obsoletus* and *L. xanthochilus* displayed the lowest values in the southern area, and *L. mahsena* had the highest. In the northern region, *L. nebulosus* had the greatest hepatosomatic index, whilst *L. lentjan* and *L. obsoletus* had the lowest. The hepatosomatic index was highest in *L. mahsena* and lowest in *L. xanthochilus* and *L. obsoletus* in the southern region.

Biochemical Blood Analysis

The biochemical analysis of fish serum from the North and South areas revealed significant differences across several liver function markers, protein metabolites *(Table 5)*. Fish from the North area exhibited higher mean values for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) compared to those from the South area, suggesting increased hepatic enzyme activity and possible hepatocellular stress or damage in the North. In addition, albumin, bilirubin and total protein concentrations were slightly higher in the North area, further supporting altered liver function or protein metabolism in the North relative to the South.

Hematological Parameters of Blood

The values of WBCs, RBCs, platelets, hemoglobin, neutrophils, lymphocytes, monocytes, eosinophils, and basophils in blood fish samples are shown in *Table 6*. In this study, the average hematological parameters during the study period were higher in the northern

Table 5. *Mean ± SE of biochemical parameters in fish serum from two areas*

Parameter		Unit	North Area	South Area	P Value
Liver Function Markers	ALT	U/L	30.48±4.32*	23.94±8.92	<0.05
	AST	U/L	41.29±5.91*	30.16±10.25	<0.05
	ALP	U/L	70.56±10.21*	63.20±13.80	<0.05
	GGT	U/L	35.89±6.87*	26.50±9.75	<0.05
Protein and Metabolites	Albumin	g/dL	4.60±0.52	3.86±0.78b	0.75 NS
	Bilirubin	mg/dL	1.06±0.18	0.88±0.14	0.62 NS
	Total Protein	g/dL	6.63±0.39	6.50±0.74	0.81 NS

*Two- sample t-test; * P<0.05; ** P<0.01; NS: Not significant*

Table 6. *Mean ± SE of hematological parameters in fish blood from two areas*

Hematological Parameters	North	South	P Value
WBCs (cells/cubic millimetre)	$12.26 \pm 0.97 \times 10^3$	$9.05 \pm 1.06 \times 10^3$	0.67 NS
RBCs (cells/cubic millimetre)	$4.67 \pm 0.60 \times 10^6$	$6.32 \pm 0.58 \times 10^6$	0.58 NS
Platelets (cells/cubic millimetre)	783.42±76.71*	630.5±60.19	<0.05
Haemoglobin (g/dl)	10.7±0.53	12.23±0.59	0.15 NS
Neutrophil (%)	31.83±1.87	45.00±3.78*	*<0.05*
Lymphocyte (%)	53.17±2.50*	42.83±2.07	*<0.05*
Monocyte (%)	11.00±1.2	9.33±1.43	0.11 NS
Eosinophil (%)	3.52±0.61	2.17±0.60	0.23 NS
Basophil (%)	0.5±0.15	0.67±0.21	0.74 NS

*Two- sample t-test; * P<0.05; ** P<0.01; NS: Not significant* area for some parameters. Conversely, levels of RBCs, hemoglobin, neutrophils, and basophils were higher in the southern area.

Although white blood cell counts were greater in the northern region than in the southern region, this difference did not reach statistical significance (P>0.05). Relative to this, there was no discernible difference between the north and the south in terms of RBC levels; nonetheless, southern levels were higher. Northern platelet counts were significantly greater than southern ones (P<0.05). While there was no statistically significant difference, northern regions had lower hemoglobin levels and southern regions had greater ones. In the south, there was a noticeable and statistically significant rise in neutrophil counts when contrasted with the north (P<0.05). On the other hand, there was a slight but noticeable increase in lymphocyte counts in the north. The north had somewhat higher numbers of monocytes and eosinophils, whereas the south had somewhat higher levels of basophils. But these variations did not reach a statistically significant level.

Heavy Metal Determination in Liver

Table 7 presents an analysis of heavy metal concentrations in the liver tissue. The accumulation pattern varied between the two areas: the North area showed higher concentrations of most metals, including Iron (Fe), Zinc (Zn), Manganese (Mn), Cadmium (Cd), and Nickel (Ni), may suggesting a broader and more intense source of industrial or multiple pollution sources in that region. Conversely, the South area had higher levels of Copper (Cu) and Lead (Pb), which could point to different contamination sources, such as agricultural practices (e.g., copper-based pesticides) or historical use of leaded fuel. The high levels of Cd are especially concerning because this metal has no biological role and is known to cause extensive cellular damage, impair neurological functions, and lead to organ failure. This data provides a clear and compelling explanation for the physiological changes previously observed in these fish. The significantly elevated liver enzymes (ALT, AST) are classic biomarkers of liver cell damage and necrosis, directly linked to the toxic effects of these accumulated metals.

Table 7. *Mean ± SE of heavy metal concentrations (mg/kg wet weight) in liver tissue of fish from two areas*

Heavy Metal	North Area	South Area	P Value
Fe (mg/kg)	2.31±0.30**	1.40±0.11	<0.01
Cu (mg/kg)	0.86±0.10	1.25±0.12*	<0.05
Zn (mg/kg)	2.79±0.15**	1.55±0.16	<0.01
Pb (mg/kg)	1.59±0.11	2.19±0.16*	<0.05
Mn (mg/kg)	2.01±0.12	1.68±0.10	0.88 NS
Cd (mg/kg)	4.26±0.22**	2.79±0.20	<0.01
Ni (mg/kg)	2.44±0.26**	1.29±0.11	<0.01

*Two- sample t-test; * P<0.05; ** P<0.01; NS: Not significant*

Histological Examination of Fish Liver

The liver of fish from south area: The largest portion of the liver consists of hepatic tissue, while a smaller portion includes pancreatic tissue, blood sinusoids, and bile ducts. In some specimens, vacuolated hepatocytes with deeply stained nuclei, exocrine pancreatic tissue surrounding the portal vein, and connected to blood sinusoids were observed *(Fig. 2-A)*. Their cytoplasm is filled with lipids in the form of large lipid droplets. Additionally, histological examination revealed blood congestion in the blood sinusoids of most of the studied fish. Bile ducts, mostly located near the central vein in the liver tissue and close to exocrine pancreatic tissue *(Fig. 2-B,C)*, were also observed. Furthermore, some specimens displayed exocrine pancreatic tissue surrounding the portal vein, separated from hepatocytes by wide spaces

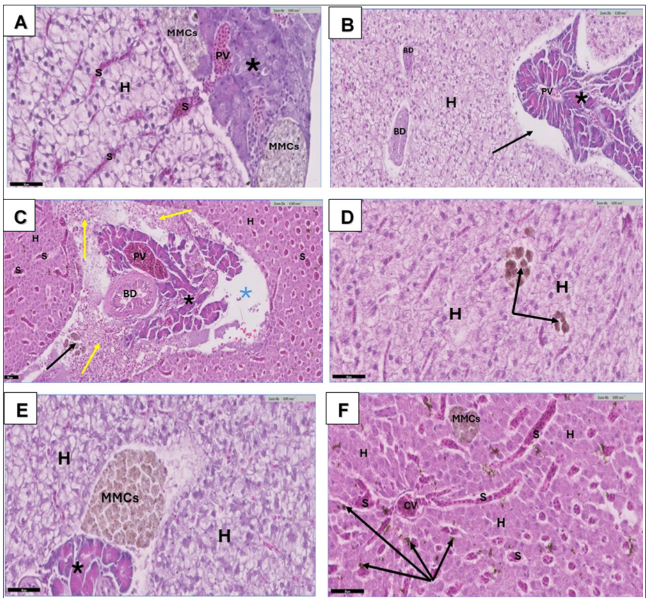

Fig 2. Histological sections of liver of south area showing: (A) Vacuolated hepatocytes (H) with deeply stained pyknotic nuclei, exocrine pancreatic tissue (*) surrounding portal vein (PV) and connected with blood sinusoids (S). Note: the melano–macrophage center (MMCs) inside the exocrine pancreas (X 400 H&E). (B): Another liver section with numerous congested blood sinusoids (s) between deeply stained acidophilic hepatocytes (H), exocrine pancreatic tissue (*) surrounding portal vein (PV) and bile duct (BD). Note: The exocrine pancreatic tissue separated from hepatocytes by wide space (blue star), blood exudate (yellow arrows) and brown pigments (black arrow) (X 200 H&E). (C): numerous congested blood sinusoids (s) between deeply stained acidophilic hepatocytes (H), exocrine pancreatic tissue (*) surrounding portal vein (PV) and bile duct (BD). Note: The exocrine pancreatic tissue separated from hepatocytes by wide space (blue star), blood exudate (yellow arrows) and brown pigments (black arrow) (X 200 H&E). (D): accumulation of brown pigments (black arrows) between the vacuolated hepatocytes (H) (X 400 H&E). (E): vacuolated hepatocytes with deeply stained pyknotic nuclei (H), melano–macrophage center (MMCs)and exocrine pancreas (X 400 H&E). (F): numerous congested blood sinusoids (s) between deeply stained acidophilic hepatocytes (H) and surrounding the central vein (CV). Note: the presence of brown pigments (black arrow) between the hepatocytes (X 400 H&E)

containing blood exudate and brown pigments *(Fig. 2-C)*. The bile ducts are lined with cuboidal cells and are visible between the hepatocytes *(Fig. 2-B)*. The presence of hemosiderin pigments was noted on hepatocytes, near blood sinusoids of fish from the southern area, and between the vacuolated hepatocytes *(Fig. 2-D,F)*. Numerous congested blood sinusoids were observed between deeply stained acidophilic hepatocytes *(Fig 2F)*. The melano-macrophage center (MMC) is a special type of macrophage that contains various pigments, appears as a pigment cluster, and is surrounded by a capsule of simple squamous epithelium. Moreover, some MMCs were found surrounded by exocrine pancreatic tissue within the interstitial tissue, as well as located in the hepatic parenchyma *(Fig. 2-A,E,F)*. These macrophages have small peripheral nuclei and contain various pigments in their cytoplasm, including brown melanin pigments.

The Liver of Fish From the Northern Area

The liver of fish from the northern area shows that the parenchymal hepatocytes are radially arranged around a central vein in cords of two cells thick. Narrow, straight blood sinusoids stemming from the central vein separate each cord. These sinusoids are covered by typical elongated endothelial cells with flattened nuclei *(Fig. 3-A)*. The H&E-stained sections reveal hepatocytes with a normal appearance of vacuolar cytoplasm, likely due to numerous lipid droplets, with small amounts of cytoplasm and rounded, vesicular nuclei *(Fig. 3-B)*. The pancreatic exocrine tissue is distributed within the liver as part of the hepatopancreas. Microscopic observations indicate that pancreatic cells are differentiated from hepatic tissue by their basophilic basal part and eosinophilic apical cytoplasm *(Fig. 3-B,D)*. The epithelium of bile ducts is simple *(Fig. 3-C)*. Hepatocytes that are highly vacuolated and degenerated, showing nuclear karyolysis, along with a dilated and congested central vein with a thick wall and clumps of brown pigments, were observed *(Fig. 3-E)*. In some specimens, a C-shaped hyaline, acidophilic structure, possibly a worm, surrounded by a connective tissue capsule and located near the central vein, was seen *(Fig. 3-F)*. Cells from the MMC aggregations are situated close to the hepatic blood vessels, bile ducts, and alongside the hepatopancreas *(Fig. 3-A,D)*. Liver histology findings showed that the North area had a mean liver damage score of 2.0, indicating moderate hepatocellular degeneration, congestion, and prominent melanomacrophage centers. Conversely, the South area had a lower mean score of 0.5, reflecting mainly normal or mild changes and a relatively intact liver structure.

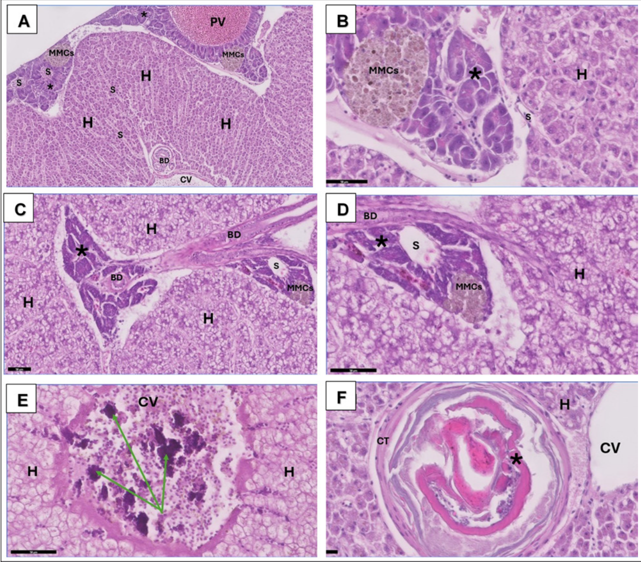

Fig 3. Histological sections of liver of north area showing: (A): Hepatic cell cords (H) radiating from the central vein (CV) and separated by blood sinusoids (S), exocrine pancreatic tissue (*) surrounding portal vein (PV) and blood sinusoids (S). Note: the melano–macrophage center (MMCs) and bile duct (BD) (X 100 H&E). (B): vacuolated hepatocytes (H) with vesicular nuclei and exocrine pancreatic tissue (*) surrounding the melano-macrophage center (MMCs). Note: the blood sinusoid (S) between the hepatocytes (X 400 H&E). (C): highly vacuolated hepatocytes (H) with nuclear karyolysis, degenerated exocrine pancreatic tissue (*) and bile ducts (BD). Note: the melano-macrophage center (MMCs) and the blood sinusoid (S) (X 200 H&E). (D): A higher magnification of the previous section shows highly vacuolated hepatocytes (H) with nuclear karyolysis, degenerated exocrine pancreatic tissue (*) and bile ducts (BD). Note: the melano-macrophage center (MMCs) and the blood sinusoid (S) (X 400 H&E). (E): highly vacuolated and degenerated hepatocytes (H) with nuclear karyolysis, dilated and congested central vein (CV) with thick wall and contained clumps of brown pigments (green arrows) (X 400 H&E). (F): vacuolated hepatocytes (H) with deeply stained pyknotic nuclei and appearance of C- shaped hyaline acidophilic structure (may be worm) (*) surrounded by connective tissue capsule (CT) located adjacent to the central vein (CV) (X 400 H&E)

Discussion

In this study, we measured physicochemical parameters and heavy metal concentrations in seawater and examined the metabolic responses of emperor fish in northern and southern Jeddah, Red Sea. The results show that the northern area had higher average values for electrical conductivity (EC), total dissolved solids (TDS), chloride, sulfate, and turbidity, while the southern area had higher pH, total alkalinity, total hardness, and nitrate levels. Statistically significant differences were found for pH, turbidity, sulfate, and total alkalinity ($P<0.05$).

All regions have mean pH values within the WHO-recommended 6.5-8.5 range. The north has lower pH levels (6.82-8.00) could be due to organic matter decomposition, whereas the south has higher pH values (up to 8.15) could be due to algal photosynthesis reducing CO_2 concentrations. CO_2 absorption from saltwater raises pH [23]. Many investigations in contaminated Al-Kumrah found pH levels of 6.62 and 7.00. In Jeddah city, various studies found pH of 7.84 in Rabigh and 8.31 in Al-Shoaibah [24]. The average TDS in two locations was greater than 30,000 mg/L, indicating that they were saline water, which ranged from 30.000 to 40.000 mg/L. The Red Sea's high salinity and geographical location caused TDS concentrations to rise in the Shuaiba region to 38.600 to 48,400mg/L [25]. In addition, EC analyzes water's dissolved ions and increased ion content from wastewater discharges drove EC similarly [26]. The average EC at Safaga along the Egyptian red sea was 60.6 mS/cm. Elevated chloride, nitrate, and phosphate concentrations in sewage flow may explain the observed increases in electrical conductivity and temperature in these two regions [27].

The low ammonia levels (<0.1 mg/L) in both regions may suggest minimal sewage impact. Rising nitrate levels in the south may indicate human-caused fertilizer loading, which may boost phytoplankton productivity. Younis et al.[25] discovered minimum ammonia concentrations in Shuaibah, Red Sea water samples, ranging from 0.03 to 0.11 mg/L. Nitrogen forms, released effluents, phytoplankton uptake rate, and nitrification or denitrification in the research region may be affected by human activities. The north had turbidity levels above 5 NTU, which might be due to sedimentation and effluents, whereas the south met the clarity norms. Human impact appears to have decreased in the south. The northern spike may be due to more sewage and soil particles entering the sea [27].

Natural geochemical characteristics and mineral-rich discharges may have created increased overall hardness and alkalinity in the north [28]. Higher alkalinity in both places may raise carbonate concentrations, which buffer water. GoA's coastal waters have increased buffering ability due to the high concentration of calcium carbonates in the water column [29].

Both regions have chloride and sulfate levels above the WHO recommendations, which are ≤250 and ≤500 mg/L, respectively. High chloride levels indicate environmental salinity and possibly industrial inputs, especially near desalination plants. Sulfate levels, however, are lower than in other studies, suggesting a lack of anthropogenic impacts. Al-Taani et al.[29] found that Cl concentrations ranged from 13.500 to 35.000 mg/L and sulfate concentrations from 2.600 to 8.900 mg/L, indicating that desalination plant discharge may cause a significant concentration of chloride ions in the water, which is similar to the current study in the south.

In general, the north of Jeddah had the highest mean values for most metrics. The discharge of treated and untreated sewage and industrial effluents from enterprises and factories may cause these results. This is comparable to Red Sea areas affected similarly [30]. In the present study, the means of Cd, Pb, Cu, and Zn were below the detection limit in two areas. In addition, Ni, Fe, and Mn were higher in the north area compared to the south area. The concentration of most heavy metals in seawater in the north and south areas was below the WHO standards, except Mn and Ni. The concentrations of Mn, and Ni in the water of the south area were slightly higher than permitted ones, while those in the water of north area had greatly exceeded them.

The rise in heavy metal levels in seawater is directly linked to human activities. This correlation is supported by the findings of Al-HasawiHassanine [24], which reported elevated levels of heavy metals in the Al-Khamra area of Jeddah, likely attributed to anthropogenic sources. The concentrations of Cd, Cu, Fe, Mn, Zn, Ni, and Pb collected from were significantly higher in this study compared to the current study. ICP-MS is selected for water analysis due to its ultra-trace sensitivity, which allows reliable measurement of elements at extremely low concentrations (down to parts per trillion), matching the low analyte content and simple matrix of water samples. In contrast, AAS is well-suited for biological samples like liver because it effectively manages moderate-to-high analyte concentrations and performs reliably in less complex organic matrices, while also being cost-effective for routine single-element quantification.

Fish morphological parameters varied widely. These variations in total length, body weight, and condition factor are normal and due to species, age, sex, and size. Fish size may fluctuate due to high water metal levels [31]. *Lethrinus lentjan* in the north and xanthochilus in the south had the highest mean overall length and weight in this study. In Lethrinus obsoletus, overall length and weight averaged lowest. The current study matches GabrMal [32], who found a link between length and weight for the top ten coral reef fish species from southern Jeddah. Shellem et al.[33] showed an average total length of 27.2 cm for Jeddah Lethrinus xanthochilus, supporting the current study. Lethrinus lentjan averaged 27.31 cm in Egypt [34], similar to the current study.

The condition factor K values indicate fish health, and greater K values reduced illnesses [35]. A condition factor greater than one indicates fish fitness, which is supported by the current study's K value of 1.30-1.70, indicating healthy body weights. The hepatosomatic index (HIS) is the main metabolic indicator in animals. Varea et al.[36] reported 1.42 and 0.47 condition factors and HSI in Viti Levu, Fiji's *Lethrinus harak* in the wet season.

The livers of emperor fish from two regions have higher ALT, AST, ALP, and GGT. The north region fish have higher liver enzymes than south area fish, may indicate persistent toxicity from ambient habitat toxicants. This contradicts Al-HasawiHassanine [24] who analyzed liver enzymes in Jeddah-trapped emperor fish Lethrinus harak. Results indicate greater amounts of total protein, albumin, and bilirubin in the northern area, with no significant difference (P<0.05). Liver structural alterations that reduce aminotransferase and deamination may raise plasma protein levels. Total protein and albumin are essential fish metabolites. Fish total protein and albumin levels are important indicators for aquatic ecosystem toxicity [37]. Serum biochemistry ranges vary by species and are regulated by seasonal changes, water temperature, nutrition, fish age, and sex [38]. Thus, greater liver functions in the north may indicate higher pollution exposure than in the south.

Elevated liver enzymes, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT), are commonly observed in individuals exposed to heavy metals like lead (Pb), cadmium (Cd), and mercury (Hg). These enzymes are released into the bloodstream when hepatocytes are injured or undergo necrosis, often as a result of cellular stress and the toxic effects of heavy metals. The primary mechanism behind this association is the induction of oxidative stress: heavy metals increase the production of reactive oxygen species (ROS), disrupt antioxidant defense systems, and damage cell membranes, leading to hepatocyte dysfunction and death. This oxidative stress induces the leakage of intracellular enzymes into the circulation, which serves as a sensitive indicator of liver injury and early hepatic toxicity in both epidemiological and experimental studies

Hematological parameters were selected based on their recognized sensitivity and importance in toxicological research. The results of this study are similar to those of El-Hamed et al.[39] which they assessed many blood parameters (WBCs, hemoglobin, neutrophil, monocyte, and eosinophil) in *Lethrinus harak* fish from Safaga, Egypt. However, in contrast to this study, the RBCs values were lower, whereas the lymphocyte values were higher. In the current study, the means of WBCs, platelets, lymphocyte, monocyte, and eosinophil were higher in the north area. On the other hand, the means of RBCs, hemoglobin, neutrophil, and basophil were higher in the southern area. Stressful conditions often lead to decreased RBC counts and hemoglobin levels in fish. This stress-induced anemia serves as a valuable indicator for assessing environmental stress. This finding agrees with RBCs and hemoglobin levels in the current study where its level was less in the northern area. According to Javed et al.[40] who observed increased WBC *in Channa punctatus* fish. Leukocytosis may be indicative of the extent of tissue damage and stress caused by heavy metals, potentially reflecting an activated immune response [41]. So, this finding agrees with WBC levels in the current study where its level was high in the northern area.

Fish growth is negatively affected by contaminated food that contains high levels of heavy metals. A clear indicator of metal toxicity in fish is the stunted growth [42]. The mean concentrations of Fe, Zn, Mn, Cd, and Ni in liver tissue of fish from the north area were higher than fish from south area. On other hand, the mean of Cu and Pb were high in the south area. A significant difference was found in all concentrations of heavy metals in the emperor liver between the two areas at (P<0.05) expect Mn. In the present study, the concentration of most heavy metals at two areas were below the FAO standards expect Cd and Mn in two areas and the Pb concentration on south area. Several studies have shown the effect of heavy metal pollution on liver tissue in emperor fish collected from the Red Sea, Jeddah Coast, Saudi Arabia [43]. The liver is essential for detoxification and storage, serving as a key organ for both the accumulation and elimination of heavy metals from the body. The liver tissues tend to accumulate higher levels of heavy metals compared to muscle tissue, which is the primary edible portion and typically exhibits lower concentrations. This occurrence can happen even when the levels of heavy metals in water are low or not detectable [44].

Thus, south-area emperor fish livers have better histological structure than north-area fish. This study found MMCs in the hepatic parenchyma of all fish species from two regions. MMCs aggregates contain Kupffer-like phagocytic cells. Al-Khumrah and Sudanese Red Sea investigations have found MMCs. Large MMC densities are usually linked to degenerative and necrotic liver diseases [23,24]. Several northern specimens had extremely vacuolated and degraded hepatocytes with karyolitic nuclei. Many vacuolar structures and dark particles were found. Histological changes in fish liver tissue may be due to Kupffer cells, which detoxify contaminants or fat buildup in hepatocytes [31]. Also, in Jeddah fish like Lethrinus harak [23,24] and *Naso hexacanthus* [45]. Lipid droplets were observed in both locations, but the southern area's cytoplasm had larger droplets, indicating more lipid buildup. Severe fatty infiltration caused hepatomegaly and pale liver tissue. Histologically, hepatocytes had extensive lipid droplets. In necrotic hepatocytes, these droplets showed as empty vacuoles as Mohammed et al.[46]. Some current study specimens have blood sinusoids and central venous congestion. This study agrees with Jenjan et al.[47], who found central venous congestion caused by larger hepatocytes with hydronic degeneration. Dilatation congests *Siganus rivulatus* hepatic blood sinusoids and arteries. Agree with Mohamed et al.[48] in *S. rivulatus* and MahmoudAbd El Rahman [49] in *Mugil ca*pito fish. Hepatocyte deterioration and blood sinusoidal congestion were found. Jasim Aldoghachi et al.[50] also found liver deterioration and sinusoidal congestion in Pb and Cd-exposed fish. in conclusion, the results of this study show that Lethrinus fish, which were collected the northern shore of Jeddah, are negatively affected by heavy metal pollution in terms of their liver function, blood parameters, and tissue structure. There may be dangers to human and environmental health from the elevated concentrations of Mn, Ni, Pb, and Cd, which surpassed international safety limits. The results highlight the critical need for monitoring and controlling coastal contamination and provide support for the idea that Lethrinus species can be useful bioindicators of marine pollution.

Declarations

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author (SAA) on reasonable request.

Competing Interests: The author declared that there is no conflict of interest.

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Ethical Approval: The animal study has been reviewed and approved by ZU-IACUC committee. was performed in accordance with the guidelines of the Egyptian Research Ethics Committee and the guidelines specified in the Guide for the Care and Use of Laboratory Animals (2025). Ethical code number ZU-IACUC/3/F/284/2025.

Author Contributions: Sahar J. Melebary, and Soha A. Alsolmy: conceptualization, project administration, funding acquisition: Raoum MominKhan, writing the original draft, writing - review, and editing.

References

1. Babayev FF: Food security in Azerbaijan in the context of global challenges. Role of business competitiveness. *Rev Univ Soci,* 15, 352-362, 2023.

2. Gupta A, Kaicker A: Food security and human health. *J Ecophysiol Occup,* 23 (3): 99-104, 2023. DOI: 10.18311/jeoh/2023/34445

3. ACTION B: The State of World Fisheries and Aquaculture. Food and Agriculture Organization (FAO), Rome, Italy, 2020. DOI: 10.4060/ca9229en

4. Dongyu Q: The State of World Fisheries and Aquaculture - Blue Transformation in Action. The State of World Fisheries and Aquaculture, Rome, Italy, R1-232, 2024. DOI: 10.4060/cd0683en

5. Van Anrooy R, Espinoza Córdova F, Japp D, Valderrama D, Gopal Karmakar K, Lengyel P, Parappurathu S, Upare S, Tietze U, Costelloe T, Zhang Z: World review of capture fisheries and aquaculture insurance - 2022. Food & Agriculture Org. 682, 2022. DOI: 10.4060/cb9491en

6. Organization WH: Population nutrient intake goals for preventing diet-related chronic diseases. http://www. who. int/nutrition/topics/, 2007.

7. Kuppusamy S, Maddela NR, Megharaj M, Venkateswarlu K, Kuppusamy S, Maddela NR, Megharaj M, Venkateswarlu K: Fate of total petroleum hydrocarbons in the environment. **In,** Total Petroleum Hydrocarbons: Environmental Fate, Toxicity, and Remediation, 57-77, 2020.

8. AbuQamar SF, El-Saadony MT, Alkafaas SS, Elsalahaty MI, Elkafas SS, Mathew BT, Aljasmi AN, Alhammadi HS, Salem HM, Abd El-Mageed TA, Zaghloul RA, Mosa WFA, Ahmed AE, Elrys AS, Saad AM, Alsaeed FA, El-Tarabily KA: Ecological impacts and management strategies of pesticide pollution on aquatic life and human beings. *Mar Pollut Bull,* 206:116613. 2024. DOI: 10.1016/j.marpolbul.2024.116613S

9. Saad AM, Sitohy MZ, Sultan-Alolama MI, El-Tarabily KA, El-Saadony MT: Green nanotechnology for controlling bacterial load and heavy metal accumulation in Nile tilapia fish using biological selenium nanoparticles biosynthesized by *Bacillus subtilis* AS12. *Front Microbiol,* 13:1015613, 2022. DOI: 10.3389/fmicb.2022.1015613

10. Saldarriaga-Hernandez S, Hernandez-Vargas G, Iqbal HM, Barceló D, Parra-Saldívar R: Bioremediation potential of *Sargassum* sp. biomass to tackle pollution in coastal ecosystems: Circular economy approach. *Sci Total Environ,* 715:136978, 2020. DOI: 10.1016/j.scitotenv.2020.136978

11. Alsulami MN, El-Saadony MT: The enhancing effect of bacterial zinc nanoparticles on performance, immune response, and microbial load of Nile tilapia *(Oreochromis niloticus)* by reducing the infection by *Trichodina heterodentata. Pak Vet J,* 44 (3): 599-610, 2024. DOI: 10.29261/pakvetj/2024.243

12. Mawed SA, Centoducati G, Farag MR, Alagawany M, Abou-Zeid SM, Elhady WM, El-Saadony MT, Di Cerbo A, Al-Zahaby SA: *Dunaliella salina* microalga restores the metabolic equilibrium and ameliorates the hepatic inflammatory response induced by zinc oxide nanoparticles (ZnO-NPs) in male zebrafish. *Biology,* 11:1447, 2022. DOI: 10.3390/biology11101447

13. Abd El-Hack ME, El-Saadony MT, Elbestawy AR, Ellakany HF, Abaza SS, Geneedy AM, Salem HM, Taha AE, Swelum AA, Omer F, AbuQamar SF, El-Tarabily KA: Undesirable odour substances (geosmin and 2-methylisoborneol) in water environment: Sources, impacts and removal strategies. *Mar Pollut Bull,* 178:113579, 2022. DOI: 10.1016/j.marpolbul.2022.113579

14. Shah AI: Heavy metal impact on aquatic life and human health - An over view. **In,** *Proceedings of the IAIA17 Conference Proceedings| IA's Contribution in Addressing Climate Change 37th Annual Conference of the International Association for Impact Assessment,* pp. 4-7, 2017.

15. Kotalova I, Calabkova K, Drabinova S, Heviankova S: Contribution to the study of selected heavy metals in urban wastewaters using ICP-MS method. **In,** *Proceedings of the IOP Conference Series: Earth and Environmental Science,* 012028, 2020.

16. Kotelevych V, Huralska S, Honcharenko V: Veterinary and sanitary assessment of fish and seafood by quality and safety indicators. *Sci Prog Innovations,* 26, 103-112, 2023. DOI: 10.31210/spi2023.26.03.19

17. Younis EM, Abdel-Warith A-WA, Al-Asgah NA, Elthebite SA, Rahman MM: Nutritional value and bioaccumulation of heavy metals in muscle tissues of five commercially important marine fish species from the Red Sea. *Saudi J Biol Sci,* 28, 1860-1866, 2021. DOI: 10.1016/j.sjbs.2020.12.038

18. Djedjibegovic J, Marjanovic A, Tahirovic D, Caklovica K, Turalic A, Lugusic A, Omeragic E, Sober M, Caklovica F: Heavy metals in commercial fish and seafood products and risk assessment in adult population in Bosnia and Herzegovina. *Sci Rep,* 10:13238, 2020. DOI: 10.1038/s41598-020-70205-9

19. Abu Shusha T, Kalantan M, Al-Nazry H, Al-Ghamdi Y: Fishes from Territorial Saudi Arabian Red Sea Water. Marian Research Center-Jeddah 225, 2011.

20. Svobodova Z, Pravda D, Palackova J: Unified methods of haematological examination of fish; Research Institute of fish Culture and Hydrobiology, 1991.

21. Neugebauer E, Cartier GS, Wakeford B: Methods for the determination of metals in wildlife tissues using various atomic absorption spectrophotometry techniques. Canadian Wildlife Service.

22. Suvarna KS, Layton C, Bancroft JD: Bancroft's Theory and Practice of Histological Techniques. E-Book; Elsevier Health Sciences. 2018.

23. Al-Wesabi EO, Zinadah OAA, Zari TA, Al-Hasawi ZM: Comparative assessment of some heavy metals in water and sediment from the Red Sea coast, Jeddah, Saudi Arabia. *Int J Curr Microbiol Appl Sci,* 4, 840-855, 2015. DOI: 10.13140/RG.2.2.19628.82569

24. Al-Hasawi Z, Hassanine R: Effect of heavy metal pollution on the blood biochemical parameters and liver histology of the Lethrinid fish, Lethrinus harak from the Red Sea. *Pak J Zool,* 55 (4): 1771-1783, 2022. DOI: 10.17582/journal.pjz/20220223170218

25. Younis AM, Elnaggar DH, El-Naggar M, Mohamedein LI: Assessment of heavy metal contamination and pollution indices in *Avicennia marina* of Nabq mangrove forest, the Red Sea, Egypt. *Egypt J Aquat Biol Fish,* 27, 361-385, 2023. DOI: 10.21608/ejabf.2023.329221

26. Alnashiri HM: Assessment of physicochemical parameters and heavy metal concentration in the effuents of sewage treatment plants in Jazan region, Saudi Arabia. *J King Saud Univ Sci,* 33:101600, 2021. DOI: 10.1016/j.jksus.2021.101600

27. Al-Alimi AQA, Saleh SM, Al-Mizgagi MA: Distribution of nutrients in surface seawater from Red Sea Coastal Area in Hodiedah Governorate, Yemen. *South Asian Res J Eng Tech,* 3 (6): 166-176, 2021.

28. Emarah DA, Seleem SA: Swelling soils treatment using lime and sea water for roads construction. *Alex Eng J,* 57, 2357-2365, 2018. DOI: 10.1016/j.aej.2017.08.009

29. Al-Taani AA, Rashdan M, Nazzal Y, Howari F, Iqbal J, Al-Rawabdeh A, Al Bsoul A, Khashashneh S: Evaluation of the Gulf of Aqaba coastal water, Jordan. *Water* 12:2125, 2020. DOI: 10.3390/w12082125

30. Zaghloul GY, El-Sawy MA, Kelany MS, Elgendy AR, Halim AMA, Sabrah MM, El-Din HME: A comprehensive evaluation of water quality and its potential health risks using physicochemical indices in coastal areas of the Gulf of Suez, Red Sea. *Ocean Coast Manag,* 243:106717, 2023. DOI: 10.1016/j.ocecoaman.2023.106717

31. Omar WA, Saleh YS, Marie MAS: Integrating multiple fish biomarkers and risk assessment as indicators of metal pollution along the Red Sea coast of Hodeida, Yemen Republic. *Ecotoxicol Environ Saf,* 110, 221-231, 2014. DOI: 10.1016/j.ecoenv.2014.09.004

32. Gabr MH, Mal AO: Trammel net fishing in Jeddah: species composition, relative importance, length-weight and length-girth relationships of major species. *Int J Fish Aquat Stud,* 6, 305-313, 2018.

33. Shellem CT, Ellis JI, Coker DJ, Berumen ML: Red Sea fish market assessments indicate high species diversity and potential overexploitation. *Fish Res,* 239:105922, 2021. DOI: 10.1016/j.fishres.2021.105922

34. Osman YA, Samy-Kamal M: Diversity and characteristics of commercial Red Sea fish species based on fish market survey: informing management to reduce the risk of overfishing. *J Fish Biol,* 102, 936-951, 2023. DOI: 10.1111/jfb.15339

35. Bakhraibah AO, Bin Dohaish AJA: Histopathological changes caused by parasites in carangoides bajad fish in the Red Sea, Jeddah. Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Veterinary Medicine 80, 2023.

36. Varea R, Paris A, Ferreira M, Piovano S: Multibiomarker responses to polycyclic aromatic hydrocarbons and microplastics in thumbprint emperor *Lethrinus harak* from a South Pacific locally managed marine area. *Sci Rep,* 11:17991, 2021. DOI: 10.1038/s41598-021-97448-4

37. Inyang IR, Obidiozo O, Izah S: Effects of *Lambda cyhalothrin* in protein and Albumin content in the kidney and liver of *Parpohiocephalus obscurus. Pharmacol Toxicol,* 2, 148-153, 2016.

38. Kavadias S, Castritsi-Catharios J, Dessypris A: Annual cycles of growth rate, feeding rate, food conversion, plasma glucose and plasma lipids in a population of European sea bass *(Dicentrarchus labrax* L.) farmed in floating marine cages. *J Appl Ichthyol,*19, 29-34, 2003. DOI: 10.1046/j.1439-0426.2003.00346.x

39. El-Hamed A, Hala A, El-Shaer WA: Study on clinicopathological and biochemical changes in some marine water fishes infested with internal parasites in Red Sea. *Egypt J Chem Environ Health,* 1, 1017-1031, 2015. DOI: 10.21608/ejceh.2015.253996

40. Javed M, Ahmad I, Ahmad A, Usmani N, Ahmad M: Studies on the alterations in haematological indices, micronuclei induction and pathological marker enzyme activities in *Channa punctatus* (spotted snakehead) perciformes, channidae exposed to thermal power plant effluent. *SpringerPlus,* 5 (1):761, 2016. DOI: 10.1186/s40064-016-2478-9

41. Witeska M, Kondera E, Bojarski B: Hematological and hematopoietic analysis in fish toxicology - A review. *Animals,* 13:2625, 2023. DOI: 10.3390/ani13162625

42. Zaynab M, Al-Yahyai R, Ameen A, Sharif Y, Ali L, Fatima M, Khan KA, Li S: Health and environmental effects of heavy metals. *J King Saud Univ Sci,* 34:101653, 2022. DOI:10.1016/j.jksus.2021.101653

43. Younis EM, Al-Asgah NA, Abdel-Warith A-WA, Al-Mutairi AA: Seasonal variations in the body composition and bioaccumulation of heavy metals in Nile tilapia collected from drainage canals in Al-Ahsa, Saudi Arabia. *Saudi J Biol Sci,* 22, 443-447, 2015. DOI: 10.1016/j.sjbs.2014.11.020

44. Younis AM, Mostafa AM, Elkady EM: Assessment of bioaccumulation and health risks of heavy metals in selected fish species from Red Sea coastal waters, Saudi Arabia. *Egypt J Aquat Res,* 50, 348-356, 2024. DOI: 10.1016/j.ejar.2024.07.007

45. Montaser M,Mahfouz ME, El-Shazly SA, Abdel-Rahman GH, Bakry S: Toxicity of heavy metals on fish at Jeddah coast KSA: Metallothionein expression as a biomarker and histopathological study on liver and gills. *World J Fish Mar Sci,* 2, 174-185, 2010.

46. Mohammed S, Idris OF, Sabahelkhier M, El-Halim A, Musa MI: Hepatic enzymes and tissues responses of rabbit fish *(Siganus rivulatus)* against heavy metals Pb and Cd at Red Sea Coast, Sudan. *Red Sea Univ J Basic Appl Sci,* 2 (Special Issue-1): 425-442, 2017.

47. Jenjan HB, Efekrin SM, Alghamari AA, Eldurssi IS, Gheth EM, Elgabaroni AS: Histopathological changes induced by heavy metals (lead and cadmium) in *Siganus rivulatus* fish collected from the Benghazi Sea Port. *Al Qalam Med Appl Sci,* 6, 439-449, 2023.

48. Mohamed SA, Elshal MF, Kumosani TA, Mal AO, Ahmed YM, Almulaiky YQ, Asseri AH, Zamzami MA: Heavy metal accumulation is associated with molecular and pathological perturbations in liver of *Variola louti* from the Jeddah Coast of Red Sea. *Int J Environ Res Public Health,* 13:342, 2016. DOI: 10.3390/ijerph13030342

49. Mahmoud S, Abd El Rahman A: Eco-toxicological studies of water and their effect on fish in El Manzalah Lake. *Res J Pharm Biol Chem Sci,* 8, 2497-2511, 2017.

50. Jasim Aldoghachi MA, Abdullah AHJ, Hussein DA: Bioaccumulation, histological and structural changes in the gills of the Nile Tilapia *(Oreochromis niloticus)* exposed to heavy metals. *Egypt J Aquat Biol Fish,* 29 (2):2333, 2025. DOI: 10.21608/ejabf.2025.422694