

# Effect of Heavy Metal Pollution on the Blood Biochemical Parameters and Liver Histology of the Lethrinid Fish, *Lethrinus harak* from the Red Sea

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## ABSTRACT

Heavy metals in aquatic ecosystems are a matter of serious concern, and adversely affect the fish health. Herein, 44 specimens of the fish *Lethrinus harak* were trapped in the Red Sea, Saudi Arabia, from a severely polluted site. Another 32 specimens of this fish were trapped from an unpolluted site (reference site). Our results revealed that Co, Cd and Pb were undetectable in water, sediment and fish liver samples from the unpolluted site, while their corresponding concentrations were significantly high in the polluted site. Mean concentrations of other metals (Mn, Fe, Ni, Cu, Zn) in water, sediment and fish liver samples from polluted site were significantly much higher than those from the unpolluted one. In contaminated fishes from polluted site, mean levels of blood biochemical parameters, serum liver enzymes (ALT, AST, ALP, GGT), serum glucose, triglycerides and urea were significantly higher than those in uncontaminated fishes from the unpolluted site, but mean level of serum total protein was significantly lower. Only, significant correlations were found between mean levels of blood biochemical parameters and mean concentrations of Zn, Cd and Pb in the liver of contaminated fishes from the polluted site. Liver of fish from the unpolluted site appeared normal, while that of fish from polluted site appeared abnormal due to sever histopathological alterations.

## INTRODUCTION

Heavy metals such as copper (Cu), nickel (Ni), zinc (Zn) and iron (Fe) are biologically essential and play important roles in the metabolic activities of organisms, while metals such as mercury (Hg), cadmium (Cd), lead (Pb) and arsenic (As) are non-biologically essential or toxic metals and mostly toxic, even in traces (Demirezen and Uruc, 2006; Wakawa *et al.*, 2008). Excessive accumulation of biologically essential metals in organisms can result in a variety of toxic effects under certain conditions (Robert, 2016). Heavy metal pollution in aquatic environments

adversely affects the fish health, as well as fish consumers. Contamination of these environments with metals, especially the toxic ones, leading to their accumulation in many organs of the fish, such as gills, muscle, intestine, liver, and kidneys (Jezierska and Witeska, 2006; Fazio *et al.*, 2014). This accumulation can lead to tissue damage, various biochemical and functional abnormalities, and dangerous chronic diseases in these organs (Jezierska and Witeska, 2006; Al-Busaidi *et al.*, 2011; Rahman *et al.*, 2012; Fazio *et al.*, 2014; Moustafa and El-Sayed, 2014; Robert, 2016; Javed and Usmani, 2019). Liver has a variety of important metabolic functions in the body, it is the major detoxifying organ, and similar to kidney, its tissue has strong affinity to accumulate heavy metals in higher levels than any other tissue in the body (Kojima and Kagi, 1978; Buckley *et al.*, 1992; Heath, 1995; Dural *et al.*, 2006; Yousafzai *et al.*, 2009; Kelle *et al.*, 2015; Hassanine *et al.*, 2019).

Currently, blood biochemical parameters of fishes are mostly used to assess their health status. For instance, levels of serum liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) are most

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## Authors' Contribution

ZAI-H and RH designed the study. ZAI-H collected, prepared and analyzed the samples. RH analyzed the data and wrote the manuscript. All authors interpreted the data, critically revised the manuscript and approved the final version. Z-AIH supervised the research work.

## Key words

Aquatic ecosystem, *Lethrinus harak*, Heavy metals, Liver, Bioaccumulation, Biochemical parameters, Red sea

widely used to assess the hepatic functions and integrity of hepatocytes (Murray *et al.*, 2003; Coz-Rakovac *et al.*, 2008), levels of serum glucose and triglycerides to assess the processes of energy metabolism (Nordlie *et al.*, 1999; Sandre *et al.*, 2017), and levels of serum total protein and urea to assess the processes of protein metabolism and growth (Schaperclaus *et al.*, 1992; Yang and Chen, 2003). All of these parameters are sensitive or useful biomarkers for water pollution, and their levels considerably changed in fishes living in metal-contaminated environments, leading to severe health troubles and diseases (Murray *et al.*, 1990; Gopal *et al.*, 1997; Shaheen and Akhtar, 2012; Abalaka, 2013).

The lethrinid fish *Lethrinus harak* is common in the Red Sea, economically important, easy to obtain, and was found resident in a severely polluted site known as “Al-Khumrah” in the Red Sea, at Jeddah City, Saudi Arabia. In the present study, the authors took the opportunity to determine the concentrations of some heavy metals in the water and sediment, and in the liver of *L. harak* from this site. Furthermore, to assess the impact of heavy metal accumulation in the liver of this fish on some of its blood biochemical parameters (liver function parameters, energy metabolism parameters, and protein metabolism parameters), and on the histological structure of its liver.

## MATERIALS AND METHODS

### Ethics

This study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the current Saudi regulations of Institutional Animal Care and Use Committee (local IACUC–King Abdulaziz University, Saudi Arabia: approval code, KAU/F/12311:5/7/2021), and with the Saudi universities guidelines for the care of experimental animals.

### Samples collection and preparation

During July of 2021, a sample of 44 specimens of the teleost fish *Lethrinus harak* (Lethrinidae), nearly equal in size ( $21.8 \pm 2.9$  cm in total length), were trapped alive by a casting net from a severely polluted site known as “Al-Khumrah” ( $21^{\circ} 22' 22''$  N,  $39^{\circ} 13' 34''$  E) in the Red Sea, at Jeddah City, Saudi Arabia (Fig. 1). This site is severely polluted due to the direct discharge of massive untreated wastewater or sewage (~100,000 cubic meters/day) into its water, and due to other maritime and anthropogenic activities. Similarly, and during the same month, 32 specimens of this fish, nearly of the same size ( $20.43 \pm 3.40$  cm in total length) were caught alive from another site with clear water in the Red Sea, at Rabigh (135 km south of Al-Khumrah). This site is far from anthropogenic activities,

and therefore was considered as a reference site.



Fig. 1. A map showing the locations of Al-Khumrah (at Jeddah City, Saudi Arabia), and the reference site at Rabigh (135 km north of Jeddah City).

In each site, water quality parameters (temperature, salinity, dissolved oxygen, pH and total dissolved solids) were measured directly by a portable computerized hydrolab (Hydrolab Multiparameter Sonde HL7, OTT Hydromet, Lindbergh Drive, Loveland, CO 80538 U.S.A.). Six readings at six different locations in each site were taken for each water quality parameter.

To reduce contamination, standard precautions such as using sterilized stainless steel dissecting instruments, clean lab plastic bags and vials for preservation of samples, sterilized containers for tissues digestion, and using high quality analytical grade reagents were taken into consideration during the collection and treatment of samples.

To detect the metal pollutants in seawater, 6 sets of water samples (each of 3 replicates) were taken from

different locations in each site. Each replicate was filtered and preserved with 2ml of  $\text{HNO}_3$  until being processed for metal analysis (Zimmermann *et al.*, 2001). Similarly, 6 sets of sediment samples (each of 3 replicates) were taken from different locations in each site, and were kept frozen at  $-20^{\circ}\text{C}$  until further processing for metal analysis (Oregoni and Aston, 1984).

Immediately after trapping, fishes were anesthetized with benzocaine (ethyl 4-aminobenzoate 80mg/L), and a blood sample was drawn from the caudal vein of each fish via a 10ml sterile disposable syringe; this step was done quickly to reduce fish stress, if occurred. Then, the blood sample was transferred into a 6ml-blood collection tube (BD Vacutainer®), left stable and allowed to clot for 30 min at room temperature ( $25^{\circ}\text{C}$ ). Lastly, the sample was centrifuged (Sigma 4-5KRL, Sigma Laborzentrifugen GmbH, Germany) at 2,000 rpm for 10 min. The obtained sera were rapidly transferred into clean polypropylene tubes, and kept frozen at  $-25^{\circ}\text{C}$  until being processed for various biochemical analyses.

Immediately after dissection, a sample of 1g was taken from the liver of each fish and kept frozen at  $-20^{\circ}\text{C}$  until being processed for metal analysis. The remaining of liver was fixed in phosphate-buffered formalin (10% neutral buffered), embedded in paraffin using a standard protocol for histological examination as recommended by Romies (1989). Sections were cut into  $4\mu\text{m}$  thick, and stained with hematoxylin and eosin (H and E). Histological images were taken with a research microscope (Olympus BH-2 Research Microscope, Shinjuku, Tokyo, Japan) connected to a computer, with the use of magnification lenses (total magnification  $100\times$ ).

#### Samples analyses

Three replicates from each obtained blood serum were used to estimate some liver function parameters (ALT, AST, ALP and GGT), some energy metabolism parameters (glucose and triglycerides) and some protein metabolism parameters (total protein and urea) in the examined fishes. All these parameters were measured by the AU5800 Automated Clinical Chemistry Analyzer (Beckman Coulter Inc., Brea CA, USA).

Each seawater sample was passed through a  $0.4\text{-}\mu\text{m}$  membrane filter and acidified with  $\text{HNO}_3$ , 65% EMPLURA® to pH fewer than 2, then analyzed directly for detection of metals in Shimadzu Inductively Coupled Plasma Mass Spectrometer (ICPMS-2030, Shimadzu Scientific Instruments Inc. Kyoto, Japan). Metal concentrations in water samples are expressed as  $\text{mgL}^{-1}$ .

Sediment samples were analyzed as recommended by Oregoni and Aston (1984). Sample was dried in an electric oven at  $110^{\circ}\text{C}$  for 8 h, and then ground in an

agate mortar. One gram of homogenized sample sieved through a 0.75-mm sieve, and digested by a mixture of concentrated acids ( $\text{HNO}_3/\text{HClO}_4/\text{HF}$ ). The residue was finally dissolved in 3% HCl (v/v), its volume made up to 50ml in a volumetric flask, and then analyzed for metal pollution in the aforementioned ICPMS. Metal concentrations in sediments are expressed as  $\text{mg kg}^{-1}$  dry weight.

From each fish liver sample, 3 replicates were analyzed for metal pollution as recommended by Zimmermann *et al.* (2001) and Nachev (2010). Immediately following thawing, 200 mg (wet weight) of homogenized fish liver was placed in 150ml perfluoralkoxy (PFA) vessel. For sample digestion, 2 ml of  $\text{HNO}_3$  65% EMPLURA® and 2.5ml of  $\text{H}_2\text{O}_2$  35% EMPLURA® were poured into the vessel, which was heated for 90 min. at about  $170^{\circ}\text{C}$  in a microwave digestion system (ETHOS™ UP, Milestone Helping Chemists, Sorisole-BG-Italy). The obtained solution was diluted to 5ml Molecular Grade Water™ in a volumetric glass flask, and then analyzed for metal pollution in the above-mentioned ICPMS. Metal concentrations in the tissue samples are expressed as  $\text{mg kg}^{-1}$  wet weight. To test the accuracy of ICP-MS analyses, 3 standard reference materials were used: (1) SRM-NIST 1640-Trace Elements in Natural Water (National Institute of Standards and Technology, Gaithersburg, MD, USA), (2) HISS-1-Marine Sediments (National Research Council, Ottawa, ON, Canada), and (3) Dogfish liver-DOLT-5 (National Research Council, Ottawa, ON, Canada).

#### Data analysis

Duncan's Multiple Range Test (1955) was applied to test for differences between levels of liver enzymes, other blood parameters, and metal concentrations; possibilities less than 0.05 were considered statistically significant ( $p < 0.05$ ). Spearman's rank correlation coefficient ( $r_s$ ) was calculated to determine possible correlations between metal concentrations in the liver of *L. harak* and levels of different blood biochemical parameters in this fish.

## RESULTS

Water quality parameters (temperature, dissolved oxygen, salinity, pH and total dissolved solids) recorded in each site are shown in Table I.

The recovered values of heavy metal concentrations recorded from the three certified reference materials, the accuracy of the analytical procedure (ICP-MS analyses), and the detection limits of each metal (Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb) are shown in Table II.

**Table I.** Water quality parameters in the reference site (at Rabigh) and in Al-Khumrah (at Jeddah City).

| Site                        | Temperature (°C) | Salinity (%) | Dissolved oxygen (mg/L) | pH        | Total dissolved solids (mg/L) |
|-----------------------------|------------------|--------------|-------------------------|-----------|-------------------------------|
| Reference site (at Rabigh)  | 24±1             | 39±2         | 6.16±0.23               | 7.84±0.21 | 34230±248                     |
| Al-Khumrah (at Jeddah City) | 29±2             | 36±2         | 2.53±0.42               | 6.62±0.15 | 48763±775                     |
| WHO guidelines              | 26-30            | 36-38        | >5                      | 6.5-8.5   | 35000                         |

**Table II.** Heavy metal concentrations recovered from the standard reference materials, the accuracy, and detection limits recorded by ICP-MS analyses.

| Standard reference material                          | Metal | Certified value (mg/L) | Recovered value | Accurate (%) | Detection limit (mg/L) |
|--|-------|------------------------|-----------------|--------------|------------------------|
| <b>SRM-NIST 1640-trace elements in natural water</b> |       |                        |                 |              |                        |
|  | Mn    | 40.07 ± 0.35           | 38.87±0.22      | 97.00        | 0.012                  |
|  | Fe    | 36.5 ± 1.70            | 35.33±1.02      | 96.79        | 0.009                  |
|  | Co    | 20.08± 0.24            | 19.02±0.14      | 94.72        | 0.006                  |
|  | Ni    | 25.12± 0.12            | 23.62±0.08      | 94.02        | 0.009                  |
|  | Cu    | 85.07± 0.48            | 81.03±0.26      | 95.25        | 0.014                  |
|  | Zn    | 55.20 ± 0.32           | 51.91±0.25      | 94.03        | 0.011                  |
|  | Cd    | 3.961 ± 0.072          | 3.883±0.023     | 98.03        | 0.005                  |
|  | Pb    | 12.005±0.040           | 11.434±0.015    | 95.24        | 0.009                  |
| <b>HISS-1-Marine Sediments</b>                       |       |                        |                 |              |                        |
|  | Mn    | 66.1±4.2               | 63.27±3.03      | 95.71        | 0.012                  |
|  | Fe    | 0.246±0.009            | 0.235±0.006     | 95.52        | 0.009                  |
|  | Co    | 0.65±0.08              | 0.61±0.04       | 93.84        | 0.004                  |
|  | Ni    | 2.16±0.29              | 2.12±0.16       | 98.14        | 0.010                  |
|  | Cu    | 2.29±0.37              | 2.22±0.31       | 96.94        | 0.008                  |
|  | Zn    | 4.94±0.79              | 4.81±0.32       | 97.36        | 0.008                  |
|  | Cd    | 0.024±0.009            | 0.022±0.006     | 92.00        | 0.006                  |
|  | Pb    | 3.13±0.40              | 2.95±0.09       | 94.00        | 0.004                  |
| <b>Dogfish liver DOLT-5</b>                          |       |                        |                 |              |                        |
|  | Mn    | 8.91 ± 0.70            | 8.74±0.54       | 98.10        | 0.009                  |
|  | Fe    | 1070 ± 80.0            | 1044±13.12      | 97.57        | 0.014                  |
|  | Co    | 0.267 ± 0.026          | 0.251±0.019     | 94.00        | 0.004                  |
|  | Ni    | 1.71 ± 0.56            | 1.66±0.07       | 97.07        | 0.008                  |
|  | Cu    | 35.0 ± 2.40            | 33.33±1.13      | 95.22        | 0.010                  |
|  | Zn    | 105.3 ± 5.40           | 100.10± 4.23    | 95.06        | 0.012                  |
|  | Cd    | 14.5±0.400             | 14.16±0.014     | 97.65        | 0.005                  |
|  | Pb    | 0.162±0.032            | 0.154±0.021     | 95.06        | 0.006                  |

**Metal pollution**

Cobalt and two toxic metals, Cd and Pb were undetectable in the water of the reference site (at Rabigh). Unlikely, mean concentrations of these metals were significantly high ( $p \leq 0.05$ ) in the water of Al-Khumrah (at Jeddah City) (Table III). Compared to those in the water of the reference site, mean concentrations of other metals (Mn, Fe, Ni, Cu and Zn) in the water of Al-Khumrah were significantly higher ( $p \leq 0.05$ ) (Table III), with multifold increase in metal concentrations followed the order Ni (109)> Fe (47)> Cu (11)> Mn (9)> Zn (6). In both sites, mean metal concentrations in the sediments were significantly much higher ( $p \leq 0.01$ ) than those in the water. Cobalt, Cd and Pb were undetectable in the sediments of reference site. Unlikely, mean concentrations of these metals were significantly high ( $p \leq 0.01$ ) in the sediments of Al-Khumrah (Table III). Compared to those in the sediments of the reference site, mean concentrations of other metals (Mn, Fe, Ni, Cu and Zn) in the sediments of Al-Khumrah were significantly higher ( $p \leq 0.05$ ) (Table III), with multifold increase in metal concentrations followed order Fe (17)> Mn (12)> Cu (9)> Ni (5)> Zn (4). Similarly, Co, Cd and Pb were undetectable in *L. harak* liver samples from the reference site (Table III). Unlikely, mean concentrations of these metals were significantly high ( $p \leq 0.05$ ) in *L. harak* liver samples from Al-Khumrah (Table III). Compared to those in *L. harak* liver samples from the reference site, mean concentrations of other metals (Mn, Fe, Ni, Cu and Zn) in *L. harak* liver samples from Al-Khumrah were significantly much higher ( $p \leq 0.05$ ) (Table III), with multifold increase in metal concentrations followed order Zn (22)> Fe (19) > Cu (16)> Mn (15)> Ni (9). Consequently, individuals of *L. harak* from the reference site were considered uncontaminated, while those from Al-Khumrah were contaminated. Generally, mean metal concentrations in the water, sediments and in *L. harak* liver samples from Al-Khumrah were significantly much higher (many folds) than those from the reference site (Table III). Therefore, our results strongly suggest that reference site is currently clean and unpolluted, while Al-Khumrah is so badly polluted.

**Table III.** Mean metal concentrations in water, sediments and *L. harak* livers sampled from the reference site (at Rabigh) and from Al-Khumrah (at Jeddah City).

| Metal | Mean metal concentration ± Standard deviation |                             |                |                                       |                             |                 |   |                             |
|-------|---|-----------------------------|----------------|---------------------------------------|-----------------------------|-----------------|---|-----------------------------|
|       | Seawater (mg L <sup>-1</sup> )                |                             |                | Sediment (mg kg <sup>-1</sup> dry wt) |                             |                 | Fish liver (mg kg <sup>-1</sup> wet wt) |                             |
|       | Reference site (at Rabigh)                    | Al-Khumrah (at Jeddah City) | WHO guidelines | Reference site (at Rabigh)            | Al-Khumrah (at Jeddah City) | NOAA guidelines | Reference site (at Rabigh)              | Al-Khumrah (at Jeddah City) |
| Mn    | 0.63±0.03                                     | 5.76±0.41                   | 0.50           | 1.64±0.07                             | 19.77±2.61                  | 10.00           | 0.48±0.08                               | 7.12±0.97                   |
| Fe    | 0.39±0.18                                     | 18.45±2.30                  | 0.30           | 2.91±0.14                             | 47.88±4.23                  | 35.30           | 1.25±0.11                               | 23.56±2.13                  |
| Co    | undetected                                    | 3.12±0.23                   | 0.50           | undetected                            | 9.85±1.09                   | 2.00            | undetected                              | 4.55±0.21                   |
| Ni    | 0.06±0.03                                     | 6.55±0.51                   | 0.05           | 4.76±0.11                             | 21.06±2.98                  | 20.90           | 0.98±0.10                               | 8.75±0.72                   |
| Cu    | 2.13±0.04                                     | 24.80±3.33                  | 2.00           | 6.91±0.43                             | 58.77±5.04                  | 34.00           | 2.45±0.09                               | 38.33±4.67                  |
| Zn    | 2.02±0.26                                     | 11.08±2.37                  | 3.00           | 8.05±0.23                             | 31.22±3.21                  | 150.00          | 0.73±0.10                               | 15.91±1.57                  |
| Cd    | undetected                                    | 5.12±0.36                   | 0.003          | undetected                            | 16.24±2.36                  | 1.20            | undetected                              | 10.25±1.93                  |
| Pb    | undetected                                    | 13.84±2.32                  | 0.01           | undetected                            | 38.02±3.31                  | 50.00           | undetected                              | 19.87±2.34                  |

**Fish blood biochemical parameters**

In the blood sera of uncontaminated *L. harak* from the reference site, mean levels of liver enzymes, ALT, AST, ALP, and GGT were 54.20±5.98, 62.14±4.37, 45.55±3.64 and 8.80±0.62 U/L, respectively, and mean levels of the other serum biochemical parameters, glucose, triglycerides, total protein, and urea were 58.37±3.09 mg/dL, 89.43±5.56 mg/dL, 24.3±2.63 g/L, and 28.81±4.54 mg/dL, respectively (Table IV). These levels were considered herein as baseline levels, since they were measured in fishes living in a clean and unpolluted environment. Compared to these levels, the corresponding ones in the blood sera of contaminated *L. harak* from Al-Khumrah were significantly higher ( $p < 0.05$ ) (Table IV), except the mean level of serum total protein which was significantly lower ( $p < 0.05$ ). These changes refer to serious abnormalities in blood biochemical parameters of *L. harak* inhabiting Al-Khumrah site.

In uncontaminated *L. harak* from the reference site, no significant correlations were found between levels of serum liver enzymes (ALT, AST, ALP, and GGT) or other blood biochemical parameters (glucose, triglycerides, total protein, and urea) and mean concentrations of Mn, Fe, Ni, Cu and Zn in the liver. Similarly, in contaminated *L. harak* from Al-Khumrah, no significant correlations was found between the level of these enzymes or other blood biochemical parameters and mean concentrations of Mn, Fe, Co, Ni and Cu in the liver, but moderate to strong correlations were found between these parameters and mean concentrations of Zn, Cd, and Pb in the liver (Table V); all correlations were positive, except the negative ones between mean levels of serum total protein and mean concentrations of Zn, Cd, and Pb in fish liver.

**Table IV.** Mean levels of serum liver enzymes (ALT, AST, ALP and GGT), glucose, triglycerides, total protein, and urea in *L. harak* from the reference site (at Rabigh) and from Al-Khumrah (at Jeddah City).

| Fish serum parameter          | Reference site (at Rabigh) | Al-Khumrah (at Jeddah City) |
|-------------------------------|----------------------------|-----------------------------|
| <b>Liver enzymes</b>          |                            |                             |
| ALT (U/L)                     | 54.20±5.98                 | 89.15±6.70                  |
| AST (U/L)                     | 62.14±4.37                 | 114.10±7.06                 |
| ALP (U/L)                     | 45.55±3.64                 | 93.65±9.73                  |
| GGT (U/L)                     | 8.80±0.62                  | 23.45±4.24                  |
| <b>Other serum parameters</b> |                            |                             |
| Glucose (mg/dL)               | 58.37±3.09                 | 109.54±5.03                 |
| Triglycerides (mg/dL)         | 89.43±5.56                 | 162.56±8.56                 |
| Total protein (g/L)           | 24.3±2.63                  | 10.13±0.06                  |
| Urea (mg/dL)                  | 28.81±4.54                 | 58.67±4.32                  |

**Histological structure of *L. harak* liver**

In uncontaminated *L. harak* from the reference site, histological structure of the liver appeared to be normal, and without any evidence of histopathological alteration (Fig. 2A), since hepatic tissue was composed of regular liver lobules surrounded by sinusoids and filled with polyhedral hepatocytes, each containing a clear round nucleus and a cytoplasm with numerous droplets. In contrast, in contaminated *L. harak* from Al-Khumrah, histological structure of the liver of appeared abnormal (Fig. 2B), with sever histopathological alteration including frank necrosis, sinusoidal dilation, degeneration of hepatocytes,

inflammatory response, adipocytes, hepatocyte vacuolization, fatty change, nuclear hypertrophy, irregular shaped-nucleus, cellular hypertrophy, cellular atrophy melanomacrophages aggregates, and vacuolated foci.

## DISCUSSION

Heavy metals in aquatic environments are mostly bound to suspended particles or adsorbed on particulate organic matter, and only tiny fractions of them are present in water as free ions (hydrated) and known as biologically available metals, i.e., can be taken up directly from water and used by living organisms. These fractions are greatly affected by several water parameters, such as temperature, pH, salinity, etc. (Merian *et al.*, 2004). Mostly, the increase in water temperature and dissolved oxygen stimulates the release or desorption of metals from the sediment into the overlying water, unlikely, the decrease in water salinity and pH also stimulates this process (Malandrino *et al.*, 2006; Haiyan *et al.*, 2013; Yanhao *et al.*, 2018; Tao *et al.*, 2021).

**Table V. Spearman's rank correlation ( $r_s$ ) matrix between mean metals concentrations in the liver of *L. harak* and levels of biochemical parameters in its blood.**

| Metal concentration in the liver of <i>L. harak</i> | Levels of serum liver enzymes |       |               |       |               |       |       |       |
|---|-------------------------------|-------|---------------|-------|---------------|-------|-------|-------|
|   | ALT                           |       | AST           |       | ALP           |       | GGT   |       |
|   | $r_s$                         | p     | $r_s$         | p     | $r_s$         | p     | $r_s$ | p     |
| Mn  | 0.341                         | 0.122 | 0.271         | 0.223 | 0.219         | 0.089 | 0.327 | 0.137 |
| Fe  | 0.362                         | 0.239 | 0.294         | 0.163 | 0.152         | 0.168 | 0.268 | 0.098 |
| Co  | 0.402                         | 0.109 | 0.185         | 0.282 | 0.149         | 0.260 | 0.159 | 0.266 |
| Ni  | 0.135                         | 0.332 | 0.322         | 0.321 | 0.284         | 0.304 | 0.148 | 0.119 |
| Cu  | 0.264                         | 0.204 | 0.211         | 0.172 | 0.364         | 0.171 | 0.234 | 0.291 |
| Zn  | 0.625                         | 0.002 | 0.752         | 0.003 | 0.592         | 0.004 | 0.668 | 0.001 |
| Cd  | 0.865                         | 0.001 | 0.906         | 0.001 | 0.709         | 0.002 | 0.872 | 0.001 |
| Pb  | 0.726                         | 0.003 | 0.792         | 0.004 | 0.877         | 0.001 | 0.765 | 0.003 |
| Levels of other blood biochemical parameters        |                               |       |               |       |               |       |       |       |
|   | Glucose                       |       | Triglycerides |       | Total protein |       | Urea  |       |
|   | $r_s$                         | p     | $r_s$         | p     | $r_s$         | p     | $r_s$ | p     |
| Mn  | 0.231                         | 0.293 | 0.143         | 0.267 | 0.187         | 0.179 | 0.245 | 0.213 |
| Fe  | 0.177                         | 0.146 | 0.088         | 0.302 | 0.301         | 0.094 | 0.283 | 0.189 |
| Co  | 0.311                         | 0.274 | 0.329         | 0.138 | 0.078         | 0.209 | 0.272 | 0.092 |
| Ni  | 0.281                         | 0.324 | 0.259         | 0.169 | 0.312         | 0.181 | 0.216 | 0.086 |
| Cu  | 0.401                         | 0.221 | 0.247         | 0.205 | 0.227         | 0.194 | 0.246 | 0.139 |
| Zn  | 0.698                         | 0.002 | 0.596         | 0.003 | -0.674        | 0.001 | 0.734 | 0.001 |
| Cd  | 0.901                         | 0.001 | 0.885         | 0.001 | -0.812        | 0.001 | 0.898 | 0.003 |
| Pb  | 0.830                         | 0.001 | 0.677         | 0.004 | -0.784        | 0.003 | 0.691 | 0.004 |

Values in bold letters show significant correlations ( $p < 0.05$ ).

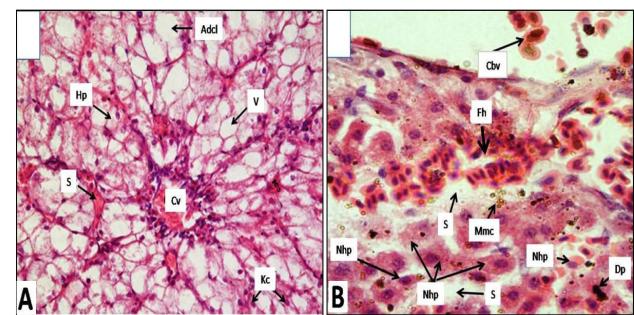


Fig. 2. (A) Normal hepatic histological structure of *L. harak* sampled from the reference site; (B) Histopathological alterations in the liver of *L. harak* sampled from Al-Khumrah: Adcl, Adipose cell, Cells with pyknosis and karyorrhexis (P and K); Cv, Centralvein; Cbv, Congestion of hepatic blood vessels; Dp, Dark brown pigments; Fh, Focal hemorrhage; Hp, Hepatocytes; Kc, Kupffer cell, Mmc, Melanomacrophages; Nhp, Necrotic hepatocytes, S. Sinusoid; V, Vacuole. (Hematoxylin and Eosin, 100 $\times$ ).

In the present study, water quality parameters in the reference site (at Rabigh) have met the WHO standards, while those in Al-Khumrah (at Jeddah City), particularly the dissolved oxygen (much lower than allowable) and total dissolved solids (much higher than allowable) have not met these standards, and clearly point to the poor water quality in this site, since in aquatic environments, low levels of dissolved oxygen and high levels of total dissolved solids can adversely affect many forms of aquatic life (Anthony, 2000).

In the current study, the accuracy of the analytical procedures carried out by the Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) ranged from 92 to 98%, which can be considered a reliable analysis.

Cobalt, Cd and Pb were undetectable in the water of the reference site, but their concentrations in the water of Al-Khumrah were significantly high. However, a significant increase was recorded in the concentrations of other metals (Mn, Fe, Ni, Cu and Zn) in the water of Al-Khumrah in comparison to those of the reference site. According to the World Health Organization WHO (2011), the maximum allowable concentrations of Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb in seawater were 0.5, 0.30, 0.5, 0.05, 2.0, 3.0, 0.003 and 0.01, respectively. Metal concentrations in the water of the reference site were nearly similar to the allowable ones, while those in the water of Al-Khumrah were greatly exceeded them.

In both sites, mean metal concentrations in the sediments were much greater than those in water. This is probably due to the high affinity of metals to bind to sediment particles, or to suspended matter particles that lastly settle and accumulate in the bottom to build up the bottom sediments (Luoma, 1990; Campbell, 1994; Dauvalter, 1998; Gurunadha *et al.*, 2007; Tekin-Ozan and Kir, 2008). So, metal concentrations are greater in sediments, and mostly 3–5 orders of magnitude greater than in the overlying water (Gurunadha *et al.*, 2007).

Cobalt, Cd and Pb were undetectable in the sediment samples from the reference site, but their concentrations in sediments from Al-Khumrah were significantly high. However, a significant increase was recorded in the concentrations of Mn, Fe, Ni, Cu and Zn in sediments from Al-Khumrah in comparison to those from the reference site. According to National Oceanic and Atmospheric Administration NOAA (2009), the maximum allowable concentrations of Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb in marine sediments were 10.0, 35.3, 2.0, 20.0, 34.0, 150.0, 1.2 and 50.0, respectively. Metal concentrations in the sediments from the reference site were much lower than the allowable ones, while those in the sediments from Al-Khumrah were greatly exceeded them, except for Zn and Pb, as per NOAA (2009).

Toxic metals, Cd and Pb were undetectable in the liver samples of uncontaminated *L. harak* from the reference site. In contrast, their concentrations in the liver samples of contaminated *L. harak* from Al-Khumrah were significantly much higher. According to WHO (2011) and Food and Agriculture Organization of the United Nations FAO (2003), the permissible concentration of Cd and Pb in fish tissues were 0.05 and 0.2 mg/kg wet wt, respectively. Metal concentrations in the liver samples of contaminated *L. harak* from Al-Khumrah were much higher (~205 and 100 folds, respectively) than the permissible ones.

Generally, our results provide strong evidences that the reference site is currently clean and unpolluted, while Al-Khumrah is severely polluted, and this has been confirmed in many previous studies (El-Sayed *et al.*, 2004; Al-Wesabi *et al.*, 2015; Salama *et al.*, 2016; Al-Mur *et al.*, 2017). Consequently, *L. harak* from the reference site were considered un intoxicated, while those from Al-Khumrah were intoxicated.

In modern ecotoxicological studies, blood levels of liver function enzymes (ALT, AST, ALP and GGT) are frequently used as sensitive biomarkers to assess the harmful consequences of polluted water on fish health (Kramer and Hoffmann, 1997; De La Torre *et al.*, 2000; Levesque *et al.*, 2002). ALT and AST are key enzymes in amino acid metabolism in the liver of teleost fishes (Yasutake and Wales, 1983; Ballantyne, 2001; Murray *et al.*, 2003; Coz-Rakovac *et al.*, 2008), hepatic ALP is a membrane-bound enzyme in hepatocytes and plays a vital role in membrane transport (Schaperclaus *et al.*, 1992), and GGT is an abundant enzyme in hepatocytes and plays a crucial role in metabolism and mediates xenobiotic detoxification in the liver (Schaperclaus *et al.*, 1992). Blood levels of these enzymes significantly elevated in fishes living in heavy metal-contaminated environments (Zikic *et al.*, 2001; Gabriel and George, 2005; Yousafzai and Shakoori, 2011; Abalaka, 2013; Gholizadeh *et al.*, 2018; Ugbomeh *et al.*, 2019). This elevation may be due to the toxic effects of these metals on fishes, and refers to histological damage in some organs, particularly the liver (Yousafzai and Shakoori, 2011; Soleimany *et al.*, 2016; Gholizadeh *et al.*, 2018; Ugbomeh *et al.*, 2019). Blood liver enzymes are normally predominantly contained within hepatocytes and are spilled into the bloodstream following cellular damage (Sallie *et al.*, 1991; Mayne, 2002; Palanivelu *et al.*, 2005; Abalaka, 2013), since toxicants such as toxic heavy metals increase the permeability of hepatocyte plasma membrane, and consequently liver enzymes can leak from hepatocytes into the bloodstream and elevate to higher levels, causing many disease, such as anorexia and fatigue (Nordlie *et al.*, 1999; Coz-Rakovac *et al.*, 2008). In our study, heavy metal concentrations in the liver of contaminated

*L. harak* from Al-Khumrah were significantly high, and led to considerable elevations in the levels of serum liver enzymes (ALT, AST, ALP and GGT) to become much greater than those in uncontaminated *L. Harak* from the reference site. Such an elevation in the levels of these enzymes in fishes due to heavy metal pollution, has been recorded in many other studies (Sallie *et al.*, 1991; Mayne, 2002; Palanivelu *et al.*, 2005; Abalaka, 2013; Al-Asgah *et al.*, 2015; Soleimany *et al.*, 2016; Ugbomeh *et al.*, 2019). In uncontaminated *L. harak* from the reference site, no significant correlations were found between levels of serum liver enzymes (ALT, AST, ALP, and GGT) and mean concentrations of all metals in the liver. Also, in contaminated *L. harak* from Al-Khumrah, no significant correlations were found between level of these enzymes and mean concentrations of Mn, Fe, Co, Ni and Cu in the liver, but unlikely, moderate to strong positive correlations were found between them and mean concentrations of Zn, Cd, and Pb in liver. These correlations strongly suggest that these metals have severe toxic effects on fish liver.

Serum glucose level in fishes is an essential indicator for environmental stress (Silbergeld, 1974; Iwama, 1998; Gagnon *et al.*, 2006), and considerably elevated (hyperglycemia) in fishes subjected to heavy metal pollution (Richard *et al.*, 1998; Levesque *et al.*, 2002; Begg and Pankhurst, 2004; Jagadesswarlu and Sunita, 2018). This elevation reduces growth, increases feed conversion ratio, and lowers sustainable growth rate. Metals toxicity mostly leads to severe liver lesions and stress conditions, both disturb carbohydrate metabolism in fish liver, and consequently blood glucose level significantly elevated (Al-Asgah *et al.*, 2015; Soengas *et al.*, 1996; Almeida *et al.*, 2001). Our results totally agree with these observations, since mean serum glucose level in contaminated *L. harak* from Al-Khumrah was significantly higher than that in uncontaminated *L. harak* from the reference site. In the latter, no significant correlations were found between level of serum glucose and mean concentrations of all metals in the liver of *L. harak*. Also, in Al-Khumrah no significant correlations was found between its level and mean concentrations of Mn, Fe, Co, Ni and Cu in the liver of *L. harak*, but unlikely, moderate to strong positive correlations were found between its level and mean concentrations of Zn, Cd, and Pb in the liver. Thus, accumulation of these metals in the liver of *L. harak* adversely affects carbohydrate metabolism in the liver (Soengas *et al.*, 1996; Almeida *et al.*, 2001; Al-Asgah *et al.*, 2015).

Serum triglycerides level is an important indicator for the metabolic status of fish, and mostly elevated (hypertriglyceridemia) in fishes inhabiting heavy metal-polluted environments (Kaplan *et al.*, 1988; Hadi *et al.*,

2009; Srivastava and Prakash, 2018; Mohamed *et al.*, 2019). This elevation builds up dangerous fatty deposits in some fish organs and triggers the blockage of blood vessels (Kaplan *et al.*, 1988; Hadi *et al.*, 2009). Considerable changes in blood triglycerides level refer to liver dysfunction and inhibition of lipid catabolism (Kaplan *et al.*, 1988; Hadi *et al.*, 2009; Srivastava and Prakash, 2018; Mohamed *et al.*, 2019). Likewise, our results revealed that mean serum triglyceride level in contaminated *L. harak* from Al-Khumrah was significantly higher than that in uncontaminated *L. harak* from the reference site. In the latter, no significant correlations were found between level of serum triglycerides and mean concentrations of all metals in the liver of *L. harak*. Also, in Al-Khumrah no significant correlations was found between its level and mean concentrations of Mn, Fe, Co, Ni and Cu in the liver of *L. harak*, in contrast, moderate to strong positive correlations were found between its level and mean concentrations of Zn, Cd, and Pb in the liver. Thus, accumulation of these metals in the liver of *L. harak* disturbs lipid catabolism in the liver (Kaplan *et al.*, 1988; Hadi *et al.*, 2009; Srivastava and Prakash, 2018; Mohamed *et al.*, 2019).

Serum total protein level in fishes is an essential indicator for liver integrity and overall health status (Nordlie *et al.*, 1999; Sandre *et al.*, 2017). This level usually decreases (hypoproteinemia) in fishes exposed to heavy metal pollution due to severe liver damage and stress conditions (Magnadottir *et al.*, 2010; Shaheen and Akhtar, 2012), and leading to slower growth, muscle atrophy, and a weakened immune system. Similarly, our results showed that mean serum total protein level in contaminated *L. harak* from Al-Khumrah was significantly lower than that in uncontaminated *L. harak* from the reference site. In the latter, no significant correlations were found between level of serum total protein and mean concentrations of all metals in the liver of *L. harak*. Also, in Al-Khumrah no significant correlations was found between its level and mean concentrations of Mn, Fe, Co, Ni and Cu in the liver of *L. harak*, but unlikely, moderate to strong negative correlations were found between its level and mean concentrations of Zn, Cd, and Pb in the liver. Thus, accumulation of these metals in the liver of *L. harak* inhibits protein production in the liver (Magnadottir *et al.*, 2010; Shaheen and Akhtar, 2012).

Blood urea level in fishes is an important indicator for their metabolic status, and mostly elevated (hyperuricemia) in fishes inhabiting polluted environments, due severe gill and liver diseases (Murray *et al.*, 1990; Stoskopf, 1993; Alkaladi *et al.*, 2015), since urea in fish is produced by liver and excreted mainly through the gills as ammonia, and a few amount is excreted by kidneys as urine (Randall

and Wright, 2005). Urea at high concentrations becomes poisonous to cells and impairs the various cellular processes (Murray *et al.*, 1990; Randall and Wright, 2005). Our results fully agree with these findings, since mean serum urea level in contaminated *L. harak* from Al-Khumrah was significantly higher than that in uncontaminated *L. harak* from the reference site. In the latter, no significant correlations were found between level of serum urea and mean concentrations of all metals in the liver of *L. harak*. Also, in Al-Khumrah no significant correlations was found between its level and mean concentrations of Mn, Fe, Co, Ni and Cu in the liver of *L. harak*, in contrast, moderate to strong positive correlations were found between its level and mean concentrations of Zn, Cd, and Pb in the liver. Thus, accumulation of these metals in the liver of *L. harak* inhibits the elimination of unwanted ammonia or urea from fish blood (Murray *et al.*, 1990; Stoskopf, 1993; Alkaladi *et al.*, 2015).

In uncontaminated *L. harak* from the reference site, metal concentrations in the liver were low, and toxic metals Cd and Pb were undetectable. In contrast, in contaminated *L. harak* from Al-Khumrah, metal concentrations in liver were significantly high, and concentrations toxic metals Cd and Pb were much higher (~205 and 100 folds, respectively) than the permissible ones. Consequently, histological structure of *L. harak* liver from the reference site appeared to be normal, and without any evidence of histopathological alteration, in contrast, histological structure of *L. harak* liver from Al-Khumrah appeared abnormal, with sever histopathological alterations including frank necrosis, sinusoidal dilation, degeneration of hepatocytes, inflammatory response, adipocytes, hepatocyte vacuolization, fatty change, and vacuolated foci. Such histopathological alteration in fish liver due to heavy metal pollution has been recorded in many other studies (Hinton and Laurén, 1990; Van Dyk *et al.*, 2009; Radhakrishnan and Hemalatha, 2010; Muthukumaravel and Rajaraman, 2013; Chamarthi *et al.*, 2014).

## CONCLUSIONS

Heavy metals such as Cu, Ni, Zn and Fe are biologically essential and play essential roles in the metabolic activities of organisms, unlikely metals such as Hg, Cd, Pb and As are non-biologically essential and mostly toxic, even in traces. Excessive accumulation of biologically essential metals in organisms can result in a variety of toxic effects under certain conditions. Due to their environmental toxicity and bioaccumulation, heavy metals in aquatic ecosystem are a matter of serious concern. Presence of these metals especially the toxic ones, as pollutants in aquatic environments leading to their

accumulation in many organs of the fish, such as gills, muscle, liver and kidney. This accumulation can cause significant histopathological alterations and dangerous chronic diseases in these organs, particularly in liver. This organ has a variety of important metabolic functions in the body, it is the major detoxifying organ, and its tissue has strong affinity to accumulate heavy metals in higher levels than any other tissue in the body. In modern ecotoxicological studies, blood biochemical parameters of fishes are mostly used to assess their health status. For instance, levels of serum liver enzymes (ALT, AST, ALP, GGT) are most widely used to assess the hepatic functions and integrity of hepatocytes, levels of serum glucose and triglycerides to assess the processes of energy metabolism, and levels of serum total protein and urea to assess the processes of protein metabolism and growth. The normal levels of these parameters considerably changed in fishes living in metal-contaminated environments, leading to severe health troubles and diseases. Generally, our results totally agree with most previous studies that all of these parameters are sensitive biomarkers for determining the adverse effects of heavy metal pollution on fish health.

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### Statement of conflict of interest

The authors have declared no conflict of interest.

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