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Research article

Impacts of Heavy Metal and Bacterial Pollution on the Common Seabream (*Pagrus pagrus*) in Benghazi Seaport, Libya

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Abstract

Background: Pollution of seawater could imply deleterious effects on marine ecosystem and pose a serious threat to fish and, then, public health. **Aim:** This study assessed the impact of water pollution on common seabream *(Pagrus pagrus*) fish in Benghazi seaport, Libya, with focus on heavy metal contamination and bacterial load. **Methods:** Common seabream fish were obtained from seaport area and dissected for tissue analysis of heavy metal content and bacteriological examination. Likewise, seaport water samples collected from three different depth points were analyzed for investigating their pollution status. Fish blood samples were used for hematological analysis. **Results:** Fish tissue samples (gills, kidneys and muscles) showed elevated levels of toxic metals such as lead and cadmium and essential metals such as iron and zinc, with gills exhibiting the highest Metal Pollution Index (5.23 µg/g). Seaport water samples contained excessive concentrations of iron (6.091 mg/l) and zinc (0.427 mg/l), exceeding recommended limits. Bacterial counts, including *Enterobacter cloacae* and *Escherichia coli*, reached 100CFU/100ml near the seaport pier (ship berth). Hematological analysis of fish revealed stress responses, such as elevated white blood cell counts (64.25×10^3/µl) and decreased blood thrombocytes (functionally equivalent to mammalian platelets) levels. **Conclusion:** These findings highlight a significant pollution from contaminated water and industrial discharges, posing risks to marine ecosystems and public health.

Keywords: *Pagrus pagrus*, pollution, heavy metals, Benghazi, *E. cloacae*

Introduction

Benghazi seaport is the largest Libyan seaport in terms of area, covering approximately 4.4 million square meters (Abmdas & Demirel, 2018). However, it is not the busiest in terms of trade volume. The port includes 18 berths, of which 11 designated for general cargo (Abmdas & Demirel, 2018).

The Mediterranean Sea faces several types of pollution, including chemical, biological, and plastic pollution. Major sources include industrial discharge, untreated sewage, agricultural runoff, and maritime traffic. These pollutants accumulate due to the sea's semi-enclosed nature, leading to serious ecological threats (UNEP/MAP, 2012).

Pollution of water bodies with heavy metals and bacteria may affect aquatic animals, such as fish, directly or indirectly (Kılıç, 2021). Heavy metals, such as zinc (Zn), cadmium (Cd), chromium (Cr), copper (Cu), and lead (Pb) are elements that cannot be destroyed or degraded. They can enter the human body through food, drinking water or inhalation of contaminated air. The presence of heavy metals in food poses a serious concern, because they contaminate the food chain and threatens public health (Azeez *et al.*, 2021).

Fish are among the products most affected by heavy metal contamination; as these metals are bioaccumulative in the food chain (*Wang et al.*, 2020). Fish accumulate heavy metals at concentrations many times higher than those in the surrounding water (Bozorgzadeh *et al*., 2021).

Bacterial contamination of water poses a serious threat to fish and public health, especially in recreational coastal areas adjacent to heavily polluted regions. Human activities increase nutrient input into aquatic ecosystems, further exacerbating the problem (Skanavis & Yanko, 2001). Coastal seawater contaminated by untreated sewage, municipal wastewater, and recreational activities often harbors various pathogenic bacteria. Isolates commonly include *Vibrio spp.*, *Pseudomonas spp.*, *Salmonella spp.*, *Entero cloacae*, *Shigella spp.*, *Escherichia coli*, and other coliform bacteria (Santhiya *et al.*, 2011).

In Libya, waste management continues to be one of the most pressing environmental challenges. To date, the country has not adopted a practical, cost-effective and environmentally sustainable strategy for sewage treatment and disposal. At present, untreated sewage is discharged directly into the sea, a practice deemed unacceptable due to the increasing volume of waste, foul odors and the escalating risks to aquatic and public health. Seaport waters in Libya are exposed to various

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pollution sources including oil spills from shipping activities, discharge of ballast water, industrial effluents, and untreated municipal wastewater. These inputs contribute to the accumulation of heavy metals, hydrocarbons and microbial contaminants, posing risks to marine biodiversity and public health (Elhachmi *et al.*, 2021).

This study aimed to assess the impact of heavy metal and bacterial pollution in Benghazi seaport on the health status of the common seabream (*Pagrus pagrus*). It is hypothesized that exposure of *P. pagrus* to heavy metal and bacterial pollution in Benghazi seaport leads to significant alterations in tissue metal accumulation, hematological parameters, and bacterial load, reflecting physiological stress and environmental degradation. Heavy metal levels and bacterial spp. loads were measured in the seaport water and fish tissue samples. In addition, some hematological parameters were investigated in fish from the seaport area. Data were benchmarked against FAO/WHO guidelines and other international water quality standards to evaluate the potential risk to human health and ecosystem.

Materials and methods

Study area

The present study was conducted at Benghazi seaport, located on the northeastern coast of Libya, along the Mediterranean Sea (32°12′54″N, 20°09′16″E). The port serves as a vital hub for commercial shipping, fishing activities and oil transport; making it one of the most strategically important harbors in the region. However, the area is subject to significant environmental pressures due to urbanization, industrial activities, and inadequate waste management.

Sampling procedures

- *Sampling of seaport water*

Water and bacterial samples were collected from three selected sites within Benghazi seaport under aseptic conditions following the standard methods for the examination of water and wastewater (American Public Health Association (APHA), 2017). Surface water samples (approximately 50 cm depth) were taken from three different points at each site using sterile glass bottles that had been autoclaved and rinsed with sample water prior to collection. Immediately after sampling, the bottles were sealed with sterilized stoppers, placed in an ice box at 4°C, and transported to the microbiology laboratory within two hours of collection. All samples were analyzed for bacterial contamination and heavy metal content using spectrophotometric methods according to standard protocols.

- *Collection of fish samples*

Specimens of *P. pagrus*, with an average body weight of 350±5 g and body length of 31cm, were collected from Benghazi Seaport. Fresh samples were obtained from the Bankina fish market under sterile conditions, where the fish had been recently caught by local fishermen using gill nets; a traditional fishing method widely practiced along the Libyan coast. Gill nets are passive fishing gears that trap fish by their gills as they attempt to swim through the mesh. This method is considered efficient for capturing demersal and pelagic species of specific sizes. The use of gill nets typically causes limited physical stress and damage to the fish, if retrieved promptly, which helps in preserving tissue integrity and ensuring reliable biological and chemical analyses.

Physical analysis of Benghazi seaport water

Water temperature (°C) and dissolved oxygen (DO, mg/l) were measured *in situ* at each sampling location using a calibrated multiparameter water quality meter, following the APHA (2017) standard methods. Measurements were taken at approximately 50 cm below the surface to minimize diel fluctuations. Temperature readings were recorded to assess possible thermal variations among sites, while DO levels were used as indicators of oxygen availability and organic load in the seaport.

Heavy metals analysis procedures

- *Heavy metals analysis of seaport water*

Water samples collected from the three sampling sites were analyzed for heavy metal concentrations following the standard procedures of the APHA (2017) and the United States Environmental Protection Agency (USEPA, 2007) guidelines. Each water sample (500 ml) was first filtered through a 0.45 µm membrane filter to remove suspended particles and subsequently acidified to pH<2 using ultrapure nitric acid (HNO_3, 65%) to preserve dissolved metals. Samples were then digested using concentrated nitric acid and hydrogen peroxide (H_2O_2) on a hot plate at 95°C until the volume was reduced to approximately 50 ml and the solution became clear. After cooling, the digest was filtered and diluted to 100 ml with deionized water.

Concentrations of iron (Fe), zinc (Zn), copper (Cu), cobalt (Co), lead (Pb), and cadmium (Cd) were determined using Atomic Absorption Spectrophotometry Atomic Absorption Spectrophotometry (AAS) (M Series, Thermo Electron Corporation, Waltham, Massachusetts, USA), under optimized analytical conditions for each element. Calibration curves were constructed using certified standard solutions, and all measurements were performed in triplicate.

Results were expressed in milligrams per liter (mg/l). Analytical accuracy and precision were checked using blank and recovery tests, and metal concentrations were compared to international permissible limits (FAO/WHO, 2011; EPA, 2007) to assess water quality.

- *Heavy metals analysis in fish tissues*

Fish were dissected on a clean and sterile surface. Tissue samples included dorsal muscle (taken from the region between the lateral line and the base of the dorsal fin), kidneys and gills. Prior to analysis, all samples were surface-dried using filter paper. Portions of tissue (0.5–1.0 g) were digested in 10 ml of concentrated nitric acid (70%) on a hot plate at approximately 100–110°C for 4–6 hours. Heating was essential as digestion at room temperature was insufficient to achieve complete tissue breakdown. After cooling, the digested mixture was filtered into a 25 ml volumetric flask and diluted to volume with deionized water. The final solution was transferred into clean plastic tubes for analysis. Concentrations of heavy metals including Cu, Pb, Fe, Zn, Co and Cd were determined using AAS (M Series, Thermo Electron Corporation, Waltham, Massachusetts, USA). All analyses were conducted at the Environmental

Laboratory of Omar Al-Mukhtar University and results were expressed in micrograms per gram of wet tissue weight (µg/g wet weight).

Metal Pollution Index (MPI) Calculation

The Metal Pollution Index (MPI) was calculated to assess the overall level of heavy metal accumulation in the analyzed fish tissues. The MPI represents the geometric mean of the concentrations of all examined metals within a given tissue sample and provides a single comparative value reflecting the degree of metal contamination.

The formula applied in this study follows the methodology proposed by Usero *et al.* (2004), Türkmen *et al* (2005) and Khan *et al.* (2014) as the following: $MPI=(C_1\times C_2\times C_3\times\cdots\times C_n)^{1/n}$, where C*fn*=concentration of n metals in the sample and n=total number of analyzed metals.

A higher MPI value indicates greater overall metal contamination within the tissue. This index is widely applied for comparative assessment of metal bioaccumulation in aquatic organisms and sediments.

Microbial examination procedures

- Bacteriological examination of seaport water

The total viable count (TVC) of microorganisms was determined in different samples by culture on nutrient agar plates with adequate sample dilution. The Colony-Forming Unit per milliliter (CFU/ml) was used to denote the results. The colonies were formed after 48 hours of incubation. The media were used to isolate different bacteria from the stool index. Total coliforms and *Enterobacter colacae* were identified using MacConkey agar and the dishes were incubated for 24 hours at 37°C. Bacterial isolations were performed following the standard microbiological examination methods for water and wastewater, as described by the APHA (1995). Identification of the isolates was carried out using biochemical tests, including Gram staining and Analytical Profile Index 20E (API 20E) identification kits (bioMérieux, Marcy-l'Étoile, France) and according to previously published methods (Patra *et al.*, 2009).

The API 20E strip consists of 20 microtubes containing dehydrated biochemical substrates. These substrates are rehydrated upon inoculation with a bacterial suspension. During incubation, bacterial metabolism induces color changes in the microtubes, either spontaneously or following the addition of specific reagents. The resulting reaction profile was interpreted using the test reading table and a bacterial species is identified based on its API.

- Bacteriological examination of fish tissues

Five specimens of *P. pagrus* were collected for bacteriological examination from the seaport of Benghazi using local traps. Sterile dissection techniques were used to collect tissue samples from the gills, intestines, and muscles of each fish. Approximately one gram of each tissue was homogenized in 9 ml of sterile normal saline (0.85% sodium chloride) to prepare a 1:10 dilution mixture. Serial dilutions were then performed and 0.1 ml of each dilution was spread onto selective agar media (MacConkey and nutrient agars) using the pour plate method. All plates were incubated at 37°C for 24-48 hours. CFUs were harvested and bacteria were identified based on colony morphology, Gram staining, and biochemical characteristics using standard microbiological techniques, as described in Cappuccino and Sherman (2014).

Fish hematological analysis

Blood samples were collected from the caudal vein of the fish using a heparinized syringe and transferred into sterile plastic tubes containing heparin as an anticoagulant. The fresh blood was immediately used for the determination of red blood cells count (RBC), total white blood cells count (WBC), Thrombocytes count (TCs), hemoglobin concentration (Hb) and hematocrit value (Ht). To ensure accuracy, five freshly caught fish were transported alive to the laboratory, anesthetized, and immediately analyzed for hematological parameters.

Ethical approval

All fish handling, sampling, and laboratory analysis procedures were conducted in accordance with internationally recognized ethical standards for the use of animals in scientific research. The study was informally reviewed and approved by academic staff at the College of Natural Resources and Environmental Sciences, University of Derna and the Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Omar Al-Mukhtar University. No endangered or protected species were used in this study and efforts were made to minimize fish suffering during handling and sampling.

Results

Physical parameters of Benghazi seaport water

The results indicated that the highest concentration of dissolved oxygen was recorded at sampling site 1, with a value of 8 mg/l (Table 1). This elevated level is likely attributed to the site's proximity to the open sea, where water is relatively unpolluted and exhibits lower biological oxygen demand (BOD). Conversely, the lowest dissolved oxygen concentration was observed at site 3, measuring 6 mg/l (Table 1). This reduction may be due to the site's location near a ship docking area, which is typically associated with increased anthropogenic activity, elevated levels of organic matter, and higher oxygen consumption.

Table 1. Physical parameters of different locations in Benghazi seaport water.

Sample	Temp. (°C)	DO (mg/l)
Site 1	26	8
Site 2	25.5	7.6
Site 3	26	6

Temp.: Temperature; DO: Dissolved oxygen

Heavy metals concentration in Benghazi seaport water

The concentrations (mean±SD) of heavy metals in Benghazi seaport water are presented in Table 2. The concentration of Fe reached 6.091 mg/l, while Co showed the lowest value of 0.011 mg/l. The

concentration of Zn was 0.427 mg/l, and that of Pb was 0.73 mg/l. The concentration of Cd was 0.011 mg/l.

These values represent the measured levels obtained from the analyzed seawater samples collected at different sites within Benghazi seaport.

Table 2. Concentration of heavy metals (mg/l) in Benghazi seaport water compared with WHO (2011) limits.

Metal	Site 1	Site 2	Site 3	Mean±SD	WHO Limit (2011)
Pb	0.070	0.074	0.075	0.073±0.003	0.01
Cd	0.010	0.011	0.012	0.011±0.001	0.003
Cu	0.090	0.094	0.092	0.092±0.002	2.00
Zn	0.410	0.430	0.440	0.427±0.004	3.00
Fe	5.900	6.100	6.270	6.091±0.011	0.30
Co	0.010	0.011	0.012	0.011±0.001	0.05

Pb: Lead; Cd: Cadmium; Cu: Copper; Zn: Zinc; Fe: Iron; Co: Cobalt

Heavy metals concentration in fish tissues

A total of twenty-five *P. pagrus* fish specimens were collected from Benghazi seaport. For each fish, three tissues (muscle, kidney, and gills) were analyzed for metal concentrations.

Results in Table 3 are presented as mean±SD based on three composite replicates (n=3) representing pooled samples from the 25 fish to determine the concentrations (µg/g) of six elements; Cu, Pb, Fe, Zn, Co and Cd. As indicated in Table 3, the highest concentrations of Fe were recorded in the gills and kidneys, while the muscle tissue exhibited comparatively lower values. Zn and Cu showed moderate levels across all tissues, whereas Co was slightly higher in the gills. Pb and Cd were detected in all examined tissues at varying concentrations. Overall, the data reveal tissue-specific variations in heavy metal accumulation.

MPI results

Among the examined tissues, the gills exhibited the highest MPI value, reflecting their role as the primary site of metal accumulation due to direct contact with the aquatic environment (Table 4).

Bacterial load in Benghazi seaport water

Following microbial testing, both the bacterial species and the number of colonies were determined. The first sample was identified as the least polluted site. Bacteria such as *E. cloacae*, *Klebsiella spp.*, *Proteus*, *Staphylococcus spp.*, and other coliform bacteria were detected using the API 20E test (Figure 1). The results of bacterial identification and total viable counts are summarized in Table 5.

In Benghazi seaport, site 1 showed the lowest contamination (77 CFU/100 ml), site 2 was moderately contaminated (80 CFU/100 ml), and site 3 exhibited the highest contamination (100 CFU/100 ml), likely due to its proximity to a wastewater outfall and commercial activities. Although all sites are below the maximum permissible limit of 200 CFU/100 ml for *E. coli* and total coliforms in recreational waters, the presence of *Klebsiella*, *Proteus*, and *Staphylococcus* spp. suggests organic/human contamination and potential health risk.

Table 3. Concentrations of heavy metals (µg/g wet weight) in different tissues of *P. pagrus* collected from Benghazi seaport.

Metal	Muscle	Kidney	Gill
Cu	15.2±1.8	19.8±2.1	18.6±2.0
Pb	0.56±0.03	0.58±0.04	0.62±0.05
Fe	95.4±5.2	125.7±6.4	132.8±7.3
Zn	47.6±3.8	52.3±4.0	55.1±4.2
Co	0.24±0.02	0.26±0.03	0.31±0.03
Cd	0.29±0.02	0.30±0.03	0.33±0.02

Values are expressed as mean±SD (n=3 composite samples). Data were obtained from composite samples of *P. pagrus* tissues (muscle, kidney, and gill) collected from three locations at Benghazi seaport. Cu: Copper; Pb: Lead; Fe: Iron; Zn: Zinc; Co: Cobalt Cd: Cadmium

Table 4. Metal Pollution Index (MPI) values in tissue samples of *P. Pagrus* collected from Benghazi seaport.

Tissue	MPI
Muscle	4.47
Kidney	4.74
Gill	5.23

Values represent mean±SD (n=3). Higher MPI values indicate greater metal contamination.

Bacterial load in fish tissues

During the microbiological analysis of *P. pagrus* samples collected from Benghazi seaport, several bacterial species were isolated, including *E. coli*, *Klebsiella spp*., *Proteus spp*., and *Staphylococcus spp.* The bacterial counts varied according to the tissue type (Table 6). The highest bacterial load was observed in the intestine, where *E. cloacae* reached (55 CFU/100ml) and *Proteus spp.* (35 CFU/100ml), followed by the gills,

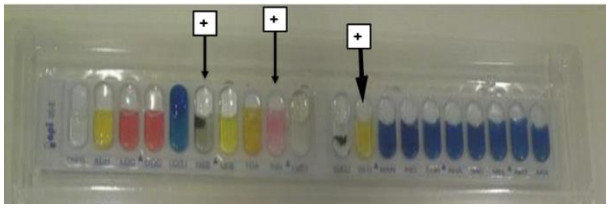


Figure1. API20E test result. The biochemical identification using the API 20E system revealed positive reactions for citrate utilization (CIT), urease activity (URE), and glucose fermentation (GLU), while other reactions such as H_2S production and indole formation were negative. This biochemical profile is consistent with the typical characteristics of *Enterobacter cloacae*, a member of the Enterobacteriaceae family. These results confirm that the isolated strain belongs to the *Enterobacter cloacae* group, which is commonly associated with aquatic environments and may act as an opportunistic pathogen in fish.

Table 5. Bacterial species isolated and total viable count (TVC) (CFU/100 ml) in water of different locations of Benghazi seaport.

Bacterial Spp.	Site 1	Site 2	Site 3
Escherichia coli	+	+	+
Klebsiella	+	+	+
Proteus	+	+	+
Staphylococcus	+	+	+
Other coliforms	+	+	+
TVC	77	80	100

+: Present

which showed *Klebsiella spp.* at 48 CFU/100ml. The lowest bacterial count was recorded in the muscle tissue, where *Staphylococcus spp.* reached 22 CFU/100ml. The total bacterial count across all tissues combined was 77 CFU/100ml. These results indicate moderate to high microbial contamination, particularly in organs directly exposed to the surrounding water, such as the gills and intestine.

Hematological parameters of P. Pagrus in Benghazi seaport

The hematological analysis of *P. pagrus* from Benghazi seaport revealed marked alterations in several blood parameters compared with typical reference values (Table 7). The red blood cell (RBC) counts averaged 2.72±0.11×10⁶/µl, which falls within the normal range (1.5–3.5×10⁶/µl), indicating possible early adaptive responses to stress. hematocrit (Ht) and hemoglobin (Hb) levels were slightly elevated, recording 38.90±3.27% and 13.48±1.4 g/dl, respectively, suggesting compensatory mechanisms to maintain oxygen transport under polluted conditions. Conversely, thrombocytes (TCs) counts were markedly reduced (12.33±2.51 ×10³/µl) compared with the reference range (20–100×10³/µl), indicating possible impairment of hematopoietic activity or toxic effects of heavy metals. White blood cell (WBC) counts were significantly elevated (64.25±4.38×10³/µl), reflecting an immune response to environmental stressors and bacterial exposure. Collectively, these hematological changes confirm that fish from the polluted site were experiencing physiological stress and possible toxicological effects associated with heavy metal and bacterial contamination .

Note: Reference ranges for fish hematological parameters vary depending on the species. However, general guidelines can be found in sources such as the FAO, EPA, and scientific literature including (Svobodova et al., 1993).

Table 6. Colony counts (CFU/100ml) in different *P. Pagrus* parts.

Bacterial Spp.	Isolation Site	Colony Count
E. cloacae	Intestine	55
Klebsiella	Gills	48
Proteus	Intestine	35
Staphylococcus	Muscle	22
Total Count	Combined tissues	77

Table 7. Hematological parameters (mean±SD) of *P. pagrus* collected from Benghazi seaport.

Index	Value	Reference Range*
RBC (×10⁶/µl)	2.72±0.11	1.5–3.5
Ht (%)	38.90±3.27	30–45
Hb (g/dl)	13.48±1.40	8–12
TC (×10³/µl)	12.33±2.51	20–100
WBC (×10³/µl)	64.25±4.38	20–150

Values represent mean±SD (n=3). RBC: Erythrocytes; Ht: Haematocrit; Hb: Haemoglobin; TC: Thrombocytes; WBC: Leucocytes. *Reference ranges for fish hematological parameters vary depending on the species. However, general guidelines can be found in sources such as the FAO, EPA, and scientific literature (e.g. Svobodova *et al.*, 1993).

Discussion

The present study was conducted to evaluate the combined effects of heavy metal and bacterial pollution on the common seabream (*Pagrus pagrus*) collected from Benghazi seaport, Libya. By assessing water quality, metal accumulation in fish tissues, and hematological parameters, the research aimed to determine how environmental contamination influences fish physiology and reflects the ecological status of the seaport. The findings indicate that Benghazi seaport ecosystem is under considerable anthropogenic pressure, leading to notable chemical and biological disturbances. The observed values of DO and temperature revealed moderate variations among the sampling sites. The highest DO concentration (8 mg/l) was recorded at site 1, whereas the lowest (6 mg/l) occurred at site 3. These differences reflect the spatial influence of organic load and hydrodynamic mixing. Lower DO levels near the ship docking area are consistent with elevated biological oxygen demand and limited water circulation, which is typical of harbors affected by urban effluents. Similar findings have been reported by Sweilum (2006) and El Zrelli *et al.* (2015), who attributed DO depletion in coastal waters to increased organic pollution and restricted exchange with open sea water. The temperature values (25.5–26 °C) fall within the normal range for Mediterranean coastal waters, indicating that thermal stress was not a major factor influencing water quality.

Analysis of heavy metals in Benghazi seaport water showed that Fe had the highest concentration (6.091 mg/l), followed by Zn (0.427 mg/l) and Pb (0.77 mg/l). Cd and Co showed lower levels (0.011 mg/l each). The concentrations of Fe, Pb, and Cd exceeded the permissible limits recommended by WHO (2011) and USEPA (2007), indicating significant contamination. These findings are consistent with the earlier results of Ahmed and Goni (2010) and Badr *et al.* (2018), who observed elevated heavy metal concentrations in industrial and harbor zones. The high Fe levels likely originate from ship corrosion and port machinery, while Pb and Cd contamination could result from fuel residues and untreated wastewater discharge. Compared with other Libyan coastal cities, such as Tripoli and Khoms (Metwally & Fouad, 2008; Ibrahim *et al.*, 2023), the present study recorded relatively higher concentrations, suggesting increasing pollution on the eastern coast. Metal accumulation in *P. pagrus* tissues followed a tissue-specific pattern; gills > kidney > muscle. The gills exhibited the highest MPI (5.23 µg/g), confirming their direct exposure to contaminated water. Fe and Zn were dominant in all tissues, with Fe ranging from 250 to 270 µg/g and Zn from 50 to 80 µg/g. Pb and Cd concentrations (0.56–0.62 µg/g and 0.26–0.30 µg/g, respectively) exceeded FAO/WHO safety limits (0.5 µg/g for Pb and 0.2 µg/g for Cd), classifying these samples as unsafe for human consumption. The high metal levels in gills and kidneys reflect their roles in respiration and detoxification, where bioaccumulation commonly occurs. Similar tissue-specific accumulation has been reported by Tayel *et al.* (2007) and Abd-Allah & Ismail (2018). Continuous exposure to metal-polluted water may impair fish metabolism and growth, ultimately affecting biodiversity and food safety. Microbiological analysis of seaport water confirmed the presence of *E. cloacae* and *E. coli*, *Klebsiella spp.*, *Proteus spp.*, and *Staphylococcus spp.* across all sampling sites. TVC ranged from 77 CFU/100ml at site 1 to 100 CFU/100ml at site 3. Although these values remain below the US EPA permissible limit (200 CFU/100 ml for recreational waters), the consistent detection of fecal indicator bacteria suggests contamination from untreated sewage and organic waste. The proximity of site 3 to a wastewater outlet explains its higher bacterial density. Comparable bacterial profiles have been reported in polluted coastal waters of Egypt and Tunisia (El-Shenawy *et al.*, 2021; UNEP/MAP, 2012). The presence of opportunistic pathogens such as *Klebsiella* and *Proteus* indicates a potential health hazard for both marine organisms and humans exposed through fish consumption or contact with seawater.

Fish tissues also exhibited bacterial contamination consistent with seaport water results. The intestinal samples showed the highest bacterial loads, with *E. coli* reaching 55 CFU/100ml and *Proteus spp.* 35 CFU/100mL. The gills recorded Klebsiella spp. at 48 CFU/100 ml, while muscle tissues contained the lowest count, with *Staphylococcus spp.* at 22 CFU/100ml. The total bacterial count across all tissues was 77 CFU/100ml, confirming moderate to high microbial contamination. These findings align with those of El-Hossadi *et al.* (1987) and El-Shenawy *et al.* (2021), who linked microbial accumulation in fish to exposure to sewage-polluted environments. The higher bacterial presence in gills and intestines is expected, as these organs are in direct contact with the external environment and play key roles in respiration and digestion.

Persistent bacterial exposure may compromise fish immune defense and reduce overall fitness. Marked hematological alterations were observed in fish from the polluted site. The Erythrocye count ($2.72\pm0.11\times10^6$/µl) remained within normal limits but indicated early adaptive stress. Hemoglobin (13.48±1.4 g/dl) and hematocrit (38.90±3.27%) were slightly elevated compared to reference ranges, reflecting compensatory mechanisms to enhance oxygen transport under polluted conditions. Conversely, thrombocyte counts ($12.33\pm2.51\times10^3$/µl) were markedly below the normal range ($20–100\times10^3$/µl), suggesting impaired hematopoietic activity, possibly due to heavy metal toxicity. The most notable change was the significant increase in white blood cell count ($64.25\pm4.38\times10^3$/µl), indicating an immune response to bacterial infection or chemical stress. Similar hematological patterns have been documented by Tayel *et al.* (2007), Sweilum (2006) and Arnaudova & Tomova (2008), confirming that hematological indices are sensitive biomarkers of environmental pollution in fish.

The concurrent presence of heavy metals and pathogenic bacteria in both water and fish tissues demonstrates a dual stressor effect on *P. pagrus*, leading to physiological and immune disturbances. Elevated metal concentrations and microbial contamination can weaken fish immunity, increase infection susceptibility, and reduce population resilience. The results highlight the urgent need for

stricter waste management and environmental monitoring in Benghazi seaport. Periodic assessments of heavy metals and microbial loads should be integrated into local environmental protection policies. Raising public awareness about the risks of consuming fish from contaminated areas is also essential. Finally, long-term studies are recommended to evaluate bioaccumulation trends and the ecological consequences of combined heavy metal and bacterial exposure in Libyan coastal ecosystems.

Given the limited number of sampling sites and the descriptive nature of the present dataset, inferential statistics were not applied. The study, however, provides valuable baseline information on the dual impact of heavy metal and bacterial pollution on marine ecosystems in Eastern Libya. Future research should include larger sample size, temporal monitoring, and advanced statistical modeling to confirm these trends and to better understand the synergistic effects of chemical and microbial stressors on aquatic organisms.

Conclusion

This study highlights the critical environmental challenges facing Benghazi seaport ecosystem. Elevated concentrations of heavy metals, particularly Cd and Pb, in both water and fish tissues, coupled with significant bacterial contamination, pose a dual threat to aquatic life and public health. Common seabream (*P. pagrus*), used as a bioindicator in this research, showed clear signs of physiological stress and toxin accumulation, particularly in its gills and blood samples.

The results demonstrate that the seaport area suffers from chronic exposure to anthropogenic pollutants, likely resulting from untreated sewage, industrial effluent, and marine activities. The presence of *E. cloacae* and coliforms highlights the urgent need for wastewater management reforms.

Conflict of interest

The authors declare no conflict of interest regarding the publication of this paper.

Author contributions

Nagi Mousa designed the study and supervised the project. Nagi Mousa and Abdulrahman Aljali collected the samples and performed the experiments. Abdulrahman Aljali and Salama I Ahmadi analyzed the data and interpreted the results. All authors contributed in writing and reviewing the manuscript.

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