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The impact of sewage water on some fish in alkhumrah area, south of jeddah governorate, saudi arabia

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Abstract. Treated sewage is a source of water and soil pollution, despite the development of treatment methods, especially the entry of tertiary treatment into use in most purification plants in the Kingdom. This pollution comes because of the high levels of salts of all kinds, especially the remnants of heavy metals that negatively affect the environment and many living organisms. In this field, the Alkhumrah station complex in the south is considered to be one of the largest sewage treatment plants in the Kingdom. It produces large quantities of treated water that are disposed of in seawater, and part of it cuts through the Al-Taawun area in Jeddah, causing pollution in some valleys and negatively affecting some living organisms, including tilapia fish that reproduce naturally in these valleys and lakes. In this context, this study aimed to determine the impact of treated wastewater on the reproduction and growth of Tilapia Fish. This study showed that water affected the growth and weight of fish during the months of May to Oct. 2023 February and Mar. 2024, as the weight of the fish ranged from 14.8 to 54.7 gm, whereas the length of the fish ranged from 84.8 mm during Jun. 2023 to 395.3 mm during Mar. 2024. These changes were attributed to the components of the water during these months, which showed a significant increase in all salts and minerals, including an increase in calcium from 23 mg/L in normal water to 90 mg/L in polluted water, while cadmium increased from 24.6 mg/L to 173 mg/L. As for the fish blood level analyses, the study showed an increase in all the criteria indicating infection and damage to some tissues, including white blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin (Hb), neutrophils (NP), and lymphocytes (LP). This increase coincides with an increase in liver enzymes that indicate liver infections, including alanine aminotransferase (ALT), which increased from 20.36 u/L in normal water to 56.08 u/L in polluted water, also for aspartate aminotransferase (AST) the normal water it was 16.98 u/L and increased in the polluted water during Feb to 35.96 u/L and during Mar to 60.96 u/L. Alkaline phosphatase (ALP) activity also increased from 51.2 to 91.2 u/L. Histological sections were used to document changes and injuries in the liver, gills, and muscles. Various histological changes have been identified, wherein liver cells exhibit an increase in macrophage aggregation within liver tissues, hepatocyte hypertrophy, and intravascular hemolysis within blood vessels, accompanied by cellular degeneration in aggregated fish from the Alkhumrah area.

Keywords: Sewage Water, Fish, Liver, Kidney, pollutants, Physiology, Histology.

Introduction

Water pollution is a significant global challenge confronting the contemporary world. The pervasive issue of water pollution has emerged as a substantial global dilemma in the present era, resonating across the entirety of the world. However, the aquatic environment has numerous adversities owing to the impact of human activities, coupled with the release of both domestic and industrial waste, further exacerbating the challenges faced by our water systems.

Water pollution is defined as the increase in the concentrations or qualities of chemical, physical, or biological attributes that pose harm to both human populations and aquatic organisms (Al-Saadi, 2000).

According to Goel (2006), water pollutants are substances or agents that alter their chemical, physical, or biological properties when introduced into water systems, thereby negatively affecting water quality. These pollutants can be categorized as chemical, biological, and suspended materials. They include substances such as pesticides and industrial chemicals, microorganisms such as bacteria and viruses, and small solid particles or organic matter suspended in water (Neerugatti *et al*., 2022).

The consequences of water pollution are profound for aquatic ecosystems, biodiversity, and the welfare of aquatic organisms and human communities. Water bodies play a pivotal role in sustaining ecological equilibrium and serve as essential habitats for a myriad of life forms. Nevertheless, the persistent discharge of contaminants into these aquatic environments has initiated a cascade of deleterious consequences, transcending the mere diminution in species abundance to infiltrate the fundamental behaviors that govern the ecological interactions and dynamics of aquatic ecosystems (Samuel *et al*., 2023).

The consequence of water pollution is the destruction of habitats in aquatic ecosystems. Contaminants in water can modify or remove physical habitats, thereby posing a threat to bottom-dwelling organisms. Sensitive ecosystems, such as coral reefs, are particularly susceptible to water pollution, leading to coral bleaching, reduced growth, and heightened vulnerability to diseases (Dubinsky and Stambler, 1996; Ellis *et al*., 2019).

Most wadis and coastal areas in the Kingdom of Saudi Arabia are contaminated by industrial and agricultural activities, especially those contributing to water pollution through the extensive use of pesticides and agricultural chemicals (Al-Akel and Shamsi, 2000). Wadi Fatimah is considered a significant example, as it is one of the primary sites for the disposal of treated and untreated sewage water, significantly affecting the quality of the surrounding water (Rehman *et al*., 2020). The southern corniche area of Jeddah receives approximately 300,000 m^3 of semi-treated sewage from AlKhumrah (Basaham *et al*., 2009). Near the industrial area of the AlKhumrah District, it is the largest wastewater treatment plant in the southern part of Jeddah city (Al-Farraj *et al*., 2012).

Many researchers have investigated the physicochemical properties of water contaminated with sewage effluent. As elucidated by many authors, the AlKhumrah area has experienced significant changes in water quality owing to the impact of sewage discharge, including elevated levels of pollutants, altered pH balance, and increased concentrations of nutrients and harmful microorganisms (Al-Farawati, 2010; Salama *et al*., 2016; Al-Zahrani and Al-Hasawi, 2018; Al-Hasawi and Hassanein, 2022).

Moreover, chemical analyses alone do not fully elucidate the impact of chemical pollution on the aquatic environment owing to the potential synergistic or antagonistic effects of intricate mixtures of chemical pollutants. In this context, alternative monitoring methods involving biomarkers have been developed to reliably assess environmental quality (van der Oost *et al*., 1996).

Hematological and biochemical parameters serve as critical biomarkers for evaluating fish health as they offer insights into the effects of pollutants. Alterations in these parameters can indicate stress and adverse effects of chemical exposure (Faggio *et al*., 2014; Oyeniran *et al*., 2021).

The present study aimed to evaluate the impact of sewage water on *tilapia* (*Oreochromis spilurus*) in the Alkhumrah area and assess its effects on fish health indicators, including hematological and physiological parameters. The study also investigated histological changes in the liver, gills, and muscles to detect potential effects not captured by standard monitoring. Additionally, physical and chemical analyses of sewage water were conducted to establish a baseline understanding of environmental pressures on fish populations in the area.

Materials and Methods

Study Area

The study area is situated along a watercourse named 'At Taawon' in Jeddah Province, Saudi Arabia, spanning a length of 3.8 kilometers and reaching depths of up to 2 meters, with geographical coordinates, Latitude 21.33691°N and Longitude 39.27446°E (Figure 1). Located within the Alkhumrah area south of Jeddah, it is located along the sewage water stream (Figure 1). The land ecosystems were dominated by Mesquite trees (Prosopis spp.).

Sample Collection

The sampling sites were selected based on the concentrations of pollutants in Alkhumrah. Fish (*Oreochromis spilurus*) samples were obtained through collaboration with local fishermen distributed across the specified study area (King Abdulaziz University Faculty of Pharmacy Ethical number Reference No: p116-2023). A completely randomized design was used in this study.

Ten *Tilapia* fish (*Oreochromis spilurus*) were collected during two periods: the first period extended from May to Oct. 2023 and corresponded to the summer period and fish growth. During this period, precipitation was always very low, and the salt concentration in the wastewater was very high. The second period in February- March 2024 corresponds to the winter-spring period. Salts Wastewater can be diluted following rainy period. Subsequently, the control group fish were obtained from aquaculture facilities at the College of Marine Sciences, King Abdulaziz University in Obhor in May 2023. These live samples were then transported to the laboratories of King Abdulaziz University in bags filled with oxygen-supplemented water. Fish from both the Alkhumrah area (polluted area) and fish farms (control) were placed in containers containing water from the collection sites and acclimated for one hour to alleviate the pressure resulting from collection before the preparation process. Subsequently, the collected fish were anesthetized using clove oil (Sarma *et al*., 2013).

Water samples were collected from the Alkhumrah area (polluted zone) and from fish farm basins (control), where the water samples were filtered and placed into clean bottles.

Blood samples for hematology were obtained from the caudal vein using a syringe and transferred into an EDTA tube. To ensure complete mixing with the anticoagulant, the contents were gently stirred 8–10 times. Additionally, the samples were placed on a roll mixer to enhance homogenization before further analysis.

Methods

An automated blood counting device (Cell Dyn 3700) was used to conduct the hematological analyses. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using the method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) enzyme activity was assessed using the method described by McComb and Bowers (1972). Total protein levels were determined using the method described by Peters (1968), while total lipid levels were measured according to the method described by Thaonnhauser (1958). The urea levels were estimated using the method described by Patton and Crouch (1977). Histological examinations were performed systematically following the procedures described by Al-Asiri (2009).

Statistical Analysis

The acquired data were systematically arranged in a tabular format for statistical analysis using the IBM SPSS Statistics 26 software. One-way ANOVA was used to assess variance and compare means. For the comparison of average blood parameters between tilapia from the contaminated area and the control group, a One-Way ANOVA statistical test was conducted to determine statistical significance. Additionally, a one-sample t-test was performed to assess the average values of the parameters in the chemical analysis of water and establish their significance level. The results are presented as the mean ± standard deviation.

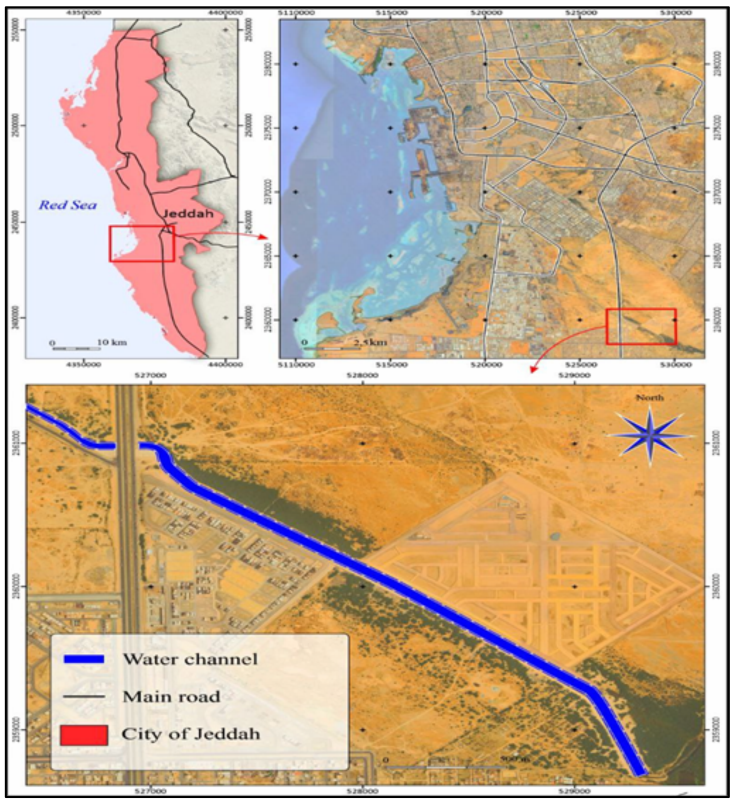

Figure 1. Location map of the study site at Alkhumrah, showing a wastewater stream in the southern region of the Jeddah governorate (By Ibrahim Al-Jaziri (2023), *personal communication*).

Results

Morphological findings

Weight and length values were analyzed using one-way ANOVA test are shown in (Table 1).

The adult tilapia fish collected from the control group had an average wight of 48.2 gm and an average length of 139 mm. Fish growing in polluted zones show a decrease in their weight ranging from 14.8 to 48.3 gm, during the hot period (May to Octobre 2023). In parallel, body length also decreases and ranges from 84.8 mm to 150.5 mm. During the moderate period (February–Mar. 2024) and because of the dilution of the treated sewage water, adult tilapia recorded high growth with a body weight ranging from 50.8 to 54.7 gm and body length ranging from 375.3 to 395.3 mm.

Table 1: Comparison of average tilapia fish body lengths and body weights between (May and Oct. 2023), including February and Mar. 2024.

Month		Body weight (gm)	Body length (mm)
Control (May-23)		48.22±16.81	139.09±27.67
Drought/hot weather	May-23	48.39±5.23	135.46±7.48
	Jun-23	14.8±4.66**	84.8±24.75**
	Jul-23	44.14±23.53	131.56±15.93
	Aug-23	25.5±10.63**	111.91±16.40*
	Sep-23	30.48±12.69*	117.5±17.55
	Oct-23	17.29±13.73**	150.59±19.61
moderate weather	Feb-24	50.82±4.74**	375.36±14.95**
	Mar-24	54.74±36.98	395.36±22.80**

Data are presented as the mean ± SD, *Indicates statistical significance; p<0.5.

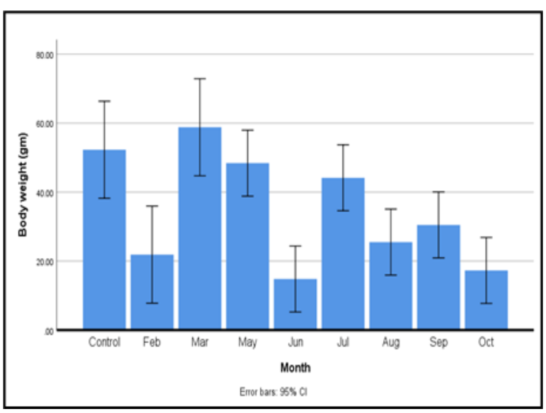

Figure (2). Comparative analysis of average tilapia fish weights across different months (May to October 2023), including the months of February and March 2024, and a control group. *Indicates statistical significance (p<0.5).

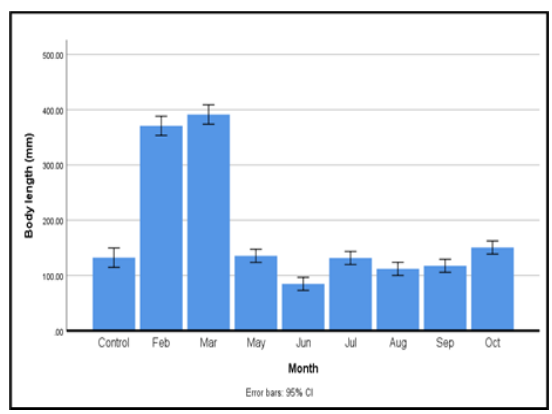

Figure (3). Comparative analysis of average tilapia fish lengths across different months (May to October 2023) including the months of February and March 2024, and a control group. *Indicates statistical significance (p<0.5).

Water analysis

Water quality parameter values were analyzed using a one-sample t-test. Water analysis showed a high concentration of all salts in the sewage water of Alkhumrah, corresponding to an electrical conductivity of 2760 µS/cm (Table 2). A sodium concentration of 676 mg/l is the highest salt concentration. However, sewage water shows also high concentration of some heavy metals such as cadmium (173 mg/l), zinc (167.6 mg/l), lithium (66.3 mg/l) and copper (23.1 mg/l) that were considered as noxious for living organisms.

Table 2. Measured concentrations of physicochemical properties in both the polluted and control groups.

Parameters	Control	Alkhumrah	Units
pH	7	6.67	
Sodium (Na)	21	676*	mg/l
Potassium (K)	4.9	49	mg/l
Calcium (Ca)	23	90.67*	mg/l
Copper (Cu)	6.2	23.17*	mg/l
Iron (Fe)	10.3	64.67*	mg/l
Lithium (Li)	13.2	66.33**	mg/l
Manganese (Mn)	18	82*	mg/l
Zinc (Zn)	14.7	167.67*	mg/l
Cadmium (Cd)	24.6	173**	mg/l
Electrical conductivity (EC)	1564	2760*	µS/cm

*Indicates statistical significance; $p<0.5$.

Blood analysis

The results of the one-way ANOVA for blood analysis are presented in (Table 3). A significant and statistically significant increase in some hematological parameters, such as WBCs, RBCs, and Hb, was recorded during the study period (May-July-October). In contrast, the numbers of neutrophils and eosinophils decreased.

Table 3. Measurement of hematological parameters in Alkhumrah area and control groups.

Parameters	Control	May	July	October
WBCs (cells/mm³)	7005±1503.28	13925±3157.40**	15456.67±1461.72**	8233.33±2374.12
RBCs (cells/mm³)	6020700±1147729.36	3692000±331787.08**	3946666.67±371124.42**	8783333.33±872028.29**
Platelets (Plt) cells/µl	13.15±1.07	10.63±0.61**	9.40±0.5**	14.80±1.21*
Hemoglobin (Hb) g\dl	5.446±10.551	10.0025±7.32**	8.896667±11.480563**	48.7067±7.922
Neutrophils (N.P) %	43±9.38	28.25±5.25**	28±2.65**	24±5.29**
Lymphocytes (L.P) %	43±7.27	53.25±6.24*	48.33±7.64	61±3.61**
Monocytes (M.C) %	9.40±2.99	12.75±4.57	13.67±4.93	13.67±6.43
Eosinophils (E.P) %	3.40±2.12	3±1.83	2.67±2.08	1.33±0.58

Data are presented as mean ± SD, * Indicates statistical significance ($p<0.5$).

Enzyme Activities

The results of the one-way ANOVA for enzyme activity analysis are presented in (Table 4).

Aspartate Aminotransferase (AST) levels were measured in three different groups: control, February, and March. In the Control group, the mean AST level was 16.98±3.98 u/l. In comparison, the Feb group exhibited a significantly elevated mean AST level of 35.96±18.77 u/l ($p>0.05$). Conversely, the Mar group demonstrated a substantially higher mean AST level of 60.96±24.35 u/l, showing a statistically significant difference when compared to the control group ($p<0.01$).

Alanine Aminotransferase (ALT) levels were assessed across the Control, Feb, and Mar groups. The Control group showed a mean ALT level of 20.36±4.73 u/l. In contrast, the Feb group exhibited a markedly elevated mean ALT level of 56.08±31.01 u/l ($p<0.05$). Similarly, the Mar group displayed a significantly higher mean ALT level of 79.28±35.37 u/l compared to the control group ($p<0.01$).

Alkaline Phosphatase (ALP) levels were analyzed in the Control, Feb, and Mar groups. The Control group demonstrated a mean ALP level of 51.2±7.66 u/l. In comparison, the Feb group displayed a notably increased mean ALP level of 91.2±36.57 u/l ($p<0.05$). Similarly, the Mar group exhibited a significantly higher mean ALP level of 110±42.81 u/l compared to the control group ($p<0.05$).

Table 4. Measurement of enzyme activity in the Alkhumrah area and control groups.

Parameters	Control	Feb	Mar	Units
AST	16.98±3.98	35.96±18.77	60.96±24.35**	U/L
ALT	20.36±4.73	56.08±31.01*	79.28±35.37**	U/L
ALP	51.2±7.66	91.2±36.57*	110±42.81*	U/L

Data are presented as mean ± SD, *Indicates statistical significance ($p<0.5$).

Biochemical Parameters

The values of Biochemical parameters were analyzed using a one-way ANOVA test. Table 5 presents the results of this analysis are shown in (Table 5). The concentration of Total Protein was examined in the Control, Feb, and Mar groups. The Control group had a mean Total Protein level of 8.82±1.21 mg/dl. In contrast, the Feb group displayed a slightly decreased mean Total Protein level of 7.94±1.63 mg/dl, albeit statistically insignificant ($p>0.05$). Likewise, the Mar group demonstrated a further decline in mean Total Protein level to 6.54±2.08 mg/dl, which also lacked statistical significance compared to the control group ($p>0.05$).

The total Lipid levels were evaluated across the Control, Feb, and Mar groups. The Control group exhibited a mean Total Lipid level of 113.4±7.89 mg/dl. Conversely, the Feb group displayed a significantly higher mean Total Lipid level of 125.8±6.3 mg/dl ($p<0.05$). However, the Mar group showed a mean Total Lipid level of 129.5±13.33 mg/dl, which was not statistically significant compared to the control group ($p>0.05$).

Urea levels were measured in the control, February, and March groups. The Control group demonstrated a mean urea level of 15.1±2.17 mg/dl. In contrast, the Feb group displayed a markedly elevated mean urea level of 128.85±190.9 mg/dl, although this difference was not statistically significant ($p>0.05$). Conversely, the Mar group exhibited a significantly higher mean urea level of 43.95±20.8 mg/dl compared than the control group ($p<0.05$).

Table 5 Measurement of biochemical parameters in Alkhumrah area and control groups.

Parameters	Control	Feb	Mar	Units
Total Protein	8.82±1.21	7.94±1.63	6.54±2.08	mg/dl
Total Lipids	113.4±7.89	125.8±6.3*	129.5±13.33	mg/dl
Blood Urea	15.1±2.17	128.85±190.9	43.95±20.8*	mg/dl

Data are presented as mean ± SD, *Indicates statistical significance ($p<0.5$).

Histopathological findings

A-Liver:

Histological examination of liver sections from the control group (G1) (Figure 4) revealed normal hepatocytes surrounding the central vein with no notable abnormalities. In contrast, the May group (G2) (Figure 5) displayed moderate histological alterations, including increased macrophage aggregation, hepatocyte hypertrophy, and intravascular hemolysis. More severe changes were observed in the July group (G3) (Figure 6), characterized by pronounced macrophage aggregation, hepatocyte hypertrophy, and significant cellular degeneration. The Oct group (G4) (Figure 7) exhibited mild histological disturbances, with intermediate features of macrophage aggregation, hepatocyte hypertrophy, and cellular degeneration compared to the other groups.

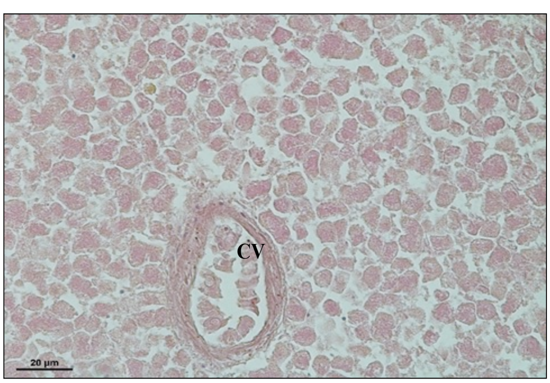

Figure 5. Section of tilapia fish liver of control group (G1) in the central vein (CV) region, showing normal hepatocytes near the central vein (CV) (H&E X400).

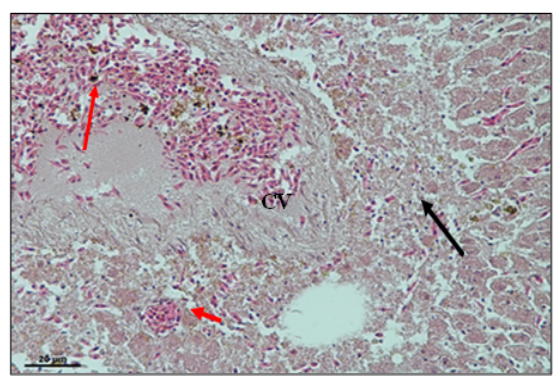

Figure 6. Section of tilapia fish liver of the May group (G2), showing central vein (CV) regions. Found moderate macrophage aggregation and hepatocyte hypertrophy (black arrows) and interavascular hemolysis in hepatoportal blood vessels and cellular degeneration (red arrows) (H&E X400).

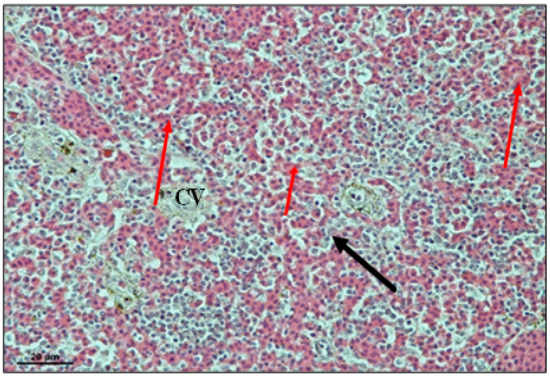

Figure 7. Section of tilapia fish liver of Jul group (G3), in the central vein region (CV). Found severe macrophage aggregation and hepatocyte hypertrophy (black arrows) and interavascular hemolysis in hepatoportal blood vessels and cellular degeneration (red arrows) (H&E X400)

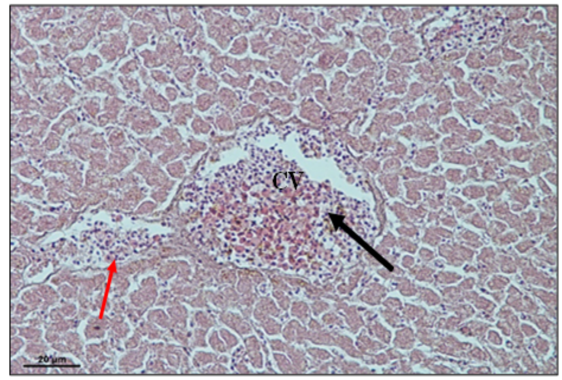

Figure 8. Section of tilapia fish liver of the Oct group (G4), in the central vein region (CV) showing mild macrophage aggregation and hepatocyte hypertrophy (black arrows) and intravascular hemolysis in hepatoportal blood vessels and cellular degeneration (red arrows) (H&E X400)

B-Gills:

Histological examination of gill sections from the control group (G1) (Figure 8) revealed normal epithelial cells and intact lamellae, which served as a baseline. In the May group (G2) (Figure 9), moderate hyperplasia and lamellar fusion were observed along with ruptures in the secondary lamellae and leukocyte infiltration, indicating a moderate inflammatory response. The July group (G3) (Figure 10) exhibited severe hyperplasia, pronounced lamellar fusions, hemorrhage, and substantial leukocyte infiltration, reflecting severe gill pathology. In the Oct group (G4) (Figure 11), mild hyperplasia and lamellar fusion, along with a mild inflammatory response, were present, suggesting an intermediate level of disturbance compared with the other groups.

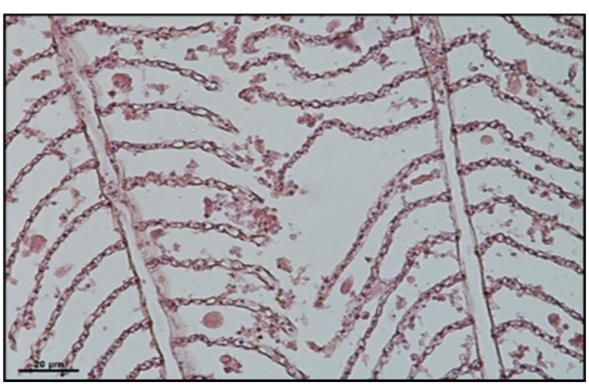

Figure 9. Section of gill regions from the control group (G1), depicting normal epithelial cells and lamellae in a healthy state (H&E X400)

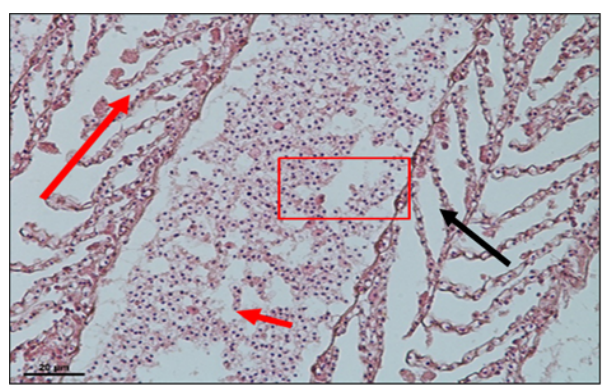

Figure 9. *S*ection of tilapia fish gill regions from the May group (G2), revealing moderate epithelial hypertrophy resulting in lamellae fusions (black arrows) and focal rupture of secondary lamellae, leading to hemorrhage and leukocyte infiltration indicative of inflammation (red arrows) (H&E X400).

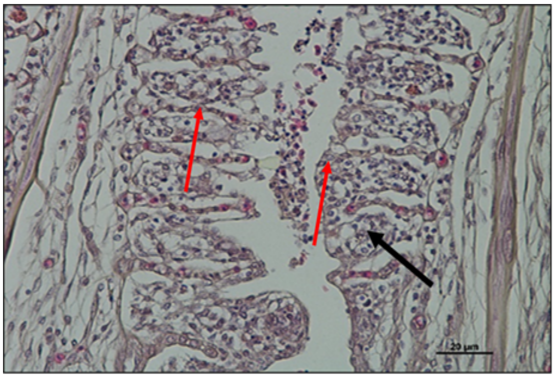

Figure 10. Sections of tilapia fish gill regions from the July group (G3), illustrating severe epithelial hypertrophy resulting in lamellae fusions (black arrows) and focal rupture of secondary lamellae leading to hemorrhage and leukocyte infiltration indicative of inflammation (red arrows) (H&E X400).

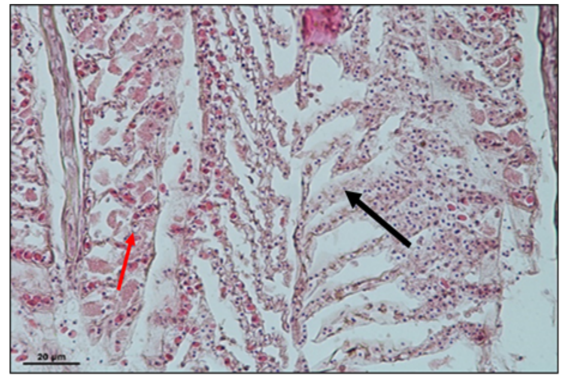

Figure 11. Histological sections of tilapia fish gill regions from the October group (G4), displaying mild epithelial hypertrophy resulting in lamellae fusions (black arrows) and focal rupture of secondary lamellae leading to hemorrhage and leukocyte infiltration indicative of inflammation (red arrows) (H&E X400).

C-Muscles:

Histological analysis of muscle tissues from the tilapia in the control group revealed a normal structure (Figure 12), with well-aligned muscle bundles and fibers showing no signs of cellular damage. In contrast, the study group from the polluted Al-Khumrah area (Figure 13) displayed significant pathological changes, including muscle bundle, necrosis, and vacuolar degeneration. Additionally, noticeable muscular degeneration and edema were observed between the muscle fibers.

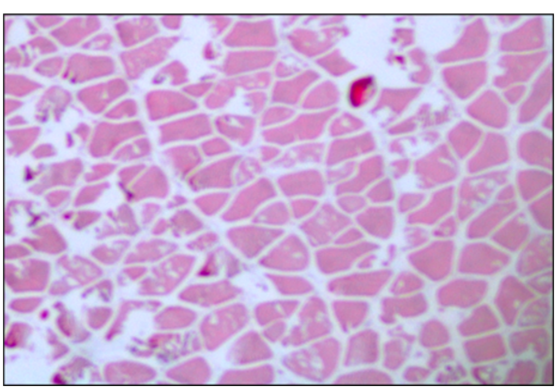

Figure 12. Section of tilapia fish muscle regions from the control group (G1), depicting normal structure and alignment in a healthy state (H&E 400).

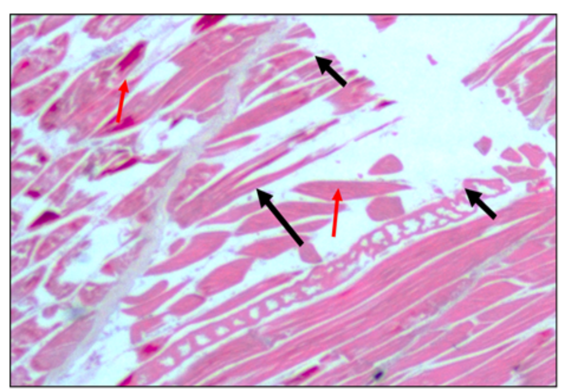

Figure 13. Section of tilapia fish muscle regions from the Al-Khumrah area show degeneration in muscle bundles, a focal area of necrosis, vacuolar degenera (black arrow), atrophy of muscle bundles, and edema between muscle bundles (red arrow) (H&E X400).

Discussion

Significant differences were noted in the water characteristics between the control and Alkhumrah areas. Substantial increases in the concentrations of several elements, such as sodium, potassium, calcium, magnesium, zinc, and cadmium, were observed in the water samples from the Alkhumrah region compared to the control group. This increase may indicate the presence of various sources of water contamination. Additionally, a noticeable increase in the electrical conductivity was observed in the water from the Alkhumrah area, indicating higher levels of dissolved substances in these waters.

In this study, elevated physical and chemical properties were observed, indicating poor water quality. There were no statistically significant differences in the pH values ($p>0.05$). This suggests relatively stable acidity or alkalinity, which is consistent with the findings of Al-Zahrani and Al-Hasawi (2018).

The electrical conductivity showed a significant increase ($p<0.05$) in the alkhumrah region compared to the control group. This result aligns with the findings reported by Al-Zahrani and Al-Hasawi (2018) and Suthar *et al*. (2010), who observed higher conductivity in sewage-contaminated areas than in non-contaminated areas. Shah and Joshi (2015) stated that electrical conductivity is a crucial metric for assessing water quality and is closely linked to the concentrations of dissolved solids, especially salts.

Body weight was lower in October, whereas body length was higher than in the other months. This might reflect the potential impact of environmental conditions on body growth and development during these periods. Variations in climatic conditions, especially in temperature and precipitation between months, can also influence morphological parameters, such as body weight and length. In accordance with these findings, Shivaraj *et al*. (2018) studied the impact of sewage water on the changes in behavior and body weight of the freshwater fish *Cyprinus carpio*. The results showed that sewage is toxic; thus, it led to altered fish physiology and affected body weight.

The water in the Alkhumrah area had much higher levels of Na, Ca, and Mg than that in the control group. The Mg concentration showed a highly significant increase. These findings are consistent with those reported by Al-Zahrani and Al-Hasawi (2018) and Martinelli *et al*. (1999), who documented increased concentrations of Mg, Ca, and Na in polluted sites compared with non-polluted sites. The concentration of K was slightly higher than that in the control group.

The present study showed significant increases in the metals such as Ag, Al, Ba, Cr, Cu, Fe, Li, Mn, Ni, Pd, Zn and Cd, while Co showed insignificant difference. These findings are consistent with those reported by Al-Zahrani and Al-Hasawi (2018), who found no statistically significant differences in Co concentrations between the polluted and non-polluted sites. These findings are consistent with those of Alghobar *et al*. (2014), who noted a significant increase ($p<0.05$) in the levels of Fe, Mn, Cu, and Zn in areas exposed to sewage water compared to those exposed to sewage water.

Hemoglobin (Hb) levels in the alkhumrah group were significantly higher than those in the control group. These findings are consistent with those of Oyeniran *et al*. (2021), who reported a significant increase in Hb levels in fish treated with liquid waste. In contrast, Maceda-Veiga *et al*. (2010)

observed a significant decrease in Hb levels in the most polluted sites. Remya *et al*. (2015) reported that changes in RBC and Hb levels are associated with iron concentration, which may potentially damage the gills, leading to oxygen depletion. According to Gaber *et al*. (2013), a significant increase in Hb levels may indicate a compensatory response that aims to improve oxygen transport to organs experiencing a deficiency.

The blood platelet (Plt) counts in the Alkhumrah group were marginally lower than those in the control group, although the difference was not statistically significant. These findings contradict those reported by Oyeniran *et al*. (2021) and Ibrahim and Elsayed (2023), who reported significant increases in platelet counts and hemoglobin levels.

The proportion of neutrophils in the alkhumrah group was significantly lower than that in the control group. These findings align with those of another study that identified a significant decrease in the percentage of neutrophils in fish exposed to zinc (Srivastava *et al*., 2011). In addition, these findings are consistent with the observations of Al-Asgah *et al*. (2015), where exposure to Cd led to a significant reduction in neutrophil counts.

The lymphocyte and monocyte percentages in the Alkhumrah group were significantly higher than those in the control group. These findings align with those reported by Oyeniran *et al*. (2021) where there were notable increases in the percentage of lymphocytes and by Cazenave *et al*. (2014) and Al-Asgah *et al*. (2015) whose findings reported significant increase in monocytes in fish exposed to sewage.

WBCs in the immune system play a crucial role, as stated by Maceda-Veiga *et al*. (2010), who reported that the percentage of different types of WBCs serves as a valuable tool for assessing the condition of fish. In the current study, the WBCs count was significantly higher in the alkhumrah group than in the control group, suggesting an immune response to environmental stressors. These results are consistent with the findings of Gaber *et al*. (2013), in which an increase in the number of WBCs was noted in fish collected from sewage drains.

The results of the liver enzyme activities were studied during February and March and exhibited significantly elevated AST, ALT, and ALP activities compared to the control group. In accordance with these findings, Ibrahim and ElSayed (2023) and Mohammadiazarm *et al*. (2023) observed significant alterations in fish blood profiles in response to changes in water quality variables. Fish exhibited elevated levels of AST and ALT, which is indicative of hepatotoxicity. Additionally, all fish displayed heightened levels of creatine and urea, suggesting nephrotoxicity, along with diminished antioxidant enzyme activity. Similar findings in agreement with the present study were demonstrated by Essien *et al*. (2015) and Al-Hasawi and Hassanine (2022) who found that, blood biochemical parameters and serum liver enzymes; ALT, AST, ALP and GGT, were significantly elevated in fishes from polluted sites.

The results of the present study showed that total protein concentrations decreased insignificantly in Alkhumrah polluted with sewage. A significant decrease in the levels of total protein and albumin has been detected in fish exposed to wastewater (Banaee *et al*., 2016; Srivastava and Punia (2011)).

In the present study, the total lipid and urea levels were significantly higher than those in the control group. These results are similar to those observed by Ibrahim and ElSayed (2023) and Banaee *et al*. (2016), who observed significant increases in urea, suggesting nephrotoxicity, along with diminished antioxidant enzyme activity.

Bhanot and Hundal (2021) studied the impact of sewage treatment plant effluents on fish health using biochemical and histopathological biomarkers in the muscular tissue of *Labeo rohita* exposed to sewage water. They found a significant decrease in protein levels and a significant increase in total lipid content in the muscular tissue of the exposed group fingerlings.

Regarding histological changes, liver tissues demonstrated an increase in clusters of immune cells within the liver tissues. These findings are consistent with those reported by Al-Wesabi *et al*. (2015), who documented similar changes in fish exposed to sewage water. They reported pathological alterations in the tissues of fish inhabiting waters affected by sewage pollution. These alterations include vacuolated hepatocytes, fatty change, adipocytes, vacuolated foci, inflammatory response, degeneration of hepatocytes, sinusoidal dilation, and frank necrosis in liver tissues.

Hepatocyte hypertrophy was observed in the fish collected from the Alkhumrah area. This is consistent with the study conducted by Macêdo *et al*. (2020), who found similar changes in fish exposed to water from the Dosí Basin after dam rupture of mining waste. The present study revealed the presence of hemolysis within the blood vessels of aggregated fish from the Alkhumrah area. This is consistent with the findings reported by El-Ghamdi *et al*. (2014), who observed hemolysis within the blood vessels of fish exposed to sewage water and industrial pollution.

Abdel-Warith *et al*. (2011) reported similar observations to the present study in fish exposed to zinc, including the presence of hepatocyte hypertrophy and the occurrence of liver cell deterioration, with a notable indication of disorganized hepatic structure. In addition, Camargo and Martinez (2007) observed alterations in the liver, including hepatocyte hypertrophy, cytoplasmic and nuclear degeneration, melanomacrophage aggregates, bile stagnation, and focal necrosis. These findings are in agreement with those of the present study.

The muscle bundles in tilapia from the polluted Alkhumrah area exhibited significant degeneration, characterized by regions of necrosis and vacuolar degeneration. In addition, there were signs of noticeable muscular degeneration and edema between muscle fibers. These results suggest that the muscle integrity and structure of the fish from the Alkhumrah region were significantly worse than those of the control group. This indicates that the muscle of tilapia is negatively affected by sewage-contaminated water. Similar findings have been reported by many researchers, who found shortening of muscle bundles, edema, hyper-vacuolization, elongation of muscle bundles, gap formation in myofibrils, degenerated myotomes, hemorrhage, inter-myofibrillar space, and necrosis

(Nagarajan and Suresh, 2005); Ramesh and Nagarajan, 2013; Bhanot and Hundal, 2021). Similarly, Mohamed (2009) observed the degeneration of muscle bundles with aggregation of inflammatory cells between them and focal areas of necrosis in *Tilapia zillii* and *Solea vulgaris*. In addition, vacuolar degeneration in muscle bundles and atrophy of muscle bundles in fish are exposed to different pollutants.

Gills are sensitive to water quality deterioration due to contact with chemical pollutants in the surrounding environment, leading to increased stress (Maurya *et al.,* 2019). In the present study, the gills of the alkhumrah group showed hypertrophy in the epithelium, evident fusion in the secondary lamellae, and focal ruptures in the secondary lamellae, leading to bleeding and infiltration of white blood cells, indicating an inflammatory response. These observations are also consistent with those reported by Da Silva Souza *et al*. (2020), who reported that fish exposed to sewage sludge showed filament epithelium proliferation, lamellar fusion, congestion, aneurysms, and lamellar disorganization, which are indicative of tissue damage.

Based on previous studies, these alterations in the gills may be associated with exposure to chemical contaminants in sewage water and poor water quality. However, these changes could represent a form of defense against exposure to pollutants, as mentioned by Al-Wesabi *et al*. (2015) and Camargo and Martinez (2007). They reported that alterations in fish gills, such as the epithelial lifting surface and hypertrophy of the epithelial cells, along with fusion in secondary lamellae, serve as examples of defense mechanisms, acting as a barrier between the external environment and blood.

In conclusion, the current study shows the negative impact of sewage water on fish growth and maturation at the morphological scale and metabolic dysfunction and tissue damage at the physiological scale. Caution should be exercised when reusing this water for agriculture, including fish farming. However, from the perspective of the current work, our future studies will focus on a wide range of ages and degrees of maturation of studying fish to determine the critical timing of fish attacks.

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تأثير مياه الصرف الصحي على بعض الأسماك في منطقة الخمرة، جنوب محافظة جدة، المملكة العربية السعودية

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مستخلص. تعتبر مياه الصرف الصحي المعالجة مصدراً لتلوث المياه والتربة بالرغم من تطور وسائل المعالجة وخاصة دخول المعالجة الثلاثية طور الاستخدام في معظم محطات التنقية بالمملكة ويأتي هذا التلوث نتيجة ارتفاع الأملاح بجميع أنواعها وخاصة بقايا المعادن الثقيلة التي تأثر سلباً على البيئة والعديد من الكائنات الحية. وفي هذا المجال يعتبر مجمع محطة الخمرة بالجنوب من أكبر محطات معالجة مياه الصرف الصحي بالمملكة والتي تنتج كميات كبيرة من المياه المعالجة يتم التخلص منها في مياه البحر وجزء منها يشق منطقة التعاون بجدة متسبباً في تلوث بعض الاودية والتأثير سلباً على بعض الكائنات الحية منها سمك البلطي الذي يتكاثر طبيعياً في هذه الأودية والبحيرات. وفي سياق هذه الظاهرة، جاءت هذه الدراسة الذي تهدف الى معرفة مدى تأثير مياه الصرف الصحي المعالجة على تكاثر ونمو أسماك البلطي (Tilapia Fish) بينت هذه الدراسة أن هذه المياه أثرت على نمو ووزن الأسماك خلال الأشهر من مايو إلى أكتوبر 2023 و خلال شهري فبراير و مارس 2024، حيث تراوح وزن السمك من 14.8غرام إلى 54.7 غرام في حين تراوح طول السمك بين 84.8 مم خلال شهر يونيو-2023 إلى 395.3 مم خلال شهر مارس-2024.وترجى هذه التغيرات الى مكونات المياه خلال هذه الأشهر و التي أظهرت ارتفاعا كبيراً في كل الأملاح و المعادن و منها ارتفاع الكلسيوم من 23 ملغ/ل في المياه العادية إلى 90 ملغ/ل في المياه الملوثة في حين ارتفع الكادميوم من 24.6 ملغ/ل الى 173 ملغ/ل. أما التحاليل في مستوى دم الأسماك فقد بينت الدراسة ارتفاع كل المعايير الدالة على إصابة و تقطع بعض الانسجة منها خلايا الدم البيضاء WBC ، خلايا الدم الحمراء RBC ،الصفائح الدموية Plt الهيموجلوبين Hb الخلايا المتعادلة N.P و الخلايا الليمفاوية L.P. ويأتي هذا الارتفاع بالتزامن مع ارتفاع إنزيمات الكبد الدالة على الإصابات الكبدية و منها Alanine Aminotransferase (ALT) التي ارتفعت من 20.36 وحدة/اللتر في المياه العادية إلى 56.08 وحدة/اللتر في المياه الملوثة أما إنزيم Alkaline Phosphatase (ALP)، فقد ارتفع بدوره من 51.2 إلى 91.2 وحدة/اللتر. هذا وقد وثقت المقاطع النسيجية التغيرات والإصابات التي حدثت في أنسجة الكبد والخياشيم والعضلات.

الكلمات المفتاحية: مياه الصرف الصحي، الأسماك، الكبد، الكلى، الملوثات، الفسيولوجيا، الأنسجة