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INVESTIGATION OF THE MICROBIOLOGICAL AND PHYSIOLOGICAL PROFILE OF NILE TILAPIA "*OREOCHROMIS NILOTICUS*"

Noha Fathy El-Tahtawi[1*], Ferial Mohamed Naser Fudllalah[1], Zahrah Ali Alhalili[2], Maryam Abdulbaqi Aljohani[1]

1. ***Department of Biology, College of Science and ARTS, Shaqra University-Sajer, Saudi Arabia.***
2. ***Department of Chemistry, College of Science and ARTS, Shaqra University-Sajer, Saudi Arabia.***

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ABSTRACT

A cross-sectional study was performed on Nile tilapia (Oreochromis niloticus) in two ponds in the Qassim region of Saudi Arabia from October 2019 to March 2020. It was carried out to isolate and identify Gram-negative and Gram-positive bacteria from pond water and different fish organs, including blood, liver, and kidney samples. Blood, liver, and kidney functions were detected in this study. Bacterial isolates linked with fish could be transmitted to humans by eating fish or through the handling of fish that cause human diseases. Different bacterial isolates were identified depending on their growth properties and biochemical reactions in a specific culture medium. *Escherichia coli, Salmonella* typhi, *Bacillus sp*., *Aeromonas hydrophila, Pseudomonas aeruginosa*, *Enterococcus faecalis,* and *Acinetobacter sp*. was isolated from water of pond A and pond B. *The most prevalent bacteria isolated from fish blood in ponds A and B were Staphylococcus aureus, Streptococcus pyogenes, and Pseudomonas aeruginosa. The most prevalent bacteria isolated from the fish liver in ponds A and B was Escherichia coli. The most prevalent bacteria isolated from the fish kidneys in ponds A and B was Staphylococcus aureus.* This study showed that bacterial infections in freshwater fish cause alterations in blood parameters such as RBCs, WBCs, Hb, Ht, and serum parameters of liver and kidney functions.

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Introduction

Fish is an important source of food for humans worldwide, accounting for roughly 60% of the world's high-quality protein supply. Fish is also very rich in minerals, vitamins, and unsaturated fatty acids. The high nutritional value and easy digestibility of fish make it an excellent food source [1]. Tilapia is the most popular species in fish farms. More than 80 countries in the world produce farm-raised tilapia [2]. *Oreochromis niloticus* is a freshwater fish that lives in various shallow streams, rivers, ponds, and lakes; however, it is less usually seen in brackish water [3]. It has several beneficial properties that make it the most resistant to adverse environmental circumstances when cultivated. It is a fast-growing, high stocking density and salinity tolerant, survives at low dissolved oxygen, efficient food conversion, and easy adaptive fish [4]. Rivers, lakes, streams, ponds, and springs are considered the main fish sources for aquaculture. Most of the time, these fish sources are probably contaminated with wastes, different organic matters, and a large number and diversity of microorganisms [5].

Aquaculture ponds are a complex ecosystem that is defined as a man-made or natural water body with a surface area of between 1 m2 and 20,000 m2 (2 hectares or -5 acres) that keeps water for at least five months of the year or all year based on geographical location [6]. They can occur naturally as part of a river system on floodplains or as isolated depressions. The sort of life in a pond has generally been defined by a combination of variables such as water level regime and nutrient levels, but other variables such as the existence or absence of streams, existence or absence of tree shading, impacts of grazing animals, salinity, and the presence of algae which act as a fertilizer factor for fishes may also play a role [7]. Pond waters, like other bodies of water, are polluted by the discharge of effluents from different industries, domestic waste, land, and agricultural

Corresponding Author: Noha Fathy El-Tahtawi; Department of Biology, College of Science and ARTS, Shaqra University-Sajer, Saudi Arabia. E-mail: N.Ahmed@su.edu.sa; noha_44000@yahoo.com.

drainage, causing water quality to degrade [8]. So that the fish which live in ponds are also exposed to degradation by chemical and microbiological polluted factors [9].

Organic manuring, which is used to increase fish productivity, causes a high concentration of pathogenic and opportunist microorganisms to be released into the ponds. Most microorganisms act as a source of posing for public health [10]. Pathogenic bacteria cause infectious diseases and fish mortality. In aquaculture, disease problems are the leading cause of economic losses [11]. There had been a steady increase in the number of new and different pathogenic bacterial species isolated from fishes [12] Since organisms utilize the surface area of the fish as a microhabitat in their ecosystem, a microbial count of various fishes revealed that the number of organisms was higher on the surface of the bodies of large fishes with higher body weight than on the surfaces of small fishes [13]. The polluted water in ponds with chemicals and microorganisms exert extensive stress impacts upon fishes. Stress can cause metabolic changes that impact cellular and molecular components like enzymes or impair functions, including metabolism, immunological response, osmoregulation, and hormone regulation [14]. The interpretation and assessment of stress-related responses of fish to pollutants are complicated by the diversity of environmental conditions in aquatic ecosystems and the cumulative interactions of such conditions [15]. Changes in specific biochemical markers in fish blood can detect acute and chronic pollution-induced damage, according to Neff (1985) [16]. Blood chemistry can be used to aid in the diagnosis of pathological, toxicological, and general clinical examinations. Following functional damage to the organs and tissues of fishes, it was demonstrated that some specific cellular enzymes could seep into the bloodstream, where they can be detected, much as they did in mammals. Relevant enzymes for assessing liver intoxication were lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, creatine kinase, and alkaline phosphatase [17]. ASAT is primarily present in the liver and may reflect the liver's physiological status [18]. ALAT has been proposed as a more sensitive indication of liver dysfunction than ASAT [19]. Urea and creatinine are important breakdown products and metabolic indicators for carbohydrates, proteins, and lipids. Hematological parameters are used to determine the nutritional status, water balance, and general health of fish [20]. As a result, hematological parameters have been utilized as indicators of fish health status in several fish species to identify physiological changes caused by stress conditions like microbial and chemical pollution exposure [21].

The Nile tilapia is the main fish in the ponds of the Qassim region - Saudi Arabia. *Oreochromis niloticus* has been chosen as an experimental model in this research because it satisfied the selection requirements for a standard bioassay fish and is among the most marketable freshwater species. This study aims to isolate and quantify the saprophytic and pathogenic bacteria in the water of two ponds and various organs such as blood, liver, and kidney of Nile tilapia that was caught from the ponds of Qassim region– Saudi Arabia, and study the different physiological changes of fish by detection some major parameters of blood, liver and kidney functions which were shown to be important in illness diagnosis were meticulously quantified and linked to numerous stressors which the changing water quality could cause due to the presence of microorganisms.

Materials and Methods

Study Area

The research has been carried out on two ponds of the Al-Mohammedia project located in the Qassim region - Saudi Arabia. The two ponds located between Unaizah and the line of Al-Zulfi Governorate, as shown in **Figure 1**.

Locations of ponds **1st pond** **2nd pond**

Figure 1. Geographical location of two ponds

Study Design

The research has been performed in a cross-sectional study design from October 2019 to March 2020.

Collection of Samples

Three water samples were collected from each pond and were transferred to the microbiological laboratory for bacterial isolation and identification. Twenty fish samples were collected from two ponds. Ten samples (5 males and 5 females) have been collected aseptically from ponds A and B. 80 collected samples (20 blood, 20 liver, and 40 kidneys) were aseptically transported to the microbiological laboratory to isolate and identify saprophytic and pathogenic bacteria. A macro and micromorphological evaluation made the identification of bacteria. The characteristics considered were size, shape, color, pigmentation, and the hemolytic nature of colonies. Also, biochemical tests were applied by using conventional methods and API.

Blood samples were collected for biochemical tests via cardiac puncture in non-heparinized tubes. An anticoagulant was used to collect a fraction of blood for hematologic parameters. Red and white blood cell counts (RBCs and WBCs), hemoglobin content (Hb), and hematocrit (Ht), according to Dacie and Lewis [22]. According to the study of Schumann and Klauke, the serum biochemical parameters alanine transaminase (ALT) and aspartate transaminase (AST) levels were determined [23]. Tobacco and Bartels showed that alkaline phosphatase was calculated according to [24] Serum urea and creatinine [25, 26].

Results and Discussion

Bacteriological Analysis

Bacteriological Analysis for the Water of Two Ponds in Al-Muhammadiyah Project in Qassim Region

Escherichia coli, Salmonella sp., Bacillus sp., Aeromonas hydrophila, Pseudomonas aeruginosa, Enterococcus faecalis, and *Acinetobacter sp.* were recorded in two ponds. *Klebsiella pneumonia, Shigella sp.,* and *Pseudomonas fluorescence* were isolated from pond B only.

Comparison between the Results of the Bacterial Analysis of Blood Samples Acquired from Fish in the Pond (A) and Pond (B)

The total bacterial counts in the blood of fish were higher in pond B (415) than in pond A (109). The percentage incidence of bacteria in blood samples collected from males and females in both ponds A and B were ranged from (41.4%-58.13%). *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Salmonella typhi,* and *Flavobacterium sp.* were found in fish blood samples from ponds A and B.

Comparison between the Results of the Bacterial Analysis for Liver Samples Which Were Collected from the Fish in the Pond (A) and Pond (B)

The total bacterial counts were higher in pond B (512) than in pond A (199). The percentage incidence of bacteria in liver samples collected from males and females in both ponds A and B were ranged from (47.5%-52.1%). *Streptococcus agalactiae, Streptococcus parauberis, Escherichia coli, Pseudomonas aeruginosa, Salmonella Typhi,* and *Aeromonas hydrophila* were recorded in liver samples of fish that collected from both ponds A and B. *Bacillus sp.* and *Enterococcus faecalis* were collected only from pond A. Still, *Staphylococcus aureus, Shigella sp.,* and *Klebsiella pneumonia* were collected from pond B. The percentage incidence of all bacteria collected from liver samples of fish in ponds A and B is nearly closed.

Comparison between the Results of the Bacterial Analysis for Kidney Samples Which Were Collected from the Fish in the Pond (A) and Pond (B)

The total bacterial counts were higher in pond B (574) than in pond A (187). The percentage incidence of bacteria in kidney samples collected from males and females in both ponds A and B were ranged from (47.4%-52.2%). *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus parauberis, Escherichia coli, Pseudomonas fluorescens, Pseudomonas anguilliseptic,* and *Aeromonas hydrophila* were recorded in kidney samples of fish collected from both ponds A and B except *Streptococcus pyogenes, Pseudomonas aeruginosa,* and *Flavobacterium sp.* were recorded in pond B only.

Physiological Analysis

Hematological and Biochemical Analysis

Comparison between the Hematological Analysis Results for Blood Samples Collected from Males and Females of Tilapia Fish in the Pond (A) and Pond (B)

Results revealed a difference in values of blood parameters (RBCs, WBCs, HB, Ht) between males and females of Tilapia Fish which were collected from two different ponds (A and B) in the Al-Muhammadiyah project of Qassim Region. The value of WBCs for females in the pond (A) was very high in comparison to the value of males in the same ponds and both males and females. The value of RBCs for females in the pond (B) was high compared to RBCs values for males in the same ponds and both males and females in the pond (A). Hb values of females in the pond (B) were very high followed by Hb values of males in the same pond and Hb values of females in the pond (A), while Hb values of males in the pond (A) was low. Ht values of males in the pond (A) were very low in comparison to Ht values of females in the same pond and Ht values of males and females values in the pond (B) as shown in **Table 1**. The parameters of WBCs and RBCs for males and females did not vary significantly within pond A and pond B.

Table 1. Comparison of Blood Cells (RBCs, WBCs, HB, Ht) between males and females of Tilapia fish from two different ponds in Al-Muhammadiyah project in Qassim region

Groups	sex	WBCs $10^3/mm^3$	RBCs $10^6/mm^3$	Hb g/dl	Ht %
Pond A	male	7.08 ±3.50	3.80 ±1.71	8.52±0.85[a]	25.84±2.74[a]

	female	10.03 ±1.95	3.76 ±0.47	11.02±0.86[a]	33.1±2.66[a]
Pond B	male	8.45 ±2.00	3.84 ±0.90	11.48±2.69	34.42 ±8.07
	female	7.98 ±1.00	4.12 ±0.61	12.02±1.82	36.45±6.29

Values are expressed as means ± S.E for 10 fishes from each pond. Data are significant at p<0.05.
a: significantly different compared to both sex in the same pond.
b: significantly different compared to one sex in two ponds.

Comparison between the Results of Liver Functions for Serum Samples Which Were Collected from Males and Females of Tilapia Fish in the Pond (A) and Pond (B)

There was a highly significant difference in AST(U/L) for both males and females of Tilapia fish in ponds (A) only ($P< 0.05$). There was a highly significant difference in AST and ALP values of the males in both ponds A and B (P<*0.05*) and highly significant in ALT (U/L) and ALP (U/L) values for both males and females in the pond (B) only ($P< 0.05$). However, the results showed no significant difference in AST of both males and females of Tilapia fish from Ponds (B), no significant difference in both males and females of ALT (U/L) and ALP from the pond (A), and no significant difference in AST, ALT, and ALP in females sex in different ponds **Table 2**.

Table 2. Comparison of liver function between males and females of Tilapia fish from two different ponds in Al-Muhammadiyah project in Qassim region

Groups	sex	AST(U/L)	ALT(U/L)	ALP(U/L)
Pond A	male	159±8.82[ab]	31.8±8.79	27.00±2.92[b]
	female	340.1±135.44[a]	24.18±5.48	27.48±3.18
Pond B	male	172.58 ±7.69[b]	31.3 ±2.65[a]	18.54±2.43[ab]
	female	192.6 ±39.00	25.92 ±2.99[a]	27.44 ±2.51[a]

Legend as in Table

Comparison between the Results of Kidney Functions for Serum Samples Which Were Collected from Males and Females of Tilapia Fish in the Pond (A) and Pond (B)

The results revealed that the urea values of males in both pond (A) and pond (B) were higher than those recorded by females. The creatinine values of males in pond (B) higher than creatinine values of females. On the contrary, the creatinine values of males in the pond (A) were lower than those recorded by females in the same pond. There is a high difference between creatinine values for males in the pond (A) and (B). In contrast, the difference between creatinine values of females in the pond (A) and (B) were very small, as shown in **Table 3**.

Table 3. Comparison of kidney functions between males and females of Tilapia fish from two different ponds in Al-Muhammadiyah project in Qassim region

Groups	Sex	Urea	Creatinine
Pond A	Male	24.26±4.51[a]	0.35±0.08[ab]
	female	9.97±0.96	0.60±0.17
Pond B	male	20.46±1.10[a]	0.94 ±0.20[ab]
	Female	8.39±1.87	0.56±0.02

Legend as in Table

This study has revealed that bacteria highly contaminate the two ponds in the Al-Muhammadiyah project of the Qassim region. Bacterial species in this study are known to present in all types of the environment of human involvement; the majority of them are human and animal pathogens. The water of two ponds analyzed in this study has clearly shown that they are loaded with different and numerous bacteria which include *Escherichia coli, Salmonella sp., Bacillus sp., Aeromonas hydrophila, Pseudomonas aeruginosa*, *Enterococcus faecalis,* and *Acinetobacter sp.* in two ponds, *Klebsiella pneumonia, Shigella sp.* and *Pseudomonas fluorescens* in pond B only. These bacteria are considered as indicator organisms which are the indication of fecal pollution and thus the human interference. These recorded results are nearly similar to those recorded by [11] who isolate *E. coli*, *Salmonella sp*., *Shigella sp*., *Proteus sp*., *Pseudomonas sp.*, *Vibrio sp*., *Klebsiella sp.*, *Enterobacter sp*., *Serratia sp.*, *Aeromonas sp*., *Streptococcus sp*. and *Staphylococcus sp*. Presence of pathogenic organisms was attributed to the presence of isolated bacterial coliforms that were considered to indicate fecal material contamination of the water. The fecal material could be due to the ponds being fertilized with animal and organic manures that are dumped directly into the ponds, or it could be excreted into the ponds by the fish [27].

In the microbiological examination of the El-quanter fishpond, similar organisms were isolated [28]. Water-borne diseases such as food poisoning, gastroenteritis, typhoid fever, and cholera could be caused by pathogenic bacteria such as Escherichia coli, Shigella, Salmonella, and Vibrio [29]. Due to the consumption of undercooked fish cultivated in such ponds. *Pseudomonas*, *Proteus*, *Staphylococcus* species were considered food poisoning bacteria. *Aeromonas spp*. were also commonly found in pond water. That organism is among the most opportunistic pathogens for freshwater fish and one of the most frequent etiological agents of diseases that cause fish mortality [30].

Different opportunistic and pathogenic bacteria were also isolated from other organs of fishes under our study. In the blood of fish, the total number of bacteria in pond B (415) was larger than in pond A (109). Percentage incidence of bacteria in blood samples collected from males and females in both ponds A and B were ranged from (41.4%-58.13%). *Salmonella typhi*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus* were collected from blood samples of fish in both ponds A and B with various percentage incidence ranging from (8.2%-25.7%) the percentage incidence of these bacteria which were isolated from fish blood samples of pond A higher than that isolated from fish blood samples in pond B. while *Flavobacterium* sp. which was isolated from fish blood samples in pond B recorded a high percentage incidence in comparison to that recorded from fish blood samples which were collected from pond A (12.3%and11. 9%, respectively*). Shigella sp*., *Enterococcus faecalis,* and *Acinetobacter sp*. were recorded only in blood samples of fish in pond B. In a similar study, different septicemic bacterial infections such as *Vibrio anguillarum*, *Vibrio alginolyticus*, *Pseudomonas fluorescens*, *Streptococcus fecalis*, *Aeromonashydrophila, Aeromonassobria*and *Staphylococcus aureus* were isolated from the blood of fish in ponds [6]. According to the study of (7), about 17–21 bacterial genera were isolated from the blood of fish in fresh ponds. The blood of fishes was highly contaminated with *Micrococcus*, *Corynebacterium sp*., *Aeromonas sp.*, *Flavobacterium sp.,* and *Pseudomonas sp.*

In this study, two important internal organs (liver and kidney) have been selected for microbial analysis: the liver, which is the central metabolic organ and is important in the upkeep of overall nutrition and homeostasis in fish, including immune responses and the kidney, that has played numerous functional roles, which include osmoregulation and immune [31].

The two internal organs of a seemingly healthy fish ought to be sterile; however, bacteria isolations from the kidney, liver, and spleen have been recorded regularly [32]. The existence of pathogenic bacteria in internal fish organs may certify that immune defense mechanisms have been weakened. Bacterial infection in fish may not be an unusual event or will inevitably result in a disease state [33].

In our study, the total bacterial count of liver samples of fish in pond B (512) was higher than that recorded in liver fish in pond A (199). The percentage incidence of bacteria in liver samples collected from males and females in both ponds A and B ranged from (47.5%-52.1%). *Streptococcus agalactiae, Streptococcus parauberis, Escherichia coli, Pseudomonas fluorescens*, *Salmonella typhi,* and *Aeromonas hydrophila* have been found in the liver samples of fish obtained from both ponds A and B with various percentage incidence ranging from (15.5% - 8%)while the percentage incidence of *Bacillus sp.* and *Enterococcus faecalis* which were isolated from pond A only were (14.5 % and 12%, respectively) and the percentage incidence of *Staphylococcus aureus, Shigella sp.* and *Klebsiella pneumonia* which were isolated from pond B only were (11%,6.6% and 10%, respectively). *Escherichia coli* which was collected from pond A and pond B, achieved a high percentage incidence (15.5% and 15% respectively), followed by *Aeromonas hydrophila* (15%), isolated from pond B. In comparison, *Aeromonas hydrophila* isolated from liver fish samples in bond A recorded (11.5%). These results are similar to those recorded by [34]. In contrast, the dominant bacteria isolated from the liver of freshwater fish were *Escherichia species*, *Aeromonas species, Flavobacteria species*, *Klebsiella pneumonia,* and *Pseudomonas species*.

In another study, five species of *Staphylococci* were isolated from freshwater fish. The dominant species were *S. saprophyticus* (29.4%), *S.epidermidis* (22%), *S. aureus* (19.1%), *S.hyicus* (17.7%), *S.intermedius* (14.5%) [35].

Pseudomonas fluorescens was isolated from liver fish in two ponds at low percentage incidence (9.5% and 8%, respectively). In contrast, in another study, *Pseudomonas anguilliseptica* was isolated at a high percentage incidence (25%) from the liver of *Oreochromis niloticus* [36].

The total bacterial count of kidney fish was higher in pond B (574) than in pond A (187). The percentage incidence of bacteria in *kidney* samples collected from males and females in both ponds A and B were ranged from (47.4%-52.2%). *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus parauberis, Escherichia coli, Pseudomonas fluorescens, Pseudomonas anguilliseptic,* and *Aeromonas hydrophila* have been found in kidney samples of fish obtained from both ponds A and B. *Staphylococcus aureus,* which was collected from ponds A and B, achieved high percentage incidence (18.1% and 13.7%, respectively) followed by different species of *Streptococcus, Pseudomonas, Aeromonas, and Escherichia. Pseudomonas aeruginosa* (9.4%), *Streptococcus pyogenes* (8.7%), and *Flavobacterium sp.* (4.87%) were recorded in kidney fish within pond B only. *Pseudomonas*, *Acinetobacter*, *Aeromonas*, *Enterobacteriaceae,* and Bacillus have been found in the kidney of *Oreochromis niloticus*. *Pseudomonas spp.* were the most common species isolated from the different fish kidneys in previous studies. *P. anguilliseptica and P. fluorescens* were continuously connected with the upper and deeper regions of the ulcers and the internal organs of the kidney and liver [37].

Pseudomonas and *Vibrio,* particularly *V. fischeri*, *V. harveyi*, *V. splendidus* and*. Pelagius* is usually found in the kidney and liver of healthy turbot [38].

Bacteria are significant pathogens for fish and cause variation in the physiological parameters of blood, kidney, and liver functions and mass losses in fish production. According to the results recorded in previous studies, most isolated bacteria in this study can cause serious diseases. Pseudomonas septicemia is one of the deadliest fish diseases produced by various Pseudomonas species, resulting in significant losses for fish farmers. In pond farmed fishes, *P. anguilliseptica* causes red spot illness and severe mortality [39]. *P. anguilliseptica* had also been isolated from O. niloticus fish in Bangladesh that had Pseudomonas Septicemia. *P. fluorescent* and *P. aeruginosa* cause pseudomonas hemorrhagic septicemia for *Oreochromus niloticus* [40].

Aeromonas hydrophila, as well as other motile aeromonads, are one of the most prevalent bacteria found in a wide range of aquatic environments around the world, and they can cause a septicemic disease in fish that is referred to as "Aeromonas septicemia," "hemorrhagic septicemia," or "ulcer sickness" [41].

Staphylocococcosis is a haemorragicsepticemic disease caused by *Staphylococcus aureus* and *S.epidermidis* isolated from the blood, brain, stomach, kidney, and liver of freshwater fish [42]. The most common Streptococcus is pathogens in *Tilapia spp. are Streptococcus parauberis, Streptococcus iniae, Streptococcus agalactiae, and Streptococcus pyogenes. S. iniae*is, a *β*-hemolytic that produces generalized septicaemia and meningoencephalitis in a range of warm-water fishes, while *S. parauberis* is an α-hemolytic that causes hemorrhagic septicemia in fishes, especially in the summer. Hemorrhage, congestion, lethargy, dark pigmentation, irregular swimming, and exophthalmos with corneal clouding are the most common pathological signs of streptococcosis in fish [43].

Bacterial-infected fish had hemorrhagic patches on various body surfaces, as well as fin and tail rot. Some fish exhibited typical ulcers. Inflammation of the vent was observed in some cases. The liver, spleen, and kidneys were all congested and enlarged, according to necropsy results. The gall bladder has been swollen [42].

The blood analysis results revealed a difference in values of blood parameters (RBCs, WBCs, HB, Ht) between males and females of Tilapia fish, which were collected from two different ponds (A and B). The parameters of WBCs and RBCs for males and females did not vary significantly within pond A and pond B. Also, there is no significant difference in parameters of Hb and Ht for males and females in pond B. In contrast, the high significance of Hb and Ht parameters were recorded for males and females in pond A only ($p< 0.05$). This variation may be due to different bacterial species, which can cause various diseases that affect the blood parameters. The number of lymphocytes, monocytes, eosinophils, and neutrophils in fish infected with pseudomonas is reduced; also, this infection causes a decrease in the level of phagocytic activity and the level of WBCs, RBCs, Hb %, and PCV % [44].

The experimental infection of *Oreochromis niloticus and Oreochromis galilas* with *P. fluorescence* and consequent progression of septicemia contributed to a significant reduction in red cell count, haematocrit, and hemoglobin, as well as generalized bacteremia in the major organs, with slight enlargement of the kidney and spleen (splenomegaly), that backs up prior results in *Oreochromis niloticus* and *Oreochromis galilas* infected both experimentally and naturally [44, 45]. Hematological parameters were used to evaluate the fish's condition, nutritional status, and overall health [46]. AST and ALT are used as biomarkers for liver function; these enzymes are nonfunctional plasma enzymes normally found in the cells of the kidneys, liver, heart, muscle, gills, and other organs. Those enzymes are also thought to be significant in evaluating the state of the liver [46]. The results of liver functions recorded in this study revealed a highly significant difference in AST (U/L) for both males and females of Tilapia fish in ponds (A) only ($P< 0.05$). There was a highly significant difference in AST and ALP values for males in both ponds A and B ($P<0.05$) and ALT(U/L) and ALP(U/L) values for both males and females in the pond (B) only ($P< 0.05$). However, there was no significant difference in AST of both males and females of Tilapia fish from ponds (B), no significant difference in both males and females of ALT(U/L) and ALP from ponds (A), and also no significant difference in AST, ALT, and ALP in female sex in different ponds. The presence of bacteria also affects the results of liver function. Infection of fresh fish with *P. fluorescence* results in anticonvulsant activity and a rise in alkaline phosphatase activity that contributes to hepatocytic destruction and imbalanced metabolism [47]. Hepatocellular and myocardial damage is assessed using serum ALT and AST activity, considered sensitive indicators [48]. Because renal proximal tubule cells can raise BUN re-absorption in the presence of heightened neurohormonal activation, serum blood urea levels provide additional information on renal function [49]. The most frequent test for assessing renal function measures creatinine in serum. Creatinine is a breakdown product of creatinine phosphate in the muscle that the body produces fairly steadily. Under normal circumstances, it is freely filtered by the glomeruli and not reabsorbed in any appreciable amount by the tubules. Active secretion occurs in a small yet significant amount because a spike in blood creatinine is only seen when the nephrons are severely damaged [50].

The results of kidney functions recorded in this study revealed a very high significant difference in urea and creatinine for both males and females in ponds A and B ($P< 0.05$). Also, a highly significant difference in creatinine was recorded for males only in ponds A and B ($P< 0.05$). There is no significant difference in urea values for males in two different ponds and creatinine values for females only in both ponds A and B. Changes in kidney function levels of three groups of *O. niloticus* as a result of bacterial infections were reported by [46]. The serum urea concentrations measured in groups one, two, and three (15.5 ± 2.38 mg/dl, 16.5 ± 1.29 mg/dl, and 17 ± 1.41 mg/dl, respectively) revealed no significant differences. Serum creatinine levels, on the other hand, had a slightly different significance in three fish (groups 1, 2, and 3) (0.275 ± 0.050 mg/dl, 0.15 ± 0.057 mg/dl and 0.25 ± 0.057 mg/dl, respectively).

When comparing the *O. niloticus* groups to the control groups, serum creatinine and serum urea exhibited no significant differences ($p\geq0.05$) in both intervals (3 and 6 days). However, after three days and six days of C. perfringens infection in *O. niloticus*, serum creatinine levels were statistically significant ($p\leq0.05$) to be slightly higher in the experimental groups. The elevation in blood creatinine occurs only when the nephrons are damaged may explain this shift in plasma creatinine [46]. Our findings suggest that bacterial infection could impact renal function, which could be explained by the fact that after bacterial infection, cells in several organs are damaged, resulting in the release of several cytoplasmic enzymes into the blood (e.g., liver and kidney enzymes), a phenomenon which can be used to make a clinical diagnosis [50].

Conclusion

This study revealed microbial contamination of water in the two ponds and fresh fish cultivated in both ponds. Any fish pond's microbial quality reflects the microbial flora of the fish. These bacteria can reduce fish yield and induce various diseases that affect fish blood and serum sample parameters. Microbial contamination of fish causes economic loss while also endangering the eventual consumers (people), especially if the fish caught from the ponds is in the processing stage.

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