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The Impact of Water Physico-Chemical Parameters on the Microbiological Quality of Algerian Red Tilapia

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ABSTRACT

The purpose of this study was to determine the effect of water physicochemical parameters on the microbiological quality of Algerian red tilapia (*Oreochromis* spp.) in selected fish farms. The research was conducted from September 2022 to October 2023 across three fish farms located in Biskra, Jijel, and Skikda. At each site, water quality parameters—including pH, water temperature (Tw), dissolved oxygen (DO), turbidity, electrical conductivity, phosphate (PO_4^{3-}), ammonium (NH_4^+), nitrate (NO_3^-), salinity, and total dissolved solids (TDS)—were measured in triplicate during each sampling using standard methods. A total of 75 fish were collected for microbiological analysis. The counts of total and faecal coliforms, coagulase-positive *Staphylococcus*, and total mesophilic aerobic flora were assessed in three body parts of each fish: the intestine, skin, and muscle. The results revealed that the intestine was the most contaminated part. Furthermore, bacterial counts were positively correlated with several water quality parameters, as confirmed by the Spearman correlation test ($P < 0.05$). Among these, water temperature, ammonium, and phosphate were identified as the most significant factors, showing strong associations with bacterial presence across all study sites. These findings highlight the importance of regular monitoring and control of water quality in fish ponds to reduce the risk of bacterial contamination and improve fish health.

INTRODUCTION

Aquaculture is a growing economic sector that plays a vital role in food security and poverty reduction **(FAO, 2020; Abd El-Hack *et al.*, 2022)**. In Algeria, the aquaculture sector has made significant progress since 2008. These advancements are largely attributed to the government's commitment to supporting continental aquaculture and implementing proactive strategies for the sustainable development of the sector across various regions, particularly in arid and semi-arid zones **(Dergal *et al.*, 2013)**. These regions are especially vulnerable to climate change due to low precipitation, high evaporation rates, and limited freshwater availability **(Mramba & Kahindi, 2023)**.

Among aquaculture species, tilapia has emerged as a globally valuable fish, farmed in over 170 countries **(McMurtrie *et al.*, 2022)**. Various tilapia strains, such as the red tilapia (*Oreochromis* spp.), are widely cultivated due to their rapid growth, resilience to a range of environmental conditions, and tolerance to poor water quality and diseases **(FAO, 2020)**. However, despite these advantages, the aquaculture sector still faces numerous challenges that can hinder its development. Chief among these is the contamination of fish with human-pathogenic microorganisms. Fish are known vectors for foodborne pathogens—introduced either naturally or through fecal contamination—which can affect the skin, gastrointestinal tract, gills, and muscle tissues **(Junior *et al.*, 2014)**.

According to **Abu-Elala *et al.* (2016)**, several pathogenic bacteria—including *Salmonella* spp., *Vibrio* spp., *Staphylococcus aureus, Bacillus cereus*, and *Escherichia coli*—can contaminate fish tissues throughout the production process and pose health risks to consumers. Contaminated water is often the primary source of these pathogens, affecting the muscles, gills, digestive tract, and kidneys of fish. The presence and proliferation of such microorganisms are heavily influenced by the physicochemical quality of water. Therefore, maintaining optimal water quality is essential for regulating growth performance, health, and disease resistance in aquatic organisms. This underscores the need for consistent monitoring and control of water parameters in aquaculture systems **(Ojwala *et al.*, 2018; Khanjani & Sharifinia, 2021)**.

In Algeria, red tilapia is commonly cultured in traditional ponds or concrete tanks, where many fish farmers lack adequate knowledge of tilapia farming practices and pond management. This gap in information regarding the basic requirements of effective aquaculture systems has negatively impacted fish production and quality. Despite the known influence of water quality on microbiological safety, there remains limited research on the relationship between physicochemical water parameters and bacterial presence in Algerian fish farms. Most previous studies have focused solely on identifying isolated bacteria from fish, rather than exploring how water quality contributes to their proliferation.

In light of this, the present study aimed to investigate the impact of physicochemical water parameters on the microbiological quality of Algerian red tilapia. To achieve this, three fish farms—among the largest producers of the red tilapia in Algeria—were selected for comprehensive analysis.

MATERIALS AND METHODS

1. Study area

This study was performed in three fish farms situated in the East of Algeria, from September 2022 to October 2023. The first fish farm is located in Jijel (36°49' North and 5°46' East), the second in Skikda (36° 87′ North and 6° 9′ East) and the third in Biskra (34° 83 North and 5° 75 East) (Fig. 1). Jijel farm covers an area of 62,38km^2, Skikda farm, covers an area of 4026km^2, while Biskra farm has a superficies of 21509km^2. The farms selection was based on several parameters: type of the pond (earthen tanks or concrete ponds), production yield, fish density and type of climatic areas. Jijel has a humid climate, Skikda a sub-humid climate and Biskra is an arid region.

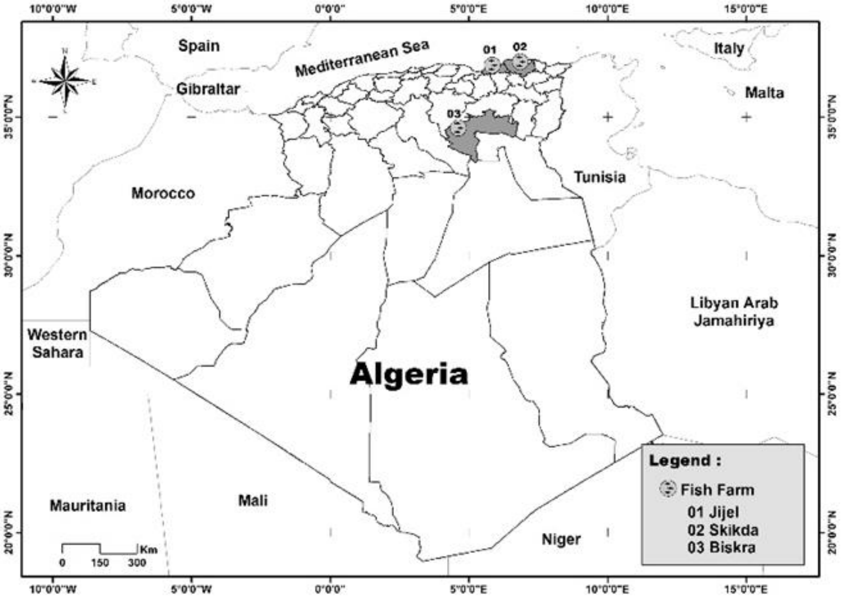

Fig. 1. Location of the three fish farms (Jijel, Skikda and Biskra) in Algeria

2. Microbiological analyses of red tilapia

To perform microbiological analyses, seventy-five red tilapia (*Oreochromis* spp.) samples were collected in sterile plastic bags. Each fish weighed between 100 and 140 grams. The fish were sacrificed, and the skin, muscle, and gut were aseptically removed using sterile scalpels. From each organ, 10 grams were separately diluted in 90 mL of saline peptone diluent (0.85% NaCl with 0.1% peptone). The mixtures were agitated for 2 minutes or until homogenization. Appropriate serial decimal dilutions were prepared using the same diluent.

For mesophilic bacteria, 1 mL of each dilution was inoculated (deep method) onto Plate Count Agar (PCA) medium (Merck, Germany). The plates were incubated at 35°C for 48 hours following **ISO 4833 (2013)**. Total and faecal coliforms were enumerated by surface plating 0.1mL of each dilution onto Violet Red Bile Lactose (VRBL) agar

(Merck, Germany). The plates were incubated at 37°C for 24 hours (total coliforms) and at 45°C for 24 hours (faecal coliforms), as described in **ISO 4832 (2006)**.

To detect coagulase-positive *Staphylococcus* spp., 0.1mL of each dilution was spread on Baird-Parker Agar and incubated at 35– 37°C for 24–48 hours. Suspected colonies were confirmed using the coagulase test according to **ISO 6888 (1999)**. After the appropriate incubation period for each analysis, bacterial counts were recorded following **ISO 7218 (1996)**. All microbiological analyses were performed in duplicate.

3. Physicochemical analysis of water

Water samples were analyzed for physicochemical parameters in accordance with **APHA (2004)** standard methods. The parameters measured included pH, water temperature (Tw), dissolved oxygen (DO), turbidity, electrical conductivity, phosphate (PO_4^{3-}), ammonium (NH_4^+), nitrate (NO_3^-), salinity, and total dissolved solids (TDS). All measurements were conducted in triplicate during each sampling session.

A total of fifteen water samples were collected in 500mL sterile bottles. Samples were transported to the laboratory in ice boxes and maintained at 4°C until analysis.

4. Statistical analyses

All results are presented as mean values ± standard deviation (mean ± SD). One-way analysis of variance (ANOVA) followed by Tukey’s HSD post hoc test was used to compare differences between sampling sites and between fish organs (for microbiological results). Spearman’s correlation analysis was used to assess relationships among water quality parameters.

To evaluate the relationship between water quality and microbial contamination in fish organs, principal components analysis (PCA) was performed. Canonical correspondence analysis (CCA) was also used to enhance interpretation of the association between bacterial presence and water quality parameters.

All statistical analyses and graphical visualizations were conducted using R statistical software.

RESULTS

1. Microbiological quality of fish

The comparison of microbiological quality across individual fish organs from different fish farms, as presented in Table (1), showed that the intestine of fish farmed in the Biskra farm was less contaminated with total aerobic mesophilic flora (FMAT) and total coliforms (TC), with no detection of faecal coliforms (FC) or coagulase-positive *Staphylococcus aureus* (ST). A similar finding was observed for skin contamination. Additionally, in muscle tissue, neither faecal nor total coliforms were detected, nor was *S. aureus*.

On the other hand, total aerobic mesophilic flora (FMAT), *S. aureus*, and both total and faecal coliforms were recorded on the Skikda farm in all three organs: intestine, skin, and muscle. For the Jijel farm, the results were intermediate between those of Biskra and Skikda and were mostly significantly different from each other.

For each fish farm, a significant difference in the level of bacterial contamination was observed between the intestine, skin, and muscle (Tukey HSD test; $P< 0.05$). However, in the case of total coliforms, no significant difference was found between skin and muscle in both Biskra and Jijel fish (Tukey HSD test; $P> 0.05$). Furthermore, significantly higher values of all bacterial counts were present in the intestine compared to the skin and muscle. Generally, bacterial counts declined in the following order: intestine > skin > muscle (Tukey HSD test; $P> 0.05$).

According to the Spearman correlation test, a high positive correlation ($P< 0.05$) was observed, notably between total aerobic mesophilic flora and total coliforms, total coliforms and faecal coliforms, and coagulase-positive *S. aureus* and faecal coliforms.

Table 1. The microbiological quality of the individual fish organs throughout the three fish farms

Part of fish		Biskra	Jijel	Skikda
FMAT (CFU/g)*	Intestine	$(4.49\times10^{4}\pm1.43\times10^{4})^{a}$	$(1.26\times10^{6} \pm 1.97\times10^{5})^{b}$	$(1.72\times10^{6} \pm2.46\times10^{5})^{c}$
	Skin	$(5.48\times10^{2} \pm1.37\times10^{2})^{a}$	$(4.31\times10^{5} \pm9.31\times10^{4})^{b}$	$(6.22\times10^{5} \pm8.79\times10^{4})^{c}$
	Muscles	$(3.38\times10^{2} \pm1.65\times10^{2})^{a}$	$(2.38\times10^{3} \pm9.03\times10^{2})^{b}$	$(3.94\times10^{3} \pm4.52\times10^{2})^{c}$
Total coliforms* (CFU/g)	Intestine	$(3.61\times10^{4} \pm1.3\times10^{4})^{a}$	$(4.07\times10^{4} \pm 1.38\times10^{4})^{a}$	$(5.05\times10^{4} \pm1.36\times10^{4})^{a}$
	Skin	$(6.7\times10^{1} \pm3.5\times10^{1})^{a}$	$(4.37\times10^{2} \pm 1.23\times10^{2})^{b}$	$(7.55\times10^{2} \pm1.23\times10^{2})^{c}$
	Muscles	Not detected	$(1.48\times10^{2} \pm2.98\times10^{1})^{b}$	$(4.13\times10^{2} \pm7.03\times10^{1})^{c}$
Faecal coliforms* (CFU/g)	Intestine	Not detected	$(3.03\times10^{2} \pm 5.9\times10^{1})^{b}$	$(6.03\times10^{2} \pm7.8\times10^{1})^{c}$
	Skin	Not detected	$(1.66\times10^{2} \pm 3.71\times10^{1})^{b}$	$(3.96\times10^{2} \pm5.51\times10^{1})^{c}$
	Muscles	Not detected	Not detected^{a}	$(3.8\times10^{1} \pm1.3\times10^{1})^{b}$
Coagulase positive *S. aureus**	Intestine	Not detected	$(1.76\times10^{2} \pm 2.6\times10^{1})^{b}$	$(2.64\times10^{2} \pm2.19\times10^{1})^{c}$
	Skin	$(1.9\times10^{3} \pm 9.91\times10^{2})^{a}$	$(1.4\times10^{3} \pm 5.14\times10^{2})^{a}$	$(1.39\times10^{3} \pm4.73\times10^{2})^{a}$
	Muscles(cfu/g)	Not detected	$(1.6\times10^{1} \pm8.9\times10^{0})^{a}$	$(6\times10^{1} \pm1.8\times10^{0})^{b}$

*Comparison is between values in the same row, where results with different letters are considered significant ($P< 0.05$) according to the Tukey HSD test.

2. Physicochemical quality of water fish pond

The findings of the physicochemical parameters of water recorded at the Biskra, Jijel, and Skikda farms are presented in Table (2). According to the results, with the exception of dissolved oxygen (DO), for which the highest value (7.1mg/ L) was recorded at the Biskra farm, all other parameters showed their highest values at the Skikda fish farm (Tukey HSD test; $P> 0.05$).

Table 2. The water quality parameters recorded in the three fish farms

Parameters	**Fish farm**		
	Biskra	**Jijel**	**Skikda**
PO_4^{-3}(mg/L)	$(2.264 \pm 2.264)^a$	$(12.28 \pm 1.804)^b$	$(19.058 \pm 5.787)^c$
NO_3^-(mg/L)	$(6.08 \pm 3.532)^a$	$(5.188 \pm 2.551)^a$	$(15.454 \pm 5.029)^b$
NH_4^+ (mg/L)	$(0.692 \pm 0.403)^a$	$(1.47 \pm 0.253)^b$	$(1.98 \pm 0.47)^b$
Conductivity(ms/m)	$(0.453 \pm 0.206)^a$	$(0.458 \pm 0.183)^b$	$(0.712 \pm 0.212)^C$
Salinity(g/L)	$(3.82 \pm 0.443)^a$	$(4.12 \pm 0.238)^b$	$(4.2 \pm 0.212)^b$
TDS(mg/L)	$(239.8 \pm 7.949)^a$	$(379.4 \pm 4.06)^b$	$(470.4 \pm 7.536)^c$
Turbidity (UNT)	$(18.42 \pm 1.51)^a$	$(26.2 \pm 8.318)^b$	$(38.2 \pm 5.76)^c$
pH	$(7.186 \pm 0.214)^a$	$(7.66 \pm 0.288)^b$	$(8.194 \pm 0.213)^c$
T_W (C°)	$(26.4 \pm 1.516)^a$	$(28.80 \pm 1.095)^b$	$(33.20 \pm 1.095)^c$
DO(mg/L)	$(7.1 \pm 1.244)^a$	$(3.8 \pm 1.205)^b$	$(2.1 \pm 0.651)^c$

*Comparison is between values in the same row, where results with different letters are considered significant ($P < 0.05$) according to the Tukey HSD test.

3. Principal component analysis of physicochemical quality of water fish pond

The two principal components, PC1 and PC2, together explained 80.57% of the total variability, with PC1 accounting for 66.54% and PC2 for 14.03% (Fig. 2). The PCA analysis also provided insights into how the quality parameters were correlated. Among the ten quality parameters, nine were positively correlated with PC1, while one parameter—dissolved oxygen (DO)—was negatively correlated with it.

In contrast, six parameters—conductivity, pH, turbidity, TDS, PO_4^{3-}, and turbidity—were positively correlated with PC2, whereas the remaining four—NH_4^+, total water temperature (TW), NO_3^-, and salinity—showed negative correlations with PC2. The PO_4^{3-} and TDS parameters showed no significant correlation with PC2.

Moreover, the analysis revealed a negative correlation between DO and the NO_3^-, NH_4^+, and TW parameters. A strong positive correlation was observed between pH and turbidity, as well as between TW and NH_4^+, and between TDS and PO_4^{3-}.

The PCA plot also revealed three distinctly separated clusters corresponding to the three farms: Skikda, Jijel, and Biskra. Samples from Skikda occupied the right side of the plot, whereas those from Biskra were positioned on the opposite (left) side. The samples from Jijel were slightly centered between the two extremes.

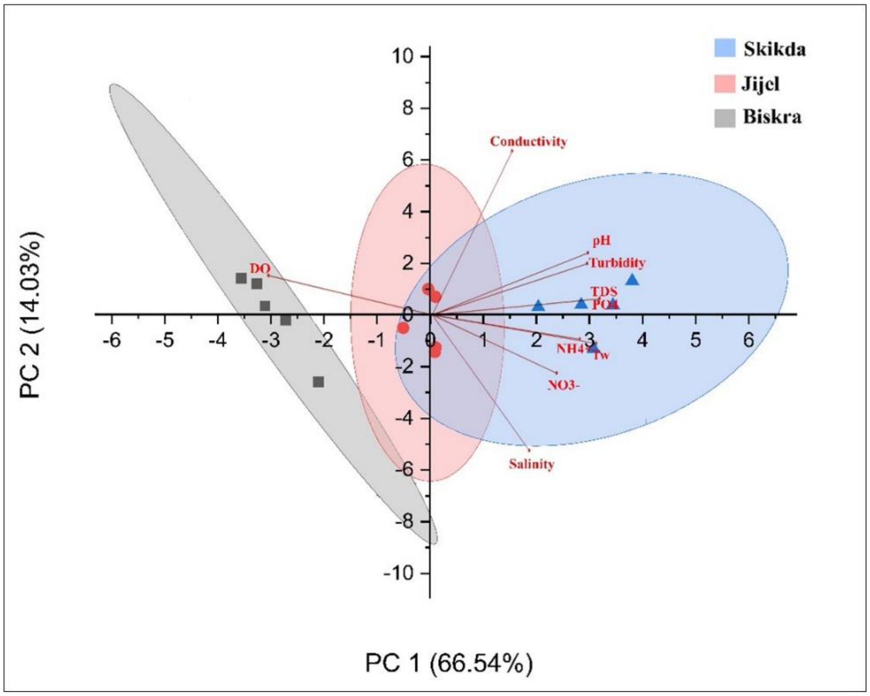

Fig. 2. Principal component analysis (PCA) score plot of biochemical quality of water fish pond from the three fish farms (Skikda, Jijel and Biskra)

4. Relationship between water physicochemical parameters and microbiological quality of fish muscles

To study the relationship between the physicochemical parameters of pond water and bacterial counts in fish muscle, a Canonical Correspondence Analysis (CCA) was performed. The CCA plot (Fig. 3) illustrated the relationships between ten water quality parameters and the distribution of bacterial groups (FMAT, FC, TC, and ST) across the three fish farms (Skikda, Jijel, and Biskra).

Among the ten physicochemical parameters, seven variables—excluding NO_3^-, conductivity, and salinity—had the greatest influence on the distribution of bacterial groups, as indicated by their longer vectors in the CCA plot. The remaining three parameters (NO_3^-, conductivity, and salinity) also influenced bacterial distribution, but to a lesser extent.

The bacterial groups total coliforms (TC), *Staphylococcus aureus* (ST), and, to a lesser extent, faecal coliforms (FC) were associated with higher values of most water parameters, except for dissolved oxygen (DO). This suggests that these bacterial groups tend to thrive in water with elevated levels of these parameters.

Conversely, total aerobic mesophilic flora (FMAT) was associated with high DO levels and lower values of the other parameters, indicating that this bacterial group prefers well-oxygenated water with fewer chemical pollutants.

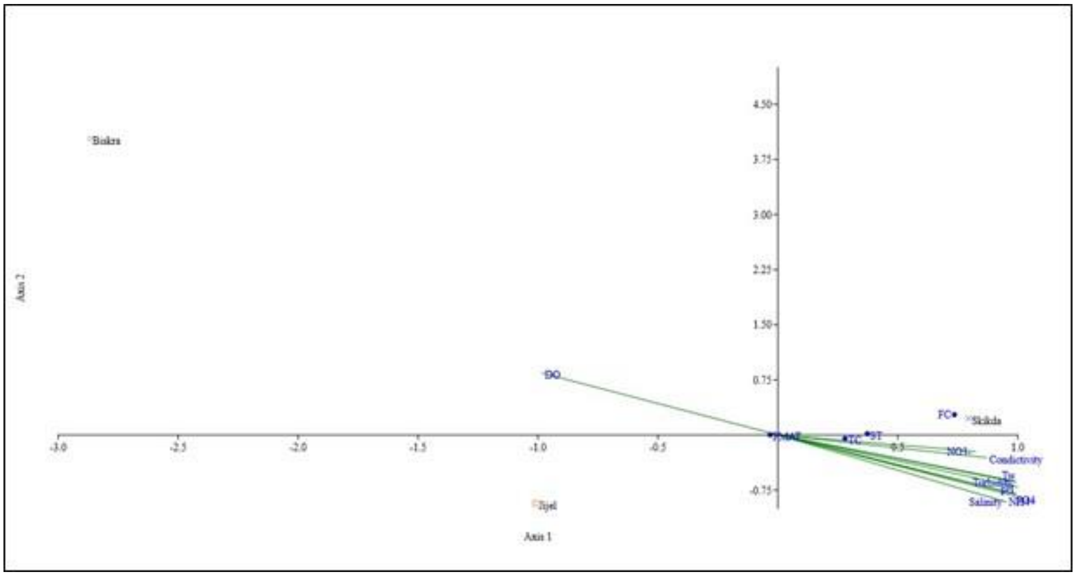

Fig. 3. Canonical Correspondence Analysis (CCA) plot showing the relationship between water physicochemical parameters and bacterial quality of fish muscles

DISCUSSION

The present study compared the microbiological quality of Algerian red tilapia (*Oreochromis* spp.) farmed at three different fish farms and examined the relationship between pond water quality and fish microbiological status. The results revealed clear differences in the microbial quality of red tilapia among the three farms. Fish from the Biskra farm exhibited the lowest levels of contamination in the intestine, skin, and muscle—contrasting significantly with those from the Skikda farm, which showed the highest contamination. Additionally, across all farms, the highest bacterial counts—including total aerobic mesophilic flora (FMAT), total coliforms (TC), faecal coliforms

(FC), and *Staphylococcus aureus* (ST)—were consistently found in the intestine, followed by the skin and then the muscle.

According to several studies, elevated bacterial levels in the intestine can be attributed to its role as a bioreactor, with faecal coliforms being a natural component of the intestinal flora. **El-Shafai *et al.* (2004)** reported that tilapia exhibited higher contamination levels in the digestive system (FC count of 9.3×10^3 CFU/g) compared to the skin (TC count of 1.3×10^3 CFU/g). The study concluded that such contamination was closely associated with the pond water environment.

To further explain the variation in contamination levels, **Junior *et al.* (2014)** emphasized that fish skin serves multiple critical roles, acting as a primary defense barrier against pathogens and environmental xenobiotics. Various antimicrobial and bioactive substances—such as peptides, lysozymes, lectins, and proteases—found in fish skin mucus exhibit antibacterial activity against *Staphylococcus* and *E. coli*.

In muscle samples from all three farms, the number of aerobic mesophilic flora remained below the threshold set by Algerian legislation (Algeria's official health standard) and the American Public Health Association (APHA). Moreover, *S. aureus* was not detected in the muscle tissue of red tilapia from Biskra, while it was present in samples from Jijel (1.6×10^1 CFU/g) and Skikda (6×10^1 CFU/g). These values remained below the Algerian microbiological standards (10^2 to 10^3 CFU/g). Faecal coliforms were detected only in samples from the Skikda farm. These findings are lower than those reported by **Mitiku *et al.* (2022)**, who found 5.1×10^4 CFU/g of total coliforms and 11.5×10^4 CFU/g of total mesophilic aerobic flora in tilapia muscle. Similarly, **Valenzuela-Armenta *et al.* (2018)** reported respective values of 1.768×10^3 CFU/g and 1.46×10^2 CFU/g.

Overall, when compared to microbiological standards set by public health authorities (Table 3), and based on average bacterial loads (total viable count, total/faecal coliforms, and *S. aureus*), red tilapia samples in this study were classified as follows: satisfactory for Biskra farm, acceptable for Jijel farm, and unsatisfactory for Skikda farm.

Conversely, water quality parameters play a vital role in the health and sustainability of aquatic organisms. Several researchers have highlighted the impact of water's physicochemical quality on bacterial presence in fish, noting that poor water conditions can weaken fish and increase susceptibility to bacterial contamination **(Ismail *et al.*, 2016; Khanjani & Sharifinia, 2021)**.

Table 3. Guidelines for microbiological quality of some ready-to-eat foods **(Gilbert *et al.*, 2000)**

Criterion	**Microbiological quality (cfu/g)**			
	Satisfactory	Acceptable	Unsatisfactory	Unacceptable
Aerobic colony count 30C/48h	$< 10^3$	$10^3 < 10^4$	10^4	Not applicable
Indicator organism				
Total coliform	< 20	20<100	≥100	Not applicable
Pathogens				
S.aureus	<20	20<100	20 < 100	$\geq 10^4$
Faecal coliform	Not detected in 25g			Detected in 25g

According to **Mramba and Kahindi (2023)**, prolonged exposure of fish to non-lethal levels of dissolved oxygen (DO), turbidity, pH, stocking density, or water temperature can lead to chronic stress, reduced feed intake, and increased vulnerability to disease. Similarly, **Evans *et al.* (2006)** confirmed that sub-lethal levels of physicochemical parameters in pond water can trigger stress responses that diminish fish resistance to pathogens. **Makori *et al.* (2017)** emphasized that earthen ponds must be carefully managed to maintain optimal levels of turbidity, dissolved oxygen, phosphate, and other water quality parameters. Their study also revealed that all measured water parameters were interdependent, collectively exerting a significant influence on fish health by inducing stress, elevating bacterial presence, and weakening immune responses.

In the present study, DO levels ranged from 2.1 to 7.1 mg/L, while the optimal level for red tilapia culture is considered to be above 5 mg/L **(Khanjani & Sharifinia, 2021)**. Ammonium (NH_4^+) concentrations ranged from 0.692 to 1.98 mg/L, and phosphate (PO_4^{3-}) levels varied from 2.264 to 19.1 mg/L. According to experiments conducted by **Elshopakey *et al.* (2023)**, ammonium concentrations above 1.14 mg/L can induce stress in fish, including elevated cortisol secretion and protein catabolism. In a related study, **Ojwala *et al.* (2018)** found that high levels of ammonia or nitrate contribute to the development of bacterial and parasitic infections.

Hisano *et al.* (2020) reported that excessive feeding—such as feeding fish six times per day—reduces nutrient conversion efficiency in pond water, leading to an accumulation of organic matter and the formation of biofloc. High-protein fish feed, often used to boost productivity, contributes to increased waste in the form of uneaten food, feces, and dissolved excretions. These are broken down by decomposers into ammonia and phosphorus. Nitrifying bacteria then convert ammonia into nitrite and subsequently into nitrate. Elevated concentrations of nitrate and phosphate can cause eutrophication, which in turn reduces dissolved oxygen levels and negatively affects aquatic ecosystems.

Increased turbidity may also be linked to the adsorption of nitrate and nitrite ions onto suspended particles. This process can stimulate the growth of microorganisms responsible for ammonia conversion, while protecting these ions from photodegradation. When nitrate, turbidity, and phosphate levels are high, DO tends to decrease, which compromises the fish immune system and increases susceptibility to pathogens. This is consistent with findings from **Mahmoud *et al.* (2016)**, who noted that such conditions can lead to disease outbreaks, as evidenced by the presence of faecal coliforms in fish organs—an indicator of faecal contamination.

Abu-Elala *et al.* (2016) demonstrated that the use of untreated organic fertilizers, along with inputs from agricultural and municipal waste, contributes to elevated nutrient levels in fish ponds. When combined with high water temperature and pH, these conditions can lead to ammonia toxicity and an increased incidence of infectious diseases such as branchiomycosis, streptococcosis, and pseudomoniasis—potentially resulting in high mortality rates and significant economic losses.

Based on our findings, all ten physicochemical parameters—except for DO—were associated with the distribution of TC, FC, and ST, suggesting that these bacterial groups thrive in water with elevated levels of these parameters. In contrast, FMAT appeared to be positively associated with high DO and negatively associated with the other nine parameters. Notably, certain measurements recorded on the Jijel and Skikda farms—including DO, NO_3^-, NH_4^+, PO_4^{3-}, and turbidity—fell outside the recommended range for optimal red tilapia culture.

CONCLUSION

The main objective of this study was to compare the microbiological quality of fresh red tilapia (*Oreochromis* spp.) farmed on different Algerian fish farms and to identify the key water quality parameters that influence bacterial presence in cultured fish. The findings underscore a critical gap in fish farmers' understanding of water quality monitoring and maintenance. Therefore, we recommend the implementation of routine water quality assessments and regular inspections by the fisheries department to ensure that pond conditions meet health and safety standards.

Additionally, the study evaluated bacterial contamination across different fish parts—intestine, skin, and muscle—in order to assess potential health risks and to inform best practices in fish handling. Notably, the intestine, being the most contaminated organ, poses a risk of cross-contamination during handling and evisceration. Spillage during gutting can contaminate the abdominal cavity, handlers' hands, and surrounding surfaces, increasing the risk of infection.

Government agencies should prioritize public awareness campaigns to educate consumers on the risks associated with consuming raw or undercooked fish, as well as on safe food handling practices. Preventing foodborne illnesses also requires targeted interventions, including the training of food handlers. Such initiatives are essential for promoting food safety and reducing the likelihood of bacterial outbreaks linked to aquaculture products.

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