**Research Paper**

# Bacteriological assessment of fish ponds in the districts and suburbs of Kirkuk Governorate

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**ABSTRACT**

The current study aimed to assess bacterial contamination in the water of fish ponds in the outskirts of Kirkuk represented by Hawija district, Daquq, Yayji, Taza and Lailan. The tests were conducted at the Department of Environment and Agriculture and included water samples of carp ponds and biological characteristics were measured for six months starting from August 2023 to the end of January 2024. The total coliform count was highest at stations two, three and five at >16 cells/ml in all months of the study, and E. coli counts varied, with the highest being 70 CFU/ml in December at station one and the lowest being 1.3 CFU/ml. 3 CFU/ml in the month of August in the third station, and it was observed from the table that the infection rates increased in the winter season in all stations, and the infection values of fecal coliform bacteria increased in the month of January compared to the rest of the seasons, as the highest rate of 90 CFU/ml was recorded in January in the third station and the lowest rate of 1. The results of the study showed a significant variation in the number of fecal coliform bacteria between the five stations, with the highest value recorded in the fifth station (13.667 cells/ml) and decreased to the lowest value in the first and fourth stations (2.1667 and 1 cell/ml, respectively).

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## 1. INTRODUCTION

Life on Earth no life exists without water as a vital ingredient. It is an essential constituent of cells, serves as their main regulator and coolant for the surrounding of other living tissues, helps absorb nutrients from food (transports them to each cell), and is a response medium in many biochemical reactions. Also, water provides important ecological services to agriculture, industry and production of energy; hence, it is an essential natural resource for sustainable development as well as for food security and health [1]. Indeed, one of the God blessings to all creatures in its central role to the majority of metabolic process and biological activities, especially in human [2]. About 70% of the human body mass is made up of water which plays an essential role in the function of all organs, including transporting nutrients and oxygen to cells, removing waste products and keeping fluid balance. It hydrates the tissue and supports joint integrity, hepatic and circulatory function (being essential for human health and activity) [3]. However, this life-saving substance is also under threat from pollution from human industrial, agricultural and urban activity, all of which have caused vast deteriorations in the quantity of water resources around the globe [4]. Water shortage is one of the most prominent issues that threaten national security, especially in arid regions that suffer from scarce water resources, as well as in areas that depend on water sources from neighbouring countries, which exacerbates climate challenges and increases strategic threats [5]. With the increasing population and industrial expansion in the Kirkuk region, the demand for water for all uses, especially for domestic, agricultural and industrial uses, has increased [6].

Fish ponds are considered closed ecological systems that rely entirely on human management to maintain their biological and chemical balance. These systems are highly influenced by water quality parameters, including temperature, pH, dissolved oxygen levels, ammonia, nitrates, and heavy metals. Environmental factors such as aeration, lighting, stocking density, and feed quality also play a critical role in determining fish health, growth, and overall system efficiency, therefore, continuous monitoring of water quality and the implementation of effective environmental management strategies are essential to maintain biological sustainability and productivity in such systems [7]. One of the most important biomarkers that reflect the quality of the water and the safety of the environment within these ponds is the concentration of bacteria, whether beneficial bacteria that contribute to the nitrogen cycle, or harmful bacteria that may threaten the health of both fish and humans. Optimal fish production is highly dependent on the physical characteristics .Effective management of fish ponds is essential and requires a thorough understanding of water quality. Water quality management is a critical factor in successful fish farming, as the majority of issues related to low growth rates, disease outbreaks, parasite infestation, and high mortality rates are due to poor water quality [8]. The need to study bacteria in fish ponds is increasing, especially in urban areas such as Kirkuk, where some farms or home ponds lack effective filtration systems or health monitoring [9]. Water contamination with pathogenic organisms, such as bacteria, viruses and parasites, can be transmitted via water to humans and economic animals, causing infections and poisoning. This contamination causes a severe impact on the health of living organisms and poses a serious threat to the environment and public health, Most studies indicate that animals are a major reservoir of organisms that cause waterborne diseases and the release of animal faeces into water and soil increases the numbers of pathogenic bacteria, leading to the potential for disease outbreaks in these habitats [10].

## 2. RELATED WORK

[11] Reported in their study on the assessment of total coliform bacteria and faecal coliform bacteria in recreational beach and marine waters in St Total coliforms were highest in deep waters on the island's western shore, wetlands, and Ghat Pier Beach, while lower levels appeared in resort irrigation water. Fecal coliforms were mostly found in deep waters and beaches during the tourist season. The study of [12] showed their study of antibiotic resistance of faecal indicator bacteria from aquariums and

nearby water sources in the Ibarwaddy Delta region of Myanmar the results showed that fish pond water contains a higher abundance of bacteria compared to other surface water sources. Study [13] indicated bacterial contamination of water sources in rural villages in Mohale Basin, Lesotho: Exposures through practices Escherichia coli bacteria were detected in all water samples, ranging from less than 30 colony-forming units (CFU)/100 mL to 4,800 CFU/100 mL in protected sources, and up to 43,500,000 CFU/100 mL in unprotected sources. The study [14] was on the evaluation of the environmental characteristics of a number of fish ponds in Kirkuk Governorate and the determination of bacterial content in the water Results: The water quality status was classified as "good," and the annual average value of the Water Quality Index increased year by year, along with improvements in water quality.

There was a clear temporal and spatial variation in water quality, with autumn showing better water quality than other seasons, and the southern region having better water quality than the northern region. Water quality was affected by the diversion of its course; during the diversion period, water quality was more stable. The study [15] The results of this study can be used to compare bacterial monitoring practices and protocols in fish farming systems, serving as a tool for discussions on the accuracy and reliability of test results, and to support the implementation of appropriate management responses.

## 3. METHODOLOGY

Kirkuk Governorate is located in the northern part of Iraq between latitude (28-35) and longitude (23-44) and is bordered by Erbil Governorate to the north, Salah al-Din to the west and south, and Sulaymaniyah to the east as shown in the Figure 1.

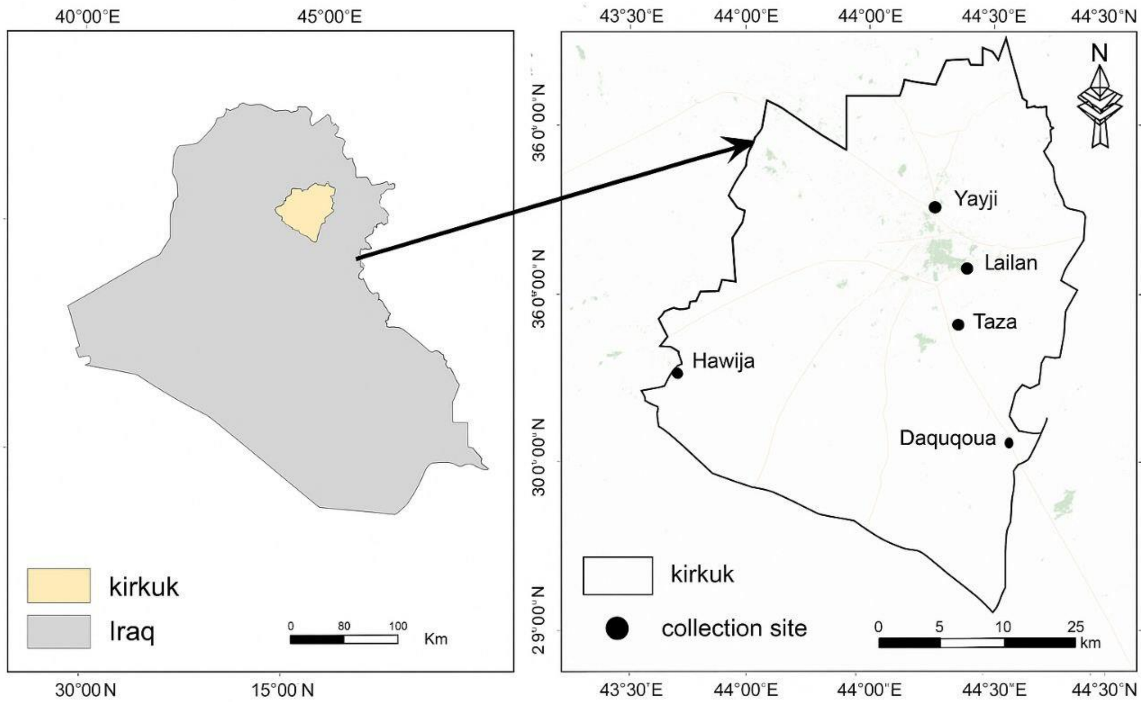


Figure 1. Map Showing the Study Sites (Kirkuk Governorate).

This bacteriological study was conducted regularly, at a rate of once per month, over a continuous period of six months. It involved the analysis of water from five fish farms located in different areas of Kirkuk Governorate, namely: Hawija District, Daquq District, Yayji Subdistrict, Taza Subdistrict, and Lailan Subdistrict. These locations were carefully selected as part of the research plan due to their importance in fish farming, in addition to the recurring health issues observed in the fish. In recent years, frequent and widespread fish deaths have been recorded in these breeding ponds, raising the need for an in-depth scientific study to determine the possible bacterial causes.

Ponds used in such farms were all earthen ponds (soil based), with the size of the pond varying from farm to farm, based on intended use and number of fish being cultured. The water flows into these ponds from the covered artesian wells that are ready with the help of special drilling machines at a considerable depth, which is 100–150 meters. The farms rely on these wells to supply their only year-round source of water, which have run 10-15 years without any significant recharging. As a matter of fact, these sites were purposefully selected based on the absence of any kind of scientific reports related to them; so this study is one valuable addition towards increasing our knowledge about bacteriological status and water quality in such fish farms.

### Sample Collection

Water samples for bacteriological examination were collected by taking clean, sterilized glass containers provided with tight-fitting lids and narrow neck of 200–250 ml. Containers were previously sterilized (24) by putting them into an electric oven at 160–180°C, for one hour to destroy any contaminants. Water samples were collected at 10–20 cm depth from the pond surface where it was free of floating microorganisms or debris during sample collection. Water sampling was done in duplicate and the collected water samples were transferred to cork-sealed chilled containers and carried to the laboratory under cold conditions (4–6°C) within 1–2 hours from collection for preservation of microbial viability, prior to being analysed using bacteriological methods The fish body samples of each species were taken with sterile swabs to be representative of different external parts of the fish. This enabled ZFIN annotators to mark different parts of the fish body, use such as mouth, gills, skin, scales, fins and tail. The swabs were carefully placed in sterile containers and transported under the same temperature and timing conditions as the water samples. All sampling and handling procedures were conducted in accordance with standard microbiological practices to maintain the reliability and validity of the laboratory results.

## 4. RESULTS AND DISCUSSION

### Total Coliform Bacteria Count

The results presented as shown in the Table 1 and Figure 2 showed that the total number of coliform bacteria in the water of the five stations ranged between 0 and more than 16 cells per milliliter. The highest counts were recorded at the second, third, and fifth stations, where values exceeded >16 cells/ml during all months of the study. This clearly indicates a significant impact of the surrounding environmental conditions at these stations, in addition to the presence of environmental stress factors, whether resulting from the accumulation of organic matter, poor aeration, or high temperatures all of which contribute to creating a favorable environment for bacterial growth and reproduction. This marked increase in coliform bacteria suggests the likelihood of continuous organic pollution in those locations, which may pose a threat to the aquatic environment and the health of the fish.

On the other hand, the lowest counts (0 cells/ml) were recorded at the first station during November and December and at the fourth station during December. The low bacterial counts in these stations are attributed to the regular use of chlorine in the breeding ponds. This substance is well known for its high effectiveness in eliminating microorganisms, including harmful bacteria, thereby reducing water contamination. The results of this study are consistent with findings from previous studies conducted in similar environments, reinforcing the reliability of these scientific results and emphasizing the need to continue using disinfectants in breeding ponds to reduce bacterial risks [16]. Based on the analysis of variance test and Duncan's test, significant differences were recorded between the coliform counts, with the first and fourth sites being significantly different ($P \leq 0.05$) from the rest of the sites.

Table 1. Monthly and Site-Specific Changes in Coliform Counts at the Study Sites Cell/Ml

| Location Season | First Station Hawija | Second Station Daquqouq | Third Station Yayji | Fourth Station Taza | Fifth Station Lailan |
|---|---|---|---|---|---|
| August | 2 | >16 | >16 | 8 | >16 |
| September | 7 | >16 | >16 | 2 | >16 |
| October | 3 | >16 | >16 | 4 | >16 |
| November | 0 | >16 | >16 | 4 | >16 |
| December | 0 | >16 | >16 | 0 | >16 |
| January | 1 | >16 | >16 | 2 | >16 |
| Average | 2.1667 a | >16 b | >16 b | 3.3333 a | >16 b |

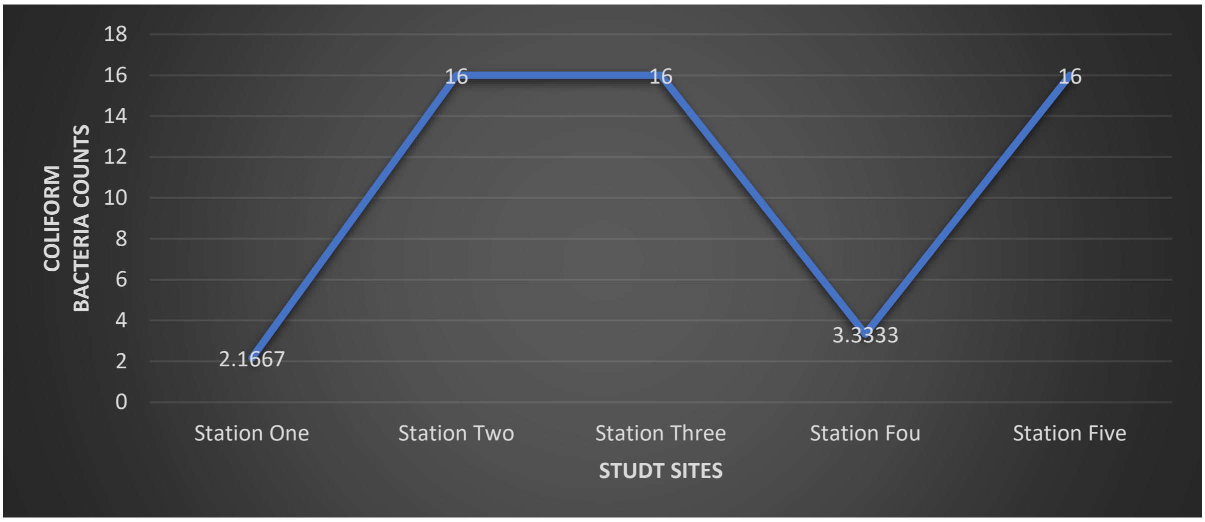


Figure 2. Coliform Bacteria Counts Levels at the Study Sites

## E. Coli Counts

The results of the current study as shown in the Table 2 and Figure 3 showed that E. coli counts varied between 1.3-70 CFU/ml, with the highest level of 70 CFU/ml recorded in December at the first station and the lowest level of 1.3 CFU/ml in August at the third station, and it was noted from the table that the infection rates increased in the winter season in all stations Due to lower temperatures and reduced ultraviolet radiation, which prolong the survival of bacteria, in addition to increased surface water runoff that carries contaminants to water sources.

Table 2. Monthly and Localised Changes of E. coli Numbers at the Study Sites CFU/Ml

| Location Season | First Station Hawija | Second Station Daquqouq | Third Station Yayji | Fourth Station Taza | Fifth Station Lailan |
|---|---|---|---|---|---|
| August | 2 | 4 | 1 | 3 | 1 |
| September | 3 | 5 | 5 | 3 | 5 |
| October | 2 | 5 | 8 | 3 | 8 |
| November | 2 | 6 | 3 | 4 | 4 |
| December | 70 | 5 | 22 | 3 | 4 |
| January | 9 | 6 | 48 | 3 | 3 |
| Average | 17a | 6a | 18b | 4a | 5a |

The results of the present study were in agreement with the study of [17] which recorded a range of 1-70 CFU/ml, higher than the findings of [18] in Nigeria which ranged from 14-40 CFU/ml, and lower than the findings of [19] which recorded 4-110 CFU/ml. The World Health Organization [20] indicates that the maximum allowable E. coli count for safe water use is 10 CFU/ml, and the high levels of E. coli bacteria are attributed to farmers' use mainly of animal manure for fish feed [12]. Environmental factors also play a role, as low temperatures help E. coli bacteria to survive longer in water up to a year [13]. These findings indicate the contamination of aquaculture water with human and organic waste, Fish with intestinal bacteria are a good indicator of contaminated faeces and contaminated water. Due to the presence of pathogenic bacteria in fish, it has been suggested that poor handling or preparation of fish can jeopardise consumer food safety [21]. Pearson's correlation coefficient showed a strong significant correlation ($p \leq 0.01$) between E. coli and Coliform bacteria values (r=542), and based on analysis of variance and Duncan's test, no significant differences were recorded between the total number of bacteria ($p \leq 0.05$) in the study sites.

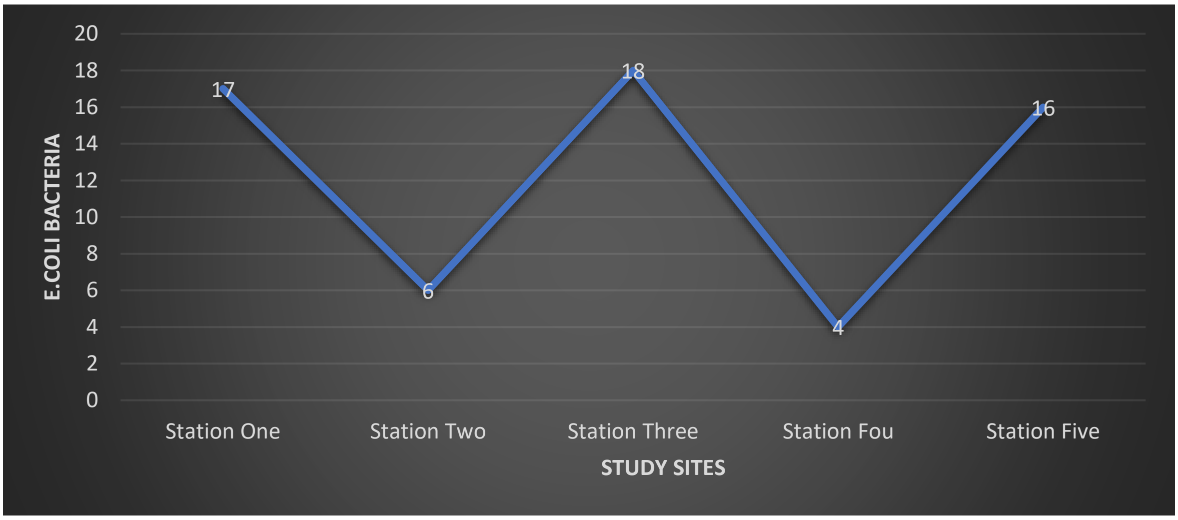


Figure 3. E.Coli bacteria levels at the study sites

### Fecal Coliform Bacteria

The results of the current study as shown in the Table 3 and Figure 4 indicated that the numbers of fecal coliform bacteria ranged between 2.1 and 90 colony-forming units per milliliter (CFU/ml). The highest recorded value was 90 CFU/ml in January at the third station, while the lowest value was 1.3 CFU/ml in August at the first station. By analyzing the data presented in the Table 3, a clear variation in infection rates among the different stations can be observed. There was a significant increase in the third station, while the second station showed a noticeable decrease in bacterial counts during the same period. This was due to what the Sebelikos also noticed, that fecal coliform bacteria bursts in early January more than just other months of the year. The high percentage at the third station is attributed to variousfactors related to climate and environment. The most outstanding among them are the low winter temperatures that increase the survival time of bacteria in water and their poor exposure to sunlight that limits the bactericidal UV effect; Furthermore, the excreta proliferation in the rainy season may increase bacterial pollution. A third station is relatively close to direct sources of pollution and might have higher bacterial levels. Given these results, future investigation into similar abiotic variables is necessary to assess their role in water quality and microbial pollution risk.

Table 3. Monthly and Localised Changes of Fecal Coliform Bacteria Counts at the Study Sites CFU/Ml

| Location Season | First station Hawija | second station Daquqouq | third station Yayji | fourth station Taza | fifth station Lailan |
|---|---|---|---|---|---|
| August | 2 | 3 | 8 | 5 | 8 |
| September | 3 | 3 | 15 | 5 | 14 |
| October | 10 | 7 | 12 | 8 | 13 |
| November | 14 | 15 | 22 | 9 | 10 |

| | | | | | |
|---|---|---|---|---|---|
| December | 12 | 12 | 20 | 12 | 12 |
| January | 12 | 7 | 91 | 15 | 8 |
| Average | 8a | 8a | 28b | 9a | 11a |

The results of the present study agreed with the study of [19] in Kirkuk with a range of 2-82 CFU/ml, and lower than the results of [22] in Mexico in his study of fish farms which reached 26.6-104 CFU/ml, and studies showed a relationship between season of the year and fecal coliform bacteria counts in aquarium water with higher fecal coliform bacteria values in winter and rainy season [23]. The accumulation of organic waste and the elevated concentrations of nutrients, such as nitrogen and phosphorus, contribute to creating a favorable environment for the growth and proliferation of fecal coliform bacteria in pond water. These conditions lead to increased bacterial contamination, especially in areas with poor aeration or limited water flow, this is a clear indicator of deteriorating water quality and its potential risk to public health and fish resources [24] and a study [25] showed the role of bacterial contaminant drift in rainy months in promoting coliform bacteria growth. Pearson's correlation coefficient showed a significant correlation ($p \leq 0.01$) between fecal coliform bacteria values and E. coli (r=542), and based on the analysis of variance test and Duncan's test, the second site recorded significant differences between the total number of bacteria ($p \leq 0.05$) in the study sites.

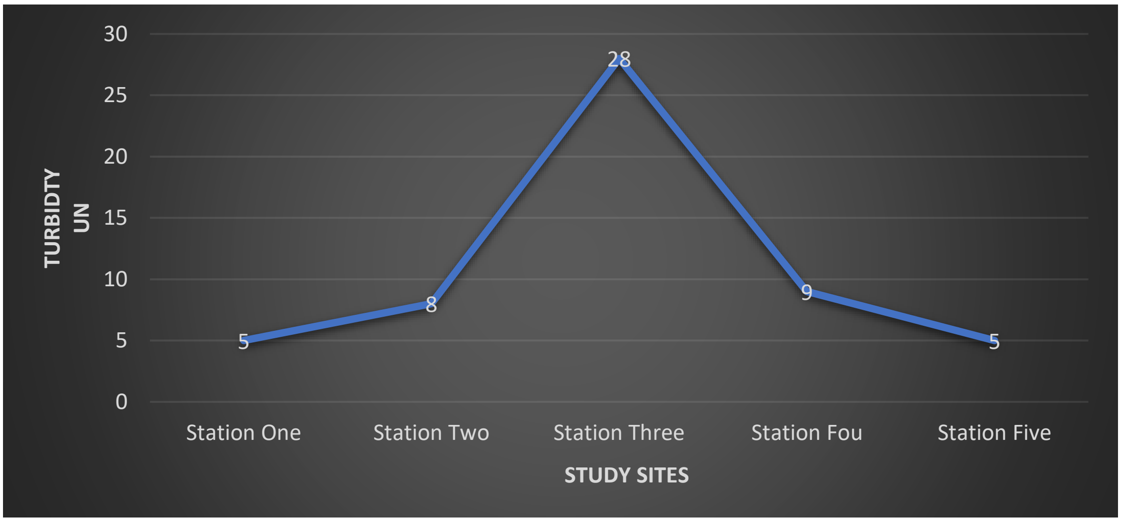


Figure 4. Coliform Bacteria Levels at the Study Sites

## 5. CONCLUSION

Large bacterial contaminants were found in fish farming ponds water across all Kirkuk areas according to the study, which represented a new step in understanding the disease. Station two, three and five showed the greatest degree of contamination which suggested local sources of pollution or else variation in pond maintenance practices. Winter detection results are interesting in terms of fecal pollution, where it was seen that Escherichia coli and the other fecal coliforms increased quite evidently in winter as recreation season has higher than the values from previous seasons due to causing effects such as temperature decrease and increase of runoff etc. These findings suggest that microbiologically, the state of bacteria existence correlates with environmental conditions and lack of proper water quality treatment in fish pond and observed significant discrepancies in THG bacteria presence among the five sample stations could be associated to disparities in anthropogenic influences, waste management strategies put in place and rate of water exchange-aeration at each pond. The findings of E. coli, a major fecal indicator bacterium raises noxious concerns about water quality and broad range of waterborne diseases transmission to fish as well as humans. In general, the results underscore the critical importance of greater regulatory control, frequent water quality check, and improved management practices in aquaculture operations. Improving sanitation, avoiding pollution and purification of pond water are three significant basic recognized actions which need to be done in order to produce fish as food processing safe and public health simultaneously.


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## Author Contributions Statement

| Name of Author | C | M | So | Va | Fo | I | R | D | O | E | Vi | Su | P | Fu |
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| Tawoos Mohammed Kamel | ✓ | | ✓ | | ✓ | ✓ | ✓ | | | | ✓ | ✓ | | ✓ |
| Omar Taha Mahmoud | ✓ | ✓ | | ✓ | ✓ | | | ✓ | ✓ | ✓ | | ✓ | | |
| Ahmed Abdulnaser Abdulla | ✓ | ✓ | | ✓ | | ✓ | ✓ | | | ✓ | | ✓ | ✓ | ✓ |

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

## Conflict of Interest Statement

The authors confirm that there are no conflicts of interest associated with this research, its results, or its publication in any form.

## Informed Consent

Several official approvals were obtained from the concerned authorities before carrying out this research, which is to evaluate bacteriological purity of fish ponds in the district and suburbs of Kirkuk Governorate. All the research procedures were approved according to ethical and professional norms of environmental studies, microbiological investigation standards and national and international guidelines for water sampling, preservation and analysis.

## Ethical Approval

This study is planned to evaluate the bacteriological quality of Fish ponds in the districts and suburbs of Kirkuk Governorate allegally, The Ethical Committee at University of Kirkuk ethically approved this research work. The researchers took care to follow all ethical considerations and established scientific norms in the execution of their study methods. The ambient water samples were then collected and analyzed through established scientific field practices. Every precaution was taken to ensure research validity and accuracy resulting in professional and ethical responsibility when conducting the study.

### Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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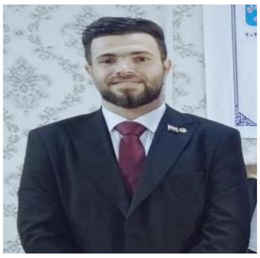
|  | **Ahmed Hussein Ahmed** has a Master of Life Sciences from Kirkuk University and is committed to having an international career in scientific research fancying for spreading development of the medical fields. He is passionate on academic advancement through teaching and scientific evidence which concentrate on methodology of a study and validity interpretation of obtained results according to scientific principles. He can be contacted at Email: Ahmedbioh1996@gmail.com |
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