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Relation of Water Quality and Bacteria Occurrence In Cage-Cultured Common Carp (*Cyprinus carpio*) In Basra, Iraq

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ABSTRACT

Aquaculture is an extreme activity of valuable and fast sector and expansion worldwide, however there are concerns related to environmental sustainability. The current study was carried out to determine the relation between water quality and the occurrence of bacteria in cage farmed common carp (*Cyprinus carpio*) in Basrah Governorate southern Iraq. Water from floating cages were randomly sampled from seven sites along the Tigris River and Shatt al-Arab estuary from December 2012 to June 2013 and from October 2018 to May 2019. The water quality of each sample was measured, including the temperature, pH, iron, chloride, sulfide, ammonia, nitrite, phosphate, total suspended solids, conductivity, biological dissolved oxygen and chemical dissolved oxygen. A total of seventeen bacteial species were identified, including *Aerococcus viridans*, *Streptococcus gordonii*, *Streptococcus thoraltansis*, *Staphylococcus lentus*, *Staphylococcus pseudintermedius*, *Staphylococcus lugdunensis*, *Kocuria kristinae*, *Lactococcus lactis* spp. *lactis*, *Leuconostoc mesenteroides* ssp. *cremoris*, *Vibrio cholera*, *Sphingomonas paucimobils*, *Aeromonus hydrophila*, *Aeromonas veronii*, *Aeromonas sobria*, *Pasteurella testudinis*, *Elizabethkingia meningoseptica*, and *Pseudomonas aeruginosa*. The results indicated that, despite the changes in the quality of the water, no association was detected between the alteration recorded and the occurrence of bacteria, whether in the water or sediment, during the period of study,

INTRODUCTION

Global population growth represents a major existential threat, and hence old farming methods may not cover the actual food requirements. In addition, major steps are needed to repair the severe environmental collapse. Therefore, innovative ideas in aquaculture could enable people to obtain nutrients without degrading the environment.

The aquaculture industry has steadily grown in response to the increasing demand for products intended for human consumption, which has allowed humanity to substantially exploit seas, lakes, and rivers, as well as maintain the quality of fish cages **(Olapoju *et al*., 2014; Karimian *et al*., 2017; Palladino *et al*., 2021;Yaghoubzadeh *et al*., 2021; https://www.ift.org, 2022)**.

Fish is the primary source of animal protein for humans in many parts of the world. Additionally, per capita fish consumption increased from 9.9kg in the 1960s to 14.4kg in the 1990s and 19.7kg in 2013, with estimates for 2014 and 2015 suggesting it may have risen to 20.3kg by 2016 **(FAO, 2014; FAO, 2016)**. As a result of rapid growth around the world, aquaculture has been affected by disease outbreaks, raising environmental concerns. It is evident that the number of new bacterial taxa associated with fish diseases and causing serious economic losses to aquaculture has been increasing **(Anamarija *et al*., 2020)**.

In Iraq, fish production depends on inland and marine fisheries for the availability of water. Rivers such as the Tigris and Euphrates and their tributaries, as well as marshes, dams, and reservoirs, comprise Iraq's main water sources. Furthermore, Iraq has an Arabian Gulf coastline with a water surface area of about 700km^2. Aquaculture was introduced to Iraq for the first time in 1955 in Baghdad, and it was limited to the culturing of the common carp (*Cyprinus carpio*). This is because this species is characterized by its hardiness, high fertility, and rapid growth. It lives on various types of foods, and it is resistant to disease; it is known for its ease of reproduction growing fast under suitable conditions. It can also live in a wide range of water temperatures, from 3-35°C **(Abdul-Razak *et al*., 2018)**.

In Basra, the first fish farm was established in Abi Al-Khaseeb district in 2008. The number of licensed farms is 37, with a total of 466 cages **(Ahmed, 2022)**. The Directorate of Agriculture in Basra officially licensed the establishment of 48 land farms with an estimated area of 3,778 acres and gave permits to establish 380 floating cage farms with a water area of 26,120m^2, representing 1,949 floating cages (the size of each cage is 3 x 4m, with a height of 2m) in 2016, alongside the Tigris River, marshes, and Shatt al-Arab estuary. The productivity of the basins ranged from 3,778 to 4,723 tons per year, and the productivity of the floating cages was estimated at 3,266 to 7,800 tons per year. Estimates of the financial return on total fish production in the Governorate of Basra range between 106.608 and 706.680 million USD, and the workers' number in this sector is more than 3000 people **(Al-Edany & Ali, 2020)**. Therefore, the present study was carried out to determine the relation between water quality and the occurrence of bacteria in cage of the cultured common carp in the Basra Governorate.

MATERIALS AND METHODS

Location sampling

The current study was carried out using cage of cultured common carp in Basra Governorate, southern Iraq. Seven sites were chosen along the Tigris River and Shatt al-Arab estuary (Fig. 1 & Table 1).

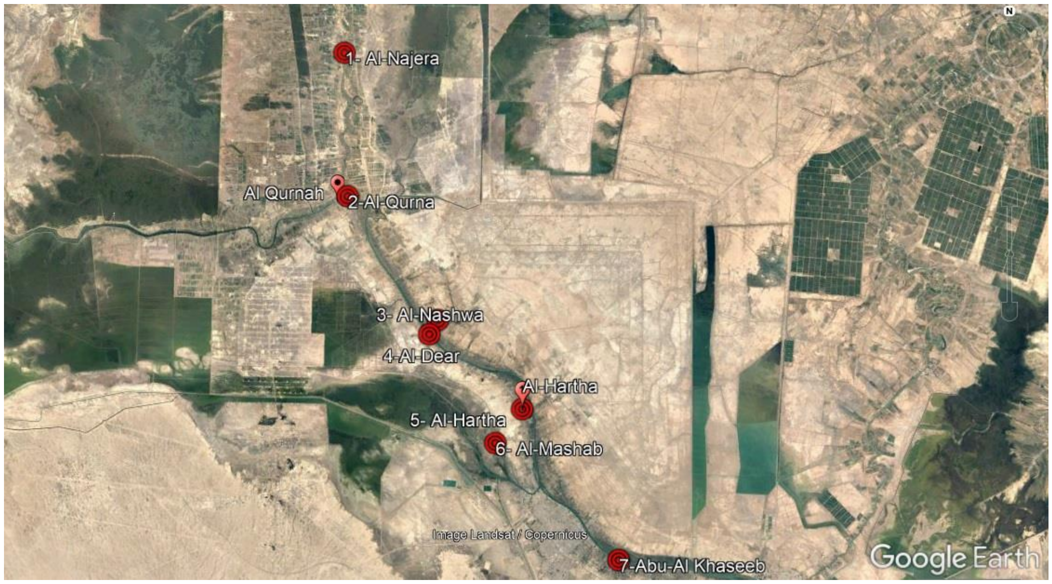

Fig. 1. locations of the studied fish cages in Basra Governorate

Table 1. The latitrude and longitude of stations

Station	Latitude	Longitude
Al-Hartha	30°41'35.96"N	47°44'20.44"E
Al-Nashwa	30°49'24.59"N	47°35'46.22"E
Al-Mashab	30°38'38.86"N	47°41'36.18"E
Al-Nagara	31°12'56.13"N	47°26'9.48"E
Abu-Al Khaseeb	30°28'25.59"N	47°54'3.54"E
Al-Qurna	31° 0'15.75"N	47°26'32.56"E
Al-Dear	30°48'9.39"N	47°34'54.11"E

Sampling

Water and sediment samples were taken monthly from December 2012 to June 2013 and from October 2018 to May 2019. The samples were selected from three sites: before, inside, and after cages (before and after being within 10m). All the water samples were collected in sterile glass bottles (500mL) and preserved at a low temperature using ice packs. Meanwhile, the sediment samples were collected using a grab sampler.

Water quality measurements

The temperature, pH, and conductivity were measured using a Multimeter 3410 (WTW- Germany). The chemical oxygen demand (COD) was measured using a Photolab S6 (WTW- Germany), while the biological oxygen demand (BOD_5) was measured using the BOD system S 606/2-i (WTW, Germany). Additonally, the colorimetric method was used to determine the nitrite (NO_2); the distillation and titration procedure was used to determine NH_3-N concentrations, and the cadmium reduction method was used to determine the nitrate (NO_3). Chloride was volumetrically determined by titration using 0.01N $AgNO_3$, while total suspended solids (TSS) was determined gravimetrically using the method of **APHA (2005)**. Moreover, potassium was determined using a flame photometer (Jenway, UK).

Bacterial isolation and identification

The same bacteriological analyses were performed for the water and sediment samples. The membrane filtration technique was used by filtering three replicates of each sample through 0.45µ pore size membrane filters (Millipore- France). For total coliforms, Petri dishes containing M- endo agar LES were used and incubated at 37± 1°C for 24h. Colonies appeared as pink to dark red spots with a metallic (golden) sheen, varying in size from pinhead-sized to complete colony coverage. For fecal coliforms, membrane filters were cultured on M-FC agar with 1% rosolic acid and incubated at 44.5°C for 18 hours in a water bath. Colonies appeared blue or light blue. Bacterial identification was performed using a VITEK 2 system with GP and GN ID cards (Biomerieux, USA).

RESULTS

As illustrated in Table (2), COD and BOD levels were high before the fish cage at all study sites but decreased in and after the fish cage. Meanwhile, other parameters were variable across the study sites, as shown in Table (3), despite the influence of the salt tide in the Shatt al-Arab River. Total and fecal coliform bacteria were studied in water and sediment, and the ln of the numbers was high at all study sites, indicating water contamination with sewage (Tables 4, 5, 6).

Seventeen bacterial species were recovered from fish cages and rivers in this study (Table 7). These bacteria are Gram-positive (50%) and Gram-negative (50%), including *Aerococcus viridans, Streptococcus gordonii, Streptococcus thoraltansis, Staphylococcus lentus, Staphylococcus pseudintermedius, Staphylococcus lugdunensis, Kocuria kristinae, Lactococcus lactis* spp. *lactis, Leuconostoc mesenteroides* ssp. *cremoris, Vibrio cholera, Sphingomonas paucimobils, Aeromonus hydrophila, Aeromonas veronii, Aeromonas sobria, Pasteurella testudinis, Elizabethkingia meningoseptica,* and *Pseudomonas aeruginosa.*

Table 2. The mean of water parameter analyses in 2013

Station	Location	COD mgL^{-1}	BOD_5 mgL^{-1}	PO mgL^{-1}	TP	TK	NO_2 μgL^{-1}	NO μgL^{-1}	EC $Mscm^{-1}$	TSS mgL^{-1}	Cl mgL^{-1}	Temp ^oC	pH
Al-Qurna	Before	190.5	5.43	0.54	0.15	0.25	60.81	6.44	1.90	106.0	2.41	21.34	8.21
	Inside	76.0	4.93	0.42	5.82	0.32	54.65	5.45	1.91	98.0	2.14	21.80	8.19
	After	93.0	5.27	0.58	7.37	0.16	3.88	10.22	1.99	88.0	4.47	21.32	8.21
	Mean	119.83	5.21	0.51	4.45	0.24	39.78	7.37	1.93	97.33	3.01	21.49	8.20
	Std. Deviation	61.79	0.26	0.08	3.80	0.08	31.24	2.52	0.05	9.02	1.27	0.27	0.01
Al-Dear	Before	248.5	6.90	0.28	0.26	0.16	6.40	7.98	1.86	128.0	2.71	22.43	8.42
	Inside	234.0	5.87	0.23	5.35	0.14	3.06	8.37	1.94	41.0	9.28	22.52	8.35
	After	270.5	4.50	0.47	7.31	0.21	52.15	5.13	1.88	27.0	1.36	22.52	8.41
	Mean	28.67	2.10	0.55	1.87	0.40	73.34	6.55	5.76	61.67	8.59	20.03	8.02
	Std. Deviation	28.04	0.19	0.27	2.69	0.36	17.42	0.94	0.28	6.66	4.63	0.23	0.06
Abu-Alkhaseeb	Before	61.0	1.90	0.79	0.27	0.82	91.99	5.66	5.48	66.0	4.43	19.87	8.07
	Inside	11.0	2.13	0.60	4.98	0.19	57.48	6.46	5.75	65.0	7.76	20.30	7.96
	After	14.0	2.27	0.25	0.36	0.19	70.55	7.53	6.04	54.0	13.58	19.93	8.04
	Mean	251.00	5.76	0.33	4.31	0.17	20.54	7.16	1.89	65.33	4.45	22.49	8.39
	Std. Deviation	18.38	1.20	0.13	3.64	0.04	27.43	1.77	0.04	54.72	4.24	0.05	0.04
Total	Mean	133.17	4.36	0.46	3.54	0.27	44.55	7.03	3.19	74.78	5.35	21.34	8.21
	Std. Deviation	102.98	1.82	0.19	3.21	0.21	32.30	1.65	1.93	32.69	4.07	1.08	0.16

Table 3. The water parameter analyses in 2018

Station		EC ms/Cm	pH	DO mgL^{-1}	BOD5
Al-Hartha		11.57	7.85	2.70	5.40
Al-Mashab		20.75	7.67	2.46	4.92
Al-Nashwa	**Before**	2.72	7.94	3.9	7.8
	Inside	2.79	7.87	2.33	4.66
	After	2.76	7.81	3.2	6.4
Mean		2.76	7.87	3.14	6.29
Std. Deviation		0.04	0.07	0.79	1.57
Al-Najera	**Before**	2.22	7.88	2.03	4.06
	Inside	2.20	7.85	3.13	6.26
	After	1.45	7.75	2.36	4.72
Mean		1.96	7.83	2.51	5.01
Std. Deviation		0.44	0.07	0.56	1.13
Abu- Al-Khaseeb		23.7	7.18	3.00	6.00
Total /	**Mean**	7.80	7.76	2.79	5.58
	Std. Deviation	8.76	0.23	0.58	1.15

Table 4. The ln of total and fecal coliform in sediment and water at 2013

Station / Location		TCs*	FCs*	TCw‡	FCw‡
Abu-Alkhaseeb /	**Before**	11.40	9.53	7.44	6.42
	Inside	8.90	7.29	7.39	5.94
	After	9.10	7.51	7.45	5.54
Mean		9.80	8.11	7.43	5.97
Std. Deviation		1.389	1.235	0.032	0.441
Daer /	**Before**	0.51	0.03	6.91	5.00
	Inside	10.92	8.80	7.03	5.16
	After	1.30	0.41	7.00	4.85
Mean		4.24	3.08	6.98	5.00
Std. Deviation		5.796	4.957	0.062	0.155
Qurna /	**Before**	9.52	8.77	5.90	4.02

Inside	1.58	1.22	5.60	6.34
After	8.82	3.62	6.13	5.11
Mean	6.64	4.54	5.88	5.16
Std. Deviation	4.396	3.858	0.266	1.161
Total / Mean	6.89	5.24	6.76	5.38
Std. Deviation	4.420	3.908	0.704	0.770

TCs*: Total coliform in sediment; FCs*: Fecal coliform in sediment : TCw‡:Total coliform in water; FCw‡: Fecal coliform in water

Table 6. The ln of total and fecal coliform in water at 2019

Site	Total coliform	Fecal coliform
Al-Hartha	4.69	2.88
Al-Mashab	8.61	4.54
Al-Nashwa- Before	4.99	3.47
Inside	9.30	4.10
After	6.96	5.42
Mean	7.08	4.33
Std. Deviation	2.158	0.995
Al-Najera- Before	7.52	3.88
Inside	9.43	4.46
After	7.42	3.56
Mean	8.12	3.97
Std. Deviation	1.13	0.46
Abu Al-Khaseeb	6.18	3.87
Total / Mean	7.23	4.02
Std. Deviation	1.725	0.732

Table 7. Bacterial species isolated from fish cages

Station		Bacterial species
Al-Meshab		*Aerococcus viridans*
Hartha		*Streptococcus gordonii, Vibrio cholera*
Al-Najera	**After**	*Sphingomonas paucimobils, Aeromonus hydrophila, Pasteurella testudinis*
	Inside	*Aeromonas veronii*
	Before	*Aeromonas sobria*
Nashwa	**After**	*Elizabethkingia meningoseptica, Leuconostoc mesenteroides ssp.cremoris*
	Inside	*Aeromonas veronii,Aeromonus hydrophila/ caviae*
	Before	*Elizabethkingia meningoseptica, Aeromonas veronii,Staphylococcus pseudintermedius*
Al-Meshab		*Staphylococcus lugdunensis,Lactococcus lactis spp lactis*
Hartha		*Streptococcus thoraltansis, pseudomonas aeruginosa*
Abu-Alkhaseeb		*Staphylococcus lentus, pseudomonas aeruginosa*
Al-najera	**After**	*pseudomonas aeruginosa, kocuria kristinae*
	Inside	*Staphylococcus lentus, kocuria kristinae*
	Before	*Streptococcus thoraltansis, pseudomonas aeruginosa*
Nashwa	**After**	*Staphylococcus lentus, kocuria kristinae*
	Inside	*Staphylococcus lentus, kocuria kristinae*
	Before	*kocuria kristinae*

DISCUSSION

The parameters of water quality may influence the growth and abundance of farmed fish. Good management and monitoring of water quality is important for the success of commercial aquaculture, which usually requires optimal environmental conditions for rapid growth and minimal resource costs.

In addition, water quality involves variable parameters that affect farmed fish, some of which play a critical role, such as temperature, suspended solids, dissolved oxygen, nitrite, ammonia, alkalinity, and carbon dioxide. However, dissolved oxygen is considered an important parameter and indicator of the oxygen demand for biodegradable pollutants **(APHA, 1998)**. Therefore, continuous monitoring of fish farms is necessary due to their requirements for dissolved oxygen.. In addition, fish farming was found to improve water quality by reducing the quantities of all elements, ions, COD, and BOD, especially during 2013 (Table 2). According to **Klein (1966)**, the water from the studied sites, based on the BOD_5 values (1mgL^{-1} = very clean, 2mgL^{-1} = clean, 3mgL^{-1} = fairly clean, $5mgL^{-1}$ = doubtful, and $10mgL^{-1}$ = bad) was classified as clean at Abu Al-Khaseeb. Meanwhile, the water from all the studied sites, based on the COD values, was classified as bad (> $7mgL^{-1}$).

In conservation nature as river and marine environments, the large water volume and exchange of water quality variables such as total alkalinity, total hardness, pH, nitrogen compounds, and hydrogen sulfide were considered of little importance**.**

In this work, the most significant changes in the quality of the water environment are described in Tables (2, 3). Based on the results, some of the water quality parameters- for example, water temperature, which is considered an important factor for bacterial diseases in fish- were at the optimum level.

Electrical conductivity is a useful indicator of both salinity and mineralization or total salt in a water sample **(Rimet, 2012; Wangui, 2014)**. According to research by **Ayers and Westcot (1985)**, its values showed slightly saline water at both Qurnah and Dear (0.7-3 $mscm^{-1}$). Meanwhile, its values ranged from slightly saline to highly saline water at Abu Al-Khaseeb (> 6 $mscm^{-1}$ and < 14 $mscm^{-1}$).

As Table (4) shows, the ln of total and fecal coliforms in both the sediment and water samples varied between the studied sites. The highest number of total coliforms was found at Abu Al-Khaseeb before the cage (11.40), and the lowest number was at Al-Dear before the cage (0.51). This variation may be explained by the proximity of the cages to the river bank, which may cause them to be affected by several factors. One of these is agricultural and domestic sewage discharged into the river, which may increase the organic content of the river water and promote the growth of various bacteria species. These results were also obtained in 2018 and 2019 (Tables 5, 6) despite the high salinity due to the salt tide coming from the Arabian Gulf toward the Shatt al-Arab estuary.

Seventeen bacterial species were recovered from fish cages and rivers in this study (Table 7), similar to the number found in previous researches **(Al-Harbi & Uddin, 2003; Al-Harbi & Uddin, 2005; Al-Harbi & Uddin, 2007; Amal *et al*., 2010; Marcel *et al*., 2013)**.

These bacteria are Gram-positive (50%) and Gram-negative (50%). Most bacterial species identified in this study were pathogenic for fish; they included *Aeromonus hydrophila*, *A. sobria*, *A. veronii*, *pseudomonas aeruginosa*, and *Pasteurella testudinis* **(Borella *et al*., 2020; Samayanpaulraj *et al*., 2020;; Duman *et al*., 2021; Ellul *et al*., 2021)**. Species like *Staphylococcus pseudintermedius* was reported to be an animal pathogen **(Lynch & Helbig, 2021)**' While, others viz. *Streptococcus thoraltansis*, *Elizabethkingia meningoseptica*, *Kocuria kristinae*, *Staphylococcus lugdunensis*, *Sphingomonas paucimobils*, and *Staphylococcus lentus* are human pathogens **(Chan *et al*., 2019; Wazir *et al*., 2019; Bernshteyn *et al*., 2020; Kinoshita *et al*., 2021; Lynch & Helbig, 2021; Crespo-Ortega *et al.*, 2022)**. The reason may be the presence of species isolated for the first time from fresh water as a result of the transfer from marine water to fresh water, especially after 2014, when the salinity rate at Shatt al-Arab increased.

Therefore, these extraordinary environmental conditions surrounding the fish cages might affect the water quality and stress the cultured fish. This would ultimately lower their immune status, eliciting bacterial infection leading to disease outbreaks **(Amal *et al*., 2015)**. According to **Al-Harbi and Uddin (2003)** and **Pakingking (2015)**, the bacterial flora of the fish may reflect the bacterial composition of the culture environment (water and sediment) and the health status of the fish.

Irianto and Ausin (2002) revealed that bacteria in fish have associations with their host by protecting them against pathogenic species through the production of antagonistic factors, which inactivate the effects of pathogenic bacterial toxins or metabolites and stimulate the immunity of the host so it can combat pathogens for nutrients or attachments sites. This evidence may explain why so many species, pathogenic or non-pathogenic, were found in the cultured fish.

CONCLUSION

In the current study, different pathogenic and non-pathogenic bacteria were isolated from fish cage of the common carp. The water quality parameters influencing the occurrence of the bacteria in the fish cage were measured. No relationship was found between the the alteration in water quality and the occurrence of bacteria, whether in the water or sediment, during the period of study, despite changes recorded in the water quality.

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