Research Article



The Effect of Alterations in Water Quality Parameters on the Occurrence of Bacterial Diseases in Different Aquatic Environments

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Abstract | The current study investigated the influence of adverse water quality parameters in different localities (Qarun Lake, Fayoum Province, Egypt; Mariotteya stream, Giza Governorate, Egypt; and Mediterranean coastal water, Alexandria Governorate, Egypt) on different fish species during the winter. Water and fish samples were collected to evaluate the physicochemical properties and heavy metal distribution in the water. Furthermore, bacteriological analysis of water and fish was performed. The physicochemical characteristics of water samples revealed high conductivity, alkalinity, and higher values of water hardness in Lake Qarun, Fayoum province and Mediterranean coastal water, Alexandria Governorate. However, the parameters in Mariotteya water were within normal limits. Heavy metals were detected in Lake Qarun and the Mediterranean Coast. However, none of the heavy metals analyzed were found in the Mariotteya water samples. Moreover, detected trace elements, cadmium and lead, exceeded the permissible limits. The bacterial load of the collected water samples revealed only the isolation of *Escherichia coli* and other coliform bacteria from Lake Qarun, while other bacteria included Vibrio spp. (2.48 log₁₀ CFU/mL), Aeromonas spp. (2.70 log₁₀ CFU/mL), and Pseudomonas spp. (3.18, 2.04 log₁₀ CFU/mL) isolated from Lake Qarun and Mariotteya stream, and Staphylococcus spp. (2.00, 1.95, and 1.00 log₁₀ CFU/mL) from Mariotteya stream, Lake Qarun, and Mediterranean Coast, respectively. Among the isolated bacteria from collected fishes, Aeromonas spp. weres detectable at a higher percentage (36.2%) followed by Vibrio spp. (31.4%), Pseudomonas spp. (16.2%), and Staphylococcus spp. (6.7%). Moreover, the highest percentage of bacterial isolates was recovered from Lake Qarun. Large shrimps from the Mediterranean Coast showed a high percentage of Vibrio spp. (40%) isolation. The log of viable microbial count and chemical parameters in water bodies had a strong correlation coefficient (r > 0.75), suggesting that the ecosystem is highly polluted by agricultural and industrial contamination.

Keywords | Aquatic environment, Water quality, Heavy metals, Bacterial contamination

Received | June 03, 2021; Accepted | August 30, 2021; Published | November 01, 2021

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Citation | Khalefa HS, Abdel-Moneam DA, Ismael E, Waziry MFF, Ali MSG, Zaki MM (2021). The effect of alterations in water quality parameters on the occurrence of bacterial diseases in different aquatic environments. Adv. Anim. Vet. Sci. 9(12): 2084-2094.

DOI | http://dx.doi.org/10.17582/journal.aavs/2021/9.12.2084.2094

ISSN (Online) | 2307-8316; ISSN (Print) | 2309-3331

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INTRODUCTION

Egypt is a leading African country in the aquaculture industry, accounting for around 1.8 million tons of total production. Fish aquaculture in Egypt has rapidly increased from 1.45 million tons in 2013 to about 1.82 million tons in 2017, with a 25% total increase (Ali et al., 2020) as a result of the rapid expansion in the application of new technologies and improved rearing and managemental strategies (Kaleem and Sabi, 2020).



Fish is a good source of low-cost and high-nutrient protein sources. Thus, the Egyptian government paid particular attention to fish meat as part of its food security policy (Elsayed et al., 2018). According to Saeed and Shaker (2008), Egyptian marine life suffers from poor water quality because water is polluted by sewage disposal and agrochemicals and exposure to high environmental influences leading to an outbreak of bacterial infection among fish. The pollution of the aquatic ecosystem by natural and inorganic chemicals is one of the most significant risks to marine life because polluted water stresses farmed fish and makes them susceptible to infectious diseases, leading to high mortality (Sarmento et al., 2004). Fish feed and live in marine ecosystems and cannot avoid the adverse effects of pollutants (Yarsan and Yipel, 2013).

Water quality, controlled by the physical, chemical, and biological properties of water, is a very sensitive problem in the aquatic environment (Sargaonkar and Deshpande, 2003; Diersing, 2009; Simpi et al., 2011). Microbiological and chemical pollutants may cause lakes' water quality to deteriorate. The marine ecosystem is exposed to a variety of pollutants with the incorporation of industrial, agricultural, and domestic wastewater.

Heavy metal contamination in the marine ecosystem has become a major health issue in recent years, it act as essential indicator of hygienic status of water, as heavy metal concentrations reflect the pollution status of those areas (Younis et al., 2020), because certain metals are not biodegradable and accumulate in various organs of animals and humans. Thus, determining trace metal concentrations in natural water systems has gained attention to monitor environmental pollution (Saad et al., 2012; Dhanakumar et al., 2015). Moreover, this accumulation is dependent on Heavy metals intake, storage, and removal from the body (Abdallah and Morsy, 2013). Lead, arsenic, and cadmium are the most hazardous metals to humans, posing significant health risks (Abd El-Hady, 2007).

Stress caused by unfavorable environmental factors leads to reduced fish immunity and paves the way to pathogens and parasites (Eissa, 2002). The members of the Enterobacteriaceae family are commonly found among the most common bacteria on freshwater fish. The coliform group and *Escherichia coli* are very important among the bacterial indicators used to define water quality and health risk (Giannoulis et al., 2005). Coliform bacteria have long been used to suggest fecal water contamination and, therefore, a health risk. Bacterial pathogens are a major threat to global fish production because of the high economic value of the diseases they cause (Wamala et al., 2018). Many previous studies have identified *Aeromonas*, *Edwardsiella tarda*, *Flavobacterium columnare*, *Yersinia ruckeri*, *Staphylococcus*, *Vibrio vulnificus*, *Streptococcus*

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agalactiae, and Pseudomonas species because they are among the most economically significant bacterial fish pathogens (Falaise et al., 2016; Zhang et al., 2018; Yaseen et al., 2020). Finally, fish in aquariums with good water quality can live longer and live healthier than fish in the wild.

Consequently, the current study aimed to assess the water quality by measuring physicochemical parameters in different surface water areas as well as determine the risk of microorganisms in the water and cultured species.

MATERIALS AND METHODS

STUDY AREA

Water and fish samples were obtained during the winter between (November 2019 and January 2020) from three sites in Egypt: Shakshouk area, Lake Qarun at Fayoum Governorate, Mariotteya drain at Giza Governorate; a 4-km distance that ranges from Shabramant to Abouseer city, and Mediterranean Coast at Alexandria Governorate as shown in Figure 1.



Figure 1: A map showing the studied areas: (1) Qaroun Lake at Fayoum governorate; (2) Mariotteya drain at Giza governorate; (3) Mediterranean coast at Alexandria governorate. The map was made using Google my Maps application.

SAMPLING

WATER SAMPLES

Water samples were collected from each of the three study areas (Figure 1). In addition, water samples were taken 30 cm below the surface of the stream in the middle of the stream. Water samples were collected in sterile 500-mL glass and 2-L bottled for bacteriological and physiochemical analysis, respectively. The samples were carefully labeled before being shipped to the testing laboratory in an icebox surrounded by ice gel packs.

FISH SAMPLES

Collected from the Shakshouk area, Lake Qarun, Fayoum were 30 Oreochromis niloticus fish (Nile Tilapia), five Tilapia

zilli, and 50 small artemia shrimps. Sample collection follows APHA (1970), and three to five shrimp samples were pooled and homogenized using 10-mL sterile alkaline peptone water and stomacher. Also, five *O. niloticus* were collected from the Mariotteya drain, Giza. Fifteen large shrimps were collected along the Mediterranean Sea's coast of Alexandria. Fish samples were held fresh and transported to the laboratory for bacteriological testing.

WATER ANALYSIS

PHYSICAL AND CHEMICAL ANALYSIS OF WATER SAMPLES

Standard methods for examining water and wastewater were used to analyze the physical and chemical parameters (APHA, 2005). An electrometric pH meter (pHep[®] HI 98107, Milan, Italy) was used to determine the pH of the water samples. A conductivity meter was used to calculate electrical conductivity (EC) at 25°C. The carbonate (CO_3^{2-}) and bicarbonate (HCO_3^{-}) ions were measured titrimetrically against 0.1 N HCl. Ion chromatography (IC; DX5000 Dionex System, Dionex Corp., Sunnyvale, CA, USA) was used to calculate major anions such as chloride (Cl⁻), sulfate (SO_4^{2-}), and phosphate (PO_4^{3-}). Inductively coupled plasma-optical emission spectrometry was used to calculate major cations such as calcium (Ca^{2+}), potassium (K⁺), magnesium (Mg^{2+}), and sodium (Na^+).

HEAVY METAL DETERMINATION

Water samples were analyzed for the presence of lead (Pb), zinc (Zn), copper (Cu), and cadmium (Cd). Nitric acid (analytical grade) was applied on collected water samples until the pH was <2. The Thermo Science iCAP 6500 ICO-OES (Thermo Fisher Scientific, Waltman, MA, USA) and the US EPA Process 2007 were used to calculate element concentrations and measured in parts per billion (in milligram per liter). The atom elements were excited with argon gas, and the blank values for each element were determined using the sample values. These tests were conducted at 'the Central Laboratory of the Faculty of Agriculture, Cairo University, Cairo, Egypt.

MICROBIOLOGICAL EXAMINATIONS OF WATER SAMPLES

Water samples were microbiologically tested 'following the guidelines of APHA (1998) guidelines. Total and fecal coliform were counted as the most probable numbers (MPN/100 mL water) using the multipletube fermentation technique with Lauryl Tryptose Broth (Biolife, Milan, Italy) for standard presumptive examination. The MPN confirmation process was carried out by moving positive presumptive tubes (gas production within 24–48 h) to 2% Brilliant green bile lactose broth (Difco, Thermo Fisher Scientific) and checking for gas production within 48 h at 35 °C and 44.5 °C for total and fecal coliforms, respectively. The poured plate method was used to determine the total bacterial count (TBC) of water

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samples. After making serial dilutions of water samples, 1 mL was transferred in triplicates into a sterile glass Petri dish. Each plate received approximately 15 mL of melted nutrient agar medium then mixed and left to solidify. The plates were incubated for 72 h at 37 °C. Following the incubation time, the number of produced colonies per plate of the same dilution was counted, and the mean value was determined. The mean (\log_{10}) of the results was given (Ronald, 2010). To isolate and maintain certain pathogenic bacteria, 1 mL of each of the last three dilutions of water samples was transferred into Petri dishes, along with approximately 15.0 mL of a particular culture media (Staphylococcus 110 medium, Aeromonas selective agar medium, thiosulfate citrate bile salt agar, TCBS (Oxoid, Thermo Fisher Scientific), and *Pseudomonas* agar medium) and hardened. Petri dishes were placed upside down in an incubator at 37 °C for 72 h.

BACTERIOLOGICAL EXAMINATION OF FISH SAMPLES

The collected fish samples were externally sterilized with 70% ethanol (APHA, 2012). Consequently, aseptic swabs of skin, kidney, and liver were inoculated in nutrient broth then streaked on tryptic soy agar (TSA) Staphylococcus 110 media, Aeromonas selective agar medium, Pseudomonas agar medium, and TCBS (Oxiod, Thermo Fisher Scientific). Both inoculated plates and broth were incubated at 29°C overnight and 35 \pm 2°C for 3 days under aerobic conditions. Following the incubation cycle, a single colony from each suspected isolate was picked up and restreaked on a new plate of its perused original culture media before being reincubated under similar conditions. A loopful of each pure culture was streaked onto slanted trypticase soy agar supplemented with 2% NaCl to serve as a stock for biochemical identification. Moreover, TSA slant and BHI broth with 15% glycerol were used to preserve the isolates for further inspection (Mahmoud et al., 2021).

BACTERIA IDENTIFICATION

The pure isolates were identified using colonial morphology, Gram staining for microscopical analysis, and descriptions of the form and arrangement of bacteria (Beveridge, 2001). The isolates were then biochemically classified using indole, Kovac's reagent, Simmon's citrate agar, MR, VP tests, triple sugar iron (Lab M Ltd., Lancashire, UK), and H₂S development for identification to genus or species level (Al-Harbi and Uddin, 2005). In parallel, the commercial API 20E strips were also used.

STATISTICAL ANALYSIS

The relationships between physico-chemical water quality variables, as well as, the microbiological loads, were evaluated using bivariate Pearson correlation coefficients (r). The correlation between bacterial isolation rates and different sites was tested using the chi-square test for

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independence (χ^2) and Fisher's exact test (FET). Statistics were considered significant when the *p* value was <0.05. The PASW Statistics (SPSS, Chicago, IL, USA), version 18.0, was used for analysis.

RESULTS

PHYSICOCHEMICAL WATER EXAMINATION

The physicochemical water analysis of the collected samples from three sites (Table 1) showed that the pH value ranged from 7.36 to 7.87. Electrical conductivity is a measurement of the water's ability to transmit electric current as well as a method for determining water purity. The Alexandria Mediterranean Coast and Lake Qarun all had electrical conductivity in the range of 38.2–39.6 ds/m, while Mariotteya stream water only had 2.11 ds/m. The results of the current study illustrated that the mean concentrations of major anions ($\rm CO_3^{-2}$, $\rm HCO^-$, $\rm Cl^-$, and $\rm SO_4^{-2}$) and major cations ($\rm Na^+, K^+, \rm Ca^{2+}$, and $\rm Mg^{2+}$) were highest in the Mediterranean Coast Alexandria, Lake Qarun, and Mariotteya stream.

Table 1: Physicochemical parameters of different surface water samples collected in winter 2019, Egypt.

Parameters	Units			Sites	Guideline FAO, 1994	
		Mariotteya stream (Giza)	Lake Qarun (Fayoum)	Mediterranean coast (Alexandria)		
pН		7.36	7.87	7.82	6.50–9.00 ^a	
EC	ds/m	2.11	39.6	38.2	-	
CO ₃	mg/L	0.00	1.20	0.00	-	
HCO ₃	mg/L	4.40	5.80	11.00	-	
Chloride	mg/L	15.00	400.00	832.00	120.00ª	
SO ₄ ²⁻	mg/L	20.48	263.31	132.09	960.00	
Ca ²⁺	mg/L	6.33	33.33	20.00	400.00	
Mg^{2+}	mg/L	6.00	90.33	153.00	60.00	
K⁺	mg/L	0.44	11.65	38.18	2.00	
Na ⁺	mg/L	27.11	534.99	763.91	919.00	
Phosphate	mg/L	2.19	2.54	2.74	2.00	

^aGuideline (CCME, 2007). *EC*: electric conductance, CO_3 : carbonate, HCO_3 : bicarbonate, *Cl*: chloride, SO_4 : sulfate, *Na*: sodium, *K*: potassium, *Mg*: magnesium, *Ca*: calcium.

Table 2: Correlations matrix of the analyzed chemical parameters in the examined water samples.

		pН	EC	CO ₃	HCO ₃	C1-	SO ₄ ²⁻	Ca ²⁺	Mg^{2+}	K⁺	Na⁺	PO ₄ ³⁻
pН	r	1.00	1.00	0.58	0.59	0.80	0.89	0.91	0.86	0.67	0.92	0.90
	P		0.036	0.610	0.594	0.411	0.306	0.272	0.336	0.537	0.253	0.291
EC	r		1.00	0.53	0.64	0.83	0.86	0.89	0.89	0.71	0.94	0.92
	P			0.646	0.559	0.375	0.342	0.308	0.300	0.501	0.217	0.255
CO ₃	r			1.00	-0.32	-0.03	0.89	0.86	0.08	-0.23	0.21	0.16
	P				0.796	0.979	0.304	0.338	0.946	0.853	0.863	0.901
HCO ₃	r				1.00	0.96	0.16	0.21	0.92	1.00	0.86	0.89
	P					0.183	0.901	0.866	0.258	0.058	0.341	0.304
Cl-	r					1.00	0.43	0.48	0.99	0.98	0.97	0.98
	P						0.717	0.683	0.075	0.125	0.158	0.121
SO4 ²⁻	r						1.00	1.00	0.53	0.24	0.64	0.59
·	P							0.034	0.642	0.843	0.559	0.597
Ca^{2+}	r							1.00	0.58	0.30	0.68	0.63
	P								0.608	0.809	0.525	0.563
Mg^{2+}	r								1.00	0.95	0.99	1.00
	P									0.201	0.083	0.045
K+	r									1.00	0.90	0.93
	P										0.284	0.246
Na ⁺	r										1.00	1.00
	P											0.038
PO 3-	r											1.00

EC: electric conductance, *Cl*: chloride, SO_{a} : sulfate, *Na*: sodium, *K*: potassium, *Mg*: magnesium, *Ca*: calcium. *r* Pearson correlation coefficient (*bold*); *p*, significance was set at *p* < 0.05.



Table 3: Heavy metals concentration in surface water samples collected in winter 2019, Egypt.

Heavy metals	Sites	Acceptable/		
	Mariotteya stream (Giza)	Lake Qarun (Fayoum)	Mediterranean Coast (Alexandria)	Permissible limit ^a
Zinc (Zn)	ND	ND	0.06	2–5 mg/L
Copper (Cu)	ND	0.03	ND	1 mg/L
Cadmium (Cd)	ND	0.04 ^b	0.04 ^b	0.01 mg/L
Lead (Pb)	ND	ND	0.11 ^b	0.05 mg/L

ND not detected; ^aAccording to Egyptian Chemical Standards (ECS, 1994); ^bExceeded the permissible limit; Cu, Cd, and Pb have comparable US Environmental Protection Agency (US-UPA, 2000) levels of 1, 0.01, and 0.005 mg/L, respectively.

Table 4: Bacterial load of surface water san	ples collected in winter 2019, Egypt.
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Bacterial isolates	Unit	Sites				
		Mariotteya stream (Giza)	Lake Qarun (Fayoum)	Mediterranean Coast (Alexandria)		
Total coliform	MPN/100 mL	0.00	12	0.00		
E. coli	\log_{10} CFU/100 mL	0.00	2.95	0.00		
Total colony count	$\log_{10} \text{CFU/mL}$	7.08	6.95	7.51		
Vibrio spp.	$\log_{10} \text{CFU/mL}$	0.00	0.00	2.48		
Aeromonas spp.	$\log_{10} \text{CFU/mL}$	2.70	0.00	0.00		
Pseudomonas spp.	$\log_{10} \text{CFU/mL}$	2.04	3.18	0.00		
Staphylococcus spp.	\log_{10} CFU/mL	2.00	1.95	1.00		

DISSOLVED HEAVY METALS

Table 3 shows that Zn, Cu, Cd, and Pb were detected in different concentrations in waters samples collected from Alexandria Mediterranean Coast and Lake Qarun. The highest mean concentration was recorded for cadmium that exceeded the permissible limit. Moreover, none of the examined heavy metals was detected in the Mariotteya stream.

MICROBIAL CHARACTERISTICS OF EXAMINED WATER

In the aspects of microbial water analysis (Table 4), the current study found the isolation of coliforms and *E. coli* only from Lake Qarun. Of all the water samples, the highest concentration of TBC was at the Mediterranean Coast (7.51 \log_{10} CFU/100 mL). Also, *Vibrio* spp. was isolated only from the same place (2.48 \log_{10} CFU/mL). While *Aeromonas* species were isolated only from the Mariotteya stream (2.70 \log_{10} CFU/mL), *Pseudomonas* spp. was found in the Mariotteya water sample and Lake Qarun (2.04 and 3.18 \log_{10} CFU/mL, respectively). *Staphylococcus* spp. was the highest in the Mariotteya stream (2.00 \log_{10} CFU/mL).

BACTERIOLOGICAL IDENTIFICATION OF FISH PATHOGENS

The occurrence of natural infection rates among moribund and freshly dead marine fishes and crustaceans is shown in Table 6. *Aeromonas* spp. was the most prevalent bacteria with a total prevalence of 36.2 %. *Vibrio* spp. was isolated with a prevalence of 31.4% followed by *Pseudomonas* spp. (16.2%) and *Staphylococcus* spp. (6.7%) with the lowest prevalence.

DISCUSSION

The aquatic environment is subjected to a variety of chemicals that have negative health effects on habituated fish and pose a threat to their survival (Mackenzie et al., 1995). Domestic wastewater pollution, especially sewage pollution, is the most common in surface water recently. This pollution degrades water quality and has been identified as a precursor to fish contamination with pathogens that have public health hazards via infecting both consumers and handlers (Omer et al., 2004).

Physicochemical water examination revealed that pH, conductivity, total suspended and dissolved solids, total alkalinity, sulfate, and phosphate were all found to affect the aquatic environment and were all increased as a result of anthropogenic factors (El-Nemaki et al., 2008; El-Amier et al., 2015).

The results of the current study (Table 1) showed that the pH value ranged from 7.36 to 7.87, and pond water with a pH of 6–9 was suitable for increased fish production (Bhatnagar and Devi, 2013). In addition, Al-Afify et al. (2019) found pH values within allowable limits (range, 7.29–8.84) in their study, with the highest value in

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autumn and the lowest value in winter, which was close to the findings of the current study. The pH value varies depending on the number of sewage emissions and the algae photosynthesis. Furthermore, lower pH values are linked to lower oxygen concentrations as a result of high levels of organic contaminants and brackish water drainage (Abdel-Halim and Aly-Eldeen, 2016). The same conclusion was reached by Ibrahim and Ramzy (2013), who found that the water lakes must be slightly alkaline, with an average pH of 8.10 ± 0.32 .

The EC distribution among sample sites revealed spatial variation, with some sites having lower EC than others. A low EC value in the Mariotteya stream was observed (2.11 ds/m), which may be due to dissolved salts adsorption on the surface of suspended particles caused by flooding. Moreover, EC and salinity reflect the dilution rate of seawater by land water discharges, revealing the degree of pollution in the aquatic environment (Zyadah et al., 2004). According to Al-Afify et al. (2019), the EC of Lake Qarun water ranged from 11.20 to 53.60 mS/cm, with a mean value of 43.60 mS/cm. The lowest EC value was found at site 1 next to the El-Batts drain. However, lower values were found during the winter, which may be due to the direct effect of drainage water dilution, especially in areas near the drains. This corresponds to Authman and Abbas (2007).

The presence of cations (e.g., sodium, calcium, and magnesium), as well as anions (e.g., chloride, phosphate, and nitrate), is one of the causes of salinity (Rani et al., 2012). It comprises the total suspended solids, and water turbidity is affected by total suspended solids (Mahananda et al., 2010).

The Mariotteya values obtained in this study are relatively low, which is good for maximum fish productivity. The Mediterranean Shore, Alexandria, had the highest concentration of Cl- (832.00 mg/L). These findings corroborate those of Ali and Fishar (2005) and Authman and Abbas (2007). While the lowest value of 15.00 mg/L was found in El Mariotteya, a similar result was found in the Nile River in Lake Nasser (Rashed and Younis, 2012). Mariotteya SO₄²⁻ value was 20.48 m g/L, while marine water ranged from 132.09 to 263.31 mg/L. Overall, the distribution of major anions (Cl⁻ and SO₄²⁻) in lake water can be primarily regulated by the evaporation rate (Abdel-Satar et al., 2010). The most common anion pattern was Cl⁻, SO₄²⁻, HCO₃₋, and CO₃⁻². These findings are consistent with those stated by Abdel-Satar et al. (2010) and Abou El-Gheit et al., (2012). Major cations (Na⁺, K+, Ca²⁺, and Mg²⁺) concentrations in marine water ranged from 534.99 to 763.91, 11.65 to 38.18, 20.00 to 33.33, and 90.33 to 153.00 mg/L, respectively, while Mariotteya

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was 27.11, 0.44, 6.33, and 6.00 mg/L, respectively. The dominant cation pattern was Na⁺ > Mg²⁺ > Ca²⁺. The high amount of agricultural runoff and domestic sewage inflow from adjacent cultivated land and neighboring villages to the drains could be explained by the overall 'concentration levels of phosphates (Sayed and Abdel-Satar, 2009). As phosphorus builds up in a lake, it can indefinitely cycle through the water column, promoting algal blooms (Edwards and Withers, 2008). The concentrations of PO₄⁻³ were found to be between 2.19 and 2.74 mg/L. This may be because most fish farmers feed their fish with artificial feeds.

The correlation coefficient (r) between different chemical parameters in the studied water samples is shown in Table 2, which shows a strong correlation between them.

Heavy metals are common trace elements in marine ecosystems, but their levels have risen because of anthropogenic effects (i.e., industrial, agricultural, and mining activities) (Kalay and Canli, 2000). The most common heavy metals that have a direct effect on marine animals and their natural habitats are mercury, cadmium, and lead (Ramamoorthy and Ballantyne, 1984; Ullrich et al., 2001).

The results of the current study (Table 3) indicated the heavy metal concentration in the studied sample. According to US-UPA and ESC standards, water samples from Lake Qarun were contaminated with Cd beyond the acceptable level, whereas water samples from Alexandria province were polluted with Cd and Pb. In addition, all 'sites' Zn and Cu levels were within acceptable limits. The findings of the current study supported the findings of other studies (Sawere and Oghenekowhoyan, 2019). However, lead was found to be the most abundant heavy metal in the water sample (range, 0.18-0.23 mg/L). Metal pollution poses environmental issues, such as possible effects on the food chain, long-term persistence, and biomagnification in the food chain (Karadede-Akin and Ünlü, 2007; Papagiannis et al., 2004), which may be dangerous to humans. Cadmium and lead are two of the most dangerous toxins in the food chain. Cadmium causes Itai-Itai disease, which is a debilitating lung disease. Lead adversely affects the blood, various tissues, and the nervous system (Malhat, 2011).

In aspects of microbial water analysis as shown in Table 4, biotyping of certain bacterial isolates revealed the existence of bacteria that pose a significant risk to human health, including *E. coli, Pseudomonas fluorescens*, and *Staphylococcus aureus*. Many studies have discussed the health risks of bacterial isolates as dangerous microorganisms that affect humans and cause a variety of diseases (Kenzaka et al., 2013). the findings of the current study showed the



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isolation of coliforms and E. coli at Lake Qarun, which can be used as bioindicators of fecal contamination (Rajasekaran, 2008). Lake Qarun is the largest reservoir of agricultural and sewage drainage in Fayoum Province, as well as the drainage from fish farms built around the lake, (Mansour and Sidky, 2003). E. coli is the best biological water predictor for public health safety (Edberg et al., 2000). Microbial water analysis at Mariotteya drain showed the isolation of Pseudomonas, Aeromonas, and S. aureus. These results follow Rokibul et al. (2013) who isolate Staphylococcus (30 × 10³ CFU/mL) and Aeromonas $(50 \times 10^3 \text{ CFU/mL})$ by count in the summer, and the lowest value throughout the winter $(0.25 \times 10^3 \text{ CFU/mL})$. No coliform count (0.0 CFU/mL) was noted in the winter. Furthermore, at Alexandria, 'Egypt's Mediterranean Sea coast has a climate close to that of 'Europe's Eastern and Southern coasts. A study in Turkey found Vibrio in 66.0% of the samples, which was nearly identical to other studies (Colakoglu et al., 2006). The total prevalence of bacterial infections in naturally infected marine fishes (40.83%) may appear to be lower than those recorded by some authors for freshwater fishes, such as Soliman (1999) who found

that TBC prevalence was 65.0%. This variation may be due to the negative impact of seawater salinity on bacterial pathogen viability. The isolated bacteria were reported during the sampling period (November 2019-January 2020) in the current sample, and this finding could be attributed to the 'areas' continued chemical and sewage contamination. More studies are needed, however, to show that this form of pollution has a seasonal impact across the year. Organic water contamination, accompanied by a decrease in dissolved oxygen content, provides a favorable environment for bacterial development. A well-established connection was noted between organic contamination of surface water and disease outbreaks. Thus, this disease may act as a warning sign of water contamination or poor water quality. The results of the current study illustrated the correlation between water chemical parameters and bacterial load (Table 5).

The negative shift in water quality parameters occasionally influences and enhances pathogenic bacterial replication and increases the susceptibility of fish to bacterial infection (El-Bouhy et al., 2016; Abdel-Moneam et al., 2021).

		Total coli- form	E. coli	Total colony count	<i>Vibrio</i> spp.	Aeromonas spp.	Pseudomonas spp.	<i>Staphylococcus</i> spp.
pН	r	0.58	0.58	0.21	0.42	-1.00	-0.07	-0.46
	Р	0.610	0.610	0.866	0.723	0.057	0.954	0.695
EC	r	0.53	0.53	0.26	0.47	-1.00	-0.13	-0.51
	Р	0.646	0.646	0.830	0.688	0.021	0.918	0.659
CO ₃	r	1.00	1.00	-0.68	-0.50	-0.50	0.77	0.46
	Р	0.000	0.000	0.524	0.667	0.667	0.436	0.695
HCO ₃	r	-0.32	-0.32	0.91	0.98	-0.66	-0.84	-0.99
	Р	0.796	0.796	0.271	0.129	0.538	0.359	0.10
Cl-	r	-0.03	-0.03	0.76	0.88	-0.85	-0.66	-0.90
	Р	0.979	0.979	0.455	0.312	0.354	0.542	0.284
SO4 ²⁻	r	0.89	0.89	-0.27	-0.05	-0.84	0.40	0.00
	Р	0.304	0.304	0.828	0.970	0.363	0.740	0.999
Ca^{2+}	r	0.86	0.86	-0.21	0.01	-0.87	0.35	-0.05
	Р	0.338	0.338	0.862	0.995	0.329	0.774	0.967
Mg^{2+}	r	0.08	0.08	0.67	0.82	-0.91	-0.57	-0.85
	Р	0.946	0.946	0.530	0.387	0.279	0.618	0.359
K+	r	-0.23	-0.23	0.87	0.96	-0.73	-0.79	-0.97
	Р	0.853	0.853	0.329	0.187	0.480	0.417	0.159
Na ⁺	r	0.21	0.21	0.57	0.74	-0.95	-0.45	-0.77
	Р	0.863	0.863	0.613	0.470	0.196	0.701	0.442
PO ₄ ³⁻	r	0.16	0.16	0.62	0.78	-0.93	-0.51	-0.80
	Р	0.901	0.901	0.575	0.433	0.234	0.663	0.405

Table 5: Correlation matrix of the examined water samples (between chemical parameter and microbiological load).

EC: electric conductance, *Cl*: chloride, SO_{a} : sulfate, *Na*: sodium, *K*: potassium, *Mg*: magnesium, *Ca*: calcium; *r* Pearson correlation coefficient; *p*, significance was set at p < 0.05.



Table 6: Rate of bacterial isolation in relation to the number of collected fish Obtained from different localities from Egypt winter, 2019.

	Mariotteya stream (Giza)	Lake Qarun (Fayoum) (N = 85)			Mediterrane- an coast (Alexandria)	Total (N = 105)	P value (FET)	
	Nile Tilapia (n = 5) (%)	Nile Tilapia (n = 30) (%)	Tilapia zillii (n = 5) (%)	Artemia shrimp (n = 50) (%)	Overall (N = 85) (%)	Large shrimp $(n = 15)$ (%)		
Vibrio spp.	0/5 (0.0)	5/30 (16.6)	2/5 (40.0)	20/50 (40.0)	27/85 (31.8)	6/15 (40.0)	33/105 (31.4)	0.285
Aeromonas spp.	4/5 (80.0)	20/30 (66.6)	2/5 (40.0)	10/50 (20.0)	32/85 (37.6)	2/15 (10.0)	38/105 (36.2)	0.020*
Pseudomonas spp.	1/5 (20.0)	5/30 (16.6)	1/5 (20.0)	10/50 (20.0)	16/85 (18.8)	0/15 (0.0)	17/105 (16.2)	0.142
Staphylococcus spp.	0/5 (0.0)	5/30 (16.6)	1/5 (20.0)	0/50 (0.0)	6/85 (7.1)	1/15 (6.6)	7/105 (6.7)	1.000
$\chi^{2}(3)$	FET	27.23	FET	25.00	26.24	FET	33.45	
P value	0.020*	< 0.0001*	1.000	< 0.0001*	< 0.0001*	0.020*	< 0.0001*	

 χ^2 : Chi-square test, *FET*: Fisher's exact test. **P* < 0.05, significant.

Concerning the results of bacterial isolates retrieved from fish samples (Table 6), the Gram-negative bacteria were more isolated than Gram-positive bacteria. Fish and shrimp samples collected from Lake Qarun and the Mediterranean Coast (Alexandria) were contaminated with all investigated microorganisms, while fish collected from the Mariotteya stream was only contaminated with Pseudomonas spp. and Aeromonas spp. The findings of the current study showed that shrimp and Nile Tilapia were the most infected Aquatic species, followed by T. zillii. This may be related to suboptimal water conditions that lead to physiological stress on reared fish and shrimps, which compromised the ability of shrimp to resist disease that consequently promotes infection (Millard et al., 2020). Moreover, the shrimp habitat as a benthic aquatic crustacean makes them more susceptible to concentrated levels of various water pollutants.

Concerning the percentages of the prevalence of the isolated bacterial groups, the results of the current study revealed that *Aeromonas* were the most prevalent species of all bacterial groups (Aboyadak et al., 2015; Abdel-Moneam et al., 2021) accounting for 80% and 37.6% in Mariotteya stream and Lake Qarun, respectively. This may be related to the ubiquitous nature of *Aeromonas* spp. in the fish gut flora and aquatic environments. Moreover, the high prevalence of *Aeromonas* spp. during winter was similar to that reported by Moustafa et al. (2010) and El-Barbary and Hal (2016) who confirm the isolation of *Aeromonas* bacteria in the late winter.

Vibriosis is one of the most common bacterial diseases affecting various marine fish species and crustaceans (Sarjito et al., 2009). In the current study, *Vibrio* species were isolated from the Mediterranean coasts water (40%) and Lake Qarun (31.8%), which is nearly similar to that reported by Abdelaziz et al. (2017) and Elgohary et al. (2020) in different marine localities. Vibrio bacterial infection may be related to the high salinity of the Mediterranean coasts water and Lake Qarun that acts as a closed saline basin that receives agricultural, sewage, and fish farms drainage wastewater of Fayoum Province (Authman and Abbas, 2007; Abdelaziz et al., 2017). The high salinity levels accompanied by decrease in dissolved oxygen content act as a predisposing stress factor that may suppress fish immunity and lead to concurrent bacterial infection.

The prevalence of infection with *Pseudomonas* spp. was 20% and 18.8% in the Mariotteya stream and Lake Qarun, respectively. Nearly similar to Hussain (2002), El-Barbary and Hal (2016) and Elgohary et al. (2020) reported an increase in infection percentage with *Pseudomonas* species in the cold months of winter, which may be attributed to the affinity of *Pseudomonas* for propagation and wide-spreading infection at low temperatures (El-Moghazy, 2004; Golomazou et al., 2006).

Staphylococcus spp. recorded the lowest infection percentage among the examined fishes in Lake Qarun and Alexandria with a total prevalence of 6.7%. Similarly, Zorrilla et al. (2003) and Moustafa et al. (2010) reported a low percentage of isolation of Gram-positive bacteria in different fish species in winter. Opposing Varvarigos (2001) who declared that the most common *Staphylococcus* spp. causing septicemia were isolated at high seawater temperature in late spring and early summer. The variation in bacterial prevalence percentage is mainly contributed to the difference in examined fish species, water quality parameters, variable environmental conditions and different localities.

CONCLUSIONS AND RECOMMENDATIONS

Monitoring the water quality in a fish facility's aquariums

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is critical for maintaining a safe fish supply. Moreover, preserving water quality is also important because contamination with pathogenic microorganisms can cause serious illness or even death in cultured water organisms.

NOVELTY STATEMENT

The current study assessed the relationship between physicochemical qualities and the occurrence of bacteria causing fish diseases in three different types of surface water bodies in Egypt: Sea water, Lake water, and water stream.

AUTHOR'S CONTRIBUTION

H.S.K.: analysis of water samples chemically and bacteriologically, D.A.A: identification and isolation of fish bacterial diseases, E.I.: statistical analysis for data, H.S.K, E.I. and D.A.A. writing and final editing, M.M.F and M.S.G. sample collecting and participate in the practical work, H.S.K and E.I. work design, M.M.Z. supervision, all authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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