

## Bacterial Load of Tilapia Fish Residing El-Ramla and Abu Simbel Khors, Lake Nasser, Egypt

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### ABSTRACT

Fishes are affected by the bacterial load and species present in the surrounding area. Therefore, bacterial load on/in fishes can be used as an indicator of aquatic pollution. Bacterial load was estimated in fish tissues of different tilapia species (*Oreochromis niloticus*, *Sarotherodon galilaeus* and *Tilapia zilli*) collected from the entrance and middle El-Ramla and Abu Simbel Khors. Results indicated that fishes of Abu Simbel Khor recorded higher bacterial loads than those of El-Ramla Khor, also fishes of entrance khors recorded higher bacterial loads than the middle. *Salmonella* sp. and *Escherichia coli* were detected in fish samples collected from Abu Simbel Khor more than fishes collected from El Ramla Khor. *Vibrio cholerae* was detected in fish samples collected from El Ramla Khor more than those collected from Abu Simbel Khor. The results reflect the exposure of Lake Nasser Khors to various pollutions, especially in its southern part. Here, it is necessary to follow up fishing and tourism boats, also coastal farms around the Khors, to reduce or prevent the causes of pollution of Lake Nasser and its Khors.

### INTRODUCTION

Lake Nasser is located at the southern border of Aswan governorate, Egypt. It is considered one of the most important sources of fish wealth for the Egyptians, especially tilapia (bolti fishes), as the most favorite fishes for Egyptians in general and Aswan people in particular (Habib *et al.*, 2014).

In Lake Nasser, there are six species of fishes (*Oreochromis niloticus* (Linnaeus, 1758), *Sarotherodon galilaeus* (Linnaeus, 1758), *Coptodon zillii* (Gervais, 1848), *Lates niloticus* (Linnaeus, 1758), *Alestes dentex* (Linnaeus, 1758), and *Hydrocynus forskalii* (Cuvier, 1819)) that have economic value (El-Haweet *et al.*, 2008). In addition, the southern part of Lake Nasser is more productive for fishes (Halls *et al.*, 2015). Also, khors (the side entrances or extensions of Lake Nasser) are essential fishing areas because they are shallow and contain abundant phytoplankton and limited water currents (Van Zwieten *et al.*, 2011; Habib *et al.*, 2014).

Some researchers and authorities are concerned about assessing fish stock in Lake Nasser (Halls *et al.*, 2015 and GAFRD, 2015). Moreover, other researchers have studied the relationships between water quality and fish abundance (Adams and Ryon, 1994 and Zaki *et al.*, 2014). Where all fish species are sensitive to aquatic pollutants, and there is a negative relationship between fish health and pollutants, for example, increased concentrations of heavy metals in the ecosystem causes damage to the fish organs (Authman *et al.*, 2008). This is because fishes cannot escape from the dangerous effects of water pollutants (Whitfield and Elliott 2002).

Microorganisms can be used as an indicator of environmental pollution because they affect and influence the surrounding environment (Hans *et al.*, 2003 and Ali *et al.*, 2015). Fish is affected by the bacterial load and the bacterial species that are present in the ecosystem, where Ali *et al.* (2015) showed that fishes collected from polluted sites (drainage) recorded higher bacterial loads (in different fish organs) than fishes collected from non-polluted sites. This is because microorganisms are located at the beginning of the aquatic food chain, while fishes are at the end.

In this study, several species of tilapia (*O. niloticus*, *S. galilaeus* and *T. zilli*) were collected from the entrance and middle of two Khors of Lake Nasser (El-Ramla Khor and Abu Simbel Khor, located in the northwest and southeast of Lake Nasser, respectively). The bacterial load in fish tissues was estimated as an indicator of aquatic pollution, and consequently evaluation of Lake Nasser.

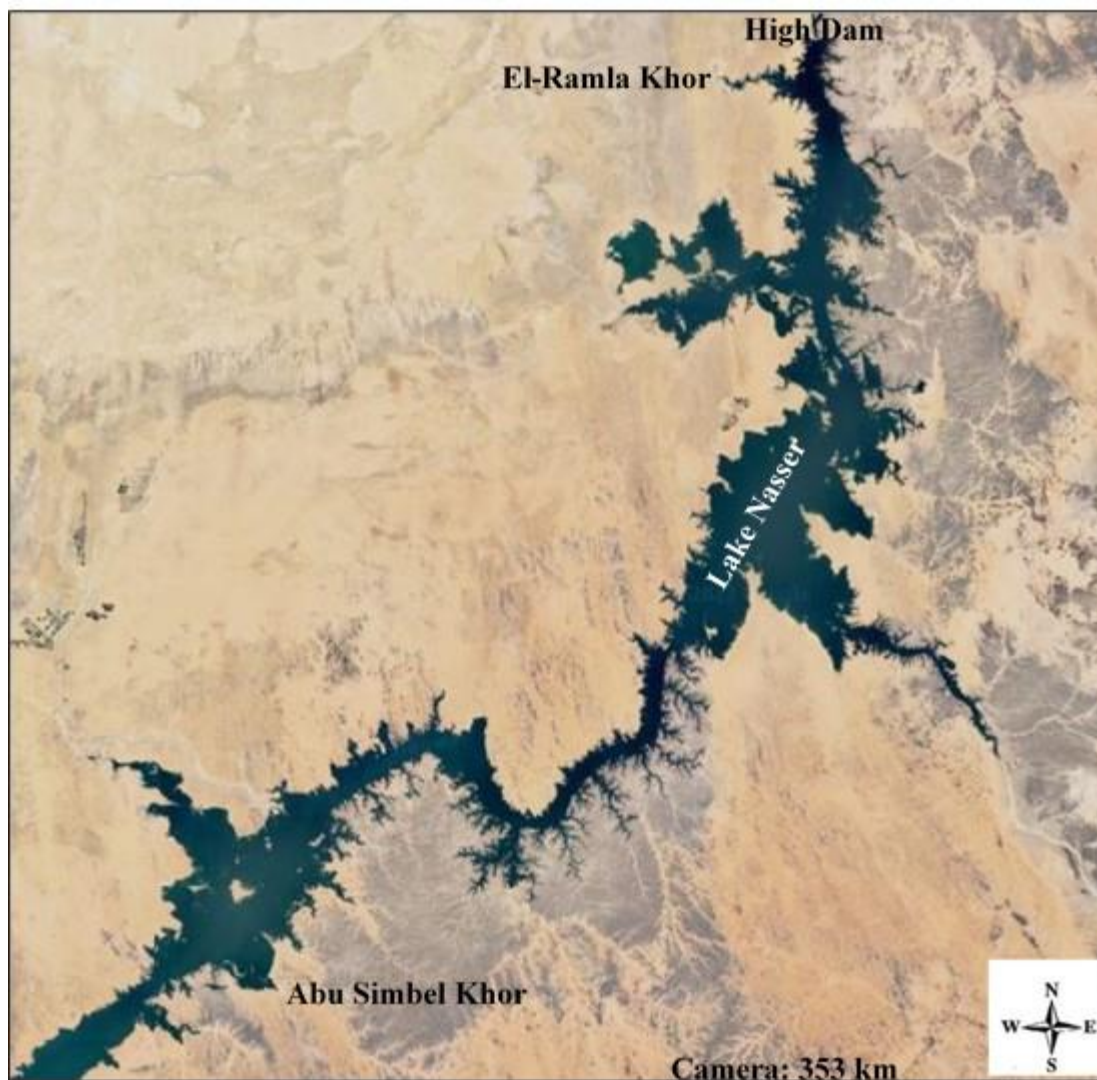
## MATERIALS AND METHODS

### 1. The study area

Fish samples were collected from the entrance and middle of two khors of Lake Nasser, one is located in the northwest of Lake Nasser (El-Ramla Khor) and the other in the southeast of Lake Nasser (Abu Simbel Khor) during March 2019 (Figure 1).

### 2. Fish samples

*Oreochromis niloticus*, Linnaeus, 1758 ( $19.4 \pm 0.5$  cm and  $249.1 \pm 7.3$  g), *Sarotherodon galilaeus*, Linnaeus, 1758 ( $19.5 \pm 1.8$  cm and  $227.3 \pm 21.3$  g) and *Tilapia zilli*, Gervais 1848 ( $19.3 \pm 1.9$  cm and  $148.2 \pm 43.1$  g) were collected from entrance and middle of El-Ramla khor. As well as *O. niloticus* ( $24.9 \pm 2.8$  cm and  $319.7 \pm 100.2$  g) and *S. galilaeus* ( $22.7 \pm 1.2$  cm and  $264.0 \pm 31.9$  g) were collected from entrance and middle of Abu Simbel khor. Fishes were collected alive and transported to laboratory in an ice-box. After dissecting the fish, 10 g of each organ (skin, gills, gut and dorsal muscles) were transferred aseptically to 90 ml of 0.85% sterile saline, homogenised and left to stand for 15 minutes at room temperature, then tenfold serial dilutions were performed up to  $10^7$ .



**Fig. 1.** Satellite image locating El-Ramla Khor and Abu Simbel Khor in Lake Nasser. (El-Ramla Khor and Abu Simbel Khor are located northwest and southeast of Lake Nasser respectively).

([https://earth.google.com/web/search/high+dam,+aswan/@23.06213554,32.32294319,341.02856194a,352570.07629238d,35y,359.63506326h,0t,0r/data=CigiJgokCdUPKV1V4ChAEVNmM\\_yDbyfAGRy5827-vVBAIfifpyrDPCZA](https://earth.google.com/web/search/high+dam,+aswan/@23.06213554,32.32294319,341.02856194a,352570.07629238d,35y,359.63506326h,0t,0r/data=CigiJgokCdUPKV1V4ChAEVNmM_yDbyfAGRy5827-vVBAIfifpyrDPCZA))

### 3. Bacteriological analyses

Various groups of microorganisms were enumerated in skin, gills, gut and muscles for several species of tilapia fish collected from the experimental sites.

**3.1. Total bacterial count:** Nutrient agar and pour plate technique was used for enumeration of total bacterial counts after incubation at 37 °C for 24 hours (APHA, 1999)

**3.2. Total Enterobacteriaceae group count:** using eosin methylene blue agar media (Atlas, 1946), 0.2 ml suspension was spread over eosin methylene blue agar media and incubated at 37 °C for 24 hours (APHA, 1999).

### 3.3. Detection of some pathogenic bacteria

Some pathogenic bacteria were detected according to APHA (1999).

- **Detection of Salmonella sp.** using spread plate technique and S-S agar (*Salmonella* and *Shigella* agar) medium at 35 °C incubation temperature for 24 hrs. Black colonies refers to a positive result.
- **Detection of Escherichia coli** using spread plate technique and eosin methylene blue agar medium at 44.5 °C incubation temperature for 24 hrs. Green metallic sheen refers to a positive result.
- **Detection of Vibrio sp.** using spread plate technique and TCBS (Thiosulfate Citrate Bile salts Sucrose) agar medium at 37 °C incubation temperature for 48 hrs. Yellow colonies refer to a positive result for *Vibrio cholera* and green colonies refers to a positive result for *Vibrio parahaemolyticus*.

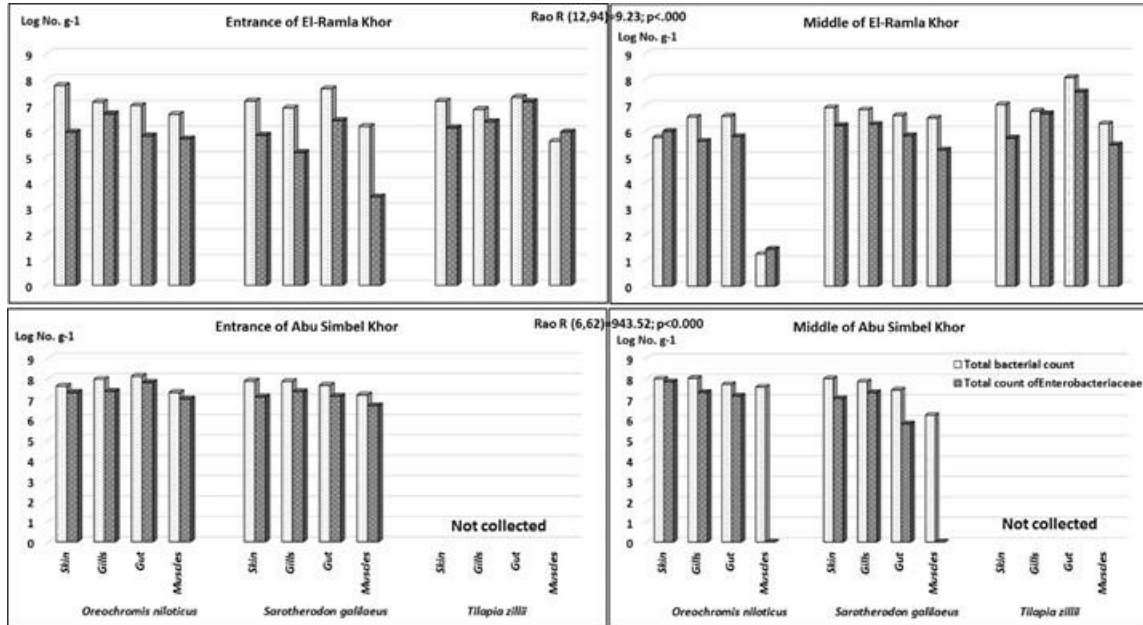
## 4. Statistical analysis

Data obtained were statistically analysed using STATISTICA 10 (StatSoft, Inc., Tulsa, USA). Analysis of variance (ANOVA) was used to examine the independent effects.

## RESULTS

Results of microbial loads (total count of bacteria and total count of Enterobacteriaceae spp.) for fish samples collected from the entrance and middle of El-Ramla and Abu Simbel khors are shown in Figure (2). Total bacterial count and total Enterobacteriaceae group varied depending upon sampling site (El-Ramla and Abu Simbel Khors) or (entrance and middle of each Khor) and species of fishes (*O. niloticus*, *S. galilaeus* and *T. zilli*). Total bacteria and total Enterobacteriaceae spp. counts ranged from 1.2 to 8.1 Log No. g<sup>-1</sup> and 1.4 to 7.5 Log No. g<sup>-1</sup> respectively for fishes of El-Ramla Khor and from 6.2 to 8.1 Log No. g<sup>-1</sup> and 0.0 to 7.8 Log No. g<sup>-1</sup> respectively for fishes of Abu Simbel Khor.

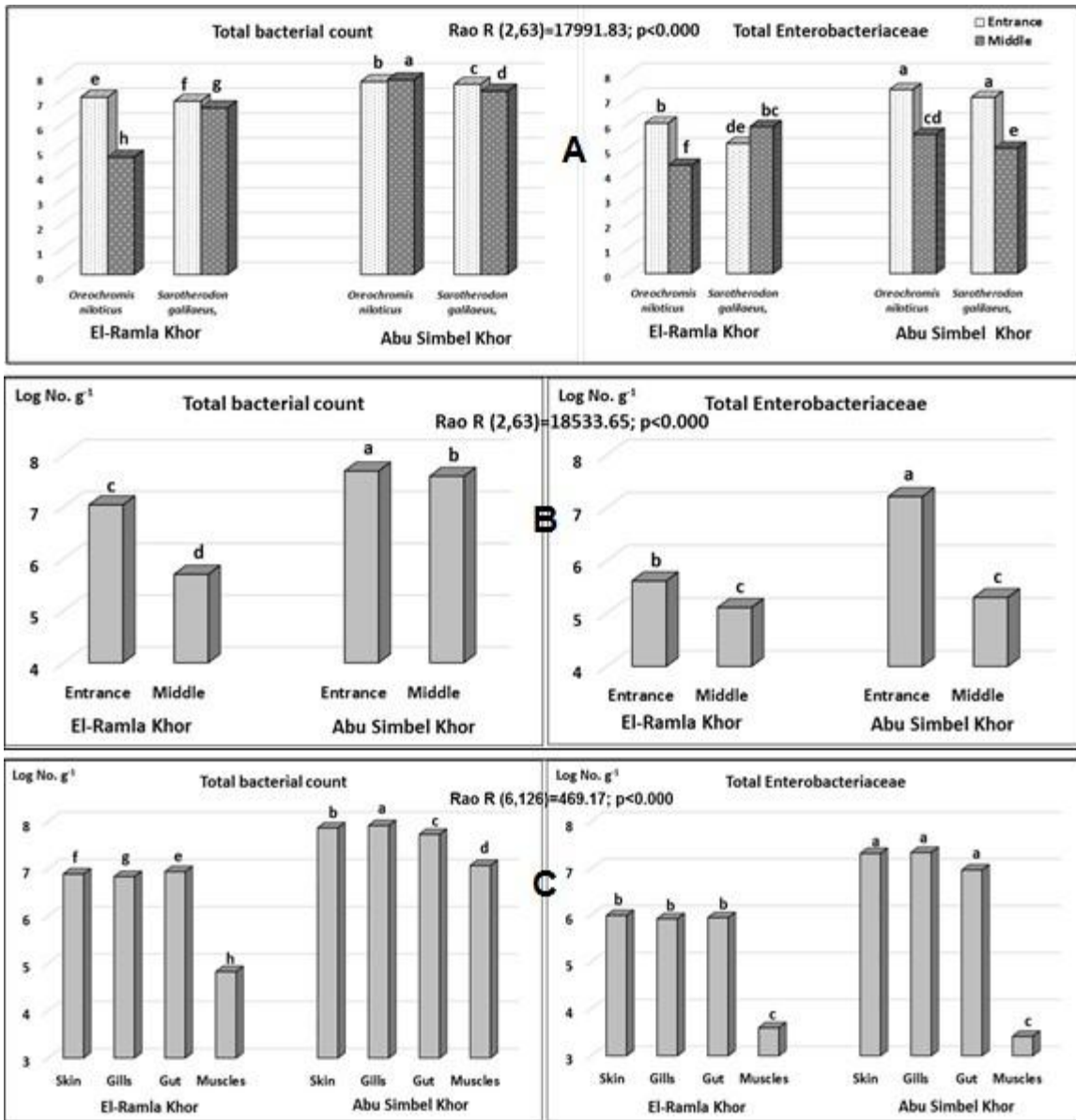
In general, combined statistical analysis for the microbial load of *O. niloticus* and *S. galilaeus* collected from entrance and middle of El-Ramla and Abu Simbel Khors are illustrated in Figure (3). The sensitivity of fishes to pollutants varied and depended on fish species where *O. niloticus* recorded higher microbial load than *S. galilaeus*, except fishes collected from the middle of El-Ramla Khor where *S. galilaeus* recorded higher microbial load than *O. niloticus* (Figure 3A).



**Fig. 2.** Microbial loads of the different organs of *Oreochromis niloticus*, *Sarotherodon galilaeus* and *Tilapia zilli* collected from entrance and middle of El-Ramla khor, as well as *Oreochromis niloticus* and *Sarotherodon galilaeus* collected from entrance and middle of Abu Simbel khor.

Also, combined statistical analysis shows that fishes of Abu Simbel Khor recorded a higher bacterial load than fishes of El-Ramla Khor, as shown in Figure (3A, B, C). Where fishes collected from the entrance and middle of Abu Simbel Khor recorded 7.7 and 7.6 Log No.  $g^{-1}$  respectively, compared to 7.1 and 5.7 Log No.  $g^{-1}$  for fishes collected from entrance and middle of El-Ramla Khor, respectively (Figure 3B). Also, fishes collected from the entrance for each Khors recorded higher counts of bacteria and total Enterobacteriaceae spp. than fishes collected from the middle (Figure 3B).

In addition, combined statistical analysis shows that the highest microbial load for various fish organs was recorded at Abu Simbel Khor and followed the order: gills>skin>gut>muscles for total bacterial count, and gills $\geq$ skin $\geq$ gut>muscles for total count of Enterobacteriaceae spp. (Figure 3C).



**Fig. 3.** Combined statistical analysis for microbial loads of *Oreochromis niloticus* and *Sarotherodon galilaeus* collected from entrance and middle of El-Ramla and Abu Simbel khors.

(A): Combined statistical analysis of microbial load for different tilapia fish species with different khors, irrespective of fish organs.

(B): Combined statistical analysis of microbial load for tilapia fishes collected from entrance and middle of El-Ramla and Abu Simbel khors, irrespective of fish species and fish organs.

(C): Combined statistical analysis for microbial load of different fish organs of tilapia fishes, irrespective of fish species and entrance or middle khors.

The different small letters above the column indicate significant differences ( $P < 0.05$ ).

Pathogenic bacteria were detected in the organs of the experimental fishes, and the obtained results are presented in Table (1). In general, pathogenic bacteria were detected in greater levels in fishes collected from Khor Abu Simbel than in fishes collected from Khor Al Ramla, where *Salmonella* sp. was not detected in Khor Al Ramla fish samples, while it was present in all skin samples of Khor Abu Simbel fish. Also, *Escherichia coli* was present in all organs of fish samples collected from the entrance and middle of Abu Simbel Khor as 100 and 87% respectively. While *Escherichia coli* was present in 16 and 41% of the fish organ samples collected from the entrance and middle of El Ramla Khor respectively. In addition, *Vibrio cholera* was detected in fish organs collected from El Ramla Khor more than those collected from Abu Simbel Khor. Where *V. cholera* was present at 50 and 42% of fish organs samples collected from the entrance and middle of El Ramla Khor respectively, while the percentage was 37% of fish organ samples collected from the entrance or middle of Abu Simbel Khor. While *Vibrio parahaemolyticus* was present in higher percentage for fish samples of the middle of El Ramla Khor (83%) and the entrance or middle of Abu Simbel Khor (62%), compared to 58% in the fish samples collected from the entrance of El Ramla Khor.

## DISCUSSION

Fish is a cold-blooded aquatic vertebrate, and it is essential to a healthy human diet because fish flesh contains high-quality protein, omega-3 fatty acids, and other essential nutrients (Rhea, 2009). Bacteria are found in/on fishes and are beneficial to fish. Bacteria can degrade complex molecules in the digestive tract (MacDonald *et al.*, 1986) and produce vitamins (Sugita *et al.*, 1991).

Fishes are sensitive to any environmental change. Therefore, many researchers use fishes as a biological indicator for toxicological metal pollution in the aquatic systems (Rashed 2001 and Abumourad *et al.*, 2014). Also, researchers studied interactions between fish and bacteria (Olafsen, 2001, Austin 2002 and Rhea, 2009). Because fishes are found in water, they are affected by the bacterial load and bacteria species present in water and sediments (Olafsen, 2001). Some researchers reported higher bacterial loads on/in fishes present in polluted waters (Zmyslowska *et al.*, 2001 and Ali *et al.*, 2015). It is difficult to differentiate between the indigenous microflora and transient microflora on/in fish, which could be present in the water film around fish (Austin, 2002), moreover extraneous bacteria are capable of surviving in fish (Del Rio Rodriguez *et al.*, 1997).

Results of the present study recorded higher bacterial load in/on fishes collected from Abu Simbel and El Ramla Khors. Also, gills and gut show that the highest bacterial load than skin and muscles. This is because microorganisms may enter the digestive tract through the mouth, and natural inhibitory compounds or microflora present on the surface

**Table 1.** Detection of some pathogenic bacteria in different organs of *Oreochromis niloticus*, *Sarotherodon galilaeus* and *Tilapia zilli* collected from entrance and middle of El-Ramla khor, as well as *Oreochromis niloticus* and *Sarotherodon galilaeus* collected from entrance and middle of Abu Simbel khor in March 2019.

Location	Fish	Organs	<i>Salmonella</i> sp.	<i>Escherichia coli</i>	<i>Vibrio cholera</i>	<i>Vibrio parahaemolytic</i>	
Entrance of El-Ramla khor	<i>O. niloticus</i>	Skin	-	-	+	-	
		Gills	-	-	-	+	
		Gut	-	-	-	+	
		Muscles	-	-	-	+	
	<i>S. galilaeus</i>	Skin	-	-	+	-	
		Gills	-	-	-	-	
		Gut	-	-	+	+	
		Muscles	-	-	+	-	
	<i>T. zilli</i>	Skin	-	+	-	+	
		Gills	-	-	+	+	
		Gut	-	-	-	+	
		Muscles	-	+	+	-	
Middle of El-Ramla khor	<i>O. niloticus</i>	Skin	-	+	+	-	
		Gills	-	+	-	+	
		Gut	-	+	-	+	
		Muscles	-	+	-	+	
	<i>S. galilaeus</i>	Skin	-	-	-	+	
		Gills	-	+	-	+	
		Gut	-	-	+	-	
		Muscles	-	-	-	+	
	<i>T. zilli</i>	Skin	-	-	-	+	
		Gills	-	-	+	+	
		Gut	-	-	+	+	
		Muscles	-	-	+	+	
Entrance of Abu Simbel khor	<i>O. niloticus</i>	Skin	+	+	+	+	
		Gills	-	+	-	+	
		Gut	-	+	-	+	
		Muscles	-	+	-	+	
	<i>S. galilaeus</i>	Skin	+	+	+	-	
		Gills	-	+	+	-	
		Gut	-	+	-	+	
		Muscles	-	+	-	-	
	<i>T. zilli</i>	Not collected					
		Not collected					
		Not collected					
		Not collected					
Middle of Abu Simbel khor	<i>O. niloticus</i>	Skin	+	+	-	+	
		Gills	-	+	-	+	
		Gut	-	+	-	+	
		Muscles	-	-	-	-	
	<i>S. galilaeus</i>	Skin	+	+	+	-	
		Gills	-	+	+	+	
		Gut	-	+	-	+	
		Muscles	+	+	+	-	
	<i>T. zilli</i>	Not collected					
		Not collected					
		Not collected					
		Not collected					

+ refer to presence, - refer to absence



of fish act as an inhibitor of microorganisms in contact with the fish surface (**Austin and Austin, 1987**). These results agree with **Naigaga et al. (2011)**, who reported that the distribution and abundance of fish species strongly influenced by water quality.

Fishes collected from the entrance of Abu Simbel Khor recorded a higher total Enterobacteriaceae spp. load than the middle, while the fishes of *S. galilaeus* collected from the middle of El-Ramla Khor recorded higher total counts of Enterobacteriaceae spp. than the entrance which means poor water quality in the entrance of Abu Simbel Khor and middle of El-Ramla Khor. These results agree with **Ali et al. (2017)**, who reported that the end of El-Ramla khor is more polluted than the beginning of El-Ramla khor. This is because the end of El-Ramla khor is narrow and far from the main channel of Lake Nasser, as well as increase the activity of fishing inside the khor. Also, increasing fishing activities in the southern part of Lake Nasser, and increased natural fish foods (**El-Shabrawy and Dumont, 2003**) where they found that the zooplankton in the upstream khors (Toushka and Korosko) was richer than the downstream khors (El-Ramla and Kalabsha).

According to the Egyptian Standard (**EOS, 2005**), which recommended that the total number of aerobic bacteria should not exceed  $10^6$  CFU g<sup>-1</sup> and the total coliform bacteria not exceed 100, the results obtained showed that total Enterobacteriaceae spp. number for all studied samples exceeded 100 CFU g<sup>-1</sup> except muscle samples of *O. niloticus* collected from the middle of Abu Simbel Khor. Although *Escherichia coli* should not be present on fresh-caught fish (**Chattopadhyay, 2000**), the results indicated pathogenic bacteria in fish organs. Furthermore, pathogenic bacteria were also detected in fish organs collected from Abu Simbel Khor more than fish organs collected from El Ramla Khor. This indicates that the southern part of Lake Nasser is more polluted than the northern part.

Pollution of the ecosystem affects the quality and quantity of fish, evident from Lake Nasser's fishing, which decreased from about 37,700 metric tons in 2009 to 18,400 metric tons in 2016 (**El-Far et al., 2020**). Also, increasing microorganisms around fishes causing accumulation of microorganisms on/in the fishes causes fish damage (**Ali et al., 2015**), reduce fish production (**Halls et al., 2015**), and rapid spoilage of fish (**Novotny et al., 2004**).

Unfortunately, accumulated bacterial load in fish tissues may be transmitted to humans, especially fishermen, through dealing with fish causing chronic or acute diseases (**Novotny et al., 2004 and Abumourad et al., 2014**).

## CONCLUSION

Bacterial load on/in fishes often reflects the quality of the surrounding water. Therefore, we can use fishes as an indicator of pollution. Where water contaminants not

only affect the quality of fish (increase fish spoilage and reduce shelf life) but also affect the health of fishermen more than consumers because fisherman deals directly with fishes, while the effects on consumers depend on consumption behavior (method of processing and cooking). This study shows that the possibility of using microbiological criteria to assess the quality of fishes and, consequently, the quality of water.

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