ISSN 1110-0354
Bull. Nat. Inst. Of Oceanogr. & Fish., A.R.E., 2000. Vol. (26): 241 - 255

BACTERIOLOGICAL POLLUTION IN LAKE EDKU

By

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Key Words: Bacteriology, pollution, Limnology, Lake Edku, Egypt.

ABSTRACT

The prevalence of fecal indicator bacteria including coliform bacteria. E. coli and fecal streptococci as well as some other pathogens were investigated in water samples collected from lake Edku and some other drainage water discharged into the lake. Examination of some Tilapia fishes. caught from the lake. was also included. In some sites. especially those located nearby the drainage outlets. high numbers of fecal indicator bacteria exceeded the guide standards the guide standards were detected. as well as vibrio and salmonella spp. Bacteriological exanimation of Tilapia fish revealed that non of the fecal indicator bacteria was found in muscles. a few numbers was detected on the skin. However. gills and alimentary canals harbored considerable numbers of these bacteria.

Results of this work indicated that continuous control of the presence of fecal pollution as well as water-borne pathogens should be taken into consideration. The wastewater should absolutely be treated before discharge into the lake.

INTRODUCTION

Water quality is of paramount importance to protect public health and no aspect of that quality is more important than the microbiology. Nowadays, the increase of pollution in natural waters has intensified the detection frequency and persistence of pathogenic microorganisms especially in areas affected by domestic sewage disposal, industrial effluent and runoff from agricultural fields and other human activities. It is also become obvious from early observations that the presence of a populated city created a potential hazard in nearby waters. This was especially true if the waters were used for human consumption or for the harvesting of fish or shellfish.

The microbiological monitoring of water is carried out by detecting and enumerating groups of bacteria, including total coliforms, *E. coli*, fecal steptococci, used as indictors of fecal contamination. It is also established that these indicators are associated with disease-causing genera of importance to public health (Godfree *et al.* 1997; Rhodes and Kator 1988). Therefore, the presence of these pathogenic microorganisms can lead to water-borne or water-borne related outbreaks (Martinez-Manzanares *et al.*, 1991, Morinigo *et al.* 1990). Also, it was found that various factors such as, temperature, sedimentation, sunlight, competition, predation, salinity, nutrient deficiencies, heavy metal, specific bacteriophages, algae and bacterial toxins affect the number of these bacteria although it is more likely that a combination of these factors were responsible (Yusef *et al.*, 1995; Davis and Evison, 1991).

The present study was done as an attempt to identify the prevalence of fecal pollution bacteria and some other possible pathogens in Lake Edku and some other drainage water discharged into the lake. The study also addresses the pollution of some *Tilapia* fish, caught from the lake. Consequently, the impact of drainage water discharged from drains, connected to the lake could be evaluated. The study is a part of the first stage of the research of the Fisheries Division, National Institute of Oceanography and Fisheries. This plan aims to management and development of fisheries in the Egyptian northern delta lakes. It is a matter of fact that the study of environmental conditions in these lakes contribute in understanding the different factors that affect the feeding, reproduction and survival of various fish species in these lakes.

MATERIALS AND METHODS

Study Area

Lake Edku

Lake Edku is a shallow brackish water situated at latitude 30° 15'N and longitude 31° 15'E east of Alexandria, Egypt. It is extending about 19 km south of Abu-Qir Bay from the east to west, with an average width of 6km and average depth of about one meter. It is directly connected with the Mediterranean Sea at the western through a narrow channel "Boughaz El-Maadya (Fig.1). The lake connection is located at the inner part of a partially sheltered bay known as Abu-Qir Bay. Lake Edku is rich in vegetation of hydrophytes and amphibious, and contains several numbers of chrysophyta, cyanophyta and chlorophyta (El-Sarraf, 1976; El-Shenawy, 1994). It receives huge amounts of drainage water (38-280 x 10^6 m^3/ day) via four main drains, Edku, Boseily, Khayry and Barzik, discharging into the eastern and southern part of the lake. This initiated an east-west flow of water in the lake, causes a slight elevation of its water above the sea level and decrease the entrance of the sea water to the lake. The fish production is greatly affected by the drainage water which carries different pollutants (Shriadah and Tayel, 1992).

Sampling

The bacteriological sampling techniques of the International Organization for Standardization, ISO 5667/1 (1980) and ISO 5667/2 (1990) were used for water samples collection. Special stainless steel sampling rod holding a sterile blue cap glass (500ml) was used. Samples were collected 25-30cm below the water surface from the stations shown in Fig. (1). More than one glass was collected for each station at the same moment when needed. Immediately, samples were transported in an ice box to the laboratory, where they kept in a refrigerator. Analysis of samples was performed within 6-8 hours of collection.

Bacteriological analysis

A: bacteriological analysis of water.

The bacteriological analysis were carried out according to ISO 9308/1 (1990) and 7899/2 (1984) using the membrane filtration technique. Quantities of (100, 10, 1 and 0.1 ml) of each sample were filtered through, 0.45 µm pore size, 47 mm diameter, grid sterile cellulose membrane (Gelman Laboratory) using, a sterile glass filtration unit (Milli Pore, Bedford, UK) and a vacuum pump

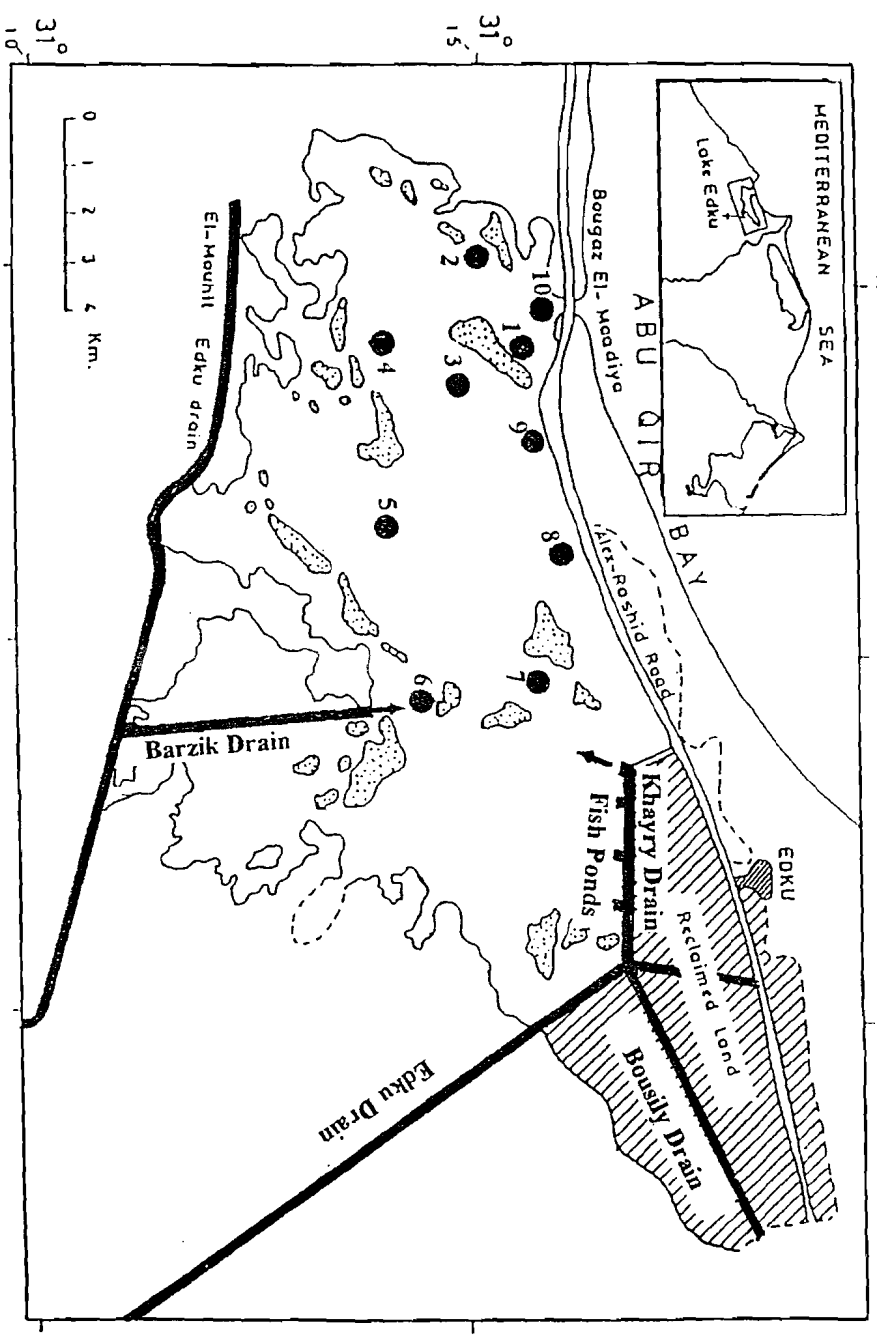

Figure 1. Lake Edku and position of sampling stations.

at a pressure of 65k Pa. The detection of total coliforms, the membranes were placed onto the surface of les-Endo agar (Difco Laboratories, Mich., U.S.A), incubated at 37°C for 24h. The dark red color with golden-green metallic sheen colonies grown on les-Endo agar was counted. Ten colonies were subcultured onto nutrient agar and the confirmatory tests including Gram-stain, oxidase test and gas production were done. The detection of thermotolerant coliforms (*E. coli*), the membranes were placed onto the surface of m-FC agar (Difco) and incubated at 44.5°C for 24h. The blue colonies grown on m-FC agar were counted and the confirmatory tests for 10 subcultured isolates including Gram-stain, gas production, indole production and oxidase test were performed. The detection of fecal streptococci, the membranes were placed onto the surface of m-Enterococcus agar (Difco) incubated at 37°C for 24h. Red, maroon or pink colonies were counted and confirmatory tests including Gram-stain, catalase test and esculin hydrolysis was performed for 10 colonies. Final counts of all the three fecal pollution indicators were calculated as cfu/100ml using the equation mentioned in ISO 9308/1 (1990) and 7899/2 (1984).

For detection of *Vibrio spp.* bacteria, the membranes were placed onto the surface of alkaline peptone agar for 6h at 25°C. The membranes were carefully transferred onto TCBS agar (Difco) and incubated at 30°C for 24h. The large green and yellow colonies were counted as *Vibrio* (El-Sersy *et al.* 1996). For detection of ***Salmonella*** and ***Shigella***, the membranes were placed onto the surface of peptone agar and incubated at 25°C for 6h. Then transferred onto S-S agar and, incubated at 37oC for 24-84h. Typical colorless, sometimes with dark center with enteric edge colonies was counted as ***Salmonella*** and ***Shigella*** spp.

B: Bacteriological examination of *Tilapia* fish.

Fresh *Tilapia* fish (100-130 gm) caught from Lake Edku were collected from El Maadya fish market in plastic bags. Fishes were transported to the laboratory in icebox. Upon arrival, organs of muscles, skin, gills and alimentary canal were separated and blended for 60s. Serial dilutions of homogenates were done using saline solution. The fecal indicator bacteria were counted by spread plating of 0.1 ml sample onto the above mentioned selective media. The bacterial counts were calculated as cfu/cm^2 for skin surface and cfu/g for muscles, gills and alimentary canal.

C: Physico-chemical parameters.

Parameters including pH, temperature, dissolved oxygen and salinity were determined by Abbas *et al*., (2001) at the same time of sampling.

RESULTS AND DISCUSSION

The microbiological quality of water is commonly determined by testing it for the presence of bacterial indicators including total coliforms, *E. coli* and fecal streptococci, which used as sanitary parameters especially if that water is used for cultivating and harvesting of fishes.

The present interpretation of the results of bacterial indicators detected during this work was made according to the European Commission (EC) guide standard (1998), which meet with the Egyptian guide standard (Ministry of Health, Egypt, 1996). Both accept the guide values of the investigated bacteria by 500 colony forming unit (cfu)/100ml of water for coliforms and 100 cfu/100ml for each of *E. coli* and fecal streptococci.

Results of the examined ten stations located in Lake Edku (Fig. 1) revealed that numbers of fecal indicator bacteria were found to exceed the Egyptian standards at stations 5,6,7 and 9 in February, 2000 (Table 1). In April 2000, only stations 6 and 7 exceeded those guide standards (Table 2), while in July 2000, the bacterial numbers exceeded the guide limits at stations 6, 7 and 10 (Table 3). Although ***Vibrio*** bacteria were not determined in winter (Feb. 2000), but in spring (April, 2000) it was detected in numbers ranged between 1 and 100 cfu/100ml (Table 2). These numbers highly increased in summer where it reached to several thousands especially at stations 6 and 7 (Table 3). Our results obtained in this work are in accordance of those of Bouchriti *et al*., 1995. They reported that during winter, this species is located in the sediments, but it is resuspended when temperature increases.

The detected numbers of fecal pollution indicator bacteria dramatically increased in Khayry and Barzik drains. Also, high counts of ***Vibrio*** as well as ***Samonella*** and ***Shigella*** *spp.* were detected (Table 4). This could be attributed to the discharge of raw domestic sewage of Edku city and many other small villages into these drains. The runoff from agricultural fields and other human activities located along these drains may be also a reason for such high bacterial counts found in these drains.

Table 1. Bacterial, chemical and physical parameters studied in Lake Edku in winter (February - 2000)

Station No.	Bacteria (cfu/100ml)							Salinity ‰	pH	Temp. °C	O_2 mg/l
	Total coliform		*E.coli*		Fecal streptococci		*Vibrio spp.*				
	Total	Confirmed	Total	Confirmed	Total	Confirmed					
1	90	40	12	6	4	2	* ND	5.9	8.74	17.4	11.7
2	110	75	80	40	20	6	* ND	2.3	8.90	15.4	8.7
3	12	11	10	10	60	45	* ND	2.3	9.36	16.6	12.1
4	160	100	100	35	50	50	* ND	2.4	9.17	15.7	13.4
5	290	230	200	180	100	85	* ND	3.3	8.11	16.1	10.7
6	1300	1000	1100	890	160	110	* ND	3.2	7.87	16.4	5.9
7	4200	2200	3000	2000	1100	970	* ND	1.6	8.28	17.3	4.7
8	100	95	90	60	80	80	* ND	2.2	8.97	16.8	10.7
9	160	130	140	130	20	12	* ND	2.7	8.90	16.4	8.6
10	100	100	100	90	40	20	* ND	2.4	8.56	16.5	7.7

* ND : Not determind.

Table 2. Bacterial, chemical and physical parameters studied in Lake Edku in spring (April - 2000)

Station No.	Bacteria (cfu/100ml)							Salinity ‰	pH	Temp. °C	O_2 .mg/l
	Total coliform		*E.coli*		Fecal streptococci		*Vibrio spp.*				
	Total	Confirmed	Total	Confirmed	Total	Confirmed					
1	80	20	10	8	25	20	15	2.6	8.64	23.9	8.9
2	60	40	50	36	10	10	1	1.8	8.28	23.5	4.7
3	2	2	1	1	25	15	10	1.8	9.91	25.2	11.3
4	30	20	15	15	20	20	60	1.9	8.87	24.1	12.9
5	120	90	100	70	60	55	40	1.9	8.68	24.6	9.1
6	1100	850	100	100	500	400	90	1.5	7.99	25.1	4.3
7	3000	1700	2100	1600	700	450	100	1.3	7.33	25.1	5.2
8	80	65	70	60	13	9	* ND	1.3	7.82	25.1	7.4
9	* ND	* ND	* ND	* ND	* ND	* ND	* ND	* ND	* ND	* ND	* ND
10	*ND	* ND	* ND	* ND	* ND	* ND	* ND	* ND	* ND	* ND	* ND

* ND : Not determined.

Table 3. Bacterial, chemical and physical parameters studied in Lake Edku in summer (July - 2000)

Station No.	Bacteria (cfu/100ml)							Salinity ‰	pH	Temp. °C	O_2 mg/l
	Total coliform		*E.coli*		Fecal streptococci		*Vibrio spp.*				
	Total	Confirmed	Total	Confirmed	Total	Confirmed					
1	17	12	8	6	100	80	100	1.9	8.60	26.0	8.3
2	40	35	25	18	10	10	50	1.7	8.38	26.0	6.0
3	12	8	8	8	7	7	60	1.8	8.84	26.5	12.3
4	90	90	80	70	30	25	120	1.7	9.20	26.5	15.6
5	90	85	80	80	70	65	420	2.5	8.06	26.5	14.5
6	1500	1100	1100	1000	720	700	4400	2.6	7.30	26.5	12.3
7	1600	1450	1400	1100	700	700	5000	1.5	7.92	26.5	6.4
8	80	80	70	60	10	6	40	1.5	8.25	26.5	7.2
9	40	20	30	30	10	10	40	1.7	8.14	26.5	6.4
10	240	190	200	160	240	210	50	4.3	8.22	26.5	5.9

Table 4. Bacterial, chemical and physical parameters studied in Khayry and Berzik drains (May - 2000)

Drains	Bacteria (cfu/100ml)								Salinity ‰	pH	Temp. °C
	Total coliform		*E.coli*		Fecal streptococci		*Vibrio spp.*	*Salmonella & shigella spp.*			
	Total	Confirmed	Total	Confirmed	Total	Confirmed					
Khayry	10000	8000	9000	6500	1000	800	2000	1000	1.3	7.49	25.7
Bersiqe	30000	22000	23000	10000	6000	2500	5000	2000	2.3	8.09	25.6

Table 5. Counts of confirmed fecal indicators bacteria detected in *Tilapias* fish. (counts are cfu/g of alimentary canal,gills or muscles and cfu/cm^2 of skin)

Date of collection	Total coliform				*E. coli*				Fecal streptococci			
	Alimentary canal	Gills	skin	muscles	Alimentary canal	Gills	skin	muscles	Alimentary canal	Gills	skin	muscles
23/01/2001	116	60	3.5	0	32	17	0.9	0	70	30	12	0
25/03/2001	80	100	1.5	0	50	65	8.3	0	150	15	0.5	0
27/03/2001	100	130	20	0	30	35	10	0	31	50	6	0

* All counts are the mean of three replicates.

These huge amounts of drainage water inter the lake initiate an east-west flow through the lake. The nearby of stations 6 and 7 to drainage discharge could be considered the reason for the presence of these high counts of the investigated bacteria in these stations. However, the dilution factors (sedimentation, UV light and combination of biological, physical and chemical factors) may lead to a reduction of bacterial numbers in the other investigated stations (Davis and Evison, 1991; Vasconcelos and Swartz, 1976; Flint, 1987).

It is well known that the microbiological quality of fishes is directly related to the quality of the water in which they are cultivated and/or harvested. Results of microbiological examination of *Tilapia* fishes (Table 5) caught from Edku lake (purchased from El-Maadya market at the same day of collecting water samples) revealed that non of the fecal indictor bacteria were detected in fish muscles. Similar results were found by Zuaretz, ***et al.***, (1993) and El-Shenawy and El-Samra (1994). On the skin, a few numbers ranged from 1.5 to 50, 0.9 to 10 and 0.5 to 12 cfu/ cm^2 were detected for total coliforms. ***E.coli*** and fecal streptococci, respectively. These numbers increased to 60-130, 17-65 and 15-[illegible] cfu/g in gills and alimentary canal for the above-mentioned bacteria, *respectively*.

In Egypt, there are no federal microbiological standards for fish. Because the indicator organisms present on fish caught from polluted waters are not generally considered part of the normal flora of the fish or their environment (Matches & Abetyla, 1983), thus these detected fecal bacteria could be considered as a localized contamination from water where the fish were cultivated and/or harvested.

CONCLUSION

The presence of fecal indicator bacteria in the water could be correlated with the presence of other some pathogens. However, some investigators have shown that no correlation was found between the presence of [illegible] of fecal coliforms and other some pathogens (Kaper ***et al.***, 1979 and Colwell ***et al***, 1981). Regardless of these opinions .and depending upon results obtained from this preliminary study, it appears that we should continue to run the fecal indicator organisms for lake Edku as evidence of a health hazard, however check for the other water/food-borne pathogens should not be neglected.

A serious cause of concern is the incidence of enteric pathogen ***Salmonella*** *spp.*. in El-Khayry and Barzik drains. As inhabitants consume the seafood collected from this environment, there exist an inherent risk of epidemic outbreak. Also, the risk to public health from direct contact of fishermen with this polluted water should put into consideration. To stop this serious contamination, the wastewater should be treated before discharge into the drains. Also, proper handling and marketing of fish as well as cleaning and well heat treating before consumption may assist to prevent such disease outbreaks.

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