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Microbial Quality of Nile Water and Drinking Water in Some Areas of Greater Cairo, Egypt

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Abstract: The aim of this study is to elucidate the microbial quality of Nile water, treated water and tap water in Greater Cairo. Four drinking water treatment plants (Giza, Embaba, Helwan and Shubbra El-Khema) were sampled for one year from June 2009 to May 2010 to evaluate the removal of bacterial indicators and some pathogenic microbes. The classical bacterial indicators (total bacterial counts at 37ºC and 22ºC, total coliforms, fecal coliforms and fecal streptococci), *Pseudomonas aeruginosa,* salmonellae groups, total staphylococci, *Candida albicans*, coliphage and free-living amoebae were determined. The counts of total bacteria in tap water samples at 37ºC and 22ºC were relatively accepted according to Egyptian Standard for Drinking Water. In addition, total coliforms were absent in all drinking water samples, while they were detected in all samples of Nile water with the highest average log count being 4.8 MPN-index/100 ml in Giza areas. Both enzymatic methods and MPN technique gave the same result concerning coliform group. The results showed that the average log numbers of fecal coliforms and fecal streptococci in intakes of the examined drinking water treatment plants ranged from 1.1 to 3.1 and 1.1 to 2.5 MPN-index/100 ml, respectively. Log average numbers of *Candida albicans*, total staphylococci, coliphage and free-living amoebae in Nile water ranged from 1.9 to 2.5, 0.6 to 2.7, 1.8 to 5.6 and 2.2 to 2.8 cfu/100 ml, respectively. The average log counts of salmonellae group and *Pseudomonas aeruginosa* in Nile water samples reached 2.5 and 2.3 cfu/100, respectively. All samples from the outlet of drinking water treatment plants were free from all tested microorganisms, except in Helwan and Giza drinking water treatment plants where coliphages were detected. Free-living amoebae were detected in some tap water samples from Helwan and Shubbra El-Khema areas. These findings indicate that further treatment of drinking water before consumption in order to avoid potential health hazards.

Key words: Nile water, drinking water, classical bacterial indicators, Enzymatic methods, *Pseudomonas aeruginosa,* total staphylococci, salmonellae groups, *Candida albicans*, coliphage and free-living amoebae.

INTRODUCTION

Water is essential to sustain life, and a satisfactory supply must be made available to consumers. Polluted water is an important vehicle for the spread of diseases. It has been estimated that 50 000 people die daily in the world as a result of water related disease (Schalekamp, 1990).

The sources of drinking water (both tap water and bottled water) include rivers, lakes, streams, ponds, reservoirs, springs, and groundwater wells. Contaminants that may be present in source water include: microbial contaminants, inorganic contaminants, pesticides and herbicides, organic chemical contaminants and radioactive contaminants (Annual Drinking Water Quality Report, 2005).

Therefore, a more logical approach is the detection of organisms normally present in the feces of humans and other warm-blooded animals as indicators of fecal pollution as well as water treatment and disinfection efficacy. The indicators should respond to natural environmental condition in water treatment processes. The indicator should be easy to isolate, identify and enumerate from the aquatic environment (WHO, 2004 and APHA, 2005). In general, the greatest microbial risks are associated with ingestion of water contaminated with human and animal excreta (Atiribom, *et al*., 2007). Most of the mortality and morbidity associated with water-related disease especially in developing countries are due directly or indirectly to infectious agents which infect man, (Obasohan, *et al*., 2010).

The presence of total Coliform and *E.coli* (fecal Coliform) in water samples could be detected by using direct measurement the activity of β-D- Galactosidase and β-D-Glucuronidase enzymes as rapid assay without selective cultivation (Noble and Weisberg, 2005; Wutor *et al*.,2007a and Fiksdal and Tryland, 2008). These enzymes hydrolyze a wide range of chromogenic and fluorogenic substrates yielding products which can be

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monitored. In this study, the chromogenic substrates Chlorophenol red β-D-galactopyranoside (CPRG), and ρ-nitophenyl β-d- glucuronide (PNPG) were employed to detect β-D- Galactosidase and β-D-Glucuronidase respectively (Wutor *et al*., 2007a,b).

The water sources must be protected from contamination by human and animal wastes which contain a variety of bacterial, viral, protozoan pathogens and helminth parasites. Failure to provide adequate protection and effective treatment will expose the community to the risk of outbreaks of intestinal and other infectious diseases. Easy diffusible assays are in demand for risk assessment of water supply systems (Fiksdal and Tryland, 2008), for example by monitoring of raw water quality, assessment of treatment efficiency, monitoring of finished water quality as well as recreational waters.

Considered primarily an aesthetic issue, discoloration is the largest cause of customer dissatisfaction associated with distribution system water quality (Piriou, 1997). The walls of the pipes in the distribution system provide ideal surfaces for microbial colonization and the biofilms formed causing a number of problems for the water companies (Charmain, *et al.,* 2003). Moreover, a wide distribution of free-living amoebae including potential pathogenic species occurred in tap water sources of western Japan (Edagawa *et al*., 2009). In addition, despite effective treatment of drinking water, microbes can enter water utility distribution systems (DS) and biofilm formation may account for the persistence of microbes in the distribution systems (Marciano-Cabral, 2010).

So, the aim of the present investigation was to evaluate the microbiological water quality of Nile water, treated water and tap water in Greater Cairo, Egypt.

MATERIALS AND METHODS

Sampling:

Water samples collection was carried out in accordance to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Two hundred and eighty-eight of water samples (Nile water [96 samples], treated water [96 samples], and tap water [96 samples]) were collected during June 2009 to May 2010 from different region (Helwan, Giza, Embaba and Shubbra El-Khema) in Greater Cairo, Egypt.

- Nile water samples (2 liters volume each) were collected in sterile brown glass bottles from the middle of the Nile River at the inlet of four drinking water treatment plants namely Helwan, Giza, Embaba and Shubbra El-Khema.
- Treated water samples (2 liters volume each) were collected in sterile glass bottles containing sodium thiosulphate crystal 18 mg/L from the outlet the same drinking water treatments plants previously mentioned.
- Tap water samples (2 liters volume each) were collected in sterile glass bottles containing sodium thiosulphate crystal 18 mg/L from some buildings fed with the distribution systems of the previously mentioned drinking water treatments plants.

Microbiological Examination:

Detection of Classical Bacterial Indicators:

Total bacterial counts (at 22ºC and 37ºC), total coliform, fecal coliform and fecal streptococci were counted using poured plate and MPN (Most Probable Number) methods according to APHA (2005).

In addition, the water samples were examined for total and fecal coliforms by rapid enzyme assay according to Wutor *et al*, 2007a&b.

β-D- Galactosidase (β-GAL) assay was used for the rapid detection of total coliform according to (Wutor *et al* (2007a&b). Chlorophenol red β-D-galactopyranoside (CPRG) 80 µg in 20 µl water was added to 90µl 0.1M sodium phosphate buffer pH (7.8) and 90µl of environmental water sample. The mixture was incubated in shaking water bath at 35ºC for one hour. Any red-magenta color appeared in the test tubes were taken as a positive result for total coliform.

β-D-Glucuronidase (β-GUD) assay was used for the rapid detection of fecal coliform according to Wutor *et al* (2007a). Assay buffer 50µl and 90µl water sample were mixed and the reaction was initiated by the addition of 110µl ρ-nitophenyl β-D- glucuronide (PNPG). The assay mixture was incubated in shaking water bath at 44ºC for 30 min. Any yellow color appeared in the test tubes were taken as a positive result for fecal coliform (*E.coli*). The enzyme substrates were obtained from Merck (Darmstadt- Germany) and the buffers were prepared according to Gomori (1955).

Detection and Enumeration of Some Microbes:

Most Probable Number (MPN) values for *Pseudomonas aeruginosa*, salmonellae group, *Staphylococcus aureus*, *E. coli, Candida albicans* and coliphage were determined according to APHA, 2005. All detected microorganisms (except coliphage) were confirmed by the membrane filter technique and specific chromogenic media (Himedia, India).

Detection and Enumeration of Free Living Amoebae:

Free-living amoebae were detected in Nile water and drinking water according to the method of Ali and Al-Herrawy (2001). Briefly, water samples (one liter volume each) were filtrated through membrane filters (1.2 µm pore size and 142 mm diameter). The membrane was inverted face to face on the surface of non-nutrient (NN) agar plates previously seeded with 0.1 ml living *E. coli*. The inoculated plates were incubated at 37ºC for 7 days with daily examination for the presence of any amoebic growth (Al-Herrawy, 1992).

Statistical Analysis:

The statistical analyses (the mean) were carried out according to procedure of Snedcor and Chochran (1973). The mean data values were using (MSTAT) computer software package version 2.1.

RESULTS AND DISCUSSION

The Nile is the main source of water in Egypt especially for the governorates allocated on the river shore and its branches. The microbiological criteria of water are considered one of the most important water quality issues consequently leading health risk problems.

The results of total bacterial counts at 37ºC and 22 ºC were expressed as an average log numbers in Table (1). Little variations in the log number of total bacterial count (cfu/100 ml) at 37ºC and 22ºC were noticed in all Nile water samples at different sites.

The highest average log count of Nile water at 37ºC reached 6.4 cfu/100 ml in El-Giza district, followed by Helwan, Shubbra El-Khema and lastly Embaba being 5.8, 5.63 and 6.4 cfu/100 ml, respectively. On the other hand, the highest average log count of Nile water at 22ºC reached 6.2 cfu/100 ml in both Helwan and El-Giza regions, while Shubbra El-Khema and Embaba regions recorded lower count, being 5.42 and 2.9 cfu / 100 ml, respectively. Other workers, in Egypt (El-Taweel & Shaban , 2003) reported that the log total bacterial count of Nile water ranged from 4.1, to 7.4 cfu/100 ml at 22ºC, while it reached from 4.1 to 7.3 cfu/100 ml at 37 ºC. on the other hand, Ali, *et al.,* (2008) reported nearly similar results to our results as they found that the log total bacterial counts at 22ºC and 37 ºC in Nile at El-Giza region reached 5.8 and 5.5 cfu/100 ml, respectively. Also, Rifaat (2008) found that the log total bacterial count of Nile water at Greater Cairo was 5 cfu /100 ml.

Concerning bacterial indicators of pollution, the highest average log numbers in Nile water were found in El-Giza region being 4.8, 3.1 and 2.5 MPN-index/100ml for total coliform, fecal coliform and fecal streptococci, respectively. In contrast, the lowest average log numbers of bacterial indicators were recorded in Embaba region being 2.2, 1.1 and 1.1 MPN-index/100ml for total coliform, fecal coliform and fecal streptococci, respectively (Table 1).

These results were in accordance with those obtained by Ali *et al*., (2008) they found that the log counts of total coliform, fecal coliform and fecal streptococci were 4.1, 2.3 and 2.5 MPN-index/100 ml, respectively, in Nile water samples at El-Giza Drinking Water Treatment Plant. Other works found that the log count of total coliform in Nile water reached 4 - 6 MPN-index/100 ml. These relatively high counts might be due to pollution by 34 industrial facilities discharging to the Nile water between Aswan and Cairo (Saleh, 2009). In another conducted by Shash *et al*., (2010) total and fecal coliforms were detected in Nile water at Greater Cairo in 100% of the tested samples reaching 10^4 and 10^3 cfu/100 ml respectively. Moreover, Niemi and Niemi (1991) reported that domestic and industrial wastewater, agriculture waste environment are sources of fecal bacterial to rivers.

The results of rapid enzyme assay techniques for monitoring total coliform and *E. coli* (fecal coliform) were shown in Table (2). The results indicated that, total coliforms were observed in all Nile water samples (red color in test tubes) due to the hydrolysis of CPRG. While the samples from outlet of Water Treatment Plants and distribution systems did not have total coliform (no change in the color). The same results were observed when PNPG was used as substrate for β-D- glucuronidase. The positive results (yellow color in test tubes) indicated the presence of *E.coli* (fecal coliform) in Nile water samples, while in outlet of treatment plants and distribution system samples no fecal coliforms were observed. These results were compatible with the results obtained using traditional methods.

The applications of rapid enzyme assay techniques for monitoring of microbial water quality were accelerated in the last decade (George *et al.,* 2000, Farnleitner *et al.,* 2002, Garcia-Armisen *et al.,* 2005 and Wutor *et al.,* 2009). The results for river samples were agreement with those obtained by Wutor *et al*., 2009 they used CPRG as chromogenic substrate for total coliform β-D-galactosidase enzyme activity. While Wutor *et al*., 2007a found that β-D-glucuronidase activity was easy and rapid way to estimate the abundance of viable *E.coli* in fresh waters, and this method was more sensitive than membrane filter technique. On the other hand, the absence of β-D-galactosidase and β-D-glucuronidase in water outlets of Drinking Water Treatment Plants and distribution systems might be due to the presence of chloride ions and ferrous at higher concentrations (Wutor *et al.*, 2007b).

Generally, the direct enzyme assay is considered an excellent early warning sign for the potential presence of fecal material in the water.

The presence of different bacterial genera in the water of the Nile at Cairo is due to direct contamination caused by human activities and indirect effect caused by ecological disturbances (Rifaat, 2008).

With respect to some pathogenic microorganisms of Nile water samples, the highest average log numbers of salmonellae groups, *Pseudomonas aeruginosa*, total staphylococci and *Candida albicans* were detected in El-Giza region being 3.1, 3.1, 2.7 and 2.5 cfu /100 ml, respectively and followed in Helwan site samples being 2.6, 2.9, 2.3 and 2.2 cfu / 100 ml, respectively. While the lowest average log number was detected in Embaba region samples being 1.9, 1.1, 0.6 and 1.9 cfu/100 ml, for salmonellae groups, total staphylococci, *Pseudomonas aeruginosa* and *Candida albicans*, respectively. Potentially pathogenic free-living amoebae were presented in 100% of the tested samples with log number being 2.6, 2.8, 2.2 and 2.3 pfu/100ml for Helwan, El-Giza, Embaba and shubbra El-Khema region, respectively. Also, coliphages were present in all Nile water samples of Greater Cairo. The log counts of coliphages were being 4.6, 5.6, 2.2 and 1.8 pfu/100 ml in Helwan, El-Giza, Embaba and shubbra El-Khema regions, respectively.

These results are in line with those obtained by El-Taweel and Shaban (2001) whom counted others indicators; total yeasts, *Candida albicans,* salmonella group and total staphylococci ranged from 10 to 10^5, 10^2 to 10^5, 10^2 to 10^3 and 10^2 to 10^3 cfu/100 ml, respectively. Also, in our result was in agreement with those obtained by Ceballos., *et al.,* (2003) who found that somatic coliphages (1×10^3 - 6.9×10^5 PFU/100 mL) and F-specific bacteriophages (65 - 5.8×10^4 PFU/100 mL) as well as *Salmonella sp.* and *Listeria sp.* were isolated from all sampling points of an urban river in north-east Brazil. In addition, Obi, *et al.,* (2003) isolated some pathogenic bacteria (*Salmonella, Shigella, Vibrio cholerae, Campylobacter, Aeromonas* and *Plesiomonas*) as well as coliphage (0-13 pfu/100 ml) from eight rivers in South Africa. In Egypt, Hikal, (2005) detected free-living amoebae in river Nile water by 100% of the total tested samples. In another study in Osaka (Japan), free-living amoebae were detected in 86.6 % out of 67 water samples collected from 13 different rivers (Edagawa, *et al.* 2009). Also, in Egypt corresponding results obtained by Saleh, (2009) demonstrated that the mean log counts of *Staphylococcus sp. and Salmonella sp*. were 3.0 and 4.0 cfu/100ml respectively, while coliphage was 3.0 pfu/100 ml in Nile water samples from different sites at Greater Cairo, Egypt.

From the aforementioned results, it could be noticed that there is no remarkable change in the microbiological profile of the Nile water. This may be attributed to dense pollution human activities and industrial centers as a major pollution sources in the Nile.

Concerning the outlet water samples of Drinking Water Treatment Plants, the average of total viable bacterial counts at 37 ºC were 7, 29, 13 and 33 cfu/ml at Helawn, El-Giza, Embaba and Shubbra El-Khema, respectively. Higher average total viable bacterial counts were obtained from tap water samples of the same districts being 18, 39, 15 and 38 cfu/ml for Helawn, El-Giza, Embaba and Shubbra El-Khema regions, respectively (Table 4). Similarly, in the present study, the average total viable bacterial counts of tap water samples at 22 ºC were 16, 32, 12 and 28 cfu/ml at Helawn, El-Giza, Embaba and Shubbra El-Khema regions, respectively. Lower average total viable bacterial counts at 22 ºC were obtained from outlet water samples the examined Drinking Water Treatment Plants being 4, 18, 11, and 22 cfu/ml in Helawn, El-Giza, Embaba and Shubbra El-Khema, respectively.

Moreover, all samples from outlet Water Treatment Plants and tap water were free from total coliform, fecal streptococci, total staphylococci and salmonellae groups. These results indicate the safety of tap water according to the Egyptian Standard, (2007) for drinking water which declared that potable water must be free from total and fecal coliforms as well as fecal streptococci, while total bacterial counts must be less than 50 cfu/ml.

The average counts of additional microbial indicators like *Pseudomonas aeruginosa* were detected in outlet of Drinking Water Treatment Plants, recording 4.1 and 2.2 cfu/100ml at El-Giza and Shubbra El-Khema Drinking Water Treatment Plants, respectively, while they were absent in Helawn and Embaba Drinking Water Treatment Plants. On other hand, the average number of *Candida albicans* at Embaba and Shubbra El-Khema Drinking Water Treatment Plants were 0.1 and 2.3 cfu/100ml, respectively. Also, in our results, *Candida albicans* was in other sites. Data given in Table (4) from tap water samples show that, the average counts of coliphage were 0.7 and 1.2 pfu/100ml at Helawn and El-Giza sites, respectively, while, they were absent in other sites. Also, free-living amoebae, were absent from outlet of Drinking Water Treatment Plants, while they were detected in some tap water samples. Data recorded in Table (4) from tap water samples show that the same average counts (0.8 pfu /mL) of free-living amoebae at Helawn and Shubbra El-Khema sites, while not detected in others sites.

This phenomenon is may be due to the bio-film which presents in distributed systems tubes.

The results were agreement with those obtained by Behrends, (2003) in German, testing the piping of a new hospital showed that the drinking water was contaminated with *Pseudomonas aeruginosa*. Also, these results are in line with those obtained by Edagawa, *et al*., (2009) found that free -living amoebae were isolated from 64.4% out of 307 tap water samples in Japan. In addition, Marciano-Cabral, *et al*., (2010) reported that despite effective

treatment of drinking water in USA, microbes can enter water utility distribution systems and hence the plumbing within building premises. Additionally, biofilm formation may add account for the persistence of microbes in the distributing system.

In conclusion, the present work highlights on the rapid enzymatic detection of microbial water contamination method as well as the microbial re-growth in the distribution system. So continuous monitoring of Nile water must be done to ensure the production of safe drinking water.

Table 1: The log average counts of classical bacterial indicators in Nile water samples during 2009 and 2010 at different sites of Greater Cairo.

Site	Log number of cell forming unit (cfu) / 100 ml				
	Total viable bacterial count at:-		Bacterial indicators (MPN-index)		
	37 °C	22 °C	TC	FC	FS
Helwan	5.8	6.2	3.34	2.9	2.2
El-Giza	6.4	6.2	4.8	3.1	2.5
Embaba	4.2	2.9	2.2	1.1	1.1
Sh. El Khema	5.63	5.42	3.9	2.8	2.1
Average	5.5	5.2	3.6	2.5	2.0

TC: Total coliform ; FC: Fecal coliform ; FS: Fecal streptococcl ; Sh: Shubbra ; MPN: Most Probable Number ; cfu: colony forming unit.
These log numbers are averages for 24 samples.

Table 2: Total and fecal coliforms in Nile water by using enzymatic methods.

Enzyme assay	Sites											
	Helwan			El-Giza			Embaba			Shubbra		
	NL	OL	DS	NL	OL	DS	NL	OL	DS	NL	OL	DS
Enzyme(1)	+	-	-	+	-	-	+	-	-	+	-	-
Enzyme(2)	+	-	-	+	-	-	+	-	-	+	-	-

Enzyme (1): β-D-galactosidase for indicate the presence of total coliform.
Enzyme (2): β-D-glucuronidase for indicate the presence of fecal coliform.
NL: Nile water samples
OL: Out let from water treatment plant
DS: Distributed systems (tap water samples)
+: Present -: Absent

Table 3: The log average count of some pathogenic microorganisms in Nile water during 2009 and 2010 in Grater Cairo.

Site	Log number of cell forming unit (cfu) / 100 ml				Total count (pfu / 100ml)	
	Salmonellae Group	*Pseud. sp.*	Total Staph.	*Cand. albicans*	FLA	Coliphage
Helwan	2.6	2.9	2.3	2.2	2.6	4.6
El-Giza	3.1	3.1	2.7	2.5	2.8	5.6
Embaba	1.9	1.1	0.6	1.9	2.2	1.8
Sh. El Khema	2.5	2.1	1.1	2.1	2.3	2.2
Average	2.5	2.3	1.7	2.2	2.5	3.6

Pseud. sp.: *Pseudomonas aeruginosa ; Cand. albicans: Candida albicans ;* Staph.: Staohylococci ; FLA: Free-living amoebae
Sh: Shubbra ; pfu: plaque forming unit ; cfu: colony forming unit.
These log numbers are averages for 24 samples.

Table 4: Classical bacterial indicators and some pathogenic microorganisms in drinking water during 2009 and 2010 at different in Greater Cairo.

Microorganism		Average counts							
		Helwan		Giza		Embaba		Sh. El- Khema	
		OL	DS	OL	DS	OL	DS	OL	DS
Total bacterial count / ml at :-	37ºC	7	18	29	39	13	15	23	38
	22ºC	4	16	18	38	11	12	22	28
Coliform MPN-index/ 100ml	Total	0	0	0	0	0	0	0	0
	Fecal	0	0	0	0	0	0	0	0
Fecal streptococci MPN-index/ 100ml		0	0	0	0	0	0	0	0
Salmonellea groupes MPN-index/ 100ml		0	0	0	0	0	0	0	0
Pseud. aeruginos MPN-index/ 100ml		0	2.2	4.1	5.2	0	2.1	2.2	3.7
Total staphylococci cfu/ 100ml		0	0	0	0	0	0	0	0
Candida albicans cfu/ 100ml		0	0	0	0.8	0.1	2.3	2.3	3.3
Coliphage (PFU / 100ml)		0	0.7	0	1.2	0	0	0	0
Free-living amoebae (PFU/L)		0	0.8	0	0	0	0	0	0.8

OL: Out let of drinking water treatment plant.
DS: Distribution systems (tap water samples).
PFU: Plaques forming unit cfu: colony forming unit.
Pseud: Pseudomonas MPN: Most Probable Number Sh: Shubbra.
N.B. These numbers are averages for 24 samples.

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