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Ecomicrobiological Study on River Nile at Kafr El-Zayat Industrial Area

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Abstract

The total microbial count including fungi, yeasts and total bacterial counts were recorded as indicators for faecal pollution in the River Nile (Rosetta branch) at Kafr El-Zayat Industrial Area. The highest counts for yeasts were recorded during winter in front of and at about 1 km far from El-Mallh and Soda Company (113 x10^2 and 148 x10^2 CFU ml^{-1}). The most common identified yeast organisms were *Saccharomyces cerevisiae* and *Kloeckera* spp. Fungi highest count was recorded during winter at El-Mallh and Soda Company at 37°C and represented by *Trichoderma* spp. *Candida albicans* and *Candida magnoliae* were the most abundant species during spring and summer at 27°C. The highest counts of total and faecal coliform bacteria were 11x10^3 CFU ml^{-1} during summer and winter. Gram-negative bacteria (i.e. pathogenic bacteria) had the highest count during winter, where *Klebsiella pneumoniae*, *Serratia liquefaciens, Sallmonella choleraesuis* and *Escherichia coli* were the most common isolated organisms. All microbiological parameters indicated that Rosetta Branch was subjected to sewage pollution. In addition, some environmental parameters such as temperature, conductivity and pH were monitored. The role of yeasts and bacteria in nitrogen cycle in the water revealed that a proposed waste water treatment unit could be beneficial before the discharge of the industrial wastes that will help in solving ammonia problem.

Keywords: River Nile, Rosetta Branch, Faecal pollution, Bacterial indicators, Pathogenic bacteria Yeasts, Fungi, Ammonification, Nitrification and Denitrification.

1. Introduction

The problem of the limited amount of water resources in Egypt acquires more attention to the problem of over population and the inability to implement the projects of increasing water resources of the River Nile (Ahmed *et al*., 1995). The Nile is the main source of water for drinking, agriculture, industry, navigation, recreation and fish production. Thus, it is of dominating influence on the economic, cultural, public health, social and political aspects of the country (Mahrous, 1997 and Rabeh, 2007). Concerning the pollution of Rosetta Branch, there are three main sources which potentially affect and deteriorate its water quality. El-Rahawy drain pours more than 400,000 m^3 daily including 398,000 m^3 liquid sewage and 2,000 m^3 of sludge from Giza Governorate (Ghallab, 2000). At the same time, Soble drain at Menofyia Governorate pours its agricultural wastes directly into this branch. The second source is Kafr El-Zayat Industrial Area, which includes the industrial effluents from the factories of super-phosphate and sulfur-compounds (e.g. El-Malia Company), oil and soap industries (e.g. El-Mallh and Soda Company) and pesticides factory, which pour their effluents directly into the branch without any treatment. The third source is many agricultural drains that discharge their waters into this branch, in addition to the sewage discharged from many cities and villages that are distributed along its two banks (Abdo, 2002).

The use of normal intestinal organisms as indicators of faecal pollution rather than the pathogens themselves is a universally accepted principle for monitoring and assessing microbial safety of water supplies. Organisms commonly used as bacterial indicators for faecal pollution are the coliform group including faecal coliforms and faecal streptococci (Anne-Marie *et al*. 1991; Hecter *et al*. 1998 and Rabeh, 2000). Total yeasts, especially *Candida albicans,* were identified and counted to determine the microbiological profile of River Nile at Cairo (EL-Taweel, 1998). Therefore, the present study aims at evaluating the eco-microbiological characteristics of River Nile water at Kafr EL-Zayat Industrial Area (Rosetta Branch) through counting some yeasts (e.g. *Candida* spp and *Trichosporon* spp), fungi and total bacteria as microbial indicators in relation to some physico-chemical parameters.

2. Materials and Methods

2.1. Study Area

River Nile is considered as the longest river in the world. Its length is approximately 6740 km. Twenty five km North of Cairo at EL-Qanater Barrage, the River Nile bifurcates into two branches (Damietta and Rosetta). Rosetta Branch width varies from 150 to 200 m, with an average depth of 2 to 3.5 m (Abdo, 2002). The water level fluctuates between 10.6 and 13.8 meter above sea level (Sayed, 2002). Rosetta Branch begins from El-Qanater El-Khyria Barrage at the south and ends at the Rosetta Estuary in the Mediterranean Sea at the north. It is of great vital importance as a source for municipal, industrial and agricultural water, and for navigation and fish farms.

2.2. Sampling

Sampling process was carried out seasonally (November 2005 - August 2006). Five stations were selected for the collection of water samples from Rosetta Branch at Kafr El-Zayat (Figure 2): station 1 in front of El-Mallh and Soda Company, station 2 in front of El-Malia Company, station 3 in front of Benover Village, station 4 at about 1 km downstream El-Malia Company and station 5 at about 1 km downstream El-Mallh and Soda Company. For pesticides determination, water samples were collected in front of El-Malia Company during winter 2006 as it produces super-phosphate and sulfur-compounds and its effluents might include chemicals used in the production of pesticides.

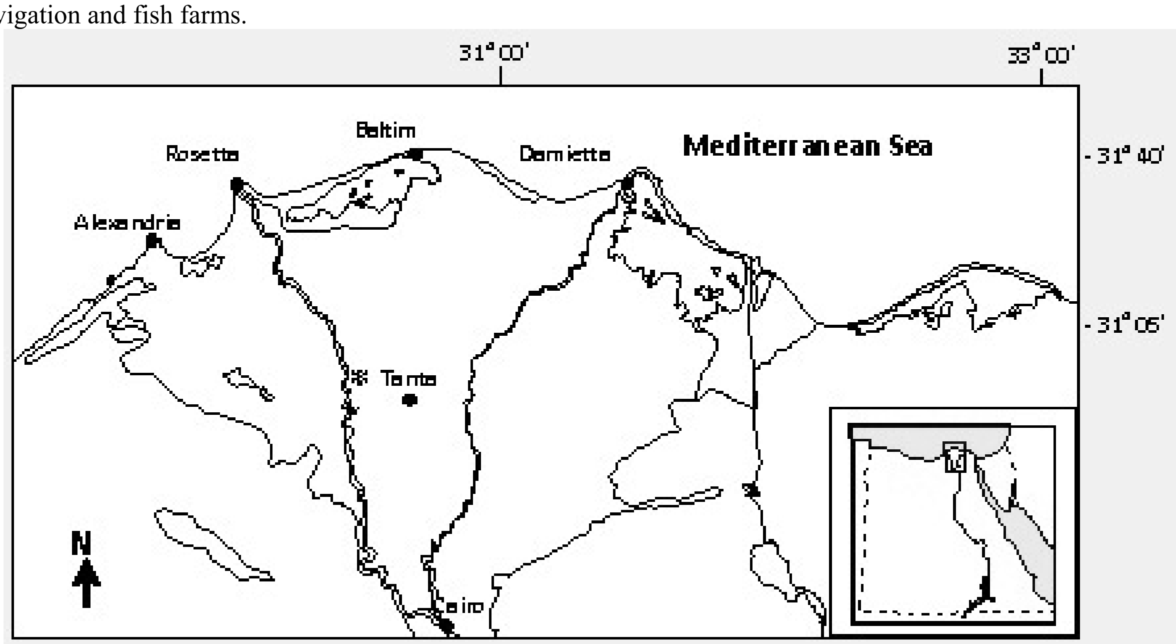

Figure 1. Map of Egypt indicates Kafr El-Zayat Industrial Area close to Rosetta Branch (*).

Figure 2: Sampling sites in the front of El-Malia (left side) and Mallh & Soda Companies (right side) at Kafr EL-Zayat Industrial Area.

2.3. Physical and chemical water analysis

Water sampling for chemical analysis were collected using plastic bottles. The samples were stored, refrigerated and analyzed immediately after arrival to the laboratory. For analysis of heavy metals, samples were preserved immediately after collection by acidifying with concentrated HNO_3 to pH < 2 using 5 ml nitric acid for 1 liter sample, then the samples were stored in a refrigerator at 4°C or in a deep freezer to prevent change in volume due to evaporation. Samples might be filtered if necessary, and a portion of the filtered sample was taken for the required determination. For pesticides analysis, samples were collected using plastic bottles (about 2 liters) and stored in a refrigerator at 4 °C. Air and water temperatures (°C), electrical conductivity (µS cm^{-1}), hydrogen ion concentrations (pH values) and salinity (EC) of water were measured directly at the sampling stations using water quality checker U-10 HORIBA. Dissolved oxygen was carried out using the modified Winkler method, Ammonia using Nesslerization method, Nitrate-Nitrogen using ultraviolet spectrophotometric screening method, and some heavy metals (Lead, Zinc, Copper, and Cadmium) using nitric acid digestion method (APHA, 1998). Some pesticides were measured using Gas chromatography (GC/ECD) according to UNEP/IOC/IAEA (1991) and IOC (1993).

2.4. Microbiological analysis

Sterile 100 ml plastic bottles were used in this analysis (Rabeh, 1993). Surface plate method (Rabeh, 1996) was used for determination of the total bacterial count at 22°C for 72 h and at 37°C for 48 h using Plate Count Agar medium. Most probable number method (MPN) was used for determination of total coliform (TC) and faecal coliform (FC) on MacConkey Broth (at 37°C for 48 h and 44.5°C for 24 h, respectively). Azide dextrose broth was used for determining MPN of faecal streptococci (FS) at 37°C for 48 h (APHA, 1998). Gram negative bacteria were enumerated in the examined water on MacConky agar medium using pour plate method (see Rabeh and Azab, 2006). All isolated Gram-negative bacteria were identified using API 20E (bioMerieux). From each plate containing specific medium, one colony was added to 5ml sterile water. The solution was inoculated into the strip, containing mini-test tubes, and incubated for 18-24 h at 37°C. The coloration reactions were read and the reaction converted to a digital code which compared to the Reading table provided by bioMerieux.

The plate count technique was used for the isolation and enumeration of fungi (Dutka, 1989). Using Aureomycin Rose Bengal Glucose Peptone Agar (ARGPA), 0.1 to 1 ml of the sample was plated out, and left to be dried. After drying, the Petri dishes were inverted and incubated aerobically at 27°C for 3 days. Incubation was also carried out at 37°C for 1 or 2 days, and then the number of colonies were counted on each plate and recorded as CFU ml^{-1}. After enumeration, fungal colonies were transferred to Peptone Dextrose Agar or Sabouraud's Dextrose Agar plates for identification purposes. The identification process was according to Barnett (1972) for the genera of fungi imperfecti, Barron (1968) for the genera of *Hyphomycetes*, Domsch *et al.* (1980) for compenedium of soil fungi, Ramirez (1982) for *Penicillia* and Raper and Fennell (1965) for *Aspergillus* species. The plate count technique was used for the isolation and enumeration of yeast (Dutka 1989). Suspected colonies of yeast were streaked onto sabouraud dextrose agar plates, and incubated for 48 h at 28°C. These cultures were used as the source of inoculum for the yeast confirmatory tests. The isolated yeasts were identified according to Lârone (1995) using API 20 CAUX from Temco Technology Company (Cairo).

Determination of the MPN of ammonifying and nitrifying microorganisms was carried out and calculated using tables of Cochran (1950). The culture nitrite and nitrate were determined after 21 days incubation by diphenylamine indicator. Also, MPN method was used for counting nitrate-reducing bacteria. The culture nitrate was tested on nitrate-reducing medium after 14 days incubation using sulfanilic acid and Naphthylamine solutions as indicators for the presence of nitrite (Naguib, 1961). Ammonification, nitrification and nitrate-reduction processes were carried out according to Rheinheimer (1959). Rate of ammonia production was determined using 5 ml of media for yeast (consisting of 10 g asparagine and 40 g glucose dissolved in 1 liter distilled water) with 50 ml of water sample. After 4, 8, 12 and 15 days of incubation at 25°C, the amount of the released NH_4-N was determined calorimetrically using Nessler's reagent (APHA, 1998). These processes were controlled by the same amount of the medium with the addition of 50 ml of the examined water, previously sterilized in autoclave and distilled water, in order to make sure that the NH_4-N at the end of the experiment is not produced as a result of atmospheric diffusion.

3. Results

The highest water temperature was recorded during summer (30.3°C), while the lowest was during autumn (19.4°C). The maximum electrical conductivity (EC) was recorded in front of El-Malia Company (723 µS cm^{-1}), while the minimum was at about 1 km downstream of El-Malh and Soda Company (559 µS cm^{-1}). The maximum value of dissolved oxygen was recorded in front of El-Malia Company (4.8 mg l^{-1}), while the minimum was in front of Benover Village (3.8 mg l^{-1}). The results showed that COD fluctuated between 11.2 in winter and 20.8 mg l^{-1} in summer (Tables 1 and 2). Generally, estimation of NH_3-N values indicated high values during winter. On the other hand the maximum value of nitrite was recorded during spring (0.35 µg l^{-1}), The average values of nitrate varied from 4.65 to 6.07 µg l^{-1} between seasons, and from 5.4 to 4.9 µg l^{-1} between stations. The maximum value of copper was recorded in front of Benover Village during spring (0.04 ppm), while the maximum of lead (0.33 ppm) was recorded at about 1 km downstream El-Malia Company. The values recorded for zinc were 0.13, 0.17, 0.02 and 0.06 ppm, while those of cadmium were 0.10, 0.19, 0.01 and 0.06 ppm in autumn, winter, spring and summer, respectively. The total concentration of all measured pesticides was 316.4 ng l^{-1}.

3.1. Counts of total (TBC) and Gram-negative bacteria (G-N)

The highest values of TBC at 22°C (600 x10^3 CFU ml^{-1}) and at 37°C (1000 x10^3 CFU ml^{-1}) were recorded during summer (Table 3). On the other hand, the lowest TBC at 22°C was recorded in front of Benover Village and at about 1 km downstream El-Malia Company during summer and autumn (14 x 10^3 CFU ml^{-1}), while the lowest at 37°C was recorded in front of El-Malia Company during spring (2 x 10^3 CFU ml^{-1}). The highest value of G-N was recorded in front of El-Malah and Soda Company during winter (31000 x 10^2 .100 ml^{-1}). Four isolates, namely *Escherichia coli, Salmonella choleraesuis, Serratia liquefaciens* and *Klebsiella pneumoniae,* were commonly found in all stations during the four seasons.

Table (1): Seasonal variation of the physico-chemical characters (mean ± SD) of the River Nile water at Kafr El-Zayat Industrial Area during 2005-2006. Seasonal maximum values are underlined.

Character		2005 Autumn	2006 Winter	2006 Spring	2006 Summer
Temperature (°C)		19.40 ± 0.40	19.60 ± 1.00	24.90 ± 0.40	30.30 ± 1.30
EC (µS cm^{-1})		752.00 ± 94.00	681.00 ± 45.00	487.00 ± 33.00	598.00 ± 138.00
pH		7.80 ± 0.25	7.60 ± 0.10	7.40 ± 0.08	7.80 ± 0.13
Salinity (%)		0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
D Oxygen (mg l^{-1})		3.20 ± 0.60	2.10 ± 0.50	4.00 ± 0.60	7.00 ± 0.70
COD (mg l^{-1})		19.20 ± 11.50	11.20 ± 5.20	14.40 ± 7.30	20.80 ± 6.60
Ammonia	µg l^{-1}	2.80 ± 0.05	3.60 ± 0.28	0.80 ± 0.10	0.30 ± 0.18
Nitrite		0.12 ± 0.02	0.13 ± 0.01	0.35 ± 0.02	0.21 ± 0.01
Nitrate		4.65 ± 0.93	4.92 ± 0.44	6.07 ± 0.23	4.87 ± 0.87
Cu^{+3}	ppm	0.01± 0.01	0.03 ± 0.02	0.07 ± 0.05	0.01 ± 0.01
Pb^{+2}		0.06 ± 0.06	0.19 ± 0.27	0.41 ± 0.39	0.13 ± 0.09
Zn^{+}		0.13 ± 0.09	0.17 ± 0.34	0.02 ± 0.05	0.06 ± 0.06
Cd^{+3}		0.10 ± 0.09	0.19 ± 0.39	0.01 ± 0.01	0.06 ± 0.05

Table (2): Spatial variation of the physico-chemical characters (mean ± SD) of the River Nile water at Kafr El-Zayat Industrial Area during 2005-2006. Station 1: in front of El-Mallh & Soda Company, Station 2: in front of El-Malia Company, Station 3: in front of Benover Village, Station 4: at about 1 km downstream of El-Malia Company, Station 5: at about 1 km downstream of El-Mallh & Soda Company. Seasonal maximum values are underlined.

Character		Station 1	Station 2	Station 3	Station 4	Station 5
Temperature (°C)		24.50 ± 6.00	23.80 ± 5.00	23.10 ± 5.00	23.10 ± 5.00	23.20 ± 5.00
EC (µS cm^{-1})		686.00 ± 118.0	723.00 ± 144.00	614.00 ± 152.00	566.00 ± 104.0	559.00 ± 95.0
pH		7.70 ± 0.28	7.70 ± 0.18	7.60 ± 0.28	7.70 ± 0.24	7.60 ± 0.19
Salinity (%)		0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
D Oxygen (mg l^{-1})		4.40 ± 2.00	4.80 ± 2.30	3.80 ± 2.40	3.90 ± 1.90	4.40 ± 2.10
COD (mg l^{-1})		17.00 ± 7.00	13.00 ± 5.00	19.00 ± 12.00	11.00 ± 9.00	22.00 ± 5.00
Ammonia	µg l^{-1}	1.70 ± 1.70	1.80 ± 1.40	1.90 ± 1.60	1.90 ± 1.50	1.80 ± 1.40
Nitrite		0.20 ± 0.10	0.20 ± 0.10	0.20 ± 0.10	0.10 ± 0.10	0.20 ± 0.10
Nitrate		5.30 ± 0.90	5.20 ± 0.60	5.40 ± 1.00	4.90 ± 1.00	4.60 ± 0.70
Cu^{+3}	ppm	0.03 ± 0.04	0.02 ± 0.03	0.04 ± 0.04	0.03 ± 0.03	0.02 ± 0.02
Pb^{+2}		0.25 ± 0.20	0.05 ± 0.10	0.24 ± 0.30	0.33 ± 0.40	0.15 ± 0.10
Zn^{+}		0.07 ± 0.06	0.26 ± 0.35	0.07 ± 0.11	0.02 ± 0.03	0.06 ± 0.09
Cd^{+3}		0.03 ± 0.05	0.06 ± 0.07	0.06 ± 0.09	0.24 ± 0.43	0.04 ± 0.08

Table 3: Total bacterial counts (CFU × 10^3 ml^{-1}), total count of Gram-negative bacteria (G-N x 10^2.100 ml^{-1}) of the River Nile water at Kafr EL-Zayat Industrial Area during 2005-2006. Station 1: in front of El-Mallh & Soda Company, Station 2: in front of El-Malia Company, Station 3: in front of Benover Village, Station 4: at about 1 km downstream of El-Malia Company, Station 5: at about 1 km downstream of El-Mallh & Soda Company. G-N: Gram-negative bacteria.

S	Autumn 2005			Winter 2006			Spring 2006			Summer 2006		
	22ºC	37ºC	G-N	22ºC	37ºC	G-N	22ºC	37ºC	G-N	22ºC	37ºC	G-N
1	400	276	1000	164	172	31000	128	232	3000	600	1000	4700
2	80	31	1000	56	180	13000	28	2	5000	84	4	4200
3	172	141	0.0	36	56	100	56	72	3000	14	60	1600
4	14	44	1000	164	400	17000	96	32	1000	23	36	3000
5	17	12	0.0	28	108	0.0	22	4	21000	22	17	1900

3.2. Total coliforms (TC), faecal coliforms (FC) and Faecal streptococci (FS)

The highest counts of TC were recorded in most stations during summer and winter (11000 CFU.100 ml^{-1}), while the lowest was recorded in spring (90 CFU 100 ml^{-1}). FC counts fluctuated between nil in station 3 during autumn and 11000 CFU.100 ml^{-1} in front of El-Malia Company during summer. Compared with the other stations, the highest counts of FC were recorded in front of El-Malia Company during summer and winter (11000 and 4600 CFU 100 ml^{-1}). FS varied from 150 during winter to 110000 CFU 100.ml^{-1} during autumn and spring (Table 4).

3.3. Yeast and fungal species as microbial indicator of water pollution

Highest count of yeasts (148x10^2 CFU ml^{-1}) was recorded during winter at 37°C at about 1 km downstream of El-Malah & Soda Company (Tables 5). Most of the isolated yeasts were *Kloeckera* spp and *Saccharomyces cerevisiae*. On the other hand, the highest count of fungi (15x10^2 CFU ml^{-1}) during winter at 37°C in front of El-Mallh & Soda Company (*Trichoderma* spp was the most abundant). The isolated yeasts were *Rhodotorula glutinis*, *Cryptococcus laurentii, Kloeckera* spp, *Saccharomyces cerevisiae, Candida albicans, Candida magnoliae, Trichosporon mucoides* and *Candida glabrata*. The most common identified fungi were *Aureobasidium pullulans, Cladosporium carinioii, Asperigullus tamari, Xylohypha bantiania, Trichoderma* spp, *Blastomyces* spp and *Penicillium mareiffei*.

3.4. Bacteria involved in nitrogen cycle

Ammonifying, nitrifying and denitrifying bacteria in River Nile water were counted during winter in front of Benover Village (it had the highest ammonia concentration during 2006). The most probable number of ammonifying bacteria was 240 colony ml^{-1}, and that of nitrifying bacteria was 39 colony ml^{-1}during winter. On the other hand, denitrifying bacteria were completely absent from the studied station. High value of 148 µg l^{-1} of water ammonification process was measured (Table 6).

3.5. Role of Yeasts in Nitrogen Cycle*:*

The result of the laboratory experiment on ammonification process by yeasts, which inhabiting River Nile water after different incubation periods, indicated that the value of ammonification process was higher, after 4 days of incubation, in non-autoclaved water sample (28.2 µg l^{-1}) than in autoclaved water sample, 2.9 µg l^{-1} (Table 7).

Table 4: Most probable number (MPN) of bacterial indicators of sewage pollution (CFU.100 ml^{-1}) of the River Nile water at Kafr EL-Zayat Industrial Area during 2005-2006. Station 1: in front of El-Mallh & Soda Company, Station 2: in front of El-Malia Company, Station 3: in front of Benover Village, Station 4: at about 1 km downstream of El-Malia Company and Station 5: at about 1 km downstream of El-Mallh & Soda Company.

S	Autumn 2005			Winter 2006			Spring 2006			Summer 2006		
	TC	FC	FS	TC	FC	FS	TC	FC	FS	TC	FC	FS
1	3900	3900	110000	4600	4600	2300	4600	230	46000	2300	2300	93000
2	2100	2100	1500	11000	4600	2300	230	230	110000	11000	11000	4300
3	400	0.00	7500	11000	930	7500	90	90	46000	11000	200	46000
4	11000	90	4300	430	230	3900	430	90	15000	2400	70	21000
5	1200	230	3900	430	230	150	150	90	9300	2100	210	15000

Table 5: Total yeast and fungi counts at 27 ºC and 37 ºC (× 10 ^{2} ml^{-1}) of the River Nile water at Kafr EL-Zayat industrial area during 2005- 2006. Station 1: in front of El-Mallh & Soda Company, Station 2: in front of El-Malia Company, Station 3: in front of Benover Village, Station 4: at about 1 km downstream of El-Malia Company, Station 5: at about 1 km downstream of El-Mallh & Soda Company.

Season	Autumn 2005				Winter 2006				Spring 2006				Summer 2006			
	Yeast count		Fungal count		Yeast count		Fungal count		Yeast count		Fungal count		Yeast count		Fungal count	
	27	37	27	37	27	37	27	37	27	37	27	37	27	37	27	37
	ºC															
1	2	1	2	0	113	128	3	15	7	30	1	0	3	2	0	0
2	1	1	0	1	0	24	1	0	2	9	0	0	0	1	0	0
3	0	0	2	2	97	29	0	0	5	10	0	1	2	0	1	1
4	0	0	0	0	2	143	1	0	4	1	1	0	0	1	0	5
5	0	1	0	0	0	148	0	1	9	2	0	0	1	1	0	0

Table 6: Ammonification, nitrification and denitrification processes (µg l^{-1}) in the River Nile water at Kafr El-Zayat Industrial Area during winter 2006. C: autoclaved water sample, S: non-autoclaved water sample, ND: not detected.

Process	Ammonification		1st step of Nitrification		2nd step of Nitrification		Denitrification	
	C	S	C	S	C	S	C	S
NH_3	9.30	148	ND	ND	ND	ND	2.15	0.90
NO_2	ND	ND	0.16	0.93	ND	ND	0.18	0.15
NO_3	ND	ND	ND	ND	24.98	25.07	ND	ND

Table 7: Ammonification processes (µg l^{-1}) by yeasts inhabiting the River Nile water at Kafr El-Zayat Industrial Area during winter 2006.

Time (day)	Autoclaved water sample	Non-autoclaved water sample
4	2.9	28.2
8	3.9	24.6
12	1.7	12.5
15	0.9	13.5

4. Discussion

Variety of wastes effluent discharge, either without or with partial treatment into the Nile, resulting in changes in its physical and chemical conditions and microbial quality. Water temperature is an important factor which affects the biological activities of aquatic organisms (Bojanic *et al*. 2001), and the amount of oxygen that can be dissolved in water. In the present study, elevation of temperature in front of EL-Mallh and Soda Company allover the year could be attributed to the discharge of hot waste water into the Nile, which consequently induces thermal pollution leading to to the discharge of hot waste water into the Nile, which consequently induces thermal pollution leading to elevation of temperature. This finding agrees with Bedair (2006). In addition, the high value of EC in the Nile water may be attributed to the discharge of El-Rahawy drain which pours more than 400,000 m^3 daily including 398,000 m^3 liquid sewage and 2,000 m^3 of sludge from Giza Governorate. Soble drain pours a great amount of agricultural effluents containing large amounts of dissolved ions and high amount of organic and inorganic constituents. This was in accordance with the study of Elewa and Mahdi (1988). The present results indicated also an obvious increase in the water salinity during autumn and summer. The increase in water salinity in summer may be due to the increase in temperature and consequently increasing evaporation (Rabeh 1993). pH of the River Nile water is always in the alkaline side with slight variation between different stations (El-Bassat 1995 and 2002, Heikal 2000, Abdo 2002 and Bedair 2006).

Dissolved oxygen (DO) is the best parameter to show the effect of pollution on a water system unless it contains toxic compounds. Oxygen is also needed for all oxidation, nitrification and decomposition processes and is controlled by three factors: photosynthesis, respiration and exchange at the air-water interface (Krom *et al*. 1989, Erez *et al*. 1990). In the present investigation, values of dissolved oxygen ranged between 1.5 mg l^{-1} during winter in the front of Benover village and 8.1 mg l^{-1} during summer in the front of El-Malia Company. Generally, DO concentrations in the Nile are always higher than 7.0 mg l^{-1}, indicating high assimilation capacity (Ezzat *et al*. 2002). According to APHA (1998), the chemical oxygen demand (COD) is used as a measure of the oxygen equivalent toorganic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. The highest COD value (36 mg O_2 l^{-1}) was recorded in the front of Benover village during autumn. This might be due to low water flow which reflects the high load of organic matter at this station. Generally, during winter the COD value was higher at some stations, this may be due to the winter closure which started by the end of December causing decrease in the level of Nile water (this leads to elevate the organic load of the Nile during this period). Also the relative increase of COD values during summer season may be attributed to the increase in water temperature which facilitates the decomposition of organic matter in water that leads to increase COD values (Ali, 1998).

Reid (1961) reported that ammonia-nitrogen in excess of 1 mg l^{-1} has been given as an indicator of organic pollution and can be toxic to aquatic species in concentration over 2.5 mg l^{-1}. Higher concentration of ammonia in the area of the present study could be attributed to organic pollution resulted from domestic sewage, industrial waste and fertilizer runoff. The high values of NH_3 recorded during hot seasons especially in spring (ranged between 0.63 and 0.91 mg l^{-1}) may be due to high evaporation rates depending on the elevated temperature, in addition to the denitrification process by the reduction of NO_2 and NO_3 to NH_3 under lower values of pH and DO (Elewa *et al*. 2001). The pronounced increase in NH_3 content during winter, especially in the front of Benover village, may be attributed to the decomposed organic matter and dying off of algal blooms resulted in increasing of ammonia concentration (Krom *et al*. 1989).

During winter and autumn, NH_3 had positive correlations with NO_3, total count of yeasts and total coliform.. This indicates that high concentration of ammonia was correlated with high level of faecal pollution. Generally, surface water not influenced by human activities contains nitrate concentration less than 5 µg l^{-1}. Excess of 5 µg l^{-1} usually indicates pollution by human or animal waste or/and fertilizers run off (Chapman 1992). Nitrate is generally considered the only thermodynamically stable form of nitrogenous compounds in absence of oxygen (Horna 1972). In case of nitrate, the highest value was recorded during summer (6.41µg l^{-1}). This agrees with Heikal (2000) who found that nitrate value ranged between 3.5-6.8 µg l^{-1} in River Nile water. Most of the maximum values of NO_3 were recorded in the front of El Mallh & Soda and El-Malia Companies which mainly attributed to the effects of agricultural and industrial effluents poured close to these stations. Comparable results were reported by Siliem (1984). On the contrary, the relative decrease in NO_3- contents recorded during autumn may be attributed to the drought period in addition to the denitrification of NO_3 into NO_2 and NH_3 by denitrifying bacteria.

Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate and in the reduction of nitrate. The relative increase of NO_2 concentrations during summer and spring may be due to the increase in temperature and the oxidation of ammonia, while the sharp decrease during autumn may be due to the increase of the oxidation rate of nitrite to nitrate. Generally, nitrite is present in water as an ion or free acid and both are toxic (Russo *et al*., 1981). NO_2 concentrations during summer and spring were high at the front of El-Mallh & Soda Company and this may be attributed to the industrial effluent from this factory, while the lowest value (in most seasons) was recorded at about 1 km downstream El-Malia and El-Mallh & Soda Companies.

Heavy metals have environmental persistence, almost of them have no toxicity at low concentration, but they have ability to incorporate into food chain and thus it is possible to be concentrated to toxic levels by the aquatic organisms. They are usually divided into two subclasses: Co, Cu, Fe, Mn and Zn which are essential for the correct functioning of biochemical processes; and Hg, Cd, Cr, and Pb that have no established biological function and represent the most important contaminants in the aquatic environment. In the study area, during autumn, the relative decrease of copper concentration may be attributed to its adsorption by humic materials that deposited to bottom, in addition to dilution effect of flood period (Elewa, 1991). On the other hand, the high values of copper contents were recorded during spring and summer, this may be due to the high evaporation rate under high temperature of water and air during hot seasons, and the release of Cu from sediment to the overlying water (Abdo, 1998). The high average values of Cd content in the study area were observed during winter, while the low values were during spring and summer. Comparable results were reported by (Bedair, 2006).

DDT compounds are used in fighting insects, mosquitoes and flies in the environment; they are converted to DDE compound by the effect of metabolic processes. Concerning the present study, the concentration of DDT compound was higher than that of DDE. This indicated that the DDT was recently present compared with DDE, which may be due to the discharge of El-Malia Company (it produces super-phosphate and sulfur-compounds that include chemicals used in the production of pesticides). However, the total concentration of all measured pesticides in the area of investigation was 316.4 ng.l^{-1}. This value was within the permissible limits ($2x10^6$ng.l^{-1}) according to the Egyptian Environmental Law no. 4 in 1994.

Total bacteria developing at 20-22°C are saprophytic types and non-pathogenic to human beings, while those developing at 37°C are mainly or potentially parasitic types derived from the soil, sewage, or excretal materials (APHA, 1998). The total count of bacteria at 22 and 37°C in the present study were high in summer and began to decrease in autumn at 22°C. This pattern was coincided with water temperature during the same seasons. The same observations were previously reported by Rabeh (2007) and Rabeh *et al.* (2007). However, Abo-Sedra (1990) reported that the effect of temperature seems to be of little influence on the question of bacterial changes in River Nile waters. In Rosetta Branch, Rabeh (2007) reported that the highest bacterial counts (66.8×10^7 and 65.6×10^7 CFU ml^{-1}) at both incubations temperatures (22 and 37°C) were recorded during summer. On the other hand, the highest counts were recorded at Sobol Drain in Menofyia Governorate, while the lowest were at Rosetta Estuary (0.3×10^7 and 0.2×10^7 CFU ml^{-1}).

The ratio of colony counts at the two incubation temperatures (22 and 37°C) helps to explain any sudden fluctuation in the bacterial count. This ratio is usually more to one in non-polluted waters, while in polluted waters it is below one. It was noticed that the ratio of bacteria recovered at 22 and 37°C was almost constant in all the examined water samples. In other words, an increase or decrease in the counts of bacteria developing at 22°C was associated with a similar trend to those developing at 37°C. The ratio of the count at 22°C to that at 37°C was slightly higher than 1, except in winter (0.51). So, the investigated area of the River Nile would be considered heavily polluted with sewage (APHA, 1998).

Total and faecal coliforms and faecal streptococci are indicators that have been used for decades to infer the presence of other potentially harmful pathogens in water (Noble *et al.,* 2004, Rabeh, 2007 and Sabae and Rabeh, 2007). The highest value of most probable number of coliform bacteria was recorded during summer in the front of El-Malia Company, which discharges nitrogenous wastes that may be encourage the growth of indicator bacteria, in addition to the sewage wastes in River Nile water which also cause the high count of these indicator bacteria in the front of Benover Village. This result agrees with that reported by Rabeh (2007). On the other hand, total coliform count increased also during winter at the same stations, which may be due to the low flow period that leads to high concentration of organic matter, sewage and industrial wastes. Also high positive correlation between NO_3 and most of faecal indicator parameters might be due to the use of NO_3 by these organisms as nitrogen sources.

Several investigators prefer faecal streptococci (FS) instead of the coliforms in determing the level of sewage pollution (Dutka, 1989, Dan and Koppel, 1992). During this investigation, FS counts were higher during autumn then spring in the front of El-Mallh & Soda and El-Malia Companies as they discharge industrial wastes contains, super-phosphate, nitrogenous and sulfur compounds. These discharges encourage the bacterial growth. FS counts not only have their own indication to pollution, but also their ratio to faecal coliforms (FC) points to the source of faeces whether it is human (> 4) or animal (< 0.7) (Geldreich 1974). Accordingly, the FC/FS ratio in the present study indicates human pollution (> 4) in winter and summer and of mixed source during autumn (3.0), while it was from animal source during spring (0.05). There is a positive correlation in the present study during spring between the two types of enteric bacteria, faecal coliform and faecal streptococci which supports the view that coliform and faecal streptococci are useful indicators for the presence of entero pathogens.

Bacterial indicators are often associated with disease-causing microorganisms of importance to public health (Godfree *et al.*, 1997) as these pathogens can lead to water-borne or water-borne related outbreaks (Martinez-Manzanares *et al.*, 1991). The highest total Gram-negative bacterial counts were recorded during winter, while the lowest were in autumn. This might be due to the high load of industrial, agricultural, human activities, and recreational activities which were discharged directly into the Nile. Comparison of the results obtained for characterization of our four isolates with those described in Holt *et al.* (1994), the isolates of the present study could be identified as *Escherichia coli*, *Salmonella choleraesuis*, *Serratia liquefaciens* and *Klebsiella pneumoniae*. *Candida albicans* is a known component in the humans and animals bodies, commonly found in the feces of humans and animals, thus it is an indicator of faecal contamination (Dutka 1989). Buck (1977) noted that *Candida albicans* was not found in treated sewage effluent. Shaban and El-Taweel (2003) counted *Candida albicans* and total yeast as indicators of fecal pollution in River Nile. Concerning yeast species contamination to water, Wójcik *et al*. (2003) revealed 28 yeast species representing seven genera: *Candida, Cryptococcus, Geotrichum, Kloeckera, Rhodotorula, Saccharomyces* and *Trichosporon* in the littoral zone of the Sulejów Reservoir of Poland. Uden and Ahearn (1963) had recorded many species of yeasts such as *Cryptococcus laurentii, Rhodotorula glutinis, Trichosporon mucoides* and *Cryptococcus albidusi* which were related to their founding species. *Aspergillus tamarii* and *Penicillium mareiffei* were recorded at different seasons during the period of investigation; these species were also appeared in the River Nile water near Sohag (El-Sharouny, 1989). Kiziewicz (2004) in his study on *Candida albicans* and *Candida tropicalis* found that their occurrence was associated with high concentration of ammonium nitrogen and suspended substance.

High rate of ammonification during winter, as indicated in the present study, may be the reason for ammonia accumulation resulting in death of fish. High count of ammonifying bacteria was accompanied with low count of denitrifying bacteria (see Jǿrgensen, 1989). Nitrifying bacteria is another important group of bacteria engaged in nitrogen cycle, which oxidised reduced nitrogenous matter to nitrite and then to nitrate (Hesselsǿe, 2001), and make link between reduced and oxidized nitrogen compounds (Prinčič *et al.*, 1998). Variation in the number of nitrite and nitrate bacteria usually correspond to variations in the rate of NH_4-N and NO_2-N oxidation (Niewolak *et al.*, 1979).The present study indicated that River Nile at Kafr El-Zayat industrial area is considered a highly contaminated one due to the presence of high numbers of indicator bacteria and yeasts. Furthermore it demonstrated a difference in the distribution of bacteria involved in nitrogen cycle which played a significant role in nitrogen removal, promoted ammoniation, nitrification and denitrification in river. In conclusion, the present study suggests pretreatment of wastewater through microbiological unit before the discharge of industrial wastes of El-Malia and El-Mallh & Soda Companies which may solve the accumulation of NH_3, that leads to fish death in Nile.

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دراسة بيئية ميكروبيولوجية على نهر النيل بمنطقة كفر الزيات الصناعية

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تم دراسة العدد الميكروبى الكلى للفطريات والخمائر والبكتريا لمياة نهر النيل (فرع رشيد) بالقرب من منطقة كفر الزيات الصناعية كمؤشر للتلوث العضوي . وجد أن أعلى قيمة للخمائر كانت في الشتاء أمام مصب شركة الملح و الصودا وعلى بعد كيلو متر واحد منها (113 × 10^2 و149× 10^2 مستعمرة على التوالي)، وكان *Kloeckera* spp و *Saccharomyces cerevisiae* أكثر الكائنات ظهورا في هذا الموسم. حققت الفطريات أعلى عدد لها خلال الشتاء أمام مصب شركة الملح والصودا أيضا عند درجة حرارة 37 ° (*Trichoderma* spp). ومن الملاحظ أن *Candida albicans* و *Candida magnoliae* كانا أكثر الكائنات ظهورا خلال الربيع والصيف عند درجة حرارة 27°. سجلت بكتريا القولون الكلية وبكتريا القولون البرازية أعلى عدد لها (11× 10^3 مستعمرة) في الصيف والشتاء. وقد لوحظت الزيادة الهائلة في العدد الكلى للبكتريا الممرضة خلال موسم الشتاء، وكان من أهم الكائنات التي تم عزلها *E. coli* ، *Sallmonella,* *Serratia* و *Klebsiella* spp. وتم فى هذه الدراسة أيضا تقدير بعض الخواص البيئية للماء مثل درجات الحرارة والتوصيل الكهربى والاس الهيدروجينى. وعموما، تشير كل الخواص الميكروبيولوجية إلى أن فرع رشيد يتعرض للصرف العضوى، ومن خلال الدورالذى تقوم به الخمائر والبكتريا في دورة النيتروجين بمياه النهر، يتضح أن عمل وحدات معالجة ميكروبيولوجية للصرف الصناعي الناتج من الشركات التي تصب مباشرة فى نهر النيل ذو فائدة عالية للتغلب نسبيا على مشكلة ارتفاع الامونيا فى هذه المياه.