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Seasonal and Regional Variation of Physicochemical and Bacteriological Parameters of Surface Water in El-Bahr El-Pherony, Menoufia, Egypt

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Abstract: Al-Bahr El-Pherony is considered one of the important sources of fisheries in Menoufia Government. Their level of pollution is swelling day by day due to non-availability of proper drainage system. Twenty-eight water samples were collected seasonally during January – September 2013. Some environmental parameters such as temperature, transparency, dissolved oxygen; biological oxygen demand and pH were monitored. In addition, the bacteriological analyses involved total viable bacterial counts (TVBCs), Total Coliforms (TC), Fecal Coliforms (FC), *E. coil*, Fecal Streptococci (FS) and some pathogenic bacteria were counted. The results of physicochemical parameters showed that the temperature values varied from 21.4°C to 32°C and transparency from 10 cm to 65 cm. TVBCs at 22 °C and 37 °C were 27 x 10^6-210 x $10^6$and 10 x 10^6– 176 x 10^6 CFU/ml, respectively. The results of the fecal indicators counts revealed that their densities increased from winter to summer along with BOD_5. In winter, CCA (Canonical Corresponding Analysis) showed strong relation of *Salmonella* with BOD_5 and negative one of *P. aeurginosa* and FS with temperature. In summer, showed positive correlation between *S. aureus* and DO. It was concluded that El-Bahr El-Pherony water was subjected to sewage pollution during the study period and had poor microbial quality.

Key words: Environmental Parameters · Indicator Bacteria · El- Bahr El- Pherony

INTRODUCTION

El-Bahr El-Pherony is an important watershed and is a crucial source of irrigation water. It covers about 30.2 Km. It is considered one of the important sources of fisheries in Menoufia Governorate. Plants and fishes living in these water bodies, when poisoned with harmful chemicals and metals can't survive. The crops and vegetables irrigated with such polluted water become harmful for human beings. The level of pollution is swelling day by day due to non-availability of proper drainage system for industrial units and housing societies established along the banks [1].

Prevention of river pollution requires effective monitoring of physicochemical and microbiological parameters [2]. In most countries, the principal risks to human health associated with the consumption of polluted water are microbiological in nature [3]. The bacteriological examination of water has a special significance in pollution studies, as it is a direct measurement of deleterious effect of pollution on human health [4]. Coliforms are the major microbial indicator of monitoring water quality [5, 6]. The detection of *Escherichia coli* provides definite evidence of fecal pollution; in practice, the detection of thermotolerant (fecal) coliform bacteria is an acceptable alternative [3].

This work aimed to assessment of the water quality of El-Bahr El-Pherony and relates the physicochemical characteristics and microbial quality of water with standard guidelines for safe consumption or usage.

MATERIALS AND METHODS

Sampling: Twenty eight water samples were collected from different locations of El-Bahr El-Pherony, Menofia Governorate during January-September 2013 as

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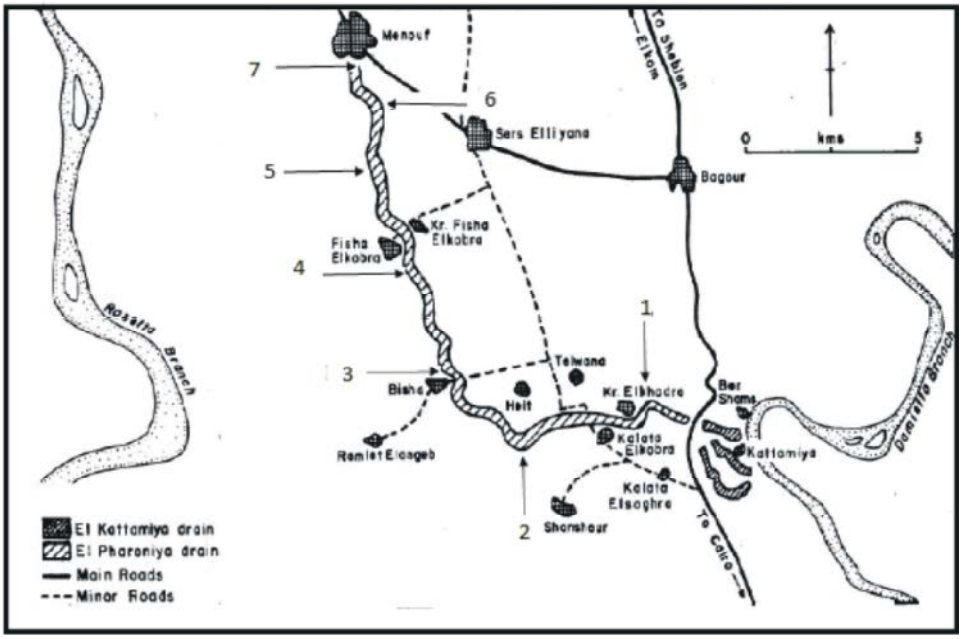

Fig. 1: A map showing the sampling sites (1-7)

Table 1: GPS data of sampling locations of El-Bahr El- Pherony

Stations	GPS data
St.1	N:30° 22` 37"; E: 31° 01` 372"
St.2	N:30° 22` 224"; E: 31° 00` 347"
St.3	N:30° 22` 27"; E: 30° 59` 47"
St.4	N:30° 23` 314"; E: 30° 58` 238"
St.5	N:30° 22` 54.5"; E: 30° 57` 585"
St.6	N:30° 26` 277"; E: 30° 56` 18"
St.7	N:30° 27` 011"; E: 30° 56` 052"

represented in (Fig. 1 and Table 1) from surface water (1 m). Glass stopped oxygen sampling bottles (300 ml capacity), for dissolved oxygen as well as biochemical oxygen demand determinations were filled carefully with water samples. Samples were aseptically collected in sterile brown bottles (500 ml capacity), transported to laboratory and stored at 4°C until bacteriological analysis completed within 6 h of sampling.

Physicochemical Analyses: The temperature, transparency and pH of the collected water samples were measured in situ. Dissolved Oxygen (DO) was determined by azide modification method as described by APHA [7]. Additionally five-day Biological Oxygen Demand (BOD_5) was estimated [7].

Bacteriological Analyses: Spread plate method was used for detection and enumeration of Total Viable Bacterial Counts (TVBCs) at 37°C and 22°C. Samples were examined for enumeration of classical indicators including; Total Coliform (TC), Fecal Coliform (FC), *Escherichia coli*, Fecal Streptococci (FS) and some pathogenic bacteria (*Staphylococcus aureus*, *Salmonella* sp. and *Pseudomonas aeurginosa*) using a membrane filtration technique [8]. Briefly, water sample (100 ml) was filtered through a gridded sterile cellulose-nitrate membrane filter (0.45 µm pore size, 47 mm diameter, Sartorius type filters) under partial vacuum (Millipore, Befrid, UK).

The membrane filters were immediately removed with sterile forceps and placed on the following media with rolling motion to avoid entrapment of air: m-Endo agar was used for TC detection after 24 hr at 37 °C. M-FC agar was used for detection of FC after 48 hr at 44.5 °C, Eosine Methylene Blue (EMB) for detection and isolation of *E. coli* after 24 hr at 37 °C. Additionally, ME (m-*Enterococcus*) agar were used for detection and enumeration of FS, after 48 hr at 37 °C. Mannitol salt agar and bismuth sulphite agar were used for detection and enumeration of *S. aureus* (yellow colonies) and *Salmonella* sp. at 37 °C for 24 hr, respectively. For detection of *Pseudomonas aeurginosa*, m-PAC agar was inoculated and incubated at 41.5 °C for 72 hr. Results were recorded as colony forming unit (CFU/100ml) using the following equation [8].

$$Bacterial\ colony\ (CFU)\ per\ 100\ ml = \frac{Bacteial\ colony\ counted}{ml\ of\ sample\ filtered} \times dilution\ factor \times 100$$

All recovered colonies were sub-cultured onto nutrient agar for purification. Isolated bacteria were identified on the basis of their colonial, morphological and

biochemical properties following Bergey's Manual of Systematic Bacteriology [9]. The biochemical tests included: Gram staining, motility, indole production, methyl red-voges Proskauer, citrate utilization, oxidase, catalase, coagulase and sugar fermentation tests.

Statistical Analysis: Standard deviation and two-way analysis of variance ANOVA test were used for correlating the data and elimination of variance is observed in results. Bacterial count was transformed prior to statistical treatment and results were analyzed by standard deviation and ANOVA test [10]. The matrix of bacterial analyses and physicochemical characteristics of the investigated samples were subjected to CCA (Canonical Corresponding Analysis) using CANOCO (Canonical Community Ordination) program [11].

RESULTS

Physicochemical Analyses: Seasonal variations of various physicochemical parameters of the collected samples during January-September 2013 were graphically represented in Figure (2). Surface water temperature degrees ranged between 21.4-32 °C, the highest values were recorded in summer (Fig. 2A). The variations in water transparency were almost local, without seasonal trends. Furthermore, the highest value of transparency was detected during winter (60 cm) and the lowest one was recorded during spring (10 cm). The pH values of water samples hardly fluctuated from sample to another but generally were falling in the alkaline side ranging between 7.85-8.72 (Fig. 2A).

The seasonal average of DO values oscillated between 3.16 mg/l in summer and 15.61 mg/l in spring (Fig. 2B). With respect to BOD concentrations, BOD of water samples ranged between 0.99-11.5 mg/l in winter and summer, respectively (Fig. 2B).

Bacteriological Analyses: Total Viable Bacterial Counts (TVBCs) at 22 °C of the collected samples during the course of study ranged between $27x10^6$ - $210x10^6$ CFU/ml, recording their maximum number in summer and minimum one during winter (Table 2). On the other hand, TVBCs at 37°C varied from $10x10^6$ to $176x10^6$ CFU/ml during winter and summer, respectively.

The regional and seasonal variations of indicator bacteria (Total Coliform; TC, Fecal Coliform; FC, *Escherichia coli* and Fecal Streptococci; FS) counts of the collected water samples during 2013 were summarized

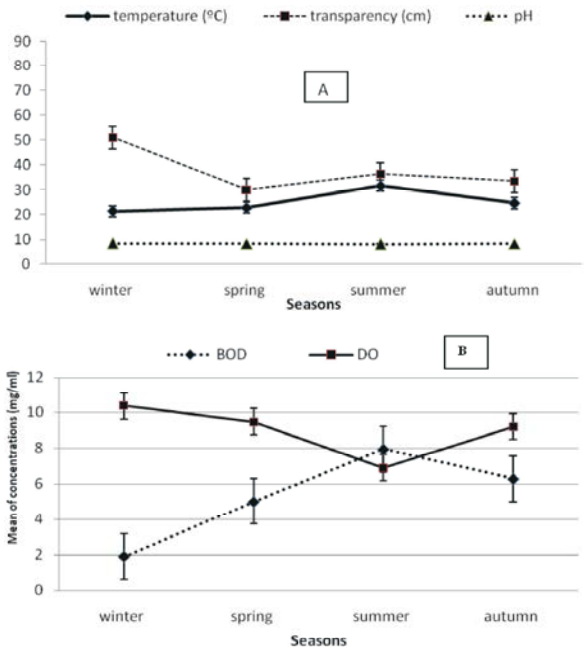

Fig. 2: Seasonal variation of physicochemical parameters of the collected samples during 2013

in Table 3. Generally, the highest counts of bacterial indicators (TC, FC, *E. coli* and FS) were detected in the warmer seasons (spring and summer), whereas the lowest number of bacteria was reported in winter. Mostly, water samples in different seasons were within the permissible limits of TC counts (2500 CFU/100 ml) stated by Law 48/1942, except St. 2, 3 and 4 during summer season were contaminated with high amount of bacterial population than the acceptable limits.

Table 4 lists the counting of pathogenic bacteria including; *S. aureus*, *Salmonella* sp. and *P. aeurginosa*. In summer collected water samples, regardless of the total mean pathogenic bacteria counts, the most abundant bacteria were *P. aeurginosa* (311 CFU/100 ml), followed by *S. aureus* (295 CFU/100 ml), whereas *Salmonella* sp. were present with lowest abundance (132 CFU/100 ml). On contrary the total mean count of pathogenic bacteria decreased during winter (43, 100, 28 CFU/100 ml for *P. aeurginosa, S. aureus* and *Salmonella* sp. respectively) (Table 2). The statistical analysis of seasonal change showed the significant data ($P<0.01$). Furthermore, all tested pathogenic bacteria showed significantly regional and seasonal variations (Table 4).

CCA Analysis: Statistical analysis of interrelationships between the seasonal variations with physicochemical parameters and bacterial abundance using CCA correlation was derived (Fig. 3). A positive correlation is

Table 2: Seasonal variations of Total Viable Bacterial Counts (TVBCs) at 22 and 37°C

	Mean of bacterial counts (CFU/ ml ×10^6) ± SD							
	22°C				37°C			
Stations (St.)	W	SP	SU	A	W	SP	SU	A
1	27±1.2	84±2.1	200±5.0	133±3.8	25±1.5	30±1.5	176±2.5	45±1.2
2	56±1.5	95±2.5	210±8.1	145±10.0	33±1.0	46±3.0	97±3.0	32±2.5
3	29±1.0	30±2.6	100±4.0	90±3.5	10±0.6	18±0.6	32±2.1	41±2.1
4	43±1.5	72±3.5	200±13.0	100±5.0	16±1.5	95±3.0	172±3.5	27±0.6
5	44±2.0	46±1.5	80±1.2	78±2.5	12±1.0	23±1.5	52±2.5	48±1.5
6	38±1.0	70±1.5	84±4.0	63±2.5	17±0.6	25±1.0	26±1.0	18±1.5
7	32±0.6	37±0.6	48±2.0	42±1.7	15±0.6	26±1.5	38±1.0	28±1.7
Station effect (F- St)	1866.3**				5105.7**			
Season effect (F- Seas.)	676.5**				1809.3**			
Station and Season effect (F-St.*Seas.)	145**				429.4**			

W: Winter, SP: Spring, SU: Summer, A: Autumn.

**Indicates significant at P= 0.01 using two-way Analysis of Variance (ANOVA)

Table 3: Seasonal and regional variations of indicator bacteria of the collected samples (CFU/100 ml ×10^2)

	TC				FC				*E. coli*				FS			
Stations (St.)	W	SP	SU	A	W	SP	SU	A	W	SP	SU	A	W	SP	SU	A
1	6.0±0.2	12.5±0.7	17.5±1.5	8.8±0.2	0.76±0.04	2.5±0.02	4.5±0.2	1.04±0.01	0.6±0.03	1.0±0.01	3.8±0.032	0.7±0.01	0.4±0.02	0.94±0.06	1.5±0.08	0.7±0.03
2	7.3±0.4	18.0±0.8	68±3.0	8.4±0.4	3.4±0.03	6.0±0.35	7.6±0.1	5.6±0.21	2.97±0.015	3.0±0.02	6.0±0.25	2.5±0.15	0.35±0.02	1.04±0.01	4.0±0.15	0.8±0.02
3	12.5±0.9	23.0±1.0	50±1.1	18±1.1	5.0±0.35	9.7±0.3	18.9±0.9	5.0±0.15	2.5±0.01	6.0±0.15	8.0±0.35	1.2±0.01	0.1±0.01	0.53±0.04	1.0±0.12	0.45±0.03
4	4.9±0.15	17.0±1.6	53±2.8	6.0±0.4	2.3±0.2	8.4±0.15	9.0±0.4	3.3±0.25	1.17±0.001	5.0±0.2	5.7±0.07	1.6±0.01	0.31±0.02	0.7±0.03	1.0±0.05	0.42±0.015
5	3.0±0.3	13.7±1.1	21±2.0	5.9±0.9	1.2±0.01	3.1±0.25	5.0±0.3	2.6±0.15	0.56±0.001	1.1±0.01	1.5±0.01	1.3±0.01	0.0	0.3±0.006	0.9±0.04	0.1±0.01
6	2.5±0.1	9.8±0.5	13±0.4	3.0±0.2	1.0±0.01	9.6±0.3	11.0±0.65	2.6±0.01	0.78±0.012	5.0±0.16	7.7±0.068	1.0±0.006	0.45±0.02	0.62±0.01	1.0±0.015	0.49±0.03
7	6.0±0.3	10 ±0.06	12±0.6	7.0±0.2	1.4±0.015	4.4±0.2	6.2±0.29	2.7±0.021	0.65±0.01	1.4±0.01	3.0±0.042	1.2±0.01	0.2±0.02	1.82±0.02	2.0±0.01	0.3±0.02
Total mean	6.03±0.3	14.9±0. 9	33.5±1.6	8.2±0.5	2.2±0.09	6.3±0.21	8.9±0.41	3.3±0.12	1.3±0.01	3.2±0.08	5.1±0.12	1.4±0.03	0.3±0.01	0.9±0.02	1.6±0.07	0.5±0.02
Station effect (F- St)	2436.3**				2219.5**				6031**				3663**			
Season effect (F- Seas.)	571.2**				857.3**				1806.9**				967**			
Station and Season effect (F-St.*Seas.)	240.4**				160.2**				426.3**				430.9**			

Tc: Total coliform, FC: Fecal coliform, FS: Fecal streptococci, W: Winter, SP: Spring, SU: Summer, A: Autumn.

Values are presented in mean (CFU/100ml) ± Standard deviations.

**Indicates significant at P= 0.01 using two-way Analysis of Variance (ANOVA)

Table 4: Seasonal and regional variations of pathogenic bacteria of the collected samples (CFU/100 ml)

	P. aeurginosa				*Salmonella* sp.				*S. aureus*			
Stations (St.)	W	SP	SU	A	W	SP	SU	A	W	SP	SU	A
1	60±2.52	120±5.5	470±3.1	240±5.5	47±1.5	90±3.5	104±4.5	188±2.5	56±3.5	90±3.0	315±8.0	62±2.5
2	40±1.5	146±4.0	620±9.5	146±4.0	0	76±2.1	71±4.6	60±5.0	260±6.5	272±4.0	380±6.5	116±4.0
3	20±0.58	67±3.5	380±8.5	76±2.5	76±1.0	122±2.5	97±3.0	70±3.5	124±4.0	320±5.5	330±7.5	100±3.0
4	30±1.5	45±2.5	310±1.5	280±5.5	30±2.5	69±1.7	110±5.5	80±7.5	98±4.5	330±8.0	250±4.0	104±3.5
5	20±0.6	100±6.0	140±4.5	116±4.5	10±1.0	160±1.5	172±6.5	120±8.0	46±3.5	86±4.0	140±4.0	120±7.5
6	49±1.2	79±3.5	126±2.5	92±2.5	32±1.9	170±2.0	134±6.1	89±6.6	69±4.5	300±6.0	360±3.5	80±0.6
7	79±4.0	100±7.0	128±9.0	120±3.5	0	98±1.5	236±4.0	78±3.0	45±3.0	80±1.5	290±6.5	76±2.0
Total mean	43±2.0	94±5.0	311±6.0	153±4.0	28±1.0	112±2.0	132±5.0	98±5.0	100±4.0	211±5.0	295±6.0	94±3.0
Station effect (F- St)	13312**				2573.6**				8305**			
Season effect (F- Seas.)	2133.5**				394.9**				1735.5**			
Station and Season effect (F-St.*Seas.)	1394.7**				334.3**				570.3**			

W: Winter, SP: Spring, SU: Summer, A: Autumn.

Values are presented in mean (CFU/100ml) ± Standard deviations.

**Indicates significant at P=0.01 using two-way Analysis of Variance (ANOVA)

expressed by relatively long vector which roughly pointed into the same direction, whereas arrow pointing into the opposite direction indicates a negative correlation. In winter, *Salmonella* sp. was strongly influenced by BOD whereas, *P. aeurginosa* and FS were negatively correlated with temperature (Fig. 3A).

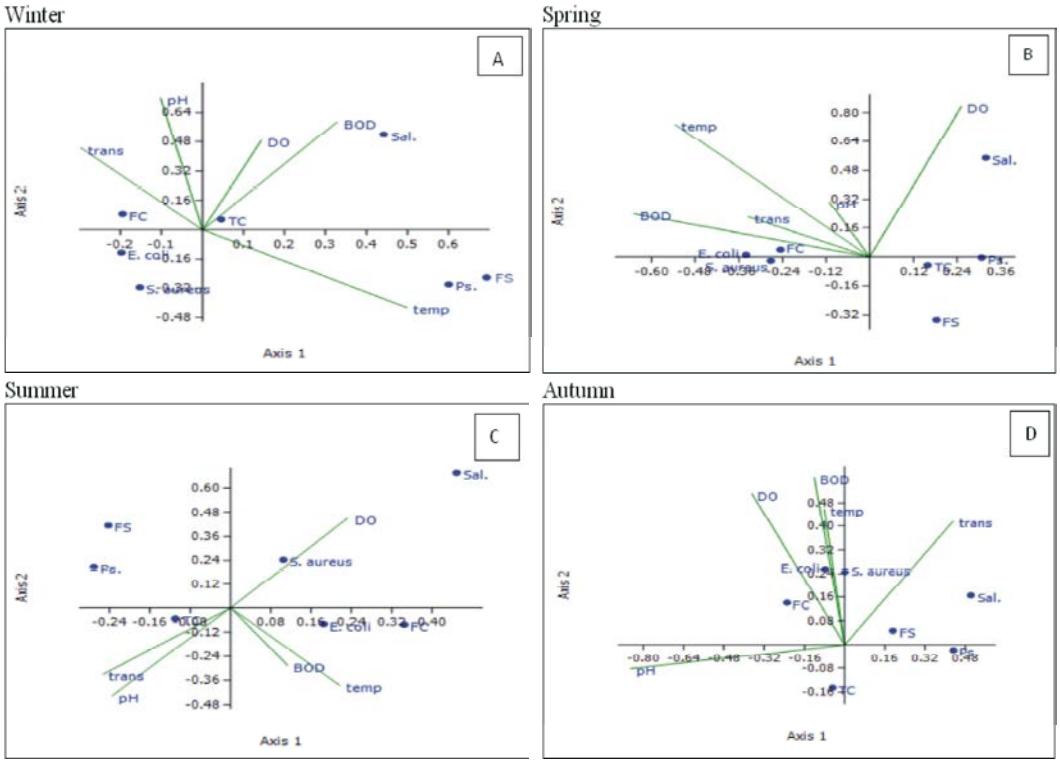

Fig. 3: Biplot of Canonical Correspondence Analysis (CCA) showing the relationships between bacterial indicators and physicochemical water characters. Symbols (Tc): Total Coliform, (FC): Fecal Coliform, (FS): Fecal Streptococci, (Ps.): *Pseudomonas* sp., (Sal.): *Salmonella* sp., (*E. coli*): *E. coli*, (St.): *Staphylococcus aureus*

In summer a positive correlation was observed between *S. aureus* and DO, in contrary a negative correlation was observed between TC with pH and transparency (Fig. 3C). In autumn, the strongest positive correlation was between *E. coli* with temperature and BOD (Fig. 3D).

DISCUSSION

The quality of natural water is generally governed by various physicochemical parameters. Contaminated water is a major source of gasterointestinal microbial pathogens and causes numerous water borne disease outbreak. The occurrence of water-borne enteropathogenic microbes in surface water is associated with fecal contamination of surface water sources [12].

Water temperature is one of the most important environmental parameters that play a prominent role in regulating nearly all physicochemical characteristics of water as well as biological productivity [13] and also in controlling the nutrient input and turnover. Temperature changes depend mainly on the climatic conditions, sampling times and the number of sunshine hours [14]. Recorded water temperature showed obvious seasonal variation with minimum in winter and maximum in summer. These findings go in harmony with Ghannam *et al*. [1].

The transparency of natural waters is an indicator of productivity. The extent to which light can penetrate depends on the transparency of standing water column. Further, transparency of water is inversely proportional to turbidity, created by suspended inorganic and organic matter [15]. The transparency of water body is affected by the factors like planktonic growth, rainfall, sun's position in the sky, angle of incidence of rays, cloudiness, visibility and turbidity due to suspended inert particulate matter. Our results revealed that the lowest water transparency value was recorded during spring (10 cm) while the highest value (65 cm) was obtained during winter. These results agree with those reported by Balkhi *et al.* [16]. This could be attributed to different factor: plankton population [17], setting of materials in calm weather [18], suspension of phytoplankton in water [19], glacial silt [20].

pH value represents the instantaneous hydrogen ion activity and affects biological and chemical reactions in a water body and an important factor that determines the

suitability of water for various purposes including toxicity to animals and plants. In our study, pH values for the collected samples were within the permissible limits of law 48/1982 (7-8.5) except for all stations in winter season. The increase in pH could be related to photosynthesis and growth of aquatic plants where photosynthesis consumes CO_2 leading to arise in pH values [21].

Dissolved oxygen (DO) is one of the key ecological factors. Its deficiency directly affects the ecosystem of a river due to bioaccumulation and biomagnifications. In our collected samples DO concentrations exceeded the permissible limits of law 48/1942 (> 5 mg/l) where they ranged from 3.16 -15.61 mg/l. Depletion in DO might indicate high organic matter and nutrients load as reported by El-Gamel and Shafik [22]. The depletion of DO could be anticipated to the higher rate of microbial decomposition of the excessive organic matter discharged directly into water body which in turn is controlled by temperature [23, 24].

Biological oxygen demand (BOD) is a measure of the amount of dissolved oxygen removed from water by aerobic bacteria for their metabolic requirements during the breakdown of organic matter [25]. Measurement of BOD is used to determine the level of organic pollution in water. Obtained data recorded higher BOD values in most investigated sites, ranging from 0.99-14.09 mg/ ml that violate permissible limits of 10 mg/ml as stated by law 48/1942. High BOD values may be due to the high level of organic matter loaded from sewage, industrial or urban discharge as reported by Chapman [26].

The maximum TVBCs at both 22°C and 37°C were detected during summer compared with other seasons, reflecting the effect of high content of organic matter. These agree with previously reported by several authors El-Hawarry *et al*. [27]; El-Taweel *et al*. [28]; Shaban and El-Taweel [29]; Sabae and Rabeh, [30] and Abdo *et al*. [31]. Total bacteria developing at 22°C are saprophytic types and non-pathogenic to human beings, while those developing at 37°C are mainly parasitic types derived from the soil, sewage, or excreta [32]. This may explain why the numbers of bacteria counted at 22°C were much higher than those determined at 37°C.

The occurrence of indicator bacteria is used as sanitary parameters for evaluation of water quality [33]. Coliform group have been served as the traditional indicators of the presence or absence of enteric viruses in water and wastewater [34]. As regards seasonal variations showed that the high counts of bacterial indicators (TC, FC, *E. coli* and FS) were detected in the warmer seasons (spring and summer), Abuo-Shady *et al*. [35] and Isobe *et al.* [36] attributed increasing of bacterial counts in warmer seasons to high temperature and the discharged waste water during these seasons. According to the guideline criteria for fecal indicator organisms of WHO [37] which accept the guide values of the investigated bacteria up to 500 CFU/100 ml for total coliforms and 100 CFU/100 ml for both fecal coliforms and fecal streptococci. The survey of the indicator bacteria along the studied area of Al-Bahr El-Pherony waters revealed that the water is subjected to sewage pollution.

Counts of pathogenic bacteria in the examined surface water were very high especially in summer season. The high number of *Salmonella, P. aeurginosa* and *S. aureus* in the collected water samples were in agreement with many researchers Pianetti *et al*. [38] and Clark *et al*. [39]. This might be due to that the surface water received high amounts of human sewage and/ or due to the presence of small animal farms. Higher levels of studied pathogenic bacteria were during the warmest seasons. Such finding is in agreement with that reported by Pianetti *et al*. [38]. The seasonal variation in counts of pathogenic bacteria may be due to biological, physical and chemical factors which in turn correlate to many environmental and climatic factors.

The application of multivariate techniques (CCA) revealed and confirmed that the seasonal variation of physicochemical factor controls the dominance of the tested bacteria in El-Bahr El-Pherony water. Many authors have reported correlations of physicochemical parameters in water meeting the bacterial regulations [40-44].

CONCLUSION

The obtained results of the present study concluded that the water quality along the studied area in El-Bahr El-Pherony was remarkably influenced by wastewater discharge from drains located on its sides regarding both physicochemical and bacteriological characteristics. Agricultural and sewage wastes are the key factors in this environmental problem. The water of Al-Bahr El-Pherony is subjected to fecal pollution and continuous monitoring of microbial quality of water is recommended to control the spreading of pathogens transmitted by contaminated water.

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