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Impact of lining material on chemical and microbial irrigation water quality of Nubaria canal, Egypt

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ABSTRACT

Objective: To assess the effect of lining material (cement) of Nubaria canal (Beheira Governorate, Egypt) on its water quality.

Methods: Trace metal ions (Fe, Zn, Cu, Pb, and Cd) and bacterial indictors for water samples collected from two types of stations (lined and unlined) during successive four seasons were analyzed. The effect of lining on bacterial indicators; total viable bacterial count at 22 and 37 °C, total coliform, fecal coliform and fecal streptococci and presence of some bacterial species were studied.

Results: Bacterial indicators and trace metals showed seasonal variations, where the highest values were recorded during summer. A significant reduction for Cu (P < 0.05), Zn (P < 0.01) and Cd (P < 0.001) was recorded in lined stations compared to those of unlined ones. Bacterial indicators recorded the lowest counts in lined stations during all seasons, while there was a significant reduction (P < 0.05) between total coliform values (1.70 \pm 0.50) in lined stations and unlined ones (3.57 \pm 1.01) during summer. *Escherichia coli* bacteria were predominant in water samples of Nubaria canal, where it recorded 34.4% of bacterial isolates.

Conclusions: Lined material plays a role for reducing the bacterial growth and metals concentration, therefore the lining of canal helps in preventing the discharge of sewage pollution into canal.

1. Introduction

Water quality monitoring has one of the highest priorities in environmental protection policy. Its main objective is to control and minimize the incidence of pollutant-oriented problems, and to provide water of appropriate quality to serve various purposes such as potable and irrigation water. The water quality is identified in terms of its physical, chemical and biological parameters[1]. Most available water resources in the world are used for agricultural irrigation. Whilst this level of water use is expected to increase due to rising world population and land use, available water resources are expected to become limited due to the climate change and uneven rainfall distribution[2].

Natural and human factors are causing changes in the physical, chemical and biological of water quality, so the science of water quality will be an issue remained for engineers and scientists for the next years. Water is not found in nature in its pure form but on the

health. There are several compounds in water that are influenced in the physical and chemical quality. Chemical analysis of water samples provides a large number of data that must be analyzed for specific purposes[3].

Therefore, a more logical approach is the detection of organisms normally presenting in the feces of humans and other warmblooded animals as indicators of fecal pollution as well as water treatment and disinfection efficacy. The indicators should respond to natural environmental conditions in water treatment processes. The indicator should be easy to isolate, identify and enumerate

other hand always has some salts, suspended solids and dissolved gases and it causes that water has various features in different

regions. Some of the minerals in water are essential for human

health, while the amount over the limit would endanger human

from the aquatic environment [4.5]. Microbial pollution in aquatic environments is one of the crucial issues with regard to the sanitary state of water bodies used for drinking water supply, irrigation and recreational activities due to a potential contamination by pathogenic bacteria, protozoa or viruses [6].

The total coliforms (TC) have been used to evaluate the general quality of water. These bacteria can be found in the gut of both warm and cold-blooded organisms. Fecal coliform (FC) is a subset

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of this group. They have been characterized to grow at elevated temperatures and specifically associated with the fecal material of warm-blooded animals[7]. These indicator organisms are not necessarily pathogenic, but indicate possible contamination of the water by different pathogens. TC count is commonly used to assess contamination level of water, especially with pathogenic bacteria of intestinal origin[8]. The group TC includes Gramnegative, non-spore forming, rod-shaped bacteria, comprising the genera *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella*. These indicate the general sanitary level of water and indicate the level of faecal contamination[9]. *Escherichia coli* (*E. coli*) is a commensal bacterium in the large intestine of human and other warm blooded animals. The presence of *E. coli* is widely used as faecal pollution indicator in water quality assessment[10].

Water quality impacts from lining leachate are still a concern. When comparing the cost of alternative lining materials available for water main rehabilitation, it is necessary to consider the total cost of the lining material, including the water quality impact cost. The potential for water quality impact of each lining material may vary depending on the characteristics of the lining material, the water, and the effectiveness of lining construction[11]. The impact of concrete liners on bacterial growth as compared to plastic pipes were carried out by Niquette *et al.*[12], asbestos-cement and cemented cast iron, as well as polyvinyl chloride and polyethylene pipes were exposed to different water sources, after which bacterial biomass was measured.

They showed that steel and iron pipes had the greatest biomass, polyethylene and polyvinyl chloride pipes the least, and asbestoscement and cemented cast iron fell in the middle range. This indicated that pipe material can impact biomass growth and therefore water quality. Cement-mortar based pipes also fell between plastic and metal pipes in a study carried out by Camper *et al.*[13] who examined the effects of distribution system materials on bacterial regrowth. A similar investigation in South Africa compared the effect of various pipe materials on bio-film formation[14].

The aim of the present study is to evaluate the effect of lining material of Noburia canal on its chemical and microbiological water quality.

2. Materials and methods

2.1. The study area

Nubaria canal is the largest main canal in West Delta region. It irrigates 470 thousand hectares. The water supplies mounted to roughly 254 m³/s of which 228 m³/s are received from the Nile, and 26 m³/s are from drainage water[15]. Nubaria canal extends 100 km and has an intake barrage and two intermediate barrages at kilometers 28 and 60. This distance of barrages was divided to six stations (three successive unlined and three successive lined) (Figure 1).

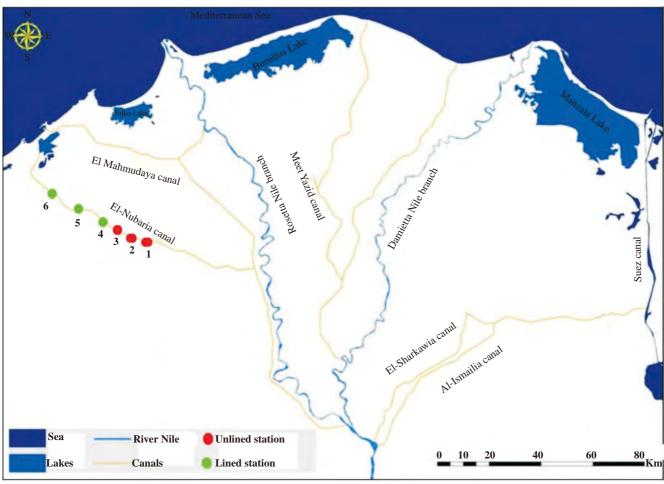


Figure 1. Map showing the sampling stations of Nubaria canal.

2.2. Samples collection

Water samples were collected seasonally during successive four seasons from autumn of 2014 to summer of 2015 from six stations and each station was 3 km with three replicate samples. Samples were collected from subsurface layer by using two sterile plastic containers for chemical and bacteriological analysis. Samples were transported in an icebox to the laboratory to be examined and analyzed.

2.3. Microbiological analysis

2.3.1. Detection of classical bacterial indicators

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 TC, FC, fecal streptococci (FS) and some pathogenic bacteria by using most probable number (MPN) technique by multiple fermentation tube. Briefly, one milliliter from undiluted sample, 1/10 and 1/100 dilution was added to each of triplicate tubes containing 9 mL of MacConkey broth. These tubes were incubated for 48 h at 37 °C for TC and at 44 °C for FC, positive tubes indicated by turbidity in the culture media and/or presence of gas. Positive tubes were confirmed by microscopic examination and biochemical tests. FS was determined by using azide dextrose broth at 37 °C for 48 h, positive tubes was indicated by dense turbidity and formation of purple button[5].

2.3.2. Isolation and identification of Gram-negative bacteria

One hundred and twenty two isolates of Gram-negative bacteria were isolated. Isolates were identified by biochemical characteristics using API 20E strip system (BioMerieux). The isolates were inoculated in strip and incubated for 24 h, the results were recorded to classify isolate[16].

2.4. Chemical analysis of water samples

2.4.1. Physico-chemical parameters

Physico-chemical parameters of water samples; hydrogen ion concentration (pH), electric conductivity (EC), total dissolved salts (TDS) and dissolved oxygen (DO) were measured in sampled station sites by using portable microcomputer meter electrode (HANNA HI 9024, HANNA HI 9635 and HANNA HI 9146).

2.4.2. Metals contents

The concentration of heavy metal ions, Fe, Zn, Cu, Pb and Cd in water samples were determined according to American Public Health Association by using the atomic absorption spectrophotometer (Solar M 600531 v1.27) equipped with deuterium arc background corrector[5].

2.5. Statistical analysis

For statistical analysis, Student's t-test was used to compare

different physico-chemical parameters of different sites representing lined and unlined water bodies, and to compare the mean TVBCs and the MPN of TC, FC and FS in different seasons in lined and unlined watercourses. Means were considered significantly different at P < 0.05, P < 0.01 and P < 0.001.

3. Results

3.1. Chemical analysis

Results in Table 1 revealed that the canal water was slightly alkaline. Also, seasonal variations in pH values ranged from 7.0 to 8.4, 8.0 to 8.3, 8.2 to 8.2 and 8.0 to 7.9 during autumn, winter, spring and summer seasons, respectively. Moreover, mean pH of unlined stations was slightly alkaline (8.2) than lined ones (7.8). In addition, results showed that, the mean DO of lined stations were higher than unlined ones, where it ranged from 4.2 to 5.3 mg/L and 3.8 to 4.4 mg/L for lined and unlined stations, respectively. Our results revealed that the ranges of EC and TDS were from 756 to 919 μ mhos/cm and 530 to 641 mg/L, respectively for lined stations, while for unlined ones ranged from 826 to 953 μ mhos/cm and 537 to 632 mg/L, respectively.

3.1.1. Iron

The iron concentration was high in autumn and low in winter and was ranged from 0.21 to 1.10 mg/L for lined stations and from 0.23 to 1.57 mg/L for unlined ones (Figure 2A).

3.1.2. Zinc

The concentrations of Zn in unlined and lined stations were the maximum (431.2 and 240.5 μ g/L, respectively) during summer, and the minimum (122.9 and 30.3 μ g/L, respectively) during winter (Figure 2B). Moreover, there was a significant reduction (P < 0.01) of Zn during winter in lined stations compared to those of unlined ones.

3.1.3. Copper

The maximum permissible limit for Cu in water according to Environmental Protection Agency (EPA) and World Health Organization (WHO) standard is 1300 and 1500 μ g/L, respectively. The study revealed that the Cu concentration ranged from 19.3 to 57.4 μ g/L for lined stations and 7.2 to 95.2 μ g/L for unlined ones (Figure 2C).

3.1.4. Lead

In most of the time, Pb concentration was higher than the detection limit 50 μ g/L WHO. Only the detectable concentration was found to be 21.6 μ g/L at unlined stations in spring (Figure 2D). Also, the mean levels of Pb in lined and unlined were 144.3 μ g/L and 110.9 μ g/L, respectively.

3.1.5. Cadmium

Levels of Cd concentrations were higher than the permissible level of EPA and WHO (5 and $10 \mu g/L$, respectively), except mean

Table 1

Mean of physico-chemical parameters of collected water samples from lined and unlined stations of Nubaria canal.

Type of	Season	pН	DO (mg/L)	EC (µmhos/cm)	TDS (mg/L)	Fe (mg/L)	Zn (µg/L)	Cu (µg/L)	Pb (μg/L)	Cd (µg/L)
substratum										
Lined	Autumn	7.0 ± 1.4	4.4 ± 0.8	919.0 ± 12.0	641.0 ± 13.0	0.63 ± 0.56	118.1 ± 7.5	34.8 ± 4.2	84.5 ± 3.7	12.4 ± 6.8
	Winter	8.0 ± 0.5	5.3 ± 0.7	880.0 ± 92.0	592.0 ± 74.0	0.21 ± 0.04	$30.3 \pm 5.6^{**}$	19.3 ± 4.7	163.2 ± 60.4	29.3 ± 5.6
	Spring	8.2 ± 0.4	4.2 ± 0.9	756.0 ± 28.0	530.0 ± 19.0	0.79 ± 0.51	185.0 ± 5.6	$23.5 \pm 5.3^*$	$155.5 \pm 22.8^{***}$	7.2 ± 2.6
	Summer	8.0 ± 0.3	4.3 ± 0.6	775.0 ± 13.0	548.0 ± 16.0	1.10 ± 0.22	240.5 ± 5.6	$57.4 \pm 10.7^*$	174 ± 60.4	$710 \pm 0.6^{**}$
	Mean	7.8 ± 0.0	4.6 ± 0.0	832.5 ± 0.0	577.8 ± 0.0	0.68 ± 0.0	143.5 ± 0.0	33.8 ± 0.0	144.3 ± 0.0	189.8 ± 0.0
Unlined	Autumn	8.4 ± 0.2	4.2 ± 1.4	953.0 ± 23.0	632.0 ± 32.0	1.57 ± 1.43	283.2 ± 126.5	76.3 ± 38.4	50.5 ± 24.2	5.1 ± 2.5
	Winter	8.3 ± 0.3	4.4 ± 1.3	887.0 ± 40.0	537.0 ± 152.0	0.23 ± 0.03	122.9 ± 36.2	12.2 ± 9.4	189.0 ± 55.3	27.3 ± 5.6
	Spring	8.2 ± 0.2	3.9 ± 1.2	861.0 ± 85.0	601.0 ± 57.0	0.25 ± 0.07	176.5 ± 60.4	7.2 ± 3.7	21.6 ± 12.1	6.7 ± 0.3
	Summer	7.9 ± 0.3	3.8 ± 0.8	826.0 ± 34.0	578.0 ± 21.0	0.96 ± 0.25	431.2 ± 155.6	95.2 ± 15.1	182.5 ± 48.3	814 ± 32.2
	Mean	8.2 ± 0.0	4.1 ± 0.0	81.8 ± 0.0	587.0 ± 0.0	0.75 ± 0.0	253.5 ± 0.0	47.7 ± 0.0	110.9 ± 0.0	213.3 ± 0.0

Values are represented as mean \pm SD. *: Significant (P < 0.05) relative to unlined sites; **: Highly significant (P < 0.01) relative to unlined sites; ***: Extremely highly significant (P < 0.001) relative to unlined sites.

during spring in lined stations (7.2 μ g/L), unlined ones (6.7 μ g/L) and during autumn in unlined stations (5.1 μ g/L) (Figure 2E). Moreover, there was a significant reduction (P < 0.01) of Cd during summer in lined stations (710 μ g/L) compared to those of unlined ones (814 μ g/L).

3.2. Bacteriological analysis

Present results of the effect of lining material of Nubaria canal on TVBCs at 22 °C and 37 °C of water samples collected from lined and unlined stations during different seasons were presented in Figure 3. Results revealed that the TVBCs of water samples that collected from lined stations showed lower counts than unlined ones during all seasons. Moreover, mean log of TVBCs of lined stations (1.8 × 10⁷ CFU/mL) at 22 °C showed significant reduction (P < 0.05) relative to unlined ones (3.5 × 10⁷ CFU/mL) during autumn season. The lowest log values of TVBCs at 22 °C and 37 °C were recorded during winter in lined stations (1.1 × 10⁷ and 1.9 × 10⁷ CFU/mL, respectively) and unlined ones (2.4 × 10⁷ and 1.9 × 10⁷ CFU/mL, respectively). Meanwhile, the highest TVBCs at 22 °C and 37 °C were detected during spring in lined stations (2.7 × 10⁷ and 2.5 × 10⁷ CFU/mL, respectively) and unlined ones (4.2 × 10⁷

and 3.9×10^7 CFU/mL, respectively).

Present data in Figure 4 declared obvious bacterial contamination in all sampling stations. Among the three primary bacterial indicators, TC represented the highest count, followed by FC and FS for both lined and unlined stations during all seasons. In unlined stations, the mean log of MPN bacterial count per 100 mL of TC during autumn, winter, spring and summer seasons [(3.66 \pm 0.70), (2.20 \pm 0.70), (4.60 \pm 1.20) and (3.57 \pm 1.20) log MPN/100 mL, respectively] were higher than those for lined ones [(1.87 \pm 0.40), (1.20 \pm 0.10), (2.80 \pm 0.50) and (1.70 \pm 0.70) log MPN/100 mL, respectively].

The present investigation showed that bacteriological data obtained throughout four seasons survey revealed the role played by seasonal variations in pollution level. The highest counts were recorded in warm seasons (spring > summer > autumn), while the lowest values were found in winter. Generally, present results indicated that TC, FC and FS values were lower in lined sites than those in unlined ones during all seasons (Figure 4). There was a significant reduction between TC value [(1.70 \pm 0.50) log MPN/100 mL] in lined sites and those in unlined ones [(3.57 \pm 1.01) log MPN/100 mL] during summer (P < 0.05). Meanwhile, during spring, a significant

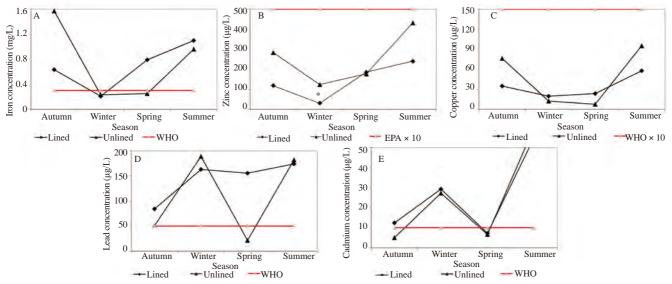
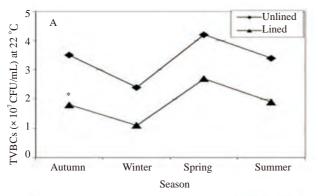


Figure 2. Seasonal variation of heavy metal ions in water samples at different stations of Nubaria canal and standard of WHO and EPA. A: The seasonal variation of Fe; B: The seasonal variation of Cu; D: The seasonal variation of Pb; E: The seasonal variation of Cd. *: *P* < 0.01 relative to unlined stations.

reduction (P < 0.05) of FC value was observed in lined sites [(1.0 ± 0.6) log MPN/100 mL] compared to those in unlined ones [(2.5 ± 0.6) log MPN/100 mL]. However, it was found that during winter, the mean values of the three types of bacteria TC, FC and FS were significantly lower in lined sites [(1.20 ± 0.10), (0.16 ± 0.02) and (0.07 ± 0.02) log MPN/100 mL, respectively] than those in unlined sites which represented by (2.20 ± 0.70), (0.95 ± 0.40) and (0.40 ± 0.10) log MPN/100 mL (P < 0.05, P < 0.05 and P < 0.01) respectively.



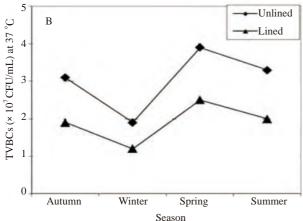


Figure 3. The mean TVBCs at different tempratures of unlined and lined stations of Nubaria canal.

A: The mean TVBCs at 22 °C; B: The mean TVBCs at 37 °C. $^{\circ}$: P < 0.05 relative to unlined stations.

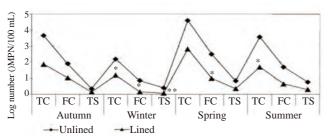


Figure 4. Mean log of MPN of TC, FC and FS/100 mL water of unlined and lined stations of Nubaria canal.

*: P < 0.05, **: P < 0.01 relative to unlined stations.

In the present study, one hundred and twenty two Gramnegative bacterial isolates were isolated and identified from collected water samples from lined and unlined stations, *E. coli* bacteria was predominant in water samples of Nubaria canal, where it recorded 34.4% bacterial isolates, while *Klebsiella pneumonia*, *Proteus penneri*, *Proteus vulgaris*, *Citrobacter* sp., *Pseudomonas aeruginosa* (*P. aeruginosa*), *Acinetobacter* sp. and

Aeromonas jandaei were 20.5%, 13.9%, 11.5%, 9.0%, 6.6%, 2.5% and 1.6% of bacterial isolates, respectively (Figure 5).

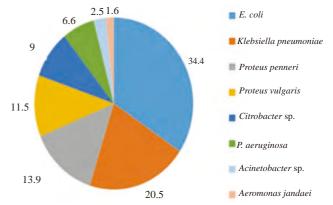


Figure 5. Percent of identified Gram-negative bacterial isolates from water samples collected from unlined and lined stations of Nubaria canal.

4. Discussion

The primary goal of water quality management from a health perspective is to ensure that consumers are not exposed to heavy metals and pathogens that are likely to cause disease.

Results revealed that the canal water was on the alkaline side and seasonal variations in pH values ranged from 7.0 to 8.3. Moreover, mean pH of unlined stations was slightly alkaline (8.2) than that of lined ones (7.8). Results showed that the highest values of DO were recorded in both lined and unlined during winter due to the decrease in water temperature. Generally, the DO of canal water was in normal values of United State Environmental Protection Agency for aquatic life[17].

EC is a function of TDS, it has a similar trend as TDS. Our results revealed that the EC and TDS showed non-significant variation between lined and unlined stations, Nikos *et al.*[18], stated that EC is a good estimator of the total amounts of mineral salts that dissolved in water. It is often used to measure salinity problems related to irrigation of crops and it is known that soils irrigated with saline water will contain a similar mix but usually at a higher concentration than in the applied water. The permissible limits for classes of irrigation water according to WHO guideline for EC revealed that all Nubaria canal stations at any recording time were in class 3 category $(750-2\,000\,\mu\text{mhos/cm})$ [19], permissible water which leaching is necessary process when using this kind of water for irrigation.

Chemical analysis of water samples that collected from unlined stations recorded the highest mean concentrations for Fe, Zn, Cu and Cd than samples of lined ones. But, mean concentrations of Pb for lined stations was highest than unlined ones, which may be attributed to lining material. Generally, there were seasonal variations in the concentrations levels of all tested trace metals in both lined and unlined stations. The highest values were during summer in lined stations. The concentration of Fe was found remarkably higher than other metals at all of the stations of Nubaria canal throughout the study period. The iron concentration was high in autumn and low in winter. The mean concentrations of Fe for lined and unlined stations were higher than the maximum permissible limit for Fe in water (0.3 mg/L) according to WHO and EPA standard[4,20]. The concentrations of Zn in unlined and lined stations were the maximum during

summer, and the minimum during winter. Moreover, there was a significant reduction (P>0.01) of Zn during winter in lined stations compared to those of unlined ones. Jeniffer and Lester worked on the water samples of the Stour River, UK and found the concentration of Zn was within the range of 10 to 325 µg/L[21]. Present levels of the highest concentration of Zn were lower than the limit of maximum permissible level (5000 µg/L) of EPA standard[20]. The maximum permissible limit for Cu in water according to EPA and WHO standard is 1300 and 1500 µg/L, respectively. The study revealed that the Cu concentration ranged from 19.3 to 57.4 µg/L for lined stations and 7.2 to 95.2 µg/L for unlined ones which were lower than the permissible standards.

In most of the time, Pb concentration was higher than the detection limit 50 μ g/L WHO[4]. Only the detectable concentration was found to be 21.6 μ g/L at unlined stations in spring. Also, the mean levels of Pb in lined and unlined stations were much higher than the permissible level for irrigation water as recommended by FAO[22]. Study showed that the levels of Cd concentrations were higher than the permissible level of EPA and WHO standard, except mean during spring in lined stations and during autumn in unlined stations. Moreover, there was a significant reduction (P < 0.01) of Cd during summer in lined stations compared to those of unlined ones.

Present results of the effect of lining material of Nubaria canal on TVBCs revealed that water samples collected from lined showed lower bacterial counts than unlined ones during all seasons, it suggest that the lined material may have properties for decreasing the bacteria growth, and may be attributed to the lining of canal helps for preventing the discharge of sewage pollution into canal. Thus the present results are in agreement with those obtained by Eissa et al. who found that the variations in the bacterial contamination of water was affected by seasonality and potential external sources of pollution during its course in the rural areas of canal[23]. For both workers in agriculture and consumers of crops, microbiological contaminated water constitutes a possible health risk. Irrigation of vegetables and fruits with raw wastewater can serve as a major pathway for bacteria, viruses and protozoa[24]. For this reason, WHO has developed guidelines for maximum allowable number of bacteria in water used for irrigation (≤ 1000 geometric mean number per 100 mL)[19].

Among existing and widely used methods, the presence of TC, FC and FS can be taken as a signal to potential danger of health risks[25]. Present data declared obvious bacterial contamination in all sampling stations. Among the three primary bacterial indicators, TC represented the highest count, followed by FC and FS for both lined and unlined stations during all seasons. In unlined stations, the mean log of MPN bacterial count per 100 mL of TC during autumn, winter, spring and summer seasons were higher than those for lined. Moreover, mean log of TC of the three seasons autumn, spring and summer for unlined stations were higher than the permissible limit (3.50 log MPN/100 mL) of international standard (EPA)[20], while the lined stations showed accepted counts. Concerning FC, the mean log counts for unlined and lined stations were in permissible limit of the international standard recommendation for FC. Bacterial contamination recorded in this study could be attributed mostly to domestic sewage pollution as well as agricultural runoff[16,26].

The present investigations showed that bacteriological data obtained throughout four seasons survey revealed the role played by seasonal variations in pollution level. The highest counts were recorded during warm seasons, while the lowest values were in winter season. These results go well with others which showed that the mean surface water temperature during warm periods is suitable for survival of bacteria[27,28]. Generally, present results indicated that TC, FC and FS values were lower in lined sites than those in unlined ones during all seasons. According to the guidelines in British Columbia, Canada, the geometric mean of five sampling events per month for various indicator organisms in irrigation water should be: $FC < 2.3 \log MPN/100 mL$, E. $coli < 1.8 \log MPN/100 mL$ and faecal enterococci < 1.3 log MPN/100 mL[29]. Also, in the guidelines set by Alberta, Canada, the geometric mean of five sampling events per month for indicator organisms should be: TC < 3.0 log/100 mL, E. coli < 2.3 log MPN/100 mL and enterococci < 1.5 log MPN/100

In the present study, *E. coli* bacteria was predominant in water samples of Nubaria canal, this may be due to domestic solid waste and sewage from various human activities. *E. coli* can be used as bio-indicators of aquatic ecosystem dynamics and determination of their occurrence may help to assess the water quality[10]. Hence the present data showed that the canal water was considered to be unfit for drinking purposes but, it is in accepted values for irrigation purposes. Abdo *et al.* reported that the pathogenic bacteria that isolated and identified from Ismailia canal, Egypt were *E. coli*, *Salmonella* sp., *Choleraesuis*, *Streptococcus faecium* and *P. aeruginosa*[31].

In conclusion, present study of bacterial indicators of water samples collected from lined sites showed lower counts than unlined ones. Moreover, according to the permissible limits for classes of irrigation water in WHO guideline, Nubaria canal water recorded class 3 category (permissible). Chemical analysis of water samples that collected from unlined stations recorded highest mean values for Fe, Zn, Cu and Cd than samples of lined ones. So, lined material of Nubaria canal may play a role for reducing the bacterial growth and heavy metals pollution, where lining of canal helps in preventing the discharge of wastewater into canal.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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