Water Quality, Microbial Assessments and Antibiotic Susceptibility of Pathogenic Bacteria of Ismailia Canal, River Nile, Egypt

[1]Abdo, M. H.; [1]Sabae, S. Z.; [2]Haroon, B. M.; [2]Refaat, B.M. and [2]Mohammed, A. S.

1: National Institute of Oceanography and Fisheries, Inland Waters and Aquaculture Branch.
2: Faculty of Science, Al-Azhar University
mh_omr@yahoo.com

Abstract: 32 water samples were collected during four successive seasons to covering the area under investigation which represents the effect of the effluent factories discharged on the Ismailia Canal water quality. Physical parameters (air and water temperature, transparency and electrical conductivity) and chemical parameters (pH, DO, BOD, COD, CO_3^-, HCO_3^-, SO_4^{--}, NO_2^-, NO_3^-, NH_3 and PO_4^{3-}) were measured to identify the Ismailia Canal water quality. These measurements showed slight variations during different seasons at different stations. On the other hand, the bacteriological analyses included the total variables bacterial counts at 22 and 37 ^{0}C and the bacterial indicators of faecal pollution (*total coliformas, faecal coliforms* and *faecal streptococci*). The pathogenic bacteria were identified as *E. coli, Salmonella, Choleraesuis, Streptococcus faecium and Pseuedomones aeruginosa.* Antibiotics susceptibility testing selected the families Beta-lactams (amipicillin & cefeprime), Aminology cosides (gentamycin & Kanamycin), Macrolides (erythromycin, spiramycin, tylosin and spectinomycin), Tetracyclines (oxytetracycline base, doxycycline HCl and chlorotetracycline HCl) and amino acides (neomycin & streptomycin). In addition to, all pathogenic bacterial isolates which revealed resistance against most applied antibiotics were subjected to fifteen herbal extracts. The test herbal extract extent antimicrobial activity, *P. aeruginosa* was sensitive to coriander and *E. coli* was sensitive to Cinnamon.

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Key words: water quality, microbial assessments, antibiotic susceptibility, Ismailia Canal, Egypt.

1. Introduction

Ismailia Canal is one of the most important irrigation and water supplies canal in Egypt. It was constructed in 1862. It transports fresh water from River Nile at north of Cairo at El-Mazalat region to Ismailia, Port Said and Suez governorates. The original canal dimensions are average 2.1 m depth and 18 m width; it extends for about 128 km and has more than one regulator bridges constructed along the total length. It is worthy to note that some factories are constructed in this area throw its wastes into the canal, which cause dramatic changes in its water quality **(Abdo, 1998).**

The quality of water is now the concern in all countries of the world. The decision of WHO's 29th session (May, 1976) emphasized that the water delivered to the consumer should meet the high requirements of modern hygiene and should at least be free from pathogenic organisms and toxic substance **(Voznaya, 1983 and Abdo, 2005).**

Aquatic ecosystem are much too complex and integrated to be simply, regulated by single nutrient. Metabolism, growth, productivity and behavior are certainly regulated by many organic compounds in addition to traditional macro factors controls e.g. nutrients **(Wetz *et al.*, 2000).**

In fact, attention has been paid for monitoring and assessing the microbiological quality of water resources all over the world **(Lindskog and Lindskog, 1988; Fernandez-Alvars *et al.*, 1991; Khalafalla *et al.*, 1993).** The industrial waste water represented the main source of water pollution in different parts of the world, e.g. Egypt **(Abu-Shady *et al.*, 1996; Sabae *et al.*,2006; Sabae *et al.*, 2008)**; Poland **(Niewolak, 2000);** Nigeria **(Ekhaise and Anyasi, 2005; Akaninwor *et al.*, 2007);** Brazil **(Gunkel *et al.*, 2007).**

A powerful monitoring program is needed to provide reliable information about the current water quality. Thus, the present study aimed to evaluate the physico- chemical and microbiological water quality of Ismailia Canal and to estimate the antibiotic sensitivity against the isolated pathogenic bacteria. Besides, screening for its sensitivity against some herbal extracts.

2. Materials and Methods

Collection water samples:-

Water samples were collected seasonally from Ismailia Canal during the period starting from August 2005 to April 2006. Four stations were selected to represent different effluents discharged into the canal. Eight water samples were collected during each season (one sample before and the other

after each station to know the effect of the effluents on the water quality of the canal). These stations are representing on the map of the Ismailia Canal, Fig. (1). The location stations as follows:-

Station I: The mouth of the canal branched from River Nile at El-Mazalat square.
Station II: Next to mouth of the canal, in front of Aghan Khan Region.
Station III: In front of Aboud terminal bus station.
Station IV: Opposite to Aboud central police camp.
Station V: Before Al-Amyeria water purification plant.
Station VI: After Al-Amyeria water purification plant.
Station VII: Before the petroleum pipes companies at Mostoroud area.
Station VIII: Next to the petroleum pipes companies at Mostoroud area.

The water samples were collected from the subsurface layer of the midstream of the Canal (at depth 30 cm) by using polyvinyl Van Dorn plastic bottle. The water samples were kept in well stopper polyethylene plastic bottles transferred immediately in an ice-box to be analyzed.

All physical and chemical parameters of the water samples were measured according to **(APHA, 2002).** CO_3^{--} and HCO_3^- were measured by titration against 0.025 N H_2SO_4 and using phenolphethaline and methyl orange indicators. SO_4^{--} was determined by turbidity method. The dissolved oxygen (DO) was performed by azide modification, COD by potassium permanganate oxidation and BOD by 5 days incubation method. The concentrations of NO_2^-, NO_3^-, NH_3 and PO_4^{3-} were determined by colorimetric techniques with formation of reddish purple azo-dye, copper and hydrazine sulphate reduction, nessler and stannous chloride reduction methods.

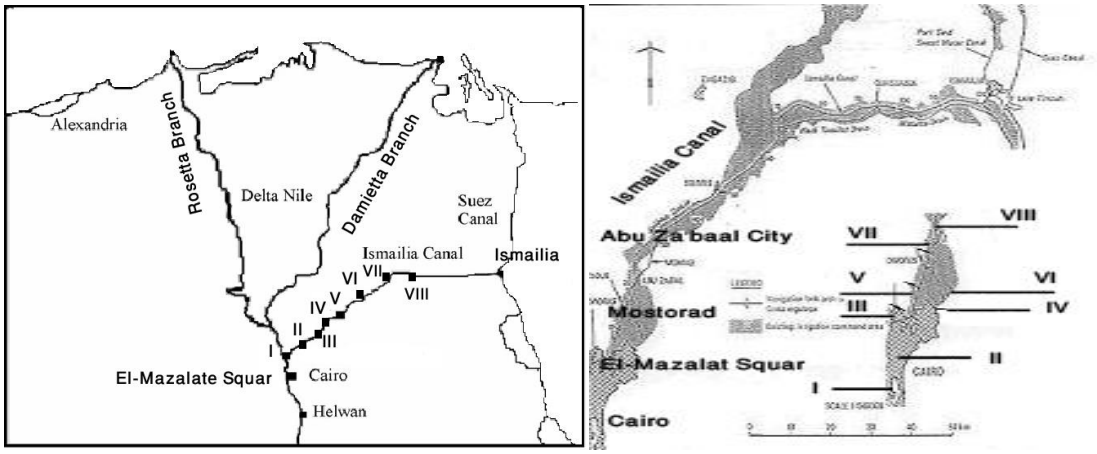

Fig. (1). The sampling locations of Ismailia Canal.

All absorbance measurements were determined using spectrophotometer Model LKB Biochem Ultraspec II.

Electrical conductivity, air and water temperatures as well as pH were measured using Hydro-Lab., Model "Multi 340 II SET". Transparency of water measured using Secchi-Disc diameter 25 cm in the field.

Bacterial Examinations:

Water samples which would be used for bacteriological analyses were collected in 500 ml sterilized glass bottles. The sterilized bottle was opened under the water surface to avoid contamination with air. The collected samples were transferred immediately in an ice-box to the laboratory to be analyzed within a maximum period of 8 hours delay.

The total viable bacterial count was detected using spread-plate technique **(APHA, 2002).** The viable bacterial count was determined using plate count agar medium. The media was sterilized and poured into Petri dishes (20 ml/plate) under aseptic conditions, after solidifications the plates were inverted and kept till use. Each water sample diluted serially (10 fold) to cover the range of 10^{-1} to 10^{-8} in sterile diluents (0.1% peptone W/V). Tenth ml of each dilution was spread on each plate surface using glass spreader. Three replicate of each dilution was used. The plates were incubated at 22°C and 37°C for 24 and 48 hrs, respectively.

The total and faecal coliforms were enumerated in MacConky broth medium, according to the procedure recommended by **Eaton *et al.*, (1995).** For presumptive test, 3 sets of tubes were prepared: five tubes each containing 10 ml of double strength broth **(APHA, 2002)** were inoculated with 10 ml water sample, five tubes containing 5 ml of single strength broth were inoculated with 1ml of water sample, and

the remaining 5 tubes containing 5 ml of single strength broth were inoculated with 0.1 ml of water sample. After incubation at 37 °C for 48 hours, the MacConky broth tubes were observed for acid and gas production. The total coliform numbers were estimated using the MPN index. Tubes with a positive presumptive reaction were submitted to the confirmed stage. Sub-cultures from positive tubes were incubated in a water-bath at 45.5 ^{0}C for 24 - 48 hr.; such tubes were again observed for acid and gas production. A number of positive tubes were used to calculate the MPN for faecal coliforms. Completed test using eosin methylene blue (EMB) agar was performed. Plates were incubated at 37 ^{0}C for 24 - 48 hr.; metallic sheen or pink with dark centre colonies on EMB agar indicated positive results.

The recommended method of **APHA (2002)** for detection and counting faecal streptococci was applied. Azide dextrose broth medium in tubes was inoculated with the suitable serial decimal dilutions of water samples, following the same procedure of total coliform. Inoculated tubes were incubated at 37 ^{0}C for 48hr. A confirmation test was made by transferring three loops from the positive tubes (those developed bacteria growth turbidity) to ethyl violet azide broth and incubated at 37 ^{0}C for 72 hr. Positive tubes were those having a slight turbidity accompanied with purple bottom.

The MPN of *P. aeruginosa* was determined using three replicate tubes containing L-asparagine broth medium double and single strengths were used for 10, 1 and 0.1 m respectively. All tubes were inoculated and incubated at 37^{o}C for 7 days. Positive results scored as green fluorescent pigment due to the growth of *P. aeruginosa*.

Enumeration of S.S bacteria (Salmonella and Shigella) using S.S agar medium, inoculate 1 ml of the sample and incubate at 37 ^{0}C for 48 hours. Typical Salmonella and Shigella colonies are pink with or without black centers.

Antibiotic sensitivity method:

Three similar colonies were transferred to 4 – 5 ml of Müeller Hinton broth. The culture was incubated at 35 ^{0}C for 2 – 6 hours to develop a turbidity of approximately 1 to 2 x 10^8 cfu/ml. Müeller Hinton agar plates were incubated with the organism grown in the broth by streaking with sterile swab, and the plates were left for 15 minutes to allow the complete absorption for the incubation before applying the antibiotic discs. The plates were incubated for 16 - 18 hours, after incubation the minimal inhibitory concentration **(MIC)** was detected for each antibiotic that makes inhibition zone.

Three successive ten-fold dilutions were used of each antibiotic 100, 10 and 1 µg/ml. The antibiotic discs prepared by saturating a disc of filter paper Watman No.1 of 0.6 cm diameter with the antibiotic, the discs then dried at 45 ^{0}C for 15 minutes.

Different antibiotic families were applied against all bacterial isolates. The antibiotic families were **Beta-lactams** (ampicillin & cefepime), **Aminoglycosides** (gentamycin & kanamycin), **Macrolides** (erythromycin, spiramycin, tylosin and spectinomycin), **Tetracyclines** (oxytetracyline base, doxycycline HCl and chlortetracycline HCl), and **Amino acids** (neomycin & streptomycin).

Some antibiotic mixtures are also, applied against all isolates these mixed antibiotics are; Ticarcillin/Clavulanic acid, Pipracillin/Tazobactam, Ampicillin/ Sulbactam and Ceftazidime/Clavulanic acid.

Identification of the bacterial isolates:

The most resistant bacterial isolates Nos.3, 18, 43 and 56; were identified according to the keys of the Bergy's Manual of Systematic Bacteriology, **(Holt *et al.*, 1986).** These tests including: Gram staining, spore staining, motility and biochemical tests; Indole, citrate utilization, urease, gelatinase, catalase, methyl red…etc.

Herbal study:

Plant extracts from some herbs are used against the most resistant isolates for the detection of antibacterial activity of these extracts. The plants used are; **Dill** (*Anethum graveolens*), **Parsley** (*Petroselinum crispum*), **Peppermint** (*Menthax piperta*), **Cinnamon** (*Cinnamomum verum*), **Anise** (*Pimpinella anisum*), **Hibiscus**, **ginger, Absinth**, **Coriandrum**, **Black Pepper**, **Chamomile**, **Nigella**, **Licorice**, **Sage, Rosemary**. The extraction method was carried out by grinding the plant well using electrical blender, add 20 ml pure ethyl alcohol on 5 gm of the plant (except for; Sage, Chamomile, Rosemary add 50 ml of ethyl alcohol), soak for 2 hours with shaking and then filter the mixture using filter paper Watman No.1. Filter paper discs 0.6 cm diameter was exposed for saturation with the extracts and let them to dry.

Three similar colonies were transferred to 4 - 5 ml of Müeller Hinton broth. The culture was incubated at 35 ^{0}C for 2 - 6 hours to develop a turbidity of approximately 1 to 2 x 10^8 cfu/ml. Müeller Hinton agar plates were incubated with the organism grown in the broth by streaking with sterile swab, and the plates were left for 15 minutes to allow the complete absorption for the incubation before applying the plant extracts discs. The plates were incubated for 16 - 18 hours. Clear zone around the disc indicates that the plant has antibacterial activity, otherwise it has no effect.

Statistical analysis:-

Correlation coefficient "r" between physico-chemical, bacteriological as well as between

investigated bacteria and hydrological parameters was calculated for testing the relationships between variables using Microsoft Excel (2003).

3. Results

Seasonal variations of physico-chemical characteristics of Ismailia Canal water were recorded in Tables (1 – 4).

Seasonal variations in air and water temperatures were found to be 30.5 - 33.9 & 26.5 - 35.4 and 19 - 23 & 19 - 27 and 18 - 19.5 & 17.5 - 29 and 23.8 - 30 & 23.8 – 32.4 ^{0}C for air and water during summer, autumn, spring and winter seasons respectively.

Seasonal variations of water transparency in illustrated that the values were ranged between 65 - 130, 50 - 125, 70 - 140 and 60 - 100 cm during summer, autumn, winter and spring respectively. The highest value reached to 140 cm at station II during winter while the lowest value was 50 cm at station VIII during autumn.

Table (1): Variations of physical and chemical characteristics of Ismailia Canal water during summer 2005

Stations / Parameters	I	II	III	IV	V	VI	VII	VIII	Regional Average
Air Temp. (^{0}C)	33.9	33.9	30.8	30.5	30.5	34.2	34.0	32.5	32.53
Water Temp.(^{0}C)	26.5	26.3	27.1	27.8	28.8	28.7	27.3	35.4	28.36
Trans. (cm)	120	130	90	85	95	75	85	65	93
EC (µmohs/cm)	324	264	317	295	337	320	367	412	329.5
pH	7.98	7.70	7.98	7.94	7.95	7.99	7.99	7.96	7.93
DO (mg/l)	10.2	10.2	9.6	10.4	11.0	11.4	12.6	10.2	10.70
BOD (mg/l)	1.3	0.8	2.6	3.2	2.8	6.0	4.7	4.4	3.22
COD (mg/l)	11.1	4.2	8.8	17.8	14.7	19.9	15.5	27.4	14.92
CO_3^{--} (mg/l)	ND	ND	ND	ND	ND	ND	ND	ND	ND
HCO_3^- (mg/l)	190	199	207	195	200	211	166	253	202
SO_4^{2-} (mg/l)	32.7	21.8	29.1	34.6	31.1	36.7	30	33.1	31.13
NO_2^- (µg/l)	7.19	5.10	8.06	6.54	14.38	9.15	4.8	7	7.76
NO_3^- (µg/l)	15.40	13.02	16.03	19.56	21.20	18.42	27.83	23.12	19.31
NH_3 (mg/l)	1.93	1.25	1.99	2.20	2.16	2.24	1.81	1.95	1.94
PO_4^{3-} (µg/l)	26.6	28.6	35.8	57.2	23.5	18.4	24.5	60.6	30.650

ND: Not Detected

Table (2): Variations of physical and chemical characteristics of Ismailia Canal water during autumn 2005

Stations / Parameters	I	II	III	IV	V	VI	VII	VIII	Regional Average
Air Temp. (^{0}C)	21.0	21.8	20.5	19.0	21.0	20.5	21.6	23.0	21.05
Water Temp.(^{0}C)	19.0	19.5	18.5	18.8	18.0	17.9	17.6	27.0	19.53
Trans. (cm)	110	125	90	80	90	70	95	50	88.75
EC (µmohs/cm)	356	311	326	318	339	341	398	464	356.62
pH	8.15	7.92	8.10	8.16	7.94	8.15	8.00	7.84	8.03
DO (mg/l)	10.0	9.2	11.2	10.4	11.2	11.9	13.6	10.8	11.03
BOD (mg/l)	3.2	1.0	3.6	3.6	3.0	3.9	3.6	4.40	3.28
COD (mg/l)	10.8	6.7	11.0	17.6	16.1	21.3	18.1	32.5	16.88
CO_3^{--} (mg/l)	11.5	3.8	11.0	13.0	11.8	12.4	11.6	15.1	10.01
HCO_3^- (mg/l)	219	271	255	250	241	272	261	233	190.6
SO_4^{2-} (mg/l)	37.0	39.9	37.0	36.6	40.1	45.2	41.3	47.7	31.1
NO_2^- (µg/l)	9.80	4.36	11.65	10.24	11.11	11.37	11.80	11.77	10.26
NO_3^- (µg/l)	21.10	18.02	18.65	16.60	20.0	28.42	27.80	23.12	22.84
NH_3 (mg/l)	1.18	0.91	1.00	1.28	0.83	0.80	0.67	1.64	1.042
PO_4^{3-} (µg/l)	49	87	75	104	80	95	101	131	90.25

Table (3): Variations of physical and chemical characteristics of Ismailia Canal water during winter 2005

Stations / Parameters	I	II	III	IV	V	VI	VII	VIII	Regional Average
Air Temp. (^{0}C)	18.0	18.3	19.2	19.5	19.0	18.6	18.3	19.0	18.73
Water Temp.(^{0}C)	17.0	17.2	16.5	17.0	17.5	18.5	18.5	29.0	18.9
Trans. (cm)	120	140	100	110	100	70	85	70	99.27
EC (µmohs/cm)	310	284	319	300	327	313	337	407	324.6
pH	8.12	8.06	8.08	8.00	8.07	8.10	7.98	7.81	8.03
DO (mg/l)	12.40	12.8	11.6	12.4	10.5	10.0	11.2	8.4	11.16
BOD (mg/l)	2.0	1.20	4.0	5.20	3.3	3.6	2.90	3.8	3.25
COD (mg/l)	18.9	8.40	9.8	17.3	12.7	20.4	20.5	35.6	18.00
CO_3^{--} (mg/l)	3.1	1.1	1.0	3.3	2.8	3.9	2.4	4.0	2.7
HCO_3^- (mg/l)	200	186	194	202	212	184	200	208	198.25
SO_4^{2-} (mg/l)	37.4	40.9	34.5	41.5	31.3	42.1	34.7	40.2	37.87
NO_2^- (µg/l)	9.10	6.30	7.40	10.5	9.32	10.0	9.53	12.14	9.30
NO_3^- (µg/l)	18.10	16.53	18.10	19.15	14.14	15.22	18.10	17.40	17.10
NH_3 (mg/l)	0.93	1.21	0.91	1.05	1.14	0.94	0.97	1.05	1.025
PO_4^{3-} (µg/l)	79.70	62.3	115.5	128.8	71.5	83.8	76.6	103.2	90.17

Table (4): Variations of physical and chemical characteristics of Ismailia Canal water during spring 2005

Stations / Parameters	I	II	III	IV	V	VI	VII	VIII	Regional Average
Air Temp. (^{0}C)	23.8	27.0	30.0	30.0	24.0	25.0	27.0	26.0	26.6
Water Temp.(^{0}C)	23.9	23.8	23.40	24.0	24.0	25.0	24.6	32.4	25.13
Trans. (cm)	90.0	100	85	90	85	60	80	70	82.5
EC (µmohs/cm)	332	291	315	304	344	321	366	510	347.87
pH	8.11	8.06	8.10	8.10	8.15	8.00	8.10	7.80	8.05
DO (mg/l)	10.5	10.7	11.3	11.5	10.9	11.0	11.8	9.20	10.86
BOD (mg/l)	2.5	1.0	3.2	3.40	2.40	3.5	2.10	3.9	2.70
COD (mg/l)	13.4	6.6	8.7	11.5	10.2	18.3	16.70	34.9	15.0
CO_3^{--} (mg/l)	ND	ND	ND	ND	ND	ND	ND	ND	ND
HCO_3^- (mg/l)	225	220	238	242	229	231	245	211	230.75
SO_4^{2-} (mg/l)	34.3	24.9	21.6	27.20	29.6	38.30	30.6	43.0	31.20
NO_2^- (µg/l)	8.26	2.36	7.66	6.89	8.56	9.48	9.21	11.24	7.95
NO_3^- (µg/l)	16.25	15.36	19.45	12.28	18.96	19.41	20.60	19.65	17.75
NH_3 (mg/l)	1.11	1.32	1.26	1.63	1.69	1.79	1.81	2.00	1.58
PO_4^{3-} (µg/l)	55.2	30.0	60.0	86.6	71.6	80.9	61.6	100.0	67.41

ND: Not Detected

Seasonal variations in EC values revealed that the ranges were fluctuated between 264 - 412, 311 - 464, 284 - 407 and 291 - 510 µmohs/cm during summer, autumn, winter and spring seasons respectively. The highest value was recorded at station VIII in autumn, where the lowest value recorded at station II in summer.

Seasonal variations in DO concentrations showed that the values were ranged between 9.6 - 12.6, 9.2 - 13.6, 8.4 - 12.8 and 9.2 - 11.8 mg/l during summer, autumn, winter and spring respectively. The highest value 13.6 mg/l was observed at station VII and the lowest value 9.2 mg/l at stations II and VIII.

Seasonal variations in BOD concentrations represented that the ranges were found to be 0.8 – 6.0, 1.0 – 4.4, 1.2 - 5.2 and 1.0 – 3.9 mg/l during summer, autumn, winter and spring seasons respectively. The minimum and the maximum values are 0.8 & 6.0 mg/l at stations II and VI during summer respectively.

Seasonal variations in COD concentrations were fluctuated between 4.2 - 27.4, 6.7 - 33.5, 8.4 - 35.6 and 6.6 - 34.9 mg/l during summer, autumn, winter and spring seasons respectively. The maximum value was 35.6 mg/l at station VIII and the minimum 4.2 mg/l at station II.

Seasonal variations in pH values were found to be 7.7 - 8.17, 7.84 - 8.16, 7.81- 8.12 and 7.8 - 8.15 during summer, autumn, winter and spring seasons respectively. The highest and lowest values recorded in summer at station V and station II respectively.

The carbonate concentrations were not detected during summer and spring seasons. The ranges of CO_3^{--} during autumn and winter were found to be 3.8 -15.0 and 1 - 4 mg/l respectively. The maximum and minimum values 15 & 1 mg/l were recorded at stations VIII and III respectively.

The seasonal variation of HCO_3^{--} concentrations were fluctuated between 116 - 251, 219 - 272, 186 - 208 and 211 - 245 mg/l during summer, autumn, winter and spring seasons respectively. The maximum and minimum values 272 and 166 mg/l at stations VI and VII during autumn and summer seasons respectively, Tables (1 - 4).

The seasonal variations in sulphate concentrations were found to be in the ranges of 21.8 - 36.7, 36.6 - 47.7, 31.3 - 42.1 and 21.6 - 43.0 mg/l during summer, autumn, winter and spring respectively. The lowest value reached to 21.6 mg/l at station IV in spring and the highest 47.7 mg/l was recorded at station VIII during autumn, Tables (1 - 4). Seasonal variations in nitrite concentrations were fluctuated in the ranges of 5.1 - 14.38, 4.36 - 11.77, 6.30 - 12.14 and 2.36 - 11.24 µg/l during summer, autumn, winter and spring seasons respectively. The minimal value was recorded in spring at station II 2.36 µg/l, while the highest value 14.38 µg/l at station V during summer.

Seasonal variations in nitrate concentrations illustrated that, the ranges of NO_3^- were found to be 13.02 - 27.83, 18.02 - 29.0, 14.14 - 19.15 and 12.28 – 19.65 µg/l during summer, autumn, winter and spring seasons respectively. The minimal value was found 13.02 µg/l at station II and the maximal value 27.83 µg/l at station VII during summer.

Seasonal variations in ammonia concentrations of the area under investigation represented that, the ranges were fluctuated between 1.25 - 2.24, 0.67 - 1.64, 0.93 - 1.21 and 1.11 – 2.00 mg/l during summer, autumn, winter and spring seasons respectively. The highest concentration value 2.24 mg/l and the lowest 0.67 mg/l were recorded at stations VI, VII during summer and autumn seasons respectively.

The seasonal variations in orthophosphate concentrations present in Tables (1 - 4) illustrated that the ranges were found to be 18.4 - 57.2, 49.0-131.0, 62.3 - 128.8 and 30.0 - 100.0 µg/l during summer, autumn, winter and spring respectively. The minimal value 18.4 µg/l was recorded in summer at station VI, and the maximal value 131.0 µg/l at station VIII during autumn.

Bacterial investigations:

Fig. (2) showed that, the bacterial counts of the area under investigation at 22 ^{0}C ranged from 2.9 x10^6 to 78.2 x10^6 cfu/ml, 13.6 x 10^6 to 93.5 x 10^6 cfu/ml, 111.3 x 10^6 cfu/ml to 360 x 10^6 cfu/ml and 41.0 x 10^6 cfu/ml to 481.8 x 10^6 cfu/ml during summer, autumn, winter and spring respectively. The minimum count is 2.9 x10^6 cfu/ml at station II in summer, and the maximum count recorded 481.8 x10^6 cfu/ml in spring at station IV. Also, the bacterial counts at 37 ^{0}C ranged from 10 x10^6 cfu/ml to 75 x10^6 cfu/ml, 15.6 x10^6 cfu/ml to 280 x10^6 cfu/ml, 90 x10^6 cfu/ml to 620 x10^6 cfu/ml and from 24.9 x10^6 cfu/ml to 413 x10^6 cfu/ml during summer, autumn, winter and spring respectively. The results showed that the lowest count recorded in summer at station II is (10.0 x10^6 cfu/ml), while the highest count was detected in spring (413.0 x10^6 cfu/ml), Fig. (3).

Counts of total coliforms by most probable number represented illustrated in Fig. (4). Total coliforms densities varied between 7 – 110000, 93l – 110000, 4 - 110000 and 460 - 4300 cfu/100ml during summer, autumn, winter and spring seasons, respectively. The lowest count is 4 cfu/100 ml was recorded at station II in winter, and the highest count (11x10^4 cfu/100 ml) was detected at station VIII.

Seasonal variations of the fecal coliforms at the area under investigation illustrated in Fig. (5). The ranges fluctuated between 3 - 46 x10^3, 0.0 - 3.9 x10^3, 0.0 - 7.5 x10^3 and 120 - 4.3 x10^3 cfu/100 ml during summer, autumn, winter and spring respectively. The minimum count was recorded during autumn and winter, and the maximum one during summer.

The most probable numbers of fecal streptococci represented in Fig. (6). The ranges were found to be from 0.0 - 460, 11 - 460, 3 - 210 and 7 - 120 cfu/100 ml in summer, autumn, winter and spring respectively.

The counts of *P. aeruginosa* were represented in Fig. (7). The estimated counts of *P aeruginosa* ranged between 0.0 - 93, 7 - 120, 0.0 - 53 and 6 - 93 cfu/100 ml during summer, autumn, winter and spring seasons respectively.

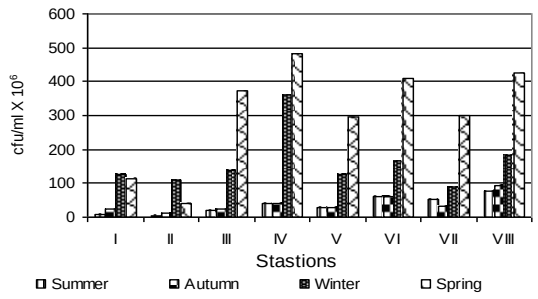

Fig. (2): Seasonal variations of bacterial count x10^6 cfu/ml in water of the area under investigation of Ismailia Canal at 22 ^{0}C.

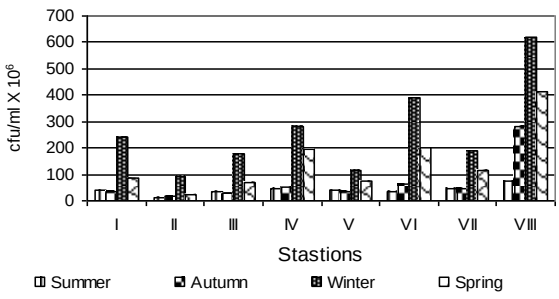

Fig. (3): Seasonal variations of bacterial count x10^6 cfu/ml in water of the area under investigation of Ismailia Canal at 37 ^{0}C.

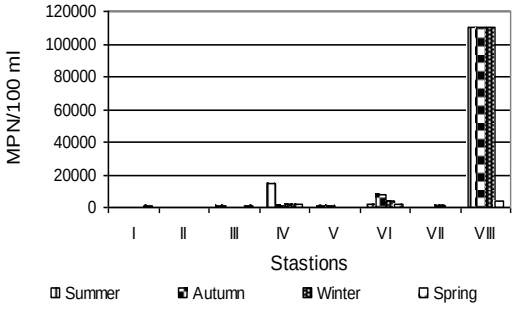

Fig. (4): Seasonal variations of total coliform bacteria MPN / 100 ml in water of the area under investigation of Ismailia Canal.

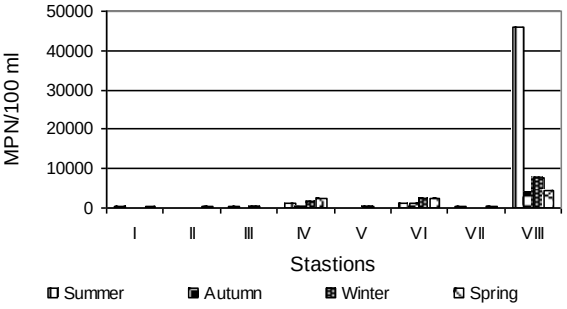

Fig. (5): Seasonal variations of fecal coliform bacteria MPN / 100 ml in water of the area under investigation of Ismailia Canal.

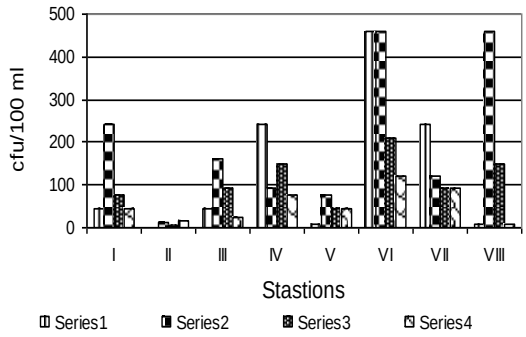

Fig. (6): Seasonal variations of fecal streptococci bacteria MPN / 100 ml in water of the area under investigation of Ismailia Canal.

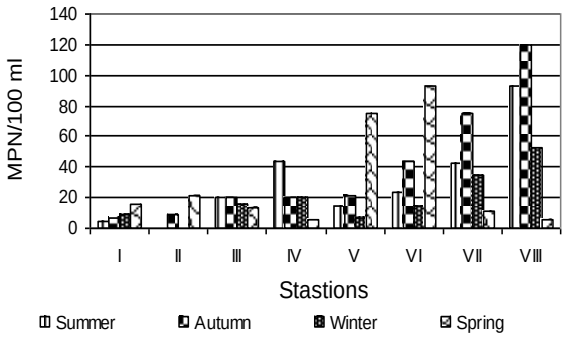

Fig. (7): Seasonal variations of *P. aeruginosa* bacteria MPN / 100 ml in water of the area under investigation of Ismailia Canal.

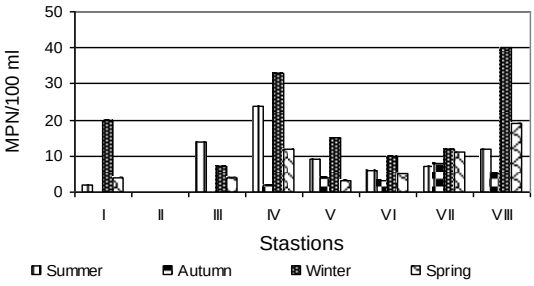

Fig. (8): Seasonal variations of *S. shigella* bacteria MPN/100 in water of the area under investigation of Ismailia Canal.

The results of salmonella–shigella bacteria were illustrated in Fig. (8). The S.S. bacterial counts ranged between 0.0 - 24, 0.0 - 8, 0.0 - 40 and 0.0 - 19 cfu/ml during summer, autumn, winter and spring seasons respectively.

Identification of most resistant isolates:

The most resistant isolates from the above antibiotic screening were characterized using Bergy's Manual. The results of characterization and identification declared that the isolated indicators of pollution are E. *coli, P. aeruginosa, S. faecium and S. choleraesuis* the applied tests represented in Tables (5 - 8).

Table (5): Characteristic properties of isolate No. 56. (*E. coli*).

Characteristics	Results
Morphological characteristics	
Gram stain	-ve
Cell shape	Rods
Motility	+ve
Biochemical characteristics	
Catalase test	+ve
KOH reaction	+ve
Fermentation of * D-Manitol * D-Glucose * Lactose	+ve (acid & gas) +ve (acid & gas) +ve (acid & gas)
Oxidase test	-ve
Indole test	+ve
Methyl red test	+ve
Citrate utilization	-ve
Gelatin hydrolysis	-ve

Table (6): Characteristic properties of isolate No. 18 *(P. aeruginosa)*.

Characteristics	Results
Morphological characteristics	
Gram stain	-ve
Cell shape	Rods
Motility	+ve
Biochemical characteristics	
Catalase test	+ve
KOH reaction	+ve
Fermentation of Lactose	-ve
H_2S production	+ve
Oxidase test	+ve
Production of diffusible pigment	+ve
Hydrolysis of Arginine	+ve
Growth on : * D-Glucose * D-Manitol	 -ve -ve
Growth at : * 4 ^{0}C * 41 ^{0}C	 -ve +ve

Table (7): Characteristic properties of isolate No. 43. *(Streptococcus faecium)*.

Characteristics	Results
Morphological characteristics	
Gram stain	+ve
Cell shape	coccus
Endospore	-ve
Motility	-ve
Biochemical characteristics	
Catalase test	-ve
KOH reaction	-ve
Citrate utilization	-ve
Ammonia utilization	-ve
Growth in 6.5 % NaCl	-ve
Growth in Sodium azide 0.02%	+ve
Growth on :	-ve
* Sucrose	+ve
* Lactose	

Table (8): Characteristic properties of isolate No. 3. *(Salmonella choleraesuis)*.

Characteristics	Results
Morphological characteristics	
Gram stain	-ve
Cell shape	Rods
Motility	+ve
Biochemical characteristics	
H_2S production	+ve
KOH reaction	+ve
Citrate utilization	-ve
Arginine hydrolysis	+ve
Growth in Sodium azide 0.02%	+ve
Growth on :	
* D-Manitol	-ve
* Maltose	+ve
* D-xylose	+ve

Herbal extracts:

Table (9), represent that, the applied herbal extracts against the identified pathogenic isolates. The present results declared that *P. aeruginosa* was sensitive to Coriandrum, Black Pepper, Chamomile, Nigella, Ginger, Sage and Rosemary. *E. coli* isolate was sensitive only against Cinnamon and Licorice extracts. Also, *S. faecium* isolate revealed sensitivity against Sage and Rosemary extracts. *Salmonella choleraesius* isolate was sensitive against Ginger and Licorice extracts. On the other hand dill, Absinth, Peppermint, Anise, Parsley and hibiscus extracts didn't affect any of the subjected isolates.

Table (9): Antibacterial activity of plant extracts against the identified isolates

S.	Plant	*E. coli*	*P. aeruginosa*	*S. faecium*	*S. choleraesius*
1	***Coriandrum sativum* (Coriander)**	- Ve	+Ve	- Ve	- Ve
2	***P. aromatic* (Black Pepper)**	- Ve	+Ve	- Ve	- Ve
3	***Anethum foeniculum* (Dill)**	- Ve	- Ve	- Ve	- Ve
4	***Absinithium vulgare* (Absinth)**	- Ve	- Ve	- Ve	- Ve
5	***Chamomilla officinalis* (Chamomile)**	- Ve	+Ve	- Ve	- Ve
6	***M. piperita* (Peppermint)**	- Ve	- Ve	- Ve	- Ve
7	***Grostemma githago* (Nigella)**	- Ve	+Ve	- Ve	- Ve
8	***Amomum zingiber* (Ginger)**	- Ve	+Ve	- Ve	+Ve
9	***Laurus cinamonum* (Cinnamon)**	+Ve	- Ve	- Ve	- Ve
10	***Anisum vulgare* (Anise)**	- Ve	- Ve	- Ve	- Ve
11	***Liquiritia offecinalis* (Licorice)**	+Ve	- Ve	- Ve	+Ve
12	***Salvia triloba* (Sage)**	- Ve	+Ve	+Ve	- Ve
13	***Rosmarinus offecinalis* (Rosemary)**	- Ve	+Ve	+Ve	- Ve
14	***C. petroselinum* (Parsley)**	- Ve	- Ve	- Ve	- Ve
15	***H . abelmoschus* (Hibiscus)**	- Ve	- Ve	- Ve	- Ve

4. Discussion

Drinking water quality had been decreased during this century due to discharge of wastewater into water sources as well as environmental pollutant. This is considered as the major global health problems, cross adaptation of microbial population to structurally related chemicals may play an important role in the practical development and application of bioremediation techniques **(Liu and Jones, 1995).**

The decrease or increase in water temperature of the Canal depends mainly on the climatic conditions, sampling times, the number sunshine hours and also affected by specific characteristics of water environment such as turbidity, wind force, plant cover and humidity **(Mahmoud, 2002 and Tayel, 2002).**

Air and water temperatures gave strong positive correlation (r = 0.73) during different seasons, indicating the importance on the heat budget of the canal water. Also, there is a positive correlation between water temperature and total coliform, faecal coliform and *P. aeruginosa* (r = 0.44, 0.47 and 0.30) this indicate the strong effect of water temperature on bacterial growth. This result coincident with that reported by **Sabae *et al.*, (2006).** The significant elevation in water temperature at station VIII during all seasons especially summer is due to the cooling

water discharged to the canal from the petroleum companies located on the Canal bank.

Turbidity in water caused by suspended matter such as clay, silt, finely divided organic and inorganic matters, soluble colored organic compounds, planktons and water microscopic organism. The clarity of a natural body of water is a major determinant of the condition and productivity of the sustain **(APHA, 2002).** The degree of turbidity of stream water is an approximate measure of the intensity of the pollution **(Siliem, 1995).** Seasonal variations in water transparency, Tables (1 – 4), showed that the lower values were recorded during spring may be due to the flourishing of phyto-zooplankton during this season. The high values during winter may be related to the decrease in water level during drought period **(Abdo, 1998).** Lower transparency values during different seasons at station VIII mainly attributed to the effluents discharge from petroleum companies at this area.

EC is a measure of the ability of aqueous solution to carry electric current. Solutions of most inorganic compounds and more abundant ions are higher conductivity **(APHA, 1995).** The EC values were increased at station VIII, Tables (1 - 4) which reflects the strong effect of petroleum Companies pipelines effluent discharge at this area. EC were positively correlated with different studied anions e.g. SO_4^{2-}, CO_3^{2-} and HCO_3^- (r = 0.44, 0.56 and 0.32) respectively. As well as with TVC at 37 ^{0}C, TC, *P. aeruginosa* (r = 0.45, 0.56 and 0.43) respectively. This is in accordance with that reported by **Sabae *et al.*, (2006).**

pH is the master that controls all aquatic chemical and biological processes. Changes in pH values beyond the optimum range may affect microbial physiology **(Hassanin, 2006).** Also, the pH of natural waters affects biological and chemical reactions, control the solubility of metal ions, and affect natural aquatic life. The desirable pH for fresh - water aquatic life is in the range of 6.5 - 9.0, and 6.5 - 8.5 for aquatic life **(Chin, 2000).** In view of pH values of Ismailia Canal water, Tables (1 – 4) revealed that, the canal water were on the alkaline side during the investigation period (pH > 7) as pointed by **Goldman and Horne, (1983).** The pH values slightly fluctuated at most stations during different seasons. However, the seasonal variations in pH were mainly affected by temperature, carbonate and bicarbonate system, rather than the photo synthetic activity of the primary procedures **(Ezz El-Din, 1990 and Abdo, 2005).**

DO is very important factor to the aquatic organisms, because it affects their biological processes, respiration of animal and oxidation of the organic matter in water and sediments. In this latter process, complex organic substances are converted to simple dissolved inorganic salts which could be utilized by the micro - and macrophyte **(Okbah and Tayel, 1999).** The results showed that, the Ismailia Canal water was oxygenated during all seasons, Tables (1 – 4). The highest values were recorded in both winter due to the decrease in water temperature, and spring, corresponding to the flourishing of phytoplankton **(Anon, 2007).** Generally, the DO at most stations of canal water within normal guideline values cited by **USEPA, (1999)** for the protection of aquatic life [for warm water biota: early life stage = 6 mg/l, other life stages = 5.5 mg/l. For cold water biota: early life stages = 9.5 mg/l, other life stages = 6.5 mg/l].

The minimum value of BOD at station II and this may be due to the treated water discharged in the canal in this area which decreases the bacterial load in water in this area. On the other hand the maximal BOD concentrations recorded usually at stations VI & VIII and this is related to the high bacterial load in the water at VI opposite to the discharge source of Al-Amyeria water purification plant. At station VIII due to the high organic matter discharged into the canal from petroleum companies and also due to the relative high temperatures which enhance the enumeration of bacteria. The higher regional average was in autumn and winter 3.3 mg/l may be due to the increase in the dissolved oxygen content as a result of decreasing in water temperature, and this is agreed with the results finding by **Sabae *et al.*, (2006)** on River Nile water.

The maximum values of COD were recorded during winter Table (3) mainly related to drought period effect during this season **(Abdo, 1998).** The maximum values 35.6 and 21.3 mg/l at stations VIII and VI respectively. This may be attributed to the effluents discharge of petroleum pipelines and Al-Amyeria water purification plant at these areas. These results are accordance with that finding by **El-Haddad, (2005)** on the same area. Correlation coefficient "r" between COD with TVC at 37 ^{0}C, TC, FC, FS and *P. aeruginosa* (r = 0.26, 0.57, 0.37, 0.49 and 0.57 respectively), these significant correlations revealed that the important role of organic matter controlled in bacteriological parameters.

The carbonate was not detected during hot seasons Tables (1&4) may be due to the flourishing of phytoplankton during these seasons **(Abdo, 1998).** On the other side the detection of CO_3^{2-} during could seasons, Tables (2&3) could be attributed to the decaying and decomposition of phytoplankton during drought period and liberation of CO_2 **(El-Haddad, 2005).**

The increase in bicarbonate concentrations during hot seasons, Tables (1&4) may be attributed to the increase in temperature accelerate the organic matter accessible to bacterial decomposition, where the

HCO_3^- is the final product of the decomposition **(Abdo, 2002).** CO_3^{2-} and HCO_3^- were dependent on each other, however (r = 0.7), as well as, CO_3^{2-} was positive correlation with FS and *P. aeruginosa* (r = 0.61 and 0.50) respectively which reveled that the important role of CO_3^{2-} and HCO_3^- controlled in bacteriological parameters.

The relative increase in sulphate concentration at all stations during winter Table (3) may be due to the death and decomposition of aquatic microorganisms then oxidation of liberated sulphur into sulphate in presence of high dissolved oxygen concentration during drought period effect in this season. These results are coincident with that reported by **Abdo, (1998) and El-Hadad, (2006)** on the same area. The positive correlation "r" between bacteriological parameters e.g. TVC at 37 ^{0}C, TC, FS and *P. aeruginosa* with SO_4^{2-} (r = 0.43, 0.28, 0.47 and 0.31) respectively showed that the important role of sulphate concentration controlled in bacterial count in the area under investigation.

The results of NO_2^- concentrations Tables (1 – 4) showed that decreased during hot seasons (summer – spring) 7.76 and 7.95 µg/l and increased during cold seasons (winter – autumn) 9.20 and 10.26 µg/l respectively. The increase in NO_2^- during cold seasons might be attributed to low consumption by phytoplankton as well as the oxidation of ammonia by nitrifying bacteria and biological nitrification **(Rabeh, 2001).** The relative increased in NO_2^- concentration at stations VI & VII during different seasons. This is related to the effluents discharge of Amyeria water purification plant and petroleum companies. Also, may be attributed to decomposition of organic matter present in waste water where nitrosomonas bacteria oxidized ammonia into nitrite **(Mason, 1991).**

NO_3^- concentrations were increased at all stations during autumn season and the regional value reached to 22.84 µg/l. This mainly attributed to the leaching water effect during this season. Also, the high values of NO_3^- were increased at stations VII & VIII mainly attributed to the effluents discharge of petroleum companies at this area. These results are in accordance with that finding by **Abdo, (1998) and Sabae *et al.*, (2006)** on the same area. The correlation coefficient "r" was found slightly positive between total coliform (TC), faecal coliform (FC) and *P. aeruginosa* with NO_3^- (r = 0.37, 0.20 and 0.27) respectively.

The average values of ammonia concentrations, Tables (1 – 4) revealed that the minimal value in winter 1.03 mg/l and the highest value is 1.94 mg/l in summer, this is due to the high temperature accelerate the reduction rate of nitrate into ammonia. The regional average showed that the lowest average value is 1.17 mg/l at station II and the highest 1.66 mg/l at station VIII, this is due to the accumulations of organic matter in the sediment as the discharge of petroleum companies where the transformation process taking place in sediment cause increasing in ammonia **(Kepinska & Wypch, 1990, Bolalak & Frankowaski, 2003 and Engy, 2005).** The toxicity of ammonia is pH dependent, so the average values of pH in present study (7.93 – 8.05) and ammonia concentrations (1.025 – 1.94 mg/l) were found with normal limits guidelines (1.27 – 3.88 mg/l) at pH (8.0 – 8.1) cited by **USEPA, (1999).**

The cycling of phosphorus within lakes and rivers is a dynamic and complex, involving adsorption and precipitation reactions, interchange with sediments and uptake by aquatic biota. PO_4^{3-} represent the major content of dissolved phosphorus in aquatic environment and the other inorganic phosphorus are not well soluble and their solubility is pH dependent **(Broberg and Persson, 1988).** The increase in PO_4^{3-} concentrations at most stations during cold seasons Tables (2& 3) can be related to the complete mixing of the water column and more phosphorus release from the bottom sediment to water in presence of high dissolved oxygen as reported by **Abdo, (2005).** On the other side the decrease in PO_4^{3-} concentration during hot seasons, Tables (1, 4) were probably due to the distinct drop in phytoplankton biomass, on which nutrient regeneration process depends **(Lehman, 1980).** The maximum values of PO_4^{3-} were recorded at station VIII e.g. 100, 30.6, 131 and 103.2µg/l could be attributed to the effluents discharged of petroleum companies at this area. Also, at station IV e.g. 86.6, 57.2, 104 and 128.8µg/l mainly related to the domestic wastes discharge from the police company into Ismailia Canal water at this area. The significant correlation coefficient "r" was positive between bacteriological parameters e.g. TVC at 22 ^{0}C and 37 ^{0}C with PO_4^{3-} (r = 0.45 and 0.57) respectively, which a good evidence to strong relationship between PO_4^{3-} concentration and total bacterial count at the points discharge e.g. IV and VII.

Seasonal average values of the total bacterial counts were minimal count 35.8 $x10^6$ cfu/ml in summer and this is due to flood period which dilute the organic matter used as food for the bacteria, while maximal mean count was 251.7 $x10^6$ cfu/ml in spring. The regional averages ranged from 42.2 x 10^6 cfu/ml at station II to 229.8 $x10^6$ cfu/ml at station IV , this may be explained by the effect of domestic waste discharged from water station treatment. This agrees with the results of **Sabae and Rabeh (2006).**

The highest values of total coliform bacteria were recorded in summer with seasonal mean value 16139 cfu/100 ml. In contrast with summer, spring showed the lower mean value of total coliforms 1529 cfu/100 ml, this might be attributed to the high temperature

and the effluents discharge into the canal, this is agree with the results of **Sabae and Rabeh (2006).**

With respect to faecal coliform bacteria and faecal streptococci. The station II recorded the minimal regional average value 61 cfu/100 ml, while station VIII recorded the maximal regional average value 15425 cfu/100 ml. The data showed that there is gradual increase in the bacterial indicators from up-to-down stream, which might be attributed to the domestic and agriculture effluents discharge into the canal, this agree with the results of **Sabae and Rabeh (2006).** Also, the pathogenic bacteria (*P. aeruginosa* and *Salmonella- shigella*) increased from up-to-down stream, this increasing might be due to the effect of the sewage, agricultural and industrial effluents discharged into the canal.

A lot of antibiotics of different families and combinations were used to carry out the assay. The inhibitory actions of the used antibiotics varied from one organism of the isolated genera to the other according to the mechanism of action of the antibiotic and the susceptibility of the isolated organism towards the antimicrobial agents.

Susceptibility of pathogenic bacteria to antibiotics is an important problem because of the diversity of resistant mutant among bacterial pathogens due to high rates of the prescriptions of antibiotics. The increased prescriptions of ampicillin lead to lowering its effect of pathogenic bacteria **(Nascimento *et al.*, 2003 and Orrett, 2004).**

All pathogenic bacterial isolates which revealed resistance against most applied antibiotics are subjected to fifteen herbal extracts. The investigation was ended to the following result; *P. aeruginosa* was sensitive to Coriander and this is in line with the results of **Ayfer and Ozlem (2003)** Chamomile, Nigella, Ginger, Sage, Rosemary and this is agreed with **Tamara *et al.*, (2006),** and Black Pepper and this is agreed with the results of **Mazia and Perween (2006).** *E. coli* isolate was sensitive only against Cinnamon and Licorice extracts this is agreed with the results of **Sema *et al.*, (2007),** and **Suree and Pana (2005),** while, *S. faecium* isolate revealed the sensitivity against Sage and Rosemary extracts. *Salmonella choleraesius* isolate was sensitive against Ginger and Licorice extracts and this is disagreed with the results of **Suree and Pana (2005)** and **Onyeagba *et al.*, (2004).**

Results of antibiotic combinations against isolates of *P. aeruginosa* as follows; Ticarcillin/Clavulanic acid (75/10 µg/ml) has an effect on all isolates. Pipracillin/Tazobactam (100/10 µg/ml) has an effect on all isolates. Ceftazidime/Clavulanic acid (30/10 µg/ml) affected 16 isolates and ampicillin/Sulbactam (10/10 µg/ml) has an effect on 15 isolates, and is in line with the results of **Hassanin, (2006).**

Isolates of FS bacteria gave different responses against antibiotic mixes and the result showed that all FS bacterial isolates were sensitive to Ticarcillin/clavulanic acid and Pipracillin/Tazobactam at concentrations of (75/10 µg/ml) and (100/10 µg/ml) respectively. While 9 isolates were sensitive against Ceftazidime/Clavulanic acid (30/10 µg/ml), only one FS isolate was sensitive to Ampicillin/Sulbactam (10/10 µg/ml).

Isolates of FC bacteria gave different responses against antibiotic mixes and the result showed that all FC bacterial isolates were sensitive to Ticarcillin/clavulanic acid, Pipracillin/Tazobactam and Ceftazidine/Clavulanic acid at concentrations of (75/10 µg/ml), (100/10 µg/ml) and (30/10 µg/ml) respectively. However, seven FC bacterial isolates were sensitive against Ampicillin/Sulbactam (10/10 µg/ml).

All pathogenic bacterial isolates which revealed resistance against most applied antibiotics are subjected to fifteen herbal extracts. The investigation was ended to the following result: *P. aeruginosa* was sensitive to Coriander and this is in line with the results of **Ayfer and Ozlem, (2003)** Chamomile, Nigella, Ginger, Sage, Rosemary and this is agreed with **Tamara *et al.*, (2006),** and Black Pepper and this is agreed with the results of **Mazia and Perween, (2006).** *E. coli* isolate was sensitive only to Cinnamon and Licorice extracts this is agreed with the results of **Sema *et al.*, (2007),** and **Suree and Pana, (2005).** While *S. faecium* isolate revealed the sensitivity to Sage and Rosemary extracts. *Salmonella choleraesius* isolate was sensitive against Ginger and Licorice extracts and this is disagreed with the results of **Suree and Pana, (2005)** and **Onyeagba *et al.*, (2004).**

Conclusion

- The main pollution sources of Ismailia Canal at the area under investigation due to the domestic and effluents of police camp and petroleum Companies. Therefore the wastewater effluents should be treated before its drainage in the canal.
- Because of resistance of bacterial isolates against most applied antibiotics, the use of traditional antibiotics should be decreased.
- The expansion of using some herbal extracts is a recommended substitution instead of traditional antibiotics.

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