Bacteriological Studies on Wastewater in Some Cities at Egypt

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ABSTRACT: Comparative assessments of drinking water quality and wastewater were carried out at different cities of El Gharbia Governorate, Egypt. The results noted that the presence of bacterial contaminations were found in samples collected from water. These bacteria contain enteric and pathogens genera when identified. Therefore, in this study was carried out to compare the levels, total counts and identification bacteria of wastewater at the same cities of previous collected drinking water samples. The results wastewater showed that the highest number of total bacteria counts were found in summer season at El mehala Elkobra. And the total bacterial counts were tested on different selective media. The bacterial isolates belonging to*: Aeromonas sobria, Staphylococcus aureus, Salmonella* sp., *Proteus mirabilis, Serratia plymuthica* and *Pantoea agglomerans.* These isolates are recorded as enteric and pathogenic in bacterial taxonomy. The results indicated that the drinking water must be safe and with high quality for human life and the presence of these enteric bacteria here are the source to contaminate drinking water by wastewater. Finally, water contamination is widely used to evaluate the quality of microbiological status of drinking water and as a parameter for estimating water pollution by wastewater

Keywords: wastewater, bacteriological characters, antibiotic susceptibility

INTRODUCTION

Wastewater is known as any domestic, industrial, as well as stormwater runoff, or commercial sewage or any combination thereof carried by water. The importance of adequate wastewater management is one of the key steps in protecting and ensuring the supply of safe water from pathogenic bacteria and the maintenance of public health (Shalinee and Ademola, 2014). Groundwater is an important source of drinking water and its quality is currently threatened by the combination of chemical pollution and microbiological contamination, especially microbes of sewage origin (Reid et al., 2003). In general, European Union regulations require that the heterotrophic plate count (HPC) be assessed at two recovery temperatures: 22°C for 72h and 37°C for 24h. Plate counts at 37°C are considered to be fast-growing bacteria more likely to be associated with pathogenic species and at 22°C. Plate counts were used to enumerate water-specific bacteria that tend to grow slowly (Ramalho et al., 2001).. The heterotrophic plate count (HPC), gives a valuable indication of general microbiological quality of water (WHO, 2001). Drinking water may be contaminated by bad bacteria resulting in health problems. The presence of different bacterial genera in the water is due to direct contamination caused by human activities and an indirect effect by ecological disturbances. The World Health Organization (WHO) reported that nearly half of the population in developing countries suffers from health problems associated with a lack of drinking water or with microbiologically contaminated water (WHO, 1992). The enteric bacteria are perhaps the most common pathogens present in wastewater. These microorganisms maintain their viability in wastewater, and it has been reported (Akin et al., 1978) that these entric bacteria may be found in raw sewage. Enteric bacteria are defined as rod-shaped Gram-negative organisms which ferment lactose. Afify et al., (2021) showed that there is a difference between bacterial isolates sensitivity and different groups of antibiotics.

In a previous study for the survey bacterial pollution of drinking water there were contaminated by enteric and pathogenic bacterial genera. This work aims to indicate that the source of these enteric and pathogenic bacteria is wastewater.

MATERIALS AND METHODS

Source of wastewater samples

Wastewater samples were collected for microbiological examinations. The samples were collected from five different cities: Tanta, Elmehala Elkobra, Mehalet Abo Ali, Samanod and Mehalet Roh during four seasons (summer, autumn, winter and spring) at El Gharbia Governorate, Egypt. Samples were collected in liter sterile glass bottles and then transferred from

the sites to the lab in an ice box and examined within 8 hours. All analyses were carried out in the microbiological laboratory of the Microbiology Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt (Abdallah, 2016).

Total bacterial count

Collected wastewater samples were analyzed for total viable bacterial count using the poured plates on nutrient agar medium (OXOID, CM0003, UK). The plates were incubated at 37º C for 24 hrs and other plates at 20º C for 48hrs (AHPA, 2005) with three plates for replicates.

Identification of bacterial isolated

Bacterial isolates which obained from plates were purified and identified according to morphological, biochemical and physiological characteristics as follows:

Morphological characterizations of isolated bacteria

The bacterial isolates were examined for their cell size, shape, G- stain, capsulation, endospore formation and motiltity according to Bergey's Manual of Systematic Bacteriology 2005.

Biochemical, physiological characterizations and antibiotic susceptibility of bacterial isolates

All the isolates were examined for different biochemical, physiological characters and antibiotic susceptibility by using the biomerieux VITEK® 2 System David H. Pincus bioMerieux, Inc Hazelwood, MO, USA (Barnett et al., 2000). In the VITEK System there are currently four reagent cards available for the identification of bacteria as follows groups:

1-GN: Gram-negative fermenting and non-fermenting bacilli.
2-GP: Gram-positive cocci and non spore-forming bacilli.
3-YST: Yeasts and yeast-like organisms.
4-BCL: Gram-positive spore-forming bacilli.

Total count of bacterial indicators

Count of *Aeromonas* sp.

Aeromonas was counted in a hydrophila agar plate according to Rippey and Cabelli, 1979.

Count of *Staphylococcus* sp.

Staphylococci count was determined by medium staph 110 (Murray et al., 2007).

Count of *Salmonella* sp.

The enumeration of *Salmonella* was performed using the bismuth sulfite agar medium. Colonies producing diffusible black pigment across the membrane filters with or without metallic sheen were counted and considered as salmonella count (Engelbrecht et al., 1977).

Count of *Proteus* sp.

Proteus was counted by used selective medium heart infusion agar Difeco plates (Baird-Parker 1962).

Count of *Serratia* sp.

Serratia was counted by used selective SD medium (Grimont et al., 1988).

Count of *Pantoea* sp.

Pantoea was counted by used nutrient agar (NA) medium (Walcott et al., 2002).

RESULTS AND DISCUSSION

The bacteriological determinatins of the wastewater samples collections were examined by counting the colonies as (colony forming unit, cfu) (total bacteria, enteric and/or pathogenic bacterial indicators) and identification of these bacteria to appear the source of bacterial pollution for drinking water (Afify et al., 2016) at the five cities of El Gharbia Governorate, Egypt.

Total bacterial counts

The results in Figs. (1&2) show that, the counts determination at 20ºC and 37ºC. Generally, the maximum value of total bacterial counts at 20ºC (Fig.1) during summer at El mehala El kobra (6.1 cfu x 10^9 /100ml) and the minimum value was in the spring at Samanod (0.4 cfu x 10^9 /100ml). On the other hand, total bacterial counts at 37ºC (Fig.2) varied from 0.02 to 5.7 cfu x 10^{10} /100ml, the maximum value was in summer at Mehalet Roh while, the minimum value was in spring at Samanod city. All wastewater samples from sites the detected total counts of bacteria at 20ºC and 37ºC were greater in summer which might be attributed to high temperature and the discharged wastewater during this season.

To the nutrients availability for growth of the bacteria may be by this phenomenon. Niemi and Niemi (1991) & Putheti and Lebure (2009) studied that sources of bacteria are domestic and industrial wastewater, agriculture waste environment. In Uruguay (Laguna de Rocha) Piccini et al., (2006) found that bacterial counts were higher of the lagoon than the freshwater, and they suggested that this may be a consequence of better growing conditions.

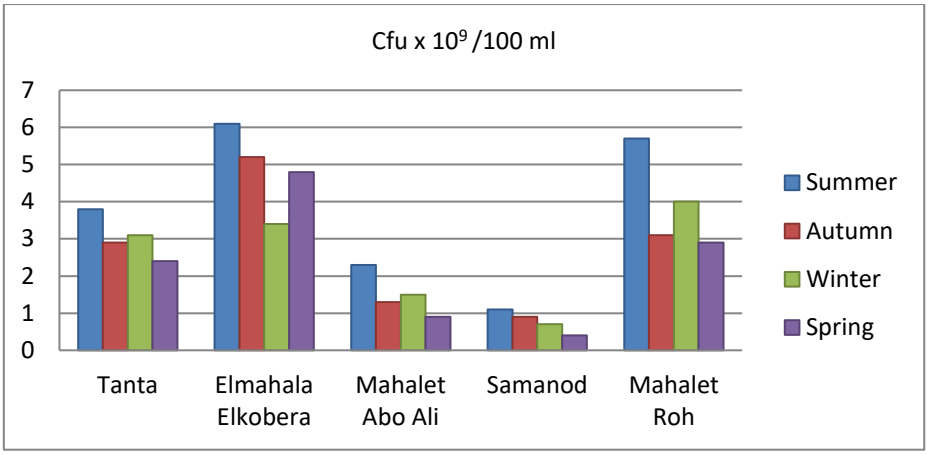

Fig. 1: Total bacterial counts (cfu/100ml) in wastewater at 20ºC of some cities at ElGharbia Governorate.

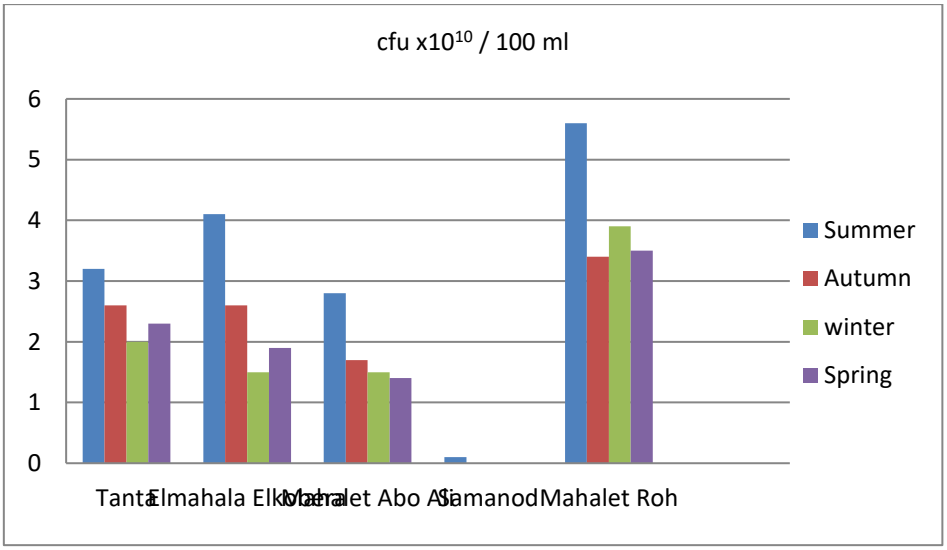

Fig.2: Total bacterial counts (cfu/100ml) in wastewater at 37ºC of some cities at El Gharbia Governorate.

Identification of bacterial isolates

Morphological characterizations

Results in Table (1) showed six representative pure bacterial isolates to examine for their morphological characters: cell size, cell shape, gram stain, capsulation, endospore formation and motility (Bergey's Manual of Systematic Bacteriology 2005).

Table 1: Morphological characterizations of bacterial isolates from wastewater

Bacterial isolates No.	Cell size µm	Cell shape	Gram stain	Capsulation	Endospore formation	Motility
1	0.3 x 1.0	Rod	-	-	-	+
2	0.5 – 1.0	Cocci	+	-	-	-
3	0.5 x 2.0.	Rod	-	-	-	+
4	0.3 x 1.5	Rod	-	-	-	+
5	0.4 x 1.0	Rod	-	-	-	+
6	0.8 x 0.4	Rod	-	-	-	+

(+): Positive ; (-): Negative

Biochemical, physiological characterization and antibiotic susceptibility

Through the different biochemical, physiological characters and antibiotic susceptibility by using the biomerieux VITEK® 2 System David H. Pincus bioMerieux, Inc Hazelwood, MO, USA (Barnett et al., 2000), tests were performed as follows:

Isolate No. 1 is positive with catalase, indole, citrate, acetate, V.P., gelatin, and lipase. While, negative with urea, malate and does not produce acid from cellobiose, inositol, melibiose, raffinose and sorbitol. Table (2) presented the results of antibiotic susceptibility. These results indicated that the isolate is identified as *Aeromonas hydrophila*. The results were found by (Schubert, 1991) conculoded the presence of entric pathogens with *Aeromonas hydrophila* in water reflecting contamination of the environment.

Table 2: Antibiotic susceptibility of isolate No.1

Antibiotic	**MIC**	**Interretation**
Ticarcillin	> = 128	R
Piperacillin / Tazobactam	<= 4	S
Cefepime	<=64	R
Ceftazidime	>=64	R
Aztreonam	>=64	R
Ertapenem	<=0.5	S
Amikacin	<=2	S
Gentamicin	<=1	S
Tobramycin	<=1	S
Ciprofloxacin	<=0.25	S
Minocycline	<=1	S
Colistin	>=16	R
Trimethoprim/ sulfamethoxazole	<=20	S
Pefloxacin	<=0.25	S

(MIC): Minimum Inhibitory Concentrations; **(R)**: Resistant; **(S)**: Sensitivity

Isolate No.2 colonies representing white yellow or orange colored, cells arranged in clusters and positive with catalase, phosphatase, fermented sucrose, lactose, mannitol, mannose, maltose and trehalose. But negative with ribose, raffinose, proline, xylose, lactose, urease, galactose and growth at 6.5% NaCl. Table (3) shows the results of antibiotic susceptibility. The isolate was identified as *Staphylococcus aureus*. *Staphylococci* are every now determined in the gastrointestinal tract and can be determined in sewage. *Staphylococcus aureus* can release by way of human contact into water environments. It has additionally been presented in drinking water supplies (Anti, 1987).

Table 3: Antibiotic susceptibility of isolate No. 2

Antibiotic	MIC	Interpretation
Benzylpenicillin	>=25	R
Oxacillin	1	R
Gentamicin	<=0.5	S
Levofloxacin	0.5	S
Moxifloxacin	<=0.25	S
Erythromycin	<=0.25	S
Clindamycin	<=0.25	S
Quinupristin/Dalfopristin	<=0.25	S
Linezolid	1	S
Vancomycin	<=0.5	S
Tetracycline	<=1	S
Tigecycline	<=0.12	S
Rifampicin	<=0.5	S
Trimethoprim/sulfamethoxazole	<=10	S
Nitrofurantion	<=0.16	S

(MIC): Minimum Inhibitory Concentrations **; (R)**: Resistant; **(S)**: Sensitivity

Isolate No.3 can grow in the presence of NaCl 0.4 to 4.0%, grow at temperature range from 5 to 47 ºC but is sensitive to heat killed at 70ºC or above and facultative anaerobe, optimum pH 6.7-7.5, negative with gelatinase and urease while, positive with oxidase and catalase. No fermented lactose, sucrose, use citrate as sole of carbon source and reduced nitrate to nitrite. The results in Table (4) shows that antibiotic susceptibility. Therefore, the isolate identified as *Salmonella* sp. *Salmonella* spp. are the main source of pathogens for human. Human waste are the infection sources due to ingestion of food or water contaminated (Scherer and Miller, 2001).

Table 4: Antibiotic susceptibility of isolate No. 3

Antibiotic	MIC	Interpretation
Tetracycline	>6	R
Chloramphenicol	>7	R
Ampicillin	>0	R
Cephaloexin	>=7	R
Amofluxin	>=0	R
Vancomycin	<=0.5	S
Nitrofurantion	<=0.16	S
Rifampicin	<=0.5	S
Linezolid	1	S
Quinupristin/Dalfopristin	0.25	S
Trimethoprim/sulfamethoxazole	<=10	S
Colistin	<=1	R
Tobramycin	>=16	S
Gentamicin	<=1	S
Ertapenem	<=0.5	S

(MIC): Minimum Inhibitory Concentrations **; (R)**: Resistant; **(S)**: Sensitivity

Isolate No. 4 grows on several media, reduced nitrate and production H_2S, positive with phosphatase, lipase, urease, catalase but not oxidase and fermented glucose, while negative with proline, sucrose, lactose, glycine, maltose, mannitol, trehalose, mannose, citrate, galactose, cellobiose and sorbitol. In addition, the antibiotic susceptibility in Table (5) results indicated that the isolate was identified as *Proteus mirabili*s (Janak, 2012). Ali et al., (2008) recorded, the characteristic spectrum of bacterial strains in water such as enteric and fecal bacteria and showed that these bacteria were the most contaminated source of drinking water.

Table 5: Antibiotic susceptibility of isolate No.4

Antibiotic	MIC	Interpretation
Ampicillin	<=2	S
Ampicillin/sulbactam	<=2	S
Cefazolin	<=4	S
Ceftriaxone	<=1	S
Cefepime	<=1	S
Aztreonam	<=1	S
Ertapenom	<=0.5	S
Imipenem	4	S
Meropenem	<=0.25	S
Amikacin	<=2	S
Gentamicin	<=1	S
Tobramycin	<=1	S
Ciprofloxacin	<=0.25	S
Moxifloxacin	<=0.25	S
Tigecycline	4	R
Nitrofurantion	128	R
Trimethoprim/sulfamethoxazle	<=20	S

(MIC): Minimum Inhibitory Concentrations **; (R)**: Resistant; **(S)**: Sensitivity

Isolate No. 5 comparison of biochemical tests the following: H_2S production, positive with urease, phosphatase, lipase positive for glucose, sucrose, inositol and arabinose fermentation, citrate utilization and no fermented proline, lactose, maltose, trehalose, mannose, cellobiose, lactose, sorbitol and galactose, variable for raffinose. The strain was identified in parallel using the MicroScan Walk Away system and by standard reference procedures (Nieto *et al.*,1984 & Amos, 1985). When compared between the micro Scan WalkAway System and routine laboratory tests for the evaluation of biochemical characteristics important for the identification of S. plymuthica strains, all tests showed correlations. 100% except for the raffinose test (91%) (Jose et al., 2000). Table (6) shows antibiotic susceptibility and strains were conventionally cultured on trypticase soybean agar at 37°C for 24 h and stored on TSA inclined trays at 4°C in mineral oil and frozen at 70 °C with 15% glycerol., Serratia plymuthica.

Table 6: Antibiotic susceptibility of isolate No. 5

Antibiotic	MIC	Interpretation
Cefazolin	<=4	S
Ceftriaxone	<=1	S
Cefepime	<=1	S
Aztreonam	>=64	R
Ertapenom	<=0.5	S
Imipenem	<=0.25	S
Meropenem	<=0.25	S
Amikacin	<=2	S
Gentamicin	<=1	S
Tobramycin	<=1	S
Ciprofloxacin	<=0.25	S
Moxifloxacin	<=0.25	S
Tigecycline	<=0.5	S
Nitrofurantion	<=16	S
Trimethoprim/sulfamethoxazle	<=20	S

(MIC): Minimum Inhibitory Concentrations; **(R)**: Resistant; **(S)**: Sensitivity

Isolate No. 6 isolate was positive with catalase, glucosidase, xylosidase, galactosidase, urease fermented sucrose, maltose, mannitol, trehalose, mannose, sorbitol and cellobiose. But negative with H_2S production, lipase, oxiase, proline, lactose, decarboxylase, citrate, urease, lactate and phosphatase. *Pantoea agglomerans* is a member of Enterobacteriaceae that inhabits

plants, soil, water and such species include bacteria reported as both commensal and pathogen of animals and humans (Gavini et al., 1989). This isolate in Table (7) shows the antibiotic susceptibility and identified according to USFDA (2002) as *Pantoea agglomerans.*

Table 7: Antibiotic susceptibility of isolate No. 6

Antibiotic	MIC	Interpretation
Ticarcillin	>=128	R
Piperacillin	8	S
Piperacillin/ Tazobactam	<=4	S
Ceftazidime	16	R
Cefepime	<=1	S
Aztreonam	16	S
Pefloxacin	<=0.25	S
Imipenem	<=25	S
Meropenem	<=25	S
Amikacin	<=2	S
Gentamicin	<=1	R
Tobramycin	<=1	R
Ciprofloxacin	<=0.25	R
Minocycline	<=1	R
Colistin	>=16	R
Trimethoprim/sulfamethoxazle	<=20	S

(MIC): Minimum Inhibitory Concentrations **; (R)**: Resistant; **(S)**: Sensitivity

Previous reportes have study that some pathogenic and entric bacteria can presence in wastewater as sources of contamination (Cabral, 2010). Those bacteria that include manyl species of the family Enterobacteriaceae and live in the human and animal intestine (Ashbolt, 2004). The identification of either Staphylococci or namely *Staph. aureus* appears the water quality level as a useful index **(**Kamel, 2005).

Total count of bacterial indicators

***Aeromonas sobria* and *Staph. aureus* counts in wastewater**

In addition, the determination of total bacterial count which is considered new indicator of pollution of water, the water is also tested to determinate occurrence of *Aeromonas sobria* and *Staph. aureus* bacterial counts.

Figs. (3&4) shows the comparison between the results of *Aeromonas sobria* and *Staph. aureus* bacterial counts at El Gharbia Governorate. Among of all wastewater samples Fig. (3) counts of *Aeromonas sobria* were almost in the summer (2.1 $x10^5$ cfu/100ml)**.** However, the winter samples contained the minimum value (1.6 $x10^5$ cfu/100ml). In the city, Tanta recorded the highest values of *Aeromonas sobria* (2.9 $x10^5$ cfu/100ml)**.** These results are in agreement with El-Taweel (2003) studied that high counts of *Aeromonas* spp. in aquatic environments might be referred to the water polluted by surface seepage of sewage from land applications. At the Fig. (4) *Staph. aureus* counts are recorded minimum value in autumn (1.5 $x10^7$ cfu/100ml) and maximum value in summer (6.4 $x10^7$ cfu/100ml) at El mehala Elkobra. The means values ranged between 4.3 and 3.5 $x10^7$ cfu/100ml in two seasons (summer and spring) respectively. The obtained data were in harmony with Vaerewijck et al (2005).

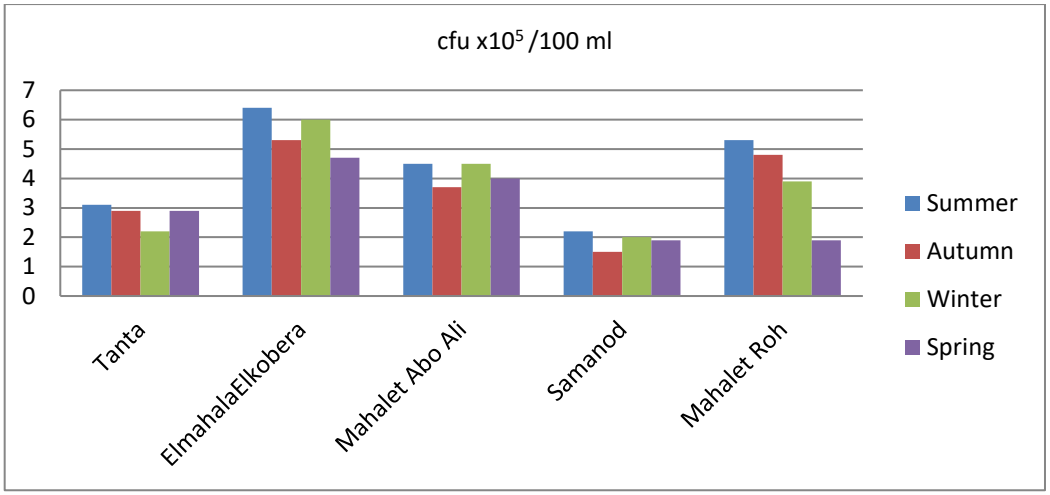

Fig.3: Total counts of *Aeromonas sobria* in wastewater of some cities at El Gharbia Governorate.

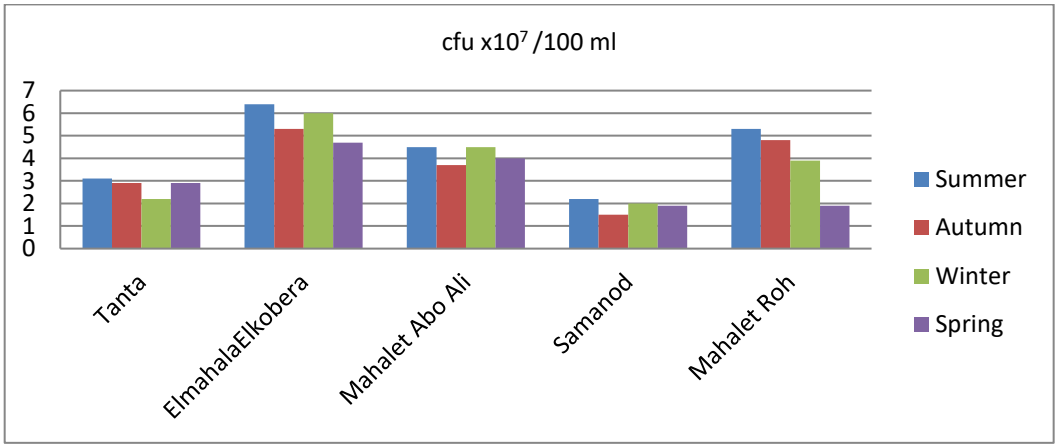

Fig.4: Total counts of *Staph. aureus* in wastewater of some cities at El Gharbia Governorate.

Counts of pathogenic bacteria as indicators in wastewater

Counts of *Salmonella* sp. and *Proteus mirabillis* were detected as indicators of pathogenic bacteria.

Fig. (5) presents values of *Salmonella* sp. counts in wastewater. The minimum value was 1.7cfu x 10^4/100ml, and it was in the cold season at Samanod, but the high data was 4.5 cfu x 10^4/100ml during summer at Tanta. Means value fluctuated between (3.6 and 2.7 cfu x 10^4/100ml) in summer and spring respectively. The enteric bacteria are perhaps the most main pathogens present in wastewater, and the mainely species, *Salmonella*. Salmonella highly densities may be found in raw sewage and it has been reported that maintain their viability in wastewater (Akin et al., 1978).

Fig. (6) shows the maximum count of *Proteus mirabillis* was 4.2 cfu x10^7 /100ml, in summer at Mehalet Roh while, the minimum count was 0.8 cfu x 10^7/100ml, in winter at Samanod, respectively. Genus Proteus is a source of enteric bacteria.

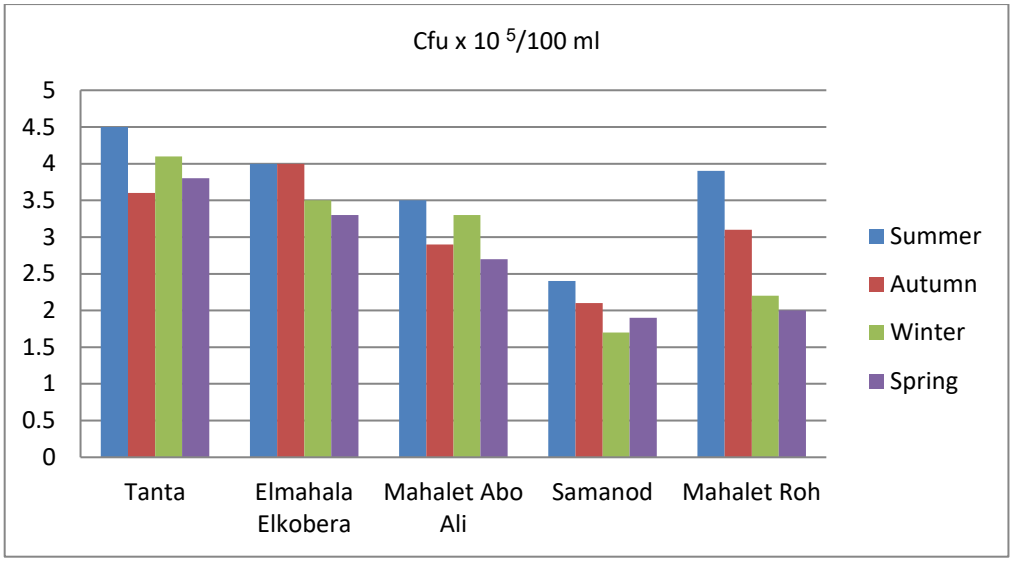

Fig.5: Total counts of *Salmonella* sp. in wastewater of some cities at El Gharbia Governorate.

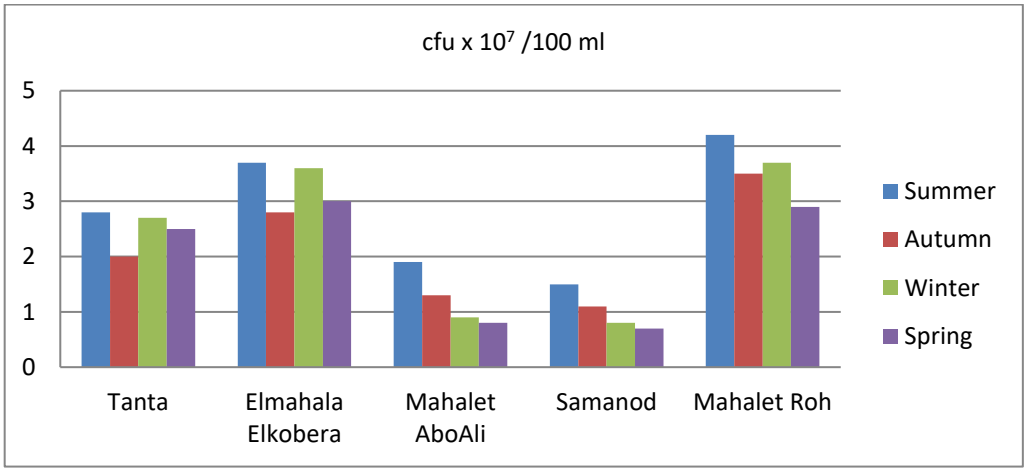

Fig.6: Total counts of *Proteus mirabillis* in wastewater of some cities at El Gharbia Governorate.

Also, *Serratia plymuthica* and *Pantoea agglomerans* counts were determined in this investigation as pathogenic bacteria. Fig. (7) indicated that *Serratia plymuthica* values of counts in wastewater. The minimum value (0.7 cfu x 10^5/100ml), it was in the spring at Samanod. While, the highest data was 3.1 cfu x 10^5/100ml during summer at Tanta. Means values were fluctuated between 2.4 and 1.7 cfu x 10^5/100ml during summer and winter respectively.

In Fig. (8) shows the maximum count of *Pantoea agglomerans* was 1.2 cfu x 10^6/100ml in the summer at Mehalet Abo Ali and the minimum count was 0.01 cfu x 10^6/100ml during autumn at Tanta. Means values fluctuated between 0.4 and 0.1 cfu x 10^6/100 ml during summer and autumn respectively.

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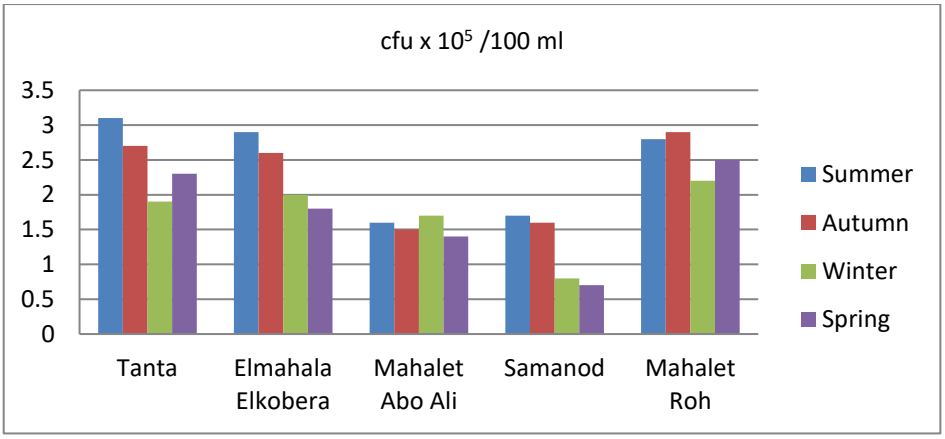

Fig.7: Total counts of *Serratia plymuthica* in wastewater of some cities at El Gharbia Governorate.

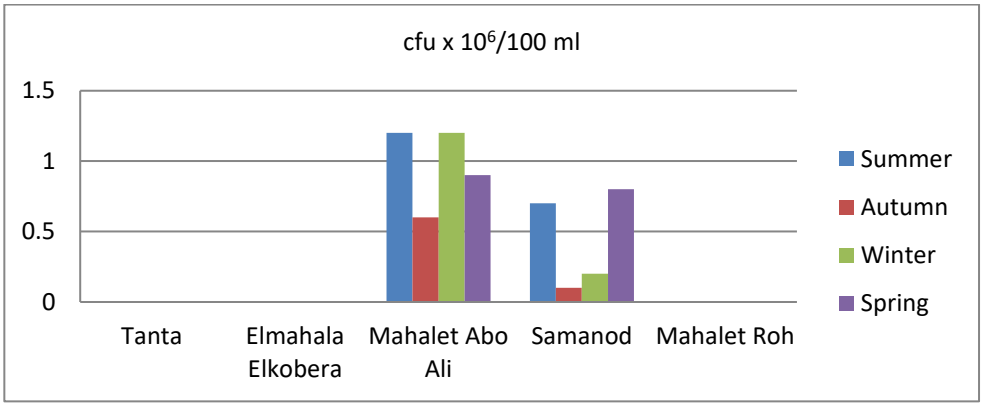

Fig.8: Total counts of *Pantoea agglomerans* in wastewater of some cities at El Gharbia Governorate.

Our results are consistent with recently published data, and the highest bacterial risk occurs when drinking water contaminated with human or animal feces. Wastewater flowing into freshwater and coastal seawater is a major source of microbial faeces, including pathogens (WHO, 2008).

In general terms, show that all water sources were grossly polluted, and water was not fit for drinking (Madhab et al.,2010). Neweigy et al., (2010) reported that groundwater contamination is always with the result of human activities. When ground water becomes to make it pure, it is very expensive. Liquid waste is discharged above ground, this may be due to the mixing of wastewater with drinking water due to pipelines. Regular monitoring of water quality to improve not only prevents diseases and dangers, but also prevents further contamination of the water source.

(Manjula et al., 2011). Also, Abd-elhameed et al., (2021) reported that in rivers some studies such as domestic and industrial wastewater, agriculture waste environment are sources of fecal bacteria.

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الملخص العربى

دراسات بكتيريولوجية على مياه الصرف الصحى فى بعض مدن مصر

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فى دراسة لقياس جودة مياه الشرب بكتيريواوجيا ثم إجراء مقارنه بمياه الصرف الصحى لمعرفة مصدر التلوث البكتيري وذلك فى مدن مختلفه بمحافظة الغربية (طنطا– المحله الكبرى– محلة أبو على– سمنود– محلة روح) بمصر . ففى هذه الدراسة تم إجراء عد كلى للبكتيريا فى عينات مياه الصرف الصحى وكذلك عد لبعض الأنواع البكتيرية المعوية والمرضية على بيئاتها المتخصصة وذلك فى فصول السنه الأربعة (الصيف والخريف والشتاء و الربيع) وثم تعريف تلك العزلات البكتيرية مورفولوجيا وفسيولوجيا وكيميائيا مع إجراء اختبارات تأثير المضادات الحيويه بجهاز الفيتك . أظهرت النتائج أن أكبر عدد للبكتيريا كان فى فصل الصيف فى مدينة المحلة الكبرى وبتعريف العزلات البكتيرية حصلنا على الأنواع التالية :

Aeromonas sobria, Staphylococcus aureus, Salmonella sp., *Proteus mirabilis, Serratia plymuthica* and *Pantoea agglomerans* .

وعند إجراء العد لهذه البكتيريا في فصول السنه الأربعة كانت أعلى متوسط الأعداد البكتيرية في فصلى الصيف والربيع ربما يرجع ذلك لمناسبة درجات حرارة الفصلين لنمو مثل هذه البكتيريا . من هذه الدراسة يمكن القول أن مصدر التلوث الميكروبي لمياه الشرب يكون مصدره الرئيسي مياه الصرف الصحي وبالتالي لابد من المحافظة على المصادر الرئيسية لمياه الشرب بعدم إلقاء مخلفات المنازل وكذلك مخلفات المصانع والمخلفات الزراعية في المصادر الرئيسية لمياه الشرب للمحافظة على صحه الإنسان بالإضافة إلى المحافظة على البيئة من التلوث .