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Impact of Domestic Wastewater Treatment Plants on the Quality of Shallow Groundwater in Qalyubia, Egypt; Discrimination of Microbial Contamination Source Using Box-PCR

Ayman Y.I. Ewida[(1)#], Mary S. Khalil[(2)], Alaa M.A. Mahmoud[(1)]
[(1)]Microbiology Department, National Water Research Center, Cairo, Egypt; [(2)]Department of Botany and Microbiology, Faculty of Science, Cairo University, Giza, Egypt.

MUNICIPAL sewage, septic tanks, fertilizers, animal feedlots and irrigation wastewater are the common sources of groundwater pollution in rural areas. Sedimentation pools of wastewater treatment plants are possibly suggested to be another source of pollution. Two wastewater treatment plants located at Qalyubia governorate named Qalyub (QWTP) and Shubra Al-khayma (SHWTP) were selected to carry out the present study; two samples from influent and effluent were collected seasonally from both plants. Furthermore, 6 groundwater samples were collected from nearby rural houses of both plants. The physicochemical and microbiological properties were evaluated for all samples. Moreover, Box-PCR for 12 strains isolated from wastewater and groundwater at Qalyub district was carried out. Water quality assessment studies proven that the effectiveness of the two wastewater treatment plants under investigation is questionable, especially QWTP. The percentages of removing of TSS, COD, BOD, ammonia, total coliform and fecal coliform counts were 87, 74, 88, 66, 94 and 89%, respectively, for QWTP, and 85, 89, 93, 86, 94 and 83%, respectively, for SHWTP. The groundwater at Qalyub district was contaminated with Mn, Fe, ammonia, BOD, coliform bacteria, fecal streptococci, *E. coli, Aerumonas hydrophilla* and *Pseudomonas aeruginosea,* while the groundwater at Shubra Al-khayma district was contaminated with Mn, coliform bacteria, fecal streptococci and *P. aeruginosea*. Microbial and chemical evaluation of groundwater, as well as, Box-PCR results proven that, the contamination of the tested groundwater might be of other source than water treatment plants.

Keywords: Bacteria, Box-PCR, Groundwater, Treatment, Wastewater.

Introduction

Groundwater represents over 97% of all freshwater on earth. Historically, it has been considered as a safe source of water that could be used by human beings (Mckeon et al., 1995). In recent years, many authors reported the contamination of groundwater with bacteria, viruses, nitrates, organic chemicals and other pollutants (Ewida et al., 2012). When talking about groundwater quality; it concerns the physical, chemical and microbiological properties of a given source, which is mainly depending on the characteristics of the recharge water, and the on-situ human activities (Kumar et al., 2006). Nowadays, there is a global awareness of groundwater quality impairment due to human-induced contamination (Bathrellos et al., 2008; Rouabhia et al., 2009; Gaber et al., 2013; Katuva et al., 2020).

Chemical contaminants of groundwater including ammonia, nitrate, BOD, some cations and anions, as well as, heavy metals, are predominantly detected by many authors; high ammonia and BOD values were reported in shallow groundwater contaminated with organic matters due to seepage of tankers or due to livestock animals (Hassanein et al., 2012).

[#]Corresponding author email: ayman_ibrahim@nwrc.gov.eg, Tel (+2) 01289573053, Fax (+2) 42174663
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Nitrate contamination usually occurs due to agricultural activities and applying fertilizers (Wakida & Lerner, 2005). The presence of high concentrations of heavy metals like Fe, Al, Mn, Pb, Cu and Ni, as well as, many cations and anions in groundwater was excessively reported (Gaber et al., 2013; Li, 2016; SubbaRao et al., 2019; Katuva et al., 2020). As contaminants and ions contained in soils dissolve through the recharge water, they reach finally the groundwater aquifer, causing deterioration of groundwater quality (SubbaRao et al., 2018).

Potential sources of pathogenic microorganisms in groundwater include municipal sewage, septic tanks, fertilizers, animal feedlots and irrigation by wastewater (Spalding & Exner, 1993; Wilhelm et al., 1996). The most virulent source is seeping from septic tanks, both constructed by villagers (Khan et al., 2018) and those of wastewater treatment plants (Obeidat et al., 2013).

Evaluation of groundwater quality is an essential tool in realizing the role of anthropogenic influences on the groundwater system (Gorgij et al., 2019). Furthermore, BOX-PCR is the most frequently used technique to evaluate the origin of a given microbial type (Safiulah et al., 2009; Galloway & Levett, 2010). So, The present study was accomplished to: (1) Evaluate the effectiveness of two main wastewater treatment plants at Qalyubia Governorate; Shubra Al-khayma and Qalyub. (2) Assess the quality of shallow groundwater at the vicinity of both wastewater treatment plants, and (3) Investigate the impact of wastewater treatment plants on shallow groundwater quality, by detecting the origin of microbial contamination, using BOX-PCR technique.

Materials and Methods

Study sites

Qalyubia governorate is located in the Nile Delta, north of Cairo, at lower Egypt. Its area extended between latitudes 30°07’30” to 30°32’92” N and longitudes 31°3’30” to 31°34’30” E (Fig. 1). It is characterized by a lot of agricultural activities including planting of apricot, banana, citrus, wheat and a lot of vegetables. Two wastewater treatment plants located in two different districts in Qalyubia governorate were chosen to check their impact on shallow groundwater wells at the nearby houses. One located at the east (Qalyub Wastewater Treatment Plant QWTP) and the other at the south (Shubra Al-Khayma Wastewater Treatment Plant SHWTP), as shown in Fig. 1.

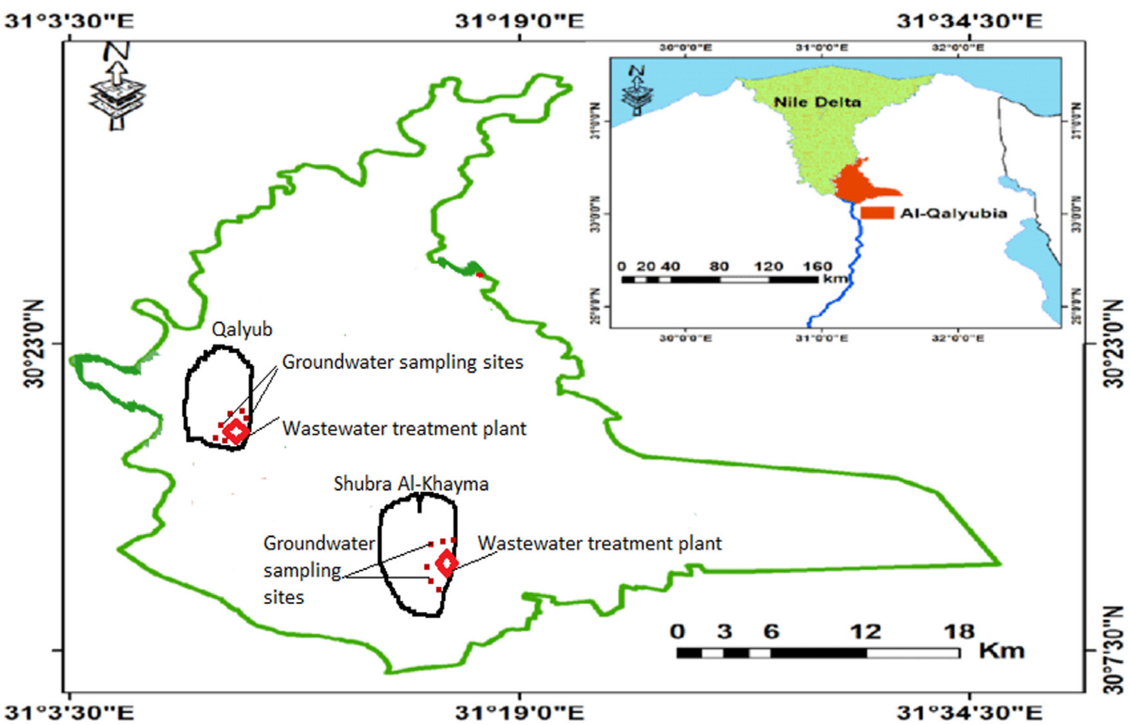

Fig. 1. Locations of sampling sites at Qalyub and Shubra Al-khayma districts, Qalyubia Governorate

Qalyub district

It is a rural district in Qalyubia governorate, located north of Cairo, near the right bank of Nile River, with a population of 110,156 (Egypt population of 2018/ 2019), it is characterized by a lot of agricultural activities. The main water treatment plant is QWTP which allocated on Qalyub road at Alhadtha square; it was constructed in 2002, over an area of 142,800m^2 with a capacity of 95,000m^3/day. In the present study, 2 samples were collected seasonally, one from raw sewage (as influent) and the other after chlorination tanks (as effluent). Also, 6 groundwater samples were collected from nearby houses to emphasize the impact of QWTP on sub-surface groundwater at Qalyub district, named Q1 to Q6. The depths of those groundwater wells ranged between 20 and 40 m, operated within 1 to 5 years. Some of them are used for domestic purposes and the others used for livestock animals.

Shubra Al-khayma district

It is one of the four big cities in Egypt, with a total area of 270km^2 and a population of 1,073,000 (Egypt population of 2018/ 2019), it is characterized by a lot of industrial activities. The main wastewater treatment plant is located on Mustorod road, named Shubra Al-khayma wastewater treatment plant (SHWTP). It was constructed in 1982, over an area of 352,800m^2 in two phases; primary and secondary treatment, each of capacity 600,000m^3/day. In the present study, 2 samples were collected seasonally, one sample from raw sewage, before sedimentation tanks (as influent) and the other after chlorination tanks (as effluent). Also, 6 groundwater samples were collected from nearby houses to investigate the impact of SHWTP on the sub-surface groundwater at Shubra Al-Khayma, named Sh1 to Sh6. The depths of those groundwater wells ranged between 20 and 30m, operated within 1 to 2 years. Some of them are used for domestic purposes and the others used for livestock animals.

Water quality assessment

Physico-chemical analyses

The following physico-chemical analyses were carried out for both types of the collected water samples according to APHA (2017). The pH was measured at 25^{o}C using pH meter (InoLab WTW level 1 electrode, with ATC probe WTW Sentix 4). Total Dissolved Solids (TDS) was determined by weighing the solid residue obtained by evaporating a measured volume of filtered water sample to dryness at 103-105 ^{o}C. Total Suspended Salts (TSS) of filtered water samples was determined gravimetrically at 105 ^{o}C. Alkalinity; Carbonates (CO_3^{--}) and bicarbonates (HCO_3^-), was measured using the titrimetric method. Electrical Conductivity (EC) was measured at 25^{o}C using a conductivity meter (InoLab Cond level 1). Major cations; calcium (Ca^{++}), magnesium (Mg^{++}), sodium (Na^+) and potassium (K^+) were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES) Perkin-Elmer product, model Optima 5300 DV. Major anions; chloride (Cl^-), nitrate (NO_3^-), phosphate (PO_4^{---}) and sulfate (SO_4^{--}), were measured using Ion Chromatography (IC), Dionex product, model DX5000. Concentrations of some heavy metals as (Al, Cu, Fe, Mn, Ni, Sr and Zn) were measured using ICP- MS (Inductively Coupled Plasma-Mass Spectrometry) Perkin-Elmer (model SCIEX Elan 9000). Chemical Oxygen Demand (COD) was measured, using the dichromate reflux method; the intensity of the formed complex was measured by the visible Spectrophotometer (HACH 2000). Biochemical oxygen demand (BOD) was measured using (OxiTop system WTW) at 20 ºC incubation in a thermostatic incubator chamber for 5 days. Total ammonia nitrogen (NH_3) was measured using the Nessler method.

Microbiological analyses

Microbiological analyses were carried out according to APHA (2017) as follows; pour plate method was used for the total bacterial count (TBC) at 22°C and at 35°C, as well as, for detection of *Aeromonas hydrophila*, using plate count agar (Difco, U.S.A) and starch ampicillin agar media, respectively. Enumeration of total coliforms, fecal coliforms, fecal streptococci, *E. coli, and Pseudomonas aeruginosa* was performed by membrane filter technique using the following media (Merk, Germany): M - Endo agar LES, M - FC agar, M - *Enterococcus* agar, M-TEC agar, and M-PA-C agar and *Pseudomonas* selective agar, respectively. The detection of *Salmonella* and *Shigella* (presence/ absence test) was carried out by membrane filter technique through the following steps; *i) Concentration;* by filtering 100mL - 1L of each water sample), *ii) Enrichment;* using tetrathionate broth and nutrient broth (pH 8), respectively, iii) *Selective growth;* using Bismuth sulfite agar and X.L.D agar, respectively, and iv) *Biochemical confirmation;* black colonies of *Salmonella* grown on Bismuth sulfite agar plates and red colonies with black centers of *Shigella* grown on X.L.D agar plates were picked up, purified and subjected for some biochemical tests as described by APHA (2017).

Discrimination of microbial contamination of groundwater by BOX-PCR technique

Isolation, purification and identification of bacterial species

The results of microbiological assessment indicated the contamination of shallow groundwater at Qalyub district with *E. coli* and some other bacterial groups. BOX-PCR technique was used to prove wither such contamination was originated as a result of sewage leakage from Qalyub wastewater treatment plant's sedimentation ponds, or not. A total of 12 bacterial isolates were selected to carry out the test. They were isolated from M-TEC and M-PA-C agar plates (the selective media for isolation of *E. coli* and *Pseudomonas aeruginosa*, respectively, as described in APHA (2017)) in the following pattern; 3 *E. coli* and 3 *P. aeruginosa* isolates from the plates of QWTP sewage samples and the same from the plates of groundwater samples of nearby houses. So, there were two groups of bacteria; 6 belong to *E. coli* (3 from sewage/ 3 from groundwater) and 6 belong to *P. aeruginosa* (3 of sewage/ 3 of groundwater). All the isolates were grown up on nutrient agar plates at 35°C for 24hrs and subjected to the next step.

Genomic DNA extraction and BOX-PCR

Genomic DNA and BOX-PCR was carried out as mentioned by (Bilung et al., 2018). The DNA was extracted using Wizard™ Genomic DNA Purification Kit (Promega, USA) as instructed by the manufacturer. The reaction mixture of a total volume of 25µL was consists of 5µL of 5x PCR buffer, 400µM of deoxynucleoside triphosphate mix, 0.4µM of primer BOXA1R (CTACGGCAAGGCGACGCTGACG), 3mM of magnesium chloride, 2.5 U of Taq DNA polymerase (Promega, USA) and 5µL of DNA template. The PCR was running as follow; initial denaturation at 94°C for 5min, followed by 35 cycles of denaturation at 94°C for 1min, primer annealing at 40°C for 2min, and extension at 72°C for 2min, with a final extension at 72°C for 10min. PCR amplification products were detected by electrophoresis of 12µL aliquots through 1.4% agarose gels in Tris-borate-EDTA (TBE) buffer which were stained with ethidium bromide 1.25mg/L, visualized under UV light, and printed image through Bio-Print (BioRad, USA). DNA standards (1-kb DNA ladder Gibco BRL) were included in each electrophoresis gel. All of the amplifications were performed at least twice in separate assays, to ensure the reproducibility of the patterns, and only bands common to the replicate amplifications were scored. DNA fingerprints of each isolate were first compared for similarity by visual inspection of band patterns. They were considered identical when all scored bands in each pattern had the same apparent migration distance, even if a slightly different molecular weight was assigned to the same band over two or three different electrophoreses. Identification of *E. coli* and *P. aeruginosa* isolates was confirmed by BOX fragments and biochemical tests. Dendrograms were established using Gel1 program for each group of bacterial species for related phylogeny (Marques et al., 2008).

Results and Discussion

Water quality assessment

Water quality assessment results for all the collected samples (wastewater and groundwater) indicated that there is no seasonal variation, the concentrations of physicochemical parameters and the counts of different types of the tested bacterial groups, for each site, were in the same range ± 5%. So, the average was calculated and recorded in Tables 1 to 4.

Assessment of Water Treatment Plants

The effectiveness of the two wastewater treatment plants under investigation is questionable, especially QWTP. The percentages of removing of TSS, COD, BOD, ammonia, total coliforms and fecal coliforms counts were 87, 74, 88, 66, 94 and 89%, respectively, for QWTP, and 85, 89, 93, 86, 94 and 83%, respectively, for SHWTP, as recorded in Tables 1 to 4. The performance of both sewage treatment plants of removing COD, ammonia and fecal coliform bacteria is low, in comparison with EPA guidelines (EPA, 2012). Ramadan et al. (2017) reported the low efficacy potential of some wastewater treatment plants in Gharbia governorate, especially those operated by activated sludge (the same case, as in the present study).

Physico-chemical assessment of shallow groundwater

The physical and chemical analyses of shallow groundwater samples collected from Qalyub and Shubra Al-khayma districts were given in Tables 1 and 2. The quality of shallow groundwater wells tested at both districts, Qalyub and Shubra Al-khayma is inconstant. The pH values ranged from 7.45 to 7.8 and from 8 to 8.17, respectively. TDS and EC values ranged from 592 to 905mg/L and 1.18 to 1.55mmhos/cm, for Qalyub district, and from 236 to 990mg/L and 0.38 to 1.82mmhos/cm, for Shubra Al-khayma, respectively. TSS in both cities ranged between 5 and 10mg/L. In accordance with such findings, El-Sayed (2018) has been recorded that the pH, TDS and EC values of 34 shallow groundwater wells tested at northeast Cairo ranged from 7.6 to 8.6, 117 to 1200mg/L, and 360 to 1900µmhos/cm, respectively.

As it is known that calcium and magnesium are responsible for water hardness, their values in both areas of study were under the permissible limits, so, the groundwater in such areas is not hard. Relevant to such results, a study carried out on a total of 144 shallow groundwater wells at Qalyubia governorate, the authors found that the mean values of calcium and magnesium were 34.3 and 19.8mg/L, respectively (El-Fakharany et al., 2017).

TABLE 1. Physical and chemical properties of wastewater of QWTP and groundwater wells of nearby houses at Qalyub district

Parameter	Unit	Water treatment plant		Groundwater wells						WHO guidelines
		Influent	Effluent	Q1	Q2	Q3	Q4	Q5	Q6	
Physical properties (average ± 5%)										
pH		7.82	7.65	7.7	7.7	7.8	7.6	7.75	7.45	**6.5 – 8.5**
TDS	mg/L	980	622	592	600	745	650	905	655	**1000**
TSS	mg/L	226	48	6	8	9	5	7	9	
CO_3	mg/L	0	0	0	0	0	0	0	0	
HCO_3	mg/L	223	188	123	138	132	115	142	155	
Total Alkalinity	mg/L	223	188	123	138	132	115	142	155	
EC	mmhos/cm	2.1	1.12	1.18	1.2	1.4	1.55	1.22	1.32	
Cations (average ± 5%)										
Calcium	mg/L	82	58	84	72	64	36	72	80	**200**
Magnesium	mg/L	32	27	19	52	53	41	36	52	**150**
Sodium	mg/L	185	125	130	110	124	145	115	125	**200**
Potassium	mg/L	22	20	7	10	9	8	8	9	**12**
Anions (average ± 5%)										
Chloride (Cl)	mg/L	190	127	138	122	133	155	125	136	**250**
Nitrate (NO_3)	mg/L	187	172	28	59	5.9	26.5	50.5	5.9	**45**
Phosphate (PO_4)	mg/L	11	9	0.9	0.7	0.5	0.3	0.7	0.8	
Sulphate (SO_4)	mg/L	99	94	88	99	65	85	128	89	**250**
Heavy metals (average ± 5%)										
Aluminum	mg/L	0.12	0.09	0.08	0.01	0.07	0.04	0.02	0.16	**0.2**
Copper	mg/L	0.06	0.02	0.02	0.05	0.03	0.05	0.06	0.04	**1**
Iron	mg/L	0.21	0.18	0.13	0.19	0.14	0.12	0.19	0.15	**0.3**
Manganese	mg/L	0.42	0.3	1.05	1.4	0.9	1.24	1.5	1.1	**0.4**
Nickel	mg/L	0.03	0.02	0.01	0.01	0.02	0.02	0.03	0.03	**0.05**
Strontium	mg/L	0.5	0.27	0.39	0.12	0.22	0.56	0.13	0.26	
Zinc	mg/L	ND	ND	0.05	0.03	0.08	0.03	0.02	0.04	**3**
Organic content (average ± 5%)										
COD	mg/L	620	160	26	13	15	17	14	14	
BOD	mg/L	490	60	8	7	10	12	3	2	**5**
Ammonia	mg/L	67	14	1.7	0.66	0.74	1.75	0.37	0.45	**0.5**

TABLE 2. Physical and chemical properties of wastewater of SHWTP and groundwater wells of nearby houses at Shubra Al-khayma district

Parameter	Unit	Water treatment plant		Groundwater wells						WHO guidelines
		Influent	Effluent	Sh1	Sh2	Sh3	Sh4	Sh5	Sh6	
Physical properties (average ± 5%)										
pH		7.5	8.2	8.13	8.17	8.11	8.10	8	8	**6.5 – 8.5**
TDS	mg/L	709	662	895	934	236	890	990	240	**1000**
TSS	mg/L	385	60	8	10	7	9	7	6	
CO_3	mg/L	0	0	0	0	0	0	0	0	
HCO_3	mg/L	255	181	158	123	161	154	124	115	
Total Alkalinity	mg/L	255	181	158	123	161	154	124	115	
EC	mmhos/cm	1.11	1.03	1.6	1.82	0.38	1.6	1.82	0.49	
Cations (average ± 5%)										
Calcium	mg/L	69.5	61	60	72	34	58	70	40	**200**
Magnesium	mg/L	23.3	11.2	28	31	11	27	24	12	**150**
Sodium	mg/L	117	107	160	170	26	180	185	30	**200**
Potassium	mg/L	20	19	11	10	6	11	11	7	**12**
Anions (average ± 5%)										
Chloride (Cl)	mg/L	140	130	173	182	36	190	195	40	**250**
Nitrate (NO_3)	mg/L	101	43	8	13	6	11	8	7	**45**
Phosphate (PO_4)	mg/L	11	6	2	1	1	2	2	3	
Sulphate (SO_4)	mg/L	174	132	23	44	43	24	28	42	**250**
Heavy metals (average ± 5%)										
Aluminum	mg/L	0.066	0.024	0.06	0.07	0.03	0.049	0.061	0.037	**0.2**
Copper	mg/L	0.03	0.065	0.02	0.08	0.026	0.014	0.06	0.022	**1**
Iron	mg/L	0.36	0.02	0.02	0.41	0.009	0.015	0.4	0.009	**0.3**
Manganese	mg/L	0.177	0.027	0.87	0.77	0.58	0.67	0.7	0.55	**0.4**
Nickel	mg/L	0.067	0.047	0.02	0.03	0.02	0.021	0.02	0.01	**0.05**
Strontium	mg/L	0.352	0.341	0.78	0.87	0.254	0.68	0.87	0.27	
Zinc	mg/L	0.015	ND	0.05	0.02	ND	0.04	0.011	ND	**3**
Organic content (average ± 5%)										
COD	mg/L	551	60	7	9	15	8	8	12	
BOD	mg/L	430	30	3	4	2	3	3	2	**5**
Ammonia	mg/L	70	10	0.44	0.48	0.47	0.35	0.29	0.33	**0.5**

The concentration of nitrate exceeded the permissible limits in two sampling points at Qalyub city, while in Shubra Al-khayma it is acceptable in all sampling points. Phosphate and sulfate values in both cities were acceptable. Heavy metals concentrations in all the tested wells, in both study areas, are under the guidelines except for manganese which exceeded the permissible limits (0.4mg/L) in all the tested wells for both cities, while Fe exceeded the permissible limits (0.3mg/L) in 2 sampling points in Shubra Al-khayma. The BOD and ammonia values also exceeded the permissible limits in 4 wells out of 6 tested wells in Qalyub City.

TABLE 3. Microbiological properties of wastewater of QWTP and groundwater wells of nearby houses at Qalyub district

Parameter	Unit	Water treatment plant		Groundwater wells						WHO guidelines
		Influent	Effluent	Q1	Q2	Q3	Q4	Q5	Q6	
Pollution indicators (average ± 5%)										
TBC 22 ºC	CFU/ mL	2200000	140000	100	210	170	520	850	155	**100**
TBC 35 ºC	CFU/ mL	690000	50000	30	70	65	430	665	83	**50**
Total coliforms	CFU/ 100mL	15200000	900000	120	30	25	220	320	15	**0**
Fecal coliforms	CFU/ 100mL	9000000	960000	30	15	15	110	115	6	**0**
F. streptococci	CFU/ 100mL	560000	250000	20	35	13	45	88	10	**0**
Pathogenic bacteria (average ± 5%)										
A. hydrophila	CFU/ mL	50000	5500	24	10	3	30	5	8	
P. aeruginosa	CFU/ 100mL	1450000	140000	150	40	10	50	15	20	**0**
E. coli	CFU/ 100mL	5500000	880000	12	8	5	67	84	2	**0**
Salmonella	Presence/absence	+	+	-	-	-	-	-	-	**0**
Shigella	Presence/absence	+	+	-	-	-	-	-	-	**0**

TABLE 4. Microbiological properties of wastewater of SHWTP and groundwater wells of nearby houses at Shubra Al-khayma district

Parameter	Unit	Water treatment plant		Groundwater wells						WHO guidelines
		Influent	Effluent	Sh1	Sh2	Sh3	Sh4	Sh5	Sh6	
Pollution indicators (average ± 5%)										
TBC 22 ºC	CFU/ mL	1500000	980000	18	300	700	320	650	48	**100**
TBC 35 ºC	CFU/ mL	530000	340000	7	100	400	130	365	30	**50**
Total coliforms	CFU/ 100mL	5300000	300000	0	21	165	22	6	0	**0**
Fecal coliforms	CFU/ 100mL	900000	220000	0	2	4	0	0	0	**0**
F. streptococci	CFU/ 100mL	1350000	10000	0	0	15	41	0	0	**0**
Pathogenic bacteria (average ± 5%)										
A. hydrophila	CFU/ mL	49000	500	5	0	0	0	0	0	
P. aeruginosa	CFU/ 100mL	120000	20000	10	132	120	65	95	12	**0**
E. coli	CFU/ 100mL	600000	100000	0	0	0	0	0	0	**0**
Salmonella	Presence/absence	+	+	-	-	-	-	-	-	**0**
Shigella	Presence/absence	+	+	-	-	-	-	-	-	**0**

Many authors reported the presence of Mn and Fe, as well as, high BOD and ammonia in shallow groundwater at Delta, Egypt (Ziedan, 2016; El-Fakharany et al., 2017; El-Sayed, 2018). Salem and co-workers mentioned that the main problem related to water in Delta is the reddish color caused by the presence of ferrous and manganese. They recorded concentrations for iron and manganese in most of the tested groundwater wells up to 1.33 and 1.45mg/L, respectively. Finally, they confirmed that fertilizers used in fruit farms and petrochemical, as well as, industrial activities are the main sources of Fe and Mn pollution (Salem et al., 2012).

Microbiological assessment

Microbial contamination of a given water resource always detected by some groups of bacteria named, pollution indicators. They include heterotrophic, coliforms, fecal coliforms and fecal streptococcus bacteria. The bacteriological quality of groundwater wells under investigation at Qalyub and Shubra Al-khayma cities was summarized in Tables 3 and 4. It was clear that bacteria were found in all the tested wells, where the heterotrophic bacterial count ranged from 100 – 850 CFU/mL at 22°C and 30 - 665 at 35°C for Qalyub groundwater, and from 18 - 700 CFU/mL at 22°C and 7 – 400 at 35°C for Shubra Al-khayma groundwater.

Coliform bacteria are a large group of different species of bacteria, including those naturally found in the intestines of warm-blooded animals. Fecal coliform bacteria (thermotolerant coliforms) are a group of bacteria that originate in feces, e.g. *Escherichia*, as well as genera not of fecal origin, e.g. *Citrobacter*, *Enterobacter* and *Klebsiella* (Doyle & Erickson, 2006). The presence of fecal coliform bacteria in a given water resource indicates the presence of sewage materials; which confirm the presence of harmful disease-causing organisms (Ewida et al., 2012). The results given in Table 3 indicated that 100% of the tested wells in Qalyub were contaminated with total coliform and fecal coliform bacteria with counts ranged from 15 to 320 CFU/100mL, and 6 to 115 CFU/100mL, respectively. Furthermore, about 66% of the tested wells at Shubra El-khayma were found to be contaminated with total coliform bacteria with counts ranged from 6 to 165 CFU/100mL and 33% were contaminated with fecal coliform bacteria with counts ranged from 2 to 4 CFU/100mL (Table 4).

The group of fecal streptococci includes several species of the genus *Streptococcus*, which have been isolated from the feces of warm-blooded animals and human intestine. The data in Tables 3 and 4 illustrated that 100% and 33% of the tested wells were contaminated with fecal streptococci, with counts ranged from 10 to 88 and 15 to 41 CFU/100mL at Qalyub and Shubra Al-khayma, respectively. That might be due to the presence of livestock animals in rural houses at the sampling locations.

Aeromonas hydrophila is a natural inhabitant of water environments, especially groundwater (Massa et al., 2001) and in a single well, the same strain can persist for years (Kuhn et al., 1997). It is a pathogenic microorganism; causes some infrequent human and animal diseases (Handfield et al., 1996; Zong et al., 2002). Detection of *A. hydrophila* in shallow groundwater is very important due to the possibility of its presence even if coliforms were absent. Data given in Tables 3 and 4 showed that *A. hydrophila* was found in 100% of groundwater wells under investigation with counts ranged from 3 to 30 CFU/mL at Qalyub city while it was present in one well only from the six tested wells at Shubra Al-khayma city in count of 5 CFU/mL.

Pseudomonas aeruginosa is commonly a normal inhabitant of soil, it has a simple nutritional requirement, so, it is suspected to isolate this organism from different water resources even under low nutritional content (Todar, 2004). Some strains have been reported as opportunistic pathogens (they may cause diseases for immune-suppressed persons) (Romling et al., 1994), so, the presence of such bacterium in groundwater wells used in domestic purposes makes it unsafe to be used. In the present work, *P. aeruginosa* was detected in all the tested groundwater wells for both cities in counts ranged from 10 to 150 CFU/100mL. It was noticed that the groundwater well named (Sh1) at Shubra Al-khayma was free from coliform bacteria, nevertheless, it still non-potable due to the presence of *A. hydrophila* and *P. aeruginosa*.

E. coli is the most famous bacterium commonly used for the detection of water contamination with sewage, the presence of such microorganism in a given water resource confirms its contamination with human faces. The data presented in Tables 3 and 4 indicated the contamination of all the tested wells of Qalyub with *E. coli* in counts ranged from 2 to 84 CFU/100 mL, while it was completely absent from all the investigated wells of Shubra Al-khayma.

Some of the enteric pathogenic bacteria may occur in groundwater wells, especially when indicator bacteria were present. *Salmonella* and *Shigella* were chosen as representative for pathogenic bacteria that have been transmitted by wastewater. Fortunately, both of them were absent from the tested groundwater wells in both cities as illustrated in Tables 3 and 4.

Discrimination of microbial contamination source of Qalyub groundwater wells using BOX-PCR

A total of 12 bacterial isolates were selected, (6) from M-TEC and (6) from M-PA-C plates of QWTP sewage and groundwater samples of nearby houses. Using biochemical reactions, the twelve isolates were identified and differentiated to (3) *E. coli* and (3) *P. aeruginosa* from the plates of QWTP sewage samples and the same from the plates of groundwater samples of nearby houses. So, there were two groups of bacteria; 6 belong to *E. coli* (3 from sewage/ 3 from groundwater) and 6 belong to *P. aeruginosa* (3 of sewage/ 3 of groundwater). BOX-PCR technique was carried out for each group to ensure the origin of *E.coli* and *P. aeruginosa* in groundwater. The results of gel electrophoresis running for both groups were illustrated in Fig. 2. The fingerprinting pattern of the DNA of each isolate confirmed the biochemical identification up to species level. The genomic bands separated by gel electrophoresis running for *E. coli* species (Fig. 2) and the phylogenetic tree of the 6 isolates (Fig. 3 a) indicated that *E. coli* species isolated from the groundwater are closely related to each other. Fortunately, they are not identical with the finger-print genotypic bands of those species isolated from the sewage of Qalyub wastewater treatment plant. On the other hand, the genotypic bands and phylogenetic tree of *P. aeruginosa* (Fig. 2 and 3 b) for both groundwater and sewage were so close to each other, it seems like they all originated from the same source, which creates little doubts about the possibility of seepage from Qalyub water treatment plant to sub-surface groundwater. Even so, the overall view of the concentrations of chemical pollutants detected in wastewater samples colleacted from Qalyub wastewater treatment plant in comparison with those measured in the groundwater wells indicated that sewage collected in QWTP didn't affect the chemical quality of groundwater in the tested wells at all (Table 1). Furthermore, the genotypic bands of *E. coli* (the specific bacterium confirming sewage-water contamination) didn't give evidence that the *E. coli* detected in groundwater was the same as those isolated from sewage. Otherwise, the presence of *E. coli* in groundwater could be explained by the concept of "point source contamination" (Tuthil et al., 1998; Fusconi & Godinho, 1999) where there some human activities were noticed close to the tested wells like, irrigation, septic tanks and livestock animals. Moreover, *P. aeruginosa* is mainly a normal inhabitant of soil, so, it might reach the sewage during collection, and it can reach the groundwater during well drilling (Ewida et al., 2012; Gaber et al., 2013). So, we can confirm that the two checked wastewater treatment plants; Shubra Al-khayma and Qalyub have no negative impact on the quality of shallow groundwater in Qalyubia Governorate.

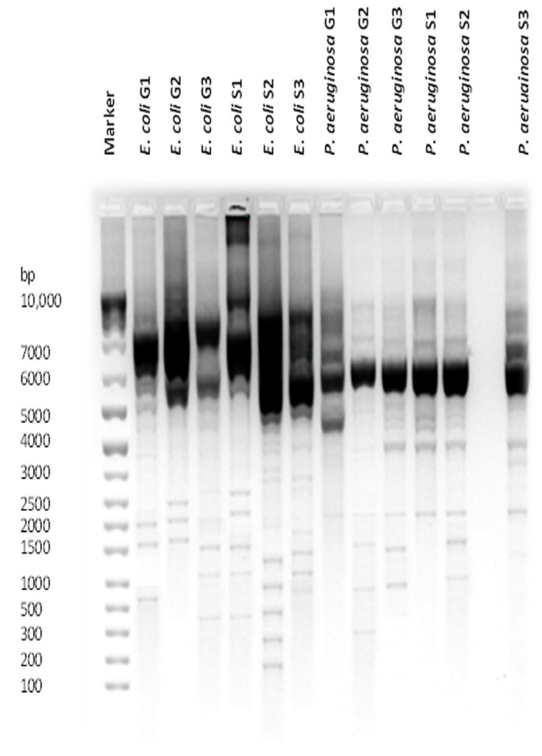

Fig. 2. Negative image of agarose gel electrophoresis of BOX-PCR fingerprinting pattern from genomic DNA of two bacterial groups of species; 6 belong to *E. coli* and 6 for *P. aeruginosa*, G (groundwater) and S (sewage).

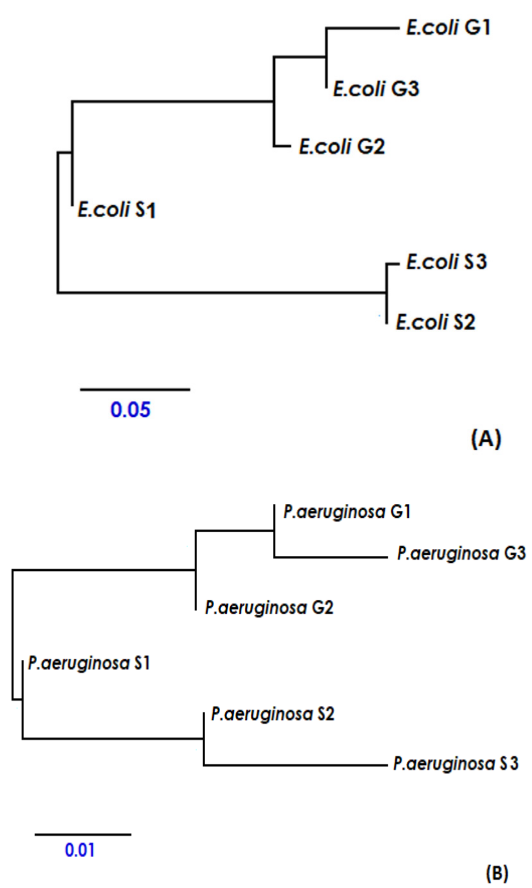

Fig. 3. Phylogenetic trees discriminating the relativeness of two bacterial groups of species; (A) for *E. coli,* (B) for *P. aeruginosa*

Conclusion

The impact of two wastewater treatment plants (QWTP and SHWTP) on shallow groundwater at Qalyubia governorate was studied. Water quality assessment studies indicated the deficiency of the two wastewater treatment plants under investigation, especially QWTP. The percentages of removing of TSS, COD, BOD, ammonia, total coliforms, and fecal coliform counts were 87, 74, 88, 66, 94 and 89%, respectively, for QWTP, and 85, 89, 93, 86, 94 and 83%, respectively, for SHWTP. The groundwater at Qalyub district was contaminated with Mn, Fe, ammonia, BOD, coliform bacteria, fecal streptococci, *E. coli, Aeromonas hydrophila and Pseudomonas aeruginosa,* while the groundwater at Shubra Al-khayma district was contaminated with Mn, coliform bacteria, fecal streptococci and *P. aeruginosa*. Microbial and chemical evaluation of groundwater, as well as, BOX-PCR results confirmed that both wastewater treatment plants have no negative impact on the quality of shallow groundwater.

Conflicts of interest: The authors declare no conflict of interest.

Author contributions: A.Y.I.E. contributed the conception of the research idea, methodology design and some laboratory works, as well as, prepared the manuscript for publications. M. S. K. contributed for interpretation of the results. A.M.A contributed for the laboratory work. All the authors confirm that they have read and agreed to the published version of the manuscript.

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References

American Public Health Association (APHA) (2017) "*Standard Methods for the Examination of Water and Wastewater*", 23rd ed. DOI:10.2105/SMWW.2882.193.

Bathrellos, G.D., Skilodimou, H.D., Kelepertsis, A., Alexakis, D., Chrisanthaki, I., Archonti, D. (2008) Environmental research of groundwater in the urban and suburban areas of Attica region, Greece. *Environmental Geology,* **56**, 11-18.

Bilung, L.M., Pui, C.F., Su'ut, L., Apun, K. (2018) Evaluation of BOX-PCR and ERIC-PCR as molecular typing tools for pathogenic *Leptospira*. *Disease Markers,* ID 1351634, 1-9.

Doyle, M.P., Erickson, M.C. (2006) Closing the door on the fecal coliform assay. *Microbe,* **1**(4), 162-163.

ElFakharany, M.A., Mansour, N.M., Yehia, M.M., Monem, M. (2017) Evaluation of groundwater quality of the Quaternary aquifer through multivariate statistical techniques at the southeastern part of the Nile Delta, Egypt. *Sustainable Water Resources Management*, http://doi.org/10.1007/s40899-017-0087-6

El-Sayed, S.A. (2018) Study of Groundwater in Northeast Cairo Area, Egypt. *Journal of Geoscience and Environment Protection,* **6**, 229-251.

Environmental Protection Agency (US-EPA) "*Guidelines for Water Reuse*". EPA/600/R-12/618 Sept, 2012.

Ewida, A.Y.I., Sami, S.S., Zayied, A. (2012) Microbiological and Hydrogeological studies on the contamination sources on groundwater at Rafah-Zuwayd, North Sinia, Egypt. *Engineering Research Journal,* **133**, 118-131.

Fusconi, R., Godinho, L. (1999) Bacteria and protozoa populations in groundwater in a landfill area in Sao Carlos. Brazil. *Revista-de-Microbiologia*, **30**, 196-202.

Gaber, S.E., Jahin, H.S., Ewida, A.Y.I. (2013) Impact of human activities on the quality of water resources at Zefta village, in Egypt. *Water Science J*ournal, **53**, 78-87.

Galloway R.L., Levett, P.N. (2010) Application and validation of PFGE for serovar identification of *Leptospira* clinical isolates. *PLoS Neglected Tropical Diseases*, **4**(9), 1-7.

Gorgij, A.D., Wu, J., Moghadam, A. A. (2019) Groundwater quality ranking using the improved entropy TOPSIS method: A case study in Azarshahr plain aquifer, east Azerbaijan, Iran. *Human Ecology Risk Assessment,* **25**, 176-190.

Handfield, M., Simard, P., Couillard, M., Letarte, R. (1996) *Aeromonas hydrophila* isolated from food and drinking water: hemagglutination, hemolysis and cytotoxicity for a human intestinal cell line (HT-29). *Applied and Environmental Microbiology,* **62**(9), 3459-3461.

Hassanein, N.M., El-Baghdady, K.Z., Farid, A.K., Tawfik, T.A., Ewida, A.Y.I. (2012) Microbial communities and water quality of some groundwater systems in Egypt. *Egyptian Journal of Experimental Biology,* **8**(1), 25-42.

Katuva, J., Hope, R., Foster, T., Koehler, J., Thomson, P. (2020) Groundwater and welfare: A conceptual framework applied to coastal Kenya. *Groundwater for Sustainable Development*, **10**, 1-12.

Khan, K., Lu, Y., Saeed, M.A., Bilal, H., Sher, H., Khan, H., Ali, J., Wang, P., Uwizeyimana, H., Baninla, Y., Li, Q., Liu, Q., Nawab, J., Zhou, Y., Su, C., Liang, R. (2018) Prevalent fecal contamination in drinking water resources and potential health risks in Swat, Pakistan. *Journal of Environmental Sciences (China),* **72**, 1-12.

Kuhn, I., Huys, G., Coopman, R., Kersters, K., Janssen, P. (1997) A 4-year study of the diversity and persistence of coliforms and *Aeromonas* in the water of a Swedish drinking water well. *Canadian Journal of Microbiol*ogy, **43**, 9-16.

Kumar, M., Ramanathan, A.L., Rao, M.S., Kumar, B. (2006) Identification and evaluation of geochemical processes in the groundwater environment of Delhi, India. *Environmental Geology,* **50**, 1025-1039.

Li, P. (2016) Groundwater quality in Western China: Challenges and paths forward for groundwater quality research in Western China. *Exposure and Health,* **8**(3), 305-310.

Massa, S., Altieri, C., D'Angela, A. (2001) The occurrence of *Aeromonas* spp. in natural mineral water and well water. *International Journal of Food Microbiology,* **63**, 169-173.

Marques, A.S.A., Marchaison, A., Gardan, L., Samson, R. (2008) BOX-PCR-based identification of bacterial species belonging to *Pseudomonas syringae - P. viridiflava* group. *Genetics and Molecular Biology, São Paulo***, 31**(1), 106-115.

Mckeon, D.M., Calabrese, J.P., Bissonnette, G.K. (1995) Antibiotic resistant Gram negative bacteria in rural groundwater supplies. *Water Research,* **29**, 1902-1908.

Obeidat, M.M., Awawdeh, M., Al-Mughaid, H. (2013) Impact of a domestic wastewater treatment plant on groundwater pollution, north Jordan. *Revista Mexicana de Ciencias Geológicas,* **30**(2), 371-384.

Ramadan, A.M., Abdel-Rahman, A., Abdullah, A.M.,Eltawab, O.A. (2017) Evaluation of wastewater treatment plants in El-Gharbia Governorate, Egypt. *Organic Chemistry Current Research,***6**(2), 1-12.

Romling, U., Wingeneder, J., Muller, H.,Tummler, B. (1994) A major *Pseudomonas aeruginosa* clone common to patients and aquatic habitats. *Applied and Environmental Microbiology,* **60**, 1734-1738.

Rouabhia, A., Fehdi, Ch., Baali, F., Djabri, L., Rouabhi,

R. (2009) Impacts of human activities on quality and geochemistry of groundwater in the Merdja area, Tebessa, Algeria. *Environmental Geology,* **56**, 1259-1268.

Safiulah, S.A., Saleh, A.A., Munwar, S. (2009) Laboratory methods for diagnosing leptospirosis: a review, *Bangladesh Journal of Medical Microbiology,* **3**(1), 39-43.

Salem, M.G., El-Awady, M.H., Amin, E. (2012) Enhanced removal of dissolved Iron and Manganese from nonconventional water resources in Delta district, Egypt. *Energy Procedia,* **18**, 983-993.

Spalding, R.F., Exner, M.E. (1993) Occurrence of nitrate in groundwater, a review. *Journal of Environmental Quality,* **22**, 392-402.

SubbaRao, N., Sunitha, B., Rambabu, R., NageswaraRao, P.V., Surya Rao, P., Deepthi, S. B., Sravanthi, M., Deepali, M. (2018) Quality and degree of pollution of groundwater, using PIG from a rural part of Telangana State, India. *Applied Water Science,* **8**, 227-233.

SubbaRao, N., Srihari, Ch., Deepthi, S. B., Sravanthi, M., Kamalesh, T., Abraham, J.V. (2019) Comprehensive understanding of groundwater quality and hydrogeochemistry for the sustainable development of sub-urban area of Visakhapatnam, Andhra Pradesh, India. *Human Ecology Risk Assessment*, **25**, 52-80.

Todar, K. (2004) "*Todar's Online Textbook of Bacteriology*." University of Wisconsin, Madison, Wisconsin. www.textbookofbacteriology.net.

Tuthil, A., Meikle, B., Alavanja, R. (1998) Coliform bacteria and nitrate contamination of wells in major soils of Fredrick, MaryLand. *Journal of Environmental Health*, **60**, 16-20.

Wakida, T.F., Lerner, D.N. (2005) Non-agricultural sources of groundwater nitrate: a review and case study: *Water Research,* **39**, 3-16.

Wilhelm, S.R., Schiff, S.L., Robertson, W.D.(1996) Biogeochemical evolution of domestic wastewater in septic systems: Application of conceptual model in sandy aquifers: *Journal of Ground Water,* **34**, 853-864.

Zeidan, B.A. (2016) Groundwater degradation and remediation in the Nile Delta aquifer. In: "*The Nile Delta, Hand Book of Environmental Chemistry*". Springer International Publishing, DOI 10.1007/698_2016_128.

Zong, Z., Lü, X.,Gao, Y. (2002) Aeromonas hydrophila infection: Clinical aspects and therapeutic options. *Reviews in Medical Microbiology*, **13**(4),151-162.

تأثير محطات معالجة مياه الصرف الصحي على جودة المياه الجوفيه الضحله في بعض المناطق بمحافظة القليوبيه؛ تحديد مصدر التلوث الميكروبي باستخدام تقنية BOX-PCR

أيمن يوسف ابراهيم عويضه[1]، ماري صبحى خليل[2]، علاء محمد احمد محمود[1]
[1]قسم الميكروبيولوجي - المركز القومي لبحوث المياه - القاهره - مصر، [2]قسم الميكروبيولجي - كلية العلوم- جامعة القاهره - الجيزه - مصر.

تعتبرمياه الصرف الصحي ومياه الري والحيوانات المنزليه من المصادر الشائعة لتلوث المياه الجوفية في المناطق الريفية، وايضا محتمل أن تكون احواض الترسيب في محطات معالجة مياه الصرف الصحي مصدرًا آخر للتلوث. لذلك تم اختيار محطتين لمعالجة مياه الصرف الصحي بمحافظة القليوبية؛ محطة قليوب (QWTP) ومحطة شبرا الخيمة (SHWTP) لإجراء الدراسة الحالية. تم تجميع عينتين من كل محطه واحده من الصرف الخام والاخرى من المياه الناتجه بعد المعالجه، علاوة على 6 عينات من المياه الجوفية من البيوت الريفية القريبة من كلا المحطتين. تم تقييم الخواص الفيزيائية والميكروبيولوجية لجميع العينات، بالاضافه الى تنفيذ Box-PCR لـ 12 عزله بكتيريه مجمعه من مياه الصرف الصحي لمحطة قليوب والمياه الجوفية المجمعه من المنازل المجاوره. أثبتت دراسات تقييم جودة المياه أن كفاءة محطتي معالجة مياه الصرف الصحي قيد الدراسة ضعيفه، خاصة محطة قليوب. و كانت النسب المئوية لإزالة المواد الصلبه العالقه (TSS) ، الاكسجين الكيميائي الممتص (COD)، الاكسجين الحيوي الممتص(BOD) ، الأمونيا(NH_3) ، والبكتيريا البرازيه(Total coliforms) والبكتيريا القولونيه البرازيه (Fecal coliforms) 87، 74، 88، 66، 94 و89٪، على الترتيب، لمحطة قليوب، و85، 89، 93، 86، 94 و83%، على الترتيب، لـمحطة شبرا الخيمه. وكانت المياه الجوفية بمنطقة قليوب ملوثه بالمنجنيز والحديد والامونيا و BOD والبكتيريا القولونيه، والبكتيريا العنقوديه البرازية، وبكتيريا *E. coli* و *Aeromonas hydrophila* و *Pseudomonas aeruginosa*، بينما اوضحت الدراسه تلوث المياه الجوفية في منطقة شبرا الخيمة بالمنجنيز والبكتيريا القولونية والبكتيريا القولونية البرازية والبكتيريا العنقوديه البرازية و *P. aeruginosa*. واثبت التقييم الميكروبي والكيميائي للمياه الجوفية، وكذلك نتيجة BOX-PCR أن تلوث المياه الجوفية المختبرة قد يكون من مصدر آخر غير احواض الترسيب بمحطات معالجة مياه الصرف الصحي.