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Environmental impact assessment of different water resources in Egypt in comparison with antibiotic resistance activity of their bacterial communities

Ayman Y. I. Ewida[1]*, Amira F. Hegab[1], Rashed A. Zaghloul[2], Ahmed A. Salem[2], Hany M. Abdel-Rahman[2]

[1]Department of Microbiology – National Water Research Center – Cairo Egypt.
[2]Agricultural Microbiology Department – Faculty of Agriculture – Benha University – Egypt.
*Corresponding Author: ayman_ibrahim@nwrc.gov.eg , ORCID; 000 – 0001 – 6913 – 6748.

Abstract: Pollution of water resources is one of the most important global environmental and economical concern. In developing countries, the lack of waste management, both industrial and domestic, is the cause of water quality deterioration. The present work is concerning the evaluation of different water resources in Egypt, in addition, evaluation of pollution risk factor by measuring the multiple antibiotic resistance (MAR) index of the bacterial community of each water resource. Water samples have been collected from seven different water resources; shallow groundwater at different rural areas, River Nile water at some different sites, agricultural drainage water (El-Husseiniya drain), sewage contaminated water (Bahr El-Baqr drain), industrial wastewater (Al-khadrawiya drain), fresh water Lake (Nasser Lake) and brackish water Lake (Qaroun Lake). The chemical and microbiological features of all water types have been evaluated, the microbial community of each water resource have been identified by means of biochemical reactions and 16S rDNA sequencing. The integrated MAR index of each bacterial community has been calculated by sensitivity test, using ten commercially available antibiotics. Water quality assessment indicated the acceptable quality of groundwater (at few tested sites), Nile River water (at most of tested sites), El-Husseiniya drain water and Nasser Lake. In contrary, Bahr El-Baqr drain showed high loads of organic contaminants and pathogenic bacteria, Al-khadrawiya drain showed contamination with some heavy metals (Al, Fe, Pb and Zn), and Qaroun Lake showed contamination with sulfate, iron and some pathogenic bacteria. The MAR index for each water resource was 0.13, 0.215, 0.24, 0.52, 0.65, 0.165 and 0.277, respectively. The contamination of a given water resource with sewage materials increases the resistance of the whole bacterial community to antibiotics by the function of plasmid exchange characterizing some members of enterobacteriacea group, which predominate in such cases. In addition, heavy metals contamination increases the virulence of bacterial community to resist antibiotics, and categorize the water resource as a high risk polluted water. Therefore, evaluation of water quality and MAR index for different water resources is recommended.

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1. Introduction

Pollution of water resources is a serious environmental issue in developing countries due to the uncontrolled urbanization. The lack of waste management leads to disposal of sewage and industrial effluents to water bodies (Balthazard et al. 2019). Water bodies can be characterized by the physico-chemical and biological features. A complete assessment of water quality is based on appropriate monitoring of these features (WHO, 1996). Many authors have been used this pattern for assessing water quality of different water bodies in Egypt. For examples, the groundwater in nine different governorates in Egypt was evaluated by (Hassanein et al., 2012) they recorded microbiological contamination of shallow groundwater at Sohag, Qena and Dakahlia. Ewida and co-workers (2021) reported the contamination of shallow groundwater in Qaluybiya governorate with Fe and Mn. The Nile River water quality at two different governorates Damietta and Dakahlia was assessed by Badr and co-workers (2013). They reported the presence of high concentration of COD, BOD, ammonia, nitrate and phosphate in the Nile at Damietta district compared to Dakahlia district. They also detected some heavy metals (Fe, Mn and Pb) in high concentration than the permissible limits. Wastewater of Bahr El-Baqr drain pose serious environmental hazards, and it has been abundantly assessed, it is characterized by high loads of organic contaminants (Abdel-Fattah and Helmy, 2015). As much as water quality assessment studies for different water resources are, there is none cover all types of water resources in the same article.

On the other hand, microorganisms in the contaminated water environment have acquired some mechanisms for adapting the presence of heavy metals (Rajbanshi, 2008). Researchers have been evident that antibiotic resistance activity in bacterial community is frequently associated with metal resistance (Bell, 1983). The respectable increase of Multiple Antibiotic Resistant (MAR) bacteria are observed in various aquatic systems. Bacteria in a given community showed MAR activity are considered virulent bacteria, and infections caused by such bacteria could be difficult to be treated (Chandrasekaran, 1998).

So, the objectives of the present study are; i) water quality assessment for seven different water resources, ii) identification of the bacterial community of each water resource, iii) evaluation of MAR index of the bacterial community using sensitivity test and iv) comparing the results of water quality assessment studies with antibiotic resistance activity of the bacterial community, to determine the health risk factor associated with the types of contaminants.

2. Materials and Methods

2.1 Samples locations with sites descriptions

Water samples were collected seasonally according to the standard methods for examination of water and wastewater (APHA, 2017). Four different water resources have been selected to carry out the study; i) shallow groundwater samples from private hand pumps at some villages of Qaluybia and Menufia Governorates, ii) River Nile water at Aswan, Beni Suef, Qaluybia and Kafr El-Sheikh Governorates, iii) agricultural drainage water from Sahl El-Husseiniya drain, Sharqia Governorate, industrial wastewater from Al-Khadrawiya drain, Menufia Governorate, sewage contaminated water from Bahr El-Baqr drains, Port Saied Governorate, iv) Lakes water samples from Nasser lake, as a fresh water lake, Aswan Governorate, and Qaroun lake as a brackish water lake.

2.2 Water quality assessment studies for all types of water resources

Some of physico-chemical and microbiological analyses for all types of water resources under investigation were carried out according to APHA (2017) to evaluate the water quality of each water resource. The pH was measured at 25^{o}C using pH meter (InoLab WTW level 1 electrode, with ATC probe WTW Sentix 4). Electrical Conductivity (EC) was measured at 25^{o}C using a conductivity meter (InoLab Cond level 1). Total Dissolved Solids (TDS) was determined by weighing the solid residue obtained by evaporating a specific volume of filtered water sample to dryness at 105^{o}C. Total Suspended Salts (TSS) was determined gravimetrically at 105^{o}C. Biochemical oxygen demand $(BOD)_5$ was measured using (OxiTop system WTW) at 20 ºC incubation in a thermostatic incubator for 5 days. 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). Total ammonia nitrogen (NH_3) was measured using the Nessler method. Major cations; calcium, potassium, magnesium and sodium were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES) Perkin-Elmer, Optima 5300 DV. Major anions; sulfate, nitrate, phosphate and chloride were measured using Ion Chromatography (IC), Dionex product, model DX5000. Concentrations of some heavy metals as (Al, As, Cd, Fe, Mn, Pb, Ni, Sr and Zn) were measured using ICP- MS (Inductively Coupled Plasma-Mass Spectrometry) Perkin-Elmer (model SCIEX Elan 9000). Microbiological analyses were carried out as follows; pour plate method was used for enumeration of total bacterial count (TBC) at 22°C and at 35°C, using plate count agar (Merck, Jermany). Enumeration of total coliforms, *E. coli,* fecal streptococci and *Pseudomonas aeruginosa* was performed by membrane filter technique using the following media (Merk, Germany): M - Endo agar LES, M-TEC agar (Difco, U.S.A.), M - *Enterococcus* agar and M-PA-C agar and *Pseudomonas* selective agar, respectively.

2.3 Isolation, purification and identification of bacterial community for each water resource

Bacterial isolates from each water resource were collected through the study time from plates of the total viable bacterial counts. The growing colonies of each plate were differentiated and categorized .according to the cultural characteristics, and then they were purified and maintained for further identification. The purified isolate was subjected to identification by means of; culture characteristics, Gram's stain, biochemical characterization as described by Krieg and Holt (1986) and Sneath (1986). Finally, the pre-identified isolates were confirmed by 16S rDNA. Bacterial DNA was extracted and purified then used as template to amplify the 16S rDNA gene by polymerase chain reaction (PCR). Universal primer 27F of (5`-AGA GTT TGA TCC TGG CTC AG-3`) and 1492R (5`-CGG YTA CCT TGT TAC GAC TT-3`) was used to amplify the 16S gene. The PCR cycles were run as the follow; initial denaturation for 5 min at 94°C, denaturation for 1 min at 94°C, annealing for 1 min at 55°C and extension for 2 min at 72°C and the final extension for another 1 ml of first and 10 min at 72°C. The previous cycles were repeated for 30 times. The amplification products have got sequencing by Biomolecular Research Services (GATC Biotech, Konstanz, Germany) using ABI 3730 l×DNA sequencer with the same primers. The sequences obtained were aligned to the GenBank database with the maximum similarity and identity using gene alignment database.

2.4 Studying the antibiotic resistance activity of each bacterial community of each water resource

Antibiotic resistance behavior of the purified isolates of each bacterial community was determined by the standardized Kirby - Bauer disc diffusion method on Mueller–Hinton agar using the following antibiotics: (amikacin as Amikin AK 30 µg), (amoxicillin as Amoxil AMX 25 µg), (ampicillin AM 10 µg), (cefotaxime as Claforan CTX 30 µg), (chloramphenicol C 30 µg), (erythromycin E 15 µg), (ofloxacin as Tarivid, OFX 5 µg), (cefadroxil CFR 30 µg), (ampicillin/ Sulbactam 10/ 10, SAM 20) and (amoxicillin/ clavulanic acid 20/ 10 AMC 30 µg). Mueller– Hinton agar medium was prepared and poured out into Petri-dishes, after solidification, plates were incubated overnight at 35°C to remove excess moisture from the surface. A 0.1 ml of freshly prepared broth culture appropriately diluted in normal saline solution (OD_{620} nm=0.1–0.08) was spread evenly on the Mueller–Hinton agar plates, and left at ambient temperature for 10 min. Antibiotic discs were mounted on the surface, then the plates were placed at 4°C for 2 h to allow the diffusion of antibiotics; thereafter, the plates were incubated overnight at 35°C. Control Mueller-Hinton agar plates were incubated without antibiotic discs. Each bacterial isolate was scored for resistance or sensitivity after 18 h by comparing the chart on the inhibitory zone diameter as given by the disc manufacturer.

Calculation of multiple antibiotic resistance (MAR) index for each bacterial community of each water resource

MAR activity is defined as the ability of a bacterial isolate to resist more than three antibiotics, the determination of such activity for a given environmental bacterial population helps in evaluation of water resource quality. MAR index was suggested by Krumperman (1983) and Hinton *et al.* (1985) according to the following formula

$MARI = a/bc$ ……………………… Eq (1).

Where (a) is the aggregate of antibiotic resistance score of all isolates in a given community, (b) is the number of antibiotics tested and (c) is the total number of isolates in the community.

3. Results and Discussion

3.1 Water quality assessment studies of different water resources

The analyses of all water samples collected through the study from all water resources were recorded seasonally, and the ranges of all records were statistically calculated and illustrated in tables 1 (for chemical analyses) and table 2 (for microbiological analyses). The results of water quality parameters tested for groundwater at different sites were mostly within the permissible limits (comparing with the Egyptian guidelines for drinking groundwater, low of health Minister for year 2005). Two sites only (Al-Baradaa and Aghour villages) were suffering from high ammonia and BOD values 0.53, 9 and 1.2, 16 mg/l, respectively. Also, iron and manganese concentrations were exceeding the permissible limits (0.3 and 0.1 mg/l) in most sites, which is a common findings in shallow groundwater in rural areas in Egypt due to some industrial activities and fertilizers used in fruits farms (Ewida et al. 2021). The bacterial contamination of groundwater was also detected in all the tested sites exceeding the permissible limits which may be due the lack of sewerage systems at some rural areas (Ewida et al. 2021).

The Nile river water was generally clear and of acceptable quality, especially at Aswan passing through Beni Suef to Qalyubia governorates. The water quality at Kafr El Sheikh Governorate, was unacceptable (comparing the results with EPA guidelines for fresh water, EPA, 2012) where the concentrations of total hardness, DO, BOD, COD, ammonia, potassium and manganese were 277, 3.8, 18, 22, 2.6, 13 and 0.422 mg/l, respectively. Bacterial pollution was also detected at the same site. That might be due to the organic loads discharged from some contaminated drains at Rosetta branch (Ewida and Mohmmed, 2019).

Regarding the quality of drains' waters tested; Sahl El-Husseiniya drain (which receives agricultural wastewater from Al-Sharqia governorate), Bahr El-baqr drain (which receives agricultural and domestic wastewater, and considered as one of the most polluted drains in Egypt (Abdel-Shafy and Aly, 2002). It is passing through four highly populated Governorates; Qalyubia, Al-Sharqia, Ismailia and Port Said) and Al-Khadrawiya drain (which receives mainly industrial wastewater from factories pipes extended from Quesna industrial zone, Munofia Governorate). The water quality assessment studies explained their pollution with high concentrations of TDS and TSS, where their recorded 3400 and 142, 2143 and 96, and 2911 and 78 mg/l, respectively, which is a consequence impact of agricultural wastewaters (Saad et al. 1984).

Table (1): Chemical assessment of different water resources

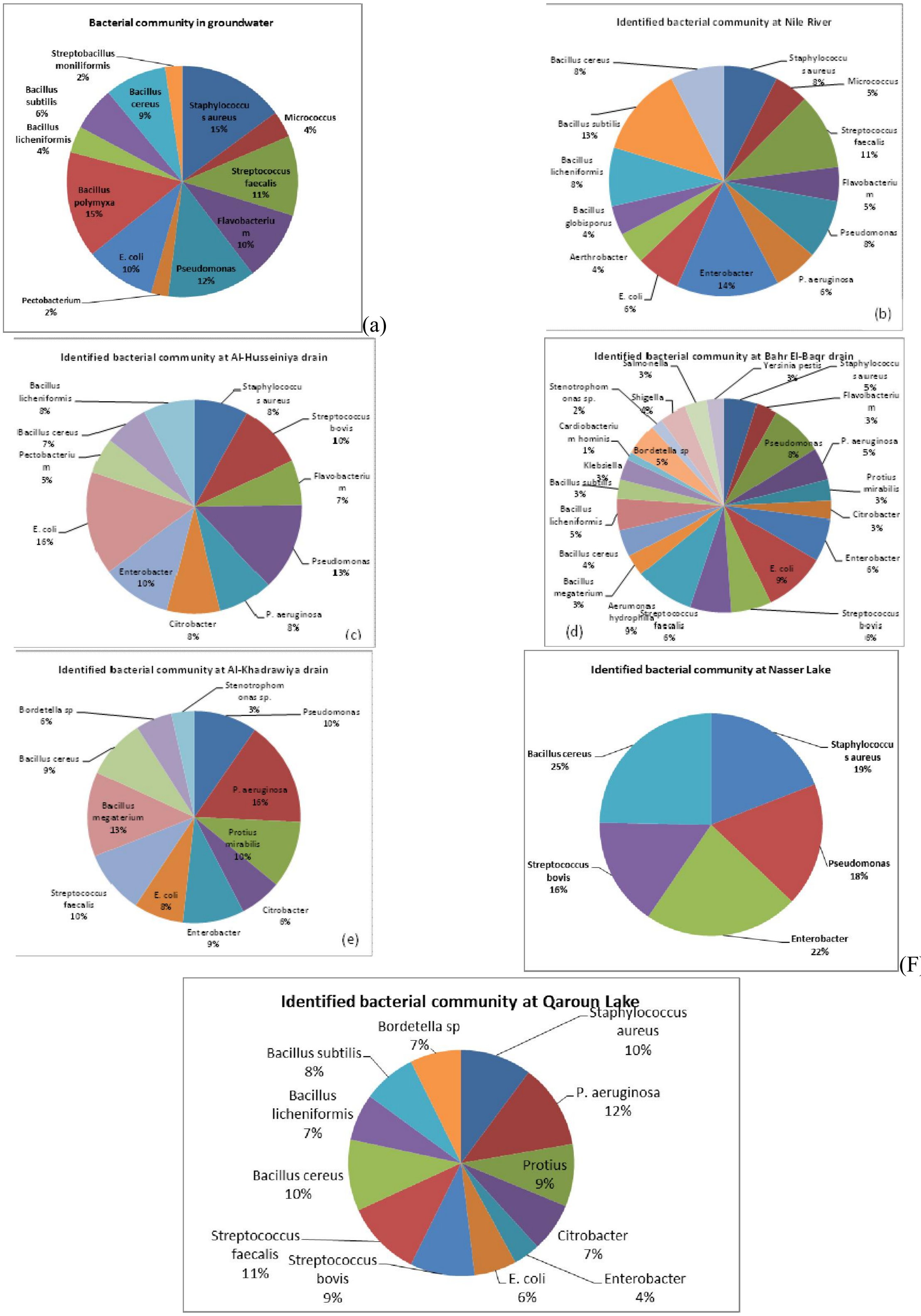
Water resource		Ground-water	Nile River water	Sahl El-Husseiniya drain	Sewage contaminated drains	Al-Khadrawiya drain	Nasser Lake	Qaroun Lake
pH		7.2-8.2	7.5-8.2	8 - 8.2	7.8-8.1	7.9 - 8.3	7.5 - 7.6	7.2 - 7.5
EC	mmhos/cm	0.65-0.91	0.28-0.9	5.3 - 5.5	1.3-3.1	3.9 - 4.2	0.25 - 0.28	3.8 - 4.5
TDS	mg/l	400-680	179-578	2200 - 3400	2100-2200	2800 - 2910	160 - 175	2900 - 3200
TSS	mg/l	11-18	3-19	120 - 142	100-260	66 - 78	3 - 4	170 - 190
Ammonia	mg/l	0.05-1.2	0.05-2.6	3.2 - 3.7	10-16	2.4 - 4.6	0.01 - 0.05	1.5 - 2.5
BOD	mg/l	3-16	2-18	24 - 34	70-110	170 - 190	3 - 5	15 - 25
COD	mg/l	7-21	5-22	35 - 56	90-190	190 - 210	6 - 9	20 - 35
Calcium	mg/l	30-61	17-71	160 - 179	65-150	130 - 155	25 - 28	280 - 300
Potasium	mg/l	5-11	4-13	20 - 24	17-27	18 - 20.6	3 - 5	22 - 31
Magnesium	mg/l	14-30	7.4-24	50 - 66	31-75	45 - 49.4	8 - 11	29 - 35
Sodium	mg/l	31-90	14-96	500 - 720	175-490	500 - 556	16 - 18	490 - 570
Sulfate	mg/l	<0.2	11-79	320 - 390	62-120	40 - 50	11 - 15	440 - 660
Nitrate	mg/l	<0.2	0.42-2.2	3.8 - 4.3	12-15	2 - 3.2	<0.02	1.5 - 2.5
Phoshate	mg/l	1.1-4.8	0.02-2.1	1.8 - 2.1	2.3-2.7	2.8 - 3.2	0.23 - 0.27	1.1 - 2.5
Chloride	mg/l	35-198	6-99	800 - 1080	270-580	620 - 756	6 - 8.2	550 - 720
Aluminum	mg/l	0.007-0.04	0.014-0.09	0.007 - 0.06	0.06-0.5	1.1 - 1.22	<0.007	0.25 - 0.35
Arsenic	mg/l	<0.006	<0.006	<0.006	0.06-0.42	<0.006	<0.006	<0.006
Iron	mg/l	0.028-0.31	0.011-0.05	<0.006	0.16-0.45	1.1 - 1.31	<0.006	0.25 - 0.38
Manganese	mg/l	0.117-0.25	0.3-0.45	0.1 - 0.15	0.26-0.28	0.5 - 0.8	<0.007	0.1 - 0.15
Nickel	mg/l	<0.007	<0.007	<0.007	0.006-0.013	<0.007	<0.007	<0.007
Lead	mg/l	<0.007	<0.007	<0.007	0.004-0.008	0.15 - 0.253	<0.007	<0.007
Strontium	mg/l	<0.002	<0.002	<0.002	0.3-0.45	0.03 - 0.05	<0.002	<0.002
Zinc	mg/l	0.007-0.04	0.007-0.17	0.01 - 0.013	0.3-0.55	1.12 - 1.22	0.007 - 0.12	0.11 - 0.15

Table (2): Microbiological assessment of different water resources

Water resource	TBC 22^{o}C	TBC 35^{o}C	Total Coliforms	Fecal coliforms	*E.coli*	Fecal streptococci	*P. aeruginosa*	*S. aureus*
	Count (CFU/ mL) log		Count (CFU/ 100mL) log					
Groundwater	2.17- 2.83	2.11 – 2.47	1 – 1.3	0.69 – 1	0.30 – 0.90	0.47 – 0.69	0.30 – 0.90	0 – 1.36
Nile River water		2.46 – 3.23	1.3 – 3.14	0.69 – 2.77	0 – 1.60	0.47 – 2.07	0.69 – 2.21	0.90 – 2.50
Sahl El-Husseiniya drain	3.95 – 4.07	3.77 – 3.95	4.60 – 4.74	4.25 – 4.51	4.07 – 4.34	3.77 – 4.04	3.84 – 4.04	4.14 – 4.25
Bahr El-Baqar drain	7.65 – 7.99	7.34 – 7.82	7 – 7.20	6.60 – 6.77	6.30 – 6.60	5.3 – 6.07	5.30 – 5.90	7 – 7.32
Al-Khadrawiya drain	5.5 – 5.6	5.14 – 5.34	5.50 – 5.65	5.14 – 5.44	5.04 – 5.27	5.25 – 5.44	6.46 – 6.53	6.57 – 6.62
Nasser lake, Aswan	2.2 – 2.3	2.07 – 2.20	1.34 – 1.47	0.60 – 0.90	0 – 0.30	0.30 – 0.77	0.30 – 0.69	1.07 – 1.25
Qaroun lake, Al-Fayoum	4.10 – 4.17	3.91 – 3.99	2.44 – 2.55	2.06 – 2.09	1.44 – 1.60	1.50 – 1.74	1.14 – 1.34	2.41 – 2.49

In addition, the sewage and industrial wastes receiving drains were suffering from high BOD and COD concentrations as they recorded 70 and 88, and 186, 210 mg/l, respectively, which might be due to the high organic content of contaminants (Ewida, 2014). Furthermore, the industrially contaminated drain (Al-Khadrawiya drain) was characterized by the presence of high concentrations of some heavy metals (Al, Fe, Mn, Pb and Zn) where they recorded 1.21, 1.3, 0.5, 0.25 and 1.22 mg/l, respectively, which is in accordance with what was found by Tamil Selvi and co-workers whom recorded high concentrations of heavy metals from industrial effluents (Tamil Selvi et al. 2012).

The bacterial contamination of the three drains under investigation was gradient according to the sewage loads, where, for example, the coliform counts in Sahl El-Husseiniya drain ranged from 40 to 55 X 10^3, and increased to 10 - 16 X 10^6 at Bahr El-Baqr drain (sewage loads) and recorded 32 – 45 X 10^4 in Al-khadarwiya drain (industrial loads) and that was with all the determined groups of bacteria. *P. aeruginosa* count was higher with industrial wastes load than sewage wastes loads which might be due to its ability of such bacterium to survive chemical toxicity (Mena and Gerba, 2009).



(G)

Figure (1): Bacterial communities in different water resources

The water quality assessment of Nasser Lake indicated its purity where all investigated chemical and microbiological features were under the permissible limits mentioned by EPA for fresh water (EPA, 2012). On the other hand, brackish water of Qaroun Lake was characterized with high TDS, TSS, sulfate concentrations, where they recorded 3168, 886 and 590 mg/l, respectively, which may be due to the discharge of agricultural, industrial and sewage wastes from El-Bats and El-Wadi drains without prior treatments (Derbala, 2018). The microbiological contamination was also detected in moderate counts for the same reason.

3.2 Identification of the bacterial community in each water resource

The bacterial community of each water resource was identified by means of biochemical reactions and 16s rDNA sequence analyses. The identified strains and percentage of each of them in the community of a given water resource was illustrated by pie charts (figure 1 from (a) to (g)). The predominant genus in groundwater, Nile River and both types of lakes was *Bacillus*, while *Pseudomonas* was predominate in agricultural and industrial wastes' contaminated drains. The sewage contaminated drain was predominated with *Escherichia*.

3.3 Antibiotic resistance activity of each bacterial community in each water resource

The hypothesis suggested by Krumperman in (1983) to calculate the MAR (multiple antibiotic resistance) index for each group of bacteria in a given community was used in the present study to evaluate the results of environmental impact assessment studies of different water resources. Actually, the hypothesis includes that; any group of isolates inside the same genus in a given community show resistance to three or more antibiotics have to considered of MAR activity. Furthermore, the calculation of MAR index was applied for all genera inside a given water resource as integrated MAR index, with considering that any water resource with a bacterial community of MAR index more than 0.25 is of high risk contamination. The integrated MAR index of groundwater tested (table 3) was 0.13. About 55%, 60%, 25%, 41% and 43% of isolates of *Streptococcus feacalis, Pseudomoas, E.coli, B. polymyxa* and *B. cereus,* respectively have showed MAR activity, however the percentage of isolates having MAR activity in all the community was only 27%, which may be due to the lack of heavy chemical contamination in most tested sites of groundwater (Kozdro and Van Elsas, 2001).

Table (3): Antibiotic resistance pattern of the bacterial community identified from groundwater

Identified bacterial community	No. of isolates	Antibiotics										% of MAR isolates	Integrated MAR
		AK	AMX	AM	CTX	C	E	OFX	CFR	SAM	AMC		
		No. of resistant isolates											
Staphylococcus aureus	***12***	0	0	0	0	0	0	0	0	0	0	0	
Micrococcus	***3***	0	0	0	0	0	0	0	0	0	0	0	
Streptococcus faecalis	***9***	5	5	2	0	3	5	0	0	7	0	55	
Flavobacterium	***8***	0	0	0	0	0	0	0	0	0	0	0	
Pseudomonas	***10***	0	5	10	0	6	7	0	0	2	0	60	
Pectobacterium	***2***	0	0	2	0	0	2	0	0	0	0	0	**0.13**
E. coli	***8***	2	4	5	0	0	2	0	0	0	0	25	
Bacillus polymyxa	***12***	0	0	12	0	5	8	0	0	0	0	41	
B. licheniformis	***3***	0	0	0	0	0	0	0	0	0	0	0	
B. subtilis	***5***	0	0	0	0	0	0	0	0	0	0	0	
B. cereus	***7***	3	5	7	2	0	2	0	0	2	0	43	
Streptobacillus moniliformis	***2***	0	2	2	0	2	2	0	0	0	0	100	
Total	**81**	**10**	**21**	**40**	**2**	**16**	**28**	**0**	**0**	**11**	**0**	**27**	**128**

The integrated MAR index of Nile River water (Table 4) was 0.215 (accepted but it is slightly high). Indeed, most of the tested sites through the Nile River showed acceptable chemical and microbiological features, the high levels of contaminants have been detected at Rosetta branch due to the discharges of wastewater from some drains. So, the isolates collected from such sites have elevated MAR index (due to contact with contaminants) which lead to increasing the integrated MAR index of the Nile River. Moreover, about 36%, 58%, 75%, 40%, 26%, 52%, 61%, 32% and 68% of the isolates of *S. aureus*, *S. feacalis, Pseudomoas, P. aeruginosa, Enterobacter, E.coli, B. subtilis* and *B. cereus,* respectively have showed MAR activity, most of them were isolated from the contaminated sites at Rosetta branch.

Regarding water quality results of Sahl El-Husseiniya drain, it was noticed the acceptable quality of its water. Comparing such findings with the integrated MAR index of the bacterial community isolated from the drain water which is (0.24) confirming the same results (accepted as an agricultural drain (< 0.25). Furthermore, the percentage of bacterial isolates inside such community having MAR activity was 35%, indicating the moderate virulence of those bacterial isolates.

Due to the high loads of sewage in Bahr El-Baqr drain, the members of enterobacteriacea were predominated the bacterial community where *Protius, Citrobacter, Enterobacter, E. coli, Klebsiella and Yersinia* constituting more than 27% of the total number of the community (Fig. 1(d)). The calculation of the integrated MAR for all the bacterial members of the community of Bahr El-Baqr drain was 0.52 which categorize such water resource as a high risk contaminated water. In addition, about 75% of the bacterial isolates of the community have MAR activity (Table 6) which indicating the distribution of MAR isolates all over the water body. The excessive increase in MAR activity might be explained by the concept of plasmid exchange, occurring in the sewerage systems (Krumperman, 1983; Moghannem et al. 2015).

Table (4): Antibiotic resistance pattern of the bacterial community identified from Nile River

Identified bacterial community	No. of isolates	Antibiotics AK	AMX	AM	CTX	C	E	OFX	CFR	SAM	AMC	% of MAR isolates	Integrated MAR index
		No. of resistant isolates											
S. aureus	***22***	0	12	12	0	8	7	0	0	0	0	36	
Micrococcus	***14***	0	0	0	0	0	0	0	0	0	0	0	
S. faecalis	***31***	13	15	31	0	18	24	0	0	9	12	58	
Flavobacterium	***14***	0	0	0	0	0	0	0	0	0	0	0	
Pseudomonas	***24***	18	20	24	0	9	9	3	0	8	0	75	
P. aeruginosa	***18***	10	10	18	0	10	11	0	0	8	0	26	
Enterobacter	***42***	12	22	25	0	8	23	0	0	14	0	52	**0.215**
E. coli	***18***	8	12	16	0	6	11	0	0	0	0	61	
Arthrobacter	***13***	0	0	0	0	0	0	0	0	0	0	0	
Bacillus globisporus	***12***	0	0	0	0	0	0	0	0	0	0	0	
B. licheniformis	***24***	0	0	12	0	0	0	0	0	0	0	0	
B. subtilis	***37***	0	12	35	0	0	0	0	0	12	12	32	
B. cereus	***22***	0	15	22	0	0	0	0	0	15	15	68	
Total	**291**	**61**	**118**	**195**	**0**	**59**	**85**	**3**	**0**	**66**	**39**	**39**	**626**

Table (5): Antibiotic resistance pattern of the bacterial community identified from Agricultural (Sahl El Husseiniya) drain

Identified bacterial community	No. of isolates	Antibiotics AK	AMX	AM	CTX	C	E	OFX	CFR	SAM	AMC	% of MAR isolates	Integrated MAR index
		No. of resistant isolates											
S. aureus	**34**	0	12	22	0	10	7	0	0	0	0	29	
S. bovis	**41**	12	15	35	0	0	12	0	0	0	0	29	
Flavobacterium	**28**	0	0	14	0	12	0	0	0	0	0	0	
Pseudomonas	**55**	7	30	44	0	9	9	3	22	18	18	0.4	
P. aeruginosa	**34**	18	28	30	15	10	22	0	20	16	14	0.64	
Citrobacter	**33**	0	11	33	3	5	9	0	0	0	0	27	**0.24**
Enterobacter	**44**	12	22	25	0	8	11	0	0	5	0	27	
E. coli	**65**	18	41	45	0	32	35	0	0	9	0	54	
Pectobacterium	**22**	0	0	16	0	0	0	0	0	0	0	0	
B. cereus	**28**	12	16	26	12	0	12	12	14	12	12	50	
B. licheniformis	**32**	8	8	22	0	8	0	0	0	0	0	25	
Total	**416**	**87**	**183**	**312**	**30**	**94**	**117**	**15**	**56**	**60**	**44**	**35**	**998**

The integrated MAR index of the bacterial community of Al-Khadrawiya drain was 0.65 (the highest record in all the studied water resources). Most of the members of the identified bacterial community showed high tendency for multiple antibiotic resistance (Table 7). In addition, 85% of the tested isolates have MAR ability, which might be attributed to the contamination of such industrial wastewater with heavy metals (as mentioned above). Moghannem and co-workers in (2015) reported the occurrence of antibiotic resistance activity with heavy metals resistance activity in some bacterial communities. Many authors reported the association of resistance to heavy metals and antibiotics by carrying plasmids and or transposons encoding genetically linked metal and antibiotic resistance (Edward et al. 2006; Matyar et al. 2008; Ewida, 2020).

The integrated MAR index for Qaroun Lake water was 0.277 (Table 8) which considered as high a risk contaminated water (<0.25). Actually, Qaroun Lake suffers the discharge of agricultural, industrial and sewage wastes from El-Bats and El-Wadi drains, it is a renewable source of pollution. Such findings are confirming the water quality assessment results mentioned above. Nasser Lake is the only fresh water Lake in Egypt, the calculation of the integrated MAR index for its water confirming the purity of such important water resource. Also, the identified bacterial community members are common inhabitants of row water; few of them showing MAR activity (28%) indicating non-virulence (Table 9).

Table (6): Antibiotic resistance pattern of the bacterial community identified from Sewage contaminated drain (Bahr El-Baqr drain)

Identified bacterial community	No. of isolates	Antibiotics										% of MAR isolates	Integrated MAR index
		AK	AMX	AM	CTX	C	E	OFX	CFR	SAM	AMC		
		No. of resistant isolates											
S. aureus	**34**	18	22	34	0	3	17	0	0	0	0	50	
S. bovis	**41**	28	22	41	0	0	36	0	22	15	22	68	
S. faecalis	**43**	33	13	43	0	38	38	12	13	22	23	88	
Flavobacterium	**21**	0	0	14	0	0	0	0	0	0	0	0	
Pseudomonas	**55**	18	25	55	12	9	45	15	25	28	28	51	
P. aeruginosa	**34**	10	14	34	14	10	34	15	14	15	19	44	
Protius mirabilis	**22**	15	18	22	0	0	12	12	15	12	14	68	
Citrobacter	**19**	11	8	19	4	6	6	0	11	11	11	58	
Enterobacter	**44**	12	22	44	0	12	36	0	12	12	26	59	
E. coli	**65**	30	45	65	36	34	65	65	30	30	36	100	
Aerumonas hydrophila	**61**	0	53	61	45	32	57	0	32	53	47	86	**0.52**
Shigella	**28**	0	16	28	8	0	26	0	26	28	16	93	
Salmonella	**23**	9	16	23	18	0	23	0	22	18	22	95	
Klebsiella	**22**	20	18	22	0	22	17	22	0	22	18	100	
Stenotrophomonas sp.	**12**	0	0	12	0	12	5	0	0	12	12	100	
Yersinia pestis	**18**	6	4	18	2	11	4	0	0	18	9	61	
Cardiobacterium hominis	**8**	0	0	8	0	8	8	0	2	8	4	100	
Bordetella sp	**33**	11	16	33	8	33	33	33	0	33	23	100	
B. subtilis	**20**	14	10	20	3	20	16	20	8	20	20	100	
Total	**603**	**235**	**322**	**596**	**150**	**250**	**478**	**194**	**232**	**357**	**350**	**74.6**	**3164**

Table (7): Antibiotic resistance pattern of the bacterial community identified from industrial wastes' contaminated drain (Al-Khadrawiya drain)

Identified bacterial community	No. of isolates	Antibiotics: AK	AMX	AM	CTX	C	E	OFX	CFR	SAM	AMC	% of MAR isolates	Integrated MAR index
		No. of resistant isolates											
Pseudomonas	**33**	30	28	33	28	33	33	28	30	30	30	100	**0.65**
P. aeruginosa	**55**	35	42	55	32	55	55	45	42	45	35	100	
Protius mirabilis	**35**	22	30	35	22	30	35	22	0	35	0	100	
Citrobacter	**22**	10	15	22	15	20	22	0	0	15	0	90	
Enterobacter	**32**	22	29	32	16	18	32	18	16	32	0	100	
E. coli	**26**	16	22	26	16	26	26	26	0	26	16	100	
S. faecalis	**33**	12	22	33	22	22	25	0	12	22	12	66	
B. megaterium	**44**	22	26	44	12	22	26	0	0	12	0	59	
B. cereus	**31**	8	21	31	21	21	31	8	8	21	8	67	
Bordetella sp	**19**	13	15	19	13	15	13	15	13	15	0	79	
Stenotrophomonas sp.	**12**	0	0	12	0	6	6	8	0	12	0	50	
Total	**342**	**190**	**250**	**342**	**197**	**268**	**304**	**170**	**121**	**265**	**101**	**85**	**2208**

Table (9): Antibiotic resistance pattern of the bacterial community identified from fresh water Lake (Nasser Lake)

Identified bacterial community	No. of isolates	Antibiotics: AK	AMX	AM	CTX	C	E	OFX	CFR	SAM	AMC	% of MAR isolates	Integrated MAR index
		No. of resistant isolates											
Staphylococcus aureus	17	0	0	5	0	0	0	0	0	0	0	0	**0.165**
Pseudomonas	16	0	8	16	0	0	0	0	0	0	0	0	
Enterobacter	20	0	7	7	0	10	10	0	0	0	0	33	
Streptococcus bovis	14	7	0	14	0	7	7	0	0	2	0	50	
Bacillus cereus	22	0	11	22	3	0	0	0	0	11	0	50	
Total	**89**	**7**	**26**	**64**	**3**	**17**	**17**	**0**	**0**	**13**	**0**	**28**	**147**

Table (8): Antibiotic resistance pattern of the bacterial community identified from brackish water Lake (Qaroun Lake)

Identified bacterial community	No. of isolates	Antibiotics: AK	AMX	AM	CTX	C	E	OFX	CFR	SAM	AMC	% of MAR isolates	Integrated MAR index
		No. of resistant isolates											
S. aureus	**32**	0	0	16	0	8	8	0	0	8	0	**25**	**0.277**
S. bovis	**29**	12	15	15	0	12	0	0	0	12	0	**41**	
S. faecalis	**34**	0	24	34	0	0	8	0	0	8	0	**23**	
P. aeruginosa	**38**	0	18	28	18	18	28	0	0	18	0	**47**	
Bordetella sp	**23**	3	15	23	13	13	23	0	0	15	0	**65**	
Protius	**28**	10	15	28	0	0	0	0	0	8	0	**35**	
Citrobacter	**22**	8	12	22	0	8	8	0	0	8	0	**36**	
Enterobacter	**12**	0	8	12	2	0	0	0	0	2	0	**16**	
E. coli	**19**	8	10	15	0	12	10	0	2	8	0	**52**	
B. cereus	**32**	0	12	24	16	16	12	0	6	6	0	**50**	
B. licheniformis	**21**	0	11	20	0	11	0	0	0	21	0	**52**	
B. subtilis	**24**	8	18	24	0	12	12	0	0	12	0	**50**	
Total	**314**	**49**	**158**	**261**	**49**	**110**	**109**	**0**	**8**	**126**	**0**	**38.5**	**870**

Conclusion

The contamination of a given water resource with sewage materials increase the resistance of the whole bacterial community of such water resource to antibiotics by the function of plasmid exchange characterizing some members of enterobacteriacea group, which predominate in such cases. On the other hand, the contamination of a given water resource with heavy metals stimulate the bacterial community to adopt their toxicity, so, increasing their ability to resist antibiotics, and become more virulence, consequently, this water resource categorized as a high risk polluted water on human health. Therefore, evaluation of water quality and MAR index for different water resources is recommended.

References

[1]Abdel-Fattah, M.K. and Helmy, A.M. (2015) Assessment of water quality of wastewaters of Bahr El-Baqar, Bilbies and El-Qalyubia drains in East Delta, Egypt for irrigation purposes. Egypt. J. Soil Sci. 55 (3): 287-302.

[2]Abdel-Shafy, H.I. and Aly, R.O. (2002) Water Issue in Egypt: Resources, Pollution and Protection Endeavors. Central European J of Medicine, 8(1):3 – 21.

[3]Badr, E.A., El-Sonbati, M.A. and Nassef, H.M. (2013) Water quality assessment in the Nile River, Damietta branch, Egypt. CATRINA, 8 (1): 41-50.

[4]Balthazard, K.A., Emmanuel, E., Agnamey, P. and Raccurt, C. (2019) Pollution of water resources and environmental impacts in urban areas of developing countries: In ”Environmental Health- Management and Prevention Practices” IntechOpen, DOI: 10.5772/intechopen.86951.

[5]Bell, J.B., Elliot, G.E.and Smith, D.E. (1983) Influence of sewage treatment and urbanization on selection of multiple faecal coliform populations. Applied Environmental Microbiology, 46:227-232.

[6]Chandrasekaran, S., Venkatesh, B. and Lalithakumari, D. (1998) Transfers and expressions of a multiple antibiotic resistance plasmid in marine bacteria. J. Current Microbiology, 37:347-351.

[7]Derbala, R.S.M. (2018) Environmental impact assessment of main drains on water and fish quality of Lake Qaroun at Fayoum Governorate. M.Sc Faculty of Science, Fayoum University.

[8]Edward R.C., Anbazhagan, K., Selvam, G.S. (2006) Isolation and characterization of a metal-resistant *Pseudomonas aeruginosa* strain. World J Microbiol Biotechnol, 22:577–586.

[9]Environmental Protection Agency (EPA) (2012) Guidelines for water reuse, office of wastewater management, Washington, D.C., USA.

[10]Ewida A.Y.I and Mohamed W.S.E. (2019) Isolation and characterization of biosurfactant producing bacteria from oil-contaminated water. Biosci Biotech Res Asia, 16(4).

[11]Ewida, A.Y.I. (2014) Oil Spills: Impact on water quality and microbial community on the Nile River, Egypt. *International J. of Environment*. 3(4):192 – 198.

[12]Ewida, A.Y.I. (2020) Bio-treatment of maize processing wastewater using indigenous microorganisms. Sustain Environ Res 30, 3.

[13]Ewida, A.Y.I., Khalil, M.S. and Mahmoud, A.M. (2021) Impact of domestic wastewater treatment plants on the quality of shallow groundwater in Qalyubia, Egypt; Discrimination of microbial contamination source using Box-PCR. Egyptian J Botany, 16(1).

[14]Farhat, H.I. and Aly, W. (2018) Effect of site on sedimentological characteristics and metal pollution in two semi-enclosed embayments of great freshwater reservoir: Lake Nasser, Egypt. J Afr Earth Sci 141: 194–206

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

[16]Hinton, M., Hedges, A.J. and Linton, A.H. (1985) The ecology of *E. coli* in market calves fed a milk substitute diet. J. Appl. Bacteriol. 58, 27–35

[17]Kozdro, J.J. and Van Elsas, J.D. (2001) Structural diversity of microbial communities in arable soils of a heavily industrialized area determined by PCR- DGGE fingerprinting and FAME profiling. Appl Soil Ecol 2001; 17:31–42.

[18]Krieg, N.R. and Holt, J.G. (1986) Bergey's Manual of Systematic Bacteriology. (Vol. 1). Williams and Wilkins, Baltimore, U.S.A

[19]Krumperman, P.H. (1983) Multiple antibiotic resistance indexing of *E. coli* to identify high risk sources of fecal contamination of foods. Appl. Environ. Microbiol. 46, 165–170.

[20]Matyar, F., Kaya, A. and Dinçer, S. (2008) Antibacterial agents and heavy metal resistance in Gram-negative bacteria isolated from seawater, shrimp and sediment in

Iskenderun Bay, Turkey. Sci Total Environ, 407:279– 285.

[21]Mena, K.D. and Gerba, C.P. (2009) Risk assessment of *Pseudomonas aeruginosa* in water. Rev Environ Contam Toxicol., 201:71-115.

[22]Moghannem, S.A., Refaat, B.M., El-Sherbiny, G.M., El-Sayed, M.H., Elsehemy, I.A. and Kalabaa, H.M. (2015) Characterization of heavy metal and antibiotic-resistant bacteria isolated from polluted localities in Egypt. Egyptian Pharmaceutical Journal, 14:158–165

[23]Rajbanshi, A. (2008) Study on heavy metal resistant bacteria in Guheswori sewage treatment plant. J. Our Nature, 6:52-57.

[24]Salih, S.Q., Allawi, M.F., Yousif, A.A., Armanuos, A.M., Saggi, M.K., Ali, M., and Chau, K.W. (2019) Viability of the advanced adaptive neuro-fuzzy inference system model on reservoir evaporation process simulation: case study of Nasser Lake in Egypt. Eng Appl Comput Fluid Mech 13(1):878–891

[25]Sneath, P.H. (1986) Bergey's Manual of Systematic Bacteriology, Volume 2. Williams and Wilkins Baltimore, U.S.A.

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

[27]Tamil-Selvi, A., Anjugam, E., Archana-Devi, R., Madhan, B., Kannappan, S., and Chandrasekaran, B. (2012) Isolation and characterization of bacteria from Tannery treatment plant and their tolerance to heavy metals and antibiotics. Asian J. Exp. Biol. Sci., 3 (1).

[28]World Health Organization (WHO) (1996) Water quality assessments - A guide to use of biota, sediments and water in environmenta l monitoring. (2nd ed.).