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Enumeration and Detection of Main Pathogenic Bacterial Genera in Rahawy and Bilbeis Agriculture Drains

Hoda Kabary [1,*], Nawal Hassanain [2], Mohamed Saber [1], Alaa Zagloul [3]

1. Agricultural Microbiology Department, National Research Centre, Egypt.
2. Department of Zoonotic Diseases, National Research Centre, Egypt.
3. Soils and Water Use Department, National Research Centre, Egypt.

[*]Corresponding Author: hoda_kabary@yahoo.com

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ABSTRACT

Pathogens in agricultural drainage water represent a major threat to the public health of humans and livestock, food safety and ecosystem quality as well. The microbiological intensities of pathogenic bacteria including, Total fecal bacteria, *E. coli*, *Salmonella* sp., *Shigella* sp., *Campylobacter* sp. *streptococci* sp. *E. coli O157* and *Listeria* sp., in two sites of each of Rahawy and Bilbeis agriculture drains were characterized by selective media culturing technique. Selective Media applied for detection and counting were MacConkey agar (MAC), Salmonella Shigella agar (SS Agar), Campylobacter Blood free selective medium (CCDA), Modified Tryptic soy agar (TSA), MacConkey sorbitol agar (SMAC), and Listeria oxford agar for total fecal bacteria, *E. coli*, *Salmonella* sp., *Shigella* sp., *Campylobacter* sp., *streptococci* sp., *E. coli O157* and *Listeria* sp. count respectively. The strains were identified and selected by the reaction obtained after full growth on the differential agar media. The obtained results confirmed that, Rahawy and Bilbeis water samples exhibited high intensities with pathogenic bacteria compared to River Nile water. River Nile water samples were free from either *Salmonella* or *Shigella*, with lowest numbers of *Campylobacter* sp., *E. coli* and *E. coli* O157 ($3x10^2$, 30 and 1, respectively). Rahawy water samples (after Hadar site) had the highest density of *E. coli* ($5x10^4$) and *Salmonella* sp. ($4x10^3$), while Bilbeis (at Bridage site) had a lower density of *Salmonella* (10) and *Shigella* sp. (20) and the highest density of *Campylobacte*r sp. ($6x10^4$).

INTRODUCTION

Pathogens in agricultural drainage water represent a major threat to the public health of humans and livestock, food safety and ecosystem quality as well. Agriculture drainage water had been implicated as a significant source of health risk for chronic, low-grade gastrointestinal disease as well as outbreaks of more acute diseases (**Hassanain *et al.*, 2021**). The transmission of pathogens might occur through surface run-off, aerosols, and groundwater as well as with direct contact between agriculture drainage water and raw edible harvests **(WHO, 2004**).

Livestock excreta contain many zoonotic microorganisms that could be harmful to human health. Pathogenic microorganisms could be water-borne or food-borne especially if the food has been irrigated with polluted agriculture drainage water or with untreated or partially treated low quality water (**Saber *et al.*, 2021**). Some pathogens could survive for days or weeks in animal feces that have been discharged onto a given soil ecosystem and they might later pollute water resources via runoff **(FAO 2006; WHO 2012)**. Pathogens from livestock that are detrimental to public health include bacteria such as *Campylobacter* sp., *Escherichia coli* O157:H7, *Salmonella* sp., all of which cause hundreds of thousands of infections every year **(Christou, 2011)**.

The aim of the research is to quantitively assess the existence of main microbial pathogenic groups in two drains located in Egypt (Bilbeis and Rahawy) in two geographically different sites for each and compare the results with control water samples from River Nile.

MATERIALS AND METHODS

Collection of water samples: October 2020, two water samples were collected from two agricultural drains under study (Bilbeis and El Rahawy) as well as one sample from river Nile (Control) at Tanash village, Warak (Figure 1). The site of the first sample from Bilbeis drain was before the barrage at abu-Hamad village and the second one was after the barrage (Figure 2). The first sample from El-Rahawy drain was collected at the entrance pan of the drain after El-Rahawi barrage and second one was after the fall close to the ending point of the drain as shown in Figure (3). Chemical analyses of water samples were detected according to (**APHA, 2005**).

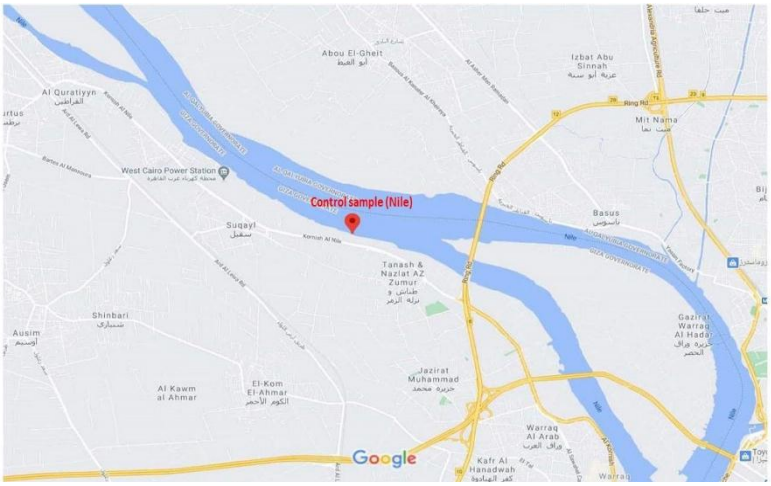


Figure (1) Location of water sample collected from river Nile water (control)

Figure (2) Locations of water samples collected from Bilbeis drain (Sample 1 (Bridge Site) and sample 2 (Mosque Site))

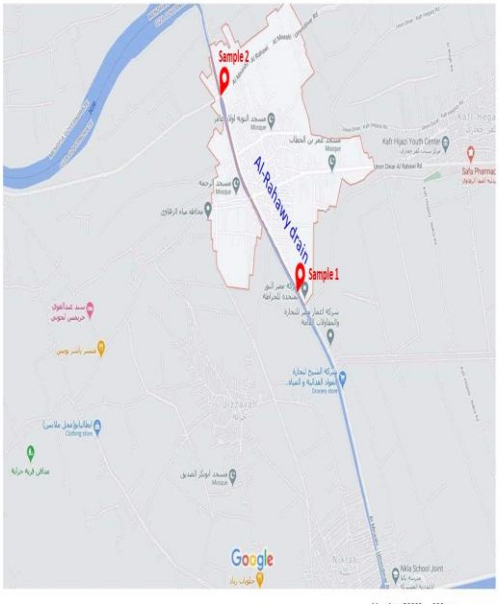


Figure (3) Locations of water samples collected from El-Rahawy drain (Sample 1 (El-hod Site) and sample 2 (El-hadar Site))

Table 1: Methodology and specific media for enumeration and detection of pathogenic bacteria in Rahawy and Bilbeis agriculture drains

	Medium type	**Culture condition**
Total fecal coliforms	MacConkey agar	Aerobic, 37^{o}C for 48 hrs
E. coli		Aerobic 37^{o}C for 48 hrs
Salmonella sp.	Salmonella Shigella agar	Aerobic, 37^{o}C for 48 hrs
Shigella sp.		Aerobic, 37^{o}C for 48 hrs
Campylobacter sp.	Blood free selective medium for Campylobacter sp. isolation, charcoal-cefazolin-sodium deoxycholate (CCDA) agar.	Microaerophilic, 37^{o}C for 48 hrs
Streptococci sp.	Modified Tryptic soy agar (selective streptococcus agar)	Anerobic, 37^{o}C for 48 hrs
E. coli 0157	MacConkey sorbitol agar	Aerobic, 37^{o}C for 48 hrs
Listeria. sp	Listeria oxford agar medium	Microaerophilic, 37^{o}C for 48 hrs

Detection of pathogenic bacteria: serial dilution method was used from the original samples till dilution factor 10^{6}. Targeted pathogens (e.g. *Salmonella, Escherichia coli* & *E. coli O157:H7, Campylobacter, Shigella* and *Listeria*) were detected using enumeration

on selective media technique (Table 1) and identified by the reaction obtained after full colonies growth (CFU). All selective media were purchased from HiMedia Company, India. Water samples were analyzed according to **APHA (2005) and Merck for Manual Microbiology 12th Edition 2010.**

RESULTS AND DISSCUSION

The colony reaction appearance on each specific medium after 24-48hrs growth summarized on Table 2. Results given in in table 3 show the microbiological intensities of pathogenic bacteria in both sites of each of Rahawy and Bilbeis agriculture drains including total fecal bacteria, *E. coli, Salmonella* sp., *Shigella* sp., *Campylobacter* sp. *streptococci* sp. *E. coli O*157 and *Listeria* sp.

Table 2: Microbial reaction specific for each group on differential media

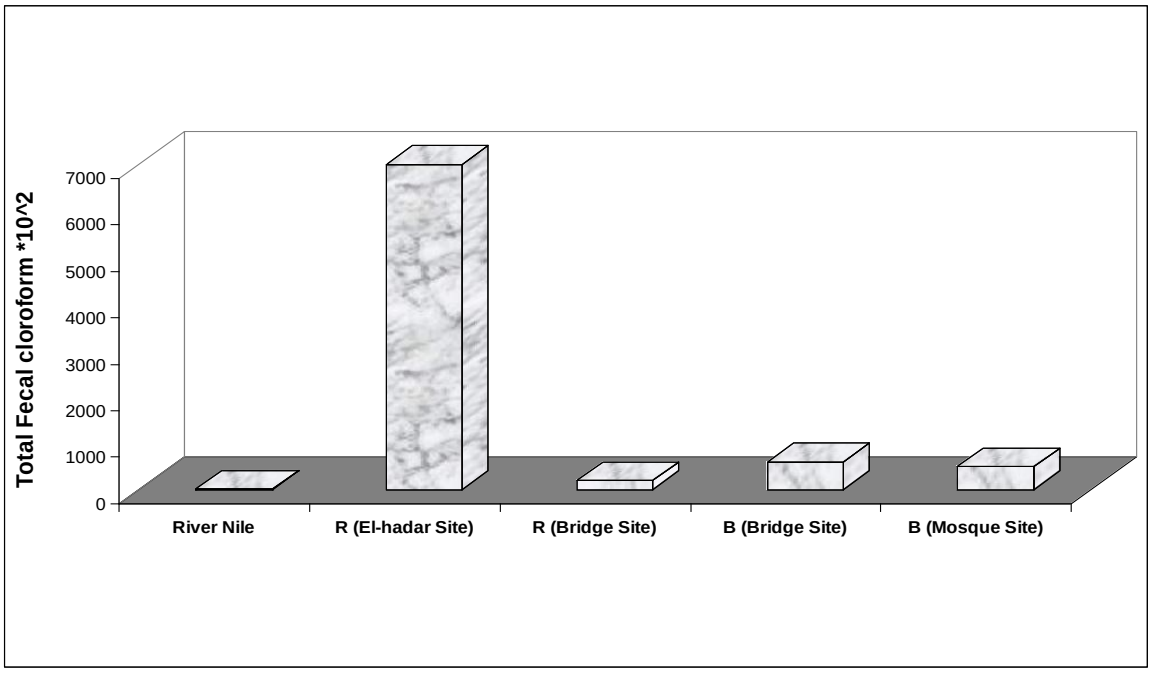
Bacterial isolate	**Growth appearance**	**References**
Total fecal coliforms	All colony	(**Morency-Potvin *et al.*, 2017; Jung and Hoilat, 2021**)
E. coli	Red to pink color	
Salmonella sp.	Colorless colony with black center	(**Winn *et al.*, 2006; ISO/TS 11133-1: 2009**)
Shigella sp.	Colorless colony	
Campylobacter sp.	White to creamy color	(**Bolton *et al.*, 1984; Handbook of Culture Media for Food and Water Microbiology 2012**)
Streptococci sp.	Creamy rounded with hemolytic activity	(**Pacifico *et al.*, 1995; Wan *et al.*, 2002**)
E. coli 0157	Colorless colony, no sorbitol fermentation	(**Novicki *et al.*, 2002; Jung and Hoilat, 2021**)
Listeria. sp	Brown colony with brown pigmentation on agar surface.	(**ISO 11290-1:1997; Yousef *et al.*, 2020**)

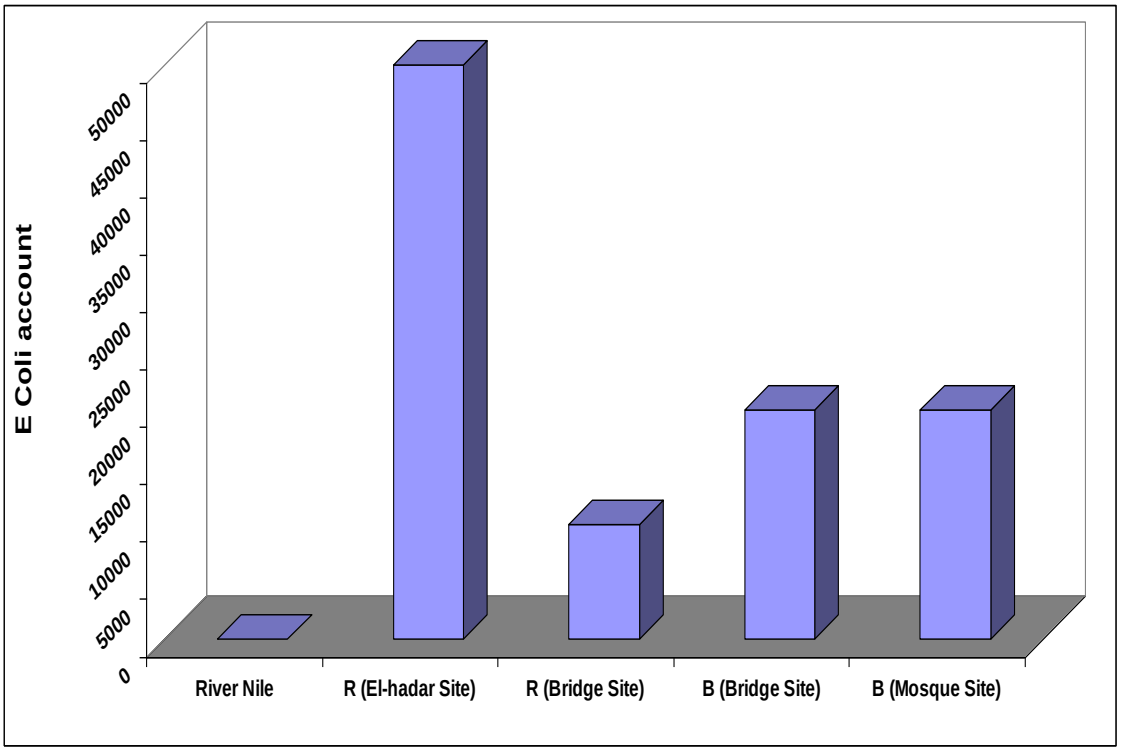
Chemical analysis of the water samples revealed that, the two drains are polluted with PTE's (Potential toxic elements) and high intensities of fecal bacteria with no detection of Persistent organic pollutants (data not presented). Rahawy and Bilbeis water samples exhibited high pollution with pathogenic bacteria compared to river Nile water (Table 3 & figure 4). River Nile water samples were free from either *Salmonella* or *Shigella*. They showed the lowest numbers of *Campylobacter sp., E. coli* and *E. coli O157* ($3x10^2$, 30 and 1, respectively). Rahawy (after Hadar site) water had highest density of *E. coli*

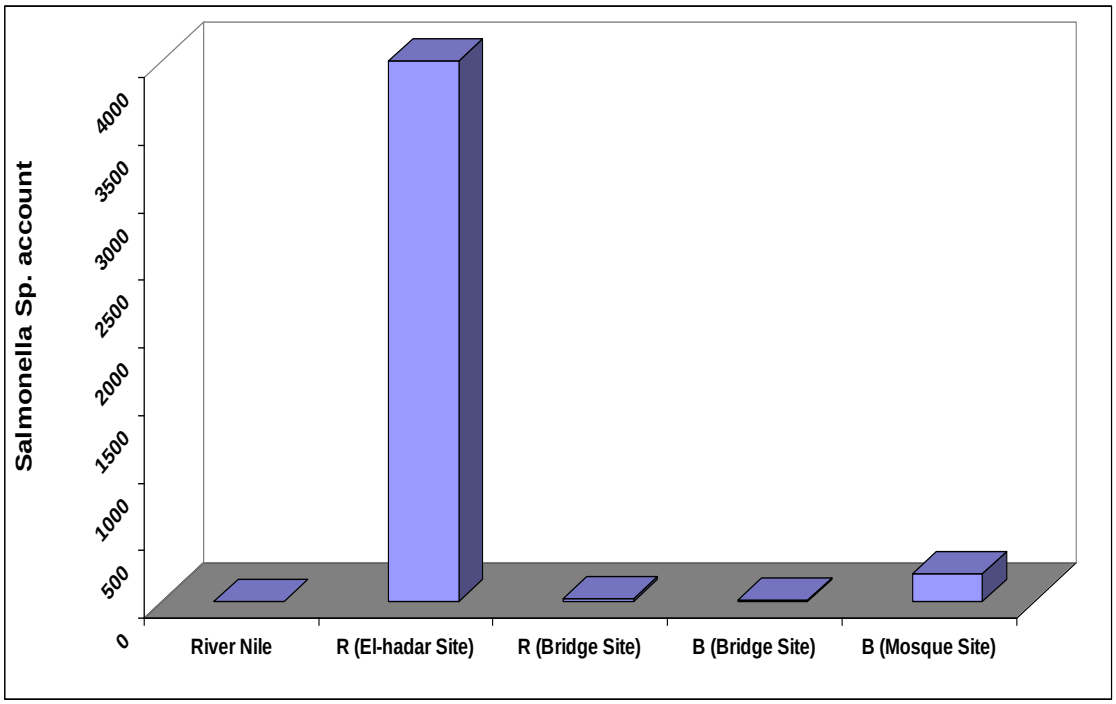
($5x10^4$) and *Salmonella* sp. ($4x10^3$), while Bilbeis (Bridage site) had lower density of *Salmonella* (10) and *Shigella* sp. (20) and highest density of *Campylobacter* sp. ($6x10^4$). Table 3 and figure 4 illustrate the presence of total fecal coliform bacteria at high densities in Rahawy (Hadar and Bridge sites) and Bilbeis (Bridge and Mosque sites)) water samples. Generally, it is unacceptable for fecal coliforms bacteria to be present at any concentration in low quality water. However, **WHO (1989)** reported that less than 10 viable fecal coliforms cells per gm or ml might be considered as a safe level.

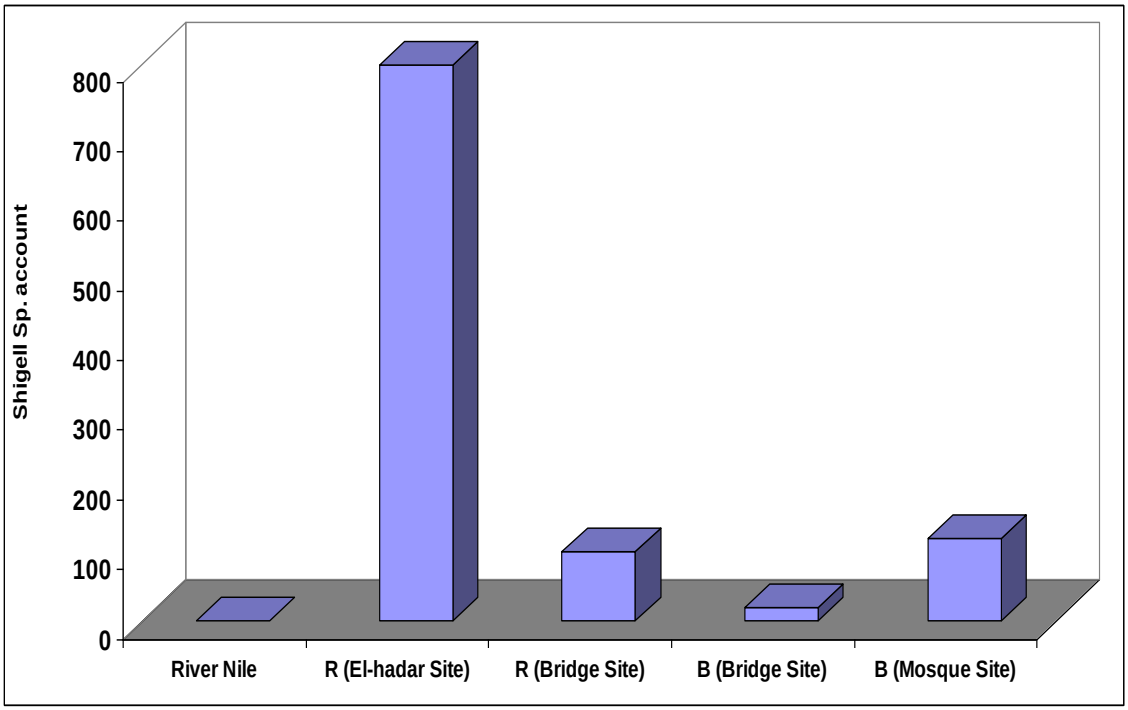
Table 3: Microbiological characterization of Rahawy and Bilbeis wastewater drains

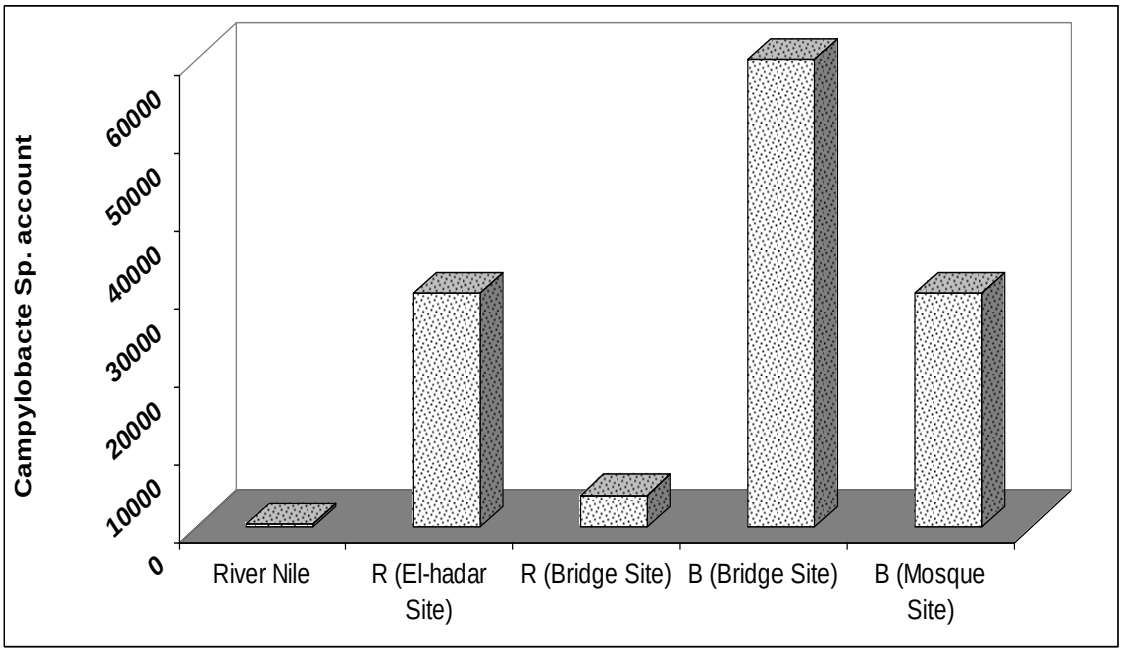
Microorganisms	**River Nile**	**El-Rahawy Drain**		**Bilbeis Drain**	
		El-hadar Site	**Bridge Site**	**Bridge Site**	**Mosque Site**
Total fecal coliforms	$8x10^2$	$7x10^5$	$2x10^4$	$6x10^4$	$5x10^4$
E. coli	30	$5x10^4$	$1x10^4$	$2x10^4$	$2x10^4$
Salmonella sp.	0	$4x10^3$	20	10	$2x10^2$
Shigella sp.	0	$8x10^2$	$1x10^2$	20	$1.2x10^2$
Campylobacter sp.	$3x10^2$	$3x10^4$	$4x10^3$	$6x10^4$	$3x10^4$
Streptococci sp.	$5x10^4$	$2x10^6$	$2x10^5$	$2x10^6$	$3x10^5$
E. coli 0157	1	$1x10^3$	$1x10^3$	$2x10^2$	$5x10^2$
Listeria. sp	20	$2x10^3$	$1x10^3$	$3x10^4$	$2x10^4$

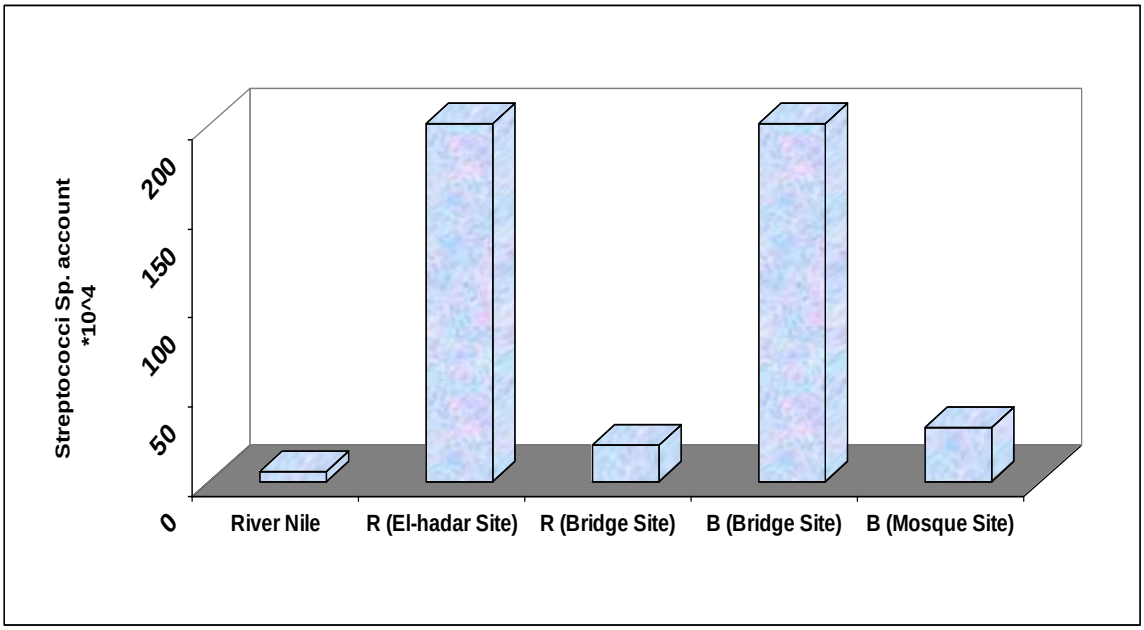


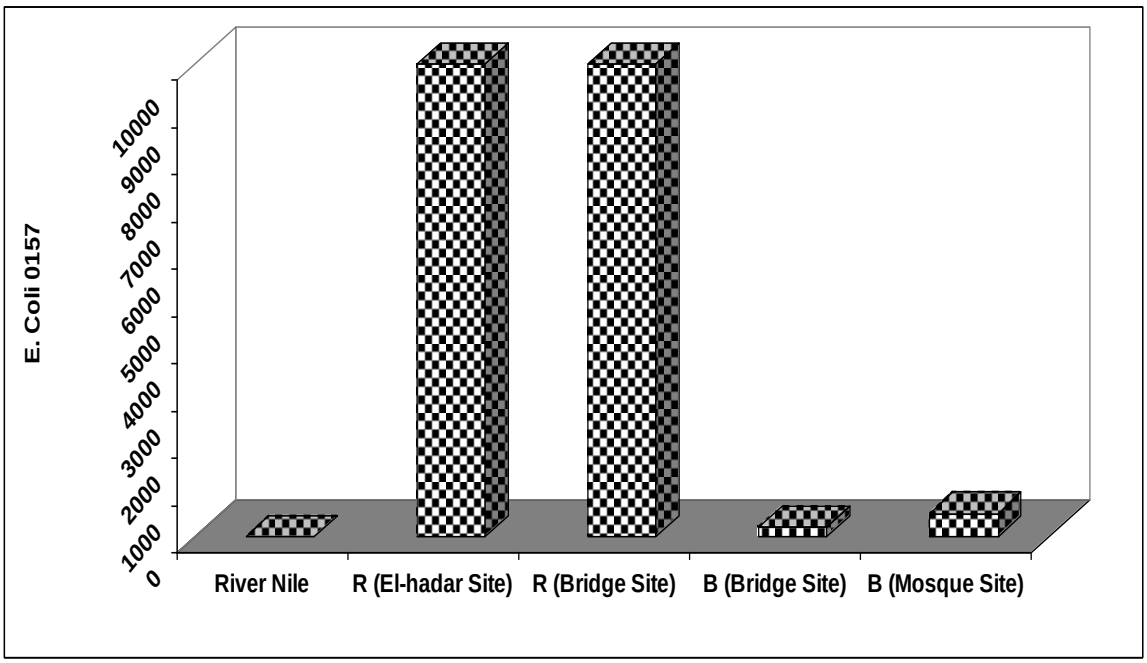

E Coli account
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
River Nile
R (El-hadar Site)
R (Bridge Site)
B (Bridge Site)
B (Mosque Site)


Salmonella Sp. account
4000
3500
3000
2500
2000
1500
1000
500
0
River Nile
R (El-hadar Site)
R (Bridge Site)
B (Bridge Site)
B (Mosque Site)


Shigell Sp. account
800
700
600
500
400
300
200
100
0
River Nile
R (El-hadar Site)
R (Bridge Site)
B (Bridge Site)
B (Mosque Site)


Campylobacte Sp. account
60000
50000
40000
30000
20000
10000
0
River Nile
R (El-hadar Site)
R (Bridge Site)
B (Bridge Site)
B (Mosque Site)


Streptococci Sp. account
*10^4
200
150
100
50
0
River Nile
R (El-hadar Site)
R (Bridge Site)
B (Bridge Site)
B (Mosque Site)


E. Coli 0157
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
River Nile
R (El-hadar Site)
R (Bridge Site)
B (Bridge Site)
B (Mosque Site)

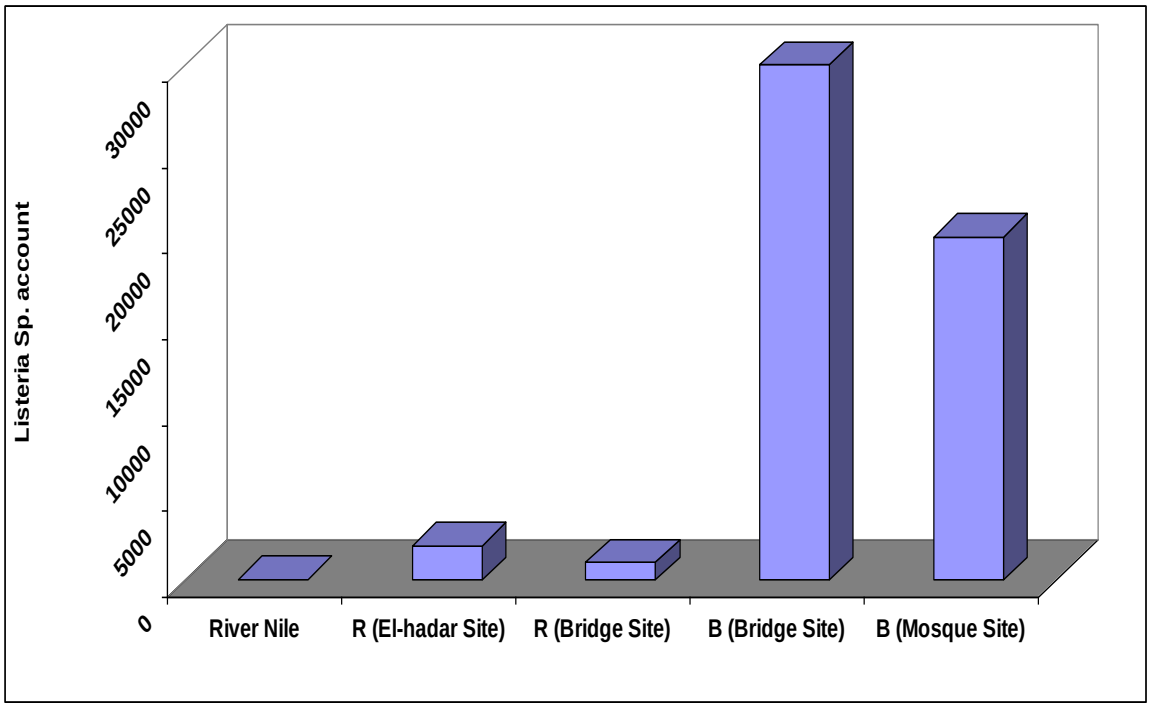


Figure (4) Microbiological characterization of Rahawy and Bilbeis wastewater drains

Microbial water quality along river Nile varies with location and depends on flow rate, water use, population density, sanitation systems, domestic and industrial discharges, demands for navigation, and agricultural runoff. **Dumont (2009**) stated that the microbial conditions in the Egyptian sector of river Nile often meet established water quality standards, yet some areas are polluted by inputs as industrial facilities discharging to the Nile between Aswan and Cairo. He concluded that the deterioration is rapid in front of Cairo and in delta at both Damietta and Rosetta branches, especially during low flow mainly due to the disposal of municipal and industrial effluents and agricultural drainage water as well, the bulk originate from treated and untreated domestic low quality wastewater discharged to agricultural drains. Worthy coliforms that could grow and ferment lactose with the production of acid and gas at 44.5 ^{o}C in the presence of bile salts are grouped as fecal coliforms (FCs) **(WHO 2006; Staradumskyte and Paulauskas 2012**). For this reason, the Thermo-tolerant Coliforms would be the scientifically more accurate term representing such for group (**Figueras *et al.*, 2008**). Thus, the bacteria of this coliform subgroup exhibited a positive correlation with fecal pollution of warm-blooded animals (**Toranzos *et al.*, 2007)**. The physiological basis of the elevated temperature phenotype in FCs has been described as thermo-tolerant adaptation of proteins. Therefore, their stability at temperatures found in the enteric tracts of animals is both constant and higher than the temperature in most aquatic and terrestrial ecosystems **(Clark, 1990)**. However, some thermo-tolerant coliform bacteria that conform to this definition also belong to the genus *Klebsiella* and had been isolated from varied ecosystems in the apparent absence of fecal pollution **(Toranzos *et al.*, 2007; Figueras *et***

***al.*, 2008)**. These were also associated with regrowth events in DWDS **(O'Reilly *et al.*, 2007; Collado *et al.*, 2010)**. However, the potential for re-growth or multiplication was less than that of the TCs. Furthermore, FCs display a survival pattern similar to those of bacterial pathogens **(Figueras *et al.*, 2010)**. Yet, their usefulness as an indicator of protozoans and viral pollution is limited and hence tends to be replaced by *E. coli* in several legislations **(WHO, 2006**). *Escherichia coli,* mostly non-pathogenic, is the most reliable indicator of enteric pathogens. (**Payment *et al.*, 2003)**. However, only some strains of *E. col*i are capable of causing disease **(Tallon *et al.*, 2005)** but at present, *E. coli* appears to provide the best bacterial indicators of fecal pollution in DW **(WHO, 2006)** This is based on the following: (a) the prevalence of thermotolerant (fecal) coliforms in temperate ecosystems compared to the rare incidence of *E. coli*; (b) the prevalence of *E. coli* in human and animal feces and generally not elsewhere in the ecosystem; and (c) the availability of affordable, fast, sensitive, specific and easier test methods to detect *E. coli*. Therefore, *E. coli* is the best and commonest microbial indicator available to date to inform public health risks associated with the consumption of polluted DW **(Staradumskyte and Paulauskas 2012; Odonkor and Ampofo, 2013**). Several European and American countries included this organism in their regulations as a primary indicator of fecal pollution in agriculture drainage low quality water. Moreover, new data had shown that *E. coli* could also survive for an extended period in lake sediments **(Byappanahalli *et al.*, 2003)**.

Fecal *streptococci, enterococci* and intestinal *enterococci* are the three synonyms used to describe the members of genus *Enterococcus* comprising different species of sanitary significance. However, the survival characteristics and the proportions of the species of this group are not the same in animal and human feces **(Borrego *et al.*, 2003; Figueras *et al.*, 2008)**. It is advantageous to use these microorganisms as a useful indicator of the microbiological quality because they show a close relationship with the health risks due to the consumption of polluted agriculture drainage water mainly for gastrointestinal symptoms; they are always present in the feces of warm-blooded animals; their inability to multiply in sewage-polluted water resources; they are not ubiquitous as coliforms and their die-off rate is slower than that of coliforms in water as well as their persistence pattern being similar to that of potential waterborne bacterial pathogens **(Figueras *et al,*. 2008; Layton *et al*., 2010).**

Saber *et al.* (2015) collected low quality water samples regularly at monthly intervals from Belbeis and Bahr El-Bakar agricultural drains as well as from river Nile and analyzed them for their microbial and pathogenic biomass. Their results confirmed the being of high densities of microbial biomass as well both classical and new indicator of pathogenic bacteria, yet at higher densities were at found in Bahr El-Bakar Drain. As expected, the intensities of all studied biomass were all the time higher in low quality water collected from both Belbis and at Bahr El-Bakar drains compared to River Nile.

Generally, the uppermost intensities of the studied biomass were detected in summer samples and the lowest ones were found in winter samples.

CONCLUSION

The microbial water quality along river Nile varies with location and depends on flow rate, water use, population density, sanitation systems, domestic and industrial discharges, demands for navigation, and agricultural runoff. . Agriculture drainage water had been implicated as a significant source of health risk for chronic, low-grade gastrointestinal disease as well as outbreaks of more acute diseases. The Rahawy and Bilbeis water samples exhibited high pollution with pathogenic bacteria compared to river Nile water. River Nile water samples were free from either *Salmonella* or *Shigella*. They showed the lowest numbers of *Campylobacter sp., E. coli* and *E. coli O157* ($3x10^2$, 30 and 1, respectively). Rahawy (after Hadar site) water had highest density of *E. coli* ($5x10^4$) and *Salmonella* sp. ($4x10^3$), while Bilbeis (at bridage site) had lower density of *Salmonella* (10) and *Shigella* sp. (20) .and highest density of *Campylobacter* sp. ($6x10^4$).

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