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OCCURRENCE SEASONAL VARIATIONS IN THE DISTRIBUTION OF SOME INDICATOR BACTERIA AND COLIPHAGES IN POTABLE WATER IN ZAGAZIG CITY

Abd El-Rady M. Ali*, Tahia A. Khater, Fatma I. El- Zamik and H.I. Abd-El Fattah

Agric. Microbiol. Dept., Fac. Agric., Zagazig Univ., Egypt

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ABSTRACT: The study aimed to determine the seasonal changes and the distribution of some fecal indicator bacteria and coliphages in inlet and outlet groundwater samples that used as drinking water in Zagazig city. The samples were taken two times every month for 12 months in triplicates from all selected six wells (El-Zeraa, El-Sagha, Wapour El-Nour, Abo-Aamer, El-Hesania and Gamal Abd El-Naser wells). The inlet and outlet groundwater samples of wells tested during one year period were clostridia free. Higher *Salmonella* and *Shigella* positive percentage was recorded in El-Zeraa (58.3%) and El-Sagha (50%) wells in inlet samples. While Wapour El-Nour and Abo-Aamer wells were *Salmonella* and *Shigella* free. The inlet groundwater samples of all six tested wells were *Aeromonas* spp. free, except El-Sagha well where the positive percentage reached 25.0%. The range of coliphages density during three months was from zero to 5.1 x 10^3 PFU/100 ml^{-1} inlet and from zero to 6.1 × 10^3 PFU/100 ml^{-1} in outlet samples. The results revealed that the coliphage counts increased in the water samples as the increasing of the environmental temperature. The relationship between total coliform/coliphage, fecal coliform/coliphage and fecal streptococci/coliphage in inlet groundwater samples of the same site were 1:3.39, 1: 4.47 and 1:3.24, respectively. Significant and positive correlation coefficients were observed between coliphage versus total coliforms (0.931 and 0.999), fecal coliform (0.609 and 0.769) and fecal streptococci (0.959 and 0.961), in inlet and outlet groundwater samples of El-Zeraa well, respectively. From the obtained results and the hygienic point of view, groundwater should be correctly chemically treated before its distribution for drinking.

Key words: Coliforms, *Salmonella, Shigella, Aeromonas,* coliphages, potable water.

INTRODUCTION

All our water comes from the water cycle, and this process controls our water resources. Groundwater circulates as a part of the hydrologic cycle (Gray, 2008). Groundwater is heavily used all over the world as the primary source of domestic drinking water supplies, and contaminated groundwater certainly enhances risk to public health. Although there are country to country differences and local variation, globally, it is estimated to provide at least 50% of current potable water supplies, 40% of the demand from those industries that do not use mains water, and 20% for water use in irrigated agriculture (Okafor, 2011).

*** Corresponding author: Tel. :** +201011787366
E-mail address: abdelradymousa02@gmail.com

Economically, groundwater is much cheaper than surface water, as it is available at the point of demand at relatively little cost and it does not require the construction of reservoirs or large pipelines. It is usually of good quality, usually free from suspended solids and except in limited areas, where it has been affected by pollution, free from bacteria and other pathogens, therefore, it does not require extensive treatment before use (Gray, 2008). Groundwater contamination come from two categories of sources: point sources and distributed or non-point sources. Landfills, leaking gasoline storage, tanks, leaking septic tanks, and accidental spills are example of point sources. In filtration from farm land treated with pesticides and fertilizers is an example of a non-point sources (Okafor, 2011).

Water- borne diseases caused by various bacteria, viruses, and protozoa have been the causes of many outbreaks (Craun *et al.,* 2006). In developing countries, such as those of Africa, water-borne diseases infect millions (Fenwick, 2006). According to World Health Organization (WHO), each year 3.4 million people, mostly children, die from water-related diseases (WHO, 2014). According United Nations Children Fund (UNICEF) assessment, 4000 children die each day as a result of contaminated water (UNICEF, 2014). WHO (2010) reported that over 2.6 billion lack access to clean water, which is responsible for about 2.2 million deaths annually, of which 1.4 million in children. Improving water quality can reduce the global disease burden by approximately 4% (WHO, 2010).

Groundwater pathogen contamination has led to numerous disease outbreaks in the United States (U.S.), for example, at least 46 outbreaks of disease occurred between 1992 and 1999, resulting in 2.739 cases of illness and several deaths (John and Rose, 2005). Also, several studies have shown that microbial pathogens, such as *Salmonella, Escherichia coli, Streptococcus faecalis,* and entero-viruses are relatively stable in groundwater (Schijven and Hassanizadeh, 2000; Hassard *et al.,* 2016). Controlling groundwater pathogen contamination has recently been emphasized in many countries, as pathogens can survive up 400 days depending on the soil temperature (Nevecherya *et al.*, 2005; Filip and Demnerova, 2009). One of the major concerns is that wetlands without lining might cause pathogen contamination of groundwater (Kay *et al.*, 2007). Similar concerns have been expressed in the United Kingdom (UK) by water regulations. The European Union (EU) has also emphasized protecting groundwater from pathogen contamination. The transport of pathogens from surface water to groundwater increases the vulnerability of groundwater (Jin and Flury, 2002). These authors reported that 70% of the water-borne microbial illness outbreaks in the U.S. have been associated with groundwater pathogen such as viruses are much smaller than bacteria and protozoa, and many can potentially reach groundwater through porous soil matrices. Also, over 50% of water-borne illnesses since 1980 have been caused by viral contamination of source water. As a results, coliphages, viruses that infect bacteria of the coliform group, were added as another fecal indicator in the 2006 Ground Water Rule (GWR) to allow direct measurement of a viral surrogate. Enteric viruses are the most likely human pathogen to contaminate groundwater. Their extremely small size (25-to100nm) allows them to infiltrate soils, eventually reaching aquifers. Depending on factors such as rainfall, temperature, soil structure, organic carbon content, soil pores, water pH, cition concentration, ionic strength, viruses can more considerable distance in the subsurface environment (US EPA, 2010). For many years, microbial indicators such as total coliforms, faecal coliforms and *E. coli* were used for predicting levels of microbiological water quality. One of the major drawbacks in using these indicators is the die off quickly in comparison to enteric viruses (Bordalo *et al*., 2002). Many studies advocated phages infecting enteric bacteria as a potential viral indicator to estimate the microbial contamination in the water environment, also as indicators of water treatment process efficacy (Sobsey *et al.,* 1995). Somatic coliphage (SOMCPH) are indicated by many studies to fill this gap depending on their correlation with enteric viruses' presence (Stetler, 1984; Payment and Franco, 1993; Wiedenmann *et al.*, 2006). Again, there are many arguments to propose coliphage as a suitable indicator for waterborne viruses due to their possible replication outside the gut in the aquatic environment; however this replication is negligible due to low density of phage and host bacteria (Jofre, 2008). Thus, ensuring the microbial quality of drinking water, watersheds process and water distribution systems is an important public health control measure designed to prevent the speed of communicable disease.

Therefore, in the light of these information, the present study was carried out to throw some light on the status and the quality of the used drinking water by the determination of coliforms, *Salmonella*, *Shigella*, *Aeromonas* and coliphage in drinking water samples of six groundwater wells in Zagazig City, Sharkia Governorate, Egypt which used for domestic purpose.

MATERIALS AND METHODS

The present study was carried out in the laboratory of Microbiology, Agricultural Microbiology Department, Faculty of Agriculture, Zagazig University, Egypt.

Ground Water Wells

Six groundwater wells in Zagazig city, namely: 1. El-Zeraa, 2. El-Sagha, 3. Abo-Aamer, 4. El-Hesania, 5. Gamal Abd El-Naser and 6. Wapour El-Nour, were selected on the basis of the heavy density of population in these areas as well as geographical distribution. The depth, length and flow rate of each single well is given in Table 1. The water samples were collected in the first period for 12 months, from all selected six wells and used for detection of pathogenic bacteria (*Clostridium* spp., *Salomonella* spp., *Shigella* spp., and *Aeromonas* spp.). The second period was for three months, the water samples were collected from three wells (El-Zeraa, El-Sagha and Abo-Aamer), which were chosen from the six wells examined during the first period, on the basis of the degree of contamination. The collected samples of the second period were used for total coliforms, fecal coliforms, fecal streptococci and coliphages.

Collection of Water Samples

From each well, inlet and outlet water samples were taken under special precautions. Inlet water sample was taken from the nearest site to the well, while outlet sample was collected from the fareast distance of water pipe from the well. The samples were collected in triplicates every 15 days interval for 12 months as first period from all tested wells, and for 3 months in the second period from only three wells as mentioned above. The water samples were collected in special sterile one - liter glass bottles with necessary precautions to avoid contamination. The monthly average values of the determined parameters of each well were came from six measurements (3 triplicates × 2 samples each month). The samples were kept in ice box and quickly transported to the laboratory and the microbiological analysis was carried out within three hours of collection. Sodium thiosulfate (N_2 S_2O_3) was used as satisfactory dechlorinating agent to neutralize any residual halogen and prevents continuation of bacterial action during samples transit.

Determination of Some Indicator Bacteria

Some fecal indicator bacteria were determined in water samples such as, total coliforms, fecal coliform and fecal streptococci according to the methods described by APHA (2005).

Determination of Some Pathogenic Bacteria

Aeromonas spp.

Presumptive the Present/Absent (P/A) test was done in bottles containing 50 ml of triple strength M-P/A broth medium (APHA, 2005). Each bottle was inoculated with 100 ml sample and incubated at 41.5±0.5°C for three days. Color change from red to yellow was taken as a positive presumptive of presence *of Aeromonas* spp. Confirmation was done by inoculating loopful onto the *Aeromonas* spp. agar plates (Oxoid lab.) and incubated for 24 hr., at 30°C. *Aeromonas* spp. colonies are typically 3 to 5 mm in diameter and yellow to honey colored.

Salmonella and *Shigella*

Salmonella and *Shigella* were counted according to the method described by APHA (2005) using *Salmonella* and *Shigella* agar medium (S.S. agar). From each positive sample, inoculating loopful onto the *Salmonella* and *Shigella* agar plates (Oxoid lab.) and incubated at'37°C±0.5°C for 48 hr.

Clostridium spp.

Presumptive P/A test was done in bottles containing 50 ml of triple strength M-P/A broth medium. The positive tubes were pasteurized at 80°C for 10 min. (APHA, 2005) before inoculation. Stained preparation from positive tubes were microscopically examined to ensure the presence of the spore-formers.

Coliphage Counts

Coliphage counts were determined using Bell's technique (Bell, 1976) with the minor modification as follows:

Bacterial Host

E. coli, strain no. 6850 was obtained from the National Research Center, Giza, Egypt. The bacterial culture was cultivated and maintained on Luria broth and Luria agar media (L.B.), (Borrego *et al.,* 1990) consists of: tryptone 10 g, yeast extract 5 g, sodium chloride 5 g and agar 15 g per/l.

Table 1. The depth and flow rate of the examined six wells in Zagazig city

No.	Well name	Depth (m)	Flow rate (m^3/hr.)	Building year
1	**El-Zeraa**	55-65	108-110	1982
2	**El-Sagha**	65-75	108-110	1985
3	**Abo-Aamer**	65-75	108-110	1994
4	**Gamal Abd El- Nasser**	70-75	108-110	1988
5	**El-Hesania**	75-80	108-110	1990
6	**Wapour El-Nour**	60-65	108-110	1992

Inoculation Procedure

Vigorous shaking of the sample were done for 3-5 min then 0.5 ml chloroform was added to 10 ml of the sample. The samples were homogenized and shacked by mechanical shaker for 30 sec., then leave to settle at 4°C until phase separation occurs. To a tube of 3 ml melted soft agar (0.7%) maintained at 45°C, 0.2 ml of host bacteria culture in exponential growth was added then 1 ml of the aqueous phase from the sample tube. Following agitation, the soft agar was poured onto a L.B. agar plate and left to solidify. For each water sample, 10 replicate plates were prepared for each bacteria host strain, thus the method is capable for detecting coliphages numbers as low as 10 per 10.0 ml water sample. The plates were incubated inverted, at 36±1°C for 12-14 hr., in spite of the fact that it is possible to detect clear areas of lytic plaques at 4-6 hr.

Statistical Analysis

Data were subjected to statistical analysis of variance to obtain the statistical differences between wells, months and site of water samples according to Snedecor and Cochran (1982). The correlation coefficients between some pathogenic bacteria with pollution indicators were calculated.

RESULTS AND DISCUSSION

Inlet and outlet groundwater samples were taken in triplicates from all selected six wells during a one year period and used for determination of pathogenic bacteria. During the second period, the groundwater samples were collected only from three wells and used for detection of coliphages. These determinations were carried out to have a clear idea on the biological status of the drinking water in Zagazig city.

Presence of Pathogenic Bacteria in Drinking Water

The specific pathogens that usually applied for justification of water quality are clostridia, *Salmonella, Shigella* and *Aeromonas* spp. Presumptive test was applied for qualitative characteristics of these pathogenic bacteria.

***Clostridium* spp.**

The presumptive test of clostridia in inlet and outlet groundwater samples of six wells during one-year period is given in Table 2. In general, the percentage of negative inlet and outlet water samples of the six wells tested was 100.0%, where the percentage of positive inlet and outlet water samples was 0.0%. This result is similar to the results observed by Sotohy and Ahmed (1996) who found that *Clostridium* spp. cannot isolated from the groundwater samples collected in Assiut city, Egypt.

On the other hand, *Clostridium perfringens* is consistently associated with human fecal wastes (Drasar, 1975). It is not used as a fecal indicator system in the US and only peripherally in Europe, especially with its irregular detection in well and river water comparing with fecal coliform. In addition, Bisson and Cabelli (1980) found that the detection of clostridia vegetative CFU would indicate an immediate, untreated fecal sources, recovery of spores indicate remote contamination of water. It is clear from the

Table 2. Presumptive test of clostridia in groundwater samples of six wells during one year period

Month	El-Zeraa		El-Sagha		Abo-Aamer		G.A. Nasser		El-Hessinia		W. El-Nour	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
July	-	-	-	-	-	-	-	-	-	-	-	-
Aug.	-	-	-	-	-	-	-	-	-	-	-	-
Sept.	-	-	-	-	-	-	-	-	-	-	-	-
Oct.	-	-	-	-	-	-	-	-	-	-	-	-
Nov.	-	-	-	-	-	-	-	-	-	-	-	-
Dec.	-	-	-	-	-	-	-	-	-	-	-	-
Jan.	-	-	-	-	-	-	-	-	-	-	-	-
Feb.	-	-	-	-	-	-	-	-	-	-	-	-
March	-	-	-	-	-	-	-	-	-	-	-	-
April	-	-	-	-	-	-	-	-	-	-	-	-
May	-	-	-	-	-	-	-	-	-	-	-	-
June	-	-	-	-	-	-	-	-	-	-	-	-
+Ve (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-Ve (%)	100	100	100	100	100	100	100	100	100	100	100	100

P/A: Present/Absent, W. El-Nour= Wapour El-Nour, G.A. Nasser= Gamal Abd El-Naser
+Ve: Positive, -Ve: Negative

present study that all the inlet and outlet groundwater samples of all six wells tested twice a month for one year were clostridia free (Table 2). In addition, the seasonal changes in the presumptive test of clostridia in the groundwater samples of these six wells during four seasons period were also clostridia free (Table 3). The present results are contradicting with the findings observed by Shaaban *et al*. (1993). The author found that the *Clostridium* spp. was present in 78.2% of total water samples tested. He also observed that the most alternative indicators in such negative coliform samples were *Clostridium* spp., followed by fecal streptococci.

Salmonella and *Shigella*

The periodical variations in the presumptive of *Salmonella* and *Shigella* in groundwater samples of six wells during one-year period are observed in Table 4. It is clear that the percentage of positive water samples ranged from 0.0 to 58.3% in the inlet and from 0.0 to 100.0% in the outlet groundwater samples, respectively. Higher *Salmonella* and *Shigella* positive percentage was recorded in El- Zeraa (58.3%) and El- Sagha (50.0%) wells than the other wells in the inlet samples. In outlet samples, the positive percentage was 100.0% in El- Sagha well.

On the other hand, the highest *Salmonella* and *Shigella* positive polluted water samples was observed in August and September months (50%) in inlet water samples, where this value was 66.7% in outlet water samples, in both months. In addition a seasonal variation in the P/A of *Salmonella* and *Shigella* was recorded (Table 5). The percentage of positive inlet water samples ranged from 0.0% in autumn and winter seasons to 33.3% in summer and spring seasons. In addition, the percentage of positive water samples was 66.7% in all seasons. In general, the present results disagree with the findings reported by Sotohy and Ahmed (1996). They found that *Salmonella* cannot be isolated from the samples collected during evaluation of groundwater in Assiut city.

Table 3. Presumptive test of clostridia in groundwater samples of six wells during four season periods

Well name	Season							
	Summer		Autumn		Winter		Spring	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
E1-Zeraa	-	-	-	-	-	-	-	-
El-Sagha	-	-	-	-	-	-	-	-
Abo-Aamer	-	-	-	-	-	-	-	-
G.A. Nasser	-	-	-	-	-	-	-	-
El-Hessinia	-	-	-	-	-	-	-	-
W. El-Nour	-	-	-	-	-	-	-	-
+Ve (%)	0.0`	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-Ve (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

W. El-Nour= Wapour El-Nour, G.A. Nasser= Gamal Abd El-Naser
+Ve: Positive, -Ve: Negative

Table 4. Presumptive test of *Salmonella* and *Shigella* in groundwater samples of six wells during one year period

Month	El-Zeraa		El-Sagha		Abo-Aamer		G.A. Nasser		El-Hessinia		W. El-Nour	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
July	-	+	-	+	-	-	-	+	-	+	-	-
Aug.	+	+	+	+	-	-	+	+	-	+	-	-
Sept.	+	+	+	+	-	-	+	+	-	+	-	-
Oct.	-	-	-	+	-	-	-	-	-	+	-	-
Nov.	-	+	-	+	-	-	-	+	-	-	-	-
Dec.	-	+	-	+	-	-	-	+	-	+	-	-
Jan.	-	+	-	+	-	-	-	+	-	+	-	-
Feb.	+	+	+	+	-	-	+	+	-	+	-	-
March	+	+	+	+	-	-	-	+	-	+	-	-
April	+	+	-	+	-	-	-	+	-	+	-	-
May	+	+	+	+	-	-	-	+	-	+	-	-
June	+	+	+	+	-	-	-	+	-	+	-	-
+Ve (%)	58.3	100	50.0	100	0.0	0.0	25.0	100	0.0	100	0.0	0.0
-Ve (%)	41.7	0.0	50.0	0.0	100.0	100	75.0	0.0	100	0.0	100	100

W. El-Nour= Wapour El-Nour, G.A. Nasser= Gamal Abd El-Naser
+Ve: Positive, -Ve: Negative

Table 5. Presumptive test of *Salmonella* and *Shigella* in groundwater samples of six wells during four season periods

Well name	Season							
	Summer		Autumn		Winter		Spring	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
E1-Zeraa	+	+	-	+	-	+	+	+
El-Sagha	+	+	-	+	-	+	+	+
Abo-Aamer	-	-	-	-	-	-	-	-
G.A. Nasser	-	+	-	+	-	+	-	+
El-Hessinia	-	+	-	+	-	+	-	+
W. El-Nour	-	-	-	-	-	-	-	-
+Ve (%)	33.3	66.7	0.0	66.7	0.0	66.7	33.3	66.7
-Ve (%)	66.7	33.3	100.0	33.3	100.0	33.3	66.7	33.3

W. El-Nour= Wapour El-Nour, G.A. Nasser= Gamal Abd El-Naser
+Ve: Positive, -Ve: Negative

On the other hand, the numerical relationships between indicator organisms (specially, total coliforms) and *Salmonella* in drinking water have both rejected and relationship the hypothesis of a positive correlation between the two variables. Venkateswaran and Hashimoto (1988) found that no supported between *Salmonella* spp. and indicator organisms concentrations in a polluted water, the present results support this hypothesis. Since, the inlet groundwater polluted samples collected from both Abo-Aamer and Wapour El- Nour wells were *Salmonella* spp. free and at the same time had total coliform densities ranged from 1.1 to 2.6 CFU/100 ml^{-1} in outlet groundwater samples of Abo-Aamer well where, these values were 1.1 to 3.0 CFU/100 ml^{-1} in inlet and from 1.1 to 8.6 CFU/100 ml^{-1} in outlet groundwater samples of Wapour El-Nour (data not shown). In contrast, Morinigo *et al*. (1990) found significant correlations between *Salmonella* and either total coliform, fecal coliform or fecal streptococci in sewage contaminated river water. This inverse between the bio-indicators (total coliform, fecal coliform and fecal streptococci) and *Salmonella* spp. might be attributed to the different conditions in which the data were collected, *e.g.* the level and type of pollution, the media applied for quantification of the tested organisms and water temperature where most of studies were made in temperate countries.

Aeromonas spp.

During the past several years, there has been increasing interest concerning the possible role of species of motile *Aeromonas* spp. as human pathogens (Burke *et al*., 1983; Janda and Duffey, 1988). The presence of *Aeromonas* spp. in drinking water indicates inefficient treatment (Grabow, 1990). In addition, *Aeromonas* spp. were more chlorine resistant than coliform bacteria (Knonchel, 1991).

The presumptive test of *Aeromonas* spp. in groundwater samples of six wells during one-year period is shown in Table 6. It is clear that the inlet groundwater samples had less positive percentage of *Aeromonas* spp. around the year as compared to the outlet groundwater samples. These values ranged from 0.0 to 25.0% in inlet and from 0.0 to 100.0% in outlet water samples. Also, both inlet and outlet groundwater samples of Wapour El- Nour were *Aeromonas* spp. free around the year, where the positive percentage reached 25.0% in inlet and 100.0% in outlet groundwater samples of El- Sagha well.

In addition, a clear seasonal changes in the *Aeromonas* spp. was observed (Table 7). The

Table 6. Presumptive test of *Aeromonas* spp. in groundwater samples of six wells during one year period

Month	El-Zeraa		El-Sagha		Abo-Aamer		G.A. Nasser		El-Hessinia		W. El-Nour	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
July	-	+	-	+	-	+	-	+	-	+	-	-
Aug.	-	+	-	+	-	-	-	+	-	+	-	-
Sept.	-	+	-	+	-	-	-	+	-	+	-	-
Oct.	-	+	-	+	-	-	-	+	-	+	-	-
Nov.	-	+	-	+	-	-	-	+	-	-	-	-
Dec.	-	+	-	+	-	-	-	+	-	+	-	-
Jan.	-	+	-	+	-	-	-	+	-	+	-	-
Feb.	-	+	-	+	-	-	-	-	-	+	-	-
March	-	+	+	+	-	-	-	+	-	+	-	-
April	-	+	-	+	-	-	-	+	-	+	-	-
May	-	+	+	+	-	-	-	+	-	+	-	-
June	-	+	+	+	-	+	-	+	-	+	-	-
+Ve (%)	0.0	100	25.0	100	0.0	16.7	0.0	91.7	0.0	91.7	0.0	0.0
-Ve (%)	100.0	0.0	75.0	0.0	100	83.3	100	8.3	100	8.3	100	100

P/A: Present/Absent, W. El-Nour= Wapour El-Nour, G.A. Nasser= Gamal Abd El-Naser
+Ve: Positive, -Ve: Negative

Table 7. Presumptive test of *Aeromonas* spp. in groundwater samples of six wells during four season periods

Well name	Season							
	Summer		Autumn		Winter		Spring	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
E1-Zeraa	-	+	-	+	-	+	-	+
El-Sagha	-	+	-	+	-	+	+	+
Abo-Aamer	-	+	-	-	-	-	-	-
G.A. Nasser	-	+	-	+	-	+	-	+
El-Hessinia	-	+	-	+	-	+	-	+
W. El-Nour	-	-	-	-	-	-	-	-
+Ve (%)	0.0	83.3	0.0	66.7	0.0	66.7	16.7	66.7
-Ve (%)	100.0	16.7	100.0	33.3	100.0	33.3	83.3	33.3

W. El-Nour= Wapour El-Nour, G.A. Nasser= Gamal Abd El-Naser
+Ve: Positive, -Ve: Negative

maximal positive percentage of *Aeromonas* spp. occurred during the spring season with values of 16.7% and 83.3% in inlet and outlet water samples, respectively. The positive percentage of *Aeromonas* spp. was gradually decreased in high temperature summer season reaching, the minimal percentage with values of 0.0% and 83.3% in inlet and outlet water samples, respectively. This general trend was detected in all examined samples indicating that *Aeromonas* spp. is markedly influenced by the seasonal temperatures. A similar trend was found by Pathak *et al*. (1988) in India where the temperature of either winter or summer are more or less comparable to this in Egypt.

Moreover, Lechevallier *et al*. (1983) found that an association between total viable counts and *Aeromonas* spp. in a potable water system, but did not demonstrate a relationship between *Aeromonas* spp. and total coliforms. On the other hand, Pathak *et al*. (1988) observed that *A. hydrophila* counts were significantly correlated with those of either fecal coliform (r = 0.81) or total coliforms (r = 0.74), but the trend was missed with the total viable counts. Also, Neves *et al*. (1990) suggested that there is a positive correlation between the number of *Aeromonas* spp. and coliform counts. Finally, the increasing perception of *Aeromonas* spp. as a human pathogen prompted many countries to evaluate its distribution in drainage water in order to assess the public health significance of its occurrence in causing any hazards during use of such water for drinking and other domestic purposes.

Coliphage as an Indicator of Fecal Pollution and Water Quality

A total of 108 inlet and outlet groundwater samples from three wells within three months were collected and quantitative analysis was applied for the presence of coliphage counts (PFU/100 ml^{-1}), total colifrom counts (CFU/100 ml^{-1}), fecal coliform counts (CFU/100 ml^{-1}) and fecal streptococci counts (CFU/100 ml^{-1}). The importance of coliphage as an indicator of water quality and its relation to coliform groups was studied.

Coliphage counts

The periodical detection of coliphage counts (PFU/100 ml^{-1}) in groundwater samples of three wells during three months period is presented in Table 8. In addition, the periodical detection of coliform groups (CFU/100 ml^{-1}) of the same groundwater samples is given in Table 9 and Fig. 1. These data show that coliphages can be found routinely in potable water samples in which coliform groups are found. The range of coliphages density was from 0.0 to 5.1×10^3 (PFU/100 ml^{-1}) in inlet and from 0.0 to 6.1×10^3 (PFU/100 ml^{-1}) in outlet samples (Table 8). The highest coliphages density was observed in El-Sagha well, where the lowest coliphages density was observed in Abo- Aamer well. These values ranged from 2.2×10^3 to 5.1×10^3 (PFU/100 ml^{-1}) in inlet, and from 3.8×10^3 to 6.1×10^3 (PFU/ 100 ml^{-1}) in outlet groundwater samples of El-Sagha well. While Abo-Aamer well was coliphage free.

Also, the lowest coliphage counts were observed in April, where the highest counts were observed in June. These counts ranged from 0.0 to 2.2×10^3 and from 4.1×10^3 to 5.1×10^3 (PFU/100 ml^{-1}) in inlet water samples of April and June, respectively. Where these counts in outlet water samples ranged from 0.0 to 3.8×10^3 (PFU/100 ml^{-1}) and from 5.6×10^3 to 6.1 x 10^3 (PFU/100 ml^{-1}) in April and June, respectively. These results revealed that the coliphage counts increased in the water samples as the increasing of the environmental temperature. The present results are almost similar to the results reported by some authors. Dhillon *et al*. (1970) found that the counts of coliphages ranged from 3.6×10 to 1.6×10^4 (PFU/ 100 ml^{-1}). Again, Ali (2000) found that the counts of coliphage were 1.2×10 (PFU/100 ml^{-1}) in seawater samples. On the other hand, several authors have been isolated the coliphages from drinking water (Grabow and Coubrough, 1986), groundwater (Dizer *et al*., 1993) wastewater (Rajala-Mustonen and Heinonen-Tanski, 1994), natural water and seawater (Pedroso and Martins, 1995).

Concerning the fecal groups (Table 9 and Fig. 1), the data show that the counts of total coliform, fecal coliform and fecal streptococci in the inlet groundwater samples were 1.6 to 12.0, 0.0 to 2.9 and 0.0 to 18.1 CFU/100 ml^{-1}, respectively. Also, the counts in the outlet groundwater samples were 2.2 to 24.0, 0.0 to 8.0 and 0.0 to 20.0 CFU/100 ml^{-1} for total coliform, fecal coliform, and fecal streptococci, respectively.

Table 8. Periodical detection of coliphages counts (PFU/100 ml^{-1}) in groundwater samples of three wells during three months period

Month	Well name					
	El-Zeraa		El-Sagha		Abo-Aamer	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
April	2.0×10^3	2.8×10^3	2.2×10^3	3.8×10^3	0.00	0.00
May	2.3×10^3	3.6×10^3	2.3×10^3	4.0×10^3	0.00	0.00
June	4.1×10^3	5.6×10^3	5.1×10^3	6.1×10^3	0.00	0.00

PFU: plaque forming unit.

Table 9. Periodical detection of coliform groups (MPN/100 ml^{-1}) in groundwater samples of three wells during three months period

Month	Well name					
	El-Zeraa		El-Sagha		Abo-Aamer	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
Total coliform counts						
April	6.9	12.5	5.1	9.1	1.6	2.2
May	5.2	16.1	8.0	12.0	2.2	2.2
June	12.0	24.0	9.2	12.6	2.2	3.9
Fecal coliform counts						
April	2.0	2.8	2.9	4.5	0.0	0.0
May	2.3	5.1	2.3	4.5	0.0	0.0
June	2.3	5.3	2.2	8.0	0.0	0.0
Fecal streptococci counts						
April	8.6	16.1	7.1	12.5	0.0	0.0
May	12.5	16.1	18.0	17.5	0.0	0.0
June	18.1	20.0	12.0	20.0	0.0	0.0

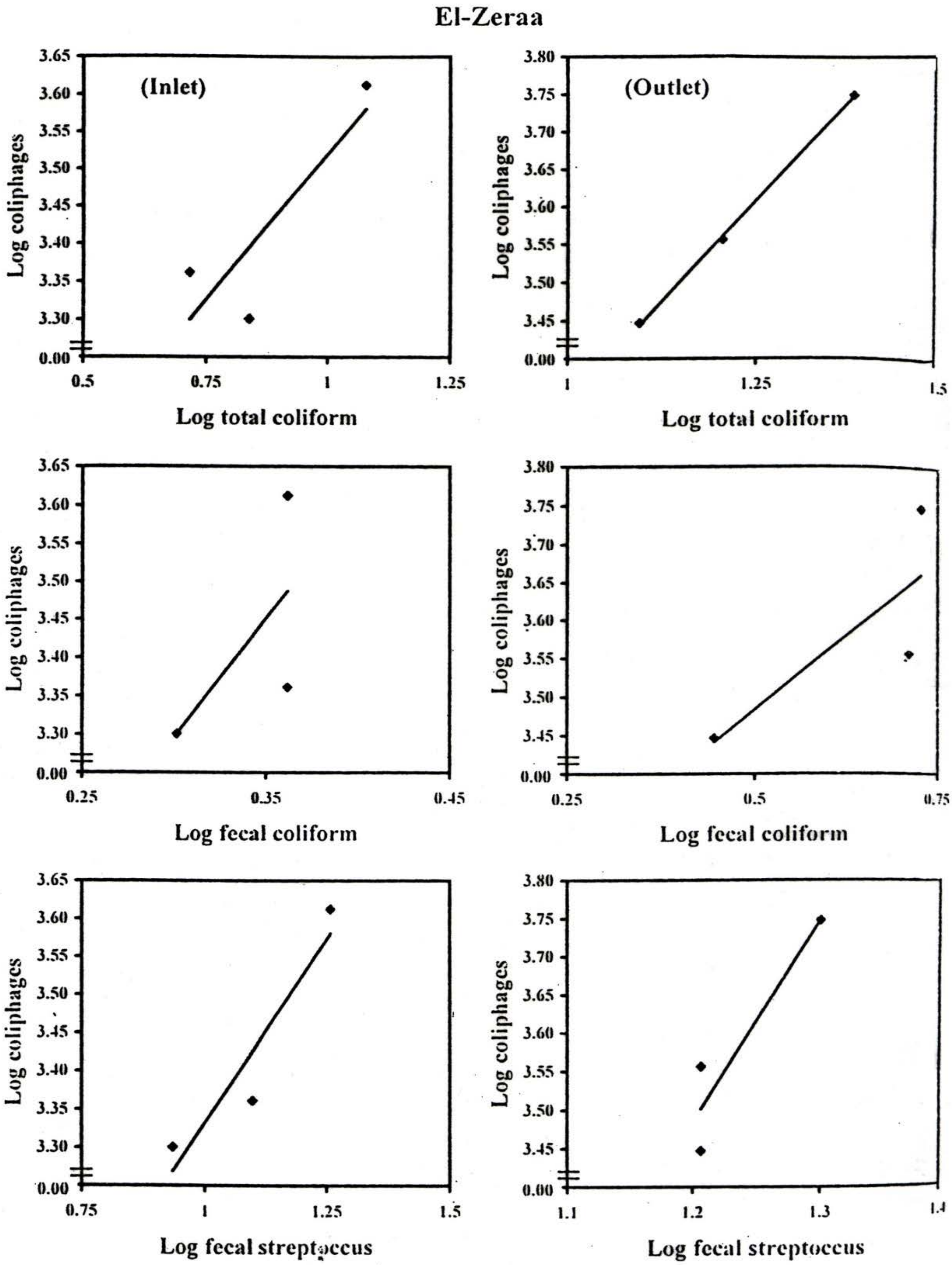

Fig. 1. Relationship between log total coliform (TC), log fecal colifrom (FC), log fecal streptococci (FS) and log coliphage in groundwater samples of three wells during three months period

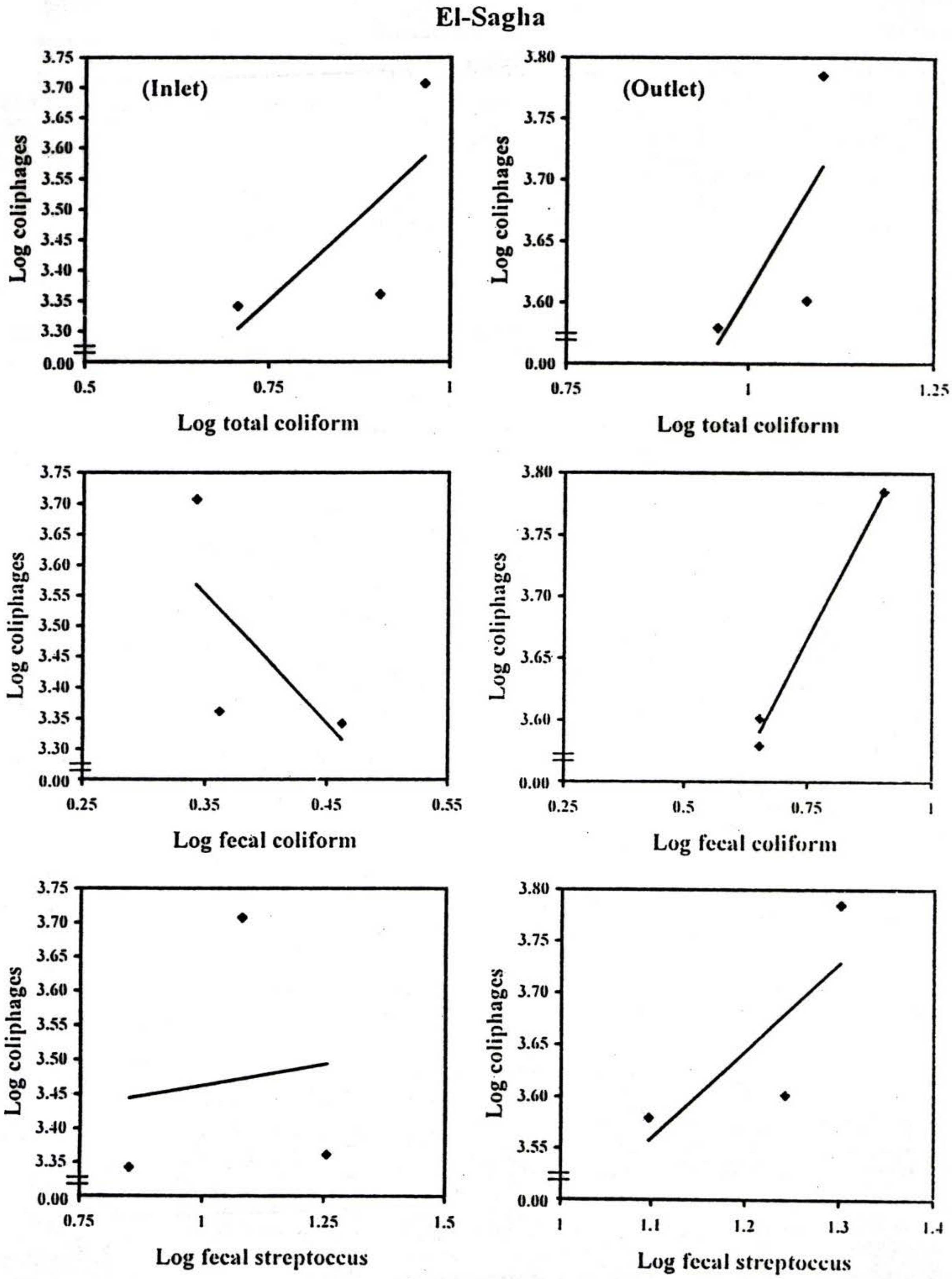
El-Sagha
(Inlet)
(Outlet)
Log coliphages
Log total coliform
Log fecal coliform
Log fecal streptoccus
3.75
3.70
3.65
3.60
3.55
3.50
3.45
3.40
3.35
3.30
0.00
3.80
0.5
0.75
1
1.25
0.25
0.35
0.45
0.55
1.5
1.1
1.2
1.3
1.4

Fig. 1. Cont.

The present results concluded that total coliforms had the highest counts where fecal coliforms was the lowest counts and fecal streptococci counts were in between in the groundwater samples of the three wells tested.

These results are in agreement with those obtained by Dutka (1973), Dufour (1977) and Kapuscinski and Michell (1983). They reported that fecal coliforms due to their low survival in adverse environments, cannot be considered as good indicators of fecal pollution.

The Relationship Between Coliform Groups and Coliphage

It is important that an indicator organism should be present in greater numbers than the pathogens. It is clear from Table 10 that coliphages had the highest counts over either total coliform (TC), fecal coliform (FC) or fecal streptococci (FS). To establish the validity of coliphages as fecal pollution indicators, these virus should be correlated with their bacterial hosts in the aquatic media. The results obtained in the present study show that the TC/ coliphages, FC/coliphages and FS/coliphages relationship in the inlet groundwater samples of the same site were 1:3.39, 1:4.47 and 1:3.24, respectively. Where, these ratios were 1:7.12, 1:8.13 and 1:7.36 in outlet groundwater samples, respectively (Table 10).

Quantitative relationships between fecal or total coliform bacteria and coliphges were established during this work. This relationship can be used to determine the number of fecal or total coliforms present in the water samples based on an enumeration of coliphages. With this method, Wentsel *et al*. (1982) determined the number of fecal and total coliforms in natural water samples based on the concentration of coliphages in the same natural water samples. On the other hand, the present obtained results support the results found by Borrego *et al*. (1987) that the coliphages are good indicators of the presence of fecal and total coliform bacteria.

The correlation coefficients between coliphage counts and total coliform (X_1X_2), fecal coliform (X_1X_3) and fecal streptococcus (X_1X_4) in groundwater samples of three wells tested during three months are shown in Table 11. The data from these tests were polluted as log-log plots of total coliform, fecal coliform or fecal streptococcus versus coliphages (Fig. 1). The results showed significant and positive correlation coefficients between coliphage versus total coliform (0.931 and 0.999), fecal coliform (0.609 and 0.769) and fecal streptococci (0.959 and 0.961), in inlet and outlet groundwater samples of El-Zeraa well, respectively. Also, significant and positive correlation coefficients in El- Sagha well were observed between coliphage and total coliform (inlet, 0.746 and outlet, 0.691), fecal coliform (outlet, 0.997) and fecal streptococci (outlet, 0.805). These values were significant ($P \leq 0.05$) or highly significant ($P \leq 0.01$), which indicating a high probability of a relationship between total coliforms, fecal coliforms or fecal streptococcus and coliphages in groundwater.

Correlation coefficients between coliform groups and coliphages also occur in freshwater as well as in the seawater (Kenard and Valentine, 1974; Scarpino, 1975; Kott *et al*., 1978; Borrego *et al*., 1987). Also, Wentsel *et al*. (1982) reported that the correlation coefficients were 0.69 for fecal coliforms versus coliphages and 0.62 for total coliforms versus coliphages. These correlations were significant at the 99.9%, indicating a high probability of a relationship between fecal or total coliforms and coliphages in natural waters.

Conclusion

Fecal pollution indicator bacteria such as total coliforms, fecal coliforms and fecal streptococci have been used as an indicator for examining the presence of pathogenic bacteria and coliphages in drinking water in Zagazig city. The present study deal with the seasonal variation and the distribution of these indicators and coliphages as well as the correlation between them in groundwater. The results revealed that there are a significant and positive correlation coefficients between coliphage versus total coliform (0.931 and 0.999), fecal coliform (0.609 and 0.769) and fecal streptococci (0.959 and 0.961), in inlet and outlet groundwater samples of El-Zeraa well, respectively. Also, significant and positive correlation coefficients in El-Sagha well were observed between coliphage and total coliform (inlet, 0.746 and outlet, 0.691), fecal coliform (outlet, 0.997) and fecal streptococci (outlet, 0.805). These values were significant ($P \leq 0.05$) or highly significant ($P \leq 0.01$), which indicating a high probability of a relationship between total coliforms, fecal coliforms or fecal streptococcus and coliphages in groundwater.

Table 10. Calculated relationship between total coliform (TC), faecal coliform (FC), faecal streptococci (FS) and coliphage in groundwater samples of three wells during three months period

Relationship	Groundwater sample*	
	Inlet	Outlet
TC/Coliphage*	1 – 3.39	1 – 7.12
FC/Coliphage	1 – 4.47	1 – 8.13
FS/Coliphage	1 – 3.24	1 – 7.36

* Based on the log number of the average of three wells during three months.

Table 11. Correlation coefficient between coliphage counts and total coliform (X_1X_2), faecal coliform (X_1X_3), faecal streptococci (X_1X_4) in groundwater samples of three wells during three months period

Correlation coefficient	Well name					
	El-Zeraa		El-Sagha		Abo-Aamer	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
X_1X_2 *	0.931	0.999	0.746	0.691	0.0	0.0
X_1X_3	0.609	0.769	-0.634	0.997	0.0	0.0
X_1X_4	0.959	0.961	-0.028	0.805	0.0	0.0

* X_1X_2: Correlation between coliphage and total coliform.
X_1X_3: Correlation between coliphage and fecal coliform.
X_1X_4: Correlation between coliphage and fecal streptococci.

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حـــدوث التغيرات الموسـمية في توزيع بعض البكتريا الدلاليــة والكوليفاج في مياه الشـــرب في مدينة الزقازيق

عبد الراضى موسى على - تحية عبد العزيز خاطر - فاطمة إبراهيم إلزامك - حسن إبراهيم عبدالفتاح

قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة الزقازيق – مصر

أجريت هذه الدراسة بهدف تقدير التغيرات الدورية والموسمية فى توزيع البكتيريا الدلالية و الكوليفاج Coli phages من عينات المياه الجوفية المستخدمة للشرب في مدينة الزقازيق و أخذت عينات المياه الجوفية من المنبع Inlet (بداية خط التوزيع) والمصب Outlet (نهاية خط التوزيع) مرتين شهرياً فى ثلاثة مكررات من الآبار الستة المختارة للدراسة لمدة ١٢ شهرا، وقد لخصت الدراسة في النتائج التالية: كانت عينات الماء من المنبع والمصب لكل الآبار الستة التي درست خلال مدة عام خالية من الكلوستريديم وسجلت النتائج وجود نسبة موجبة وعالية من السالمونيلا والشيجيلا فى بئر الزراعة (٥٨.٣%) وبئر الصاغة (٥٠.٠%) في مياه المنبع، بينما كان بئر وابور النور وبئر أبو عامر خالية من السالمونيلا والشيجيلا، كانت عينات المياه الجوفية من المنبع فى جميع الآبار الستة التي درست خالية من الأيروموناس، ما عدا بئر الصاغة حيث سجلت نسبة موجبة وصلت ٢٥.٠% وقد تم تقدير الكوليفاج Coliphage كدليل على جودة المياه: وكانت النتائج أن تراوحت كثافة الكوليفاج Coliphage من صفر حتى ٥.١ × ١٠ [٣] (خلية/١٠٠ مل) فى مياه المنبع، وتراوحت من صفر حتى ٦.١ × ١٠ [٣] (خلية/١٠٠ مل) في مياه المصب، وتوضح النتائج زيادة كثافة الكوليفاج فى عينات المياه بزيادة درجة حرارة البيئة، وكانت النسبة بين مجموعة القولون الكلية/الكوليفاج (١ : ٣.٣٩)، ومجموعة القولون البرازية/ الكوليفاج (١ : ٤.٤٧)، ومجموعة استربتوكوكس البرازية/الكوليفاج (١ : ٣.٢٤) في عينات المياه الجوفية من المنبع لنفس الموقع، أوضحت الدراسة وجود ارتباط معنوى وموجب بين الكوليفاج ومجموعة القولون الكلية (٠.٩٣١، ٠.٩٩٩)، الكوليفاج ومجموعة القولون البرازية (٠.٦٠٩، ٠.٧٦٩)، والكوليفاج ومجموعة الستربتوكوكس البرازية (٠.٩٥٩، ٠.٩٦١) فى عينات المياه الجوفية من المنبع والمصب فى بئر الزراعة على التوالى، وتتلخص نتائج الدراسة الحالية أيضا الي وجود تباين كبير فى المياه الجوفية للآبار الستة المختبرة، وكان أحسن ماء أرضى للآبار وابور النور وأبو عامر ولكن كانت أسوأ مياه جوفية للآبار الصاغة والزراعة، ولكن كانت المياه الجوفية لبئر جمال عبد الناصر وبئر الحسينية متوسط الجودة ومن النتائج المتحصل عليها ومن الناحية الصحية فإن مياه الآبار الجوفية يجب أن تعامل كيماوياً بصورة محكمة قبل توزيعها للشرب.

المحكمـــون:

١- أ.د. حامد السيد أبو علي أستاذ الميكروبيولوجيا – كلية الزراعة بمشتهر – جامعة بنها.

٢- د. ســـمير أحمد مرغني أستاذ الميكروبيولوجيا المساعد – كلية الزراعة – جامعة الزقازيق.