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Seasonal Variations in the Microbial Biomass and Pathogenic Bacteria in Low Quality Water Collected From El-Salam Canal: Case Study

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ABSTRACT

Surface low quality water samples were periodically collected at monthly intervals from El-Salam Canal at start (west Port Said) and middle (Balloza region) points. Samples were microbiologically analyzed for their microbial biomass and bacterial pathogens comparing with River Nile. Results showed that high densities of microbial biomass existed at both start and middle El-Salam Canal. Also, pathogens, represented by the classical pathogenic indicators, pathogenic bacteria, and new pathogenic indicators were present both start and middle El-Salam Canal at high densities. The intensities of the studied microorganisms were always higher at low quality water collected from El-Salam Canal compared to Nile. The counts being more or less the same at both start and middle sectors of El-Salam Canal, despite tending to be slightly higher at the start point.
Keywords: biomass, pathogenic, El-Salam Canal, water

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INTRODUCTION

Since the 1980's, efforts had been undertaken to extend available irrigation water supplies, by mixing drainage water with fresh water as represented by El-Salam Canal. The Government of Egypt implemented El-Salam Canal project to reuse drainage water, to create new communities along the Canal and to re-charting Egypt's population map (Hafez, 2008). El-Salam canal, having a mixture of such drainage water and the Nile water (1:1 ratio), crosses the Suez canal eastward to the deserts of north Sinai (Othman, et al., 2012). When this policy was first conceived, the principal goal was to supplement irrigation canal waters with drainage water to allow for better and additional irrigated lands. Despite low quality water signifies one of the weightiest renewable sources of water in Egypt; it is now contaminated with both chemical and biological constituents that have adverse impacts on sustainable farming. Low quality water use especially in unrestricted agriculture has adverse implications for the health of the stakeholders along the contamination pathway (i.e. from farm to fork). Despite the health risks, urban irrigated agriculture plays critical roles in urban poverty reduction agenda by supporting the livelihoods of farmers and many off-farm entrepreneurs (Amponsah, et al., 2015). The biological characteristics of water are judicious indicators for their uses, since they are more dynamic and often more sensitive than physical or chemical properties. They should be easy to measure, high sensitive and anticipative. In this regard, two major parameters should be cared about, microbial biomass and pathogens. Intonations, in the current are being guided to perceive seasonal variation in the biological characteristics of low quality water collected from El-Salam Canal.

MATERIALS AND METHODS

Sampling

Surface low quality water samples (0-30 cm) were periodically collected at monthly intervals during the period from December 2011 to July 2012 from start (west Port Said) and middle (Balloza region) points at El-Salam Canal as well as from River Nile (Tanash village). Samples were kept in an ice box, no more than 24 hours, before being subjected to microbiological analyses.

Microbiological methods

The key constituents that biologically differentiate low quality water in terms of agronomic value and health & environmental hazards were determined including total bacteria, fungi, *Azotobacter,* total and fecal coliform as well as *Pseudomonas, Salmonella* and fecal *Streptococci.* The studied microorganisms were counted after being grown in their specific growth media (Atlas, 2005) using the serial dilution method.

For total bacterial counts triplicate plates were prepared of each dilution using Topping medium (2.5 g peptone, 2.5 g yeast extract, and 15 g agar in one liter water with a pH 7) and incubated for five days at 30º C. Colonies were counted by means of the colony counter from plates yielding 30-300 colonies.

For fungi counts triplicate plates were prepared from each dilution using Martin medium (10 g glucose, 5 g peptone, 1 g dihydrogen potassium phosphate, 0.5 manganese sulphate, 1 part/30000 parts rose Bengal, 30 ug streptomycin, 20 g agar in one liter of distilled water) and counted after five days incubation period at 30° C.

The most probable numbers of *Azotobacter* were determined in a nitrogen free medium under aerobic condition (mannitol 10.0 g, dipotassium phosphate 0.5 g, magnesium sulphate 0.2 g, sodium chloride 0.02 g, manganese sulphate, sucrose 20.0 g, yeast extract (10%) 0.1 g, calcium carbonate 10.0 g, D.W 1000 ml). After two weeks incubation period at 30 ºC, positive tubes were distinguished by the presence of brown pellicle and *Azotobacter* cells in microscopic examinations.

Total and fecal coliforms bacteria were grown in MacConky broth (20 g/l peptone, 10 g/l lactose, 5 g/l bile salt, 0.01 g/l bromo cresol purple (pH 7.2). Each tube contained a Durham tube. The presence of gas and acid after 24 hours, incubation at 37 ºC indicated positive total coliforms tubes**.** While the presence of gas and acid after 24 hours, incubation at 44 ºC indicated positive fecal coliforms tubes.

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Fecal *Streptococci* were grown in a medium composed of Tryptose 10.0 g, Beef extract 3.0 g, sodium chloride 5.0 g, sodium azide 0.2 g 1000 DW (APHA, 1998) and were counted after 48 hour incubation period at 37ºC. Positive tubes were recognized by the presence of turbid growth.

Salmonella were grown on SS agar plates composed (per litter): Agar 18 g, Lactose 10.0 g, Bile salts 8.5 g, $Na_2S_2O_3$ 8.5 g, Sodium citrate 8.5 g, Beef extract 5.0 g, Pancreatic digest of casein 2.5 g, Peptic digest of animal tissue 2.5 g, Ferric citrate 1.0 g, Neutral Red 0.025 g, Brilliant Green 0.33 mg (Atlas, 2005) for 48 hour at 37ºC according to the scheme described by Quinn et al., (2002).

The most probable numbers of *Pseudomonas* were determined according to APHA, (1998) after 48 hour incubation period at 37ºC. The medium used for growing *Pseudomonas* is composed of Asparagine 3.0, Dipotassium hydrogen phosphate 1.0, Magnesium sulphate 0.5 g, 1000 ml DW). Positive tubes were recognized by the formation light green bluish color.

RESULTS

The present study aimed to monitor the microbiological profile of low quality water collected from the start and middle of El-Salam Canal compared to Nile water during the period from December 2011-July 2012. The microbial biomass in water was represented in the current study by the total bacterial counts, total fungal counts and the most probable numbers of *Azotobacter*. Results shown in Fig (1) indicated that total bacterial counts at the start point of El-Salam Canal ranged between $25x10^3$ CFU/1ml in February sample and $55x10^5$ CFU/1ml in June sample. While the total bacterial counts at the middle point of El-Salam Canal ranged between $30x10^3$ CFU/1ml in February sample and $35x10^5$ CFU/1ml in June sample. The total bacterial counts recorded at the start and middle point of El-Salam Canal was relatively the same and similar to counts of River Nile sample (which ranged between $15x10^2$ CFU/1ml in February sample and $30x10^5$ CFU/1ml in June sample). The main trend of bacterial counts revealed decreased numbers during cold months while it recorded relatively higher numbers during summer months.

The total fungal counts (Fig. 2) at the start point of El-Salam Canal ranged between $7x10^2$ CFU/1ml in February sample and $8x10^3$ CFU/1ml in June sample. While the total fungal counts at middle point of El-Salam Canal ranged between $5x10^2$ CFU/1ml in February sample and $3x10^3$ CFU/1ml in June sample. The corresponding total fungal counts in Nile water ranged between 14x10 CFU/1ml in February and $15x10^2$ CFU/1ml in June sample. The numbers in both start and middle points of El-Salam Canal recorded close values during the course of study except in December and January, while the fungal counts in Nile water were generally lower than in the canal.

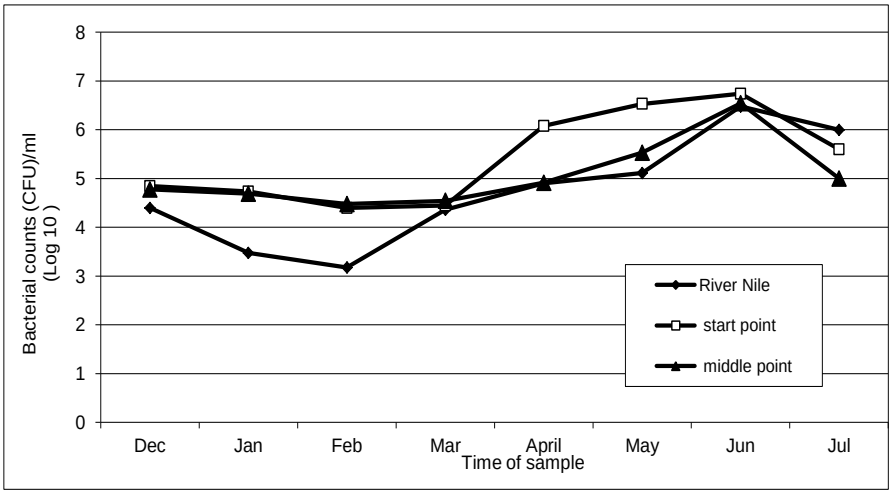

Fig (1) Seasonal variations in the total bacterial counts (CFU/ml) in the first and the middle point of El-Salam canal during the period from December 2011 to July 2012 compared to river Nile.

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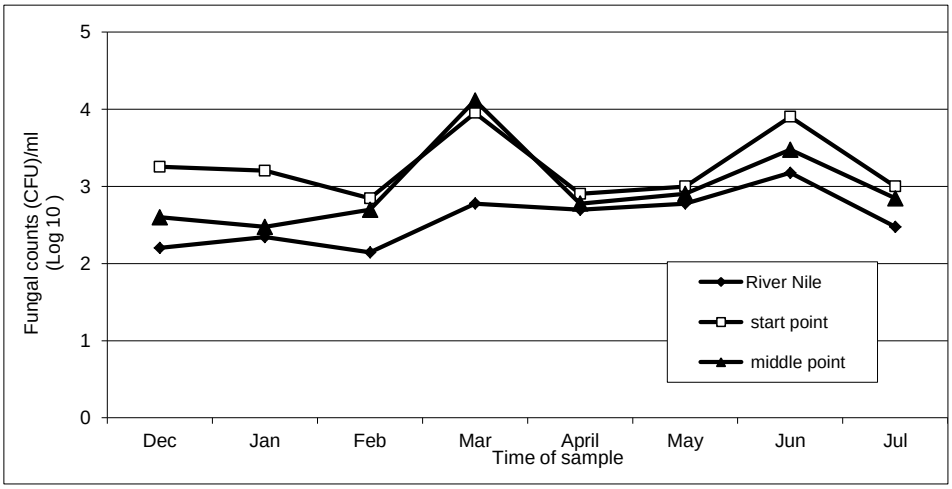

Fig (2) Seasonal variations in the total Fungal counts (CFU/ml) in the first and the middle point of El-Salam canal during the period from December 2011 to July 2012 compared to river Nile.

The most probable numbers of *Azotobacter* (Fig. 3) at the start point of El-Salam Canal ranged between $65x10^2$ CFU/1ml in February sample and $27x10^4$ CFU/1ml in June sample. While it ranged between $12x10^3$ CFU/1ml in February sample and $28x10^4$ CFU/1ml in June sample at middle point of El-Salam Canal. The corresponding most probable numbers of *Azotobacter* in Nile water ranged between $15x10^2$ CFU/1ml in February and $22x10^4$ CFU/ 1ml in June sample. The gap between *Azotobacter* counts in both start and middle points of El-Salam Canal was very small while it was clear in case of Nile water during the course of study except in June and July.

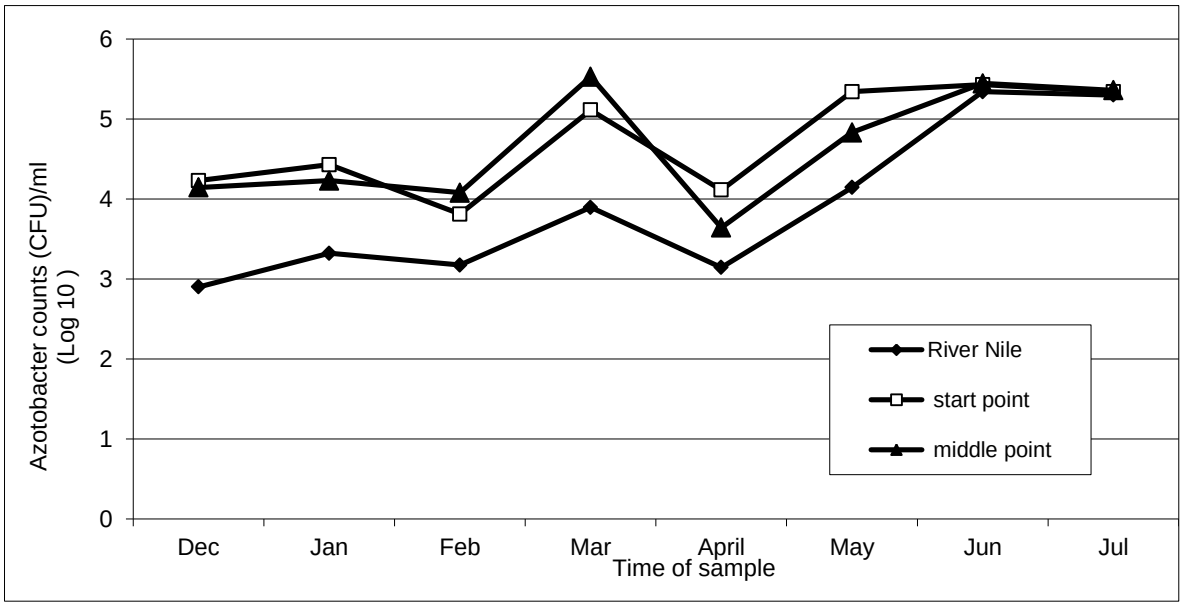

Fig (3) Seasonal variations in the total Azotobacter counts (CFU/ml) in the first and the middle point of El-Salam canal during the period from December 2011 to July 2012 compared to river Nile.

The classical infectious bacterial indicators were represented in the current study by the most probable numbers of both total and fecal coliforms. Results given in Figure (4) indicated that the most probable numbers of total coliforms at the start point of El-Salam Canal ranged between $9x10^2$ CFU/1ml in February sample and $11x10^4$ CFU/1ml in June sample, while it ranged between $9x10^2$ CFU/1ml in February sample and $48x10^3$ CFU/1ml in June sample at middle point of El-Salam Canal. The corresponding most

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probable numbers of total coliforms in Nile water ranged between $1x10^2$ CFU/1ml in February sample and $45x10^3$ CFU/ 1ml in June sample.

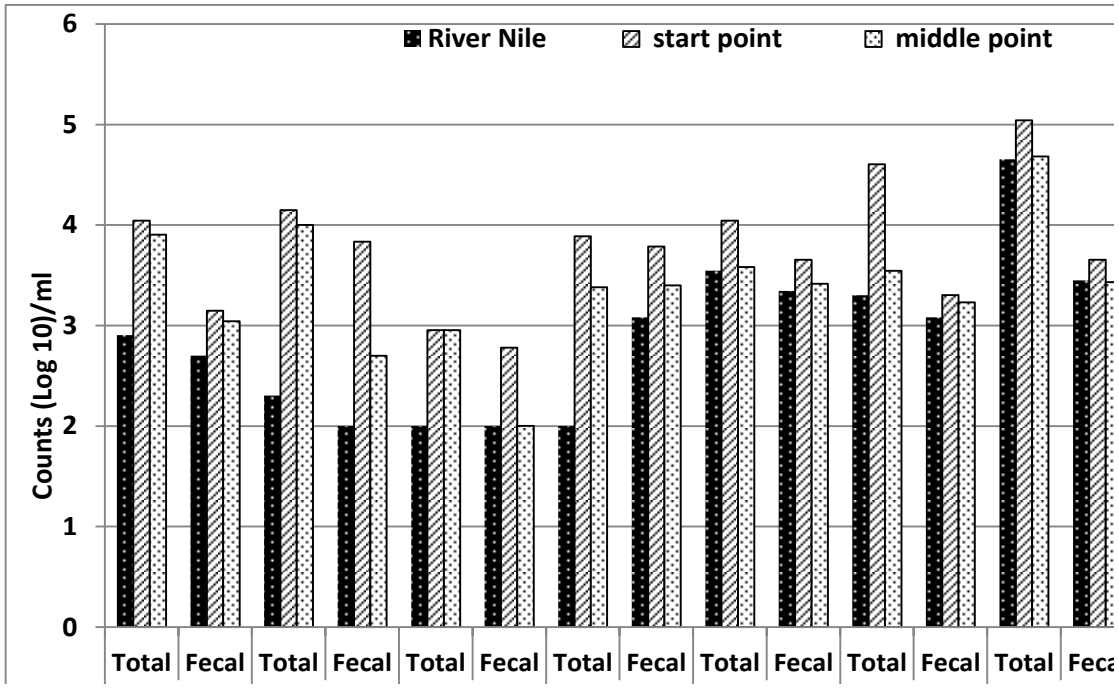

Fig. (4). Seasonal variations in the total and fecal coliform counts (CFU/1ml) as a classical pathogenic indicators at the first and the middle point of El-Salam canal during the period from December 2011 to July 2012 compared to river Nile.

For the most probable numbers of fecal coliform (Fig. 4), their counts at the start point of El-Salam Canal ranged between $6x10^2$ CFU/1ml in February sample and $45x10^2$ CFU/ 1ml in June sample. At middle point of El-Salam Canal the most probable numbers of fecal coliform ranged between $1x10^2$ CFU/1ml in February sample and $27x10^2$ CFU/1ml in June sample. While the average densities of corresponding fecal coliforms in Nile water ranged between $1x10^2$ CFU/1ml in February sample and $28x10^2$ CFU/1ml in June sample.

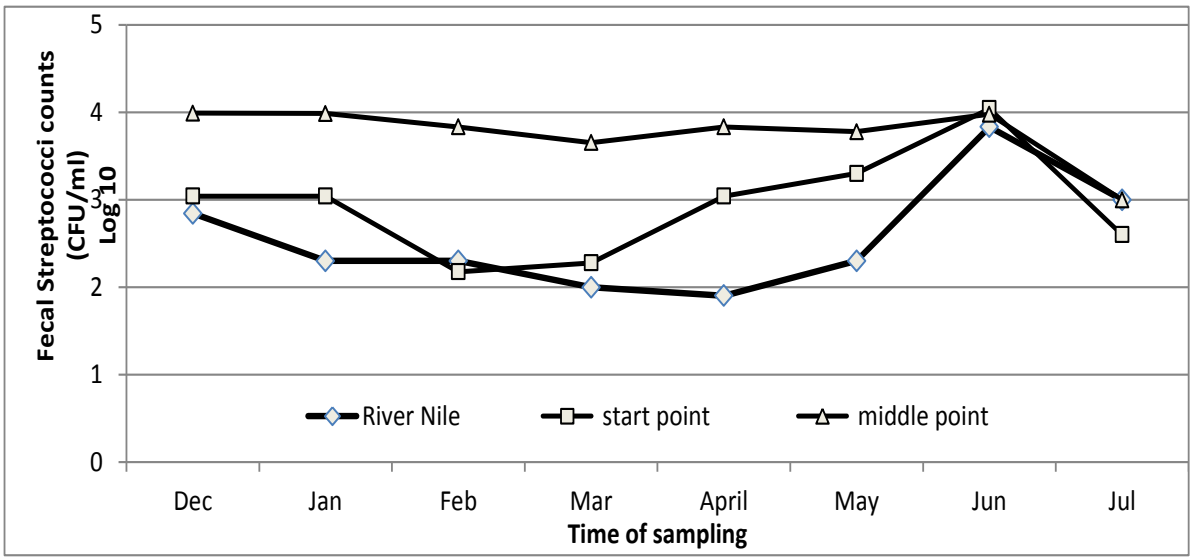

Fig. (5). Seasonal variations in the fecal *Streptococcus* counts (CFU/1ml) as a pathogenic indicator at the first and the middle point of El-Salam canal during the period from December 2011 to July 2012 compared to river Nile.

The pathogenic bacteria were represented in the current work by fecal *Streptococcus* and *Salmonella.* Results given in Figure (5) indicated that the most probable numbers of fecal *Streptococcus* at the start point of

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El-Salam Canal ranged between 15x10 CFU/1ml in February sample and $11x10^3$ CFU/1ml in June sample. While it ranged between $68x10^2$ CFU/1ml in February sample and $95x10^2$ CFU/ 1ml in June sample at middle point of El-Salam Canal. The corresponding most probable numbers of fecal *Streptococcus* in Nile water ranged between 20x10 CFU/1ml in February sample and $68x10^2$ CFU/1ml in June sample.

For the most probable numbers of *Salmonella* (Fig. 6) at the start point of El-Salam Canal, their counts ranged between 2x10 CFU/1ml in February sample and $7x10^2$ CFU/1ml in June sample. In the same time, counts of the most probable numbers of *Salmonella*, at middle point of El-Salam Canal ranged between $3x10^2$ CFU/1ml in February sample and $10x10^2$ CFU/1ml in June sample. While the average densities of corresponding *Salmonella* in Nile water were relatively low and ranged between 1x10 CFU/1ml in February sample and 4x10 CFU/ 1ml in June sample (Fig. 6).

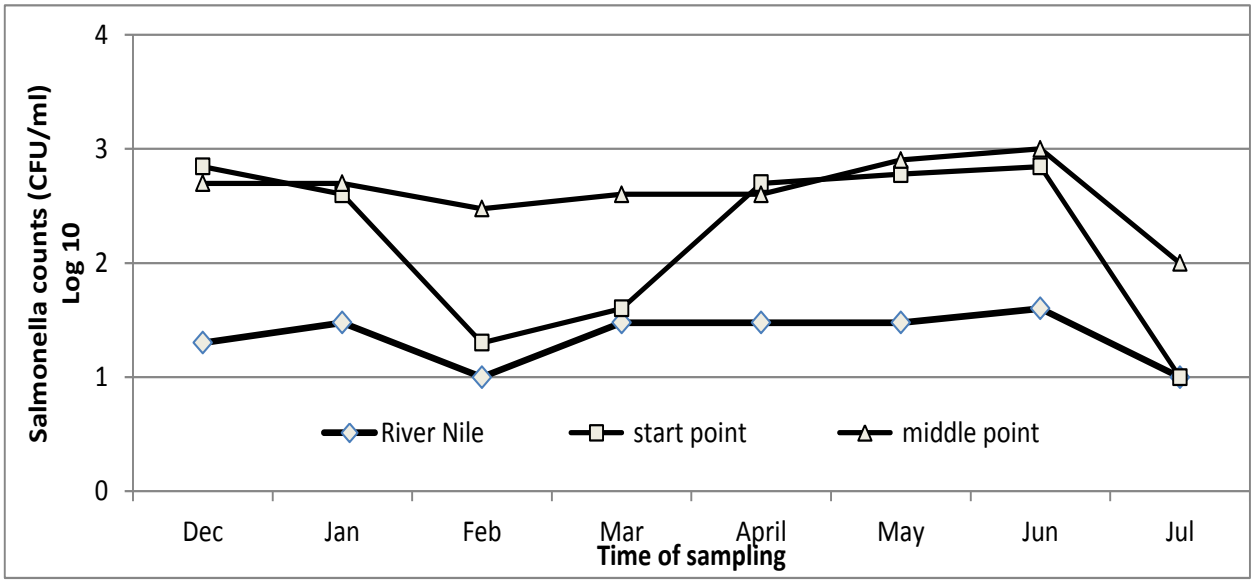

Fig. (6). Seasonal variations in the Salmonella counts (CFU/1ml) as a pathogenic indicator at the first and the middle point of El-Salam canal during the period from December 2011 to July 2012 compared to river Nile.

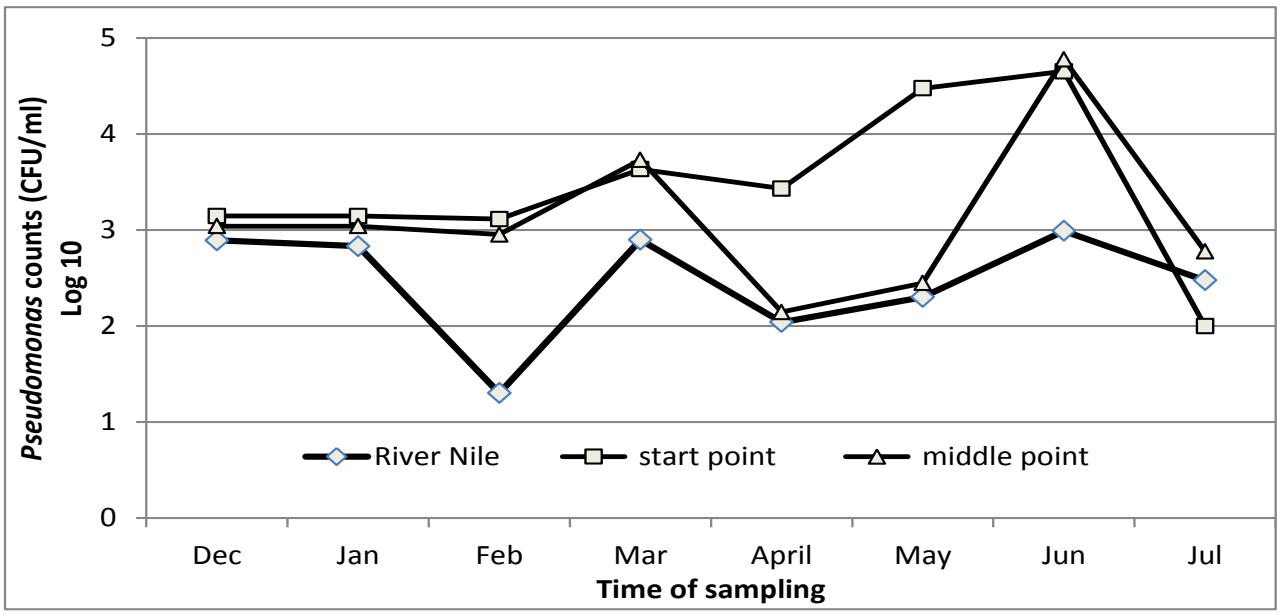

Fig (7) Seasonal variations in the counts of Pseudomonas as a new indicator of pathogenic bacteria (log CFU/1ml) at the first and the middle point of El-Salam canal during the period from December 2011 to July 2012 compared to river Nile.

The average counts of new pathogenic indicators (Fig. 7) represented by *Pseudomonas* ranged between $13x10^2$ CFU/1ml in February sample and $45x10^3$ CFU/ 1ml in June sample at the start point of El-Salam Canal. While it ranged between $9x10^2$ CFU/ 1ml in February sample and $60x10^3$ CFU/ 1ml in June sample at middle point of El-Salam Canal. The corresponding most probable numbers of *Pseudomonas* in Nile water ranged between 2x10 CFU/1ml in February sample and 98x10 CFU/ 1ml in June sample. Similar trends were previously found by Hafez, (2005) and Kamel et al., (2006).

DISCUSSION

At the time being, Egypt is suffering from an extensive shortage in water resources as far as the water demands exceed the available income. One of the key components of the water strategy in Egypt nowadays is the reuse of low quality water. Although irrigation with low quality water acclaimed to accomplish vast paces towards evoking agricultural production, from environmental and health aspects, it broadened the dissemination of pathogens and disfigured the microbial biomass in the ecosystem. These obstacles led to a strict unfriendly environmental aftermath, as well as to unsustainable agriculture.

It is quite evident that low quality water problems in Egypt vary with location and depend on factors such as climate, water flow rates, water uses, population densities, effluent discharges, leaching of agrochemicals residues etc. In this regard, a number of policies had been developed for preventing further low quality water degradation and ensuring enough water supply and sanitation coverage. Instruments targeting both industrial and agricultural sectors are of precise weight, and intend at providing the correct incentives and disincentives to water users for adopting more environmentally friendly practices.

The principal purpose of the agricultural drainage system is to sustain proper soil moisture levels in fields and to remove accumulated salts. The drainage network discharges directly to the main canals of Nile valley transferring salts, sediments and pathogens. At the time being, approximately 5 billion m^3 of drainage water is annually reused in Delta, and another 4.7 billion m^3 returns to Nile upstream of Cairo. Official reuse in the Delta is expected to rise to about 7 billion m^3 in the short term and to about 9 billion m^3 by 2017. In addition to threatened low quality water supplies, unauthorized use of polluted drainage water and raw sewage effluent to irrigate fields also contributes to growing concerns. Farmers illegally remove approximately 3 to 4 billion m^3 of drainage water annually to irrigate their fields. When contaminated with human waste, toxic elements and PTEs could impose great health risks, and also adds to rising salinity and pollution levels in the agricultural drainage water of Delta (INECO and EC (2009). This represents one of the most important water management challenges faced in Egypt today.

The mixing of Nile water with agriculture drains waters contaminated by many pathogenic microorganisms had the greatest effect on public health. A large number of epidemics due to the presence of these pathogens in the environment had been reported (Moe, 2002). In the current work, results showed that low water quality are becoming more and more polluted. Because of lack of substitute disposal options, wastes are habitually disposed into low quality water supplies, in violation of the existing legislations (Darwesh et al., 2014).

Many researchers came to the conclusion that fecal indicators bacteria failed to judge the water safety, where they found deferent opportunistic and/or pathogenic bacteria in the absence of them (El-Abagy et al., (1999) and El-Taweel and Shaban, (2001). Although detection of coliform bacteria in low quality water indicates that it might be unsafe, other bacteria had been isolated from low quality water resources that might propose some health risks through contact, ingestion, or inhalation. Generally, it is unacceptable for fecal coliform bacteria to be present at any concentration in low quality water. However, WHO, (1989) reported that less than 10 viable fecal coliforms cell per gm or ml might be considered as a safe level. Also, Housing & Building National Research Center (2004) entitled in Table 4-1 in the report of the permanent committee on reuse of treated sewage effluent in farming in Egypt part one: Code (2004) norms of sewage effluent, the permitted limit of fecal coliforms per 100 ml should not exceed 5000 according to article no 66 in the law number 48 (1982). Feachem et al., (1983) and Pescod, (1992) stated that the possible levels of *Salmonella* in sewage effluent per liter should not exceed 700. In the current work, although the counts of fecal coliforms were considered as a general indicator for the existence and survival of enteric pathogenic bacteria in low quality water ecosystems, yet to reach a valid conclusion, attention was also put on the existence of *Salmonella*, *Psedumonas and* fecal *streptococci* (APHA 1998). This is because many bacteria like *Candida*,

Aerobacter, *klebsiella etc*. are able to grew on MacConky broth producing acid and gas after 24 hour incubation at 44 ºC resembling fecal coliforms.

Gained results confirmed the presence of enteric pathogens in El-Salam Canal. It seems reasonable that the potential transfer of enteric pathogens from low quality water to humans is of real concern under Egyptian conditions due to the existence of a broad range of pathogens therein as showed in the results and the widespread use of manual labor in farming, having close contact with these water, and relatively low standards of hygiene. The recorded densities of the classical bacterial indicators in Nile water, however, were high and surpassed the values advised by WHO, (1989) and Cabelli, (1983). The densities of new indicators of contamination (fecal Staphylococci and *Pseudomonas*) exhibited the same trend of classical bacterial indicators. Kamel et al., (2006) stated that *Staphylococci* could be used as a convenient indicator of contamination as they had significant correlations with the classical bacterial indicators, physico-chemical characters and phytoplankton biomass. *Pseudomonas* is a shared environmental organism and could be found and survive in soil, water and sewage effluent. About 16% of the waterborne disease outbreaks reported between 1987 and 1996 were attributed to the bacterial pathogen *Pseudomonas aeruginosa* (de Victorica and Galvan, 2001).

With respect to pathogenic bacteria, results told that *salmonellae* existed in El-Salam Canal which might encourage epidemic outbreaks due to the multiple uses of surface water in agriculture, drinking water and food production.

Noteworthy, pathogens always tend to unsurprisingly fade away from low quality water ecosystem with time as far as they were not fortified with additional sources. Such behavior is predictable, as this group of microorganisms is naturally habituating the intestinal tract where an utterly different ecosystem subsists. Death of pathogens in general and of fecal *E. coli* in particular in low quality water ecosystems is mostly caused by saprophytic microflora, the antibiotic properties of its metabolic products as well as with the antimicrobial action of some root exudates.

It seems reasonable to state that safe use of low quality water in farming necessitates continuous evaluation of their biological, hygienic, chemical and physical as well as aesthetical characteristic. It is well known that microorganisms respond quickly to varied environmental stresses as they have intimate relations with their surroundings due to their high surface to volume ratio. The contamination of water sources with microorganisms such as pathogenic bacteria and viruses highlighted the most of rigorous monitoring (Bartram, et al., 2003).

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