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Seasonal Variations In The Microbial And Pathogenic Biomass In Low Quality Water Collected From Two Egyptian Agricultural Drains.

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ABSTRACT

Low quality water samples were regularly collected at monthly intervals from Belbeis and Bahr El-Bakar agricultural drains as well as from River Nile and analyzed for their microbial and pathogenic biomass. Results confirmed the being of high densities of microbial biomass as well as both classical and new indicator pathogenic bacteria, yet at higher densities at 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.

Keywords: Microbial biomass, Pathogenic bacteria, Agricultural drains, Low quality water.

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INTRODUCTION

By 2030, more than half of the African countries are expected to stand facing sever water scarcity tribulations (Amponsah, et al., 2015). One of the solutions to face this situation in Egypt started since 1970 using low quality water from agricultural drains in farming in an attempt to raise water use efficiency and expand cultivated area. At present, drainage water reuse is widely practiced in Delta and Upper Egypt throughout a central drainage reuse system (Masoud and Abdel Aal, 2014). Certainly, the biological characteristics of low quality water are thoughtful indicators for their use, since they are more dynamic and often more sensitive than their physical or chemical properties. Unquestionably drainage water is contaminated with both chemical and biological constituents that have adverse impacts on health, environment and sustainable farming (FAO, 2002). Improving planning and management practices to robust these low quality water uses in farming are certainly crucial instruments to sustaining future burdens in Egypt. Realistic precautions to prevent drainage water impregnated with pathogens from reaching the edible portion of crops are now prudent. The present work aims to study the seasonal variations in the microbial and pathogenic biomass in low quality water collected from two agricultural drains in Egypt.

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 both Belbis and Bahr-el-Bakr drains 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.

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

In the present study the microbiological profile of Belbeis and Bahr El-Bakar drains was followed and compared with River 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 drawn in Fig (1) indicate that total bacterial counts in Belbeis drain ranged between $10x10^5$ CFU/ml in February and $45x10^5$ CFU/ml in June and between $10x10^5$ CFU/ml in February and $42x10^5$ CFU/ml in June in Bahr El-Bakar drain. This indicate that more or less the same intensity of total bacterial counts existed in both drains fluctuating around 10^5 CFU/ml, which is much higher than those found in river Nile. The higher counts of total bacteria points to the presence of well furnished aquatic ecosystem that encouraged their proliferation mainly nutrients including contaminants. The corresponding total bacterial counts in river Nile water exhibited relatively lower values (ranged between $15x10^2$ CFU/ml in February and $30x10^5$ CFU/ml in June). It seems reasonable that the total bacteria counts in river Nile were much more affected with temperature rather than with nutrients as in case of drainage water. The lowest counts of total bacterial counts in both drains as well as in river Nile water were recorded during winter cold season (January and February) despite of the presence of enough nutrients.

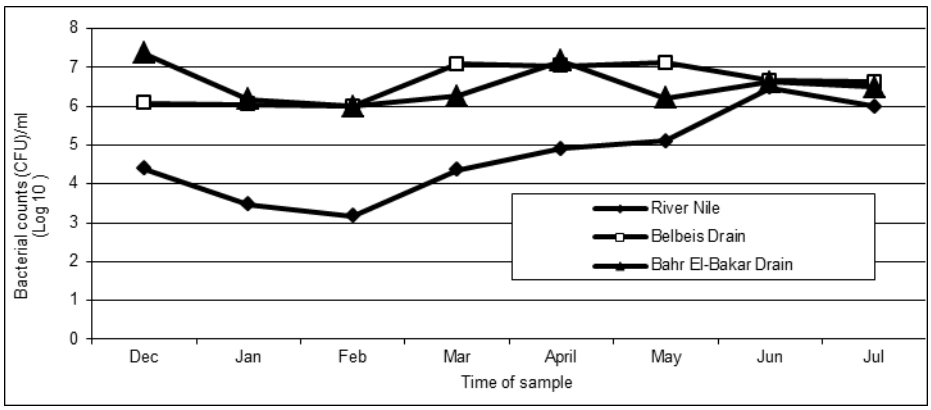

Fig (1) Seasonal variations in the total bacterial counts (CFU/ml) in Belbeis and Bahr El-Bakar drains during the period from December 2011 to July 2012 compared to river Nile

No clear variations were distinguishable in the counts of fungi between summer and winter samples in both drains where they ranged between $4x10^3$ CFU/ml in February and $39x10^3$ CFU/ml in June in Belbeis drain and between $5x10^3$ CFU/ml in February and $1x10^4$ CFU/ml in June Bahr El-Bakar drain. The matching total fungal counts in river Nile water exhibited the same trends between summer and winter samples but at an obvious lowers intensities where fungal counts ranged between 14x10 CFU/ml in February and $15x10^2$ CFU/ml in June.

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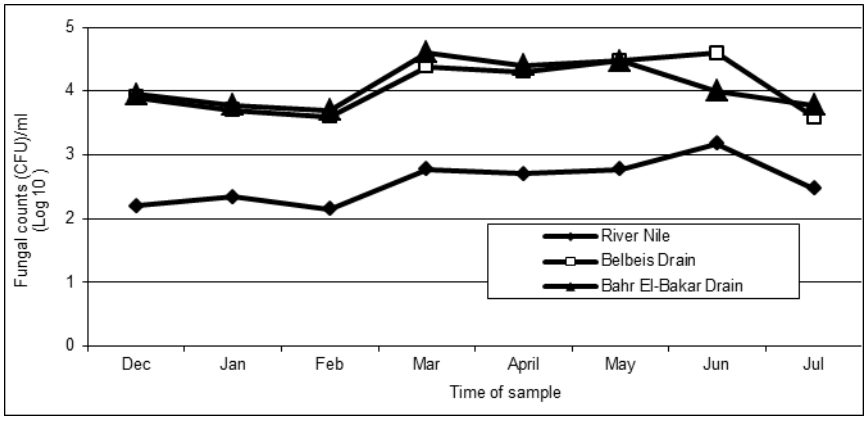

Fig (2) Seasonal variations in the fungal counts (CFU/ml) at Belbeis and Bahr El-Bakar drains during the period from December 2011 to July 2012 compared to river Nile.

As illustrated in Fig. (3), Belbeis drain water contained *Azotobacter* (estimated as most probable numbers) intensities ranging between $11x10^4$ CFU/ml in February and $18x10^5$ CFU/ml in June. Bahr El-Bakar drain water in the same period contained *Azotobacter* numbers ranging between $40x10^4$ CFU/ml in February and $62x10^4$ CFU/ml in June. The corresponding most probable numbers of *Azotobacter* in river Nile water ranged between $15x10^2$ CFU/ml in February and $22x10^4$ CFU/ml in June.

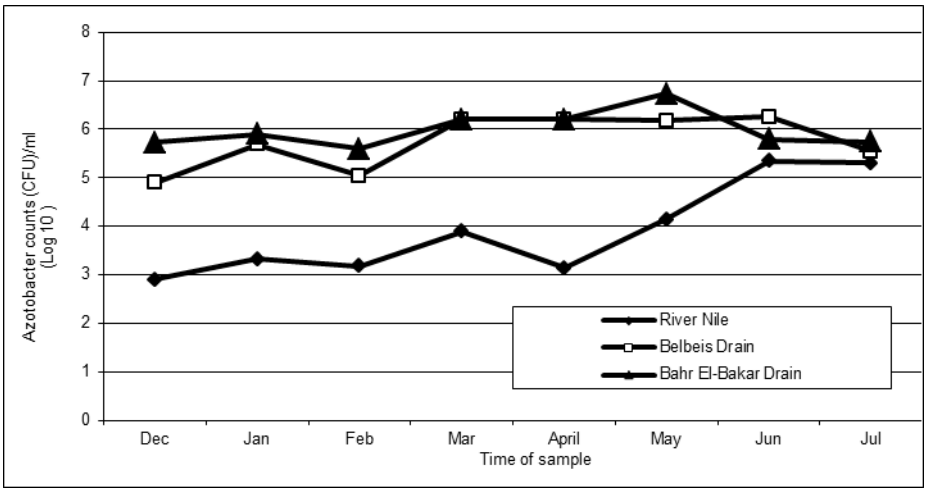

Fig (3) Seasonal variations in *Azotobzcter* counts (CFU/ml) at Belbeis and Bahr El-Bakar drains during the period from December 2011 to July 2012 compared to river Nile

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 Fig (4) indicate that the most probable numbers of total coliforms in Belbeis drain ranged between $20x10^4$ CFU/ml in February and $92x10^4$ CFU/ml in June. Bahr El-Bakar drain water contained more or less the same intensities ranging between $16x10^3$ CFU/ml in February and $39x10^5$ CFU/ml in June. The parallel most probable numbers of total coliforms in river Nile water ranged between $1x10^2$ CFU/ml in February and $45x10^3$ CFU/ml in June. For the most probable numbers of fecal coliforms (Fig. 4), their counts at Belbeis drain ranged between $20x10^3$ CFU/ml in February and $11x10^3$ CFU/ml in June. Similar findings were recorded for counts of fecal coliforms in Bahr El-Bakar drain water ranging between $6x10^3$ CFU/ml in February and $14x10^4$ CFU/ml in June. On the other hand, the average densities of corresponding fecal coliforms in river Nile water ranged between $1x10^2$ CFU/ml in February and $28x10^2$ CFU/ml in June.

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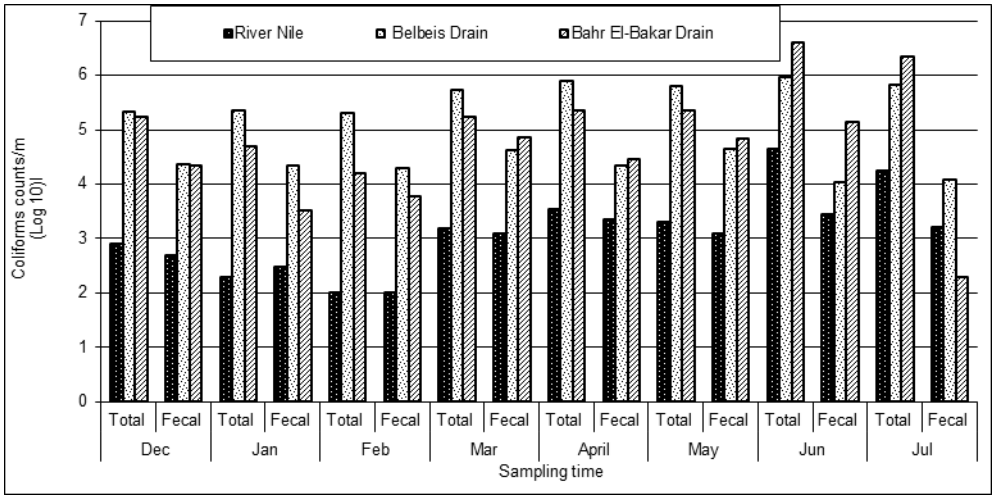

Fig (4) Seasonal variations in the Total and fecal coliform counts(CFU/ml) as a classical pathogenic indicators at Belbeis and Bahr El-Bakar drains during the period from December 2011 to July 2012 compared with river Nile.

The pathogenic bacteria were represented in the current work by fecal *Streptococcus* and *Salmonella.* Results given in Fig (5) indicate that the most probable numbers of fecal *Streptococcus* at Belbeis drain ranged between $20x10^2$ CFU/ml in February and $72x10^2$ CFU/ml in June. In Bahr El-Bakar drain counts of fecal *Streptococcus* ranged between $15x10^2$ CFU/ml in February and $47x10^3$ CFU/ml in June. The corresponding most probable numbers of fecal *Streptococcus* in river Nile water ranged between 20x10 CFU/ml in February and $68x10^2$ CFU/ml in June.

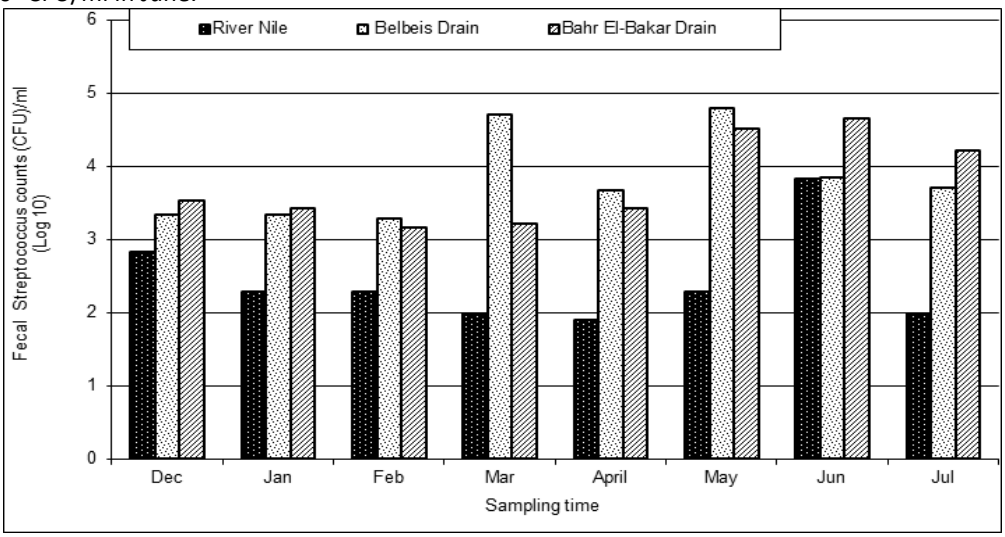

Fig (5) Seasonal variations in the fecal *Streptococcus* counts (CFU/ml) as pathogenic bacteria at Belbeis Drain and Bahr El-Bakar Drain during the period from December 2011 to July 2012 compared to river Nile.

As regards the most probable numbers of *Salmonella*, as illustrated in Fig (6), their counts ranged between $5x10^2$ CFU/ml in February sample and $31x10^3$ CFU/ml in June sample at Belbeis Drain and ranged between $7x10^2$ CFU/ml in February sample and $32x10^3$ CFU/ml in June sample at Bahr El-Bakker Drain. While the average densities of corresponding *Salmonella* in River Nile water ranged between 1x10 CFU/ml in February sample and 4x10 CFU/ml in June sample.

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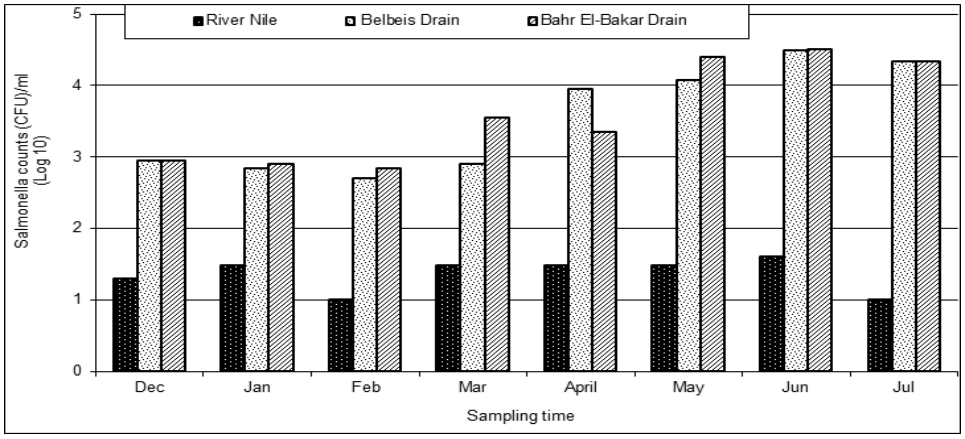

Fig (6) Seasonal variations in *Salmonella* counts (CFU/ml) as pathogenic bacteria at Belbeis and Bahr El-Bakar drains during the period from December 2011 to July 2012 compared to river Nile.

The average counts of the new pathogenic indicators (Fig 7) represented by *Pseudomonas* at Belbeis drain ranged between $39x10^3$ CFU/ml in February and $22x10^4$ CFU/ml in June. In Bahr El-Bakar drain *Pseudomonas* counts ranged between $17x10^2$ CFU/ml in February and $33x10^4$ CFU/ml in June. The corresponding most probable numbers of *Pseudomonas* in river Nile water ranged between 2x10 CFU/ml in February and 98x10 CFU/ml in June. Similar trends were previously shown by El-Ganzori *et al*., (2000).

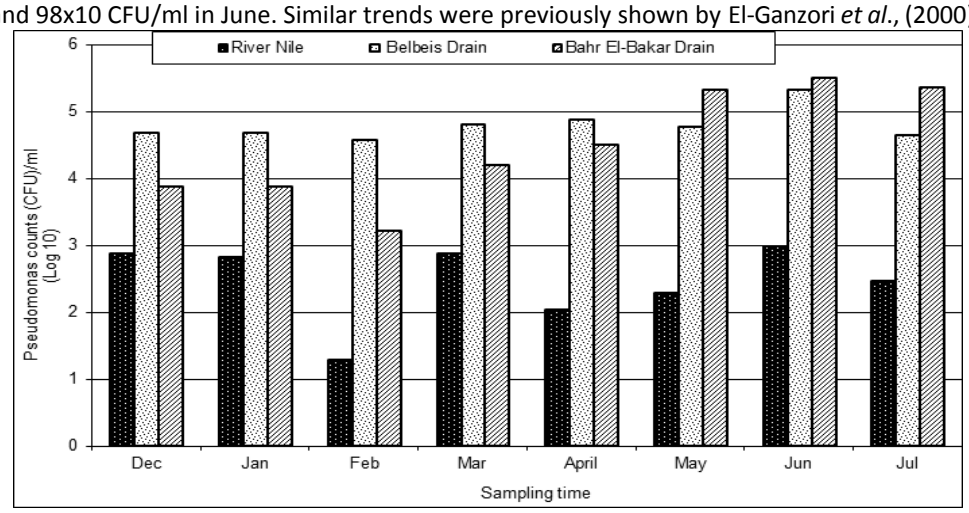

Fig (7) Seasonal variations in *Pseudomonas* counts (CFU/ml) as new pathogenic indicator at Belbeis and Bahr El-Bakar drains during the period from December 2011 to July 2012 compared to river Nile.

DISCUSSION

One of the key components of the water strategy in Egypt nowadays is the reuse of low quality water in farming. Despite irrigation with drainage 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.

It is quite evident that drainage water reuse problems varies with site and are linked to many factors. A number of policies had been developed for preventing further drainage water degradation and ensuring enough water supply and sanitation coverage. Instruments targeting both industrial and agricultural sectors are of strict weight, and intend at providing truthful incentives and disincentives to water users for adopting more environmentally friendly practices (Darwesh et al., 2014).

Certainly, mixing river Nile water with drain waters contaminated by pathogenic microorganisms had a 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 drainage water are

becoming more and more polluted, predominantly those passing through villages, towns and residential areas. Reused drainage water for sure affected the quality of entire river Nile water **(**INECO and EC, 2009).

Despite being in the decade of biological trustworthiness, several constraints are still surrounding sustainable reuse of drainage water as far as the environmental merit is vital for water reuse to survive. Reuse practices routines should be developed within the frame of the recent achievements in environmental biotechnology, which are increasingly acknowledged as a potential solution to abundant problems overlaying present situation (Saber, 2001). Sustainable management of low quality water in farming would never be achieved as far as agricultural practices continue to overstress the level of biological and/or chemical contaminants that jeopardizes both flourishing farming and sustainability and necessitates setting new farming systems devoted to following environmental and sustainable approaches.

Historically, fecal coliforms in common and *E. coli* in distinct had been used as indicators for monitoring of water quality (Clesceri, *et al*., 1998, Saber 2001 and Chigbou, *et al*., 2005). It is widely settled upon that it is not judicious to verify the existence of all types of pathogenic organism's in soils and low quality water ecosystems. For this cause, the indicator microorganism notion was highly considered since some decads ago. Regulatory agencies generally rely on tests for fecal coliforms bacteria to indicate contamination. Although fecal coliforms themselves are not pathogenic, they designate that pathogens could exist and perhaps flourish. However, no single indicator microorganism could anticipate the incidence of all enteric pathogens. If there are truthful correlations between indicator microorganisms and enteric pathogens, it would be crucial to define the scope and state of affairs these microorganisms could be used as consistent indicators of fecal contamination (Bynum , 2001 and Tyagi *et al*.**,** 2006).

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 coliforms 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 and 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). 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 drainage water ecosystems, yet to reach a valid conclusion, attention was also put on the existence of *Salmonella*, *Psedumonas and feacal 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 both investigated drains, and even in river Nile water. It seems reasonable that the potential transfer of enteric pathogens from agricultural drainage 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 both studied drains were high and surpassed the values advised by WHO, (1989) and those of 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 that could be found and survive in drainage water. 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). The presence of *Salmonella* in drainage water ecosystems could encourage the occurrence of 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 drainage water ecosystem with time unless 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 drainage 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.

Microbial indicators seem to be sensitive and detectable rather early in assessing water quality in comparable to the soil chemical and physical indicators (Karlen *et al*. 1999). The presence of a viable and diverse microbial community in irrigation water had been considered essential for sustainable farming (Kennedy and Smith 1995) as it acts as an early indicator of biological processes (Islam and Weil 2000). As far as only a small portion of water microbial biomass is cultivable, hence the microbial biomass were represented in the current work by the total counts of bacteria, and fungi and the most probable numbers of *Azotobacte*r that were considered highly sensitive bio-indicators for the biological characteristics of drainage water, within the frame that the higher the intensities the higher the bioactivity. El-Ganzori *et al*., (2000) carried out a study during the period between March 1998 to July 1999, to investigate the possibility to reuse drainage water at El-Wasat and El-Manaifa in Kafr El Sheikh and found that the bacterial count level for most of the sites complies with quality criteria to irrigate the restricted crops according to WHO guidelines (1989).

It seems reasonable to state that safe use of drainage 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. In most instances, changes in microbial populations or their activity could precede detectable changes in the water characteristics, thereby providing an early sign of soil contamination. The contamination of water sources with microorganisms such as pathogenic bacteria and viruses highlighted the most of rigorous monitoring (WHO, 2003).

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