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Seasonal Variations in the Microbial Biomass and Pathogenic Bacteria in some Egyptian Sewage Effluents.

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ABSTRACT

A case study was performed to monitor changes in microbial biomass and bacterial pathogens in the effluent of two important sewer plants that discharges their effluents in agricultural irrigation canals in Giza, Egypt. The studied effluents collected monthly for microbiologically analysis from El-Mohtamadia canal (Zenen sewer plant) and Abu-Rawash sewer plants as well as from Nile River for comparison (Tanash village). Results showed that high densities of microbial biomass existed at both studied sewer plants. Also, pathogens, represented by the classical pathogenic indicators, pathogenic bacteria, and new pathogenic indicators were present both the investigated effluent collected from both Abu-Rawash sewer plant of El-Mohtamadia canal (Zeneen sewer plant). The intensities of the studied microorganisms were always higher at low quality water collected from both sewer plants compared to Nile River. The counts being always higher in summer samples compared to winter ones.
Keywords: Microbial biomass, Pathogenic bacteria, Sewage effluents, Low quality water.

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INTRODUCTION

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 whether agricultural drainage water or treated sewage effluent. The first use of treated wastewater in Egypt was in 1915 in the eastern desert north east of Cairo. An area of 2500 acres is still under irrigation with wastewater, which receives only primary treatment. With the scarcity of water resources, it is planned to irrigate 150,000 acres with treated wastewater up to the year 2000 (El Gamal, *et al*., 2005).

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.

Sewage effluent is contaminated with both chemical and biological constituents that have adverse impacts on sustainable farming and soil ecosystems Improving planning and management practices to robust sewage effluent reuse in farming these are crucial instruments to sustaining future burdens in Egypt rest on competent use of available contemporary water resources. The biological indicators of sewage effluent contamination should be those easy to measure, highly sensitive and anticipative. Two major parameters should be cared about, the size of microbial biomass mainly enteric pathogens, bacteria, fungi as well as their activities.

MATERIALS AND METHODS

Sampling: Surface low water quality samples (0-30 cm) were periodically collected at monthly intervals from El-Mohtamadia canal (Zenen sewer plant) and Abu-Rawash sewer plants as well as from Nile (Tanansh 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, 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 µg streptomycin, 20 g agar in one liter of distilled water) and counted after five days incubation period at 30° C.

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

The biological characteristics of sewage effluent are judicious indicators for their use in farming, since they are more dynamic and often more sensitive than physical or chemical soil properties. The microbial biomass in sewage effluent was represented in the current study by the total bacterial counts, total fungal counts. Results displayed in Fig. (1) indicated that total bacterial counts in Abu-Rawash sewage effluent ranged between $10x10^5$ CFU/1ml in February sample and $90x10^6$ CFU/1ml in June sample. The total bacterial counts at El-Mohtamadia canal (Zenen sewer plant) ranged between $10x10^5$ CFU/1ml in February sample and $50x10^5$ CFU/1ml in June sample, while the corresponding total bacterial counts in Nile water ranged between $15x10^2$ CFU/1ml in February sample and $30x10^5$ CFU/1ml in June sample.

The total fungal counts (Figure 2) in Abu-Rawash sewage effluent ranged between $5x10^2$ CFU/1ml in February sample and $1x10^4$ CFU/1ml in June sample. While the counts of fungi at El-Mohtamadia canal (Zenen sewer plant) ranged between 14x10 CFU/1ml in February sample and $26x10^3$ 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.

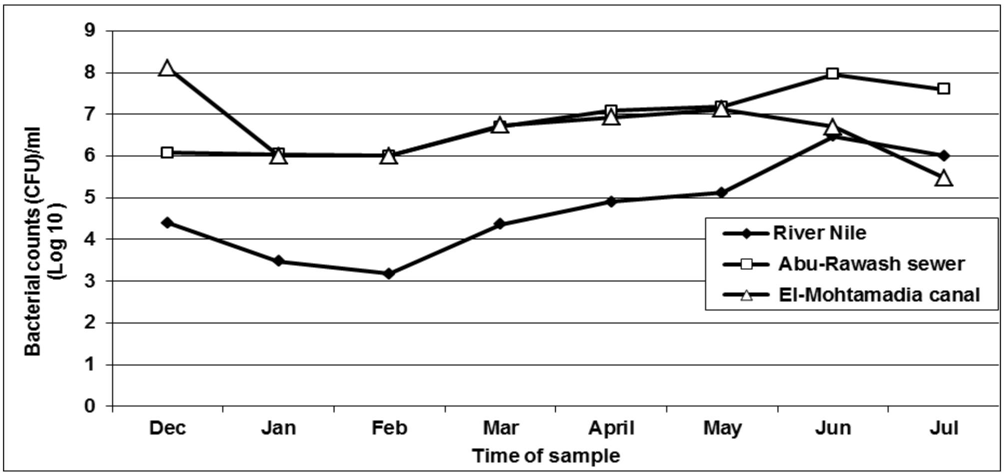

Fig 1: Seasonal variations in the total bacterial counts (CFU/ml) at Abu-Rawash sewer and El-Mohtamadia canal (Zenen sewer plant) during the period from December 2011 to July 2012 compared to river Nile

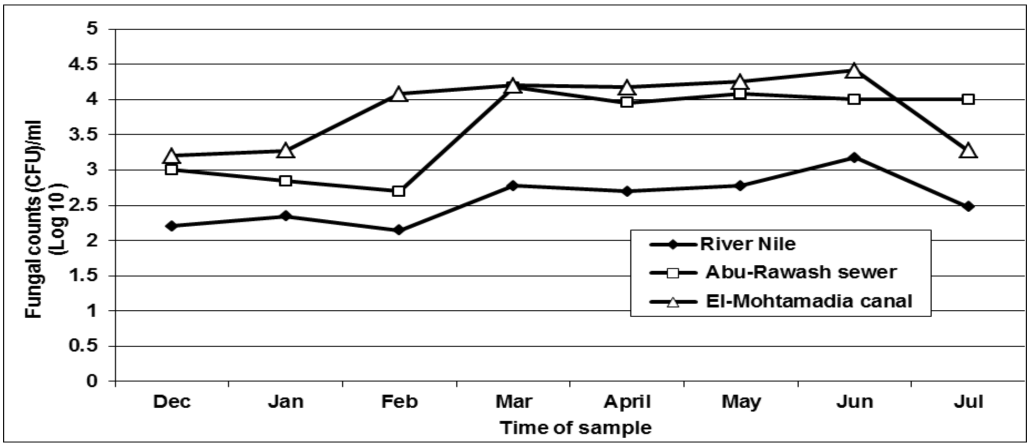

Fig 2: Seasonal variations in the fungal counts (CFU/ml) at Abu-Rawash sewer and El-Mohtamadia canal (Zenen sewer plant) during the period from December 2011 to July 2012 compared to river Nile.

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 (3) indicated that the most probable numbers of total coliforms in Abu-Rawash sewage effluent ranged between $11x10^3$ CFU/1ml in February sample and $79x10^4$ CFU/1ml in June sample. While the most probable numbers of total coliforms at El-Mohtamadia canal (Zenen sewer plant) ranged between $19x10^3$ CFU/1ml in February sample and $22x10^6$ CFU/1ml in June sample. The corresponding most 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.

For the most probable numbers of fecal coliform in Abu-Rawash sewage effluent, their counts ranged between $7x10^2$ CFU/1ml in February sample and $14x10^4$ CFU/ 1ml in June sample. At El-Mohtamadia canal (Zenen sewer plant), the counts of most probable numbers of fecal coliform ranged between $17x10^3$ CFU/1ml in February sample and $17x10^4$ 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. 3).

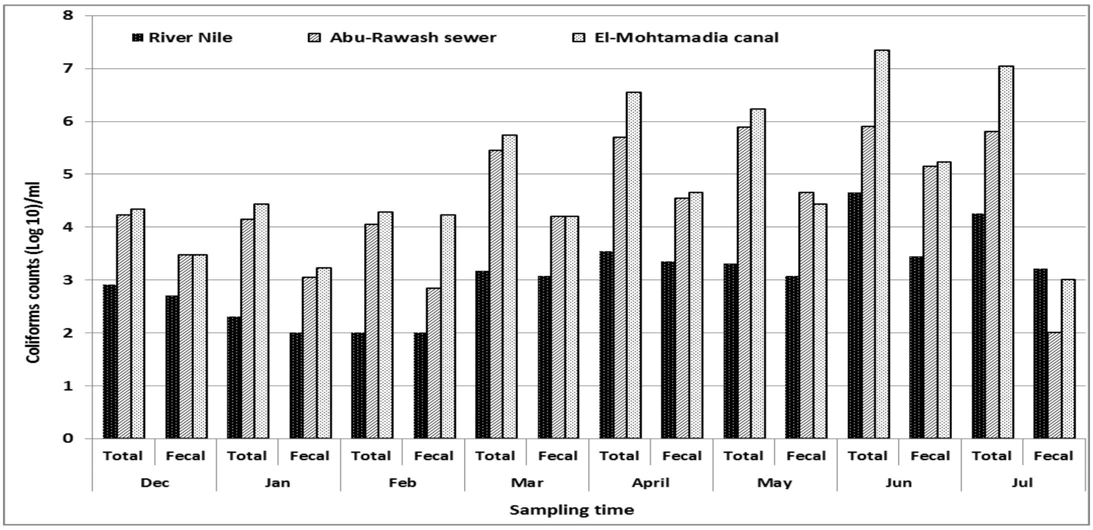

Fig 3: Seasonal variations inthe Total and fecal coliform counts (CFU/ml) as a classical pathogenic indicators at Abu-Rawash sewer and El-Mohtamadia canal (Zenen sewer plant) during the period from December 2011 to July 2012 compared with river Nile.

The pathogenic bacteria were represented in the current work by fecal *Streptococcus* and *Salmonella.* Results given in Figure (4**)** indicated that the most probable numbers of fecal *Streptococcus* in Abu-Rawash sewage effluent ranged between $10x10^2$ CFU/1ml in February sample and $68x10^3$ CFU/1ml in June sample. While the most probable numbers of fecal *Streptococcus* at El-Mohtamadia canal (Zenen sewer plant) ranged between $12x10^2$ CFU/1ml in February sample and $27x10^3$ CFU/1ml in June sample. 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* in Abu-Rawash sewage effluent, their counts ranged between $5x10^2$ CFU/1ml in February sample and $22x10^3$ CFU/1ml in June sample. The most probable numbers of *Salmonella* at El-Mohtamadia canal (Zenen sewer plant) between $4x10^2$ CFU/1ml in February sample and $22x10^3$ CFU/1ml in June sample. While the average densities of corresponding *Salmonella* in Nile water ranged between 1x10 CFU/1ml in February sample and 4x10 CFU/ 1ml in June sample. Similar trend were found by saber *et al.,* (2012) (Fig. 5)

The average counts of new pathogenic indicators (Fig. 6) represented by *Pseudomonas* in Abu-Rawash sewage effluent ranged between $13x10^3$ CFU/1ml in February sample and $1x10^4$ CFU/ 1ml in June sample. While it ranged between $14x10^3$ CFU/ 1ml in February sample and $1x10^4$ CFU/1ml in June sample at El-Mohtamadia canal (Zenen sewer plant). 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. Results are in harmony with those of Saber *et al.,* (2012).

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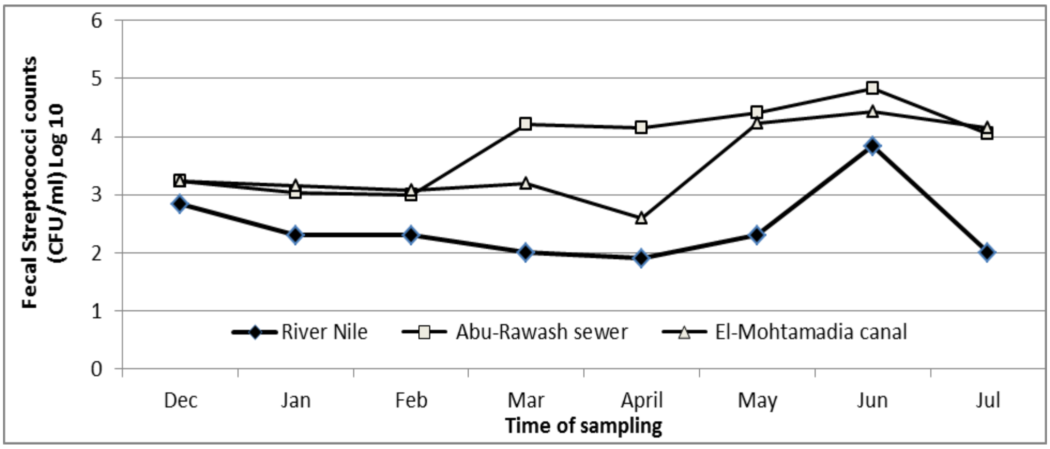

Fig 4: Seasonal variations in the fecal *Streptococcus* counts (CFU/ml) as pathogenic bacteria at Abu-Rawash sewer and El-Mohtamadia canal (Zenen sewer plant) during the period from December 2011 to July 2012 compared to river Nile.

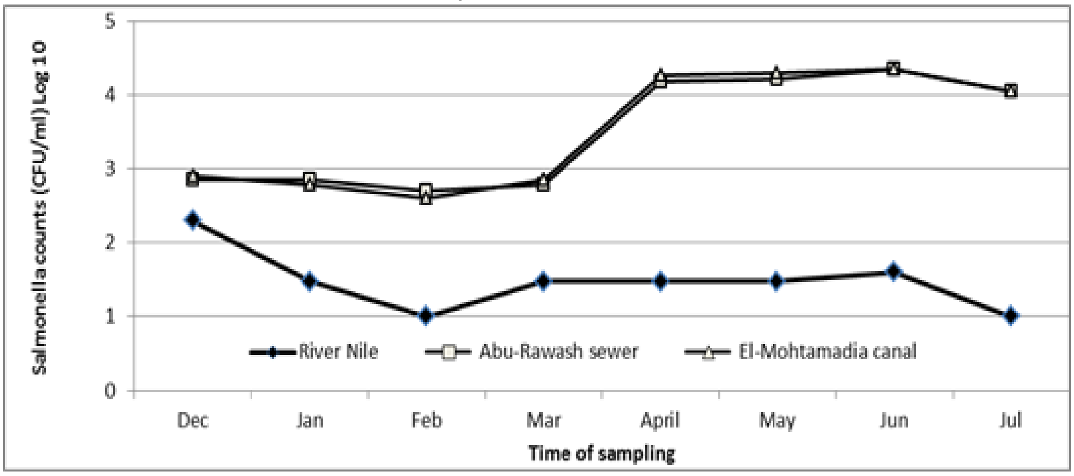

Fig 5: Seasonal variations in *Salmonella* counts (CFU/ml) as pathogenic bacteria at Abu-Rawash sewer and El-Mohtamadia canal (Zenen sewer plant) during the period from December 2011 to July 2012 compared to river Nile.

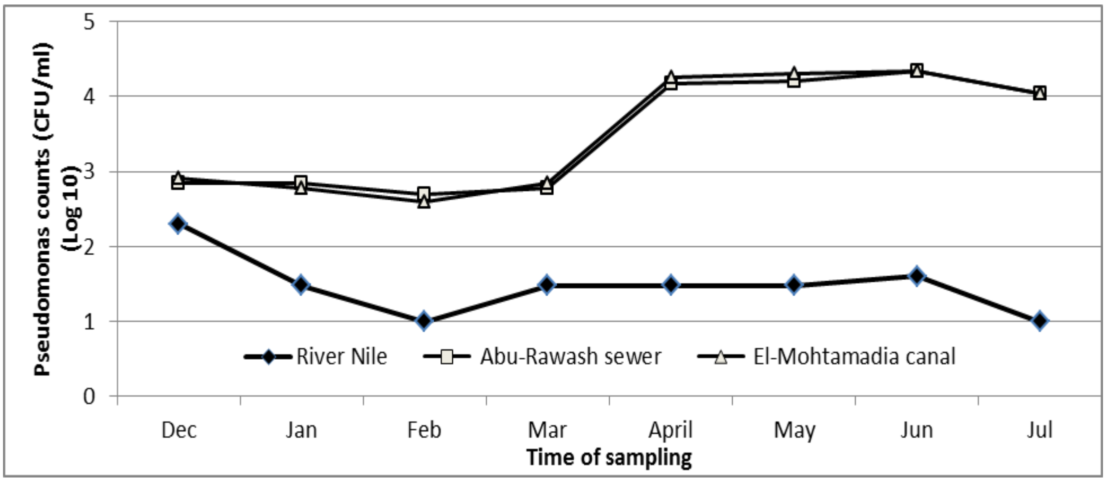

Fig 6: Seasonal variations in *Pseudomonas* counts (CFU/ml) as new pathogenic indicator at Abu-Rawash sewer and El-Mohtamadia canal (Zenen sewer plant) during the period from December 2011 to July 2012 compared to river Nile.

DISCUSSION

Despite being in the decade of biological credibility, several constraints are surrounding the notoriety of sustainable reuse of sewage effluent. Environmental and health worth is vital for reuse of sewage effluent 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 copious problems overlaying present situation (Saber 2001). Sustainable management of sewage farming would never be achieved farewell as agricultural practices persist to overstress the level of biological and/or chemical contaminants that jeopardizes both prosperous farming and sustainability and necessitates evaluating their biological characteristics to set up a new farming system devoted to following environmental and sustainable approaches.

Definitely, densely populated areas with inadequate sanitation and sewerage facilities function as a major source of pollution for low quality water supplies (UNEP 1991 and Jagals et al., 1995). Pollutants associated with sewage effluent reached many canals and drain as well a significant part of Nile itself, creating a transport mechanism to many downstream water users.

Historically, fecal coliforms in common and *E. coli* in distinct had been used as indicators for monitoring of sewage effluent (Clesceri, *et al*., 1998 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 sewage effluent. For this cause, the indicator microorganism notion was highly considered since some years 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. EPA recommended *Escherichia coli* as a responsive measure of fecal pollution. 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 (Tyagi *et al.,* 2006 and Bynum, 2011).

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 sewage effluent 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 treated sewage effluent. 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). 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 soils and low quality 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 all investigated sewage effluent samples as well as in Nile water. The recorded densities of the classical bacterial indicators in sewage effluent and Nile water, however, were high and surpassed the values advised by WHO, (1989) and Cabelli, (1983).

It seems reasonable that the potential transfer of enteric pathogens from sewage effluent 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 densities of new indicators of contamination (fecal Staphylococci and *Pseudomonas*) in sewage effluent 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 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). The presence of *Salmonellae* in sewage effluent could encourage the occurrence of epidemic outbreaks due to the multiple uses of surface water in agriculture, drinking water and food production.

Microbial indicators seem to be sensitive and detectable rather early in assessing sewage effluent quality in comparable to chemical and physical indicators (Karlen et al., 1999). The presence of a viable and diverse microbial community in sewage effluent irrigation water had been considered essential for sustainable farming (Kennedy and Smith, 1995 and Saber, 2012) as it acts as an early indicator of biological processes (Islam and Weil, 2000). As far as only a small portion of microbial biomass is cultivable, hence the microbial biomass was represented in the current work by the total counts of bacteria, and fungi.

It seems reasonable to state that safe use of sewage effluent in farming necessitates continuous evaluation of their biological, hygienic, chemical 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 sewage effluent characteristics, thereby providing an early sign of contamination. The contamination of sewage effluent with microorganisms such as pathogenic bacteria and viruses highlighted the most of rigorous monitoring (Darwesh et al., 2015 and WHO, 2003).

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REFERENCES

[1] APHA. 1998. Standard Methods for the Examination of Water and Wastewater 20th ed. New York, Inc.
[2] Atlas, R. 2005. Handbook media for environmental microbiology. CRC Press, Taylor & Francis Group.
[3] Bynum, J. 2011. Disease Causing Organisms in Your Food and Water Courtesy of EPA, FDA and USDA. (Available online with updates at http://www.thewatchers.us/myths/number-3-coliform.html).
[4] Cabelli, V. 1983. Health Effects Crieria for marine recreational water. United States Environmental Protection Agency Publication EPA-600/ 1-83-031.
[5] Chigbou, P.; Gordon, S. and Strange, T. 2005. Fecal coliform bacteria disappearance rates in a north-central Gulff of Mexico estuary. Estuar. Coast. Shelf Sci., 65:309-318.
[6] Clesceri, L.; Greenberg, A. and Eaton, A. (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed., p.9-32 and 9-75. American Public Health Association, Washington, D.C.
[7] Darwesh, O.M.; Moawad, H.; Barakat, O.S. and Abd El-Rahim, W.M. 2015. Bioremediation of Textile Reactive Blue Azo Dye Residues using Nanobiotechnology Approaches. Res. J. Pharm., Biol. Chem. Sci., 6(1): 1202-1211.
[8] De Victorica, J. and Galvan, M. 2001. *Pseudomonas aeruginosa* as an indicator of health risk in water for human consumption. Water Sci. Technol., 43:49-52.
[9] El-Abagy, M.; Shaban, A. and El-Taweel, G. 1999. Efficiency of water treatment systems in production of drinking water at Beni-Suif water treatment plant. Egypt. J. Appl. Sci., 14: 23-33.
[10] El-Taweel, G.E. and Shaban, A. M. 2001. Microbiological quality of drinking water at eight water treatment plants. International J. of Environ. Health Res., 11: 285-290.
[11] Housing and Building National Research Center. 2004. The Egyptian manual as guidelines for treated

waste water reuse in agriculture. Ministry of Housing & Utilities and New Communities, Cairo, Egypt.
[12] Islam, K. and Weil, R. 2000. Soil quality indicator properties in mid-Atlantic soils as influenced by conservation management. J. Soil and Water Cons., 55: 69-78.
[13] Jagals, P.; Grabow,W. and De Villiers J. 1995. Evaluation of indicators for assessment of human and animal's fecal pollution of surface runoff. Water Sci. Technol., 31:235-241.
[14] Kamel, M.; El-Taweel, G. and Shaban, A. 2006. Microbiological monitoring of El-Salam canal, Sinai, Egypt. J. Environmental Sciences, 31:109-127.
[15] Karlen, D.; Mausbach, M.; Doran, J.; Cline, R.; Harris, R., and Schuman, G. 1999. Soil quality: A concept, definition, and framework for evaluation. Soil Sci. Soc. Am. J., 61: 4-10.
[16] Kennedy, A. and Smith, K. 1995. Soil microbial diversity and the sustainability of agricultural soils. Plant Soil, 170: 75-86.
[17] Quinn, P., Carter, M.; Markey, B.; Donnelly, W. and Leonard, F. 2002. Antimicrobial agents. Great Britain by MPG, Books Ltd, Bodmin, Cornwall. UK.
[18] Roper, M. and Marshall, K. 1978. Effects of a clay mineral on microbial predation and parasitism on Escherichia coli. Microb. Ecol., 4: 279-289.
[19] Saber, M. 2001. Clean biotechnology for sustainable farming. Eng. Life Sci., 1(6): 217-223.
[20] Saber, M.; Turky, A. S.; Abd-El-Zaher F. H. and Abd-El-Mowla, D. M. 2012. Biological characterization of sandy soils irrigated with sewage effluent for extended periods. International Journal of Basic and Applied Sciences, 1(1): 68-76.
[21] Tyagi, V.; Chopra, A.; Kazmi, A. and Kumar, A. 2006. Alternative microbial indicators of fecal pollution: current perspective. Iran. J. Environ. Health Sci. Eng., 3: 205-216.
[22] UNEP. 1991. Water pollution control. United Nations Environmental Program, Nairobi, Kenya.
[23] WHO. 1989. Health guidelines for the use of wastewater in agriculture and aquaculture. Technical report series no. 778, WHO, Geneva.
[24] WHO. 2003. World Health Organization, heterotrophic plate counts and drinking-water safety: The significance of HPCs for water quality and human health. Bartram J., J. Cotruvo, M. Exner, C. Fricker and A. Glasmacher, WHO Geneva, Swedish IWA Publishing London, UK.