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## PHYSICO-CHEMICAL AND MICROBIAL PROFILE OF AGRICULTURE WASTEWATER SAMPLES FROM EL MARYOTIA CANAL IN EGYPT

Eman. S. Ahmed<sup>1</sup>; El-Tahan, M.H.<sup>1</sup>, Galal. M. Khalafall<sup>2</sup> and M.Z. Sedik<sup>2</sup>

<sup>1</sup>Research assistant & Senior Researcher RCFF- Agricultural Research Center Egypt

<sup>2</sup>Microbiology Department, Faculty of Agriculture, Cairo University, Egypt

### ABSTRACT

Physicochemical and microbial analyses were carried out on collected agriculture waste water samples at three different sites from the El-Maryotia canal at locations Shapramantarea, Fayesl, and Kerdasa in Giza governorate. Physicochemical parameters (Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), bicarbonates ( $\text{HCO}_3$ ) and heavy metals) total bacterial count, total fungi, total coliform counts, total faecal coliform counts, isolation of *Aeromonas hydrophila* (PCR), *Streptococcus* count, *Pseudomonas* count, *Enterococcus* count, total yeast count and enumeration of spore-forming bacteria of 36 agricultural wastewater samples were examined in different seasons.

**Keywords:** Agriculture wastewater, physicochemical parameters, BOD, COD, *Streptococcus*, *Pseudomonas*, and *Aeromonas*.

### Introduction

Water is the main source of life on our planet. Life, as we know, could not be evolved without water. Due to its vital importance, water is required in a pure form as plants, animals and humans cannot survive if it is loaded with high metal concentration, hazard chemicals or pathogenic microorganisms (Frick *et al.*, 1999). Globally, 2.1 billion people are deprived of access to clean water and about 4.5 billion have no access to adequate sanitation (Ranade and Bhandari, 2014).

A recent UN report indicates that by 2025, two-thirds of the population of the world could face water stress. The scarcity of water could be in the form of physical scarcity, where water availability is limited and demands are not met, or it could be in the form of economic scarcity, where although water is available, there are no means infrastructure to provide water of required quantity and quality (WHO and UNICEF, 2017).

The world is faced with problems related to the management of wastewater. In the last few decades, anthropogenic activities coupled with rapid urbanization and industrialization have brought about ecological pressure on the aquatic environment which directly or indirectly affects human health (Bhat *et al.*, 2018). Wastewater use is increasingly seen as an option to meet these growing needs for water. The agricultural sector is currently the largest user of water and wastewater globally, accounting for approximately 70% of water use on average (Winpenny *et al.*, 2010).

Agriculture, which accounts for 70 percent of water abstractions worldwide, plays a major role in water pollution. Farms discharge large quantities of agrochemicals, organic matter, drug residues, sediments, and saline drainage into water bodies. The resultant water pollution poses

demonstrated risks to aquatic ecosystems, human health and productive activities (UNEP, 2016). Agricultural drainage includes artificial subsurface drainage and surface drainage. Agricultural wastewater reuse can be classified into direct and indirect wastewater reuses (Rutkowski *et al.*, 2007).

The presence of high concentrations of these pollutants above the critical values stipulated by national and international regulatory bodies is considered unacceptable in receiving water bodies. This is because, apart from causing a major drawback in wastewater treatment systems, they also lead to eutrophication and various health impacts in humans and animals (EPA, 2000).

Heavy metals are one of the most persistent pollutants in wastewater. Unlike organic pollutants, they cannot be degraded, but accumulate throughout the food chain, producing potential human health risks and ecological disturbances. Their presence in wastewater is due to discharges from residential dwellings, groundwater infiltration, and industrial discharges. The accumulation of these metals in wastewater depends on many local factors, such as the type of industries in the region, way of life and awareness of the impact on the environment through the careless disposal of wastes (Silvia *et al.*, 2006).

Methods of wastewater treatment were first developed in response to the adverse conditions caused by the discharge of wastewater to the environment and the concern for public health. Further, as cities became larger, limited land was available for wastewater treatment and disposal, principally by irrigation and intermittent filtration (Wang and Chen, 2009).

A wide variety of active and inactive organisms have been employed as bio-sorbents to sequester heavy metal ions from aqueous solutions. It has been found that bio-sorbents are rich in organic ligands or the functional groups, which

play a dominant role in the removal of various heavy metal contaminants. The important functional groups are carboxyl, hydroxyl, sulfate, phosphate, and amine groups.

The concentrations of heavy metal in the water column depending on some physical and chemical factors like salinity, temperature, dissolved oxygen; pH, redox potential, conductivity and ionic strength (Goksu, 2003). These physicochemical parameters have been informed to affect biochemical reactions within water systems (Gulson *et al.*, 1997).

So that the present study the physicochemical and microbial analyses of collected agriculture wastewater samples at three different sites from the El-Maryotia canal at locations Shapramant area, Fayesl, and Kerdasa in Giza governorate.

## Material and Methods

### Study sites

The current study was carried out by collecting the wastewater agriculture samples at three different sites. Samples from the El-Maryotia canal were obtained at depth (100-199 cm) at locations Shapramant area, Fayesl, and Kerdasa in the Giza governorate, Egypt. The samples were periodically collected monthly intervals in April, August, October and December (2018). Special care was taken to obtain fair samples. Wastewater agriculture samples were collected seasonally during this period from the investigated three areas three samples.

### Physicochemical parameters of agricultural wastewater

Temperature, pH, BOD, COD, and  $\text{HCO}_3^-$  were determined according to the methods described by APHA, (1995 and 1998), and Baruah and Barthakur (1997).

### Heavy Metals

Heavy metals (Iron ( $\text{Fe}^{+3}$ ), Copper ( $\text{Cu}^{+2}$ ), Chromium ( $\text{Cr}^{+6}$ ), zinc ( $\text{Zn}^{+2}$ ), Cobalt ( $\text{Co}^{+2}$ ), Magnesium ( $\text{Mg}^{+2}$ ) and Cadmium ( $\text{Cd}^{+2}$ )) were determined using atomic absorption spectrophotometer type Buck scientific accuses 214/215 according to Wirsén and Jannasch (1976).

### Microbiological analysis of wastewater agriculture samples

#### (i) Total bacterial and total fungi counts

Asa method described by Onajite *et al.* (2018) one ml after dilution ( $10^{-4}$ ) of each collected agricultural wastewater samples were inoculated into nutrient agar media for bacteria and Potato Dextrose Agar (PDA) media for fungi. The pour plate method was used for inoculation. The inoculated bacterial plates were incubated at  $37^\circ\text{C}$  for 48 hrs. and fungi plates were incubated at  $28^\circ\text{C}$  for 72 hrs.

#### (ii) Total coliform counts and total faecal coliform counts

The coliform counts were determined by the most probable number (MPN) technique (APHA, 1995) while, total and faecal coliform groups were determined by multi-tube fermentation methods (UNICEF, 2002). Presumptive test analysis was done using MacConkey broth to enumerate total coliforms while confirmatory test analysis of the samples was done using brilliant green broth to enumerate fecal coliforms.

#### (iii) *Streptococcus* count

For faecal streptococci count, serial dilutions of water samples were made from  $10^{-1}$  to  $10^{-3}$ . Decimal volumes of 1ml and 0.1ml of each dilution were aseptically transferred to 10ml of sterile Azide dextrose tubes and incubated at  $35^\circ\text{C}$ . Tubes were examined for turbidity between 24 to 48 hours (Raji *et al.*, 2015).

#### (iv) *Pseudomonas* count

*Pseudomonas* spp. was estimated using appropriate dilutions of analyzed samples ( $10^{-1}$ – $10^{-3}$ ) for agricultural wastewater filtered in duplicate through  $0.45\text{-}\mu\text{m}$  cellulose-acetate filters (Millipore, USA) and then placed on a *Pseudomonas* Isolation Agar (PIA, BD Diagnostic Systems, USA) and incubated at  $37^\circ\text{C}$ . Typical *Pseudomonas* colonies appearing blue-green on PIA agar plates were enumerated as total *Pseudomonas* counts (TPC) (Luczkiewicz *et al.*, 2015).

#### (v) *Enterococcus* count

The count was performed by inoculating tubes containing 9.5 ml Tryptic Soy Broth (TSB) with 0.5 mL of the samples and incubated for 18 h at  $37^\circ\text{C}$ . Tubes showing bacterial growths from each site were streaked onto selective agar plates (Bile AesculinAzide Agar). The plates were incubated at  $37^\circ\text{C}$  for 24 h (Teixeira *et al.*, 2007).

#### (vi) Total yeast count

The membrane filter technique was employed for yeast and mold count. A volume of 100 ml of the samples was filtered through membranes of  $0.45\ \mu\text{m}$  pores (Millipore, Massachusetts, USA). The membranes were placed on Dichloran Rose Bengal Chloramphenicol (DRBC) Agar. The plates were incubated at  $25^\circ\text{C}$  for 5 to 7 days (Samah *et al.*, 2014).

#### (vii) Enumeration of spore-forming bacteria

The procedure of (ISO, 2002) weight 10 ml of water into a sterile blender jar and blend with 90 ml sterilized peptone (0.1%) pipet 10 ml to each of two large 22mm diameter tubes making sure sediment is transferred also. Place the thermometer in one of the tubes and cap the other loosely. Heat the tubes in the  $82\text{-}83^\circ\text{C}$  water bath with agitation, when the tube with thermometer reaches  $80^\circ\text{C}$ , hold both tubes for 20 minutes at that temperature. Serial dilutions from  $10^{-1}$  to  $10^{-9}$  were plated on plate count agar (PCA oxid CM 325) and incubated at  $30^\circ\text{C}$  for 3 days.

#### (viii) Isolation of *Aeromonas hydrophila*

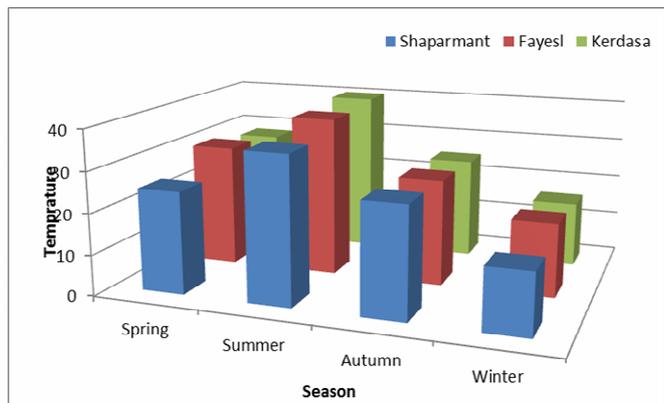
Species of *Aeromonas* was identification by following Aero-key as proposed by Carnahan *et al.* (1991) with modifications suggested by Agarwal (1997).

## Results and Discussion

### Physicochemical parameters of agricultural wastewater

Each water body has an individual pattern of physical and chemical characteristics which are determined largely by the climatic, geomorphologic, and geochemical conditions prevailing in the drainage basin and the underlying aquifer. The results of the physicochemical qualities of the wastewater samples (water temperature, pH, BOD, COD, and  $\text{HCO}_3^-$ ) of the selected sites were detected as there are important parameters that affect microbial populations.

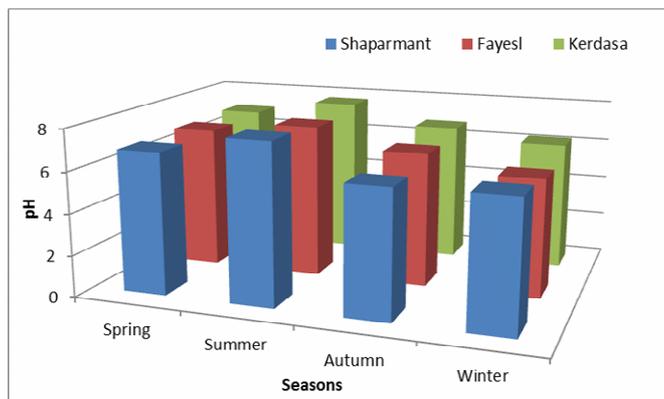
The temperature profile generally varies ranged between 15 and 40°C at all sampling points from different locations. The lowest temperature was recorded in samples 1, 2, 3 from Shapramant in winter and the highest temperatures were recorded in samples 7, 8, and 9 from Kerdasa in summer (Figure 1).



**Fig. 1:** Seasonal variation in Temperature values of the wastewater agriculture samples at three different sites in Giza Governorate.

The temperature of the effluents may pose a threat to the aqua-based organisms, which is not in conformity with the report of Igbino and Okoh (2009) but was similar to the reports of Singh *et al.* (2012). In wastewater, the temperature is basically important for its effect on other physical and chemical factors, and the rate of some reactions could be increased by the discharge of this wastewater into streams. So wastewater can reduce the solubility of oxygen and amplified odor due to anaerobic reaction (Akan *et al.*, 2009).

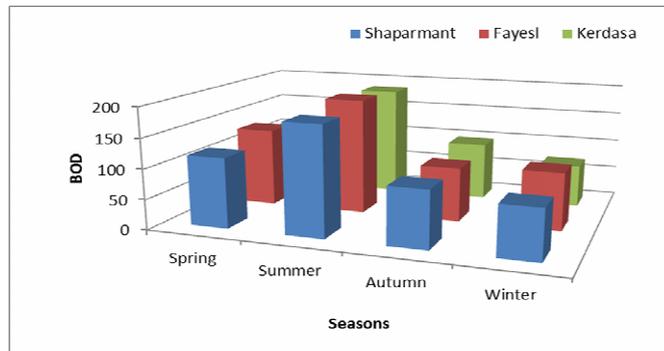
The pH of the water is known to affect the availability of micronutrients as well as trace and heavy metals. The lowest pH value 5.5 was recorded in sample 6 from Fayeel in winter while the highest pH value of 8.1 was recorded in sample 3 from Shapramant in summer (Figure 2). The pH level of water defines its utility for a different purpose. It has been established that pH is a vital characteristic in assessing the acid-base level of water. Low or high pH has a toxic effect on aquatic life and alters the solubility of other chemical pollutants as well as other important elements in surface water.



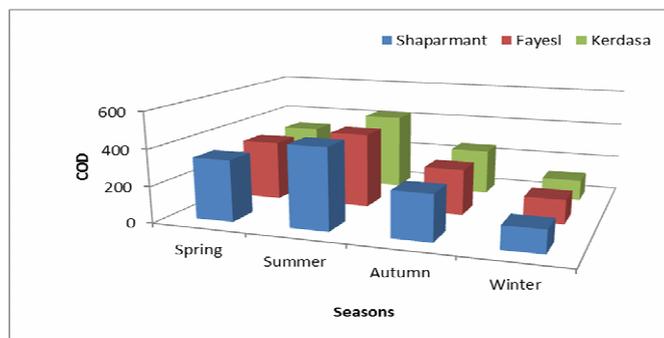
**Fig. 2:** Seasonal variation in pH values of the wastewater agriculture samples at three different sites in Giza Governorate.

This may lead to adverse effects on those that depend on it for various uses and also the ecosystem. The South African recommended limit for pH in the water for domestic use is 6 to 9 (DWAF and WRC, 2009).

The BOD profiles throughout the sampling period generally ranged from 74-196 mg/L (Figure 3), while the COD ranged from 111-450 mg/L (Figure 4). High levels of BOD can be traced to the heavy discharge of industrial effluents, domestic sewage, crops, and animal waste (Fatoki *et al.*, 2003). Anderson and Grether (2010) reported that High levels of COD in water may point to poor water standards caused by municipal or farmed effluent discharges.

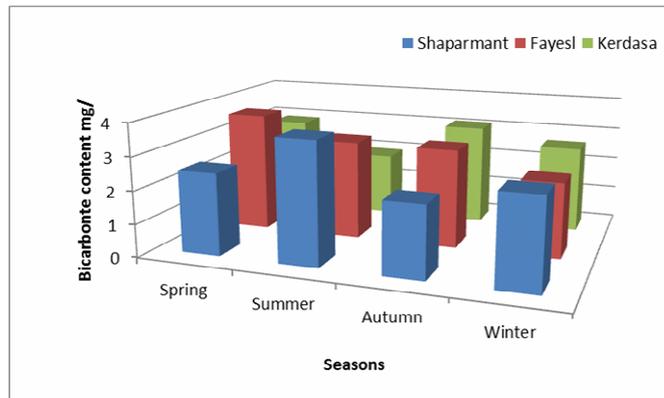


**Fig. 3 :** Seasonal variation in BOD values of the wastewater agriculture samples at three different sites Giza Governorate.



**Fig. 4:** Seasonal variation in COD values of the wastewater agriculture samples at three different sites Giza Governorate.

Bicarbonate ( $\text{HCO}_3^-$ ) lowest value 1.94 mg/l was detected in samples 3 and 6 from Shapramant and Fayeel, respectively in spring and in samples 7 and 9 from Kerdasa at summer, while, the highest value 332.45 mg/l was detected in sample 7 from Kerdasa in autumn (Figure 5).



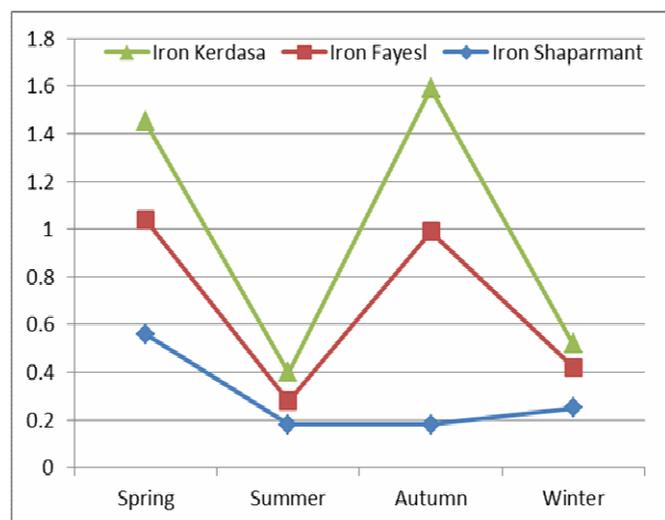
**Fig. 5 :** Seasonal variation in bicarbonate values of the wastewater agriculture samples at three different sites Giza Governorate.

**Heavy metals**

The water samples were analyzed for their total content of heavy metals, it is well known that a long-time exposure of water and sediment to heavy metals can produce considerable modification of their microbial populations, reducing their activity and their number (Doelman *et al.*,

1994). Continuous use of such water for irrigation over a longer period may cause accumulation of Cd, and Pb, up to toxic levels for plant and animal health (Adhikari *et al.* 1998).

Iron concentration varied between 1.1 ppm in sample 4 at autumn and 0.03 ppm in samples 8 at winter (Figure 6), zinc is an essential element for living organisms as it is crucial in protein metabolism since it is required for the correct functioning of many proteases (Vallee and Falchuk, 1993).



**Fig. 6 :** Seasonal variation in the Iron concentration of the wastewater agriculture samples at three different sites Giza Governorate.

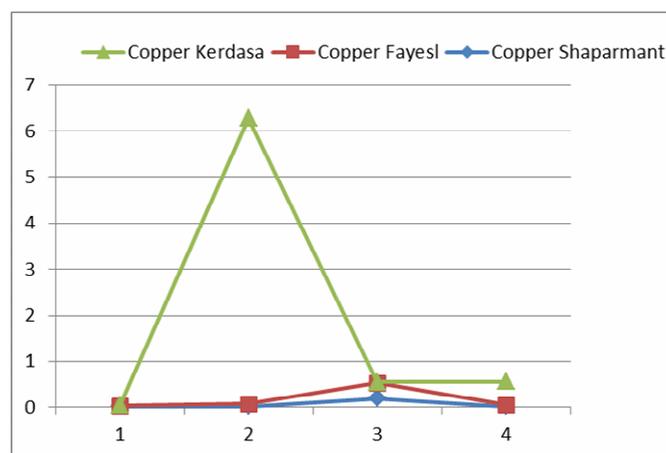
Batayneh (2010) stated that all Zn concentrations of the water samples were lower than the MPCL recommended by JISM (2008) (4.0 mg/L) for drinking water. The highest value of zinc 42.52 ppm was recorded in sample 2 at spring while the lowest value 0.001ppm in sample 2 in winter (Figure 7).



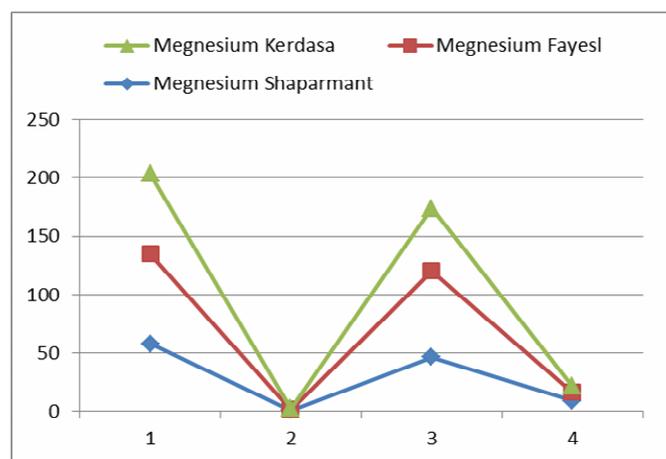
**Fig. 7 :** Seasonal variation in Zinc concentration of the wastewater agriculture samples at three different sites Giza Governorate.

Copper is a cofactor in numerous enzymatic processes and represents the third most abundant transition metal found in living organisms (Brandolini *et al.*, 2002). Copper highest value 0.42 ppm was recorded in sample 6 at autumn while copper lowest value 0.010 was recorded in sample 6,7,8 and 9 at spring (Figure 8), in addition, to sample 3 at summer and sample 1 in winter, cadmium (Cd) is one of the most toxic heavy metals and is considered non-essential for living

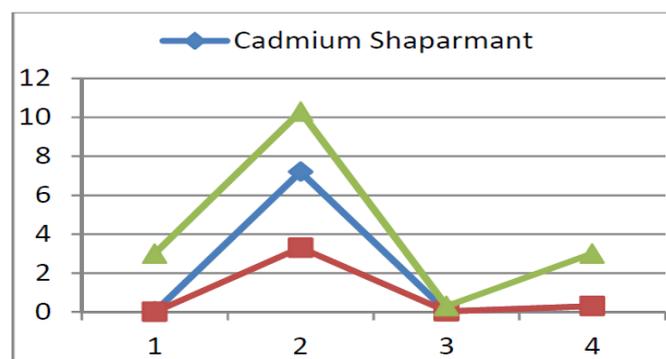
organisms (Woodbury, 1998), magnesium highest value 90.3 ppm was recorded in sample 6 in autumn and the lowest value 0.688 ppm was recorded in sample 5 at summer (Figure 9). Cadmium concentration showed variation during different season and samples the highest value 12.74 ppm was recorded in sample 9 at summer while, the lowest value 0.001 ppm was recorded in sample 3 at autumn (Figure 10), chromium highest value 12.24 ppm was recorded in sample 9 in winter and the lowest value 0.87 ppm was recorded in sample 2 at autumn (Figure 11) and finally, cobalt concentration varied between 1.41 ppm in sample 5 in summer and 0.10 in sample 5 in autumn (Figure 12).



**Fig. 8:** Seasonal variation in Copper concentration of the wastewater agriculture samples at three different sites Giza Governorate.



**Fig. 9:** Seasonal variation in Magnesium concentration of the wastewater agriculture samples at three different sites Giza Governorate.



**Fig. 10 :** Seasonal variation in Cadmium concentration of the wastewater agriculture samples at three different sites in Giza Governorate.

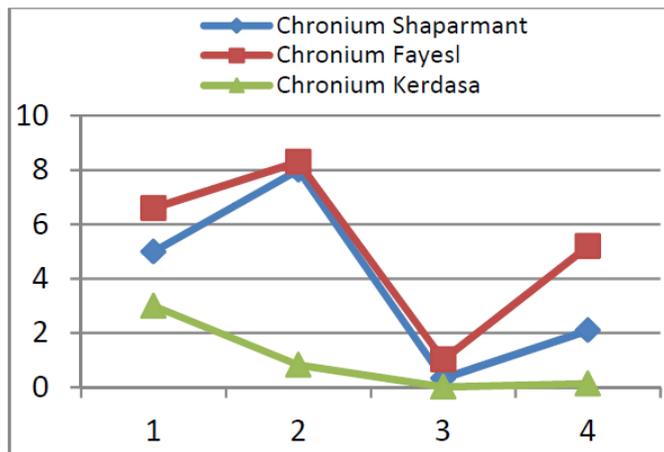


Fig. 11 : Seasonal variation in Chromium concentration of the wastewater agriculture samples at three different sites in Giza Governorate.

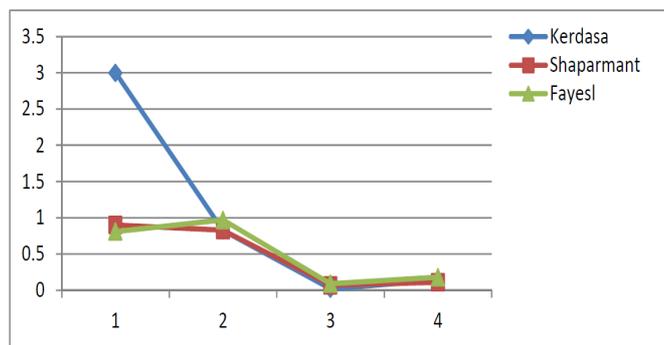


Fig. 12 : Seasonal variation in Cobalt concentration of the wastewater agriculture samples at three different sites in Giza Governorate.

**Microbiological analysis of wastewater agriculture samples**

Total bacterial count highest value  $8.0 \times 10^5$  (cfu/ml) was recorded in samples 4 and 5 in summer and sample 8 in spring while the lowest value  $1.8 \times 10^3$  (cfu/ml) was detected in sample 1 in spring (Figure 13).

Therefore, the total count is no longer used as an indicator for recreational waters as they are widespread in nature, but are still used to assess drinking water quality (USEPA, 2017). Ali *et al.* (2008) reported that the log total bacterial counts at 22°C and 37 °C in the Nile at El-Giza region reached 5.8 and 5.5 CFU/100 ml, respectively and Rifaat (2008) found that the log total bacterial count of Nile water at Greater Cairo was 5 CFU /100 ml.

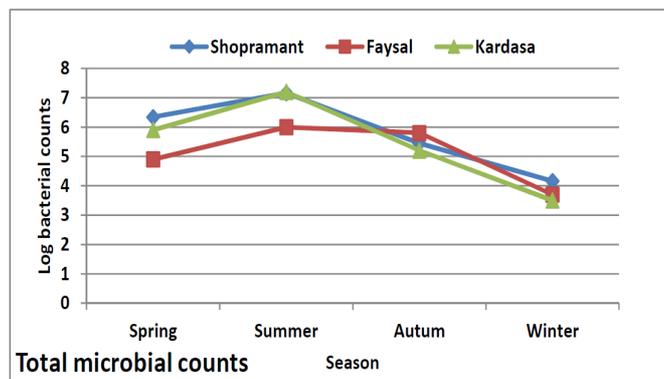


Fig. 13: Seasonal variation in Total bacterial count of the wastewater agriculture samples at three different sites (Shapramant area, Fayesl, and Kerdasa).

Webster *et al.* (2004) demonstrated the occurrence of total coliforms, faecal coliforms, heterotrophic bacteria, *Aeromonas* and *Pseudomonas* in water samples analyzed which indicated the incidence of water contamination as some of these species are indicators of faecal contamination. These organisms may harbor potential pathogens and the presence of pathogenic organisms that can pose severe health risks to consumers in general and immune-compromised individuals in particular.

Also, Osman *et al.* (2011) reported that the highest average log numbers in Nile water were found in the El-Giza region being 4.8, 3.1 and 2.5 MPN-index/100ml for total Coliform, faecal coliform, and fecal streptococci, respectively. In contrast, the lowest average log numbers of bacterial indicators were recorded in the Embaba region being 2.2, 1.1 and 1.1 MPNindex/100ml for total Coliform, faecal Coliform, and fecal streptococci, respectively.

The highest value  $6.1 \times 10^6$  (cfu/ml) of the total coliform count was detected in sample 9 in summer high total coliform count may be indicative of the presence of high organic compounds in the water, while the lowest value  $3 \times 10^3$  (cfu/ml) was recorded in sample 3 in autumn (Figure 14).

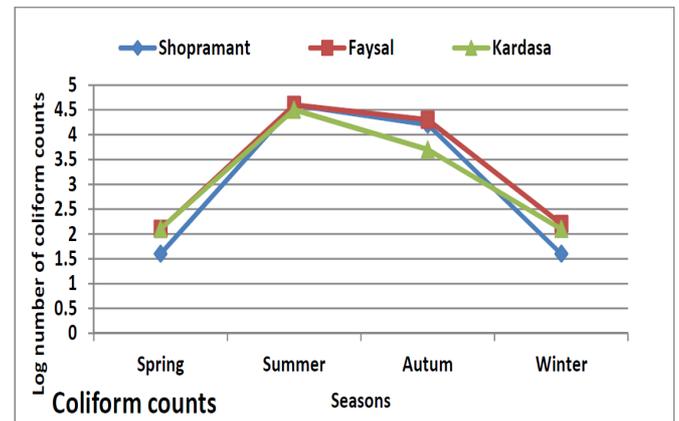
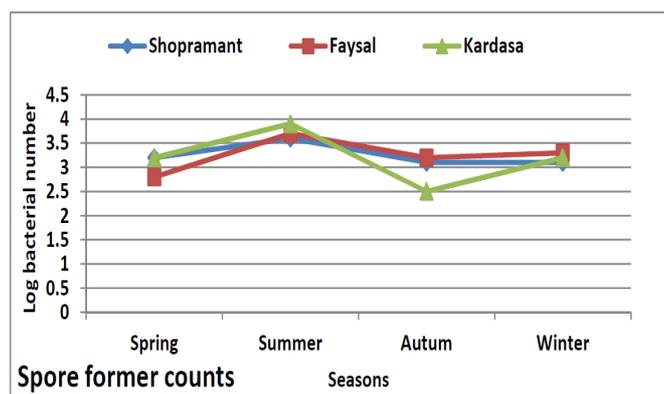


Fig. 14 : Seasonal variation in Total coliform count of the wastewater agriculture samples at three different sites (Shapramant area, Fayesl, and Kerdasa).

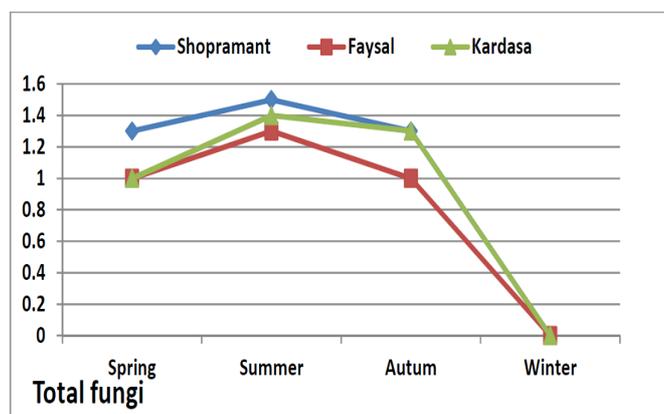
Total coliforms were considered as bacterial water quality indicators to assess fecal contamination in recreational waters in the USA, as required by the Beaches Environmental Assessment and Coastal Health Act (U.S. Congress, 2000), to reduce health risks. However, it was reported that some members of the coliform group live in the environment (*i.e.*, outside of the gastrointestinal tract), which may show a false indication for fecal contamination in water. These results were in accordance with those obtained by Ali *et al.* (2008) they found that the log counts of total Coliform, faecal Coliform, and faecal *Streptococci* were 4.1, 2.3 and 2.5 MPN index/100 ml, respectively, in Nile water samples at El Giza Drinking Water Treatment Plant.

Total spore-forming bacteria varied between  $4.0 \times 10^5$  (cfu/ml) in sample 4 in summer and  $1.3 \times 10^3$  (cfu/ml) in sample 9 in autumn (Figure 15) Nieminski *et al.* (2000), in a survey of 24 utilities, observed total aerobic spore concentrations of greater than 2 CFU/ ml in 75% of the waters. Rice *et al.* (1996) reported similar findings in a study of four utilities treating surface waters.



**Fig. 15:** Seasonal variation in Spore-forming bacteria of the wastewater agriculture samples at three different sites (Shapramant area, Fayesl, and Kerdasa).

Total fungal count ND in all summer samples and the highest value  $6.0 \times 10^2$  was detected in sample 2 in spring (Figure 16). Mohamed *et al.* (2014) reported that average counts of total fungi in studied groundwater samples ranged from 4 to 119 cfu/100 ml, Egyptian standards for drinking water have no limit for the total fungal count.



**Fig. 16 :** Seasonal variation in Total fungal count of the wastewater agriculture samples at three different sites (Shapramant area, Fayesl, and Kerdasa).

The highest value of fecal coliform was  $4.3 \times 10^4$  (cfu/ml) in sample 5 in winter and ND in most samples of autumn. Hagedorn and Liang (2011) indicated serious faecal contamination of Tiaoxi River and reported higher levels ( $2.54 \log^{10}$  CFU/mL) of *E. coli* for the water samples collected near Fengkou drinking water station. Only some faecal coliforms are pathogenic and a previous study showed that FC presence does not always correlate with pathogen presence (Schriewer *et al.*, 2010).

Total yeast count highest value  $4.8 \times 10^5$  (cfu/ml) was detected in sample 1 and 4 in summer and the lowest value was  $10^3$  (cfu/ml) in all winter samples, Mohamed *et al.* (2014) reported that average counts of total yeasts ranged from 3 to 211 cfu/100 ml.

*Streptococcus* count was not detectable in most samples of spring and the highest value  $40 \times 10$  (cfu/ml) was recorded in sample 8.

Fecal streptococci were detected more often than either thermotolerant coliforms or *E. coli* (Krapac *et al.*, 2002). Geldreich (1996) suggested that fecal streptococci bacteria are more numerous in faecal material than the other bacteria and more resilient in non-enteric environments, which may have accounted for these bacteria being more often detected

and at a larger concentration in groundwater samples than thermo-tolerant coliforms.

*Enterococcus* count ND in all spring, summer, and autumn samples while it was detected in winter and the highest value  $8 \times 10$  (cfu/ml) was detected in sample 5. *Enterococcus* spp, total coliforms, thermotolerant coliforms, and *E. coli* are bacteria whose presence indicates that the water may be contaminated by human or animal wastes (ICMSF, 1998).

*Pseudomonas* count was ND in most samples in spring and in all samples of autumn and was recorded  $10^3$  in winter. *Pseudomonas* are opportunistic Gram-negative pathogens, naturally, occur in the aquatic environment and as a part of the normal gut flora of healthy fish; it causes an outbreak when the optimum environmental conditions change. *Pseudomonas* species have been described as etiological agents of diseases in fish in Egypt (El-Nagar, 2010). Bacterial indicators such as *Pseudomonas aeruginosa* were detected once to three times only during the year (Shabaan *et al.*, 2016).

#### Isolation, identification, and classification of *A. hydrophila*

*Aeromonas hydrophila* was positive and present in all 36 samples of spring, summer, autumn, and winter. Abdel-Gawad, and Abdel-Rahman (2004) mentioned that *A. hydrophila* is a Gram-negative, rod-shaped, facultative anaerobic bacterium. It had been reported in many countries in the world and isolated from a wide range of mammals, surfaces water and sewage. Yogananth *et al.* (2009) demonstrated that the detection of virulence factors of *Aeromonas hydrophila* was a key component in determining potential pathogenicity because these factors act multifunctionally and multifactorial. Also, Shabaan *et al.* (2016) recorded that *Aeromonas hydrophila* have been recovered in abundance from lakes particularly in warm temperatures.

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