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DISTRIBUTION OF ENTERIC PROTOZOA IN THE RIVER NILE DRAINS: EGYPT USING FIELD AND REMOTE SENSING STUDIES

By
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Abstract
Polluted water is a massive international issue that needs to be monitored and evaluated at all conditions for water management policy. This study identified enteric protozoa in three drains; as well as the physicochemical characteristics, heavy metals of such freshwater bodies in seasonal biotic status in Abu Youssef, El-Rahaway, and El-Qalyubia in the River Nile, Egypt. Also, the spatial distribution of the pathogenic protozoa along the three drains was investigated using remote sensing (RS) and the geographic information system (GIS). Applying these approaches gave a useful epidemiological of protozoa in drains. The results showed *Entamoeba histolytica* and *Giardia intestinalis* in the three drains. Their survival depended on oxygen demand (COD) concentration, organic matters and coliform bacteria existence despite the heavy metal pollution. This study gave valuable information within the GIS framework to accurately quantify the spatial distribution of aquatic biota and water chemistry and thus determined the risks to water bodies and proposing the proper mitigation measure to save such vital resource.
Keywords: Egypt, Pathogenic protozoa; Pollution; Water quality; RS/GIS; Nile Delta.

Introduction

The Nile River is the Egyptian lifeline and offers a sufficient source of fresh water for almost all uses concerning drinking and irrigation (Ali *et al,* 2014). For several decades, the water quality of the Nile River has steadily deteriorated due to the dumping of untreated effluents and anthropogenic contaminants that related to the randomly urban expansion over the fertile soil of the Nile Delta and very close to the running canals and drains (Abdel-Satar, 2005). The Aswan Dam construction resulted in major changes in physical, chemical and biological properties of the downstream soil and water (Abd El-Hady, 2014).

The shortage of effective sewage facilities and coverage combined with water contamination induced by the degradation of old water networks is one of the most significant environmental issues. Besides, various problems in sewage system construction, design, and maintenance resulted in communicable and non-communicable diseases (Anwar, 2003). Also, the pollution of heavy metal in fresh water resources is one of the most serious problems. Probable causes included municipal waste-water treatment systems, industrial, residential effluent and community runoff (Badr *et al,* 2006; Helmy, 2015).

Contaminated water, weak sanitation and hygiene classified as the third leading risk factor in developing countries, including Egypt. Water-borne diseases are responsible for more than two million deaths and four billion cases of diarrhea a year. Pathogenic diarrhea is responsible for the highest risk of disease and death, as well as the most severely affected groups are children under the age of five (WHO, 2000).

Geographical Information Systems (GIS) and satellite image data can provide good data for the identification and management of both human and animal disease outbreaks. Satellite imagery can be used to monitor many environmental variables such as temperature, precipitation, humidity, wind speed and direction, etc. that affect the behavior of pathogens, vectors and their interactions with human and animal hosts (Singh *et al,* 2015). GIS data analysis can assist in many ways, such as the detection and dissemination of diseases over time, at-risk population groups, disease outbreak trends, healthcare facilities and program response preparation, and disease outbreak evaluation. Satellite monitoring has been used in many studies for various water and vector-borne diseases such as; diarrhea, cholera, typhoid, leptospirosis, Rift Valley Fever, foot and mouth disease, bluetongue, West Nile Virus disease, etc. GIS and remote sensing data analysis have been shown to be effective tools for disease detection, prediction of outbreaks and monitoring of control programs (Singh *et al,* 2015).

The water consistency was determined by the body's physical, chemical and microbiological properties. High heterogeneity is typical of the water quality properties on the planet. The productivity of the natural water sources used for different purposes should be measured in terms of the basic water quality parameters, which have the greatest effect on the potential water usage (Gómez-Gutiérrez *et al,* 2016). Heavy metals are essential elements of the surface of the Earth and cannot be depleted or damaged (Barakat, 2016). Some heavy metals are essential as trace elements to maintain the metabolism of the human body (e.g., copper, selenium, zinc), which caused toxicity at higher concentrations (El-Kowrany *et al,* 2015).

The study aimed to detect water contamination by protozoa using the integrated studies of remote sensing and GIS, as well as the physicochemical properties and heavy metals in three selected drains in the River Nile, Egypt.

Materials and Methods

Study Area: Three drains were Abu Youssef (Menoufia Governorate), El-Rahaway (Giza Governorate) and El-Qalyubia (Qalyubia Governorate), with different ecological, and environmental conditions and human behaviors (Figs. 1 & 2).

Remote sensing analysis: Two exploratory supervised classifications maps using a K-means clustering algorism (with two classes; urban and vegetation) were generated using optical bands (OLI-7 bands) of the Landsat-8 satellite data from March 2013 and April 2020 to map the changes in the urban expansion that might cause much pollution stresses on the drains (Fig. 3). Two Landsat 8 optical images were freely downloaded from website (https://earthexplorer.usgs.gov/).

Sampling: Field studies were done monthly for 18 months from March 2018 to August 2019 and expanded to 2020. The first six months passed as screening survey. In next 12 months, water samples (plastic jerry cans 10L) were monthly collected for physicochemical, heavy metals, bacteria and protozoa to evaluate spatial pattern abundance by using ArcGIS software. This was done in the three drains transported immediately to the laboratory for examination.

Protozoa were identified and counted by electron microscope and molecular biology. Then the cysts were separated from filter paper by dipping each filter in 10 ml of distilled water in a small flask and stirring gently by hand. The filter was then removed, and the solvent was ready for the next phase. Cysts were grown by cultivation in a glass tube with rice and agar dissolved in phosphate-buffered saline (PBS) 2% (autoclaved) to duplicate numbers of cysts, then cysts series transferred from 2-4 times. Using glutaraldehyde- osmium fixation, dehydrated in ethanol, cells can be fixed and embedded in Epon (Daniel *et al,* 1980).

DNA extraction and PCR amplification: Samples were strained using a 0.45µm sieve (Sartorius) and examined using a light microscope. Positive samples for *Entamoeba histolytica* and *Giardia intestinalis* cysts were determined using the PCR method. The genomic DNA was collected using liquid nitrogen to lyse the cyst wall during the freezing and thawing periods. The extraction was carried out after overnight incubation, using a DNA purification kit (QIAGEN, Germany). Depending on a small subunit rRNA specific to *E. histolytica* and *G. intestinalis* cysts, PCR primers were formulated using Tag Polymerase. In the thermocycler, the reaction was intensified by the following sequence: initial denaturation at 94°C for 3 min., 35 cycles at 94°C for 30sec, 43.5°C for 30sec and 30sec for 72°C with final extension at 72°C for 5 minutes. PCR of 185 & 1950 base pairs was given by forward and reverse primer of 0.2µm (Tab. 1).

Small subunit rRNA (SSRR) gene: Sequencing for *E. histolytica* and *G. intestinalis* isolates were performed in Clinilab, Egypt, using the originally developed dideoxyribonucleoside chain termination technique (Gupta and Verma, 2019). The electrophoresis of the cycle sequencing reaction product automatically measured the nucleotide sequences. The fluorometric scans were generated from which the sequence was assembled using the sequence analysis program (Wang *et al,* 2018).

Genetic diversity and analysis: DNA nucleotide sequences were found in the NCBI database using Simple Local Alignment Search Tool (blast). Multiple sequence alignments were made using DNAMAN software (Madison, Wisconsin, USA, version 5.2.9 & Crustal version 1.74) after Mendlovic *et al.* (2004). The nucleotide distances were calculated on the basis of alignment differences using the Juckes and Cantor technique (Tuffley *et al*, 2012) to fix superimposed substitutions with the Molecular Evolutionary Genetics Analysis (MEGA) software (version 6.0). Using the unweighing pair Group Method with Arithmetic Mean (UPGMA) by MEGA 6.0 software, phylogenetic relationships between recognized pathogens were evaluated and boot strap analysis (1000 replicates) was performed to measure the validity of the developed phylogenetic tree. Lastly, the nucleotide sequence data identified was submitted to the GenBank Database of the National Center for Bio-technology Information (NCBI), USA, and their accessories (Sayers *et al,* 2020).

Nucleotide sequence accession number: Small subunit rRNA (SSRR) gene sequences were deposited in database of National Center for Biotechnology Information (NCBI) (https:/www.ncbi.nlm.nih. gov/) with accession numbers MK 329244.1 & MK332025.1 for *E. histolytica* strain A1 &*G. intestinalis* strain A2, respectively.

Water quality determination: To find relationship between protozoa abundance, diversity and water quality of the drains; physical (pH, EC, TSS, TDS, Ca^{+2}, & Mg^{+2}), chemical (COD) and microbiological properties of water were determined. Heavy metals were determined by the Inductive Coupled Argon Plasma Optical Emission Spectrophotometric (ICP-OES). The analytic was determined by its emission intensity as the sample is aspirated into an Inductively Coupled Plasma (ICP) torch. The concentrations were directly proportional to the emission intensities. Finally, microbiological (Total and Fecal coliform) were performed. Samples were diluted and filtered in a sterile membrane filter under partial vacuum Petri dishes of m-Endo agar LES were used for total coliform each membrane filter was placed in a dish and all dishes were incubated at 35°C for 24h. But, mFC medium was used for fecal coliform, Petri dishes submerged in 24-hour water bath at 44.5°C. The standard coliform colony was pink to dark red with metallic surface sheen (total coliform) were counted and calculated. Colonies were produced by thermotolerant (fecal) coliform were various shades of blue (APHA, 2017). Coliform counted by equation: Total coliforms, No./100ml = Coliforms colonies counted×100 = No. CFU/100ml samples of filtered**.** After determination of water quality, values statically analyzed using SPSS.

Results

Protozoa cysts were detected in the three drains (Abu Youssef, El-Rahaway, & El-Qalyubia). These were: 1-*Entamoeba histolytica* cysts usually measured 12 to 15µm, with 4 nuclei characterized by central karyosomes and fine, uniformly distributed peripheral chromatin. 2- *Giardia intestinalis* cysts were oval to ellipsoid and measure 8-19 µm (average 10-14µm). Mature cysts with 4 nuclei, while immature ones with 2 nuclei.

SSrRNA gene series identification: Molecular identification of the *E. histolytica* and *G. intestinalis* isolates were based on small subunit rRNA gene sequence analysis. The results showed partial sequence of SSrRNA gene around 1946 & 183bp long, composed of variable and conserved regions. Sequence was compared with other species (International GenBank Database) by DNAMAN program and identified as *E. histolytica* strain A1 & *G. intestinalis* strain A2 with similarity (99%) and assigned with NCBI accession numbers MK329244.1 & MK332025.1.
Using the neighbor-joining process, the phylogenetic tree was mapped and eight clusters were created as compared with different nine GenBank SSrRNA partial sequences for *E. histolytica* and *G. intestinalis* strains. The highest homologous were found between *E. histolytica* strain A1 & MN307385.1 with similarity (100%). Minimum similarity (73%) was between *E. histolytica* strain A1 & other strains have accession numbers EF204915.1, FR686358.1, X89636.1 and LC259410.1. However, highest homologous were found between *G. intestinalis* strain A2 & KU939394.1 with similarity (100%). Minimum similarity (60%) was between *E. histolytica* strain A1 & other strains accession numbers DQ157272.1, AY309064.1, AF199446.1 & HQ179634.1 represented as a distinct cluster.

Protozoa: Seasonal abundance of 2 protozoa at Abu-Youssef, El-Rahaway and El-Qalyubia stations were monthly counted on Rafter cell using a light microscope (counted number ×filtered or centrifuged volume) over the 2020year. Changes in the vegetation cover and built-up areas along the three drains from March 2013 to April 2020 were mapped using the remote sensing work. The generated change detection map from remote sensing work showed a significant urban expansion around 1.94 Km^2 from March 2013 to April 2020. Urbanization was risky for the drains and aspects of the banks.

Water analysis: Quality characters were measured with the seasonal distributions of physical and chemical parameters.

Water physicochemical parameters were analyzed by a one-way analysis of variance. Analysis showed significant difference between months for EC, TDS, COD & Mg^{+2} in Abu-Youssef drain. In El-Rahawy drain, concentrations of Ec, TDS, & Ca^{+2} were significantly different. But, pH & Ec were significantly different in El-Qalyubia drain.

Heavy metals determination: Concentration of four heavy metals (Al, Cu, Fe, & Zn) in water samples from the three drains during the study period was given.
One way analysis determined the variations between four seasons, which showed significant differences ($P < 0.05$) as to Al, Cu, Fe, & Zn in the 3 drains.

Biological analysis: One way analysis of total and fecal coliform showed differences between the three water drains. Differences for total coliform were significant at El-Rahawy drain at $P. < 0.05$.

Ethics approval: The study was done after approval by Ethics Committee of the Faculty of Science, Suez Canal University, Ismailia.
Details were given in tables (1, 2, & 3) and figures (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, & 18)

Table 1: Specific primer sets of *Entamoeba histolytica* and *Giardia intestinalis.*

Primers	Sequence	T_m (°C)	% GC	Expected size (bp)
Forward	5´CCCGAGAATAGA-AAACTCTT 3´	60.01	50	1950
Reverse	5´-TCAAG-TATAGTGCACCATCT 3´	60.55	55	
Forward	5´AAGTGTGGTGCAGACGGACTC 3´	60.42	55	185
Reverse	5´CTGCTGCCGTCCTTGGATGTG 3´	60.18	60	

Table 2: Seasonal abundance of the protozoans at all stations during study period

Drain	parasites	Winter	Spring	Summer	Autumn
Abu-Youssef	*E. histolytica*	66.67±44.10	654.17±922.98	391.67±41.67	16.67±16.67
	G. intestinalis	33.33±33.33	285.00±128.68	266.67±88.19	16.67±16.67
El-Rahaway	*E. histolytica*	83.33±44.10	525.00±425.98	533.33±148.14	0.00±0.00
	G. intestinalis	0.00±0.00	401.67±162.28	433.33±212.79	0.00±0.00
El-Qalyubia	*E. histolytica*	0.00±0.00	333.33±333.33	316.67±66.67	33.33±33.33
	G. intestinalis	0.00±0.00	25.00±125.00	183.33±60.10	8.33±8.33

X =Mean number, S.E. = Standard error, Duncan's multiple range test ($P < 0.05$).

Table 3: One-way of heavy metals shows the difference between seasons at each drain

Heavy metal	Abu-Youssef drain		El-Rahawy drain		El-Qalyubia drain	
	F.	*P.*	*F.*	*P.*	*F.*	*P.*
Aluminum	1789.79	0.000*	1268.75	0.000*	2361.0	0.000*
Copper	56.75	0.000*	350.75	0.000*	117478.0	0.000*
Iron	7942.75	0.000*	4555.0	0.000*	10414.0	0.000*
Zinc	98.75	0.000*	74.72	0.000*	17002.24	0.000*

*Significant difference at $P < 0.5$.

Discussion

In aquatic ecosystems, parasites are undeniably essential and vital elements in which they drive fundamental ecological processes, such as by contributing to the biodiversity, productivity and food web structure of a system or ecosystem engineering (Poulin, 1999; Marcogliese, 2004). Like free-living organisms, parasites respond to ecosystem disruptions and can provide useful information about the efficiency, integrity and health of the environment in response to contaminants and other stressors (Brend *et al,* 2017).

The metabolic activity of microorganisms increases to maximum with temperature, while further temperature increases will decrease the metabolic activity of organisms by enzyme denaturation, and organisms will either become less active or die (Thomson *et al,* 2017). In addition, a temperature higher than the optimum can minimize protein synthesis by causing changes in ribosomal conformation (de Groot and Ventura, 2006). On the other hand, temperatures lower than the normal optima adversely affect membrane fluidity and prevent the activity of transport systems. These changes can influence the mobility of substrates in cells, thereby affecting growth rates (Barria *et al,* 2013; Jacob *et al,* 2018).

In winter the protozoa decreased in the first drain (Abu-Youssef drain) and rarely appeared in the third drain (El-Qalyubia drain) but in the second drain (El-Rahaway drain) *Entamoeba histolytica* only appeared with low count .In autumn they decreased to (16.67±16.67) for *Entamoeba* and *Giardia* in the first and third drains decreased to (33.33±33.33 & 8.33±8.33) for *Entamoeba* and *Giardia* respectively but disappeared in the second drain this may be due to the effect of water temperature on the pathogenic protozoa. The distribution was affected by land scape of the three drains Abu Youssef, El Rahaway and El Qalyubia. The two species (*Entamoeba histolytica* and *Giardia intestinalis*) was appeared clearly in thematic maps which indicate their distributions among the three drains of the research area. Urbanization expanded from 2013 to 2020 explained the prevalence the two protozoa parasites (Arnone and Walling, 2007). Generally, *E. histolytica* and *G. intestinalis* were encountered in many governorates (Abozahra *et al,* 2020; Mohamed *et al,* 2020 respectively). The infective cysts of both protozoa were acquired from raw vegetables (Kamel et al, 2014) as well as polluted water (El Shazly *et al*, 2007)

Heavy metals such as Cu, Zn, Ni, Cr, Co, Mo, Fe and Mn are essential micronutrients for various species, including microorganisms, plants and animals (Jacob *et al,* 2018). In addition to the use of parasites as accumulation steps, e.g. in determining the biological availability of pollutants, shifts in the behavior or numbers of an organism that served as an effective indicator [94] usually evaluated environmental impacts (Brend *et al,* 2017). Heavy metals that show high concentrations in the three locations among seasonal of the metals were Copper, Iron, Zinc, and Aluminum.

Pathogenic protozoa existence is associated with the concentration of heavy metals in water, usually when heavy metals increase the existed protozoa presence count is low or disappeared (Rebekka and Artz, 2002). In the present study, protozoa existed in summer and spring although heavy metals (Al, Cu, Zn, & Fe) recorded high concentration in the three locations. During summer, in Abu Youssef, Fe & Al recorded high concentrations (1.440±0.006 & 0.630±0.006, respectively), but in spring Zn recorded a high concentration (0.140±0.006). In El Rahaway the concentrations of Al, Fe, & Zn during summer were as follows: Fe>Al>Zn, (1.420-±0.006 > 0.670±0.006 >0.147±0.009) while Cu was high in spring (0.300±0.006). In El-Qalyubia Fe & Al recorded concentrations (2.040±0.006 & 0.840±0.006, respectively), and in spring Cu & Zn were high (4.280± 0.006 &1.560±0.006, respectively).

In the present study, in summer and spring heavy metals and protozoa increased in three the drains. Shrivastava (2009) reported that copper, an important engineering material, is one of the toxic metals, which caused many health hazards and harmful biochemical effects on living beings. El-Kowrany *et al.* (2015) in Gharbia Governorate examined potable water for protozoal and bacterial pathogens as well as heavy metals. They identified the protozoal contaminants in water, and showed that flow cytometry positive results were more than conventional staining, with prevalence of *Giardia* cysts and *Cryptosporidium* oocysts in water samples. They concluded that evaluation of drinking water is needed as well as formulation and implementation of an integrated plan to limit the contamination by pathogens and heavy metals. Gyamfia *et al.* (2019) in Ghana examined water (at 20cm & 40cm depths) to determine heavy metal (Fe, Pb, Zn, As, Mn, Cu, &Hg) levels, reported that groundwater in the community was potable but unsuitable for human usage, and that soil was extremely polluted with all the measured heavy metals (except Hg) from contamination factor, enrichment factor, geo-accumulation index and pollution load index assessments. De Conti *et al.* (2020) in Italy reported that Ryegrass (*Lolium perenne* L.) is a plant species that can express mechanisms of tolerance to copper (Cu) toxicity, and that agronomical approach of intercropping syst- ems with crops like vine plants could represent a promising strategy to control Cu toxicity in vineyard soils. Wu *et al.* (2020) in china reported that the use of reclaimed water for agricultural irrigation effectively reduced the use of freshwater resources including groundwater, addressing the increasingly severe challenge of water shortage.

Zinc, an essential metabolite in biological processes, is a chemical and biological interaction in plants with other components such as phosphorus, iron and nitrogen (Mousavi, 2013). Zinc is used as fertilizers for many crops like corn, rice, beans in forms of inorganic compounds such as Zinc sulphate monohydrate or organic compounds such as Disodium zinc EDTA and Zinc lignosulfonate (Mousavi *et al*, 2013). The excessive amount of zinc causes pollution through soil-water interaction can also be reached water through the discharge of plants treatment, which received industrial waste water as in third location. Besides, The pH of drains water varied with an average ranged of 7.18 to 8.3 which is acceptable and in the permissible range of the National River Water Quality Standard which ranges between 6.5 & 8.5 (Saad El-Din *et al,* 2014).

In general, water solids included suspended solids (TSS), and dissolved solids (TDS). In El-Rahaway and El- Qalyubia, TSS, TDS showed high amounts in nearly all months and also conductivity increased due to solids increass (Saad El-Din *et al,* 2014). This may be due to domestic discharges in water, and one of the main reasons for the zoonotic protozoa existence (Amer, 2012). Kistemann *et al.* (2012) in Germany reported that protozoal parasites, mainly *Giardia* cysts were detected frequently in surface waters.

Chemical Oxygen Demand (COD) estimated water contamination by measuring the amount of oxygen needed for decomposition of organic matter, not to exceed 3mg/L (APHA, 2017). The present study showed improvement in COD concentration in the three drains. In El-Qalyubia drain, there was (133.75±32.92 & 276.00±67.96) with highest concentration due to plant treatment in Qalyob City and El-Khanater discharged. This COD increase in water might be due to high concentration of organic matters from the domestic and industrial water (Azrina *et al,* 2006). Total coliform was the bacteria type present in the soil, water, human and animal wastes, but fecal coliform was the total coliform found in gastrointestinal tract of man and animals (Noble *et al,* 2003). Total and fecal coliform were indicators of water quality. In the present study, huge numbers of bacteria exceeded the preassemble range of water quality standards for total coliform bacteria 5000MPN/100ml, fecal coliform 1000 MPN/100ml (EPA, 2012). In the three drains bacteria were high than preassemble range all-year with significant difference ($P < 0.005$). Sewage polluted water, contained helminthic and protozoal infective stages and high resistance to chlorine of coliform bacteria depended on being ingested by protozoa (King *et al,* 1988). Haridy *et al.* (2006) in rural areas reported zoonotic parasites in farm animals. Abd El-Latif *et al.* (2020) in Egypt reported that the presence of same *Giardia* sub-assemblage in diarrheic children and in raw water proved the potential for waterborne dissemination of *Giardia*.

Conclusion

Recreational water contaminated with fecal pollution pave the way a great public health problem, as fecal waste including enteric protozoa cause serious waterborne illnesses. Molecular biology technique confirmed the protozoa. The removal of heavy metals is a demand of sustainable development.

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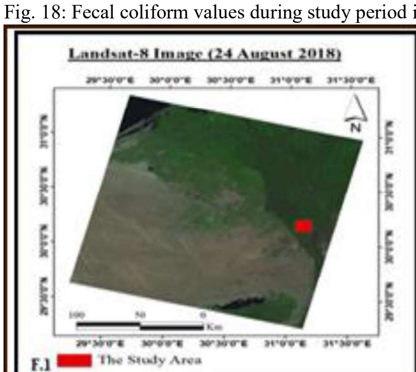
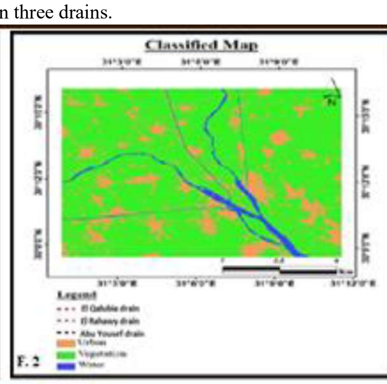
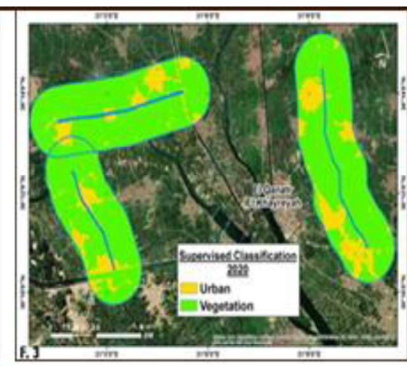
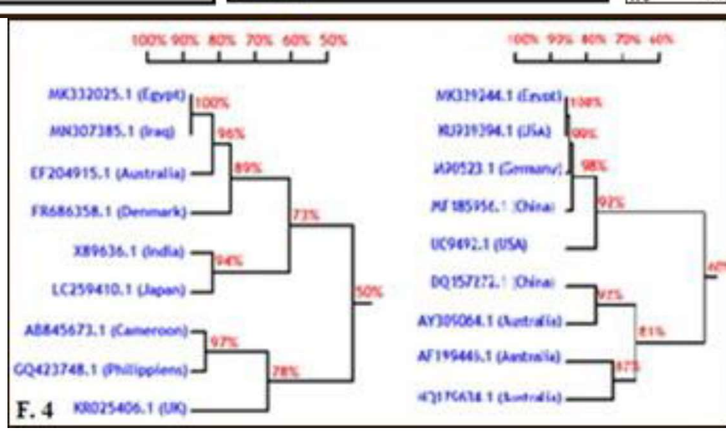
Explanation of figures

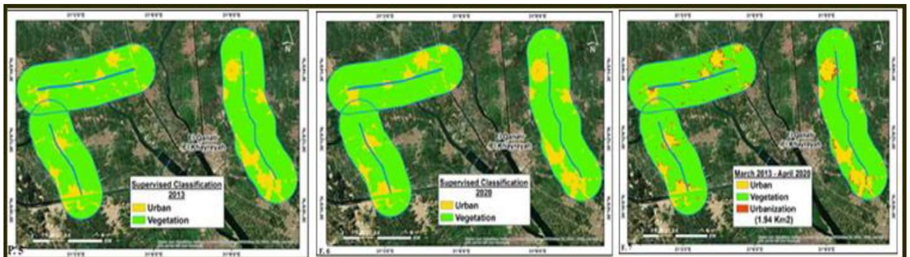
Fig. 1: Landsat-8 image of study area.
Fig. 2: Supervised classified map of research area.
Fig. 3: Supervised classification (April 2020) showed urban and vegetation surrounding three drains.
Fig. 4: Phylogenetic tree of *E. histolytica* and *G. intestinalis* strains A1 & A2, respectively.
Fig. 5: Supervised classification showed spatial distribution of urban and vegetation in three drains (March 2013).
Fig. 6: Supervised classification showed urban and vegetation spatial in three drains (April 2020).
Fig. 7: Map from remote sensing work showed significant urban expansion around 1.94 Km2 from March 2013 to April 2020.
Fig, 8: pH values during study period in three drains
Fig. 9: EC values during study period in three drains
Fig. 10: TSS and TDS values during study period in three drains
Fig. 11: COD values during study period in three drains
Fig.12: Mg+2 and Ca+2 values during study period in three drains
Fig.13: Aluminum values during study period in three drains
Fig. 14: Copper values during study period in three drains
Fig. 15: Iron values during study period in three drains.
Fig. 16: Zinc values during study period in three drains
Fig. 17: Total coliform values during study period in three drains
Fig. 18: Fecal coliform values during study period in three drains.

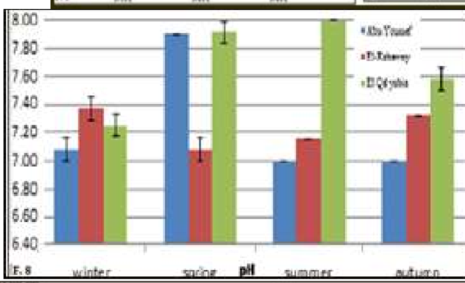


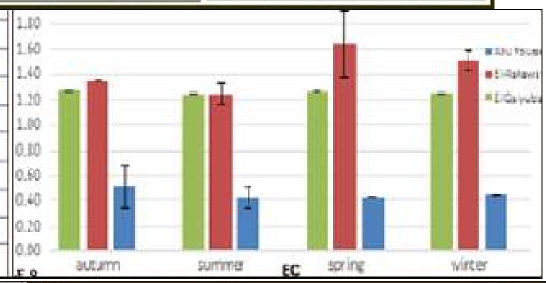


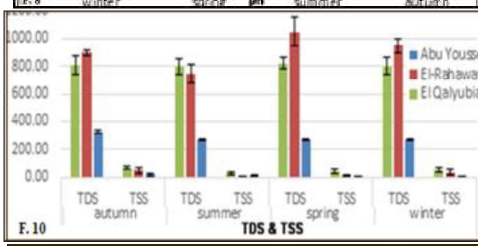


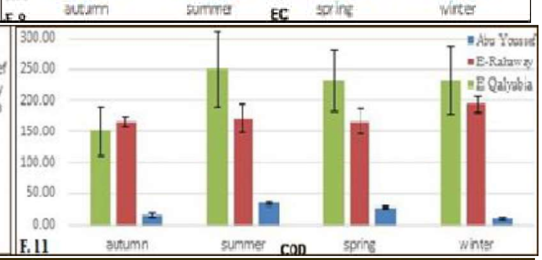


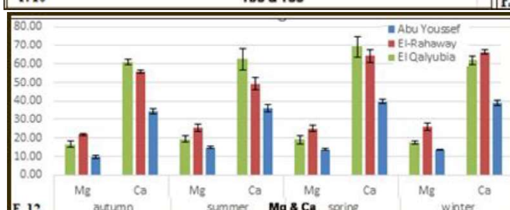

Supervised Classification 2013
Urban
Vegetation
Supervised Classification 2020
Urban
Vegetation
March 2013 - April 2020
Urban
Vegetation
Urbanization (1.94 Km2)

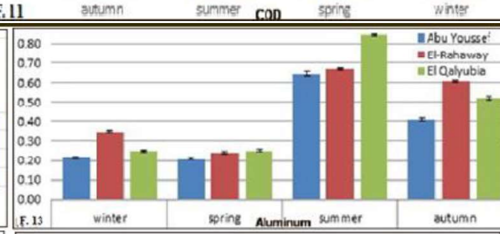

Abu Youssef
El-Rahaway
El Qalyubia
winter
spring
summer
autumn
pH
F. 8

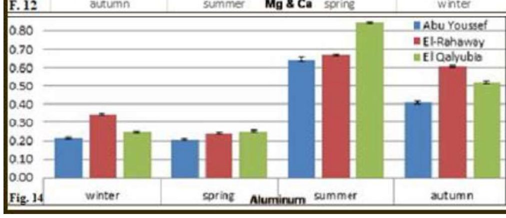

autumn
summer
spring
winter
EC

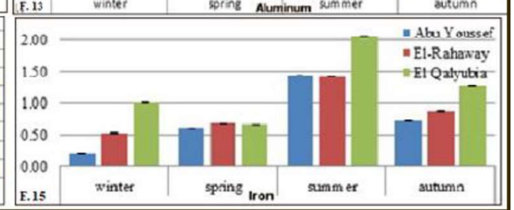

Abu Youssef
El-Rahaway
El Qalyubia
TDS
TSS
autumn
summer
spring
winter
TDS & TSS
F. 10

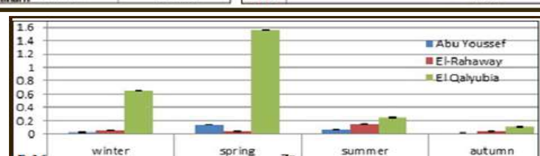

Abu Youssef
El-Rahaway
El Qalyubia
autumn
summer
spring
winter
COD
F. 11

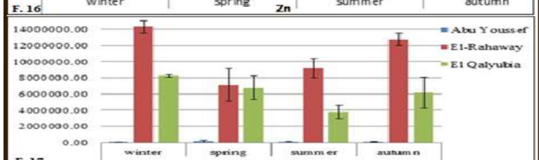

Abu Youssef
El-Rahaway
El Qalyubia
Mg
Ca
autumn
summer
spring
winter
Mg & Ca
F. 12

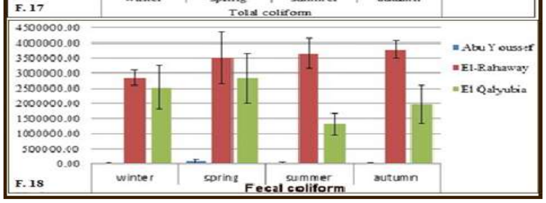

Abu Youssef
El-Rahaway
El Qalyubia
winter
spring
summer
autumn
Aluminum
F. 13


Abu Youssef
El-Rahaway
El Qalyubia
winter
spring
summer
autumn
Aluminum
Fig. 14


Abu Youssef
El-Rahaway
El Qalyubia
winter
spring
summer
autumn
Iron
F. 15


Abu Youssef
El-Rahaway
El Qalyubia
winter
spring
summer
autumn
Zn
F. 16


Abu Youssef
El-Rahaway
El Qalyubia
winter
spring
summer
autumn
Total coliform
F. 17


Abu Youssef
El-Rahaway
El Qalyubia
winter
spring
summer
autumn
Fecal coliform
F. 18