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Microbiological and Chemical Examinations of some Water Sources in Damietta City During Winter 2018

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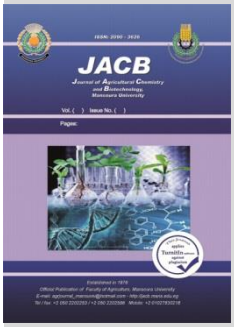

ABSTRACT

This research was carried out in Microbiology Department, Faculty of Agriculture, Damietta University, Damietta, Egypt.Water samples were collected from different sites at Damietta City for both microbiological and physico-chemical examinations during winter 2018. The microbiological results showed that virally investigated types including Poliovirus type 1, Rotavirus, HAV type IB, and H9N2 avian influenza were absent in all examined samples during winter 2018. The highest mean values of total bacterial and total fungal counts in SD and SA sites were 251.333 and 112.667 cfu/ml, respectively. There was no bacterial growth on MacConkey broth, Salmonella Shigella (SS) and Staph-110 agar media. Algae in the SA and SD sites showed the highest quantities, both motile and non-motile species were found. There was no species of protozoa in all examined sites except SA and SD sites which showed few motile ones. Obtained results of physico-chemical parameters, showed the pH values varied between 7.46 in AF site and 7.96 in SD site. Temperature also, varied between 21 and 22.4°C in EA and SD sites. The highest values of COD and BOD_5^{20} were in SeA and SD sites and were 9.6 and 18.9 mgO_2/L, respectively. In case of heavy metals, results showed that the highest values of aluminum, cadmium, iron, nickel, lead, manganese, zinc, copper and chromium were in FD,FA,SD,SD,EA,SA, SD,SeA and SD sites and were 0.13,0.019,0.117,0.054,0.046,0.030,0.387,0.007 and0.014 ppm, respectively. So, these results proved that water sources are in agreement with the ES for using of this water for human purposes.

Keywords: Microbiological examinations, Molecular investigations, Algae, Protozoa**,** Physical parameters, Heavy metals and Minerals.

INTRODUCTION

Water is essential to sustain life and a satisfactory supply must be provided to consumers. An enormous effort should be made to achieve a drinking water quality as high as practicable (WHO, 1996).

Water contamination can originate from variety of sources, including industrial or agricultural runoff, poorly treated or untreated, human and animal waste.

Contamination can also be naturally occurring with chemicals such as arsenic or fluoride, and seeps into drinking water sources from geologic strata. In developing countries, the most common form of contamination is microbiological one which comes primarily from human or animal faeces mixing with drinking water sources during transport or at the point of use. More specifically, microbial contamination refers to the introduction of any number of harmful bacteria, viruses or protozoa, collectively known as pathogens, into a water sources (Samantha, 2012).

All hepatitis A and rotaviruses positive samples were from the upstream sections of Tyhume River while noro-viruses were detected in samples from downstream sections only. Because of the low infectious dose of enteric viruses, the detection of even low concentrations of hepatitis A virus, rotaviruses and noroviruses in surface water poses a significant risk to public health (Timothy and Anthony, 2013).

Sabae and Rabeh (2007) studied the bacterial load of Damietta water from autumn 2005 to summer 2006 and they found that, *E. coli*, the main indicator of faecal pollution, constituted 16% of Gram-negative bacteria in Damietta water. However, maximum counts were recorded during summer while the minimal counts were detected in winter.

Microbial quality water along the River Nile varies with location and depends on flow rate, water use, population density, sanitation systems, domestic and industrial discharges, demands for navigation, and agricultural runoff. Microbial conditions in Egypt often meet established water quality standards, but some areas are polluted by industrial facilities discharging to the Nile between Aswan and Cairo. Total Coliform (TC) bacteria reach 10^6/100 ml in many delta drains, 200 times the Egyptian standard of 5×10^3/100 ml (Rabeh, 2009).

Among the pathogens disseminated in water sources, enteric pathogens are the most frequently encountered ones. As a consequence, sources of faecal pollution in waters devoted to human activity must be strictly controlled. Entero-pathogens, such as E. coli, are generally present at very low concentrations in environmental waters (Rompre *et al*., 2002).

The proper balance of physical, chemical, and biological properties of water in ponds, lakes and reservoirs is an essential ingredient for successful production of fish and other aquatic resources. However, only few BOD, temperature, conductivity, total alkalinity,

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total hardness and ions among tested parameters in Eleyele Reservoir fell within optimum recommended range for growth and survival Olanrewaju *et al*. (2017).

The rainy season mean values for water temperature, pH, nitrate-nitrogen were significantly higher than those for the dry season. However, for transparency, conductivity, dissolved oxygen, hardness, alkalinity, phosphate-phosphorus and total dissolved solids, the dry season mean values were significantly higher than those mean values of the rainy season. As in most other African inland water bodies, there was seasonal variation in the physico-chemical parameters (Ibrahim *et al*., 2009).

The Biological oxygen demand (BOD) is the amount of Dissolved oxygen (DO) which is used to decompose the organic matter in water by microorganisms. It depends on several factors such as: temperature, concentration of organic matter and density of phytoplankton. The BOD test is the most useful method in estimating the amount of biodegradable organic matter present in the aquatic environment. Also, BOD rapidly deplete DO content of polluted water with sewage, so it is important to estimate the amount of these pollutants in the given water body (Ahmed, 2007).

Ali *et al*. (2015) reported that, heavy metals concentrations in the River Nile water especially Ni, Pb and Cd exceeded the permissible limits and its abundance followed the order of Pb>Ni>Cd>Cu.

This study aims to examine different water samples from Damietta city for microbiological examinations, molecular investigations and physico-chemical examinations during winter 2018.

MATERIALS AND METHODS

Samples collection and preparation

Water samples were collected from different ten sites during winter 2018 of Damietta city including River Nile (Damietta branch) in front of El-Adlyah treatment station source (SA) and Damietta treatment station source (SD), inside the two water treatment stations collected from sedimentation stages (SeA and SeD) and filtration stages (FA and FD), outside the stations collected from the start point of the network of the two stations as a reservoir (RA and RD), and the end point of networks from different sites (EA and ED).

All examined sites used for samples collection are shown on map of Damietta city (photo 1). Samples were collected in 100 ml sterile glass bottles of 20 cm below the water surface to avoid floating materials, preserved in ice-box for physico-chemical examinations that were carried out in Chemical laboratory of Drinking Water and Sanitation Company in Damietta, Al-Moalmien Square; Musharrafa Street, Damietta, Egypt. Also, microbiological examinations were carried out within 6 hours after collection in the microbiological laboratory of Microbiology Department, Faculty of Agriculture, Damietta University, Damietta, Egypt. Three replicates of one ml of each sample were used for microbiological examinations.

Photo .1. Sites of collected water samples in Damietta City during winter 2018

Samples were collected in 40 L sterile polyethylene plastic bottles and then were delivered to the laboratory. Before sampling, the polyethylene bottles were washed, cleaned and rinsed thoroughly with distilled water. Samples were collected at 20 cm depth from the water surface to avoid floating materials or from networks tap water under aseptic technique. The samples were further subjected to ultrafiltration in Chemical laboratory of Drinking Water and Sanitation Company in Damietta, Al-Moalmien Square, Musharrafa Street, Damietta, Egypt. The molecular investigations of viral pathogens were carried out in GENE laboratory, Flat 4, Building 20, Farmland housing, Kafr El-Sheikh, Egypt, to experimentally investigate the viral persistence of Poliovirus type 1, Rotavirus, HAV type IB, and H9N2 avian influenza (influenza A/H9N2 subtype).

A 100 ml sample in glass bottle from each collected sample was used for determination of biological oxygen demand (BOD) using a dissolved oxygen meter (DOM Model: YK-22DO, Power: 9 V battery, Lutron Electronic Enterprise Co., LTD Made in Taiwan). Also, salinity was determined using a conductivity meter (CM Model: CD-4301, Power: 9 V batteries, Lutron Electronic Enterprise Co., LTD Made in Taiwan).

Heavy metals were determined using Varian AA240FS Fast Sequential Atomic Absorption Spectrometer, Made in USA.

Minerals were determined using Dionex ICS-3000 Varian Carry 100 Spectrometer, Made in USA.

Microbiological examinations

Molecular investigation

Ultrafiltration membrane method

In brief, suspended materials of each water sample were removed by 3 successive filtrations, through a glass microfiber filter, a 0.45 µm and a 0.2 µm polyamide membrane filters, respectively. About 75 milliliters of filtrate were then ultracentrifuged at 15, 2743 g (ultracentrifuge L-80, Beckman) for 90 min at 18°C. The resulting pellet was re-suspended in 500 µl of supernatant. An equal volume of chloroform was then added. After homogenization and centrifugation at 405 g (Sigma 3–15) for 10 min, the aqueous phase was collected and stored at -20°C until nucleic acid extraction according to Ana *et al*. (2018) using Sartorius Stedium Biotech 16277 (Made in Germany) used for ultrafiltration membrane method.

Real time PCR

Real time PCR was used to screen whether the drinking water taken from different sources and at different stages of purification (from Nile (SA, SD), at the entry (RA, RD), and exit of purification station (EA, ED)) containing some viruses including Poliovirus type 1, Rotavirus, HAV type IB, and H9N2 avian influenza. To conduct real time PCR first, total RNA was isolated from different water samples and then revised transcribed into cDNA which was used as a template for qPCR. Throughout the whole real time PCR experiment, the housekeeping gene (GAPDH) was used as an internal reference for normalization and data was expressed as mean ± SEM.

Petri dishes, test tubes, and Durham tubes, Micropipette tips: 0-1000 uL, bottles, flasks, incubator and autoclave were used for the bacterial and fungal examinations ten samples in three replicates were done.

Bacteria, fungi, algae and protozoa were determined using a light microscope (Olympus CX31 Binocular Halogen Microscope, Made in Japan) with digital camera.

Cultivation media

Nutrient agar medium was used for the cultivation of a wide variety of microorganisms, Staph-110 medium was used for isolation and enumeration of Staphylococci, Salmonella Shigella agar medium (SS agar) was used for the selective isolation and differentiation of pathogenic enteric bacilli, especially those belonging to the genus Salmonella, MacConkey broth medium was used for counting of coliform bacteria, Potato dextrose agar medium was used for isolation and enumeration of fungi (Ronald, 2010) and Eosin Methylene blue agar medium (EMB) was used for confirming of coliform counts and for isolation and selection of E. coli (APHA, 2017).

Molecular investigations (Real time PCR)

Real time PCR with SYBR Green was used to measure expression of mRNAs of target genes in water samples, with GAPDH as an internal reference. The isolated cDNA were amplified using 2X Maxima SYBR Green/ROX qPCR Master Mix following the manufacturer protocol (Thermo scientific, USA, # K0221) and gene specific primers. The web based tool, Primer 3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3 _www.cgi) was used to design these primers based on published viral sequences. To ensure primer sequence is unique for the template sequence; we checked similarity to other known sequences with BLAST (www .ncbi. nlm. nih. Gov /blast /Blast.cgi).

Total bacterial count

Poured plate method was used after preparing suitable serial dilutions of water samples, one ml was transferred into sterile glass Petri dish in triplicates. Approximate 15 ml of melted nutrient agar medium at 45-50 °C was poured in each plate, then thoroughly mixed and left for solidification. The plates were incubated at 37 °C for 72 hours in a digital incubator (Switch, MPM Instruments S.R.L. and Bernareggio /Made in Italy). After the incubation period, developed separated colonies were counted per each plate of the same dilution and the mean value was calculated. The count of total bacteria was calculated as follows: Total count of bacteria = average number of triplicate plates of the same dilution × reciprocal of the dilution used cfu/ml or cfu/g sample (Ronald, 2010).

Salmonella and Shigella count was done on SS agar medium. Also, Staphylococci count was done on staph. 110 medium (Ronald, 2010).

Total coliform count

This test was done in two stages, the first test was to detect the presence of acid and gas “presumptive test", and to ascertain the presence of coliform bacteria in the second test “confirmed test” (APHA, 2017).

Total fungal count

Poured plate method was used one ml of suitable serial dilutions of all water samples were inoculated onto three plates using poured plate method (Ronald, 2010).

Approximately fifteen ml of potato dextrose agar (PDA) medium at about 50°C was poured in each plate, then thoroughly mixed and left for solidification. The plates were incubated at 25°C for 5 days. After the incubation period, developed separated colonies were counted per each plate and the mean count of three plates was recorded.

Algal and protozoal examination

After the sample is taken it is fixed with Lugol’s iodine solution. The sample is then pressurised and a representative portion of known volume is transferred to a sedimentation tube. After a suitable settling period, which is dependent on volume, any algae or protozoal cells in the sample are directly identified and enumerated using light microscope. The method is a direct enumeration method but will use a multiplication factor, based upon the volume of sample analysed, number of microscope fields of view used, and the area of each field to calculate the final result ALS, (2017).

Physico-chemical examinations

Collected water samples were subjected to physical examination according to Standard Methods of Examinations of Water and Waste Water 23rd Edition (APHA, 2017). Salinity or electrical conductivity (EC) was determined using a conductivity meter (CM) (Model: CD-4301, Power: 9 V batteries, Lutron Electronic Enterprise Co., LTD, Made in Taiwan). This method was followed after Gloterman et al. (1978). The dissolved oxygen was determined using a dissolved oxygen meter (DOM) in each site. In this method, the dissolved oxygen was determined (BOD205) as the reduction in dissolved oxygen. BOD5 was calculated by the method described by (Stirling, 1985).

Heavy metals

To determine aluminum, cadmium, iron, nickel, lead, manganese, zinc, copper, chromium and cobalt concentrations, collected water samples were subjected to the methods of Gloterman et al. (1978) using Varian AA240FS fast sequential atomic absorption spectrometer, Made in USA.

Minerals

To determine calcium, magnesium, ammonia, nitrite, phosphates, silica, sulphate, fluoride, sodium and potassium concentrations, according to Standard Methods of Examinations of Water and Waste Water 23rd Edition (APHA, 2017) was adapted using Dionex ICS-3000 Varian Carry 100 Spectrometer Made in USA.

Statistical analyses

Data obtained throughout this study were analyzed by computer-assisted one-way ANOVA, using the software package statgraphics version 5.0 (costat). Least significance differences (LSDs) were calculated at 99% level of significance $P < 0.05$ (Murica et al., 1997).

RESULTS AND DISCUSSION

Microbiological examinations

Real time PCR was used to screen whether the drinking water coming from different sources or different stages of purification (from Nile (SA, SD), at the entry (RA, RD), and exit of purification station (EA, ED)) containing some viruses, including Poliovirus type 1, Rotavirus, HAV type IB, and H9N2 avian influenza.

Real time PCR of water

Obtained qPCR results revealed absence of all viruses examined (Poliovirus type 1 gene, Rotavirus gene, HAV type IB gene and H9N2 avian influenza at SA, RA, EA, SD, ED and RD sites during winter 2018.

Obtained results are in disagreement with that obtained by Timothy and Anthony (2013) since they found that hepatitis A virus was detected in 13% of the samples in concentrations ranging between 1.67×103 genome copies/ℓ and 1.64×104 genome copies/ℓ while rotaviruses were detected in 4% of the samples with concentrations ranging from 9×101 genome copies/ℓ to 5.64×103 genome copies/ℓ. Enteroviruses were not detected in any of the samples, while noroviruses were detected in 4% of the samples.

Bacterial and fungal examinations

All water samples were taken from different sites in Damietta City. Ten of water samples were all examined microbiologically included total bacteria, total fungi, total coliform, Salmonella sp. and Shigella sp., Staphylococci, algae and protozoa.

Results in Table 1 showing, that the highest mean value of total bacterial count was in SD site and was 251.333 cfu/ml that in disagreement with (50 cfu/ml) the Egyptian Standard No. 458/2007 according to EOG, (2007) followed by SA, FA, SeD, RA, SeA, FD, EA and ED sites which were 223, 9.667, 7.333, 6.667, 6, 3.333, 3 and 1.333 cfu/ml, respectively. The total bacterial count was in the lowest mean value in case of RD site and was 0.0 cfu/ml.

Obtained results are lower than that results obtained by Tayo et al. (2011) who reported that, the total bacterial count of the water samples ranged from 4.0 x107 - 1.42 x 108 cfu/ml.

Obtained results showing, there was no bacterial growth on MacConkey broth agar medium. There was no bacterial growth on Salmonella Shigella (SS) agar medium. Also, results showing, that there was no bacterial growth on Staph-110 agar medium.

On the other hand, the highest bacterial indicators were detected in warmer seasons which might be attributed to high temperature and the discharged wastewater during this season (Sabae and Rabeh, 2007).

The guideline criteria for faecal indicator organisms of WHO (1992) accept the guide values of the investigated bacteria up to 500/100 ml for total coliform and 100/100 ml for both faecal coliform and faecal Streptococci. So these data revealed that the Nile water at the investigated sites is subjected to sewage pollution which considered to be very serious concept.

Table 1. Bacterial and fungal evaluation (cfu/ml) of water taken from Damietta City during winter 2018

Parameter Sample	Total bacteria (cfu/ml)	Total fungi (cfu/ml)
SA	223[a]	112.667[a]
SeA	6[b]	20[b]
FA	9.667[b]	7[b]
RA	6.667[b]	0[b]
EA	3[b]	0[b]
SD	251.333[a]	64.667[ab]
SeD	7.333[b]	3.333[b]
FD	3.333[b]	4.333[b]
RD	0[b]	0[b]
ED	1.333[b]	0[b]
LSD 0.05	37.256	48.450
F	60.583	5.297
P	.0000 ***	.0009 ***

SA: Source of El-Adlyah treatment station; SeA: Sedimentation stage of El-Adlyah treatment station; FA: Filtration stage of El-Adlyah treatment station; RA: Reservoir of El-Adlyah treatment station; EA: End of network of El-Adlyah treatment station; SD: Source of Damietta treatment station; SeD: Sedimentation stage of Damietta treatment station; FD: Filtration stage of Damietta treatment station; RD: Reservoir of Damietta treatment station; ED: End of network of Damietta treatment station.

Obtained results are in good agreement with recently published data (Osman, 2006) who examined the microbiological quality of the River Nile in three different sites i.e. Helwan, El-Giza and Shoubra and the average log number of each indicator was varied from site to another according to the environmental conditions where the highest average log number of total bacterial count, total Coliform, faecal Coliform and faecal Streptococci at 37°C were 6.96, 4.38, 3.29 and 2.58, respectively.

These results are lower than that obtained by Sabae et al. (2014) who studied the total bacterial count in Al-Bahr El-Pherony (An important source of fisheries in Menoufia Government) during winter and it was 210 x 106 cfu/ml. The current results are lower than that obtained by Khalifa and Sabae (2012) who found that, total Coliforms of Damietta Branch of River Nile during winter was 40 cfu/100ml.

Table 1 also showed that, the highest value of total fungal count found in SA site was 112.667 cfu/ml, followed by SD, SeA, FA, FD and SeD sites which were 64.667, 20, 7, 4.333 and 3.333 cfu/ml, respectively. The current results are higher than that obtained by El-Fadaly et al., (2016) who found that, total fungi count in the River Nile water during winter 2016 was 0.0 ×105 cfu/ml. Total fungal counts were in the lowest values in case of RA, EA, RD and ED sites (0.0 cfu/ml).

Algal and protozoal counts

Results in Table 2 showed that, algae quantities in the SA and SD sites were the highest as it contained motile and non-motile species which in disagreement with the Egyptian Standard No. 458/2007 according to EOG, (2007).

This results is followed by SeA, FA, SeD and FD sites which contained lower quantities of algal species

that are non-motile. The other sites RA, EA, RD and ED were free from any algal species that can be seen in photo 2 according to APHA, (2017).

Results in Table 2 showed no protozoal species in all examined sites except SA and SD sites which contained few numbers of motile modes that are not pathogenic for human according to specialists in National Research Center (personal contact).

Physico-chemical examinations

All types of water samples were collected during winter, 2018 from different sites in Damietta City. Ten of water samples were all examined in this study for Physico-chemical examinations.

Data in Table 3 showed, residual chlorine determined only in FA, RA, EA, FD, RD and ED sites. The highest values were in FA, EA and FD sites and were 2 ppm, followed by RD site of 1.6 ppm, but the lowest values were in RA and ED sites which found 1.5 ppm. The highest value of turbidity was in SeA site which was 5.54 NTU that is in disagreement with (1 NTU) the Egyptian Standard No. 458/2007 according to EOG, (2007), while the lowest value was in RD site and was 0.31 NTU.

Photo .2. Some algal species appeared in some water sources in Damietta City during winter 2018

Table 2. Algal and protozoal results of water taken from Damietta City during winter 2018

Parameter	Algae			Protozoa		
Sample	Qualitative	Quantity	motility	Qualitative	Quantity	motility
SA	Positive	V .High	Motile and non-motile	Positive	Few	motile
SeA	Positive	Few	Non-motile	Negative	-----	-----
FA	Positive	Few	Non-motile	Negative	-----	-----
RA	Negative	Negative	-----	Negative	-----	-----
EA	Negative	Negative	-----	Negative	-----	-----
SD	Positive	V .High	Motile and non-motile	Positive	Few	motile
SeD	Positive	Few	Non-motile	Negative	-----	-----
FD	Positive	Few	Non-motile	Negative	-----	-----
RD	Negative	Negative	-----	Negative	-----	-----
ED	Negative	Negative	-----	Negative	-----	-----

SA: Source of El-Adlyah treatment station; SeA: Sedimentation stage of El-Adlyah treatment station; FA: Filtration stage of El-Adlyah treatment station; RA: Reservoir of El-Adlyah treatment station; EA: End of network of El-Adlyah treatment station; SD: Source of Damietta treatment station; SeD: Sedimentation stage of Damietta treatment station; FD: Filtration stage of Damietta treatment station; RD: Reservoir of Damietta treatment station; ED: End of network of Damietta treatment station.

Table 3. Physical examinations of water taken from Damietta City during winter 2018

Sample Test	SA	SeA	FA	RA	EA	SD	SeD	FD	RD	ED
R. Cl	-----	-----	2	1.5	2	-----	-----	2	1.6	1.5
Turb	3.65	5.54	0.34	0.54	0.89	3.76	3.77	0.70	0.31	0.54
PH	7.87	7.87	7.46	7.57	7.51	7.96	7.94	7.63	7.71	7.57
T°C	22	22	21.2	21.8	21	22.4	22	21.2	21.5	21.8
CND(EC)	579	488	510	581	507	576	581	584	579	581
TDS	347	293	306	348	304	346	349	350	347	348
TSS	4	6	1	1	1	4	4	1	1	1
TOC	4.896	4.759	3.011	2.674	3.121	4.901	4.814	2.342	2.765	2.674
COD	6.4	9.6	----	----	----	6.4	6.4	----	----	----
DO(initial)	25.8	14.5	20.1	16.4	21	32.3	14.0	15.5	16.8	15.2
DO(final)	10.6	11.3	10.0	11.0	9.8	13.4	10.2	10.6	11.3	8.7
BOD5	15.2	3.2	10.1	5.4	11.2	18.9	3.8	4.9	5.5	6.5
Cl	36	34	41	42	42	37	38	45	44	42
Alkalinity	172	174	164	166	166	172	174	168	166	166
T. Hard	170	176	170	166	166	172	176	172	168	166
Ca. Hard	104	106	104	102	102	104	106	104	102	102
Mg. Hard	66	70	66	64	64	68	70	68	66	64
P. Hard	UDL	2	6	UDL	UDL	UDL	2	4	2	UDL

SA: Source of El-Adlyah treatment station; SeA: Sedimentation stage of El-Adlyah treatment station; FA: Filtration stage of El-Adlyah treatment station; RA: Reservoir of El-Adlyah treatment station; EA: End of network of El-Adlyah treatment station; SD: Source of Damietta treatment station; SeD: Sedimentation stage of Damietta treatment station; FD: Filtration stage of Damietta treatment station; RD: Reservoir of Damietta treatment station; ED: End of network of Damietta treatment station; R.Cl.: Residual chlorine; Turb.: Turbidity; T: Temperature; CND: Conductivity; TDS: Total dissolved solids; TSS: Total solved solids; TOC: Total organic carbon; COD: Chemical oxygen diamond; DO: Dissolved oxygen; BOD: Biological oxygen diamond; Cl.: Chlorides; T. Hard: Total hardness; Ca. Hard: Calcium hardness; Mg. Hard: Magnesium hardness; P. Hard: Permanent hardness; UDL: Under detection limit.

The pH values varied between 7.46 in FA site and 7.96 in SD site. Temperature varied between 21°C in EA and 22.4°C in SD sites. The highest value of CND was in FD site of about 584 dSm^{-1} (mhos/cm), while the lowest value was in SeA site of about 488 dSm^{-1} (mhos/cm).

The highest values of total dissolved solids was in FD site and was 350 ppm, the lowest value was in SeA site and was 293 ppm. The highest value of total solved solids was in SeA site of 6 ppm, but the lowest values were in FA, RA, EA, FD, RD and ED sites which was 1 ppm. The highest value of TOC was in SD site and was 4.901ppm, while the lowest value was in FD site and was 2.342 ppm.

COD was determined in SA, SeA, SD and SeD sites only and the highest value was in SeA site and was 9.6 mgO_2/L, followed by the other three sites which was 6.4 mgO_2/L. The highest values of initial DO were 32.3 and 25.8 mgO_2/L in SD and SA sites, respectively, and the lowest value was 14.0 mgO_2/L in SeD site. The highest values of final DO were 13.4, 11.3 and 11.3 mgO_2/L in SD, SeA and RD sites, respectively, while the lowest values were 9.8 and 8.7 mgO_2/L in EA and ED sites, respectively.

The highest value of BOD_5^{20} was in SD site and was 18.9 mgO_2/L while the lowest value was in SeA site and was 3.2 mgO_2/L.

These results are higher than that obtained by El-Fadaly *et al*. (2016) who studied the Biochemical Oxygen Demand (BOD_5) that was 7.0 mgO_2 /L during winter in the River Nile water. The same was found by Ahmed (2007) who studied the BOD value of El-Rahawy drain and he found that BOD value was 16.5 mg/l during winter. Also, the same results obtained by EL-Shafei (2016) who showed to be higher since he found that the maximum value of DO recorded was (16 mg/l) at El-Kanater El-Khayria during December.

The highest value of chlorides was in FD site and was 45 ppm, but the lowest value was in SeA site and was 34 ppm. The value of alkalinity was higher in SeA and SeD sites and was 174 ppm, while the lowest value was in FA site and was 164 ppm.

The total hardness was higher in SeA and SeD sites and was 176 ppm, while the lowest values were in RA, EA and ED and was 166 ppm. For calcium hardness the highest value was in SeA and SeD sites and was 106 ppm, while the lowest values were in RA, EA, RD and ED sites found to be 102 ppm. The highest value of magnesium hardness were in SeA and SeD sites and was 70 ppm, while the lowest values were in RA, EA and ED sites found to be 64 ppm. The value of permanent hardness was higher in FA site and was 6 ppm, while the lowest values were in SA, RA, EA, SD and D/ E sites and was UDL<2 ppm. All hardness results were in a good agreement with the Egyptian Standard No. 458/2007, according to EOG, (2007).

The mean dry season values of transparency (12.46m), conductivity (86.41µs), dissolved oxygen (4.70mg/l), water hardness (56.07mg/l), alkalinity (53.38mg/l), phosphate-phosphorus (6.86mg/1) and total dissolved solids (43.19ppm) were higher than those of rainy seasons. Water temperature (27.65°C), pH (7.15) nitrate-nitrogen (5.21mg/l) mean values were higher in the rainy season than that of the dry season as reported by Ibrahim *et al*. (2009).

Heavy metals values

Aluminum was determined in all examined sites and the highest value was in FD site and was 0.13 ppm (Table 4), while the lowest value was in SeA site and was UDL<0.01 ppm. The highest mean value of cadmium was found in FA site and was 0.019 ppm that in disagreement with (0.003 ppm) the Egyptian Standard No. 458/2007 according to EOG, (2007), while the lowest values were in RA, EA and ED sites which found to be UDL<0.001 ppm.

These results are higher than that obtained by El-Fadaly *et al*. (2016) who studied the cadmium values in Manzala Lake during winter and it was 0.007 ppm.

The highest value of iron was in SD site and was 0.117 ppm while the lowest values were in FA, RA, EA, FD, RD and ED and was UDL<0.001 ppm. The highest value of nickel was in SD site and was 0.054 ppm in disagreement with (0.02 ppm) the Egyptian Standard No. 458/2007 according to EOG, (2007), while the lowest values were in FA and FD sites and was UDL<0.001 ppm. The highest value of lead was 0.046 ppm in EA site in disagreement with (0.01 ppm) the Egyptian Standard No. 458/2007 according to EOG, (2007) and the lowest value was 0.001 ppm in RA and ED sites.

Ali *et al*. (2015) also explained that, Pb can find its way to the water of the River Nile through the leaching of gasoline from the fishery boats and the tour ships travels. Moreover the increasing of heavy metals concentrations at River Nile can be attributed to the huge quantities of sewage and industrial wastes via drains.

The highest value of manganese was found in SA site and was 0.030 ppm, while the lowest mean value was in RA and ED sites and was 0.001 ppm. Obtained results showed that the highest value of zinc was in SD site and was 0.387 ppm, while the lowest values were recorded in SeA, FA, RA, SeD, FD and ED (UDL<0.001 ppm). The highest value of copper was found in SeA site and was 0.007 ppm, while the lowest value was in RD site and was 0.001 ppm.

Table 4. Heavy metals values (ppm) of water taken from Damietta City during winter 2018

S P	SA	SeA	FA	RA	EA	SD	SeD	FD	RD	ED
Al	0.01	UDL	0.03	0.10	0.08	0.01	0.03	0.13	0.09	0.10
Cd	0.012	0.001	0.019	UDL	UDL	0.016	0.007	0.004	0.008	UDL
Fe	0.084	0.038	UDL	UDL	UDL	0.117	0.081	UDL	UDL	UDL
Ni	0.005	0.053	UDL	0.012	0. 013	0. 054	0.008	UDL	0.001	0. 012
Pb	0.027	0.003	0.005	0.001	0.046	0.032	0.021	0.014	0.022	0.001
Mn	0.030	0.027	0.002	0.001	0.002	0.022	0.024	0.004	0.004	0.001
Zn	0.002	UDL	UDL	UDL	0. 073	0.387	UDL	UDL	0. 009	UDL
Cu	0.006	0.007	0.004	0.006	0.005	0.005	0.006	0.003	0.001	0.006
Cr	0.006	0.011	0.011	0.003	0.006	0.014	0.013	0.006	0.001	0.003
Co	UDL	UDL	UDL	UDL	UDL	UDL	UDL	UDL	UDL	UDL

S: Sample; P: Parameter; SA: Source of El-Adlyah treatment station; SeA: Sedimentation stage of El-Adlyah treatment station; FA: Filtration stage of El-Adlyah treatment station; RA: Reservoir of El-Adlyah treatment station; EA: End of network of El-Adlyah treatment station; SD: Source of Damietta treatment station; SeD: Sedimentation stage of Damietta treatment station; FD: Filtration stage of Damietta treatment station; RD: Reservoir of Damietta treatment station; ED: End of network of Damietta treatment station; Al: Aluminum; Cd: Cadmium; Fe: Iron; Ni: Nickel; Pb: Lead; Mn: Manganese; Zn: Zinc; Cu: Copper; Cr: Chromium; Co: Cobalt; UDL: Under detection limit.

The highest value of chromium was found in SD site and was 0.014 ppm, while the lowest value was in RD site and was 0.001 ppm. Cobalt was not detected in all sites that examined during winter 2018 (all results were UDL<0.001 ppm).

Heavy metals (mainly Iron; Fe &Cupper; Cu) were almost within the permissible limits (1 mg/L) according to the Egyptian Law48/1982. Concentration of lead (Pb) and cobalt (Co) were however higher than the permissible limit (0.05 and 0.05 mg/L) of Canadian water quality guidelines for the protection of aquatic life (CWQGs) and FAO (1985). Zinc experienced many peaks during the period of study that exceeded the permissible limits (1 mg/L). Heavy metals are normally added to fresh water streams, rivers and ponds, deliberately as components of herbicides or as by-products of different human activities (Elham et al. 2014).

Minerals values

Data in Table 5 showed that, the highest mean values of calcium were recorded in SeA and SeD sites being 42.4 ppm, while the lowest value was in RA, EA, RD and ED sites and was 40.8 ppm. In case of magnesium, the highest value was in SeA and SeD sites and was 16.8 ppm, while the lowest value was found in RA, EA and ED sites and was 15.36 ppm.

In case of ammonia, the highest value was found in SA site and was 0.41 ppm, while the lowest values was in FA, RA, EA, FD, RD and ED sites and was UDL<0.01 ppm. Nitrite recorded the highest value in SeD site and was 0.026 ppm, while the lowest value was found in FA, RA, EA, FD, RD and ED sites and was UDL<0.005 ppm.

The highest value of silica was in FA site and was 1.34 ppm, while the lowest value was found in SD site and was 1.13 ppm. Obtained results of ammonia and nitrite in a good agreement with the Egyptian Standard No. 458/2007, according to EOG, (2007).

The highest mean value of phosphates was in SeA site and was 0.042 ppm, while the lowest value was in RA and ED sites and was 0.031 ppm. The highest value of sulphate was in RA and ED sites and was 45.5 ppm, while the lowest value was in SeA site and was 32.5 ppm.

The highest value of fluoride was in SeD site and was 0.464 ppm, while the lowest value was in RA and ED sites and was 0.418 ppm.

Table 5. Minerals values (ppm) of water taken from Damietta City during winter 2018

S P	SA	SeA	FA	RA	EA	SD	SeD	FD	RD	ED
Ca	41.6	42.4	41.6	40.8	40.8	41.6	42.4	41.6	40.8	40.8
Mg	15.84	16.8	15.84	15.36	15.36	16.32	16.8	16.32	15.84	15.36
NH3	0.41	0.38	UDL	UDL	UDL	0.38	0.38	UDL	UDL	UDL
NO2	0.019	0.024	UDL	UDL	UDL	0.023	0.026	UDL	UDL	UDL
Si	1.18	1.16	1.34	1.28	1.28	1.13	1.16	1.25	1.24	1.28
P	0.036	0.042	0.035	0.031	0.036	0.035	0.039	0.034	0.032	0.031
SO4	33.4	32.5	36.8	45.5	40.5	38.4	34.8	42.5	38.5	45.5
F	0.427	0.451	0.443	0.418	0.427	0.431	0.464	0.457	0.426	0.418
Na	46	52	46	41	43	47	51	44	42	41
K	6.86	7.19	7.07	6.71	6.86	6.94	7.21	7.13	6.81	6.71

S: Sample; P: Parameter; SA: Source of El-Adlyah treatment station; SeA: Sedimentation stage of El-Adlyah treatment station; FA: Filtration stage of El-Adlyah treatment station; RA: Reservoir of El-Adlyah treatment station; EA: End of network of El-Adlyah treatment station; SD: Source of Damietta treatment station; SeD: Sedimentation stage of Damietta treatment station; FD: Filtration stage of Damietta treatment station; RD: Reservoir of Damietta treatment station; ED: End of network of Damietta treatment station; Ca: Calcium; Mg: Magnesium; NH3: ammonia; NO2: Nitrite; Si: Silica; P: Phosphates; SO4: Sulphate; F: Fluoride; Na: Sodium; K: Potassium; UDL: Under detection limit.

The highest value of sodium was in SeA site and was 52 ppm which in a good agreement with (200 ppm) the Egyptian Standard No. 458/2007, according to EOG, (2007), while the lowest value was in RA and ED sites and was 41 ppm. The highest value of potassium was in SeD site and was 7.21 ppm, while the lowest values were in RA and ED sites and was 6.71 ppm.

CONCLUSION

Obtained results proved that the investigated viral types including Poliovirus type 1, Rotavirus, HAV type IB, and H9N2 avian influenza (influenza A/H9N2 subtype) were absent in all examined samples during winter 2018. The highest mean value of total bacteria was in SD site (251.333 cfu/ml), the highest mean value of total fungi was in SA site (112.667 cfu/ml). The SA and SD sites contained the highest algal quantities in examined samples (motile and non-motile species) and there was no protozoal species in all examined sites except SA and SD sites (few numbers of motile ones). The highest values of aluminum, cadmium, iron, nickel, lead, manganese, zinc, copper and chromium were in FD, FA, SD, SD, EA, SA, SD, SeA and SD sites (0.13, 0.019, 0.117, 0.054, 0.046, 0.030, 0.387, 0.007 and 0.014 ppm, respectively). Cobalt was not detected in all sites that were examined during winter 2018 (UDL<0.001 ppm). The highest values of calcium and magnesium were in SeA and SeD sites (42.4 and 16.8 ppm, respectively). The highest values of ammonia, nitrite, silica, sulphate, phosphates, fluoride, sodium and potassium were in SA, SeD, FA, (RA and ED), SeA, SeD, SeA and SeD sites (0.41, 0.026, 1.34, 45.5, 0.042, 0.464, 52 and 7.21 ppm, respectively). So, these results proved that water sources are in agreement with the ES for using of this water for human purposes.

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فحوص ميكروبيولوجية و كيميائية لبعض مصادر المياه بمدينة دمياط خلال شتاء 2018

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أجرى هذا البحث بمعمل الميكروبيولوجيا، بقسم الميكروبيولوجيا الزراعية، كلية الزراعة، جامعة دمياط، دمياط، مصر. تم جمع العينات من مواقع مختلفة فى مدينة دمياط لعمل الفحوصات الميكروبيولوجية، الفيزيائية والكيميائية خلال فصل الشتاء لعام 2018. نتائج الفحص الميكروبيولوجى أظهرت أن الاختبارات التى اجريت على الفيروسات المسماه بالبوليو فيروس (فيروس شلل الاطفال)، الروتا فيروس ، فيروس الالتهاب الكبدى الوبائى أ و فيروس انفلونزا الطيور ه9ن2 اظهرت عدم وجود ايا منها خلال شتاء 2018. كما أظهرت أن أعلى قيم لمتوسط العد الكلى للبكتريا والعد الكلى للفطريات كانت فى موقع مأخذ محطة دمياط و مأخذ محطة العدلية وكانت 251.333 و112.667 وحدة مكونة للمستعمرة/ملليتر على التوالى. فى حين لم يكن هناك أى نمو على بيئات الماكونكى السائلة، بيئة السالمونيلا شيجيلا و بيئة الاستاف-110. وقد أظهرت الطحالب فى مواقع مأخذ محطتى العدلية ودمياط أعدادا كبيرة ومنها المتحرك وغير المتحرك. لم يكن هناك أى بروتوزوا فى العينات المأخوذة خلال هذا الفصل ماعدا مواقع مأخذ محطتى العدلية ودمياط والتى كانت بأعداد قليلة ومتحركة. النتائج الحالية أظهرت ان بعض الاختبارات الفيزيوكيميائية كانت، يتراوح تركيز أيون الهيدروجين مابين 7.46 فى موقع فلترة محطة العدلية و7.96 فى موقع مأخذ محطة دمياط. اختلفت درجة الحرارة مابين 21 درجة فى موقع نهاية شبكة محطة العدلية و 22.4 درجة فى موقع مأخذ محطة دمياط. أعلى قيم للاحتياج الكيميائى للاكسجين والاحتياج الحيوى للاكسجين كانت فى مواقع ترسيب محطة العدلية ومأخذ محطة دمياط وكانت 9.6 و18.9 مليجرام اكسجين/لتر على التوالى. فى حالة المعادن الثقيلة النتائج أظهرت، أعلى قيم للألومنيوم، الكادميوم، الحديد، النيكل، الرصاص، المنجنيز، الزنك، النحاس والكروم كانت فى مواقع فلترة محطة دمياط، فلترة محطة العدلية، مأخذ محطة دمياط، مأخذ محطة دمياط، نهاية شبكة محطة العدلية، مأخذ محطة العدلية، موقع مأخذ محطة دمياط، ترسيب محطة العدلية ومأخذ محطة دمياط وكانت 0.13، 0.019، 0.117، 0.054، 0.046، 0.030، 0.387، 0.007 و 0.014 جزء فى المليون على التوالى. لذا، تلك النتائج اوضحت ان مصادر المياه فى توافق مع المواصفات المصرية لاستعمال المياه للاستخدام الآدمى.