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Original Article

Biological Assessment of Water Toxicity and Fertility of Wastewater Omer Bek Drain Discharging into Receiving Water of the River Nile in Egypt

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Article Info	Abstract

Water toxicity and fertility of a drain wastewater discharging wastewater directly to the Damietta branch of the River Nile were seasonally investigated from winter to autumn, 2016. Nutrient enrichment bioassays to define the algal growth limiting nutrients, their bioavailability and heavy metal toxicity of the sampled water, were carried out with the ISO standard test alga *Pseudokirchneriella subcapitata*. The algal growth potential (AGP) test exhibited a wide range of growth potential ranging from 0.4 mg.l^{-1} to 19.42 mg.l^{-1} algal dry weight. At drain, mixing and recovery stations the algal growth was mainly limited by heavy metals toxicity with inhibition percent at day 14 of growth (%I$_{14}$) ranged between 27.9 and 58.6 while at the upstream reference station, %I$_{14}$ ranged between 20 and 37.The algal growth was also limited by N and P depending on site and season. In all seasons, but only at drain station, the expected Chl. a was significantly higher than the observed Chl. a, however, the reverse was held true for other sampling stations during this study. The relationships between the chemically analyzed nutrients (P and N) and their bioavailable concentrations were greatly affected by heavy metal toxicity. The results indicated that the observed chlorophyll a showed significant correlations with TDP(r=0.82), while the expected chlorophyll a correlated significantly with NH$_4$-N(r=0.73). The algal growth potential test proved a sensitive and reliable mean to assess water fertility and toxicity of polluting drains and the receiving waters.

1. Introduction .

For decades, the algal growth potential test(AGPT) has been evolved and accepted as a standard method for the determination of bio availability of nutrients (N and P) for microalgae and assessing the fertility and metal toxicity of different waterbodies all over the world (Skulbreg,1967 & 1975; EPA, 2002; Miller *et al*., 1978 ; Forsberg *et al*. 1978; Källqvist,1975 & 1984; Golterman, 1983; Schultz, 1985; Raschke,1987, Abdel-Hamid *et al*., 1992,1993, Lópeza and Dávalos-Lind 1998; Horvatic´*et al*. 2011&2013; Dávalos-Lind *et al*.,2013).The AGPT defined as the highest dry weight content reached under specific conditions which reflect the nutrient content of water sample and follows the premise that, the nutrient that occurs in smallest amount is the limiting factor to the growth of the test alga *Pseudokirchneriella*

subcapitata (Miller *et al*., 1978; EPA, 1978; Kviderova, 2010).Since chemical analyses alone cannot reveal the overall effects of dissolved inorganic compounds on living photosynthetic biomass. The significance of measuring algal growth potential in water samples reveals correctly the difference between the nutrients of a sample determined by chemical analysis and the nutrients that are actually bioavailable for algal growth and the addition of given nutrients to the water sample indicate which nutrient is limiting for algal growth (Miller *et al*., 1974; Lukavsky, 1992; McCormick& Stevenson 1998; EPA, 2002).

Algal growth potential (AGP) tests and limiting-nutrient assays are the most direct and effective methods for the assessment of a receiving water to determine its trophic status and sensitivity to changes in N and P loading, in addition to heavy metal toxicity. (Miller *et al*., 1974; Raschke and Schultz, 1987; Abdel-Hamid *et al.,* 1992; Lukavsky, 1992; Lopez and Davalos-Lind, 1998; Shin and Cho, 2000;Kuang*et al*., 2004; Millican *et al*., 2008)

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The selective addition of nitrogen and phosphorus singly or in combination, and the addition of EDTA to the sampled water can indicate which nutrient(s) is possible limiting for algal growth, and also determine the magnitude of heavy metal inhibition to the growth of the standard test alga (Mille *ret al.,* 1974; Greene *et al.,* 1975; Payne, 1976; Gerhold, 1976; Greene *et al.,* 1976; Donald *et al.,* 2013).In Egypt the AGP-test has been developed and employed in water quality studies since 1976 (Fayed and Shehata, 1980) and was applied mainly for assessing the fertility and toxicity of the River Nile (Zahran*et al.,* 1988; Abdel-Hamid, 1992). The present paper aims to correlate the algal growth potential (AGP) with P and N concentrations to evaluate limiting nutrient(s) of revering ecosystems receiving wastewater, and to assess the heavy metal toxicity.

2. Material and Methods

2.1. Study area

The main River Nile downstream of Delta (El-Qanater) Barrages divides into two branches, the eastern(Damietta) and the western (Rosetta) branch. Damietta branch begins at the Delta Barrage and ends220km downstream at Faraskour dam near Damietta. It receives excessive wastewater discharges from Omar Bek drain (Figure1).This drain, located in El-Gharbia governorate, starts from Zefta City and ends at Samanoud City. The wastewater is mainly composed of untreated sewage, industrial wastewater; agricultural wastewater discharged from urban areas located lengthwise the drain. The geographical location of study area is shown in (Figure 1).
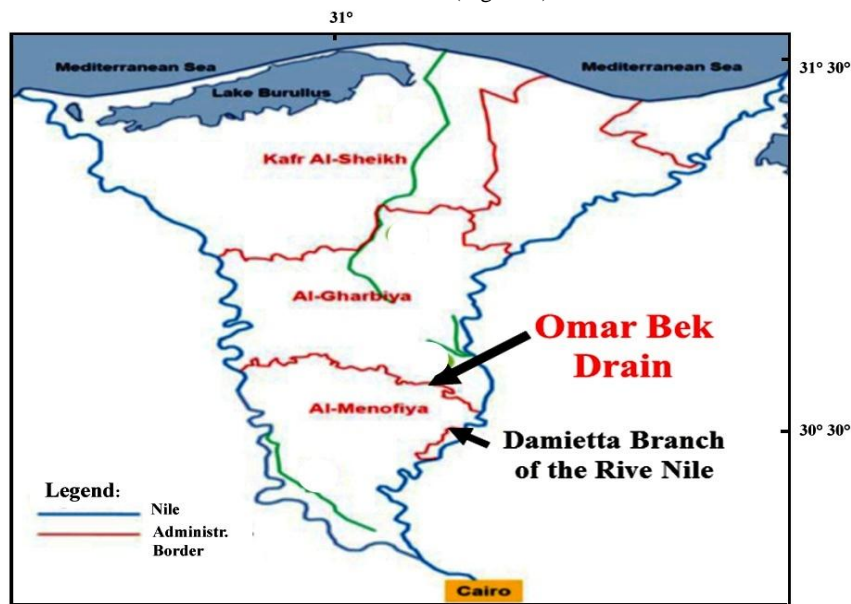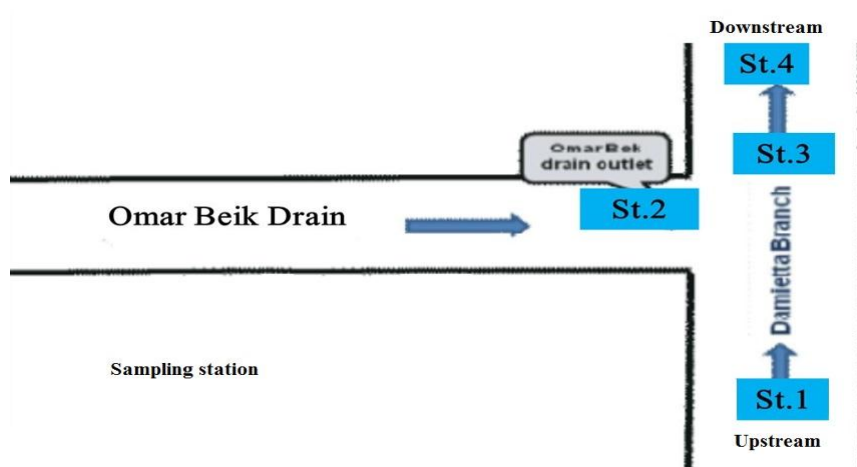

Fig.1. The geographical location of study area.

Fig.2. Schematic diagram illustrating four sampling stations

Table 1. Wastewater and water sampling stations

Sampling stations	Description
Station1 (Reference station)	It is a riverinestation, located 300 m on upstream of the outlet of the drain.
Station2	Located 20 meter before the outlet of Omar-Bek drain.
Station3	Located 2 meter downstream the outlet of Omer Bek where wastewater is mixed with Nile fresh water.
Station4	Located at 600 m downstream of the outlet of the drain.

2.2 Sampling

Water samples were seasonally collected during the period from mid-winter–mid-autumn. Sample collection, handling, and processing were conducted according to EPA (1985).

2.3 Sample preparation

Upon arrival to the laboratory, water samples were mixed. Six liters were filtered through GF/C What man glass filters. The first one liter filtrate was discarded and five liters were stored at 4 $^{\circ}$C in dark until used for different analyses.

2.4 Preparation of glassware

Glass wares were washed with non-phosphate detergent, flushed thoroughly with tap water, rinsed with 10% (v/v) HCl solution, neutralized by swirling with a saturated sodium carbonate solution, rinsed five times with tap water followed by five rinses with deionized distilled water, dried at 50 $^{\circ}$C, plugged with cotton stoppers and stored in a closed cabinets until needed.

2.5 Chemical analysis

Unless otherwise stated, chemical analysis followed standard respective methods as described in APHA (1989 and 2005).The Analysis included total dissolved phosphorus (TDP), dissolved reactive phosphorus (DRP), Nitrate - N, ammonium- N, and Nitrite - N was determined using the method described by (Barnes and Folkard,1951) and (Dewis and Freitas,1970). Analysis of the heavy metals Cd, Cu, Fe, Pb and Zn was achieved by the direct aspiration into an air-acetylene flame using atomic absorption spectrophotometer type BUCK Scientific Atomic Absorption SPECTROPHOTOMETER model ACCUSYS 211 (APHA, 1989)

2.5 Algal growth potential test (AGPT)

The test followed (Miller *et al*., 1978) with green micro algae *Pseudokirchneriella subcapitata* Strain NIVA-CHL 1, as the standard test alga(ASTM,2004). This strain was obtained from the culture collection of the Norwegian Institute for Water Research (NIVA), Oslo, Norway.

In order to determine nutrient limitation and heavy metal toxicity, calculated amount of phosphorus, Nitrogen and Na_2 EDTA were added to the GF/C filtered water samples in various concentrations according to a scheme shown in (Table 2). The test alga was grown for five days in standard Algal Assay Medium (AAM), (Mille *ret al*., 1978). Algal inoculum was prepared by centrifuging 100 ml of algal suspension at 1000 rpm for 5 minutes. The supernatant was, decanted and the residue was resuspended in sterilized distilled water. This step was repeated twice. The inoculum was left to stand for 2 hours. Three replicate culture flasks were used for each treatment listed in (Table2). The flasks were inoculated with the test alga to obtain 1000 cells ml^{-1} as final algal density. To avoid carbon dioxide limitation, the sample to flask volume ratio was kept in the range of 1:5. Flasks were incubated at 24 $\pm$ 2°C for days and continuous illumination with cool white fluorescent tubes at 4304 $\pm$ 10% lux (USEPA, 1978). The light intensity was measured adjacent to the culture flasks at the liquid level. To ensure free gas exchange, the culture flasks were plugged with cotton stoppers and shaken by hand once every day during the incubation period.

Table 2.Basic experimental design to determine nutrient limitation or algal growth potential test(AGPT)

Treatments
Control(GF/C filtered wastewater)
Control + 0.05 mg P l^{-1} as K_2HP0_4
Control + 1.00 mg N l^{-1} as $NaN0_3$
Control + 0.05 mg P l^{-1} + 1.00 mg N l^{-1}
Control + 1.00 mg Na_2 EDTA l^{-1} as Disodium Ethylene diaminetetraacetic acid
Control + 0.05 mg P l^{-1} + 1.00 mg Na_2 EDTA l^{-1}
Control + 1.00 mg N l^{-1} + 1.00 mg Na_2 EDTA l^{-1}
Control + 0.05 mg P l^{-1} + 1.00 mg N l^{-1} + 1.00 mg Na_2 EDTA l^{-1}

2.5.1. Growth parameter

The incubation period of days was found to be enough for the test alga to attain its maximum standing crop (MSC)(Miller *et al*, 1978). At the end of the incubation period, the algal cells were counted using heamocytometer with inverted microscope. The mean cell volume was calculated with a proper geometric equation .The dry weight of algal biomass was calculated according to the following formula (Kallqvist, 1990).

mg dry weight *P.subcapitata*l^{-1}= cell counts (cells ml^{-1}) ×MCV(cubic micrometers)×(3.6×10^{-7})

2.5.2 Determination of available P and N for algal growth

The bioavailable phosphorus (BAP mg l^{-1}) for the growth of *Pseudokirchneriellasubcapitata*, was derived by dividing the growth yield (MSC) obtained with 1.00 mg N l^{-1} by the phosphorus yield coefficient (430), whereas, bioavailable nitrogen (BAN mg l^{-1}) was determined by dividing the growth yield obtained with 0.05 mg P l^{-1} by the nitrogen yield coefficient equal to(38)(Miller *et al*., 1978)

2.5.3 Determination of the expected chlorophyll a biomass

The expected chlorophyll a represents the maximum mean corrected chlorophyll a biomass that the wastewater or water sample can support under optimum growth conditions. It was calculated according to the equation ($\log_{10}$ Chl. a = 1.15 $\log_{10}$ (dry weight) + 0.95), proposed by (Raschke & Schultz, 1987).

2.5.4 Determination of the observed or actual chlorophyll a

The observed chlorophyll a represents the algal standing crop biomass at the time of sampling. One liter was filtered onto GF/C filters. The filters were ground using tissue grinder in presence of cooled 90% acetone and kept in dark for 12 hours at 4 °C for extraction. Spectrophotometric determination of chlorophyll a was carried out according to the trichromatic method described in (APHA, 1985).

2.5.5 Assessment of heavy metal toxicity

The heavy metal toxicity was reported as the percent (%) of inhibition at 14 day (% I$_{14)}$ based on the difference in mg algal dry weight l^{-1} obtained in EDTA treated and control cultures.

$$\%I_{14} = \frac{MSC(C+EDTA)-MSC(C)}{MSC(C+EDTA)} \times 100$$

2.6. Statistical analysis

A proposed percent coefficient of variance guideline (Table3), which is basically adopted from (Miller *et al*., 1978) and modified by (Abdel-Hamid *et al*., 1992), was used to a certain whether or not the differences obtained in algal maximum standing crop (MSC), between different treatments and the control cultures, are statistically significant. Regression and multiple correlation analysis were carried out using STATGRAPHICS (STSC, ver 4.2) programmer. The correlation coefficient differed significantly from zero at $p < 0.05$.

Table3. A proposed % coefficient of variance guideline to ascertain whether or not the differences obtained in algal maximum standing crop (MSC) between different treatments and control cultures are statistically significant (adopted from MILLER *et al* ., 1979, and modified by Abdel-Hamid *et al*., 1992).

MSC of control cultures	% C.V	Statistical difference	Designation in text
< 1.0 mg dry wt/l	±50 - ±75	Significant	*
	±75 -±100	Highly significant	**
	>±100	Very high significant	***
>1.0 mg< 3.0 mg dry wt/l	±30 -± 45	Significant	*
	±45- ±60	Highly significant	**
	>±60	Very high significant	***
>3.00 mg< 10.0 mg dry wt /l	±20 - ± 30	Significant	*
	±30 - ±40	Highly significant	**
	>±40	Very high significant	***
> 10 mg dry wt/l	±10 - ± 15	Significant	*
	±15 - ±20	Highly significant	**
	>±20	Very high significant	***

% C.V is the percent coefficient of variance = sample standard deviation /mean ×100

3. Results

3.1. Algal growth potential (AGP)

The results of AGP (Figures. 3, 4, 5 & 6) are reported as the mean maximum standing crop (mg dry wt l^{-1}) of the test alga grown for days at different treatments (Table 2),under optimum growth conditions. The algal growth potential showed marked local and seasonal variations. It exhibited a wide range of growth potential ranging between 0.4 mg.l^{-1} and 19.42 mg.l^{-1} of algal dry weight. From the visual inspection of AGP figures (Figures 3-6) the growth of the test alga showed a number of distinct responses of the test alga towards different water qualities and treatments including the following:

1) The GF/C filtered wastewater collected from the drain station 2 supported the highest maximum standing crop (MSC) of tested alga (7.1- 9.9mg dry wt l^{-1}) followed by samples collected from the mixing zone (station 3) where wastewater mixes with freshwater (0.6-3.7 mg dry wt l^{-1}). Followed by the water sample collected from station 4(proposed recovery station) (0.93 -2.5 mg dry wt l^{-1}). The least MSC was generally supported by water samples collected from the upstream reference station (0.4 to 0.9 mgdrywtl^{-1})

2) Compared to the control cultures, the EDTA treatments resulted in an significant increase in MSC, suggesting the role of heavy metal toxicity or bioavailability as growth limiting factors (Figures 3a,4a,5a and 6a)

3) For most cases, the addition of P caused a significant increase in MSC indicating P limitation conditions of the wastewater and water samples in all station expect drain station2.

4) The increase in algal biomass, due to the combined addition of P and EDTA, was significantly higher than in case of single P addition. This finding indicates that the growth, of the test alga, was primarily limited by heavy metal toxicity and secondarily by P (Figures 3b, 4b, 5b and 6b).

5) The addition of N, singly or in combination with EDTA, to wastewater samples collected from station2 , resulted in a remarkable and significant increase in algal biomass (Figures 3c,4c,5c and 6c).In this particular case (station 2), the AGP was primarily limited by heavy metal toxicity and secondarily by nitrogen.

6) Compared to control cultures the combined P and N additions caused significant increase in algal biomass (Figures 3d,4d,5d and 6d). Generally, the combined additions of P&N and EDTA supported the maximum standing crop of test alga, in almost cases.

3.2. Relationship between chemically analyzed nutrients (P & N) and their bioavailable concentrations.

Table 4 lists the local and seasonal variations of TSIN and TDP along with their bio-available concentrations during the period of study. It is clear that the sampling station 2 (waste water discharged from Omar Bek drain into the River Nile) contained the highest concentrations of ammonia-N, Nitrate–N, Nitrite-N and total dissolved phosphorus (TDP) discharged into the downstream receiving water of the River Nile. Quantitative information about the relationship between the nutrients and their bioavailable concentrations, in presence and absence of heavy metal toxicity, are presented in (Table5) indicate that the highest concentration of BAN and BAP in N- limitation condition. Remarkable local and seasonal variations did exist in ratios between the chemically analyzed nutrients and their bioavailable concentrations calculated from the results of the algal growth potential test. For instance during summer the % BAP/TDP were (1.4 and 8.57) and %BAN/TSIN were 3.2 and 17.65 at station 1(Ref.) and station 2, respectively. These ratios at both stations exhibited clear seasonal variations. One clear observation was that, in almost all cases the addition of Na$_2$ EDTA to test culture resulted in obvious increase in bioavailability of both N and P, but with remarkable local and seasonal variations were recorded. For instance, only 10 % of the total dissolved phosphorus was available for the test alga grown in filtered water sample collected from station 4 during spring, however, upon EDTA addition the % BAP/ TDP, of the same water sample, increased to 20 (Table 5). Also, the %BAN/TSIN of the water sample, collected from station 2 (drain) during autumn, was 12.57 and 23.27 before and after EDT Aaddition respectively.
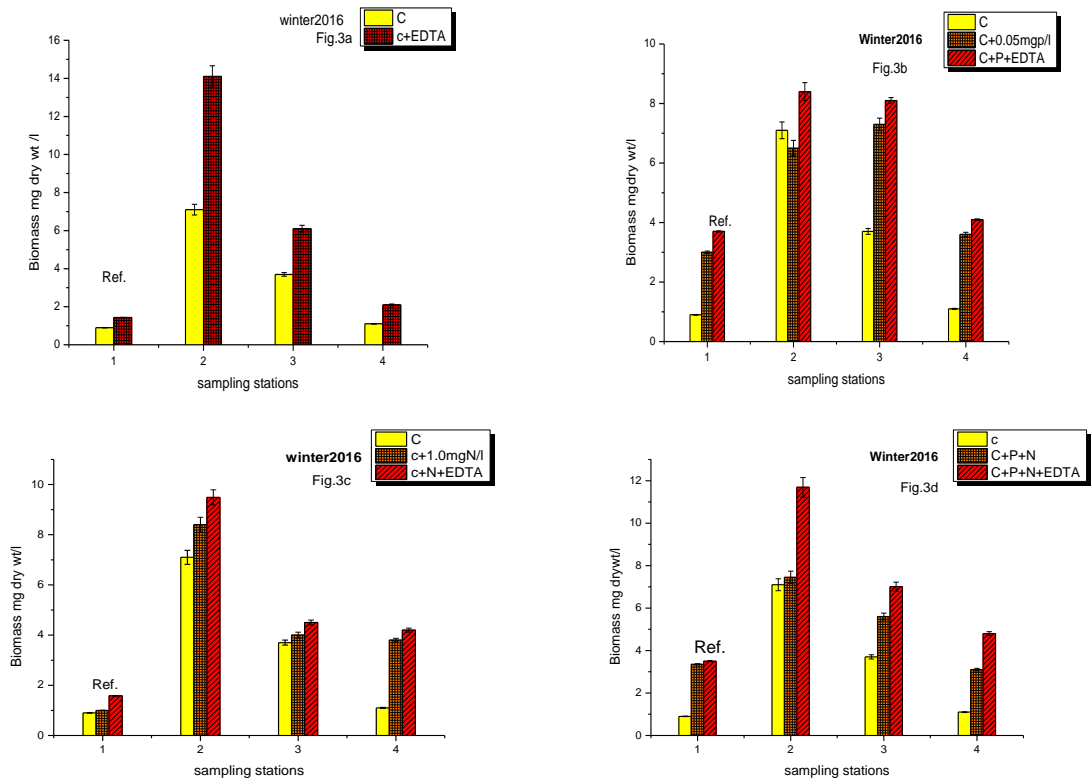
Fig.3. Algal growth potential of the study area during mid -winter (2016).
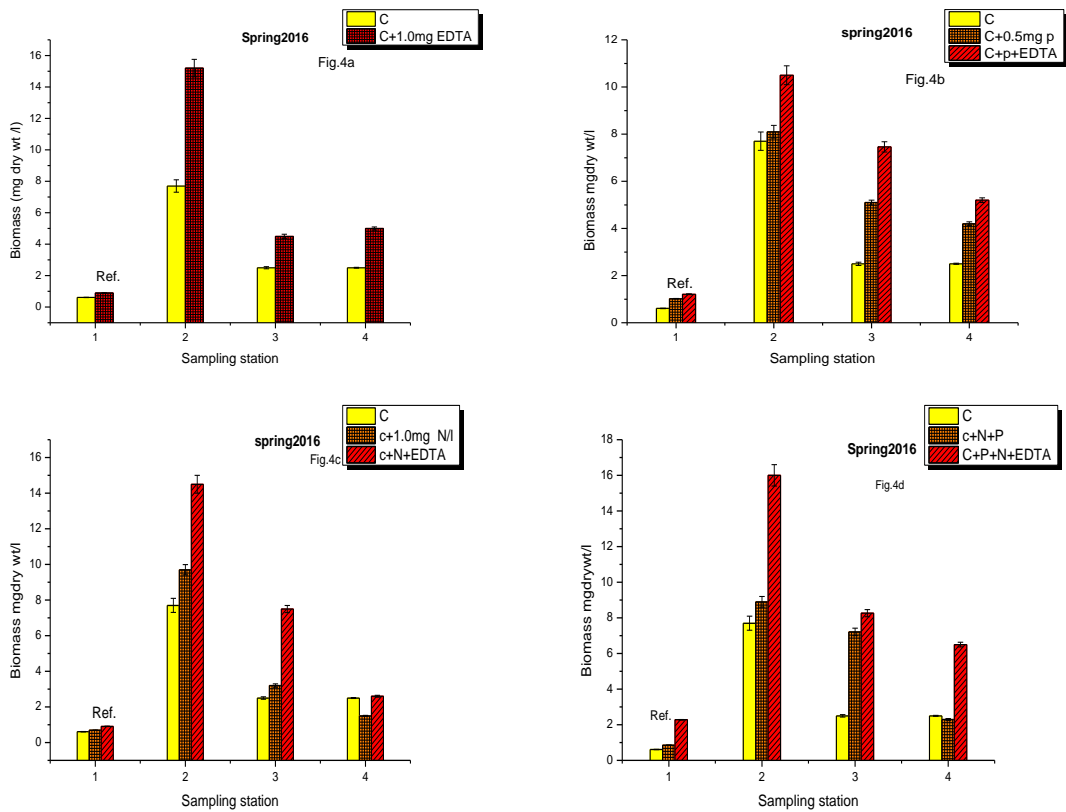

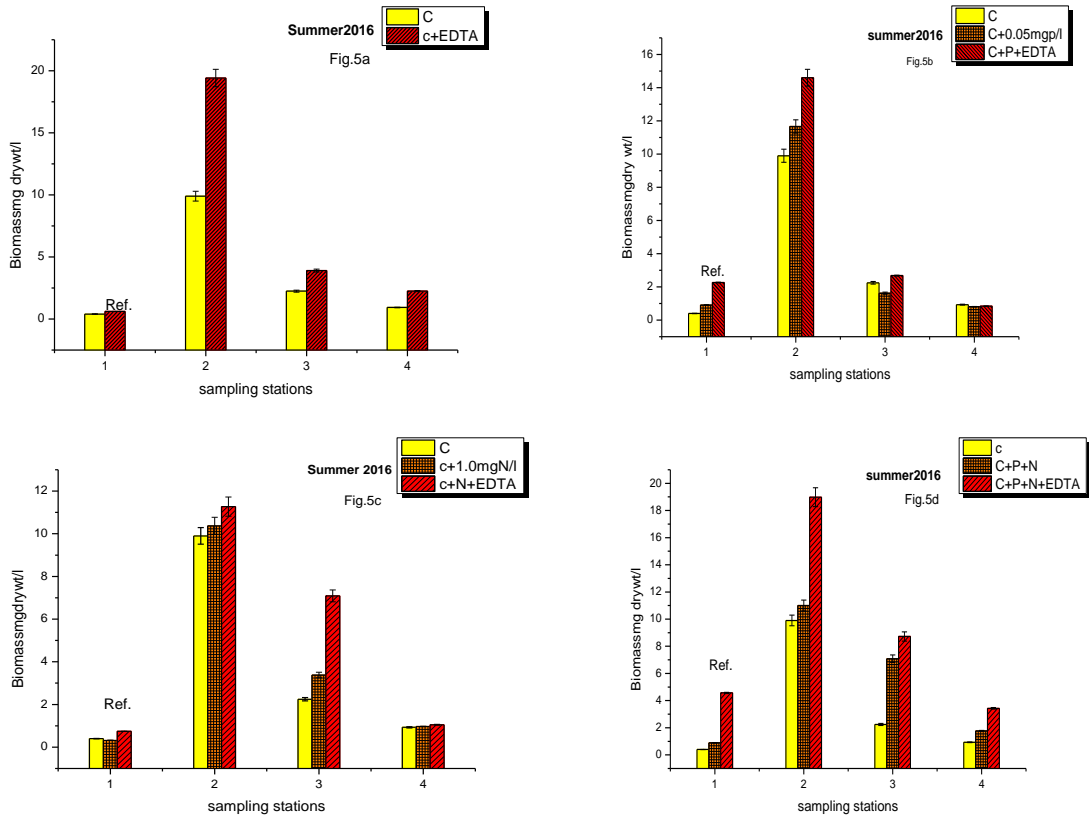**Fig. 4.** Algal growth potential of the study area during mid -spring (2016)

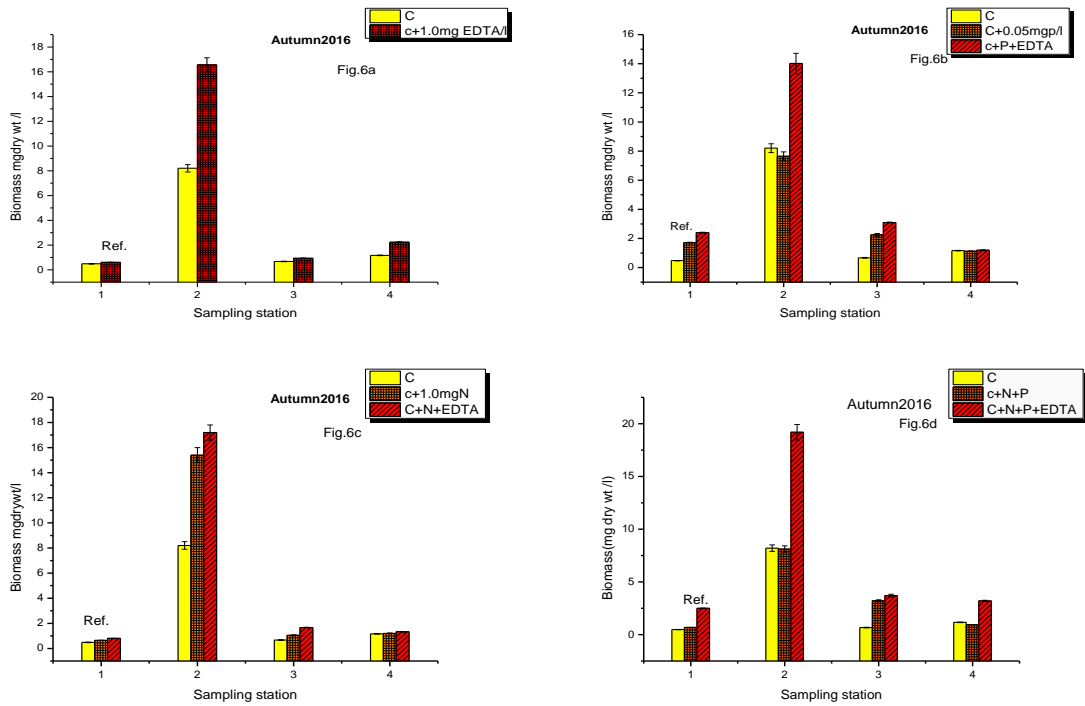**Fig. 5.** Algal growth potential of the study area during mid -summer (2016).

Fig. 6. Algal growth potential of the study area during mid-autumn 2016.

Table 4. Local and seasonal variations in concentration (mgl^{-1}, Mean ± SD) of nitrite-N, nitrate-N, ammonium–N (collectively known as total soluble inorganic nitrogen) and total dissolved phosphorus (TDP) and their bioavailable concentration.

Parameter	Stations			
	1(ref)	2	3	4
Winter				
NH4-N	0.2±0.01	1.17± 0.05	1.05 ± 0.04	0.3± 0.009
NO2-N	0.035 ±0.01	0.06± 0.003	0.05 ±0.002	0.04± 0.001
NO3-N	0.22± 0.004	0.5± 0.025	0.26±0.01	0.25±0.007
TSIN	0.45 ±0.009	1.73± 0.08	1.36± 0.05	0.59± 0.017
TDP	0.047± 0.001	0.57± 0.03	0.32 ±0.01	0.08± 0.002
BAP	0.0023 0.001	0.019 0.001	0.009 0.01	0.008± 0.0002
BAN	0.07± 0.00	0.17± 0.0008	0.19± 0.007	0.09± 0.003
Spring				
NH4-N	0.21± 0.004	0.74± 0.04	0.65± 0.03	0.4± 0.01
NO2-N	0.03± 0.006	0.04 ±0.002	0.038± 0.002	0.036 ±0.001
NO3-N	0.05 ±0.001	0.24± 0.01	0.06± 0.002	0.058± 0.002
TSIN	0.3 ±0.006	1.02± 0.05	0.72± 0.03	0.49± 0.01
TDP	0.024± 0.001	0.59± 0.03	0.23± 0.009	0.03 ±0.002
BAP	0.0016± 0.0001	0.022± 0.001	0.007 ±0.0003	0.003± 0.0002
BAN	0.026 ±0.001	0.21 ±0.01	0.13 ±0.005	0.11± 0.003
Summer				
NH4-N	0.24± 0.004	1.07± 0.05	0.92± 0.03	0.27 ±0.008
NO2-N	0.01± 0.001	0.016± 0.001	0.008± 0.003	0.015± 0.0004
NO3-N	0.45 ±0.009	0.61± 0.03	0.53 ±0.02	0.48± 0.01
TSIN	0.7± 0.0	1. 7± 0.08	1.46± 0.05	0.76± 0.02
TDP	0.06± 0.001	0.28± 0.01	0.16± 0.006	0.06± 0.001
BAP	0.0007± 0.0001	0.024± 0.001	0.007± 0.0003	0.002 ±0.0006
BAN	0.023± 0.0004	0.3 ±0.01	0.04± 0.00	0.02± 0.0006
Autumn				
NH4-N	0.13± 0.003	0.82± 0.04	0.36± 0.01	0.76± 0.02
NO2-N	0.015± 0.0003	0.02 ±0.001	0.008± 0.0003	0.018± 0.0005
NO3-N	0.24± 0.005	0.75 ±0.03	0.27 ± 0.01	0.28± 0.008
TSIN	0.4 ±0.008	1.59± 0.07	0.54± 0.02	1.06 ±0.03
TDP	0.036 ±0.0007	0.32± 0.02	0.072 ±0.003	0.049± 0.001
BAP	0.00± 0.0001	0.035± 0.002	0.0024± 0.0001	0.0027± 0.0002
BAN	0.04 ±0.0008	0.2± 0.01	0.06 ±0.002	0.03 ±0.0009

Table5. Quantitative relationship between the chemically analyzed nutrients (TDP and TSIN), and their bioavailable concentrations.

Ratio	Seasons															
	Winter				Spring				Summer				Autumn			
	1(ref)	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
%BAP/TDP	4.8	3.3	2.7	9.1	6.6	3.7	3.04	10	1.4	8.6	4.4	3.3	3.8	10.9	3.4	5.5
%BAP.EDTA/TDP	7.8	3.8	3.2	10	8.3	5.6	7.4	20	3.4	9.3	10	4.0	5.0	12.5	5.5	6.3
%BAN/TSIN	17.5	9.8	13.9	15.2	8.6	20.6	18.5	23.1	3.2	17.6	2.7	2.6	10	12.6	11.1	2.8
%BAN.EDTA/TSIN	21.6	12.7	15.6	18.3	10.6	26.4	26.3	28.7	8.5	22.3	4.7	2.9	15	23.3	15.1	2.9

1=upstream reference station; 2=Drain (wastewater); 3=Mixing zone; 4=Downstream recovery station

3.3. Assessment of heavy metal toxicity

The effects of heavy metal toxicity on algal growth was reported as % growth inhibition at 14 day (% I_{14}) based on the difference in mg algal dry weight l-1 obtained in EDTA treated and control cultures (Table 6). As shown from Table 6, the % I_{14} displayed remarkable significant (p ≤ 0.05)

variations at different sampling stations and seasons with values ranged between (20% -58.6%),where the lowest value expressing growth inhibition due to metal toxicity was observed at the upstream reference station during autumn and the highest value was recorded at station 4 during summer (Table 6).

Table6. The percent inhibition at day (%I) based on difference in mg dry wt /l obtained in EDTA treated and control cultures

Station	Algal growth inhibition (% I_{14})			
	Season			
	winter	spring	summer	autumn
1(Ref.)	37	23.7	34.4	20
2	49.6	49.34	49	50.4
3	39.3	44	42.5	27.9
4	47	50	58.6	47.9

3.4. The relationship between observed and expected chlorophyll a

Figure 7; illustrate both local and seasonal variations in concentration of both observed and expected chlorophyll a. It was clear from Figure 7 that relatively higher concentrations of both observed and expected chlorophyll a were exclusively recorded at station 2. Substantially very highly significant (p≤ 0.01) increase in expected chlorophyll a than observed Chl. awas only noticed at station 2 in all seasons, however, is reverse was held true for other stations (Figure 7). Also, as shown in Figure 7 the observed chlorophyll a fluctuated between 6.84 mgl^{-1} (at station 4 during spring) and47.43 mg.l^{-1} (at station 2 during spring), While the expected chlorophyll a varied greatly between 3.1mg.l^{-1} (at station 1 during summer) and 100 mgl^{-1}(at station 2 during autumn).

3.5. Statistical analysis

The symbolic presentation of % coefficient of variance in Table7, which lists the difference in algal maximum standing crop (MSC) between different treatment and control cultures. It is obvious that the growth of the test alga mainly limited by heavy metal toxicity particularly at drain water station (2),

mixing zone station (3) and downstream recovery station (4), while there was not significant heavy metal limited to the growth of test alga at upstream reference station (1). Also for most cases the growth of test alga was limited by P followed by N depending on sampling stations and seasons. However, usually the combined addition of P,N, and EDTA enhanced highly significant growth of test alga at all station in all seasons.

The result of correlation between chemically analyzed nutrient, their bioavailable and expected chl. a, observed chl. a (Table 8) indicate that total soluble inorganic nitrogen (TSIN) was mainly composed of NH_4-N as the two parameter showed a highly significant correlation (r=0.93).The bioavailable nitrogen (BAN) correlated significantly (p≤0.05) and strongly with ammonium-N (r=0.65) and TSIN (r=0.61). The bioavailable phosphorus (BAP) maintained significant correlation with TDP(r=0.75) and BAN(r=0.81). The observed Chl.a showed significant correlation with TDP (r=0.82), while the expected chl. a correlated significantly with NH_4-N (r=0.73), TSIN (r=0.79), TDP(r=0.86).
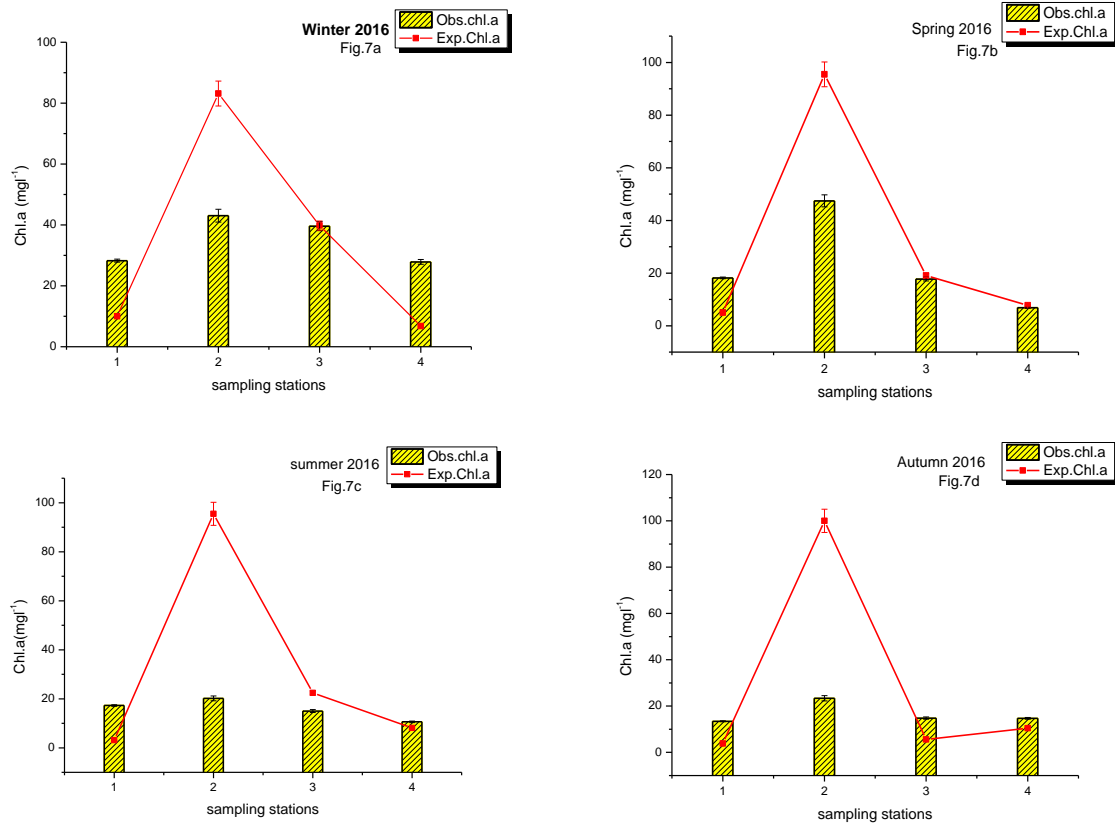
Fig.7. The relationship between the observed (Phytoplankton) and the expected (AGP) chlorophyll.

Table 7. Differences in algal maximum standing crop (MSC) between different treatments and control cultures

Stations	C+P				C+N				C+P+N				C+EDTA				C+P+EDTA				C+N+EDTA				C+P+N+EDTA			
	A	W	SP	SU	A	W	SP	SU	A	W	SP	SU	A	W	SP	SU	A	W	SP	SU	A	W	SP	SU	A	W	SU	SP
1(ref)	*	*	*	*	NS	NS	NS	NS	NS	*	NS	*	NS	NS	NS	NS	**	*	*	**	*	*	NS	*	**	*	**	**
2	NS	NS	NS	NS	***	*	*	NS	NS	NS	NS	NS	***	***	***	***	***	*	*	**	***	*	***	NS	***	**	***	***
3	*	***	**	*	NS	*	*	***	**	**	***	***	*	**	*	*	**	***	***	NS	*	NS	***	*	**	***	***	***
4	NS	***	*	NS	NS	***	***	NS	NS	***	NS	*	**	**	**	*	NS	***	NS	NS	NS	***	NS	NS	***	***	***	**

P=phosphorus; N=Nitrogen; C = Control W=winter; Sp= spring; Su= summer; A = autumn; *Significant (according to the %C.V guideline presented inTable7); ** Highlysignifcant; *** Very high significant; NS = non-significant

Table8. Correlation between NH_3-N, NO_2-N, NO_3-N, TSIN, bio-available nitrogen (BAN),total dissolved phosphorus(TDP) and bio-available phosphorus

	NH3-N	NO2-N	NO3-N	TSIN	TDP	BAN	BAP	Obs. Chl a	Exp.Chl a
NH_3-N	1								
NO_2-N	0.33	1							
NO_3-N	0.47	-0.33	1						
TSIN	0.93*	0.	0.75*	1					
TDP	0.74*	0.55*	0.33	0.69*	1				
BAN	0.65*	0.43	0.29	0.61*	0.72*	1			
BAP	0.66*	0.21	0.62*	0.75*	0.75*	0.81*	1		
Obs. Chl a	0.49	0.71*	0.09	0.42	0.82*	0.55*	0.50*	1	
Exp.Chl a	0.73*	0.29	0.57*	0.79*	0.86*	0.85*	0.95*	0.61*	1

4. Discussion

The algal bioassays are usually used to identify growth limiting nutrients and to eutrophication potential of aquatic ecosystem (Goldman, 1963; Likens, 1972; Schelske *et al.*, 1978; Paerl and Bowles, 1987; Källqvist and Berge, 1990; Xu *et al.*, 2010). The algal growth potential test seems to be a good tool to formulate a disciplinary guideline for water quality of wastewater drain and the receiving water of the River Nile. In this study, the water fertility, toxicity, and the nutrient limitation and bioavailability of nutrients of wastewater drain and the receiving waters were evaluated. The results (Figures3a-6a) indicated that the drain station 2 maintained the highest maximum standing crop of test algal, followed by mixing station and the recovery station 4 during the study period, while the lowest one was recorded at relatively unpolluted upstream reference station. This finding indicates the hypereutrophic conditions (increase of nitrogen and phosphorus concentrations) of the receiving waters due to the drainage wastewater discharge. Similar results were reported by (Paerl *et al.*, 2011). The wastewater reaching the drain station from agriculture land, domestic and industry may be the main cause for the high water fertility. However, actually more than 80% of the AGP biomass were less than or equal to 8 mg l$^-$·a result revealing, so far, a desirable water quality (Vollenweider, 1971; Raschke and Schultz, 1978)

The AGP was mainly limited by P in most stations (where the concentration of phosphorus is low)expect the drain station 2,where AGP was mainly limited by heavy metal toxicity followed by nitrogen (Table 7) This finding seems common feature in freshwater ecosystems (Miller *et al.*, 1978; Elser *et al.*, 1990,2007; Kielsina *et al.*,2001;Schindler *et al.*,2008). This may attributed due to the presence of considerable high amounts of bioavailable P at drain waters (Table 4), and the excessive phosphorus should enhance the development of nuisance algal blooms (Schultz, 1985), a condition which has been reported during this study and supported with the highest concentration of observed chl.a (Figure 7). This finding may assumes the validity of other factors controlling P bioavailability among which total alkalinity (NTAC, 1968), hardness (NAS, 1974), type and amount of suspended solids (Golterman, 1975), N:P ratio (Chiaudani and Vighi, 1974), natural complexities and organic matter content (Fayed, 1981) and heavy metals (Van Donk *et al.*, 1988), may be the most significant.

N limitation is only observed at stations 2(drain), 3(mixing zone) and 4(recovery zone) (Table 7) due to high pollution condition. The excessive loading of P-rich agricultural fertilizers, domestic wastewater into drain station is actually the main cause of raising P level and bringing the receiving waters into N limitation state. This finding, supported by the reports of (Fitzgerald, 1969), (Lee,1973) and (Rhee,1974, 1978),(Forsberg, 1976), (Miller *et al.*, 1978), and (Howarthand Marino, 2006) .

The significant increase in AGP due to combined P and N additions (Table 7) was, mainly, attributed to P addition in case of P limitation, and to N when was the growth limiting nutrient. This finding similar to (Hernandez *et al.*, 2001; Bernal-Brook *et al.*, 2003; and Harpole *et al.*, 2011). Always, the combined additions of P, N, and EDTA (Figures 3d - 6d) supported the highest algal biomass production, presumably due to the combined effects of nutrient availability and reduction in heavy metal toxicity (Miller *et al.*, 1978).

The relationships between the chemically analyzed nutrients (P and N) and their bioavailable concentrations were affected greatly by heavy metal toxicity (Table 5) Similar observations were reported by (Chuiaudani and Vighi, 1974) , (Greene *et al.* 1975, 1978), Miller *et al.* (1976, 1978), (Permazzi*et al.*, 1979), (Fayed 1981), (Van Donk *et al.*, 1988) and (Abdel-Hamid *et al.*, 1992). In many cases, the growth of the test alga was severely inhibited by heavy metal toxicity (Table 6). Generally, the EDTA treated cultures maintained significantly higher algal biomass compared to control (Figures 3a - 6a) indicating the presence of heavy metal with toxic concentration(Elster&Komarek,2003). As chelating agent EDTA may reduce metal toxicity through its binding capacity (Gächter*et al.*, 1974), keeps trace metals in concentrations available for algal growth (Dufkova, 1984) or it facilitates P uptake (Wetzel, 1975). Based on the result of %I$_{14}$ indicate that AGP was mainly inhibited by heavy metal toxicity, followed by limitation of N and P.

Similar to what was observed by Claesson (1978), Klapwijk *et al.* (1989) and Abdel-Hamid *et al.* (1992) the observed phytoplankton chlorophyll a, showed no direct interrelationship with the expected chlorophyll a (Figure 7), but showed relation with nutrient especially (TDP) (r=0.82)as the increase of phosphorus lead to high phytoplankton biomass(high observed Chl.a (Table 8) similar finding recorded by Jones *et al.* (2011). This discrepancy between the observed and the expected chlorophyll a biomass, may be due to the role of other factors governing the growth of natural phytoplankton populations like, for instance, light (Van Donk, 1983), temperature (Kopczynska, 1981), water turbidity (Smith, 1982), nutrient release from the sediments and external loadings (Golterman, 1983), and differences in chlorophyll a content of phytoplankton populations (Nicholls and Dillon, 1978).

5. Conclusion

Algal growth potential test (AGPT) proved a good, effective and sensitive mean for assessing the productivity and toxicity of the Omer Bekdrain wastewater and the receiving freshwater of the River Nile. The AGP values were highly related with the heavy metal toxicity and concentrations of P and N. The highest biomass was recorded at drain station and lowest biomass was recorded at the upstream reference station. The test also assesses heavy metal toxicity contained in water samples.

List of abbreviation:

AGP = algal growth potential

AGPT =algal growth potential test

BAN =bioavailable nitrogen

BAN.EDTA =bioavailable nitrogen in case of Na2 EDTA treatment

BAP =bioavailable phosphorus

BAP. EDTA=bioavailable phosphorus in case of Na2 EDTA treatment

Exp. Chl. a = expected chlorophyll a

MSC = Maximum standing crop (biomass of the test alga in mg dry weight l^{-1})

Obs. Chl.a = Observed chlorophyll a

DRP= dissolved reactive phosphorus

TDP = total dissolved phosphorus

TSIN = total soluble inorganic nitrogen (nitrate- N+ nitrite-N+ammonium-N)

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الـملخـص الـعـربـى
تقييم بيولوجي لسمية وخصوبة مياه الصرف الصحي فى مصرف عمر بكعلى مياه نهر النيل في مصر

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تم تقييم سمية المياه وخصوبة مياه الصرف الصحي لمصرف عمر بيك الذي يصرف مباشرة إلى فرع دمياط من نهر النيل. جرت الدراسة بين فصل الشتاء والخريف،٢٠١٦. إستخدمت إختبارات الإضافات المغذية لتقدير العناصر الغذائية المحددة للنمو الطحلبي ، وتوافرها الحيوي ، وسمية المعادن الثقيلة في عينات المياة. وذلك باستخدام طحلب الإختبار القياسي ل سيدوكرشينرلا سبكابيتاتا. تراوح نمو طحلب الإختبار معبرا عنة بالوزن الجاف بين ٠،٤ و ١٩،٤٢مجم لكل لتر. لوحظ أن في محطات الصرف والإختلاط والإستعادة كان نمو طحلب الإختبار القياسي قليلا نسبيا بسبب سمية المعادن الثقيلة حيث تراوحت نسبة تثبيط النمو عند اليوم الرابع عشر (٪ I14) بين ٢٧،٩ و ٥٨،٦ ، بينما تراوحت في المحطة المرجعية (٪ I14) بين ٢٠ و ٣٧. أوضحت النتائج ان نمو طحلب الإختبار تأثر بتركيز عنصري النيتروجين والفسفور وتباينت النتائج من محطة لأخري و من موسم لأخر. كان تركيز الكلوروفيل (أ) المقاس للطحالب العالقة أعلي من تركيزة المتوقع في جميع المحطات والمواسم فيما عدا محطة مصرف عمر بيك. تأثرت العلاقات بين المواد الغذائية التي تم تحليلها كيميائيًا (الفسفور والنتروجين) وتركيزاتها البيولوجية المتاحة بشكل كبير من سمية المعادن الثقيلة.أظهرت النتائج إرتباط الكلوروفيل المقاس ارتباطا معنويا مع (r = 0.82) TDP ، في حين أن الكلوروفيل المتوقع يرتبط بشكل كبير مع(r = 0.73) NH4-N.أثبت اختبار إمكانات نمو الطحالب أنه وسيلة حساسة ويمكن الاعتماد عليها لتقييم خصوبة المياه وسمية المصارف الملوثة والمياه المستقبلية.

143

Biological assessment of water toxicity and fertility of wastewater Omer Bek drain discharging into receiving water of the River Nile in Egypt

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