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Determination of the Growth Rates of *Spirolina* and *Cheatoceros* Algae in Urban Waste Sewage and their Capability to Deplete Nitrate and Phosphate Content in the Sewage

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ABSTRACT: The application of cyanobacterial and diatom cultures for the treatment of industrial effluents has been well recognized.In this study aimed to evaluate the effect of urban sewage on growth of *Spirolina plantensis* and *Chaetoceros muelleri.* The experiment was conducted in 6 treatments as a growth medium. Result showed that in treatment 5 maximum cell densities was ($565\times10^2\pm237.7$) at day7 thus treatment 5 has best condition for growth *S. plantensis* and in treatment 3 maximum cell density was ($825\times10^4\pm92$) at day13.Treatment 5 has best condition for growth *C. muelleri*. Total chlorophyll a, contents (µg/1) recorded in *S. Plantensi s*and *C. muelleri* was highest at treatment 3(0.21±0.07) and treatment 4(0.23± 0.10) respectively. In present investigation, both the algal species can be good potential to growth in urban sewage. The urban sewage removal efficiency of *C. muelleri* was higher as compare to *S.plantensis* which can be recommended for phytoremediation purpose. © JASEM

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Keywords: *Spirolina; Chaetoceros;*Nitrate and phosphate; Chlorophyll a;Urban sewage; Depletion.

The pollution increase, industrialization and rapid economic development, are cause of decrease the availability and quality of water resources, in many areas worldwide.Nowadays, uncontrolled discharge from industrial sectors or agriculture discharged to aquatic ecosystems and contaminated total aquatic environment which, not only cause toxic effect on human, via accumulation in aquatic animals, through food chain but also affect biodiversity (Lavajoo et al. 2015). Wastewater generated from urban or industry sewage sources contain high concentrations of organic matter, nitrogen and phosphorus, and causes eutrophication in receiving water. Urban sewages contain nutrients (which have been identified as the main causes leading to eutrophication in natural waters) must be treated before being discharged into water bodies (Mallick, 2002). Instances of the effect of sewages on microalgae growth are arrested cell division, inhibited growth rate, restrained enzyme activity and reduced photosynthesis (Chen et al., 2009; Baumann and Morrison, 2009). Compared to other aquatic organisms in marine environment, unicellular microalgae exhibit highest resistant to sewages and highly recommended as bio-indicator for the assessment of marine pollution (Rijstenbeil et al., 1994; Kapkov and Belenikina, 2003; Kapkov and Belenikina, 2007). The use of several microalgae cultures in wastewater treatment has a major advantage that allows effective utilizing of nutrients (De la Noue et al.,1992). Microalgae culture systems can be employed in different processes, such as wastewater treatment and production of animal food (De la Noue and Proulx, 1988). The wastewater treatment by microalgae and cyanobacteria is known (De la Noue et al., 1993; De la Noue and Proulx, 1988; Oswald, 1988).In fact, traditional wastewater treatment process required high operation cost to provide suitable condition for aerobic bacteria to effectively consume organic components in polluted water. However, microalgae provide an efficient low-cost approach to treat wastewater (Lananan et al., 2014; Nasir et al., 2015).Recent studies showed that many algal species, especially *Chlamydomonas, Scenedesmus* and *Chlorella* decreased nutrients under light condition (mixotrophy) and they are also capable of heterotrophic growth on simple molecules, such as acetate, glucose and organic acids in the dark (Laliberte andDe la Noue, 1993).It has been suggested that a Microalgae have been offered as bioremediation treatment to decrease (NH_4^+, $NO_3^-$and PO_4^{3-}) nutrients (Mallick, 2002).The presence of high concentrations of ammonia and urea in urban sewages inhibit algal growth and physiological activity (Przytocka-Jusiak, 1976).Therefore, in the present study the growth rates and amount of nitrate and phosphate absorptionof *Spirolina plantensis* and *Chaetoceros muelleri* on urban wastewater were determined.

MATERIALS AND METHODS

*Test Species***:** In this study the microalgae cultures of marine blue green algae (*Spirolina plantensis)* and Diatom (*Chaetoceros muelleri)* were obtained from the phytoplankton culture laboratory, of institution Persian Gulf and Omani Sea Hormozgan in Iran. The

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urban sewage was collected from discharged refinery site of Bandar Abbas.

Experimental Design: In the laboratory, the samples of sewage were filtered through a 25mm, 3µm glass microfiber filters (GF/C) mounted on a Millipore filtration unit and sterilized by autoclavingat 121°C for 15 minutes. The culture growth medium prepared with three replicates for conducting the experiments and the duration was 15 days under similar laboratory conditions at temperature (30ºC ± 2ºC) for *Spirolina plantensis*and Temperature (25ºC ± 2ºC) for *Chaetoceros muelleri*;, Light (2500 ± 500) lux for*Spirolinaplantensis*and Light (4500 ± 500) lux for *Chaetoceros*, optimum pH was between 8.0 and 11 for *Spirolina plantensis* and for *Chaetoceros muelleri* was between 9.0 and 11, that adjusted by electronic pH meter (ELICO, Model LI 120) and with constant aeration.

Treatments A:(1) *Spirolina plantensis* was cultivated in f/2 Medium based on (Guillard, 1975). (2) *Spirolinaplantensis* was cultivated in 80% of filtered seawater and 20% urban waste (3)*Spirolina plantensis* was cultivated in 60% of filtered seawater and 40% urban waste (4) *Spirolina plantensis* was cultivated in 40% of filtered seawater and 60% urban waste (5)*Spirolina plantensis* was cultivated in 20% of filtered seawater and 80% urban waste (6) *Spirolina plantensis* was cultivated in 100% urban waste. Total volume of culture and media was 200ml.

Treatments B:(1) *Chaetoceros muelleri* was cultivated in f/2 Medium based on (Guillard, 1975) (2) *Chaetoceros muelleri* was cultivated in 80% of filtered seawater and 20% urban waste (3)*Chaetoceros muelleri* was cultivated in 60% of filtered seawater and 40% urban waste (4) *Chaetoceros muelleri* was cultivated in 40% of filtered seawater and 60% urban waste (5)*Chaetoceros muelleri* was cultivated in 20% of filtered seawater and 80% urban waste(6) *Chaetoceros muelleri* was cultivated in 100% urban waste. Total volume of culture and media was 200ml.

Cell density and Growth Rate: The cell growth was monitored by measuring cell numbers by manual counting under the binocular light microscope. Growth rates were calculated as µ. day^{-1} according to the following Formula $\mu = (N1/N_0)/t$; Where, N0 and N1 represent cell density at the start and the end of the growth period, and tare the time between measurements (in days).

Physicochemical analysis: Water quality analysis of ammonia and phosphorus (orthophosphate) determination were based on Phenate Method and Vanadomolybdo phosphoric Acid Method adapted from the Standard Method for the Examination of Water and Wastewater (APHA, 2005) . Sampled water was clarified from the MA and EM biomass by centrifugation at 5000 rpm, 15 min to obtain clear supernatant which was subjected immediately to water quality analysis for the determination of ammonia and phosphorus. Nitrate and chlorophylla, were also measured by spectrophotometer UV/visible (Varian-carry 100) according to Manual of Oceanographic Observations and Pollutant Analysis Methods procedures (Marine environment assessment marine meteorology, 1999).

*Statistical analysis***:** To test whether there was statistical difference among the cell density between *Chaetoceros muelleri* and *Spirolina plantensis* in different days, we performed a Student's t-test. The mean and standard deviation values of the triplicates for each treatment were calculated. The results were analyzed statistically by using Spss 17 software and graphical analyses were performed using Microsoft Office Excel.

RESULTS AND DISCUSSION

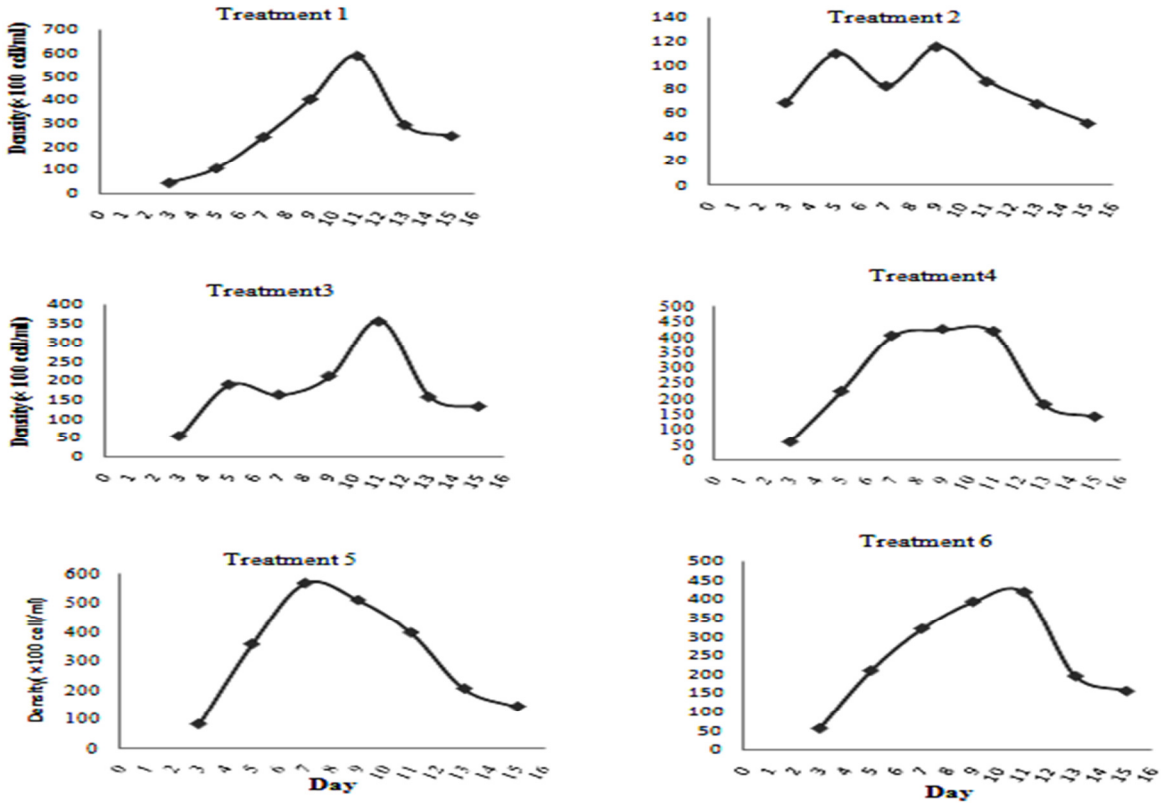
Growth of Spirolina plantensis at different treatments: In the present study, the number of *Spirolina* cells at different treatments was shown. In treatment 1 (100% f/2 Medium) cells appeared to reach their stationary phase after 11days of cultivation and started to decline thereafter. In treatment 1 maximum and minimum cell density were ($588\times10^2\pm121$) and ($48\times10^2\pm 8$) for day11 and day 3 respectively. One- way analysis of variance (ANOVA), showed significant difference between days and cell density ($P<0.05$). All analyses were performed at 5% statistical significance level. In treatment 2 (80% f/2 Medium+ 20% urban waste) cells appeared to reach their stationary phase after 9days of cultivation and started to decline thereafter. In treatment 2 maximum and minimum cell density were ($116\times10^2\pm16$) and ($52\times10^2\pm 8$) for day9 and day 15 respectively. In one- way analysis of variance (ANOVA), between days and cell density did not observed significant difference ($P>0.05$). All analyses were performed at 5% statistical significance level. In treatment 3 (60% f/2 Medium+ 40% urban waste) cells appeared to reach their stationary phase after 11days of cultivation and started to decline thereafter. In treatment 3 maximum and minimum cell density were ($357\times10^2\pm92$) and ($54\times10^2\pm 18.5$) for day11 and day 3 respectively. One- way analysis of variance (ANOVA), showed significant difference between days and cell density ($P<0.05$). All analyses were performed at 5% statistical significance level. In treatment 4 (40% f/2 Medium+ 60% urban waste) cells appeared to reach their stationary phase after

11days of cultivation and started to decline thereafter. In treatment 4 maximum and minimum cell density were ($424.7\times10^2\pm62.1$) and ($60\times10^2\pm 6.1$) for day11 and day 3 respectively. One- way analysis of variance (ANOVA), showed significant difference between days and cell density ($P<0.05$). All analyses were performed at 5% statistical significance level. In treatment 5 (20% f/2 Medium+ 60% urban waste) cells appeared to reach their stationary phase after 11days of cultivation and started to decline thereafter. In treatment 5 maximum and minimum cell density were ($565\times10^2\pm237.7$) and ($80\times10^2\pm7.1$) for day7 and day 3 respectively. One- way analysis of variance (ANOVA), showed significant difference between days and cell density ($P<0.05$). All analyses were performed at 5% statistical significance level. In treatment 6 (100% urban waste) cells appeared to reach their stationary phase after 11days of cultivation and started to decline thereafter. In treatment 5 maximum and minimum cell density were ($417\times10^2\pm190.7$) and ($56.4\times10^2\pm 15.9$) for day11 and day 3 respectively. One- way analysis of variance (ANOVA), showed significant difference between days and cell density ($P<0.05$). All analyses were performed at 5% statistical significance level (Fig.1) (Table.1).

Table.1. Cell density of *Spirolina plantensis* and *Chaetoceros muelleri* at the end of test period (day 15)

Treatments	*Spirolina*($\times10^2$ cell/ml)		*Chaetoceros*($\times10^4$ cell/ml)	
	Mean	Std.	Mean	Std.
Treatment1	275.90	181.46	324.02	250.97
Treatment2	83.07	27.78	539.66	234.47
Treatment3	180.71	100.76	664.95	236.74
Treatment4	264.71	161.86	424.16	205.67
Treatment5	333.0	222.51	475.88	328.17
Treatment6	320.61	202.18	305.80	252.74

Impact of different treatments and days on the growth of *Spirolina plantensis*: To determination of best treatment condition and lowest time for highest *Spirolina plantensis* growth we used of two- way analysis of variance (ANOVA). We observed a strong positive correlation (R^2 = 0.82) between treatment conditions and times for *Spirolina plantensis* growth (Fig.2).



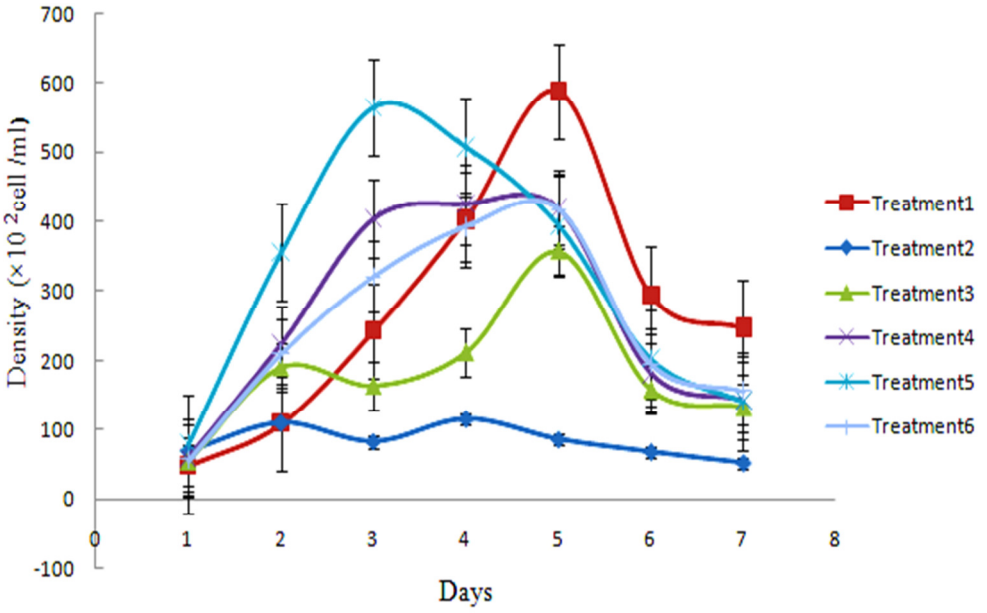


Fig.2. *Spirolinaplantensis* cell density in different treatments at 15days.

Growth of Chaetoceros muelleri at different treatments: In treatment 1 (100% f/2 Medium) cells appeared to reach their stationary phase after 9days of cultivation and started to decline thereafter. In treatment 1 maximum and minimum cell density were ($489\times10^4\pm121$) and ($179\times10^4\pm 8$) for day9 and day 5 respectively. In one-way analysis of variance (ANOVA), between days and cell density did not observed significant difference ($P>0.05$). All analyses were performed at 5% statistical significance level. In treatment 2 (80% f/2 Medium+ 20% urban waste) Cells appeared to reach their stationary phase after 9days of cultivation and started to decline thereafter. In treatment 2 maximum and minimum cell density were ($639\times10^4\pm16$) and ($439\times10^4\pm 8$) for day9 and day 3 respectively. In one-way analysis of variance (ANOVA), between days and cell density did not observed significant difference ($P>0.05$). All analyses were performed at 5% statistical significance level. In treatment 3 (60% f/2 Medium+ 40% urban waste) cells appeared to reach their stationary phase after 13days of cultivation and started to decline thereafter. In treatment 3 maximum and minimum cell density were ($825\times10^4\pm92$) and ($63\times10^4\pm 18.5$) for day13 and day 3 respectively. One-way analysis of variance (ANOVA), showed significant difference between days and cell density ($P<0.05$). All analyses were performed at 5% statistical significance level.In treatment 4 (40% f/2 Medium+ 60% urban waste) cells appeared to reach their stationary phase after 9days of cultivation and started to decline thereafter. In treatment 4 maximum and minimum cell density were ($523\times10^4\pm62.1$) and ($195\times10^4\pm 6.1$) for day9 and day 3 respectively. One-way analysis of variance (ANOVA), showed significant difference between days and cell density ($P<0.05$). All analyses were performed at 5% statistical significance level. In treatment 5 (20% f/2 Medium+ 60% urban waste) cells appeared to reach their stationary phase after 13days of cultivation and started to decline thereafter. In treatment 5 maximum and minimum cell density were ($832\times10^4\pm237.7$) and ($71\times10^4\pm 7.1$) for day13 and day 3 respectively. One-way analysis of variance (ANOVA), showed significant difference between days and cell density ($P<0.05$). All analyses were performed at 5% statistical significance level. In treatment 6 (100% urban waste) cells appeared to reach their stationary Phase after 15days of cultivation and started to decline thereafter. In treatment 5 maximum and minimum cell density were ($529\times10^4\pm190.7$) and ($58\times10^4\pm 15.9$) for day15 and day 3 respectively. One- way analysis of

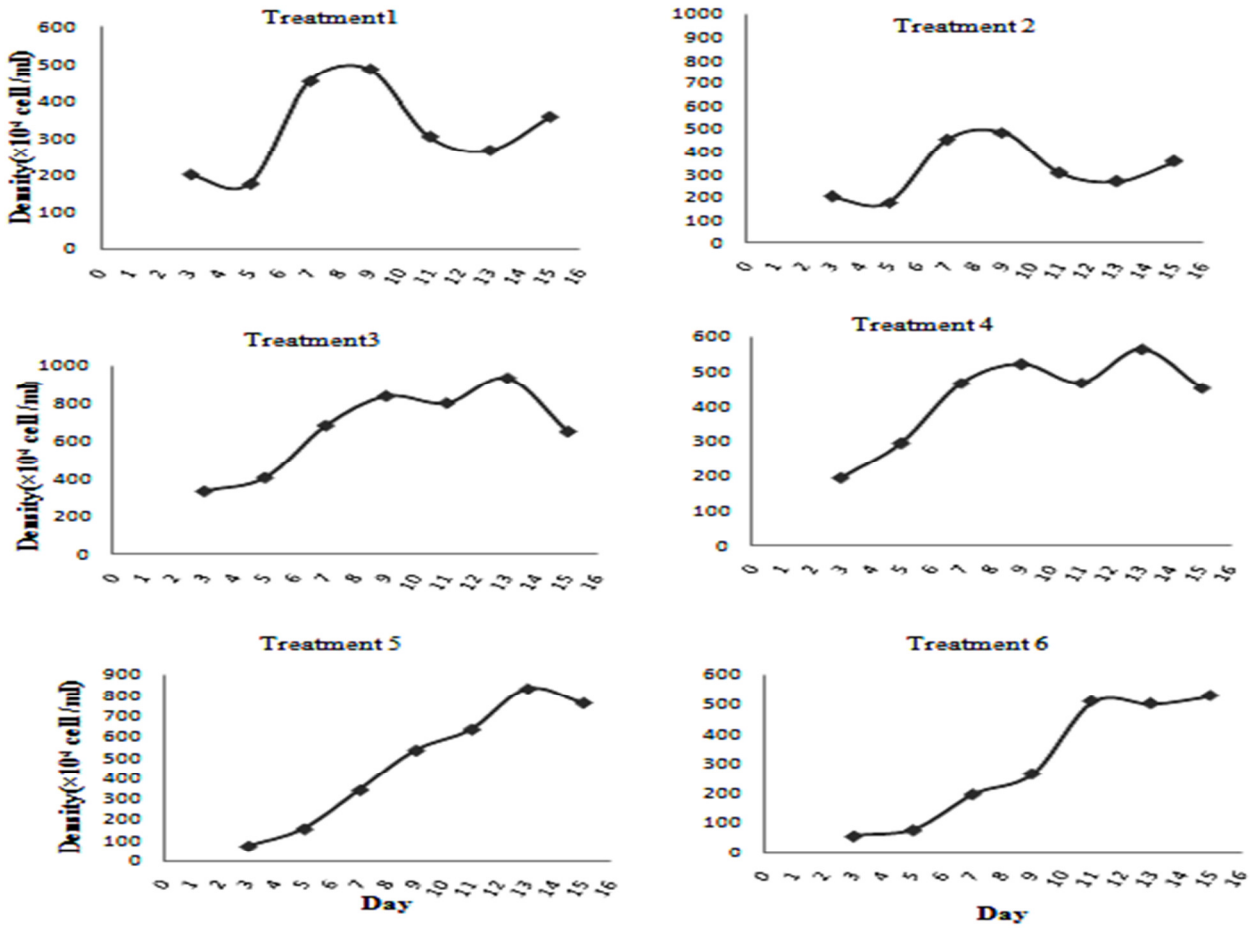
variance (ANOVA), showed significant difference between days and cell density (P<0.05). All analyses were performed at 5% statistical significance level (Fig.3) (Table.1).

Impact of different treatments and different days on the growth of Chaetoceros muelleri: Two- way analysis of variance (ANOVA) used for determination of best treatment condition and lowest time for highest *Chaetoceros muelleri* growth. We observed a strong positive correlation ($R^2 = 0.70$) between treatment conditions and times for *Chaetoceros muelleri* growth (Fig.4). The result of t-test suggested that in most of treatments (except treatment 3) at different days (except day15) did not have significant difference between both of algae (P>0.05) but there was significant difference between cell density in both of
algae (P<0.05) (Fig.4) (Table.2).

Table.2. T-test analysis for comparison between *C. muelleri* and *S. plantensis* cell density in different treatments at 15days.

Day y3 / Treatment	Day5	Day7	Day9	Day11	Day13	Day15
Treatment1 s	Ns	Ns	Ns	Ns	Ns	Ns
Treatment2 s	Ns	Ns	Ns	Ns	S	Ns
Treatment3 s	Ns	S	S	S	S	S
Treatment4 s	Ns	Ns	Ns	Ns	S	Ns
Treatment5 s	Ns	Ns	Ns	Ns	S	S
Treatment6 s	Ns	Ns	Ns	Ns	S	Ns

S: Significant difference at confidence interval 95% ($p < 0.05$).
NS: No significant difference at confidence interval 95 % (P>0.05).



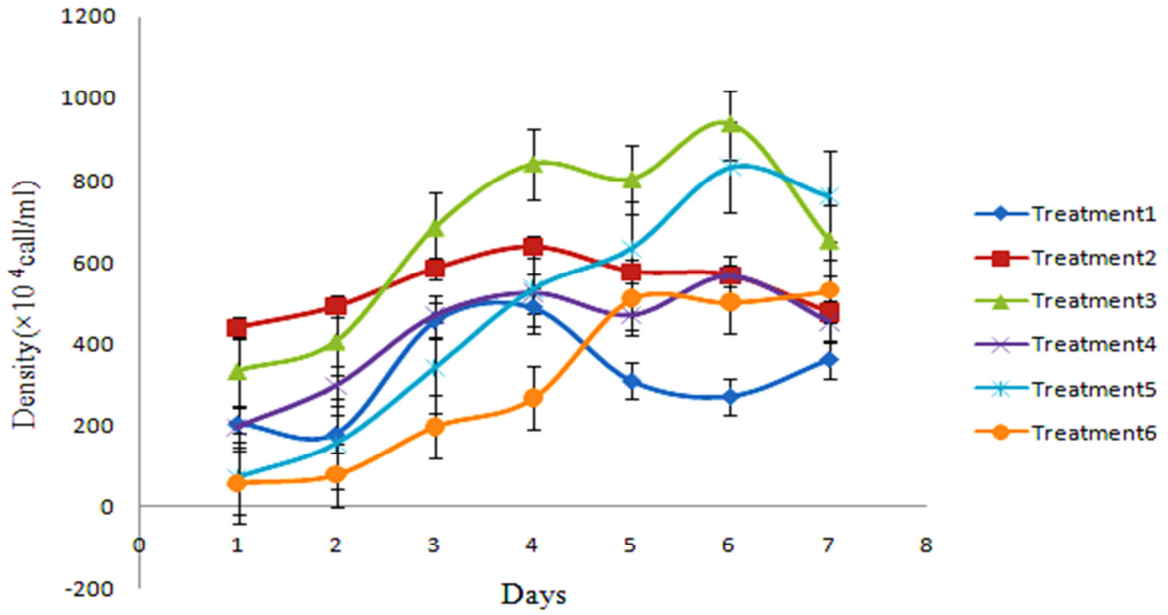


Fig.4. *Chaetoceros muelleri*cell density in different treatments at 15days

Chlorophyll a

The mean concentration of Chlorophyll a in different treatment in *Spirolinaplantensis* and *Chaetocerosmuelleri*were respectively (0.169±0.06) µg/l and (0.173±0.06) µg/l. The result of t-test between Chlorophyll a concentrations and two algae was showed no significant difference between them.Total chlorophyll a contents (µg/1)recorded in*Spirolinaplantensis*and in *Chaetocerosmuelleri*were highestat treatment 3(0.21±0.07)and treatment 4(0.23± 0.10) respectively (fig.5) (Table.3).

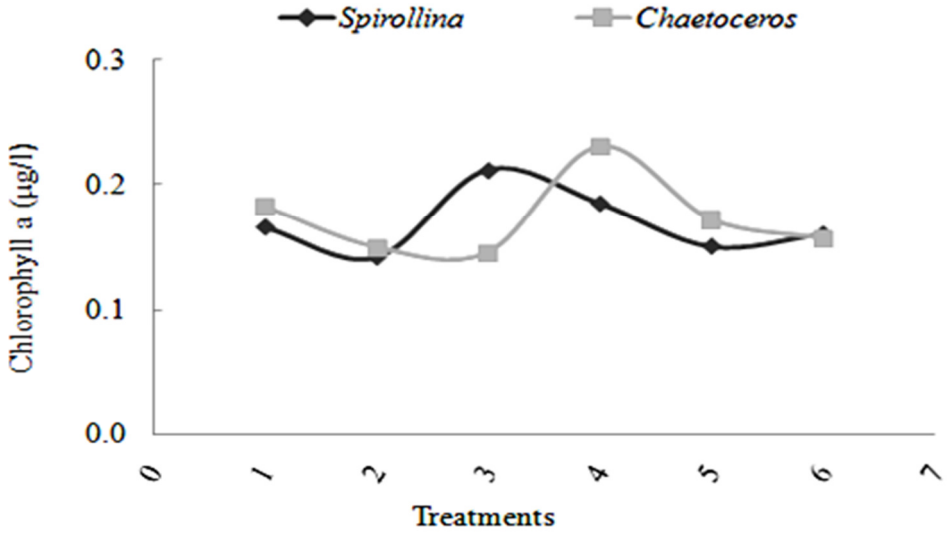


Fig.5. Concentration of chlorophyll- a, in different treatments

Table.3. Mean and standard deviation at the end of test period (day 15)

	Chlorophylla(µg/l)				Nitrate(mg/l)			
	Spirolina		*Chaetocerous*		*Spirolina*		*Chaetocerous*	
Treatment	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
Treatment1	0.16	0.04	0.18	0.03	0.23	0.23	0.55	0.18
Treatment2	0.14	0.087	0.14	0.01	0.27	0.17	0.50	0.14
Treatment3	0.21	0.07	0.14	0.10	0.30	0.21	2.83	0.14
Treatment4	0.18	0.03	0.23	0.10	0.25	0.25	0.67	0.23
Treatment5	0.15	0.01	0.17	0.04	0.27	0.17	0.62	0.17
Treatment6	0.16	0.09	0.15	0.04	0.29	0.20	0.80	0.15

Mean and standard deviation of three replicates are shown.
Underline indicated maximum concentration.

Concentration of nitrate and phosphatein*Spirolina plantensis* and *Chaetoceros muelleri:*In this study the range of nitrate concentration in*Spirolinaplantensis*and*Chaetocerosmuelleri*were respectively between 0mg/l to 0.56mg/l and 0.25mg/l to 5.0mg/l. The results of one- way analysis of variance (ANOVA) for*Spirolinaplantensis*and*Chaetoceros muelleri*, suggested that there were no significant and significant difference in nitrate concentration in different treatmentrespectively.Also the range of phosphate concentration in*Spirolinaplantensis*and *Chaetocerosmuelleri*were respectively between 2mg/l to 31.2 mg/l and 24.5 mg/l to 61.9 mg/l. The results of one- way analysis of variance (ANOVA) for*Spirolina plantensis*and *Chaetoceros muelleri*, suggested that there were significant and no significant difference in phosphate concentration in different treatments respectively (Fig.6, 7).

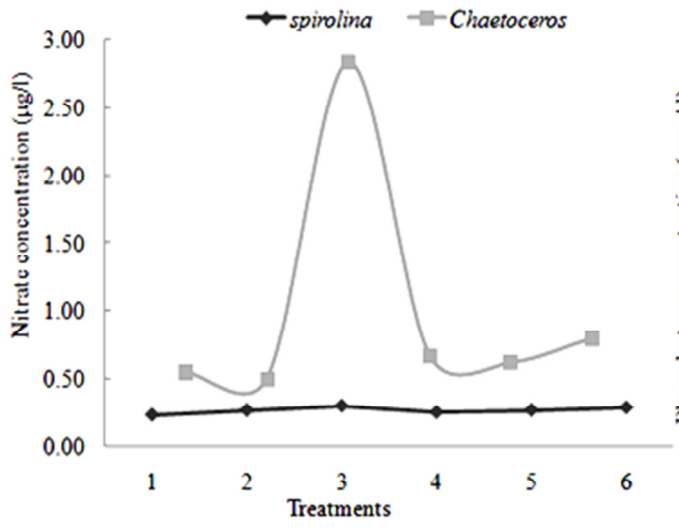


Fig.6. Concentration of nitrate in different treatments

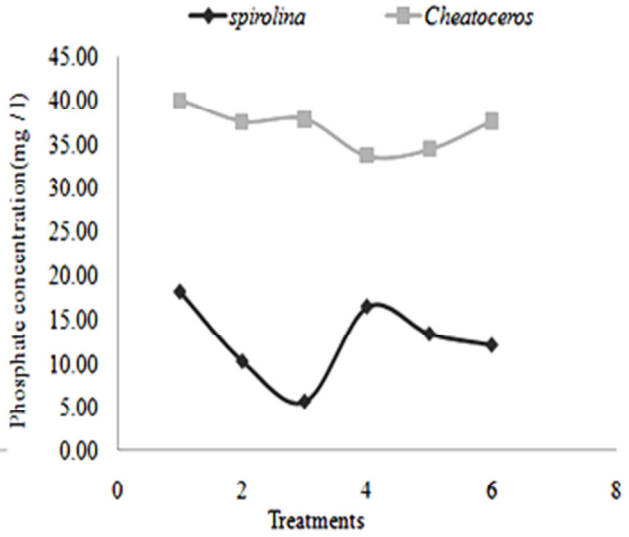


Fig.7. Concentration of phosphate in different treatments

As we know application of cyanobacterial and diatom cultures for the treatment of industrial effluents has been well recognized. Microalgae culture suggests an interesting step for wastewater treatments, because they provide a tertiary biotreatment coupled with the production of potentially valuable biomass, which can be used for several purposes. The comparisons influence two factors (treatments and days) via one-way analysis of variance (ANOVA) in *Spirolinaplantensis*was more than *Chaetoceros muelleri*. In *Spirolinaplantensis*maximum cell densityat the lowest timesnowed in treatment 5and 6. The important note in *Spirolina* sp. was in all treatments, cells appeared to reach their stationary phase at day5 to day11 then started to decline thereafter. In this study existence of nitrate and phosphate in treatments was one of the main factors on *Spirolinaplantensis* growth at different days and the growth continued to stationary phase when enough nutrients were in media but growth decreased when the concentration of nutrients were low. Therefore we could suggest in lab conditions, the growth rate

decreased when concentration of nutrient decreased thus not only optimum conditions affected on *Spirolina plantensis* but also enough nutrients are important factor to growth rate. Recent study suggested the ability of microalgae cultures in the elimination nitrogen(50.2%) and phosphorus (85.7%) in industrial wastewatertreatment Colak and Kaya (1988).Cyanobacteria such as *Spirulinaplantensis*have the great compatibility to alterations in environmental factors which could grow in wastewater and produce significant biomass. Other authors have reported the use of *Spirulina plantensis*grown on Zarrouk's medium in wastewater treatment (Amala and Ramanathan 2013; Chaiklahan et al. 2013, Ismail et al. 2013). In *Chaetoceros muelleri* the best treatment for highest growth was treatment3 with growth pick in day13. The stationary phase in treatment 6 (100% urban waste)occurred due to over the day because there was enough nutrients for growth *Chaetoceros muelleri.* Other treatments also showed an irregular growth thus the growth rate of *Chaetoceros muelleri* same *Spirolina plantensis* was depended on concentration of nutrients.Livingston et al.(2002) indicated Nitrogen was one of the chief limiting nutrients to phytoplankton through nutrient limitation experiments.

In reported papers, Lau et al. (1995)studied the higher the algal density, the better the growth and the higher the nutrient removal efficiency. The growth rate of *Chaetoceros simplex* was slightly enhanced with lower concentration and inhibited at higher concentrations of the effluent (Karthikeyan et al., 2010).The concentration of chlorophyll a, in *Spirolinaplantensis* in treatment 2 and 5 presentedexponential phase, due to increasing cell density in these treatments while *Chaetoceros muelleri* was not showed exponential phase in concentration of chlorophyll a. one of the main colures in microalgae is chlorophyll a, which have important role in photosynthesis and could used to indication for primary production (Strickland and Parson, 1972). The low concentration of nitrates in all treatments, suggested thatthere was no significant difference in nitrate concentration in different treatment in both of algae. Concentration of phosphate (mg/l) in both of algae in treatment 1(100% f/2 Medium) was higher than other treatments thus the reductions in urban waste phosphate in all algal treatments were significantly better than those without urban waste.

*Conclusion***:** The phytoremediation was very efficient, cost effective and eco-friendly indicating that microalgae has vital role in the removal of different pollutants from wastewater. In present investigation both the algal species had very good potential to growth in urban sewage but the urban sewage removal efficiency of *Chaetoceros muelleri*was higher as compare to *Spirolina plantensis* which can be recommended for phytoremediation purpose.

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