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ALGAL GROWTH AND WASTE STABILIZATION PONDS PERFORMANCE EFFICIENCY IN A SUB-TROPICAL CLIMATE

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Abstract

Both irrigation and potable water are in diminutive supply in most of the developing countries particularly those situated in tropical and subtropical regions where, often untreated wastewater is utilized for the purpose of irrigation. Treated wastewater has proved to be a potential asset serving as an alternate source for the expansion of irrigated agriculture. Waste stabilization ponds (WSP) are considered as less costly and effective substitute for the wastewater water treatment in tropics. The principle of wastewater treatment in waste stabilization pond is based on the symbiotic relationship between bacteria and various algal species. In this study, an attempt was made to relate algal growth and different extrinsic factors using multiple regression models. The predominant algal species found in WSP systems were *Chlorella*, *Euglena*, *Oscillatoria* and *Scenedesmus*. The growth of individual algal species and overall algal growth was principally governed by temperature, total sunshine hours and Total Kjeldhal Nitrogen (TKN). The study suggested that algal bacterial symbiotic relationship works well and the dissolved oxygen production through algal photosynthesis was optimum to decompose heavy organic load resulting in oxygen-rich effluent (liquid fertilizer) which could be successfully exploited for unrestricted irrigation.

Key words: Waste stabilization ponds, Algae, Wastewater, Effluent, Symbiosis.

Introduction

Availability of adequate water supply and water quality deterioration is a worldwide problem that is being intensified in the current face of climate change. Therefore, the use of marginal-quality water for agriculture is gaining importance predominantly in water-scarce developing countries (Qadir *et al.,* 2007a). Untreated wastewater is commonly used for irrigation worldwide owing to its affordability and high nutritive quality (Scott *et al.,* 2004; Keraita & Drechsel, 2004) disregarding its harmful effects on environment and human health (Qadir *et al.,* 2007b). The health hazards associated with unrestricted irrigation with wastewater are well documented. (Angelakis *et al.,* 2003). Therefore, it is imperative to treat wastewater before its reuse or discharge into the environment (Ongley, 1996). In arid and semi-arid regions with more or less permanent water shortage, recycling and reuse of treated wastewater is one of the most effective options (Ahmad & Hisham, 2008).

Since the last few decades, Pakistan is facing chronic shortage of water supply. The shortage of water is putting great stress on the agri-based economy of the country. Alternatively, untreated wastewater is frequently used for irrigation that is liable for a number of environmental health issues in the country (Khan *et al.,* 2001; Khan *et al,* 2009). In Pakistan, hardly two percent of the wastewater is treated (Minhas & Samra, 2003; Anon., 2003). Whereas, on the national scale approximately 26% of vegetable production depends on irrigation with wastewater (Ensink *et al.,* 2004). In countries like Pakistan, it is extremely important to treat wastewater in order to save water as a precious commodity and to protect the environment from pollution hazards.

Treated wastewater has proved to be a prospective asset that can be used as an alternate water resource for irrigated agriculture. Treatment of wastewater is a viable solution for the problems emerged through indiscriminate dumping which is helpful for the protection of public health and environment (Fonesca *et al.,* 2007; Papadopoulos & Savvides, 2003; Asano & Levine, 1996; Marcos do Monte *et al.,* 1996; Feigin, *et al.,* 1991).

There are many options available for wastewater treatment, but waste stabilization pond (WSP) is the most widely used option particularly in the areas where low-cost land is available (Abbas *et al,* 2006). The WSP system is affordable and dependable alternative method as compared to costly biomechanical systems of wastewater treatment particularly in hot and humid climate (Khan *et al,* 2008; Ensink *et al.,* 2004; Alcalde *et al.,* 2003; Mara & Pearson, 1998; Curtis & Mara, 1992).

However, little work has been done so far on economic exploitation of WSP effluent in Pakistan. Treated wastewater proves to be an economic asset rather than economic burden and helps in the improvement of crop yield and environmental quality (Khan *et al.,* 2014; Khan *et al,* 2012; Khan *et al.,* 2010; Khan *et al.,* 2009; Khan *et al.,* 2008; Khan *et al.,* 2007).

Prior to the selection of WSP system, climatological factors such as wind velocity, solar radiations, and temperature should be carefully considered as these factors directly influence the bacterial growth in the wastewater that contains high amount of organic matter whose biodegradation has to progress gradually through bacterial activity. Hot climate often considered as idyllic for ponds performance efficiency (Mara & Pearson,1998), but at the same time techno-economic affordability criterion cannot be ignored. The treatment of wastewater through WSP is based on symbiotic relationship between bacteria and algal species (Kayombo *et al.,* 2002). The significant factors controlling and influencing the symbiosis are temperature, solar radiation and variations in pH within the WSP system (Murakani *et al.,* 1992; Wilderer *et al.,* 1991). Ponds performance efficiency and the symbiotic relationship are also influenced by wastewater strength and the retention time (Kayombo *et al.,* 2002).

The micro-algal component in the symbiotic relationship plays a significant role (Aziz & Ng, 1993; Nurdogan & Oswald, 1995) in connection with the removal of nutrients, heavy metals and pathogens along-with the production of oxygen through photosynthesis available for heterotrophic aerobic bacteria that stabilize organic pollutants. In doing so, they release carbon-dioxide through respiration which is utilized by the algal species for photosynthesis. Production of oxygen through algal photosynthesis, particularly in the semi-arid regions helps in minimizing the operation and maintenance cost of WSP. Indeed, in these regions it is considered as one time investment in terms of capital cost (Munoz & Guieysse, 2006; Safonova *et al.*, 2004). Munoz & Guieysse (2006) developed guidelines with respect to the algal–bacterial symbiosis, operation, design and start-up, which are helpful for treatment of hazardous contaminants.

Karachi being the largest city of Pakistan and the hub of industrial activities where the water requirement is exponentially increasing for both domestic and industrial usage . The total demand of fresh water in Karachi is approximately 1000 MGD of which about 650 MGD is converted to wastewater. A sizeable amount of this water is also apportioned to industrial and agriculture sectors. This inappropriate allocation is responsible for chronic shortage of water supply for domestic consumption. Although three wastewater treatment plants are in operation in the city but most of the wastewater is discharged into the sea without any considerable treatment through Lyari and Malir Rivers. This practice has rendered various economic and environmental-health implications like, seafood contamination, marine pollution along with loss of precious water.

The principal objective of this work was to examine the growth of four predominant algal species present in the WSP; to relate their growth with the prevailing environmental factors and to determine the ponds performance efficiency in the WSP constructed and designed at Karachi University Campus (KUC).

Materials and Methods

Technical details of ponds: The particulars of WSP system at the KUC is presented in Table 1. These ponds are of equal dimensions having trapezoidal shape. The sides and bottom of the ponds are made of concrete layer to elude mosquito breeding and seepage. A set of 2 ponds, labeled as P-1 and P-2, are linked in a series. Similarly, other set of 2 ponds are connected to each other and marked as P-3 and P-4. The first set of two ponds (P-1 and P-2) is furnished with baffles. The entire ponding system is linked with influent delivery conduit that is joined with the service tank that receives wastewater from the underground influent sump that is connected to a system f underground sewerage network. Since the system of baffles was under maintenance therefore, the data related to P-3 and P-4 is given and discussed.

Table 1. Technical details of waste stabilization pond system at Karachi University Campus.

S. No.	Parameters	Characteristics
1.	Pond area (bottom)	98 m^2
2.	Pond area (WSP)	184 m^2
3.	Pond area (average)	130 m^2
4.	Outlet for effluent	1.0, 1.3, 1.5 m
5.	Influent sump capacity	13630 L
6.	Effluent sump capacity	5455 L
7.	Service tank capacity	3068 L
8.	Pond volume at 1.5m depth	198,625 L
9.	Average retention time in P-3	7.5 days
10.	Average retention time in P-4	7.5 days
11.	Average total retention time	15 days
12.	Total average hydraulic load	27000L
13.	Total average BOD_5 load	500 Kg/ha.d

(Based on average BOD_5 load of 250 mg/l; Khan and Ahmed 1992; Khan and Khan 2007)

Design criteria: Population served= 5000 population units Flow in summer = 150,000 gallons per day
The ponds are designed on the criterion that the surface BOD load on the ponds surface area should not be exceeded to 500 kg/ha. d. In the KUC the monthly average organic load of raw wastewater was 250 mg/L.

Surface area: Serial connection of two ponds generally provide enhanced performance and treatment. Therefore, total area required for pond construction is 0.4 hectare.

Depth: The suitable depth for the enhanced ponds operation was 1.5 m, that to prevent the odor problem.

Ponds feeding schedule: The WSP functioned on a plug flow system. An electric pump was installed to lift the domestic raw wastewater through the service tank that served P-3. The serving was spasmodic and once the desirable daily hydraulic load of 27000 L (BOD_5 load = approx. 500 Kg/ha.d) was attained the serving was stopped. The desired hydraulic load in the Primary pond (P-3) took nearly 12 hours (8.0 A.M till 8.0 P.M). The the total retention time of 15.0 days in P-3 (primary pond) and P-4 (secondary pond) was retained constant throughout the year (January to December).

Sample collection: Raw wastewater (influent) samples were collected in the morning when the pumping started. The samples of secondary pond (P-2) were collected from about 12 mm depth at the outlet of each pond. The samples of raw wastewater and P-2 were collected in plastic containers. Samples were collected separately in wide mouth sterile glass bottles for biological parameters. Sampling was executed twice a week and the samples were transported to the laboratory immediately, for processing.

Analysis of effluent samples: The samples were analyzed for; pH of the samples was determined using HACH sensation 156 multi parameter dissolved oxygen meter. Dissolved oxygen was determined using Jenway 630i dissolved oxygen meter. The above mentioned parameters were determined onsite.

BOD_5 estimation was performed by using azide modification method (Anon., 2005). Phosphate phosphorus (PO_4-P) and Total Kjeldahl Nitrogen (TKN) were determined by ascorbic acid method and Kjeldahl methods respectively (Anon., 2005).

Total coliforms count (TCC) was determined by MPN technique (Anon., 2005). Algal growth was ascertained by measuring Chlorophyll ''a'' and ''b'' spectrophotometrically and calculated according to Anon., (2005). For the monitoring of different types of predominant algae in the effluent approximately 10 ml of sample was collected and centrifuged at 3000 rpm for 15 minutes. The sediment was spread on a glass slide and algal cells were counted under oil immersion objective of microscope. The algal cells were identifies as *Chlorella* sp., *Euglena* sp., *Oscillatoria* sp. and *Scenedesmus* sp.

Statistical analysis: Multiple regression models were developed for the predominant algal species using STATISTICA (statistical software package) for Windows. The list of variables used for the model development is given in Table 2.

Table 2. List of variables used for model development.

S. No.	Dependent variables	Units
1.	*Chlorella*	(x 10^4/ ml)
2.	*Euglena*	(x 10^4/ ml)
3.	*Oscillatoria*	(x 10^4/ ml)
4.	*Scenedesmus*	
.	**Independent variables**	
5.	Temperature (min)	°C
6.	Temperature (max)	°C
7.	Total sunshine hours	hours
8.	W.V (wind velocity)	Knots/hr
9.	BOD_5	mg/l
10.	DO	mg/l
11.	TKN	mg/l
12.	PO_4-P	mg/l
13.	TCC	log values)

Stage-1

Correlation: Correlation between independent and dependent variables was established to classify the possible linear relationship (Tables 5-6). In the first stage of model development, correlations between 9 dependent and 4 independent variables were calculated. In addition, correlation matrix was also developed between total algal count and independent variables. The values of each correlation coefficient as positive or negative signs would disclose whether the relationship is positive (+) or negative (- reverse).

Stage-2

Selection of explanatory variables: Preliminary subsets of possible explanatory variables were obtained for stage-2 of the model development, using the stepwise forward selection (SFS) by statistical software package STATISTICA for windows. The subsets of variables were then being incorporated in next stage of model development.

Stage-3

Multiple regression models for cash and food crop area and production: The Hierarchical Multiple Regression (HMR) was used to recover the combinations of variables to develop realistic equation design. This process was accomplished by envisioning on screen the rise in R^2 and F-ratios produced by the addition of specific variable to the equation. The regression models were not always found statistically satisfactory (low F ratio, comparatively low R^2, and non-uniform distribution of the residuals).

The final models have to fulfill the following statistical criteria to be considered acceptable.

i. To produce F ratio momentous at least at the 95% significance level (P value<0.0500)
ii. The values of Durbin – Watson statistic (D.W. stat.) should be among 1 and 3 to elude complications of autocorrelation.
iii. The model should possess a random and uniform distribution of the residuals with no values beyond 3 sigma.
iv. Have no problem of multi-colinearity.

Results and Discussion

At a depth of 1.5 cm with given surface area no undesirable odor was observed which indicates the availability of adequate oxygen to maintain aerobic settings. Intermittent removal of scum assisted in sustaining good performance of the ponds throughout the study.

Ponds performance efficiency in relation to climatological parameters is given in Table 3. The mean BOD value of influent and effluent samples was 234 and 66 mg/l, respectively. The maximum BOD removal efficiency was observed in the month of October (81%). This could have been due to high temperatures which intensify the amounts of oxygen required to endure biological activity in the aerobic pond (Dalu & Ndamba 2003). It may be noted that temperature and applied loading, affect minimum and maximum removal of BOD rates range from 40% to 60% (Mara *et al*., 1992).

The mean DO value of the effluent sample was 5.63mg/l while minimum and maximum values were 4.2 (January) and 6.8 (August) mg/l. It has been noted that WSP contains significant amount of chlorophyll ranged between 500 to 2000 µg/l including chlorophyll a, which is responsible for greater DO concentration (Curtis *et al.*, 1992). This is being reiterated in the present findings (Table 3). As such, diurnal variations in the DO concentration are expected due to algal activity. After the sunrise, the concentration of DO gradually increases which is readily attributed to algal photosynthetic activity. The concentration of DO reaches its peak at the mid-day after which it gradually declines. Present findings confirm that the photosynthetic production of DO is not the only factor but it is likely to be influenced by high wind velocity which is responsible for the atmospheric dissolution of DO. After dawn the algal photosynthetic activities cease and respiration activities within the WSP consume oxygen. The surface layer of water at the WSP contains high levels of dissolved oxygen, mainly due to the dissolution of atmospheric oxygen, and algal photosynthesis which is suitable for the decomposition of organic matter by the heterotrophic organisms. Such layers of water with elevated level of DO persist for a longer period depending upon ambient temperature (Anon., 2002).

Table 3. Ponds performance efficiency (%) in relation to temperature, wind velocity and total sunshine hours.

Months (2014)	Temp°C (water)		TSH	W.V (Knot/hr)	Performance of ponds (P-3 and P-4) mg/l											
	Min.	Max			BOD (mg/l)		DO (mg/l)		TKN (mg/l)		PO_4-P (mg/l)		TCC (log values)		Chlorophyll "a" and "b" (mg/l) (effluent)	
					I	E	I	E	I	E	I	E	I	E	"a"	"b"
Jan.	9.2	28.5	8.1	2.15	225	80 (35.5%)	0.32	4.2	48	35.6 (74.2%)	4.5	2.11 (46.8%)	3.18	1.84 (57.8%)	1.24	1.86
Feb.	15.5	25.3	8.3	2.63	231	78 (33.7%)	0.41	4.8	35.2	20.9 (59.3%)	4.4	2.24 (51%)	3.24	1.92 (59.3%)	0.88	1.42
Mar.	16.4	31.8	8.2	2.38	228	75 (32.8%)	0.24	6.1	43.5	27 (62%)	5.6	3.7 (66%)	2.52	1.46 (57.9%)	1.31	1.77
April	17.3	32.5	9.1	4.21	235	79 (33.6%)	0.18	5.8	49.9	30.8 (61.7%)	6.2	3.3 (53.2%)	2.39	1.41 (59%)	1.08	0.92
May	25.3	38.4	9.3	5.41	233	67 (28.7%)	0.24	6.2	38.7	21.6 (55.8%)	5.4	3.04 (56.3%)	2.56	1.94 (75.7%)	0.47	0.86
June	25.8	37.6	9.4	8.21	229	55 (24%)	0.43	6.6	31.4	22.2 (70.7%)	4.2	3.2 (76.1%)	2.75	1.64 (59.6%)	0.62	1.01
July	27.5	37.6	9.6	7.31	238	59 (24.7%)	0.28	6.5	34.5	25.3 (73.3%)	4.6	1.4 (30.4%)	2.35	1.43 (60.8%)	0.51	1.17
Aug.	27.8	35.4	7.3	9.42	239	58 (24.2%)	0.24	6.8	32.8	21.6 (65.8%)	3.35	1.67 (49.8%)	2.28	1.57 (68.8%)	0.87	1.287
Sept.	27.3	31.3	7.4	8.81	240	61 (25.4%)	0.25	5.4	40	27.8 (69.5%)	5.2	2.1 (40.3%)	2.45	1.54 (62.8%)	0.72	1.19
Oct.	24.2	33.5	8.3	4.82	234	45 (19.2%)	0.25	6	34	22.6 (66.5%)	5.31	3.84 (72.3%)	2.63	1.34 (51%)	0.91	1.55
Nov.	25.4	34.2	6.1	3.02	220	72 (32.7%)	0.38	4.8	34.1	20.3 (59.5%)	5.44	3.63 (66.7%)	3.63	1.1 (30.3%)	0.36	1.36
Dec.	13.4	25.2	8.9	3.4	231	62 (26.8%)	0.23	4.4	37.7	30.6 (81.2%)	5.19	3.12 (60.1%)	2.56	1.25 (48.8%)	0.82	1.18
Min.	9.20	25.2	6.10	2.15	220	45	0.18	4.20	31.4	20.3	3.35	1.40	2.28	1.10	0.36	0.86
Max.	27.8	38.4	9.60	9.42	240	80	0.43	6.80	49.9	35.6	6.20	3.84	3.63	1.94	1.31	1.86
Mean	21.2	32.6	8.33	5.15	231	65	0.29	5.63	38.3	25.5	4.95	2.78	2.71	1.54	0.82	1.30
Std. Dev.	6.47	4.49	1.03	2.65	5.90	11.05	0.08	0.89	6.01	4.88	0.76	0.83	0.42	0.26	0.30	0.31
Std. error	1.87	1.30	0.30	0.77	1.70	3.19	0.02	0.26	1.73	1.41	0.22	0.24	0.12	0.08	0.09	0.08

I= influent, E= Effluent; Vertically first figure is influent value, second is effluent and figures in parentheses are percent performance efficiency; TSH= Total sunshine hours, W.V= wind velocity, TKN= Total Kjeldahl nitrogen, TCC= total coliform; Based on the analysis of minimum 8 samples per month

Kayombo *et al.* (2002) reported a direct relationship between pH and increase in DO concentration during the daytime. Hydroxyl-ions probably enhances due to increase in pH; and may reach to 11 particularly at late afternoon (Kayombo *et al.,* 2002). High pH (> 8) represents high photosynthetic activity that requires greater amount of CO_2. This also corroborates the present findings. It may be argued that algal photosynthetic activity is responsible for increased pH as carbon-di-oxide is consumed at a higher rate than it is produced by bacterial respiration through the decomposition of organic matter. However, pH above 8 represents high ammonia concentration which is toxic to algae thus hampering photosynthetic activity (Mara *et al.,* 1992). In the present study pH reaches up to 7.8 in the secondary pond (data not presented). Tadesse *et al.* (2004) also reported that the variations in pH and DO in WSP is largely dependent on the diurnal cycle of sunlight intensity. It was found that the pH and the DO concentration were higher with intensive sunlight at the mid-day. The present results are in consistent with the findings of Kayombo *et al.* (2002) and Tadesse *et al.* (2004)

The minimum and maximum values of TKN in influent samples ranged between 31.40 and 49.90 mg/l with a mean of 38.32mg/l. In an effluent samples minimum and maximum TKN ranged between 20.30 to 35.60 mg/l with a mean of 25.53mg/l. The maximum TKN removal efficiency was found in May (44%). Previous reports suggest that nutrient removal efficiency of WSP is elevated in summer than in winter (Toms *et al.,* 1975). Ferrara & Avci (1982) pointed out that the top aerobic layer of water in WSP may contain lesser number of nitrifying bacteria. Therefore, rate of nitrification is relatively slower. The nitrogen removal from WSP is mainly through the process of nitrification-denitrification, ammonia volatilization and the algal uptake. Patrick & Paul (1997) observed high nitrogen in WSP when the concentration of chlorophyll a content was highest; however, the extended retention time in WSP is significantly increases the process of nitrogen removal through nitrification. The complete removal of nitrogen can take place through denitrification process in which nitrate is converted first to nitrite and then to the nitrogen which finally escape to the atmosphere. It was also suggested that algae are directly involved in nitrogen removal from the waste stabilization ponds.

Although the removal of phosphorus from the WSP is highly variable, but poorly understood (Powell *et al.* (2008).

The maximum PO_4-P removal efficiency of WSP was 70%, obtained in July. The minimum and maximum values of PO_4-P in the influent samples fluctuated between 3.35 to 6.2 mg/l while in effluent 1.4 to 3.84mg/l. Picot *et al.* (1992) reported that removal of phosphorus in winter was low (15%) while in summer it was high (30%). The results of Picot, *et al.* (1992) validate the findings of present study.

During the algal growth when excess carbon di oxide is consumed the pH increases and higher pH values are responsible for the precipitation of phosphate with metal ions. Similar findings were also made in the present case that the pH value reaches up to 7.8.

Algal growth also consumes phosphorus for the synthesis of cellular components as vital element needed for cellular components such as nucleic acids phospholipids and nucleotides. Another method of phosphorus removal is its storage in the algae in the form of polyphosphate (Powel *et al.,* 2008). Temperature, light intensity and phosphate concentration are important factors for biological phosphorus removal in WSP (Shilton *et al.,* 2006).

The minimum log values of TCC of the influent samples ranged between 2.28 to 3.63 while that of effluent fluctuated in a narrow range of 1.10 to 1.94. The maximum (70%) removal efficiency of TCC was found in July. Marked reduction/inactivation in the microbial load of wastewater was noted with the increase in the intensity of sunlight (Sinton *et al.,* 1994; Davies-Colley *et al*., 1994). Sinton *et al.* (2002) suggested that fecal coliforms present in WSP effluent are relatively more tolerant to sunlight than those found in raw wastewater. Sunlight, along with other factors may remove up to 99.99% of microorganisms of public-health importance (Fernandez *et al.,* 1992). Khan & Khan (2007) reported about 99% coliforms removal from WSP in subtropical climate. However, in the present case TCC removal of 70 % was found in July.

Chlorophyll "a" concentrations in WSPs varies depending upon he climatological conditions. Wright *et al.* (1979) reported 963 µg /l of chlorophyll a whereas Hussainy (1979) found a maximum of 2800 µg /l of chlorophyll "a" from WSP. In the present study the maximum amount of chlorophyll "a" was 1.31 mg/l while that of Chlorophyll "b" was 1.86mg/l in March and January, respectively. Increase in chlorophyll "a" can be directly correlated with increased DO production indicating high photosynthetic activity (Kayombo *et al.,* 2003). High chlorophyll content is mainly due to high total sunshine hours, which is responsible for enhanced photosynthetic activity.

The results of algal count are reported in Table 4. The predominant algal species observed were *Chlorella*, *Euglena*, *Oscillatoria* and *Scenedesmus*. The sum of algal species were in the order of *Scenedesmus* >*Chlorella*> *Oscillatoria*> *Euglena*. Shanthala *et al.* (2009) identified 71 algal species from the WSP in Karnataka State, India with a predominance of *Scenedesmus* and Chlorella, whereas Euglenoids and Ankistrodesmus were found to be the subdominant species. Wrigley & Toerien (1990) observed flagellated algal genera including Euglena, Lepocinclis and *Chlamydomonas* appeared predominantly in small scale sewage ponds in South Africa.

It may not be out of place to mention that generally one or two species are dominant at any one time in the WSP, as reported earlier (Amengual-Morro *et al*, 2012). According to that study, the most commonly recorded genera were *Ankistrodesmus, Chlorella, Chlamydomonas, Euglena, Microcystis, Micractinium, Oscillatoria and Scenedesmus.*

The physico-chemical properties of the WSP water are directly linked with the variation in algal species. However, the usual algal succession is linked to fluctuations in organic loads and nutrients (Amengual-Morro *et al,* 2012). The ponds performance efficiency with respect to nutrient removal can be directly connected with the abundance of different types of algae found in WSP. As reported *Scenedesmus* and *Chlorella* can provide nearly complete elimination of total phosphorus, ammonia and nitrate from secondary wastewater treatment (Martinez *et al*., 2000; Ruiz-Marin *et al*., 2010; Zhang *et al*., 2008).

The maximum Chlorella count was observed in March and December (75 x10^4/ml). However, Shanthala *et al*. (2009) reported maximum algal cell count in May. The number of Euglena count was minimum as compared to other species. The minimum and maximum values of Euglena count were 2.0 to 65 x10^4/ml. In fact Scenedesmus was found in abundance throughout the year and its minimum and maximum values were 55 x10^4/ml (August) to 862 x10^4/ml (January).

The results of the first stage of model development indicate the possibility of developing expressive multiple regression models using different correlations (Tables 5-6).

Multiple regression models were developed with respect to each algal species. The validity of the equation can be judged by the coefficient of determination (R^2). The regression equation for growth of Chlorella explains 63.93% of variation in growth by the included factors (Equation 1). The main governing factors are maximum temperature, total sunshine hours and Total Kjeldahl nitrogen. The growth of Euglena is predominately controlled by minimum temperature, TKN and total coliforms (Equation 2). For the growth of *Oscillatoria* the important factors are temperature, wind velocity, TKN and total coliforms (Equation 3). The regression equation for *Oscillatoria* has an R^2 showing strong dependence on the named independent variables. Growth of *Scenedesmus* was chiefly regulated by maximum temperature, dissolved oxygen and BOD (Equation 4). The regression equation shows R^2 = 0.96 which is exceptionally high. The equation for total algal growth also has a high coefficient of determination (R^2 = 0.884). The total algal growth was principally determined by maximum temperature, wind velocity, BOD and TKN (Equation 5).

Table 4. Monthly variation in total algal count.

Months	Total algal count				
	Chlorella (x 10^4/ ml)	Euglena (x 10^4/ ml)	Oscillatoria (x 10^4/ ml)	Scenedesmus (x 10^4/ ml)	Total (x 10^4/ ml)
Jan.	75	30	7	750	862
Feb.	20	5	4	35	64
Mar.	390	20	5	16	431
Apr.	25	10	6	30	71
May.	25	5	15	103	148
Jun.	35	6	14	95	150
Jul.	25	20	13	220	278
Aug.	25	6	9	15	55
Sept.	30	Nil	11	110	151
Oct.	53	7	65	50	175
Nov.	63	3	3	62	131
Dec.	75	10	2	75	162
Mean	70.08	11.09	12.83	130.08	223.17
Min	20.00	3.00	2.00	15.00	55.00
Max	390.00	30.00	65.00	750.00	862.00
Std dev	102.79	8.53	17.00	203.19	226.00
Std Error	29.67	7.77	4.91	58.66	65.24

Equation 1
Chlorella

$$\text{Chlorella} = -323.0351 + 0.3_{\text{Min T}} - 0.55_{\text{Max T}} - 0.46_{\text{TSH}} - 1.0_{\text{W.V}} - 0.05_{\text{BOD}} + 1.37_{\text{DO}} - 0.155_{\text{PO4}} + 0.593_{\text{TKN}} + 0.087_{\text{TCC}} \pm 1186.140$$

R^2=0.6393 F ratio= 0.393 df= 9,2 P=<0.8109 D.W.= 2.95

Equation 2
Euglena

$$\text{Euglena} = -320.0142 + 2.81_{\text{Min T}} - 1.4_{\text{Max T}} - 0.17_{\text{TSH}} - 0.56_{\text{W.V}} - 0.125_{\text{BOD}} + 0.243_{\text{DO}} - 1.62_{\text{TKN}} + 0.279_{\text{PO4}} + 0.691_{\text{TCC}} \pm 160.4022$$

R^2= 0.90387 F ratio= 2.0894 df= 9,2 P=<0.1842 D.W.= 2.62

Equation 3
Oscillatoria

$$\text{Oscillatoria} = 8.6925 + 1.18_{\text{Min T}} - 0.39_{\text{Max T}} - 0.23_{\text{TSH}} - 1.5_{\text{W.V}} - 1.2_{\text{BOD}} + 0.568_{\text{DO}} - 0.738_{\text{TKN}} + 0.123_{\text{PO4}} + 0.605_{\text{TCC}} \pm 133.69$$

R^2= 0.8325 F ratio= 1.104 df= 9,2 P=< 0.9541 D.W.= 2.92

Equation 4
Scenedesmus

$$\text{Scenedesmus} = 583.739 - 0.87_{\text{Min T}} + 1.27_{\text{Max T}} - 0.34_{\text{TSH}} - 0.28_{\text{W.V}} - 0.58_{\text{BOD}} - 0.70_{\text{DO}} + 0.44_{\text{TKN}} - 0.52_{\text{PO4}} + 0.327_{\text{TCC}} \pm 780.8336$$

R^2= 0.9600 F ratio= 5.33 df= 9,2 P=< 0.167 D.W.= 2.24

Equation 5
Total algae

$$\text{Total algae} = 290.7958 - 0.59_{\text{Min T}} + 0.899_{\text{Max T}} - 0.54_{\text{TSH}} - 0.86_{\text{W.V}} - 0.65_{\text{BOD}} + 0.058_{\text{DO}} + 0.743_{\text{TKN}} - 0.42_{\text{PO4}} + 0.367_{\text{TCC}} \pm 1473.668$$

R^2= 0.884 F ratio= 1.707 df= 9,2 P=< 0.8618 D.W.= 2.59

Table 5. Correlation matrix between individual algal count and independent variables.

	CHL_	EUG_	OSCIL_	SCENE_	T_MIN	T_MAX	TSH	W.V	BOD	DO	TKN	PO4	TCC
CHL_	1.0	0.004	-0.133	-0.079	-0.331	-0.138	-0.096	-0.425	0.262	0.028	0.184	0.408	-0.168
EUG_		1.0	-0.079	0.204	0.126	-0.146	-0.218	0.332	-0.033	-0.146	0.371	-0.340	0.047
OSCIL_			1.0	-0.097	0.300	0.259	0.108	0.145	-0.692	0.299	-0.244	0.304	-0.116
SCENE_				1.0	-0.459	-0.169	0.047	-0.240	0.301	-0.443	0.633	-0.368	0.351
T_MIN					1.0	0.785	-0.121	0.796	-0.649	0.704	-0.683	-0.141	-0.184
T_MAX						1.0	0.178	0.588	-0.427	0.809	-0.465	0.004	-0.023
TSH							1.0	0.087	-0.132	0.324	0.192	-0.059	0.329
W_V								1.0	-0.649	0.686	-0.313	-0.463	0.038
BOD									1.0	-0.514	0.390	-0.017	0.278
DO										1.0	-0.474	-0.072	0.042
TKN											1.0	-0.117	-0.008
PO4												1.0	-0.401
TCC													1.0

Kayombo *et al.* (2002) reported that the diurnal fluctuation of WSP parameters are largely influenced by hourly and daily variation of light intensity. The self-purification natural process prevailed in the WSP with the help of sunlight (Tadesse *et al.*, 2004). The top water layer may be supersaturated during warm sunny afternoons in the tropical regions (Vonshak *et al.*, 1982; Picot *et al.*, 1992). Algae constituted 50–60% of the final effluent BOD_5 of secondary WSP (Mara *et al.*, 1983).

Removal of phosphate in the WSP may also be affected by the predominant algal species WSP. For example, due to high light intensity motile algae have the capability to move within the pond depth. Microalgae while synthesizing their cellular component, of which phosphorus is an essential element may results in biological phosphorus removal. The storage of phosphorus within the biomass in the form of polyphosphate is another method of biological removal of phosphorus (Powel *et al.*, 2008). It was found that algal photosynthetic production of DO in WSP was optimum for degradation of high organic load which could be easily lead to algal growth production of good quality effluent that primarily depends on rich in nutrients.

Table 5 shows correlation coefficient between algal growth and the independent variables. Chlorella was positively correlated with BOD_5 and PO_4. Euglena showed significant correlation with TKN ($p<0.001$) and PO_4 ($p<0.01$). *Oscillatoria* exhibited correlation with maximum and minimum temperature, dissolved oxygen, TKN and PO4 while negative correlation with BOD_5. Scenedesmus disclosed positive correlation with BOD_5 and TKN (p at the most 0.05) while negative correlation with minimum temperature, wind velocity (WV) and dissolved oxygen.

Regarding correlations among the independent variables most remarkable correlation between minimum temperature and wind velocity (WV). Total algal growth showed a significant negative correlation with wind velocity (WV) and dissolved oxygen ($p<0.001$) and appositive correlation with PO4 ($p<0.001$) and TCC ($p<0.05$).

Conclusions

The treated effluent produced through the WSP may serves as a liquid fertilizer. The effluent is adequately opulent in nutrients therefore; its application for irrigation may save the cost of inorganic fertilizer. The organic matter is predominantly decomposed due to the growth of algae that release oxygen for decomposition process.

The most significant benefit in the use of treated wastewater is that it can evade environmental health problems of dumping it into nearby water bodies. Thus substantial amount of fresh water can be hoarded for domestic consumption. Together with, oxygen rich effluent is produced through the WSP technology.

Table 6. Correlation matrix between total algal count and independent variables.

	Total	T_MIN	T_MAX	TSH	W_V	BOD	DO	TKN	PO4	TCC
Total	1.0	-0.5636	-0.2016	0.0175	-0.4151	0.3524	-0.3704	0.6607	-0.1303	0.2356

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