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Environmental factors controlling algal species succession in High Rate Algal Pond

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Abstract

In the last decade, studies have focused on identifying microalgal species responsible for wastewater treatment in high rate algal pond (HRAP). In this study, investigation of the microalgal community composition in Pilot-scale Race way-type high rate algal pond through study period of 24 months, chlorophyll "a" content was estimated, Physico-chemical parameters were analyzed, light intensity and temperature also measured, and the biomass productivity was calculated. It was found from the recorded results that, more than 19 genera of phytoplankton belonging to 4 divisions were identified with dominance variation throughout the study period. The presence of the species inside the pond is dependent on seasonal variation. Chemical analysis data exhibit a significant increase of COD, BOD, and TSS concentration with the temperature increase. There is an obvious impact of light intensity and temperature on the growth rate, where the highest growth response of 3.5 mg/l Chl. (a) was achieved at the highest light intensity of 1900 lux with a temperature degree of 39 °C. The average algal biomass produced from HRAP 0.99 kg algae/m3/d. This study demonstrates that the predominance of algal community structure affected by seasonal variations, and there are clear light intensity and temperature impact on the growth rate, as well as there was a promising production of algal biomass can be used in different aspects.

Keywords: Microalgal predominance, Open raceway pond, wastewater treatment

1. Introduction

High rate algal pond (HRAP) structure some portion of a propelled pond system that has been appeared to offer increasingly powerful wastewater treatment, because of higher nutrient removal rates, just as lower capital and activity costs, than conventional treatment ponds [1, 2]. High rate algal pond is a minimal cost microalga based wastewater treatment plant and is intended to accomplish two objectives: the secondary treatment of wastewater and the generation of algal biomass.

HRAPs are not quite the same as conventional oxidation ponds since they are planned in a raceway setup, increasingly shallow (0.2-1 m), continuous mixing by a paddle wheel, and can effectively work at high loading rates, and short hydraulic retention time (HRTs) [3]. The consistent mixing given by the paddle wheels presents the algal growth to quick cycles of vertical dissemination, and consequently, algal biomass thriving. Microalgae get by in earth eco-systems, showing a diversity of species present in an expansive spectrum of freshwater, brackish water, seawater, and wastewater [4].

The role of algae in the municipal wastewater treatment providing high organic removal and pathogen retraction [5, 6], this is different from the role in industrial wastewater treatment that depends on biosorption and bioaccumulation of industrial pollutants [7–12]

HRAP systems have the additional advantage of resource restoration from the wastewater. Algal biomass gathered from HRAP utilized as fertilizer, feed, or as a feedstock for biofuel production [13], with the later utilization accepting significant consideration in recent years [1, 14]. In any case, high capital expenses for large scale microalgal biofuel production presently blocks this from being a financially feasible alternative, however, this when combined with wastewater treatment, might end up reasonable in the future [14].

The primary focal points of coupling wastewater treatment with microalgae cultivation are; the creation of low-cost biomass for biofuel production,

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recuperation of valuable nutrients, and advanced wastewater treatment. In this way, wastewater treatment has been broadly applied to reuse wastewater and ensure accessible freshwater [15]. Microalgal biomass created during wastewater treatment has been reaped and effectively changed into different bio-products, for example, pharmaceuticals and bioactive compounds[16, 17]. These incorporate antibacterial, antiviral, antitumor/anticancer, antihistamine, and numerous other biologically important products [18]. Microalgae have been utilized in human health food products[19], feeds for fish and livestock [20], high-value [21–23], chemicals, pharmaceutical products [23][24] and pigments.

The algal biomass created during the treatment procedure is possibly valued collected biomass that can be utilized as composts and food and crude material for biotechnological industries [25, 26]. In this manner, the kind of the species present and the amount of the biomass are potential indicators of the treatment efficiency.

The objective of this study was to, i) investigate the effect of seasonal variation on the community structure and water characterization of the constructed HRAP, ii) determine the impact of light intensity on the algal growth, iii) estimate them for algal biomass production.

2. Experimental

This study was conducted using a pilot-scale raceway HRAP unit treating municipal wastewater installed at Zenin treatment plant, Giza Governorate, Egypt. Race way-type pond, made of glass-fiber reinforced plastics (GRP) material, with 6.5m3, and the effective wastewater depth is 0.3 m. The wastewater moves in the pond by an electric fan (paddlewheel) attached to the pond to give a flow rate velocity of 0.2 m/s. The effluent from the primary facultative pond was fed to HRAP in continuous mode. The detention time applied to HRAP was 5 days, with a Surface organic load ranged between 2.6 to 3.7 with an average of 3.32 g BOD /m2/d, the seasonal variation showed higher organic load in winter and spring and it decreased in summer and autumn **(Fig.1)**.

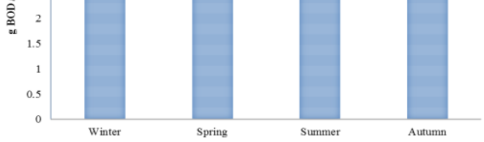

Fig. (1) varition of organic loading rate during diffrent seasons

2.1. Physicochemical characteristics of wastewater

The physico-chemical analysis of the wastewater was carried out according to [27] and covered the following parameters: total chemical oxygen demand (COD), Biochemical oxygen demand (BOD), total Kjeldahl nitrogen (TKN), nitrite-nitrogen (NO2-N), nitrate-nitrogen (NO3-N), total suspended solids (TSS), and total phosphorus (TP).

2.2. Identification of algal community structure

Lugol'-iodine solution was used to preserve the algal sample. Sub-samples were dispensed into glass Sedgwick-Rafter cells and examined using the OLYMPUS CX41 microscope. Species composition and dominance in the samples were determined semi-quantitatively. Algal identification has been done according to the main references used in phytoplankton identification[28].

2.2.1. Measurement of algal Chlorophyll "a"content

The growth rate of algal biomass was assessed by determining the chlorophyll a content. The following equation was used for calculating the concentration of chlorophyll a (as µg/L) [27].

$\mathbf{C_a}$= 11.85(OD664) – 1.54(OD647) – 0.08(OD630)
Chlorophyll a µg/L = C_a X extract volume / volume of sample L .   (Eq. 1)

Where: OD 664, 647 and 630 are the absorbance at 664,647 and 630. The proportion of algal biomass in the high rate algal pond was estimated from the chlorophyll-a concentration using the following equation [29].

[Algal biomass (mg/L)] = [chlorophyll- a (mg/L)] x100/1.5. (Eq. 2)

This equation assumes that the algal biomass has constant chlorophyll- a content of 1.5% of the dry weight. The actual chlorophyll content of algae cells varies with algal species, cell density, and growth conditions [24].

2.2.2. b. Measurement of light intensity and temperature

Light intensity was measured using a light sensor, Apogee Instruments Quantum Sensors, which was used to measure light intensity at the surface and different depths.

Table (1) Characterization of HRAP Influent and effluent

Parameters	*Units*	*Influent*				*Effluent*			
		Min	Max.	Avg.	St. Dev.	Min	Max.	Avg.	St. Dev.
pH		7	7.8	7.5	0.28	7.9	10.9	8.9	1.4
Chemical oxygen demand (COD)	mg/l	63	313	96	17	189	596	378	189
Biological oxygen demand (BOD)	mg/l	26.5	131.5	41.2	5.9	79	291	177	108.4
Total suspended solids (TSS)	mg/l	11	77	27	14	123	480	245	173
Total Kjeldahl nitrogen	mg/l	24.5	52	38	4.2	14	81	64	24
Ammonia	mg/l	18	32	26.7	0.2	0	7	1.4	2
Organic nitrogen	mg/l	5.8	21	12	3.8	11.4	81	34	13.3
Nitrite	mg/l	0.0	0.6	0.05	0.04	0.1	27	4.3	5.1
Nitrate	mg/l	0.0	0.4	0.2	0	0	7.7	2.1	1.9
Total phosphorous	mg/l	1	7	3	1.5	1.2	8.4	3.1	0.2

2.2.3. Estimation of biomass productivity

The biomass productivity of algae is a measure of its ability to produce biomass. Productivity may be expressed either in volume terms or in terms of the surface area of the culture pond. The volumetric productivity (Pv, kg m^{-3} d^{-1}) of the biomass is determined as follows:

$$P_v = \frac{X_f - X_i}{\Delta t} \qquad \text{(Eq. 3)}$$

There are several ways in which you can enter and format your text in this Where: Xi (kg m^{-3}) is the initial concentration of the biomass, Xf (kg m^{-3}) is the peak concentration of the biomass, and Δt (d) is the time interval between inoculation and the attainment of the peak biomass concentration [30].
The areal biomass productivity (Pa, kg m^{-2} d^{-1}) and the volumetric productivity (Pv, kg m^{-3} d^{-1}) of a raceway are related as follows [30].

$$P_v = \frac{P_a}{h} \qquad \text{(Eq.4)}$$

Where: h is the depth in m. productivity is high in a dilute culture but declines rapidly as the biomass concentration increases.

3. Results and Discussion

3.1. Wastewater Characterization

The HRAP was fed with municipal wastewater treated in a primary facultative pond with a detention time of 5 days. **Table (1)** summarize the characterization of the influent and effluent of HRAP. The data illustrated in **(Fig. 2)** showed that the COD and BOD concentration was higher in winter and spring than in summer and autumn. The results recorded in **Table (1)** showed a significant increase of COD, BOD, and TSS concentration in pond effluent, the average residual concentrations were 378,177 and 245 mg/l respectively.

The increase of the organic matter concentration in HRAP effluent could be due to high algal biomass production which is accepted as organic matter. These results change by changing climate temperature which affects the algal biomass production in the pond. The results illustrated in **Fig. 2 & 3** showed that as the temperature increases the COD & TSS concentration increases. In summer, as the temperature recorded 35-40°C, the COD concentration in HRAP effluent increased by 4 times its concentration in the influent as well as TSS concentrations which increased by 9 times the influent concentration. HRAP pH value ranged between 7.9 to 10.9 with an average of 8.9 (Table 1).

In summer season pH reached 10.4 and decreased to 8.5 in the winter (**Fig. 4**). The elevation of pH is closely related to the increase in algal biomass especially in summer (**Fig. 5**), as temperature ranged between 35 to 38 °C; algal photosynthesis increases and thus DO increase which raise pond pH. DO and pH increase due to algal growth associated with dissolved CO_2 consumption by algae [31]. Through photosynthesis, the utilization of dissolved inorganic carbon is accomplished by algae to produce organic matter, as shown in the following Equation.

$$6CO_2 + 12H_2O \xrightarrow{\text{Light, pigment receptor}} C_6H_{12}O_6 + 6H_2O + 6O_2 \qquad \text{(Eq.4)}$$

The nutrients removal mainly depends on two main factors, the first direct factor which is algal growth as well as subsequent biomass separation. The second factor is an indirect factor; which is resulted from raising the pH of HRAP by algal photosynthesis results stripping of ammonia and precipitation of orthophosphate causing indirect removal of nutrients. The nutrient removal efficiency in HRAP is controlled by the parameters that determine algal growth and activity, such as detention time, solar radiation, and temperature.

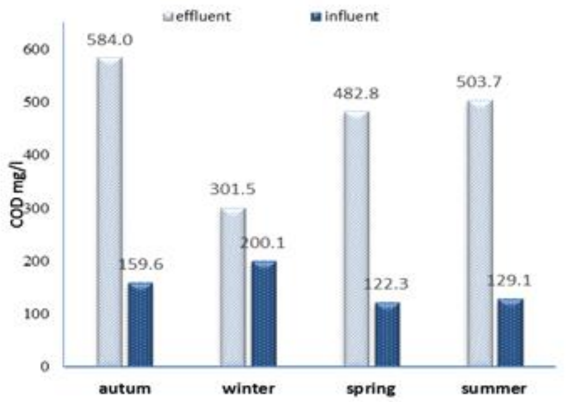

Fig. (2) COD of HRAP influent and effluent during different seasonal variation

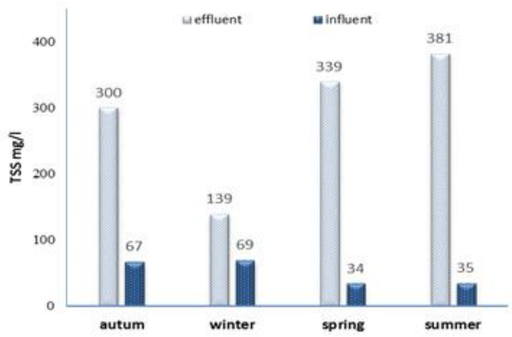

Fig. (3) TSS of HRAP influent and effluent during different seasonal variation

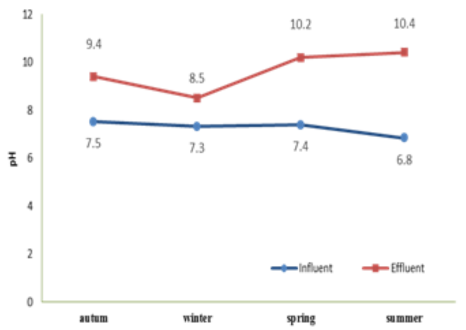

Fig. (4) pH Seasonal variation of HRAP influent and Effluent

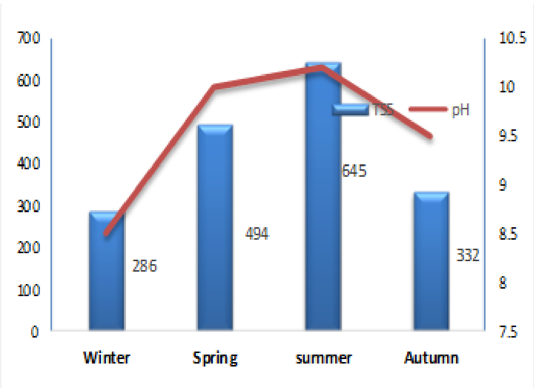

Fig. (5)The relation between TSS and pH during the diffrent seasons

3.2.Microalgae Assessment and Relative Abundance

The phytoplankton community structures of HRAP were studies through different seasons. More than 16 genera of phytoplankton were identified in the HRAP that belonging to 3 classes; Chlorophyceae, Cyanophyceae, and Bacillariophyceae. Among these genera were, *Coelosterum microporum*, *Coelastrum reticulatum*, *Selenastrum gracile*, *Oocystis parva*, *Pediastrum gracilimum*, and *Micractinium pusillum (Chlorophyceae)*, different *Microcystis sp.* (*M. flosaqua*, *M. aeruginosa*, *M. virdis) Oscillatoria limnetica* (Cyanophyceae) and *Nitzschia linearis* (**Fig.6**). Since the high rate algal pond operation started in summer 2017 (temperature may be reached to 40ºC) the HRAP algal population was predominated by different *Microcsystis sp* (**Fig. 6 and 7**). Then, the algal abundance had been changed in winter and spring (temperature degree may be ranged from 18-35ºC) where species of green were the most dominant. Hence, changes in dominant algal species were related to seasonal and temperature change where the population change according to the season and degree of temperature [32].

Therefore, in the late autumn, the community structure of HRAP completely changed where the *Microcystis* sp. appeared in minimum detectable observation and the dominance of different algal species took place. Besides, all algal species mixed and floated in the pond water column. The predominant algal species were *Scenedesmus quadricauda, Oocystisparva, Selenastrum gracilimum, Coelastrum reticulatum, Pediastrum gracilimum,* and *Micractinium pusillum* (Chlorophyta).

Since the Summer until the late autumn, the pond was dominant with *Microcystis* sp., from the late autumn to the middle of winter the pond was dominated with *Scenedesmus quadricauda*, followed by *Oocystis parva* and *Pediastrum gracilimum* which belonging to class (Chlorophyta). In addition to the presence of other species including *Microcystis* sp. This dominancy was changed since the middle of winter to be *Oocystisparva,* the most dominant species followed by *Scenedesmus quadricauda* and *Pediastrum gracilimum* (**Fig. 8**). Suddenly, *Microcystis*sp returns to be the most dominant again in the middle of spring with the presence of other microalgal species, where the same observation had been seen at the beginning of seasons.

According to [33], Small colonies of genera such as *Actinastrum*, *Micractinium*, *Scenedesmus*, and *Pediastrum* were dominated in HRPs treating domestic sewage. Also, [34] Found that, *Actinastrum sp., Micractinium sp., Pediastrum sp., as well as Desmodesmus sp. Dictyosphaerium sp*. and *Coelastrum sp. as* the main species in HRPs treating domestic sewage.

From these results, it could be concluded that since this study was carried out in a humid hot climate, the dominancy of species was affected by temperature and solar radiation.

However, it can be affirmed that the difference in solar radiation due to seasonal variation has influenced dominance, but the phytoplankton community abundance was not affected. As well as, there is no observed photoinhibition phenomenon.

[35]suggested that factors such as competition, climate, and other related types with other organisms, also the chemical production by algae, were responsible for the diversity of species and seasonal variations in the composition of the phytoplankton community observed in the carpet industry effluent. [36].

2.3. The impact of light intensity and temperature on the algal growth rate

The light intensity was seasonally measured through a year from summer to spring (**Fig. 9**) to determine the impact of available light on algal growth (i.e., Chlorophyll a content). Open raceway ponds rely on available sunlight. Seasonal variation and change in temperature have a significant impact on microalgal biomass. There was a fluctuation in light intensity through the study period that gives a good algal growth value through the middle of summer where the temperature reached 37- 40 ºC. On the other hand, high algal chlorophyll appeared through the late of summer, where it reached 2.5 mg/L with light readings 1460 Lux, then declined with decreasing the light intensity at autumn appearing a minimum value at early of winter, where chlorophyll readings reached 2.2 mg/L with light intensity 806 Lux.

One of the main controllers of microalgal performance in HRAPs is a light limitation, also it has availability impacts on both; rate and efficiency of photosynthesis and ultimately productivity [37, 38].

Starting from the middle of winter the growth increased with increasing light till reached maximum at the late of spring, where chlorophyll a reached 3.4 mg/L and light intensity reached 1900 Lux. [39] revealed that algae growth rates increase rapidly with increasing light intensity.

Light conditions influence straightforwardly the growing and photosynthesis of microalgae (Duration and intensity). A light/dark cycle was needed by microalgae for photosynthesis utilization, it needs light for a photochemical stage to deliver (ATP) Adenosine triphosphate (NADPH) Nicotinamide adenine dinucleotide phosphate-oxidase and needs dark for biochemical stage integrate basic particles for growth [40].

By investigations, it is revealed experimentally that the light duration and intensity increase is directly related to the dominancy of microalgae species. [41], placed three algae samples in different light conditions (photoperiod, intensity), it is found that there was a high difference in the growing concentration between them as the maximum biomass was recorded between 0.1 g and 2.05 g when the algae culture exposed to 62.5 µmol photons m-1 s-1 for a 16:8 h light/dark photo-period duration.

The light intensity accessible to the microalgae is represented by both the level of weakening inside the pond and internal self-shading between cells. Light going through the water column decays exponentially with depth as the microalgae absorb or disperse the light. Over 80% of the light entering a HRAP is consumed by the microalgae and the high biomass prompts strong light lessening, coming about in up to 33% of the water column getting insufficient light to support net photosynthesis [32].

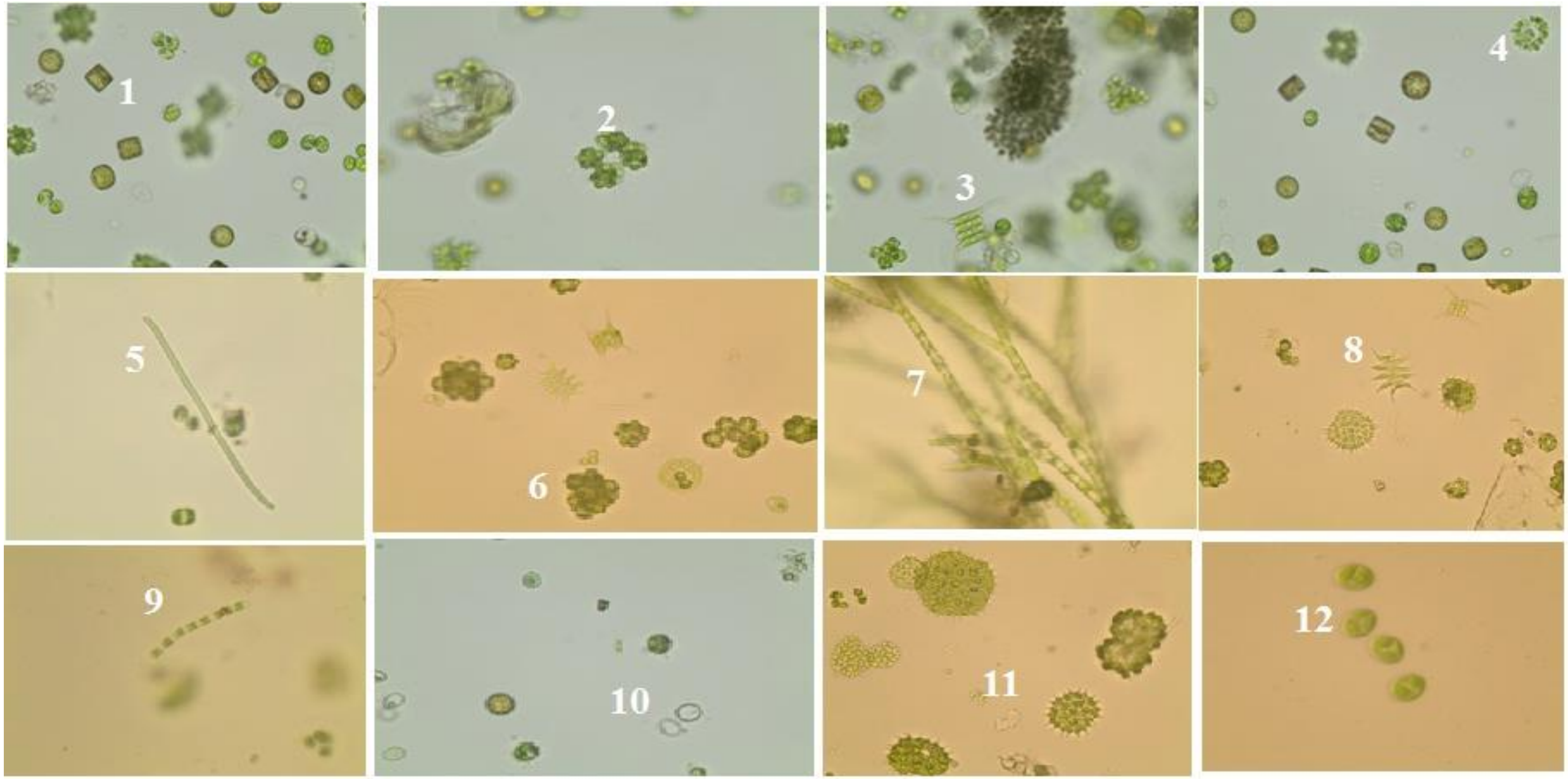

Fig. (6) Microscopic photographs of different microalgal species in HRAP 1- *Stephanodiscus sp;* 2- *Micractinium pusillum; 3- Scenedesmus quadricauda;* 4-*Dictyospharium Ehrenberg;* 5- *Oscillatoria limnetica ;* 6- *Coelosterum microporum*; 7- *Stigeoclonium tenue;* 8- *Scenedesmus obliquus;* 9- *Ulothrixsp;* 10-*Cyclotella comta;* 11- *Pediastrum gracilimum;* 12- *Siderocells elegans*

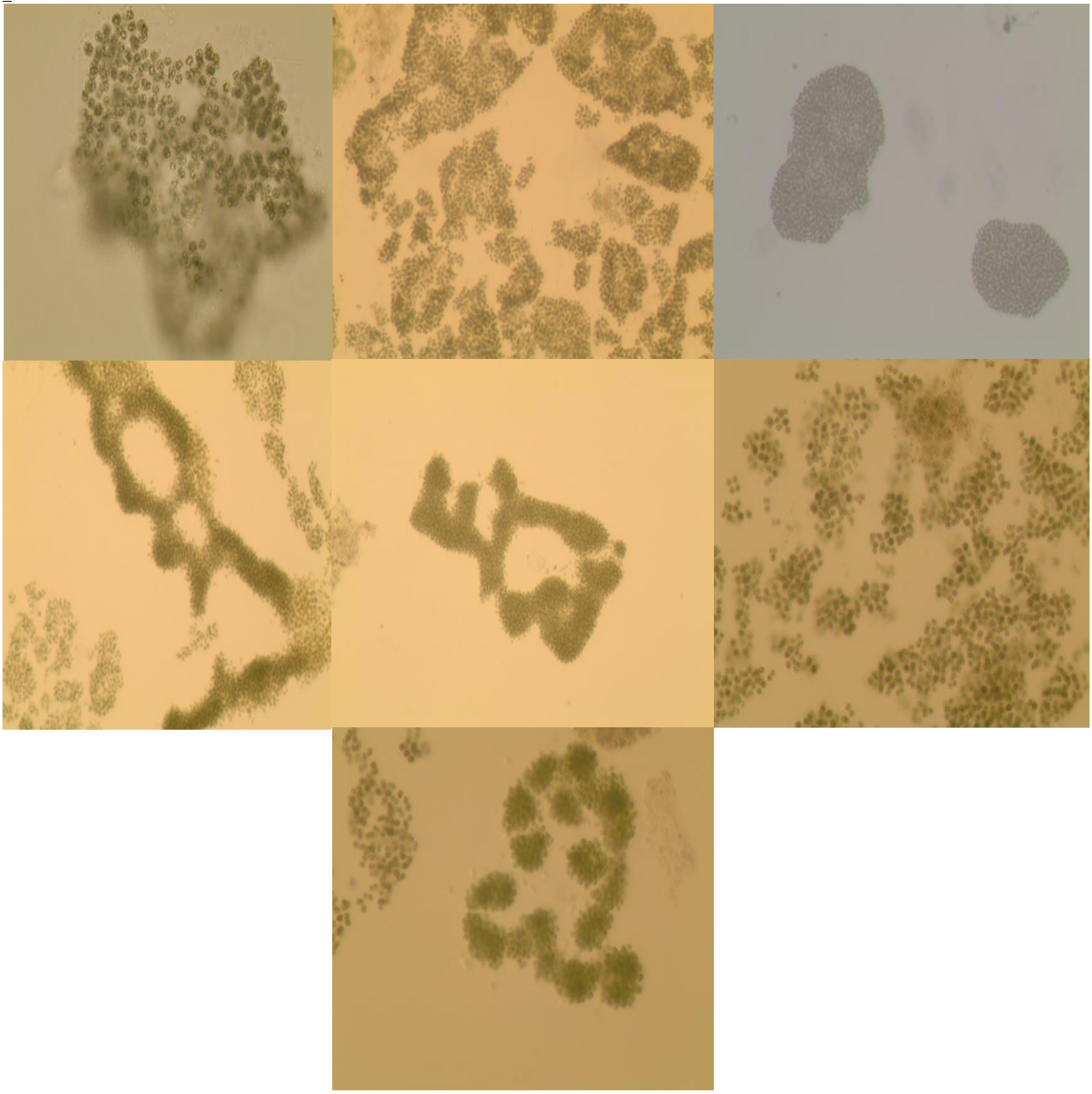

Fig. (7) Microscopic photographs of different Predominance Species of *Microsyctis* sp. in HRAP

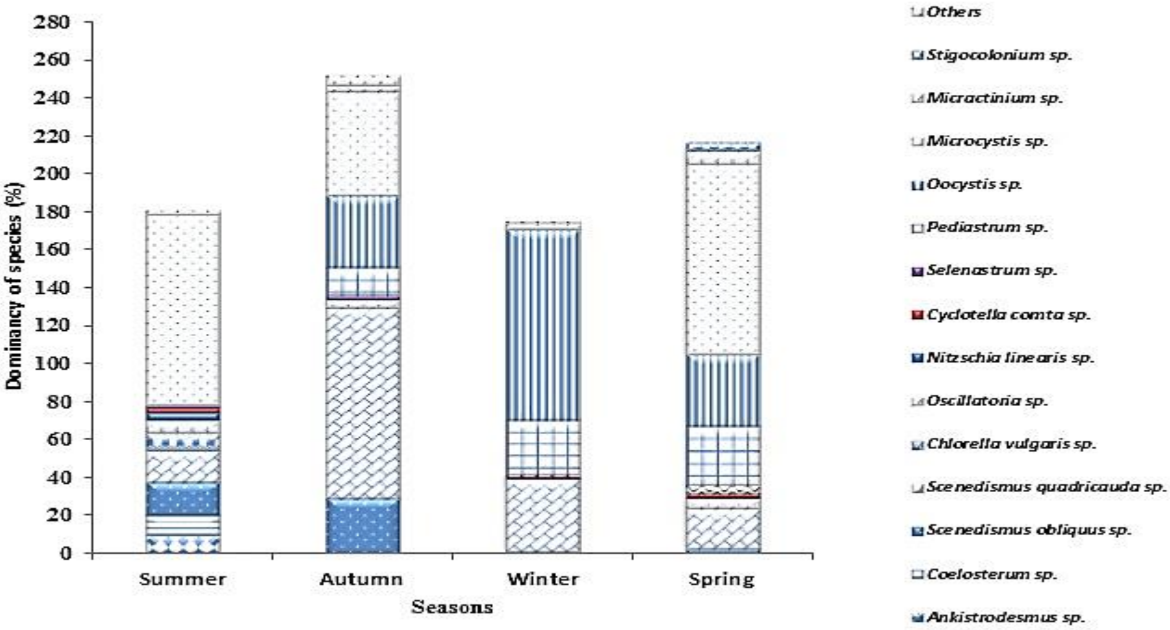

Fig. (8) Species composition in HRAP at different seasons

It is evident from the results that the growth of algae in HRAP was affected by the difference in temperature as shown in (**Fig. 10**) where it is observed that from September to November where the temperature was maximum 37 ºC the readings did not exceeds 2.8 mg/L chlorophyll. Whereas, by decreasing temperature, in the winter the readings moderately decreased to 1.5 mg/L. the growth started to increase again since the spring where the temperature increased. The growth of algae reached the maximum in the late spring, where the temperature reached 39 ºC and chlorophyll reached 3.5 mg/L.

The biomass content (calculated using Equation 1). It was obvious from the results (**Table 2**) that the dry weight increased gradually in HRAP till reached maximum in October, where the biomass detected in the pond was 308.5 mg/L. This period the pond was dominated with different *Microcystis* species and always found floated on the surface of the water. In the middle of autumn, the community structure differed as mentioned before and the algal biomass was 112.5 mg/L.

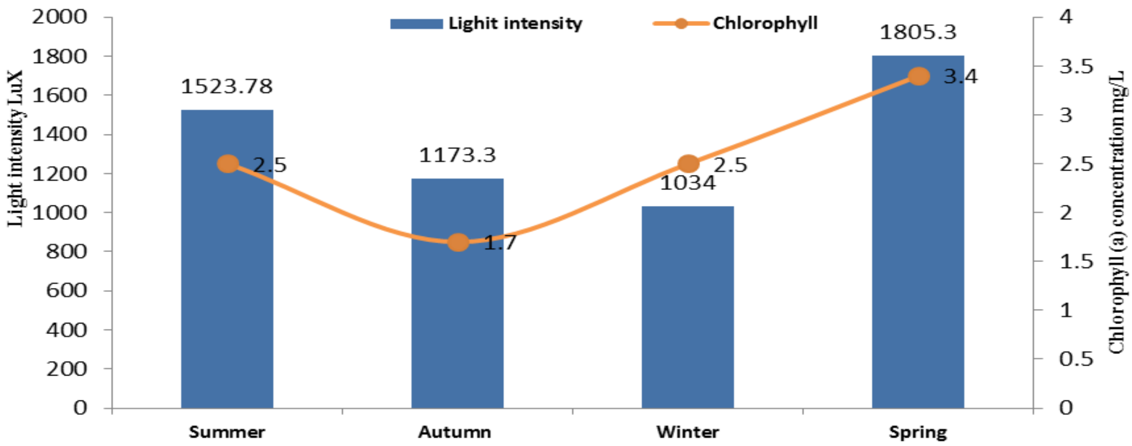

Fig. (9) Relation between light intensity and chlorophyll “a” content in HRAP at different seasons

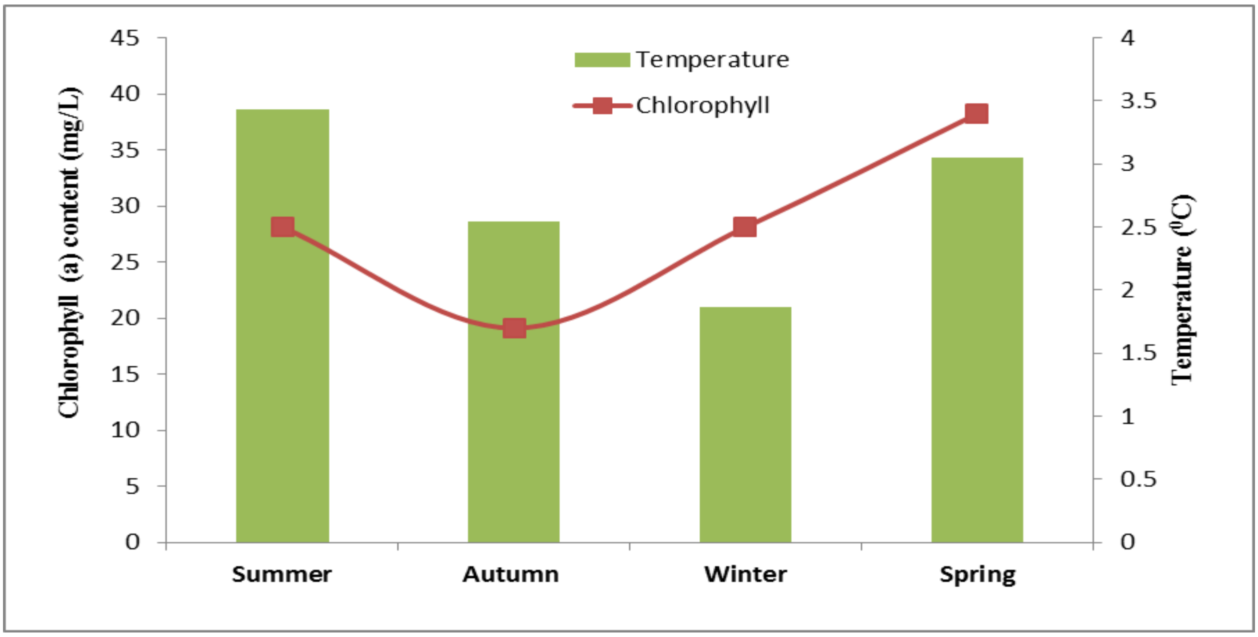

Fig. (10) The relation between temperature and chlorophyll "a" content in HRAP at different seasons

The results showed the average algal biomass and algal productivity in the HRAP (which was calculated according to Equation 2, 3) was 0.99 kg algae/m^3/d and the algal productivity was 0.3 kg/m^2/d.

Table (2) Areal biomass and algal productivity of the HRAPs during the experiment period

values	Algal biomass (kg algae/m^3/d)	Areal biomass productivity (kg/m^2/d)
Maximum	2.2±0.21	0.7
Minimum	0.57±0.14	0.17
Average	0.99	0.3

It was stated that the maximum attainable biomass concentration in a raceway is of the order of 0.5–1.0 kg/m^3, however, the results in this study showed that the average biomass concentration within the range was reported but the maximum biomass was higher. This may be due to the nature of the weather is characterized by a bright sun most of the time in Egypt [30].

4. Conclusions

It can be concluded from the data recorded in this study that, the seasonal variation changes have a great effect on the predominance of algal community structure and this indicated by the changes in the dominancy of some algal species, where the different algal species previously predominated in a definite season then disappeared in the next one started to be predominant again in the same season of the second year, while the other species disappeared gradually. Also, both factors light intensity and temperature have a clear impact on algal growth. Besides, the temperature impact reflects the chemical analysis data throughout the different seasons, as well as there is a promising production of algal biomass that can be harvested and processed in different aspects such as, biofuel production, aquaculture, and animal feeding.

5.Declaration of interest

None.

6.Acknowledgment

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