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Original Research Article

Reclamation Of Organic, Inorganic And Heavy Metal Constituents From Sewage Water Using *Chlorella Vulgaris*

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ABSTRACT
Due to the detrimental effects, the removal of xenobiotics from wastewater is crucial. Several chemical and physical methods, including ion exchange, reverse osmosis, electrodialysis, and ultrafiltration, is used to remove xenobiotics from wastewater. Due to its low operating costs and effectiveness in absorbing and/or eliminating organic and elemental contaminants from wastewater, the biological method of employing microalgae has attracted the attention of the scientific community. *Chlorella vulgaris* was utilised as a biological absorbent for sewage water (SEW) cleanup. According to the findings of this study, *C. vulgaris* successfully removed contaminants and enhanced the physicochemical properties of wastewater, such as pH, DO, and alkalinity. SEW's BOD, COD, suspended solids, heavy metals (HMs) such as iron (Fe), zinc (Zn), cadmium (Cd), mercury (Hg), copper (Cu), and lead (Pb), and nutrient load (phosphate and nitrate) were all reduced simultaneously. Cultivating *C. vulgaris* resulted in the effective cleanup of the contaminants. The investigation of Pearson's correlation revealed that physicochemical factors and algal biomass are positively correlated. For eliminating pollutants and restoring the physicochemical qualities of water, the current study supports biological wastewater treatment employing *C. vulgaris* as an efficient and environmentally beneficial approach.

Keywords: Chlorella vulgaris; Sewage water; Bioremediation; Heavy metals; Algal biomass

INTRODUCTION
Sewage wastewater (SEW) is essentially the wastewater discharged by the community after fouling by different kinds of applications.[1] The untreated water may be defined as a combination of liquid and waste that is discharged from domestic and industries uses. They also contain ground, surface and storm water.[2] The sewage water consists of 99.9% water and 0.1% solids. It is organic in composition since it contains carbon compounds from human waste, vegetable matter and paper etc. generated from domestic and industrial sources. [1] The sewage water contains environmentally persistent heavy metals. The dissolved organic matter of sewage water consists of hydrophilic acids and aquatic humic substances.[3] The presence of nutrients in raw water results in microbial proliferations and cause health hazards. Due to the presence of organic compounds and nutrients in

sewage water their disposal into aquatic ecosystem is highly dangerous and leads to eco-toxicological responses such as eutrophication and depletion of dissolved oxygen in freshwater bodies. They also cause severe negative impact on food chain.[4,5]

Sewage water being rich in nutrients might be a good option for the cultivation of crops.[6] Further, sewage water reduces the consumption and demand of freshwater. Hence in many parts of the world sewage water is being utilized for irrigation. But the use of sewage water poses a challenge in crop cultivation due to accumulation of heavy metals. These heavy metals are phytotoxic and also their entry into food chain causes chronic poisoning in humans.[7] Similarly, salinity, acidity and solidity might also affect the growth of plants. Thus recycling of sewage water to remove contaminants is highly essential. Addressing these environmental issues has become more important in a cost-effective manner. One of the most promising technologies in the treatment of sewage water is the utilization of microalgae. The use of microalgae is advantageous due to the coupling of water treatment and energy generation. Microalgae are highly effective in remediation of pollutants from sewage water, as they provide the tertiary treatment option. The microalgae assimilate nutrients present in wastewater and utilize for their growth.[8] The photosynthetic process of microalgae increases the temperature, pH and dissolved oxygen of the water in which they grow. This alteration in the physicochemical properties of water eliminates the optimal growth conditions of microbes and thus restricts the growth of pathogenic microbes.[9] The present study focuses on the batch cultivation of microalgae, *C.vulgaris* utilizing the sewage water in the growth medium. Further, the study will evaluate the biomass yield coupled with the removal of organic, inorganic, heavy metals and the alterations in physicochemical properties of the sewage water due to microalgae treatment.

MATERIALS AND METHODS

Collection of sewage water samples

Sewage water samples were collected in and around Kumbakonam city (10.97°N and 79.42°E), Thanjavur District, Tamilnadu, India. The samples were collected in containers pre-treated with acid, fixed with HNO_3, and transported to the laboratory where it was stored at 4^oC until experiments were carried out.

Microalgae growth conditions

Microalgae, *C. vulgaris*, obtained from Microalgal Mass Cultivation Centre (MMCC), Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamilnadu, India were maintained in ATCC medium: 824 ASN-III media. The composition of the medium included NaCl – 25.0g, $MgSO_4.7H_2O$ – 3.5g, $MgCl_2.6H_2O$ – 2.0g, $CaCl_2.2H_2O$ - 05.g, KCl – 0.5g, citric acid – 3.0g, Ferrous ammonium citrate – 3.0 mg, EDTA – 0.5 mg, A-5 trace metal – 1.0 ml, $NaNO_3$ – 0.75g, $K_2HPO_4.3H_2O$ – 0.75g, Na_2CO_3 – 0.02g, Vitamin B_{12} – 10.0 mcg and distilled water – 1000 ml. The composition of A-5 trace metals included H_3BO_3 – 2.86g, $MnCl_2.4H_2O$ – 1.81g, $ZnSO_4.7H_2O$ – 0.222g, $Na_2MoO_4.2H_2O$ – 0.039g, $CuSO_4.5H_2O$ – 0.079g, $CO(NO_3).6H_2O$ – 0.49g and distilled water – 1000 ml.

Chlorella vulgaris culture

Samples of *C.vulgaris* were purchased (Bill No. 103) from National Repository for Microalgae and Cyanobacteria (NRMC), Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The samples (*C. vulgaris*) were plated on an ATCC medium. The inoculated plates were maintained at 25^oC in a culture chamber, provided with a white fluorescent light source with a 12 h light/dark cycle. The growth was monitored at regular intervals. Microalgae colonies, after harvest, were transferred to a liquid ATCC medium. Uni algal cultures were produced by repeated streaking and the algae were identified by their morphological and cultural characteristics, using Biology of the Algae[10] and used for sewage wastewater treatment.

Experimental design

The experiments were carried out in a completely randomized design by Taiwo et al. (2016) with slight modifications.[11] The cultivation of microalgae, *Chlorella vulgaris* using sewage wastewater, was carried out at room temperature (34±1^{o}C) and relative humidity (65%) at the Department of Biochemistry, Government Arts College (Autonomous), Kumbakonam. 20L of wastewater samples were taken in 35 L clean and labeled bowls. To this wastewater 100 ml of ATCC medium and 0.15 g of algae, *C.vulgaris* was added. Control was maintained without growth media and microalgae. The experimental step and analysis were continued in triplicates. The algal growth was sustained for 20 days (480 h).

Collection and characterization of wastewater

The collected sewage wastewater samples were filtered, using a 0.45μm pore-sized Whatman membrane filter, to remove suspended solids and microorganisms. The physicochemical parameters of DWW such as pH, alkalinity, Dissolved Oxygen (DO), Electrical conductivity (EC), Total Dissolved Solids (TDS), Total Solids (TS), Total Suspended Solids (TSS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), phosphate and nitrate were analyzed before and after treatment of DWW with microalgae, *C. vulgaris* following the prescribed procedure of APHA, (2012) manual. The nutrient content (phosphate and nitrate) of SEW was determined spectrophotometrically. pH and EC were determined using the potentiometric method. COD, BOD, and DO were determined volumetrically. The concentration of HMs such as iron, cadmium, zinc, copper, chromium, mercury, and lead were determined, analytically with the digital UV-spectrophotometer.[12] The analysis was carried out in triplicate.

Determination of microalgae biomass productivity

The productivity of microalgae biomass was determined by UV-Visible spectrophotometer at a wavelength of 680 nm as a density indicator of microalgae, following the protocol cited in Kumar et al. (2015).[13] A standard graph was plotted against known biomass concentration (mg/ml). Different concentrations (1 mg/ml to 10 mg/ml) of microalgae were prepared and used as standard. The absorbance of the standard microalgae solution was measured at wavelength 680 nm.

Removal efficiency

The removal efficiency (RE) of pollutants by *C. vulgaris* was calculated by using the formula proposed by Taiwo et al. (2016).[11]

$$\text{RE (\%)} = \frac{Ci - Cf}{Ci\ X\ 100}$$

Where,

Ci = concentration of element in untreated sewage wastewater. Cf = concentration of element in treated sewage wastewater.

FTIR and GC-MS analysis

The functional groups of pollutants in sewage wastewater were determined, before and after treatment, using Fourier Transform Infrared Spectroscopy (model Perkin-Elmer 1725X). The wastewater samples were dissolved in a 9:1 (v/v) combination of methanol and water and vortexed overnight. Following incubation, the contents were filtered, using Whatman (No.42) filter paper. The obtained pellets were dried in a hot air oven and analyzed, using FTIR spectroscopy in the wavelength range between 400–4000 cm^{-1}.[14] The chemicals contained in wastewater were quantified, using a GC-MS Thermo MS DSQ II system, equipped with a capillary column and helium as the carrier gas (1.0 mL/min).[15]

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Statistical analysis

The descriptive means and standard deviations, Pearson's correlation analysis of physicochemical variables, HMs, and elements were carried out, using IBM software SPSS (version 25). The PCA analysis was performed with PAST software. Correlation was considered significant at $p < 0.01$ level.

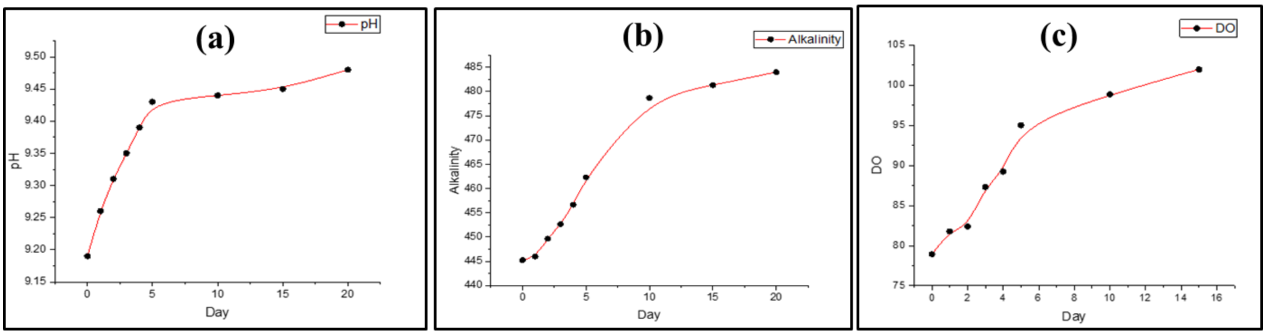
RESULT AND DISCUSSION

The characteristics of untreated sewage water before and after treatment with *C. vulgaris* are presented in Table 1. The physicochemical characteristics of sewage water after the treatment with *C. vulgaris* exhibited a maximum reduction in TDS, TSS, TS, EC, BOD and COD along with enhancement in the levels of pH, alkalinity and DO after microalgae incubation (Table 2) for a period of 20 days.[16] *C. vulgaris* utilizes the atmospheric CO_2 and involve in photosynthetic process in the presence of light to synthesis energy. This photosynthetic process of micro algae leads to the utilisation of CO_2 or bicarbonate ions as carbon source catalysed by the enzyme carbonic anhydride. The photosynthetic process increases the alkalinity and pH of the sewage water. The pH of the sewage water was increased from 9.19 to 9.45 with the growth of micro algae (Fig.1a). Similarly, the alkalinity of the sewage water used for the cultivation of microalgae was increased from 445.80 mg/L to 484.00 mg/L (Fig.1b), with 20 days of *C. vulgaris* growth. The untreated sewage water recorded a DO content of 78.94 mg/L. The growth of microalgae, *C. vulgaris* increased the concentration of DO in growth medium and a final DO concentration of 121.47 mg/1 was observed after 20 days of treatment (Fig.1c). Dinesh Kumar et al. (2017) reported a DO content of 77.84 mg/L in the untreated sewage water, which was consistent with the present study.[17]

Table 1. Characteristics of raw (untreated) and microalgae treated sewage water (SEW)

Parameters	Before treatment	After treatment
Physicochemical parameters		
pH	9.19±0.63	9.45±0.65
Alkalinity (mg/l)	445.80±31.15	484.00±31.43
DO (mg/l)	78.94±0.47	121.47±0.27
TDS (mg/l)	1072.20±2.28	675.33±12.50
TS (mg/l)	1747.60±5.75	1083.00±86.52
TSS (mg/l)	675.40±0.82	407.67±2.05
EC (ms/cm)	1.13±1.40	0.68±1.25
BOD (mg/l)	254.00±17.50	22.26±1.49
COD (mg/l)	289.00±19.81	29.53±1.29
Heavy metals		
Zinc (mg/l)	28.80±2.03	19.67±1.36
Cadmium (mg/l)	.001±1.19	0±0.22
Copper (mg/l)	18.47±0.03	2.35±0.08
Iron (mg/l)	8.00±0.56	5.20±0.35
Chromium (mg/l)	0.16±0.01	0±0.0
Lead (mg/l)	0.01±0.01	0±0.0
Mercury (mg/l)	0.016±0.01	0±0.0
Cadmium (mg/l)	0.01±1.19	0±0.0
Organic and inorganic elements		
Nitrate (mg/l)	5.80±0.35	1.50±010
Phosphate (mg/l)	16.40±0.28	3.30±0.01
Calcium (mg/l)	157.20±10.85	119.67±8.47
Chloride (mg/l)	517.20±36.05	457.33±31.85

Figure 1. Effect of C.vulgaris growth on (a) pH, (b)Alkalinity, (c) DO



Removal of solids

The TDS of sewage wager was 1072.20 mg/L, which was reduced to 675.33 mg/L (Table 2). The growth of *C. vulgaris* showed 37.01% (Fig.2a) of TDS removal from the sewage water. Though, *C. vulgaris* exhibited significant reclamation of TDS and biomass yield, it was not effective in reducing the TDS below the permissible limit of 500 mg/L. A low TDS content of 690 mg/L in sewage water was reported compared to present study.[13] Ahmad et al. (2013) reported very low percentage (1.78%) of TDS removal with the initial TDS of 4650 mg/L was reduced to 4567.2 mg/L with *C.vulgaris.*[18]

The total solids (TS) in the sewage water were 1747.60 mg/L. Most of the TS present in sewage were removed by physical sedimentation. During the treatment period a constant removal of TS was observed and at the end of microalgae treatment (20 days), the percentage of TS removal was 39.82% (Fig.2b). The concentration of TS was reduced to 1083.67 mg/L from the initial concentration of 1747.60 mg/L (Table 2). Kumar et al. (2018) reported that the sewage water to contain 780 mg/L of total solid, which was comparatively low than the TS reported in the present study.[19]

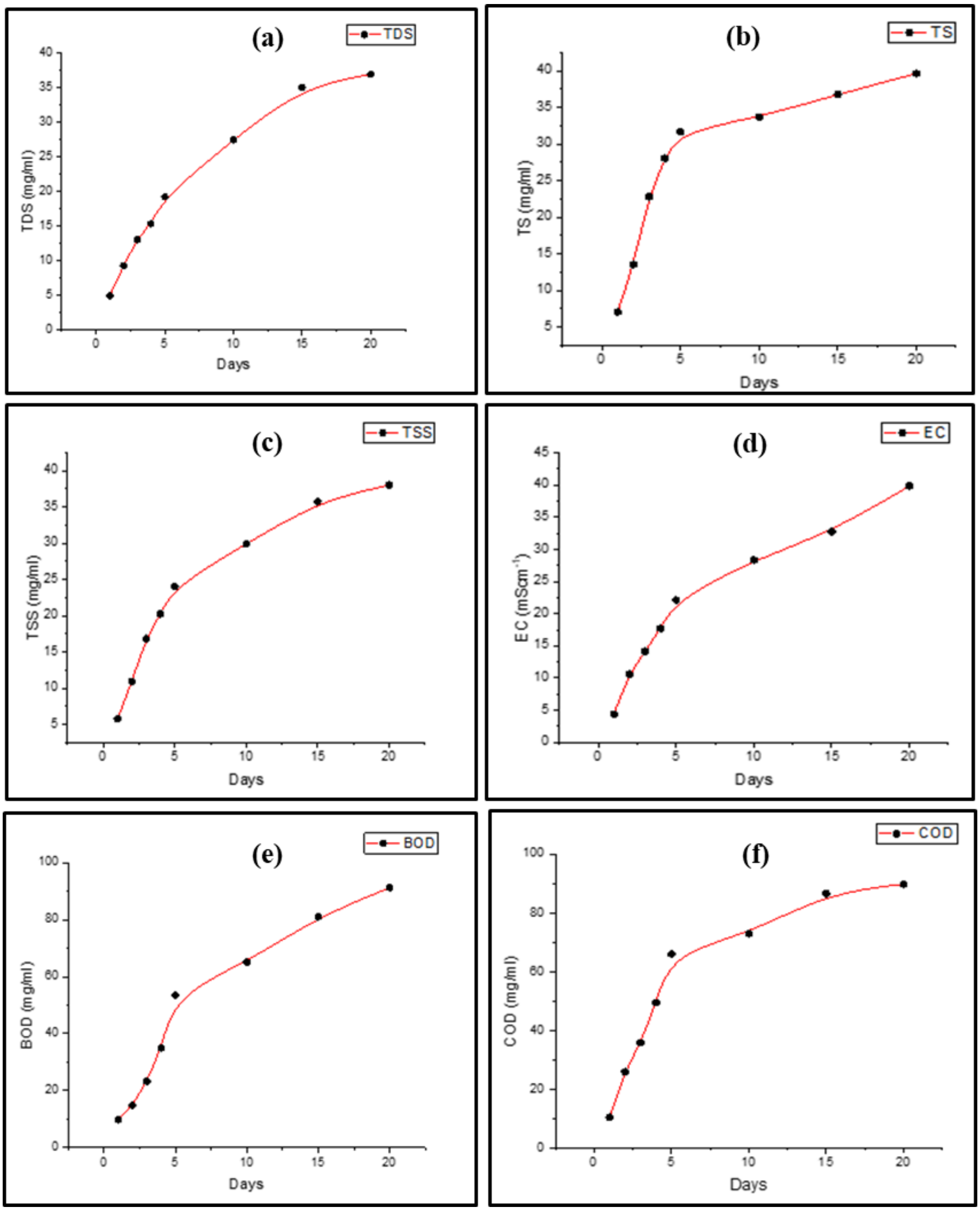
EC, BOD and COD

The EC conductivity of sewage water before treatment was observed to be 1.34 $mScm^{-1}$ (Table 1) was in agreement with the study of Renuka et al. (2013), which showed that the EC of sewage water was 1131 μScm^{-1}.[15] The removal efficiency of BOD in sewage water increases the ability of microalgae to oxidize the organic compounds into CO_2 and water with the aid of molecular oxygen as the oxidizing agent. The BOD leads to depletion of dissolved oxygen which might kill the aquatic organisms and hence its removal from the growth medium is highly essential. The BOD content of the raw sewage water was found to be 254.00 mg/L. The growth of microalgae resulted in 91.43% (Fig.2e) removal of BOD with 20 days of treatment. During the exponential growth phase of *C. vulgaris* 65.09% of BOD was reclaimed (Table 2). The COD content of sewage water was reduced to 29.53 mg/L from the initial concentration of 289.00 mg/L. The percentage of removal was observed to be 89.78% with 20 days of microalgae growth (Fig.2f). *C. vulgaris* showed 82.71 and 82.30% of BOD and COD reclamation in sewage water which did not correlate to the concentration of biomass. No significant difference in the removal of BOD and COD was observed with different concentration of *C.vulgaris*.[22] The study of Govindan (1984) observed 95% and 91% removal of BOD and COD respectively, was in agreement with the present study.[20]

Table 2. Physicochemical variables of sewage water (SEW) at different phases of microalgae treatment

Duration of Treatment (h)	Water Samples/Physicochemical variables								
	pH	Alkalinity (mg/l)	DO (mg/l)	TDS (mg/l)	TS (mg/l)	TSS (mg/l)	EC (mg/l)	BOD (mg/l)	COD (mg/l)
T1 (0 h)	9.19 ± 0.63^{c}	445.80 ± 31.15^{i}	78.94 ± 0.47^{d}	1072.20 ± 2.28^{a}	1747.60 ± 5.75^{a}	675.40 ± 0.82^{a}	1.13 ± 1.40^{a}	254.00 ± 17.50^{a}	289.00 ± 19.81^{a}
T2 (24 h)	9.26 ± 0.63^{c}	446 ± 31.15^{gh}	81.77 ± 0.39^{d}	1019.33 ± 2.52^{b}	1647.00 ± 89.60^{a}	627.67 ± 2.05^{a}	1.08 ± 4.64^{a}	229.00 ± 15.89^{ab}	258.67 ± 17.92^{b}
T3 (48 h)	9.31 ± 0.64^{c}	447.67 ± 31.20^{ef}	82.40 ± 0.99^{d}	972.67 ± 12.90^{c}	1556.67 ± 89.25^{b}	584.00 ± 2.05^{ab}	1.01 ± 1.25^{b}	216.33 ± 15.05^{bc}	214.00 ± 14.77^{ab}
T4 (72 h)	9.35 ± 0.64^{c}	452.67 ± 31.20^{e}	87.30 ± 0.39^{d}	932.33 ± 4.51^{d}	1453.67 ± 88.97^{c}	521.34 ± 1.25^{bc}	0.97 ± 8.96^{c}	195.00 ± 13.44^{cd}	185.33 ± 12.74^{b}
T5 (96 h)	9.39 ± 0.64^{cb}	456.67 ± 31.20^{d}	89.22 ± 1.21^{c}	907.67 ± 3.51^{e}	1393.62 ± 88.55^{d}	486.00 ± 2.45^{cd}	0.93 ± 3.86^{c}	165.33 ± 11.41^{de}	146.00 ± 9.94^{c}
T6 (120 h)	9.43 ± 0.65^{ab}	462.33 ± 31.29^{d}	95.00 ± 0.29^{c}	866.00 ± 5.29^{f}	1327.78 ± 88.41^{e}	461.78 ± 2.49^{de}	0.88 ± 2.94^{d}	118.00 ± 8.05^{f}	98.33 ± 6.72^{c}
T7 (240 h)	9.44 ± 0.65^{a}	478.67 ± 31.29^{c}	98.83 ± 0.21^{b}	777.00 ± 2.00^{g}	1225.00 ± 87.78^{f}	448.00 ± 0.94^{ef}	0.81 ± 4.90^{e}	88.67 ± 6.02^{g}	78.33 ± 5.11^{d}
T8 (360 h)	9.45 ± 0.65^{a}	481.33 ± 31.36^{b}	109.94 ± 0.31^{b}	696.00 ± 8.89^{h}	1123.00 ± 87.15^{f}	427.00 ± 1.70^{fg}	0.76 ± 3.68^{e}	48.33 ± 3.15^{h}	39.00 ± 2.66^{e}
T9 (480 h)	9.48 ± 0.65^{a}	484.00 ± 31.43^{a}	121.47 ± 0.27^{a}	675.33 ± 12.50^{i}	1083.00 ± 86.52^{g}	407.67 ± 2.05^{h}	0.68 ± 1.25^{f}	22.26 ± 1.49^{i}	29.53 ± 1.29^{f}

Figure 2. Effects of *C.vulgaris* on the remediation of physicochemical variables in Sewage water



Nutrient reclamation

The inorganic contaminants such as nitrate, phosphate, and chlorides play the role of nutrient and enhance the growth of microalgae.[21] Phosphate and nitrate are essential for the growth of microalgae, *C.vulgaris*. Nitrogen exists in various forms such as ammonia, organic nitrogen and nitrate. In the present study, the concentration of nitrate in the untreated sewage water 5.80 mg/L (Table 3). A low concentration of nitrate (5.80 mg/L) was observed in sewage water compared to the study of Choi et al. (2012).[22] About 74.14% of nitrate was absorbed by *C. vulgaris* from the sewage water in 20 days of growth (Fig. 3a). A rapid uptake of nitrate (58.62%) was observed during the exponential growth phase (10 days). Valderrama et al. (2002) reported 76.6% of nitrate removal by *C. vulgaris* in industrial waste water.[23] The removal efficiency of nitrate by *C. vulgaris* might be attributed to the N-dependence of microalgae from the external environmental sources for their growth.[24]

Table 3. Nutrient and element concentration of sewage water (SEW) at different phases of microalgae treatment

Duration of Treatment (h)	Organic and inorganic elements (mg/l)			
	Nitrate (NO_3^-)	Phosphate (P)	Calcium (Ca)	Chloride (Cl)
T1 (0 h)	5.80±0.35^{a}	16.40±0.28^{a}	157.20±10.85^{a}	517.20±36.05^{a}
T2 (24 h)	4.57±0.32^{ab}	15.53±0.03^{ab}	156.67±10.78^{b}	514.00±35.91^{b}
T3 (48 h)	4.40±0.28^{bc}	14.93±0.37^{ab}	154.33±10.50^{b}	510.00±35.49^{b}
T4 (72 h)	3.83±0.27^{cd}	13.50±0.03^{ab}	152.33±10.36^{c}	508.67±35.14^{c}
T5 (96 h)	3.27±0.24^{d}	12.13±0.03^{ab}	150.67±10.15^{d}	504.67±34.72^{d}
T6 (120 h)	2.80±0.20^{e}	10.03±0.03^{bc}	137.33±9.73^{e}	496.67±34.37^{e}
T7 (240 h)	2.40±0.17^{f}	9.63±0.02^{cd}	130.00±9.45^{f}	487.00±33.74^{f}
T8 (360 h)	2.00±0.14^{f}	6.53±0.02^{d}	129.00±9.03^{g}	477.67±33.11^{g}
T9 (480 h)	1.50±0.10^{g}	3.30±0.01^{d}	119.67±8.47^{h}	457.33±31.85^{h}

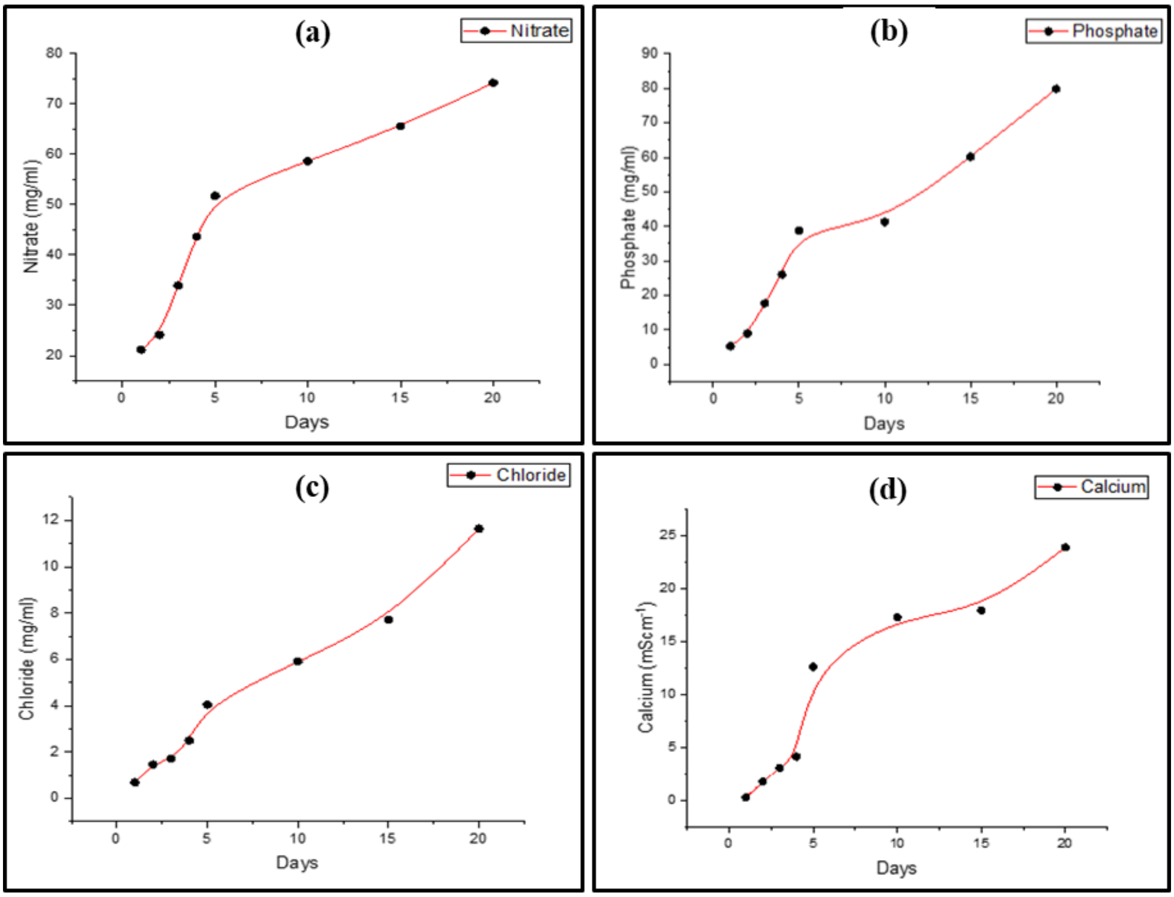
The uptake of phosphate by microalgae depends on various factors such as composition of growth media, environmental conditions, including concentration of nutrients, intensity of light, photo period and the type of algal species etc.[25] In the present study, the concentration of phosphate in sewage water was 16.40 mg/L. Treatment of sewage water with *C. vulgaris* for a period of 20 days showed a decrease in phosphate content to 3.3 mg/L with a removal efficiency of 79.88%. High phosphate absorption (41.28%) was observed during the exponential growth phase of microalgae (Fig.3b). The absorption of phosphate by *C. vulgaris* reported in the present study was comparatively high to the phosphate uptake of 28% by *C.vulgaris*.[23] Similarly 55% of phosphate uptake was reported from agro industrial wastewater by *C. vulgaris*. From the results of phosphate uptake by *C. vulgaris* it was evident that the later growth phase of *C. vulgaris* did not show significant impact compared to early days of growth.[22]

The calcium content of sewage water was found to be 157.20 mg/L. 23.87% of calcium in untreated sewage water was effectively absorbed by *C. vulgaris* and the final concentration of calcium observed in sewage water was 119.67 mg/L. Similarly, the concentration of chloride was 517.20 mg/L was reduced to 457.33 mg/L (Fig. 3) with a removal efficiency of 11.64% (Table 3). Lekhmi et al. (2015) reported 91% of chloride removal with 10% concentration of *C. pyrenoldosa*, which was significantly high compared to the removal of chloride in the present study.[26] The low uptake of chloride by *C. vulgaris* might be due to the low amount of microalgae inoculated (1g/L) compared to *C. pyrenoldosa* and due to difference in the species used for sewage water treatment. The uptake of chloride by microalgae might be attributed to the membrane that is highly permeable and helps in accumulation of chloride ions because of external osmotic pressure and low light intensity.[27]

Table 4. The heavy metal concentration of sewage water (SEW) at different phases of microalgae treatment

Duration of Treatment (h)	Heavy metals (mg/l)					
	Iron (Fe)	Zinc (Zn)	Copper (Cu)	Chromium (Cr)	Lead (Pb)	Mercury (Hg)
T1 (0 h)	8.00±0.56^{a}	28.80±2.03^{a}	18.47±0.03 ^{a}	0.16±0.01 ^{a}	0.01±0.01	0.016±0.01 ^{a}
T2 (24 h)	7.70±0.53^{b}	27.50±1.99^{ab}	16.59±0.42 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00
T3 (48 h)	7.40±0.51^{ab}	26.00±1.89^{bc}	14.77±0.43 ^{a}	-	-	-
T4 (72 h)	6.97±0.49^{abc}	25.07±1.85^{cd}	13.26±0.15 ^{a}	-	-	-
T5 (96 h)	6.67±0.47^{abc}	24.00±1.82^{cd}	10.88±0.17 ^{a}	-	-	-
T6 (120 h)	6.30±0.45^{cde}	23.03±1.75^{de}	8.54±0.17 ^{a}	-	-	-
T7 (240 h)	6.07±0.42^{de}	22.30±1.70^{ef}	5.53±0.23 ^{a}	-	-	-
T8 (360 h)	5.53±0.39^{ef}	21.00±1.40^{fg}	3.71±0.19 ^{a}	-	-	-
T9 (480 h)	5.20±0.35^{f}	19.67±1.36^{h}	2.35±0.08 ^{a}	-	-	-

Figure 3. Effects of *C. vulgaris* on the remediation of nitrate, phosphate, chloride and calcium

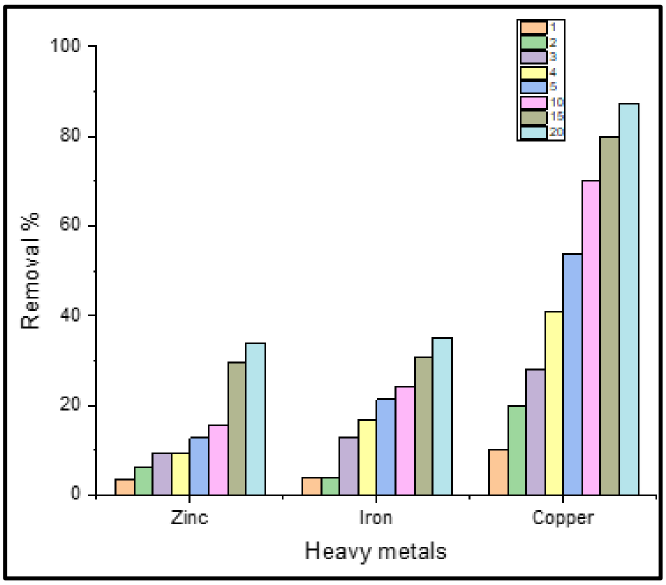


Removal of heavy metals

Sewage water is one of the major sources of heavy metals as it contains varying amount of heavy metals.[28] Hence it is essential to evaluate the impact of heavy metal pollutants on primary producers such as a micro alga which forms an important part in the food chain of aquatic organisms. The heavy metals such as copper, chromium and nickel on the growth of *C. vulgaris* showed that copper exerts maximum toxicity compared to chromium and nickel.[28] The microalgae are effective in absorbing heavy metals from waste water as they involve both absorption and adsorption mechanism. However, the binding of heavy metals depends on the species and the ionic charges of metal ions. Wong and Chang, (1991) reported that heavy metals such as copper and chromium do not inhibit the growth of microalgae below the concentration of 0.5 mg/L and 1.0 mg/L respectively.[28] In the present study, the concentration of copper and chromium was found to be 18 mg/L and 0.16

mg/L respectively. Copper was reduced by 87.20% with 20 days of microalgae treatment and chromium was completely absorbed within 24 h treatment (Table. 4). Similarly, mercury was also reclaimed completely within 24 h of microalgal growth. The study observed that *C. vulgaris* showed moderate removal efficiency against heavy metals such as iron and zinc. This reduced activity of micro algae towards iron and zinc might be attributed to the high pH of sewage water which ranged between 9.19 to 9.45 during the growth of micro algae (Fig. 4). Donmez et al. (1999) reported that the absorption of copper was effective at pH above 5. The study also reported that chromium was removed effectively by microalgal species compared to copper.[29] Greene (1984) stated that the optimum pH in the range between 6-7 was effective in removing heavy metals such as cadmium, zinc, chromium and copper.[30] Malakoottain et al. (2015) reported that pH in the range between 3 and 8 was effective in removing zinc from wastewater.[31]

Figure 4. Heavy metal removal efficiency of *C.vulgaris*



GC-MS studies and FTIR

The GC-MS analysis of sewage water showed 25 prominent peaks (Fig. 5 and Table. 5a). Dimethyl malonic acid, 3, 4-difluorobenzyl heptyl ester was the major compound that occupied 44.86% of area. The other compounds occupying significant proportion of the area are 1,4-Benzenedicarboxylic acid, dimethyl ester, Malonic acid, bis(2-trimethylsilylethyl ester, Morphinan, 7,8-didehydro-4,5-epoxy-17-methyl-3,6-bis[(trimethylsilyl)oxy]-, (5. alpha., 6.alpha.)-, Cyclononasiloxane, octadecamethyl-, Benzeneacetic acid, 2, 4, 5-tris [(trimethylsilyl)oxy]-, trimethylsilyl ester, Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15-hexadecamethyl-, Ethanol, 2-[4-(2-chloro-4-nitrophenyl)piperazin-1-yl]- and Binaphthyl sulfone. The treatment of sewage water with *C.vulgaris* showed that majority of the toxic compounds were effectively biodegraded and the GC-MS analysis of sewage water after treatment showed the presence of only 3 compounds (Fig.5b and Table 5b) Naphthalene, 2,7-dimethyl-, 1,4-Benzenedicarboxylic acid, dimethyl ester, 1,2-Benzenedicarboxylic acid, 2-ethoxy-2-oxoethyl methyl ester.

Table 5. GC-MS analysis of sewage water (SEW)
(a) Before microalgae treatment

S. No	R. time	Area %	Compound name
1	5.481	0.47	cis-5,8,11,14,17-Eicosapentaenoic acid, tetra-butyl dimethyl silyl ester
2	5.867	1.59	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene
3	6.211	0.57	n-Dodecylpyridinium chloride
4	6.286	0.63	1,4-Pentadiene
5	6.488	0.59	Bicyclo [3.3.1] non-6-en-2-ylamine
6	7.343	0.6	Spiro(6,6-dimethyl-2,3-diazobicyclo[3.1.0]hex-2-ene-4,1-cyclopropane)
7	12.712	0.59	5,6-Dihydro-4H-1-benzazonine-2,7 (3H,7aH)-dione
8	14.776	3.86	1,4-Benzenedicarboxylic acid, dimethyl ester
9	16.881	0.43	Not retrieved
10	17.796	0.38	Not retrieved
11	17.913	3.58	Hexasiloxane, tetradecamethyl-
12	18.609	0.38	1H-Imidazole, 4-methyl-5-nitro-
13	18.861	0.67	1-Decen-4-yne, 2-nitro-
14	18.937	1.26	1-[.alpha.-(1-Adamantyl)benzylidene] thiosemicarbazide
15	19.7	0.43	Bicyclo[4.1.0]heptane,-3-cyclopropyl,-7- carbomethoxy,trans-
16	20.27	3.31	Malonic acid, bis(2-trimethylsilylethyl ester
17	20.396	0.80	Benzene, pentachloronitro-
18	21.805	3.63	Morphinan,7,8-didehydro-4,5-epoxy-17-methyl-3,6- bis[(trimethyl silyl)oxy]-,(5.alpha.,6.alpha.)-
19	23.013	5.47	Cyclononasiloxane, octadecamethyl-
20	24.012	4.21	Benzeneacetic acid, 2,4,5-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester
21	24.347	44.86	Diethylmalonic acid, 3,4-difluorobenzyl heptyl ester
22	24.892	3.64	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15- hexa decamethyl-
23	25.337	1.23	Ethanol, 2-[4-(2-chloro-4-nitrophenyl)piperazin-1-yl]-
24	25.698	4.41	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
25	26.897	12.54	Binaphthyl sulfone

(b) After microalgae treatment

S. No	R. time	Area %	Compound name
1	13.098	5.09	Naphthalene, 2,7-dimethyl-
2	14.583		1,4-Benzenedicarboxylic acid, dimethyl ester
3	14.902	6.46	1,2-Benzenedicarboxylic acid,2-ethoxy-2-oxoethyl methyl ester
4	17.359	6.67	No hits retrieved
5	17.729	6.46	No hits retrieved

Figure 5. Chromatogram of sewage water (SEW)

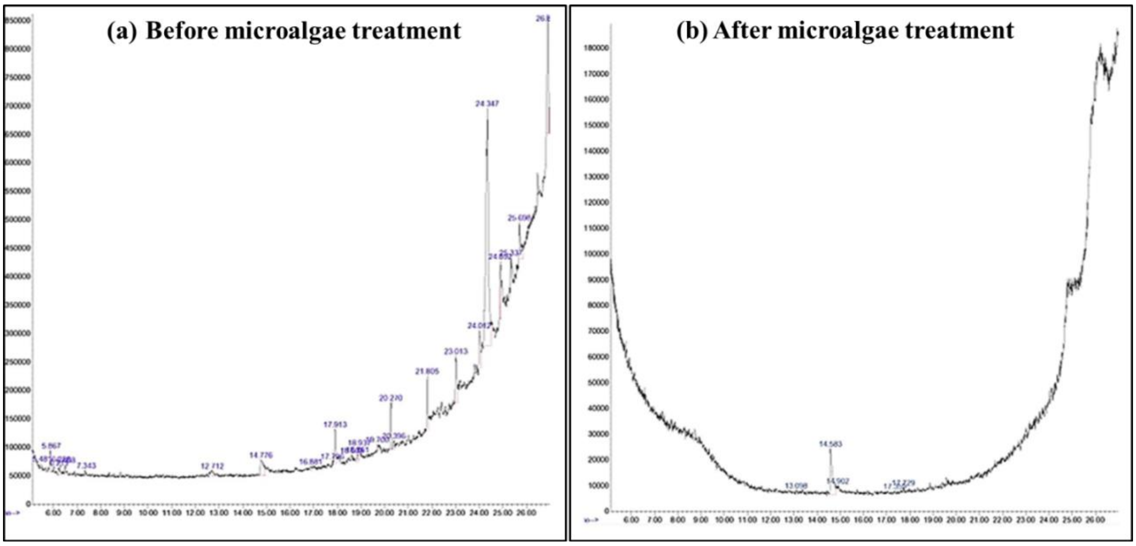
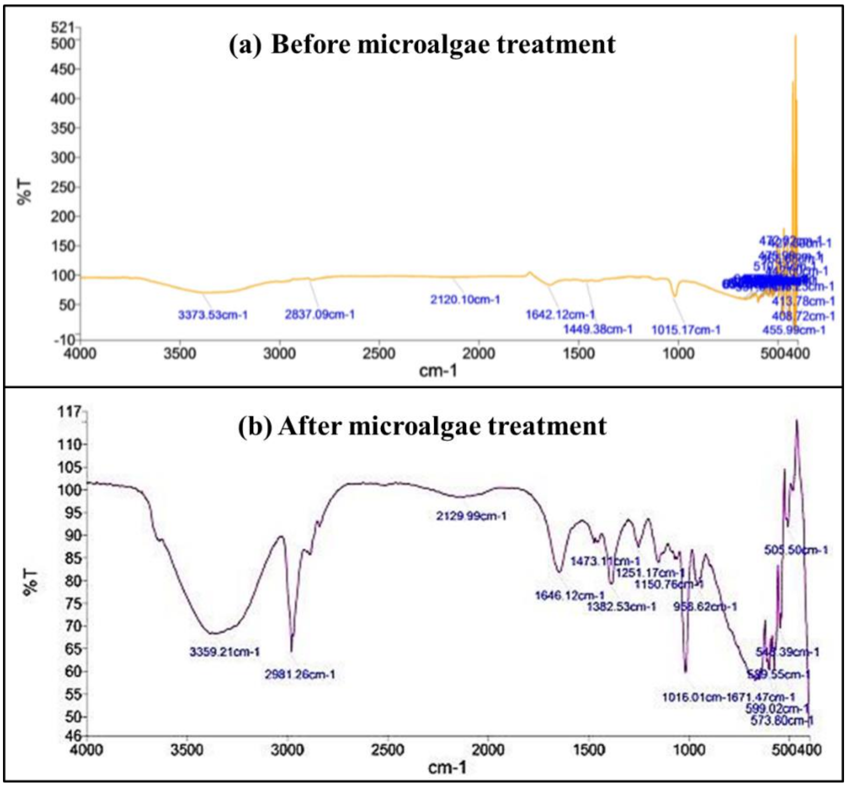


Fig. 6 represents the FTIR spectrum of sewage water before treatment with micro algae. The bands in the region 3300-3900 cm^{-1} showed the presence of O-H and N-H functional groups. The absorption band at 2981.37 cm^{-1} indicates the presence of methylene group.[32] The C=O stretching vibrations was represented with the peak at 1644.03. The protein (CH_2) and the CH_3 bending of methyl carboxylic acid were indicated with a peak at 1386.66 cm^{-1}. The phosphodiester bond stretching in nucleic acid and the ester (C-O) stretch were shown by peaks at 1252.35 and 1156.18 and 1016.26 cm^{-1} respectively. The peak at 957.64 cm^{-1} indicates –C-H and =CH_2 bending vibrations. The presence of hydrocarbons of aliphatic groups was represented with several peaks in the region 800-400 cm^{-1}.[33]

The absorption bands in the region of 3700-3300 cm^{-1} represent the characteristics peaks for O-H and N-H stretching vibrations.[34] The peak at 3373 cm^{-1} indicates the N-H stretch of aliphatic or aromatic primary amines. Increase in hydrocarbons due to the presence of aliphatic compounds was shown by several peaks in the region 400-800 cm^{-1}.[35, 36] The peak at 2837 cm^{-1} indicates the CH_2 symmetric stretching vibrations. The absorption bands between 2800 and 3000 cm^{-1} indicate the presence of lipids with the identification of methyl and methylene groups. The bands at 1015, 1149 and 1642 cm^{-1} indicate the presence of proteins and carbohydrates.[37, 38] The C=C stretching vibration was identified with the peak at 2120 cm^{-1} (Fig. 6b).

Figure 6. FTIR spectrum of sewage water (SEW)



Algal growth

Samples were recovered daily to access the growth of microalgae based on optical density observations. The result indicated a short lag phase in growth pattern of microalgae during initial 2 days. This lag phase in the growth of microalgae may be due to assimilation. An exponential growth of microalgae was observed till the 10th day. The present study observed the growth of microalgae (biomass yield) leading to the simultaneous uptake of nutrients and organic matter and heavy metals for a period of 20 days (Fig. 7). The biomass yield was high during the exponential growth phase followed by a significantly low yield during the stationary phase. Several factors influence the growth of micro algae. In the present study, the growth of *C.vulgaris* was effected with sewage water. The pH of the untreated sewage was 9.19. The production of *C.vulgaris* in sewage water was monitored using UV-vis spectroscopy at 680 nm. The micro algae showed 2.700 g/L of productivity after 10 days of growth and the productivity at the end of 20 days of growth was found to be 3.000 g/L. The linear regression equation obtained with the standard graph showed Y=0.26X+0.7556 with R^2 value of 0.9745 (Fig. 8). Mostafa et al. (2012) reported that *C.vulgaris* recorded 1.05 g/L of biomass production in sewage water with a pH value of 8.11. This reduced production of micro algal biomass may be attributed to the difference in level of pH compared to the present study.[39]

Figure 7. Algal biomass productivity with respect to cultivation days

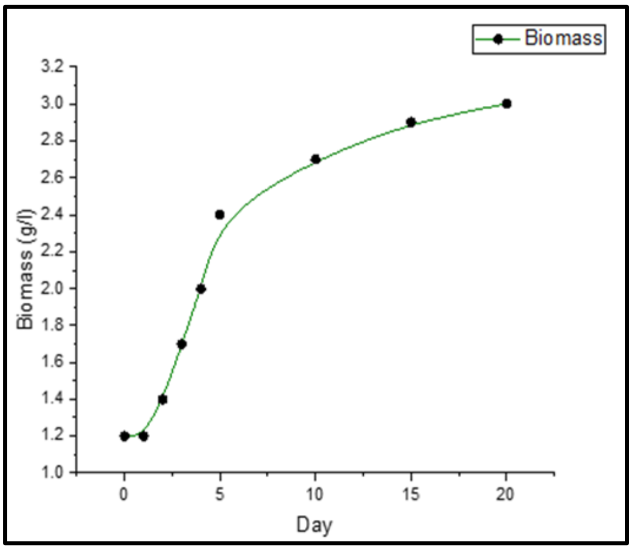
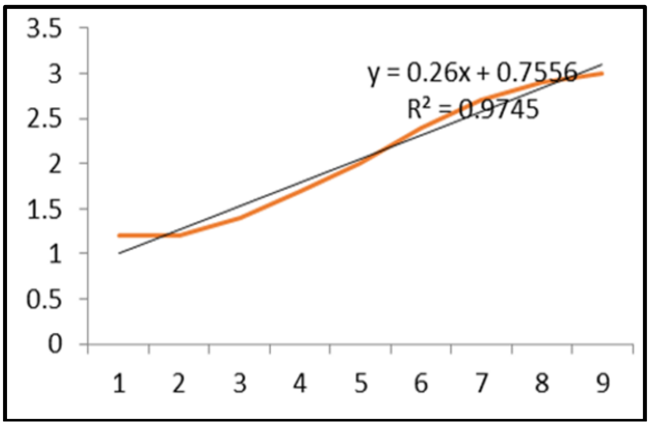


Figure 8. Linear regression of algal biomass



A positive correlation between algal biomass yield and pH (0.945), alkalinity (0.977), and DO (0.978) was observed with Pearson's correlation analysis (Table 6). The absorption of heavy metals, inorganic and organic elements had significant ($p < 0.01$) on the growth of microalgae (Table 6). Venckus et al. (2016) reported a negative correlation between nitrogen and phosphorus. In the present study, the nitrate showed a significant negative relationship with the yield of algal biomass.[40]

Table 6. Intraspecific relationship between microalgae biomass and physicochemical, heavy metal, nutrient, and elemental variables

	Biomass	pH	Ala	DO	TDS	TSS	TS	EC	BOD	COD	N	P	Cl	Ca	Cr	Pb	Hg	Cd	Zn	Fe	Cu
Biomass	1																				
pH	.945**	1																			
Ala	.977**	.880**	1																		
DO	.978**	.926**	.971**	1																	
TDS	-.977**	-.934**	-.977**	-.981**	1																
TSS	-.953**	-.995**	-.888**	-.929**	.939**	1															
TS	-.982**	-.973**	-.955**	-.975**	.990**	.978**	1														
EC	-.978**	-.958**	-.962**	-.988**	.992**	.959**	.993**	1													
BOD	-.989**	-.935**	-.979**	-.993**	.990**	.937**	.983**	.989**	1												
COD	-.988**	-.979**	-.948**	-.968**	.978**	.981**	.994**	.986**	.982**	1											
N	-0.300	-0.248	-0.438	-0.356	0.439	0.275	0.378	0.392	0.343	0.291	1										
P	-.960**	-.903**	-.950**	-.992**	.973**	.908**	.961**	.979**	.985**	.954**	0.357	1									
Cl	-.926**	-.849**	-.948**	-.977**	.957**	.849**	.927**	.961**	.964**	.911**	0.438	.985**	1								
Ca	-.969**	-.880**	-.979**	-.983**	.961**	.878**	.941**	.963**	.981**	.943**	0.308	.968**	.968**	1							
Cr	-0.445	-.681*	-0.379	-0.461	0.518	0.638	0.575	0.538	0.476	0.558	0.199	0.434	0.390	0.375	1						
Pb	-.820**	-.873**	-.725*	-.770*	.763*	.907**	.833**	.789*	.764*	.837**	0.166	.734*	0.637	.691*	0.500	1					
Hg	-0.445	-.681*	-0.379	-0.461	0.518	0.638	0.575	0.538	0.476	0.558	0.199	0.434	0.390	0.375	1**	0.500	1				
Cd	.[b]	-1**	-1**	-1**	1**	1**	1**	1**	1**	1**	1**	1**	1**	1**	1**	.[b]	1**	1			
Zn	-.915**	-.850**	-.928**	-.957**	.966**	.859**	.936**	.953**	.955**	.915**	0.466	.977**	.974**	.928**	0.432	0.662	0.432	1**	1		
Fe	-.986**	-.950**	-.959**	-.988**	.981**	.962**	.988**	.985**	.989**	.984**	0.341	.979**	.941**	.953**	0.496	.842**	0.496	1**	.945**	1	
Cu	-.993**	-.958**	-.976**	-.985**	.992**	.960**	.994**	.993**	.995**	.993**	0.361	.970**	.944**	.968**	0.520	.798**	0.520	1**	.937**	.988**	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

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