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Chlorella vulgaris DPSF 01: A unique tool for removal of toxic chemicals from tannery wastewater

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The present study investigated the removal of toxic pollutants and reduction of heavy metals from tannery wastewater using *Chlorella vulgaris*. The physiochemical parameters like pH, electrical conductivity (EC), biological oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), total dissolved solids (TDS), chloride, total hardness (TH), bicarbonate, magnesium, ammoniacal nitrogen, phosphate and heavy metals (Cu, Cr, Zn, Ni and Fe) were analysed using standard methods. Functional group of toxic chemicals in tannery wastewater and *C. vulgaris* treated wastewater were analysed by Fourier transforms infrared spectroscopy (FT-IR) and gas chromatography – mass spectrometry (GC-MS). 20 to 60% of chemicals (bicarbonate, chloride, nitrogen, phosphate and magnesium) were reduced by the treatment using *C. vulgaris* within 28 days. FT-IR and GC-MS analysis reveals that the functional group of azo compounds was not in *C. vulgaris* treated wastewater. Thus, the results obtained conclude that *C. vulgaris* can be used as a suitable tool for the removal of toxic chemicals of tannery wastewater.

Key words: *Chlorella vulgaris*, toxic chemicals, tannery wastewater, physio-chemicals, Fourier Transforms Infrared Spectroscopy (FT-IR), Gas Chromatography – Mass Spectrometry (GC-MS).

INTRODUCTION

Nowadays, industries are releasing huge amount of wastewater without treatment and causing major water pollution and diseases. There are so many conventional methods such as chemical (chlorination) and physical (sedimentation process) available for wastewater treatments but having drawbacks (Suresh et al., 2015). India is ranked third in leather production in the world and 88% of tannery industries are in Tamilnadu, Uttar Pradesh and West Bengal. The maximum tannery industries are located near river basins in Tamilnadu. During leather production, there are various toxic chemicals that are used and wastewater is directly discharged into rivers without treatment. The largest organic and inorganic pollutants present in the urban and rural wastewater is due to industrial and anthropological activities (Bernhardt et al., 2008).

The biotechnological based treatments are useful for overcoming these problems. Bioremediation is a

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worldwide acceptable technology in wastewater treatment. In bioremediation, bacteria, fungi and algae have been used. Algae are the best because it is less expensive and potential source of wastewater treatment compared to bacteria and fungi (Sheehan et al., 1998).

Microalgae are universally acknowledged in the purification of wastewater (Ayodhaya, 2013). Removal of organic and inorganic pollutants from wastewater by algae is known as phycoremediation. Phycoremediation is safe, efficient and eco-friendly for the removal of toxic materials including heavy metal from wastewater (Ding et al., 2014). The biomass of algae can be used for bio-fuel production after the wastewater treatment (Yadavalli et al., 2014). Various microalgae are used in the treatment of wastewater including *Scenedesmus* spp. (Ajayan and Selvaraju, 2012), *Chlorella marina* (Chellam and Sampathkumar, 2012), *Chlorella vulgaris* (Chu et al., 2008), *Chlamydomonas* and *Digdigma proteus* (Rehman et al., 2007), *Oscillatoria*, *Ulotrix* and *Phormodium* (Rai et al., 2005; Balaji et al., 2015).

This work aimed at evaluating the growth of *C. vulgaris* in tannery wastewater and its efficiency in reducing the pollution load of wastewater by examining the pH, EC, BOD, COD, TS, TDS, Chloride, TH, bicarbonate, magnesium, ammoniacal nitrogen, phosphate and heavy metals (Cr, Cu, Fe, Zn and Ni) of tannery wastewater before and after treatment.

MATERIALS AND METHODS

Collection of tannery wastewater and analysis of physio-chemical parameters

Tannery wastewater was collected from the wastewater outlet of tannery industries located in the Erode (Latitude - 11.3410° N, Longitude - 77.7172° E) District, Tamilnadu, India. The collected samples were stored in sterile polythene bottles at 4°C. The physio-chemical parameters of tannery wastewater such as pH, electrical conductivity, biological oxygen demand, chemical oxygen demand, total solids, total dissolved solids, chloride, total hardness, bicarbonate, magnesium, ammoniacal nitrogen and phosphate and heavy metals (Cr, Cu, Fe, Zn and Ni) were assessed using standard methods (Clesceri et al., 1989).

Collection of microalgae and chemicals

C. vulgaris DPSF01 was collected from the Department of Marine Science, Bharathidasan University, Tiruchirappalli, Tamil nadu, India. It was grown in the Bold's Basal Medium (BBM) at 20 to 23°C under fluorescent lights (with 12 h light: 12 h dark photoperiods) (Nichols, 1973). The chemicals used for the preparation of media were purchased from MERCK, Mumbai, India.

Phycoremediation

The experimental designs were T_1 (100% Raw tannery wastewater), T_2 (75% of tannery wastewater diluted with tap water), T_3 (60% of tannery wastewater diluted with tap water), T_4 (45% of tannery wastewater diluted with tap water), T_5 (30% of tannery wastewater diluted with tap water) and T_6 (15% of tannery wastewater diluted with tap water) (Cindrella et al., 2016). The culture of *C. vulgaris* was centrifuged at 10,000 rpm for 10 min and the supernatant was removed. The pellet of the algal cells were washed with sterile water and resuspended to inoculate into respective dilution. The density of *C. vulgaris* was about 30×10^3 cells/mL. The culture was grown for 28 days at a constant temperature of 15 to 20°C with the photoperiod of 12 h light and 12 h dark. At different time intervals (7^{th} day, 14^{th} day, 21^{st} day and 28^{th} day) the samples were collected and stored for further analysis (Ajayan and Selvaraju, 2011).

Analysis of algal growth

The algal growth was indirectly analysed by algal cell count method and estimated using hemocytometer during the treatment of tannery wastewater at different intervals (7^{th} day, 14^{th} day, 21^{st} day and 28^{th} day) according to Lenore (1998).

Estimation of chlorophyll

Chlorophyll (*a* and *b*) was estimated according to Arnon (1949). 20 mL of the culture was centrifuged at 10,000 rpm for 10 min. The collected pellet was mixed with 90% acetone. The mixture was centrifuged at 5000 rpm for 10 min; the absorbance value of supernatant was measured using UV-spectrometer (UV–2450, Shimadzu) at 663 nm (Chlorophyll *a*) and 645 nm (Chlorophyll *b*).

Analysis of physio-chemical properties

20 mL of sample was taken and centrifuged at 10,000 rpm for 20 min; the pellet was discarded and the pH of supernatant analysed by pH meter (ELICO Model-107). The electrical conductivity of supernatant was assessed by digital EC meter (ELICO Model-180) (Lauber et al., 2009).

The assessment of pH, EC, BOD, COD, TS, TDS, Chloride, Total Hardness, Bicarbonate, Magnesium, Ammoniacal nitrogen and Phosphate were followed by APHA (1989) method. The assessments were determined on seven days interval from 1^{st} day to 28^{th} day. Heavy metals (Zn, Cu, Fe, Ni and Cr) were assessed at different time intervals using Atomic Absorption Spectrophotometer (1983-400 HGA 900/AS 800 Perkin Elmer) and multi-Element Standard (MERCK-112837) (Fraile et al., 2005).

Statistical analysis

Experiments were carried out with three replications. Results are represented with means ± standard errors for three independent experiments.

FTIR and GC-MS analysis

The functional groups of toxic chemicals from tannery wastewater before and after treatment were analysed by Fourier Transforms Infrared Spectroscopy (Perkin-Elmer 1725_X). The treated and untreated wastewater were dissolved in methanol-water (9:1) (v/v) and kept in a shaker overnight at room temperature. After the incubation period, the sample was filtered by using filter paper (Whatman No. 42, Maidstone, England). The filtrate was dried in

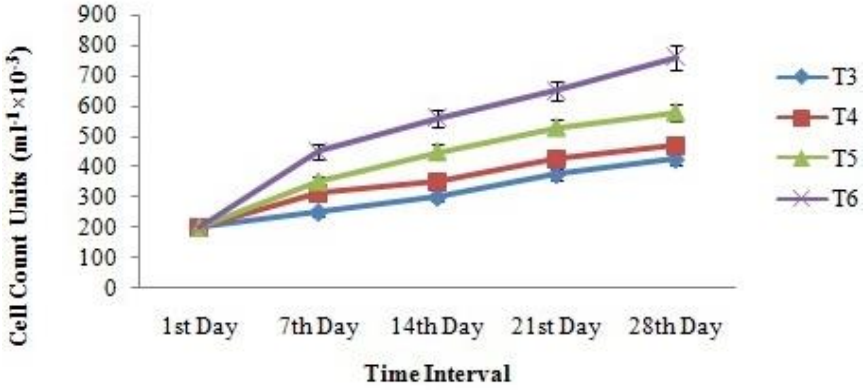

Figure 1. Cell count of *Chlorella vulgaris* on different concentration of tannery wastewater.

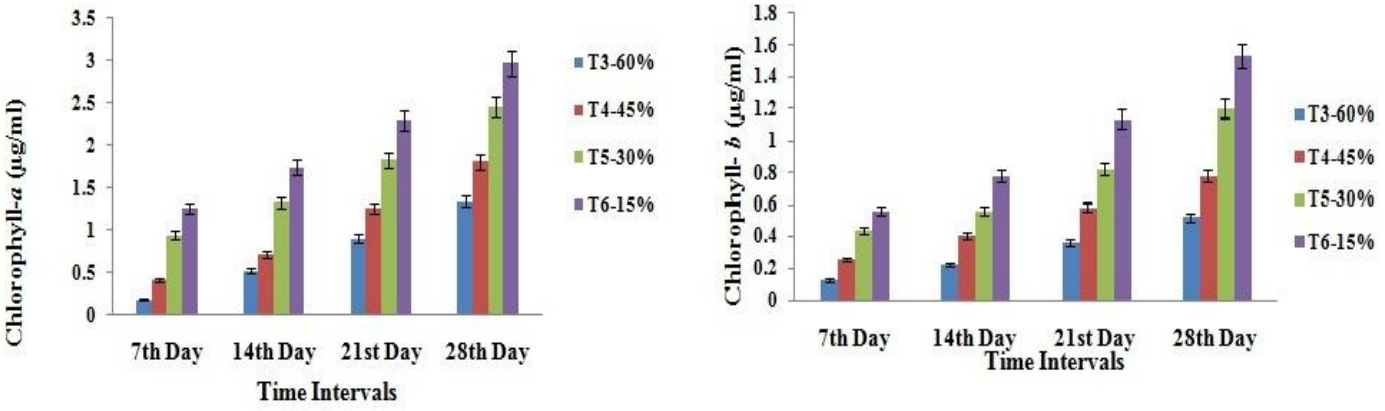

Figure 2. Analysis of chlorophyll-*a* and *b* of *Chlorella vulgaris* on different concentration of tannery wastewater.

hot air oven and the pellet was collected. The samples were analysed under the wavelength ranging between 400–4000 cm^{-1} (Kishore et al., 2015). Degradation of azo compounds was determined by GC-MS Thermo MS DSQ II, gas carrier helium (1.0 mL/min), capillary column (Ajay Kumar Pandey and Vinay Dubey, 2012).

RESULTS AND DISCUSSION

Measuring the algal growth

The maximum cell growth was found to be 760 × 10^3 cells mL^{-1} in T_6 on the 28th day whereas the lower growth was observed in T_3 and T_4 (Figure 1). The maximum growth of micro-algae in T_5 and T_6 treatment was due to heavy metal resistant mechanism and highest dilution of tannery wastewater (Rehman, 2011). The micro-algae were unable to grow in T_1 and T_2 treatments due to high amount of heavy metals and lower dilution of tannery wastewater (Ajayan et al., 2015).

Estimation of chlorophyll

The yield of chlorophyll '*a'* and '*b'* were high in T_5 and T_6 treatments on 28th day (Figure 2a and b). The Chlorophyll '*a'* reached a maximum level of 2.97 µg/mL in T_6 whereas T_5 had 2.46 µg/mL on 28th day. Chlorophyll *b* was 1.54 and 1.20 µg/mL in T_6 and T_5, respectively. The present study proves that *C. vulgaris* was able to decolourise the tannery wastewater by dominant production of chlorophyll *a* and *b* (Hanumantha et al., 2011).

Physio-chemical analysis of treated and untreated tannery wastewater

Tables 1 and 2 present the physio-chemical parameters

Table 1. Physio-chemical properties of tannery wastewater before and after treatment.

S/N	Parameters	Raw Effluent	Treated Effluent (15% dilution)
1	pH	5.5±0.3	7.78±0.20
2	EC (dsm^{-1})	13.01±0.4	2.19±0.16
3	Biological Oxygen Demand (mg L^{-1})	1560±2.64	333±4.58
4	Chemical Oxygen Demand (mg L^{-1})	2920±3.60	1314±10.39
5	Total Solids (mg L^{-1})	7152±5.03	2578±14.15
6	Total Dissolved Solids (mg L^{-1})	6370±2.88	2348±7.83
7	Chloride (mg L^{-1})	590±5.23	180±6.69
8	Total hardness (mg L^{-1})	1288±1.52	428±8.25
9	Bicarbonate (mg L^{-1})	750±5.29	177±7.26
10	Magnesium (mg L^{-1})	54±2.96	26±4.35
11	Ammoniacal Nitrogen (mg L^{-1})	17±4.48	8.12±0.60
12	Phosphate (mg L^{-1})	18±2.34	10.68±1.63

of raw tannery wastewater and algal treated tannery wastewater respectively. The pH of the tannery wastewater increased from 5.5 to 7.78 in all the treatments (T_3, T_4, T_5 and T_6). Due to the mechanism of photosynthesis, the microalgae reduced the concentration of dissolved CO_2 hence the pH of tannery wastewater rose from acidic to alkaline on treatment (Borowitzka, 1998). The electrical conductivity of tannery wastewater reduced (from 11.51 to 2.19 dSm^{-1}) after the treatment. The reduction of BOD (1560 to 333 mg L^{-1}) and COD (2920 to 1314 mg L^{-1}) in *C. vulgaris* treated wastewater confirms the carbon dioxide sequestration. Balakumar et al. (2014) reported the carbon dioxide sequestration and reduction of green house gases from tannery wastewater using algal biomass. High amounts of xenobiotics compounds contribute in increasing the COD, which was reduced to about 90% by *Chlorella* (Sharma and Khan, 2013). The reduction of total solids (7152 to 2578 mg L^{-1}) and total dissolved solids (6370 to 2348 mg L^{-1}) during the treatment increases the cell count of *C. vulgaris*.

Algae are able to reduce TDS to base level by the mechanism of biosorption and adsorption (Nandha et al., 2010). The lowest total hardness was found in T_6 treatment (428 mg L^{-1}) on the 28th day while the 10 to 50% of total hardness were reduced by the treatment of *C. vulgaris* in tannery wastewater. Similar results were observed in lake and pond water treatment using *Chlorococcum humicola* (Sivasubramanian et al., 2012). The other chemical constituents (bicarbonate, chloride and magnesium) of tannery wastewater decreased from the 7th to the 28th day using the cultivation of *C. vulgaris*. The bicarbonate (177 mg L^{-1}), chloride (180 mg L^{-1}) and magnesium (26 mg L^{-1}) were very low on 28th day, because of the utilization of nutrients by *C. vulgaris* for their growth.

50% of ammoniacal nitrogen was removed by the *C. vulgaris* which determines the denitrification and nitrification process by micro-algae (Durai and Rajasimman, 2011). Phosphate has been used for the production of ATP, phospholipids and nucleic acid hence phosphate was reduced (18 to 10.68 mg L^{-1}) by the treatment of *C. vulgaris* in tannery wastewater (Becker, 1994).

Efficiency of *C. vulgaris* on heavy metals removal

Table 3 shows the reduction of heavy metals from tannery wastewater by *C. vulgaris* during the treatment process. Heavy metals (71% of Copper, 50% of Zinc, 45% of Iron, 40% of Chromium and 20% of Nickel) were reduced by *C. vulgaris*. It shows that *C. vulgaris* is resistant to the toxicity of heavy metals in tannery wastewater. After 28 days, the reduction of heavy metals were in the following order; Copper > Zinc > Iron > Chromium > Nickel. Mehta and Gaur (2005) indicated the removal of heavy metals from wastewater by pre-treatment of algae using $CaCl_2$. The uptake of nickel by algae was stimulated by copper ions due to similar ionic properties and increased permeability of plasma membrane (Mehta et al., 2000). 50% of zinc metal was removed by *C. vulgaris* in tannery wastewater. Similar results were reported by Dinesh Kumar et al. (2015). Chromium is predominantly present in tannery wastewater and 40% of chromium was removed by *C. vulgaris.* Hammouda et al. (2015) showed that 56.3% of chromium was removed by *Chlorella* when tannery wastewater was mixed with domestic wastewater.

FTIR analysis of treated and untreated tannery wastewater

FTIR spectrum of raw and treated tannery wastewater

Table 2. Evaluation of physio-chemical parameters during phycoremediation of tannery wastewater.

Treatments	1st day	7th day	14th day	21st day	28th day
Physical parameter					
pH					
Control	5.5±0.3	5.5±0.1	5.5±0.12	5.5±0.8	5.5±0.7
T_3 (60%)	6±0.05	6.15±0.08	6.22±0.10	6.28±0.12	6.36±0.15
T_4 (45%)	6.32±0.02	6.40±0.08	6.47±0.10	6.55±0.12	6.64±0.18
T_5 (30%)	7±0.03	7.17±0.05	7.25±0.08	7.27±0.10	7.30±0.15
T_6 (15%)	7.43±0.05	7.48±0.10	7.58±0.12	7.65±0.15	7.78±0.20
Electrical conductivity (dSm^{-1})					
Control	13.01±0.4	13.01±0.2	13.01±0.4	13.01±0.3	13.01±0.6
T_3 (60%)	11.51±0.10	10.91±0.15	10.11±0.12	9.89±0.08	9.55±0.15
T_4 (45%)	9.49±0.09	8.78±0.13	8.22±0.09	7.47±0.10	6.71±0.11
T_5 (30%)	7.46±0.07	6.84±0.14	6.27±0.08	5.45±0.14	4.57±0.16
T_6 (15%)	5.33±0.15	4.65±0.13	3.92±0.15	3.05±0.14	2.19±0.16
Chemical parameter					
Biological oxygen demand (mg L^{-1})					
Control	1560±2.64	1560±2.88	1560±1.15	1560±1.73	1560±2.64
T_3 (60%)	1535±2.88	1473±2.51	1405±6.65	1336±7.93	1266±7.57
T_4 (45%)	1467±7.23	1335±6.35	1210±11.26	1080±10	954±11.37
T_5 (30%)	1379±8.14	1194±12.49	1014±3.21	836±5.29	647±8.14
T_6 (15%)	1251±11.15	1015±8.73	787±11.93	562±15.39	333±4.58
Chemical oxygen demand (mg L^{-1})					
Control	2920±3.60	2920±4.04	2920±4.72	2920±2.00	2920±6.88
T_3 (60%)	2887±8.11	2815±10.11	2744±6.08	2671±10.14	2602±13.89
T_4 (45%)	2822±8.08	2685±13.69	2543±8.14	2407±5.81	2265±6.24
T_5 (30%)	2705±8.73	2507±10.58	2301±10.97	2075±13.22	1839±9.13
T_6 (15%)	2527±9.52	2235±7.37	1922±8.62	1617±10.92	1314±10.39
Total solids (mg L^{-1})					
Control	7152±5.03	7152±5.50	7152±3	7152±3.84	7152±5.78
T_3 (60%)	6814±8.83	6356±11.05	5846±7.05	5328±6.38	4746±4.33
T_4 (45%)	6410±8.95	5858±4.09	5290±6.42	4737±9.24	4152±7.02
T_5 (30%)	5937±5.85	5304±11.56	4662±6.69	3995±4.91	3345±12.53
T_6 (15%)	5399±5.20	4673±11.93	3967±11.66	3266±7.93	2578±14.15
Total dissolved solids (mg L^{-1})					
Control	6370±2.88	6370±3.38	6370±4.37	6370±3.51	6370±5.29
T_3 (60%)	6058±3.71	5530±2.51	5047±3.75	4527±2.40	4014±7.12
T_4 (45%)	5636±5.85	5145±7.68	4647±5.60	4141±6.65	3605±5.45
T_5 (30%)	5068±4.97	4553±5.60	4067±6.00	3591±4.93	3104±7.21
T_6 (15%)	4399±7.05	3893±10.36	3411±6.65	2887±7.81	2348±7.83
Total hardness (mg L^{-1})					
Control	1288±1.52	1288±5.36	1288±3.60	1288±4.91	1288±5.81
T_3 (60%)	1265±4.84	1224±4.16	1186±4.33	1146±7.05	1106±3.92
T_4 (45%)	1179±4.58	1106±6.43	1037±6.17	969±5.81	900±4.61
T_5 (30%)	1050±5.77	958±7.05	866±8.08	774±4.35	675±6.11
T_6 (15%)	887±6.33	772±6.35	662±4.16	550±7.50	428±8.25

Table 2. Contd.

Chloride (mg L^{-1})					
Control	590±5.23	590±4.93	590±5.68	590±6.42	590±4.72
T_3 (60%)	578±8.71	566±8.71	551±5.56	538±2.51	524±7.37
T_4 (45%)	560±5.13	527±5.48	492±7.21	461±6.08	425±5.29
T_5 (30%)	535±4.58	476±4.91	426±7.75	368±5.77	311±8.38
T_6 (15%)	477±7.68	401±5.20	330±6.08	252±5.68	180±6.69
		Bicarbonate (mg L^{-1})			
Control	750±5.29	750±3.05	750±4.50	750±5.56	750±2.64
T_3 (60%)	735±5.50	715±7.68	687±7.57	661±6.65	634±4.40
T_4 (45%)	708±4.16	657±5.92	602±5.48	549±6.35	497±5.54
T_5 (30%)	643±6.35	568±5.36	495±4.09	423±5.68	348±8.08
T_6 (15%)	571±6.35	473±4.63	373±3.92	272±5.89	177±7.26
		Magnesium (mg L^{-1})			
Control	54±2.96	54±4.40	54±3.78	54±4.66	54±2.64
T_3 (60%)	53±4.93	51±4.35	48±2.60	44±4.05	41±3.78
T_4 (45%)	51±4.35	47±2.64	42±4.05	39±5.23	33±4.16
T_5 (30%)	49±2.60	43±3.48	36±3.60	31±4.04	24±4.16
T_6 (15%)	45±4.72	41±3.78	37±3.78	32±3.48	26±4.35
		Ammoniacal Nitrogen (mg L^{-1})			
Control	17±4.48	17±4.72	17±3.21	17±2.64	17±4.72
T_3 (60%)	16.5±3.06	15.62±2.98	14.39±2.34	12.5±2.01	10.48±0.36
T_4 (45%)	16.15±3.09	15.22±2.72	14.1±2.57	12.51±2.95	10.29±1.66
T_5 (30%)	16.02±2.59	14.33±2.04	12.78±1.95	11.03±1.31	9.37±1.76
T_6 (15%)	15.97±1.76	13.99±1.82	11.97±1.14	10.08±1.51	8.12±0.60
		Phosphate (mg L^{-1})			
Control	18±2.34	18±2.15	18±1.62	18±2.05	18±1.66
T_3 (60%)	17.80±1.56	17.55±0.86	17.02±1.92	16.92±1.17	16.51±3.28
T_4 (45%)	17±4.72	16.56±3.30	15.96±3.04	15.12±2.67	14.62±2.53
T_5 (30%)	16.01±3.11	15.18±2.71	14.26±2.61	13.29±1.70	12.67±3.11
T_6 (15%)	14.98±2.56	13.91±1.72	12.86±3.19	11.98±2.15	10.68±1.63

are shown in Figure 3a and b. A peak at 3510 cm^{-1} represents NH_2 group of aromatic amines. The region between 3420–3250 cm^{-1} indicates the presence of OH group of alcohols and phenols. A broad peak at 2250 cm^{-1} indicates C≡C of alkynes. The FTIR data of raw tannery wastewater (Figure 3a) shows the presence of azo group from the region between 1539 to 1580 cm^{-1}. The wave number 1315 cm^{-1} shows the presence of SO_2 in sulfones (Figure 3a).

The peak value between 3200 to 3600 cm^{-1} represents the stretching vibration of O-H and N-H group; the peak value 1258 cm^{-1} indicates the stretching of phosphodiester (>P=O) in nucleic acid of microalgae (Dilek et al., 2012); whereas the peak value of 2756 cm^{-1} indicates the stretching of OH group of carboxylic acid on treated water. A net negative charge formed by the carboxyl, hydroxyl, amino and sulphydryl groups on the cell surface confirms the high affinity for the binding of heavy metals (Deng et al., 2007; Gupta and Rastogi, 2008). The region between 1080 to 1040 cm^{-1} denotes the presence of SO_3H in sulfonic acid. Gardea-Torresdey et al. (1990) reported that the carboxyl group has a higher metal binding capacity followed by –OH, $–SO_3H$ and $–P_2O_3$.

In Figure 3b (treated tannery wastewater), there was no peak value between 1539– 1580 cm^{-1} indicating the absence of azo group. The comparative FTIR spectra analysis of treated and untreated tannery wastewater reveals that the absorption peak of azo compound groups was not present in the treated wastewater.

Table 3. Heavy metals analysis of raw and treated tannery wastewater.

Treatments	7th Day	14th Day	21st Day	28th Day
		Chromium (ppb)		
Control	98.69±4.85	98.69±4.20	98.69±3.30	98.69±4.96
T_3 (60%)	95.36±2.63	90.96±2.51	86.38±3.58	82.16±4.65
T_4 (45%)	92.56±2.77	86.93±1.65	81.16±1.23	75.66±3.64
T_5 (30%)	89.67±2.36	80.57±1.16	71.55±2.16	62.44±2.84
T_6 (15%)	85.35±3.19	74.23±2.20	66.02±3.65	51.86 ±1.35
		Copper (ppb)		
Control	70.61±2.69	70.61±2.21	70.61±2.50	70.61±1.06
T_3 (60%)	67.66±3.97	60.75±2.28	51.54±3.64	45.62±2.78
T_4 (45%)	64.70±2.43	52.23±2.05	44.36±2.38	38.27±3.86
T_5 (30%)	61.71±3.03	45.16±2.35	37.12±3.08	30.85±2.17
T_6 (15%)	58.79±2.97	35.18±1.35	30.83±2.15	20.68±0.99
		Iron (ppb)		
Control	66.32±1.76	66.32±2.43	66.32±2.49	66.32±2.53
T_3 (60%)	64.06±2.21	60.13±3.22	55.53±2.79	50.59±1.44
T_4 (45%)	61.96±2.36	57.03±3.05	51.33±3.11	43.67±1.47
T_5 (30%)	59.71±3.54	55.58±3.09	50.38±1.44	41.56±1.52
T_6 (15%)	57.25±3.10	50.51±2.50	44.27±2.31	35.52±2.24
		Zinc (ppb)		
Control	36.34±2.60	36.34±2.56	36.34±3.03	36.34±2.85
T_3 (60%)	35.22±3.10	34.10±2.28	31.26±1.42	28.66±1.77
T_4 (45%)	33.10±2.07	32.06±1.73	27.35±2.04	25.38±2.12
T_5 (30%)	34.32±2.27	33.32±1.29	25.32±2.13	21.26±1.40
T_6 (15%)	32.78±2.13	29.12±1.56	23.79±2.45	18.48±1.68
		Nickel (ppb)		
Control	12.86±1.38	12.86±1.10	12.86±0.62	12.86±0.52
T_3 (60%)	12.69±1.55	12.54±0.95	12.37±1.49	12.18±1.04
T_4 (45%)	12.50±1.08	12.16±1.46	11.80±1.30	11.46±1.35
T_5 (30%)	12.32±1.38	11.51±1.34	10.91±0.87	10.76±1.12
T_6 (15%)	12.17±1.04	11.52±1.34	10.82±1.13	10.12±1.44

Degradation of azo compounds by *C. vulgaris*

GCMS analysis confirmed the degradation of azo compounds by *C. vulgaris*. Figure 4a represents the GCMS analysis of raw tannery wastewater. Azo compounds such as 1H-1,2,4-triazole-3-yl-N-[2-(3-methylphenoxy)ethyl] carboxamide (Mol-wt–246; RT-30.99), 1,6-dihydroimidazo[4,5-d]imidazole (Mol-wt–108; RT-5.58) and 4-{4-(3,5-dimethylphenyl)-2-[4-(methylsulfonyl)phenyl]-1,3-thiazol-5-yl}-2,6-dimethylpyridine (Mol-wt–416; RT-31.45) were present in tannery wastewater. All the above compounds were absent in *C. vulgaris* treated wastewater (Figure 4b). The degradation of azo compounds by azo reductase in *C. vulgaris* was due to the breakage of N=N bond (Lin and Liu, 1992).

Conclusion

Results of this investigation concluded that in tannery wastewater, *C. vulgaris* has a remarkable potential to survive as well as uptakes nutrients and heavy metals from tannery wastewater. Appreciable reduction of BOD and COD in tannery wastewater provides a space for the survival of other aquatic organisms. The mechanism of adsorption of heavy metals and uptake of nutrients from

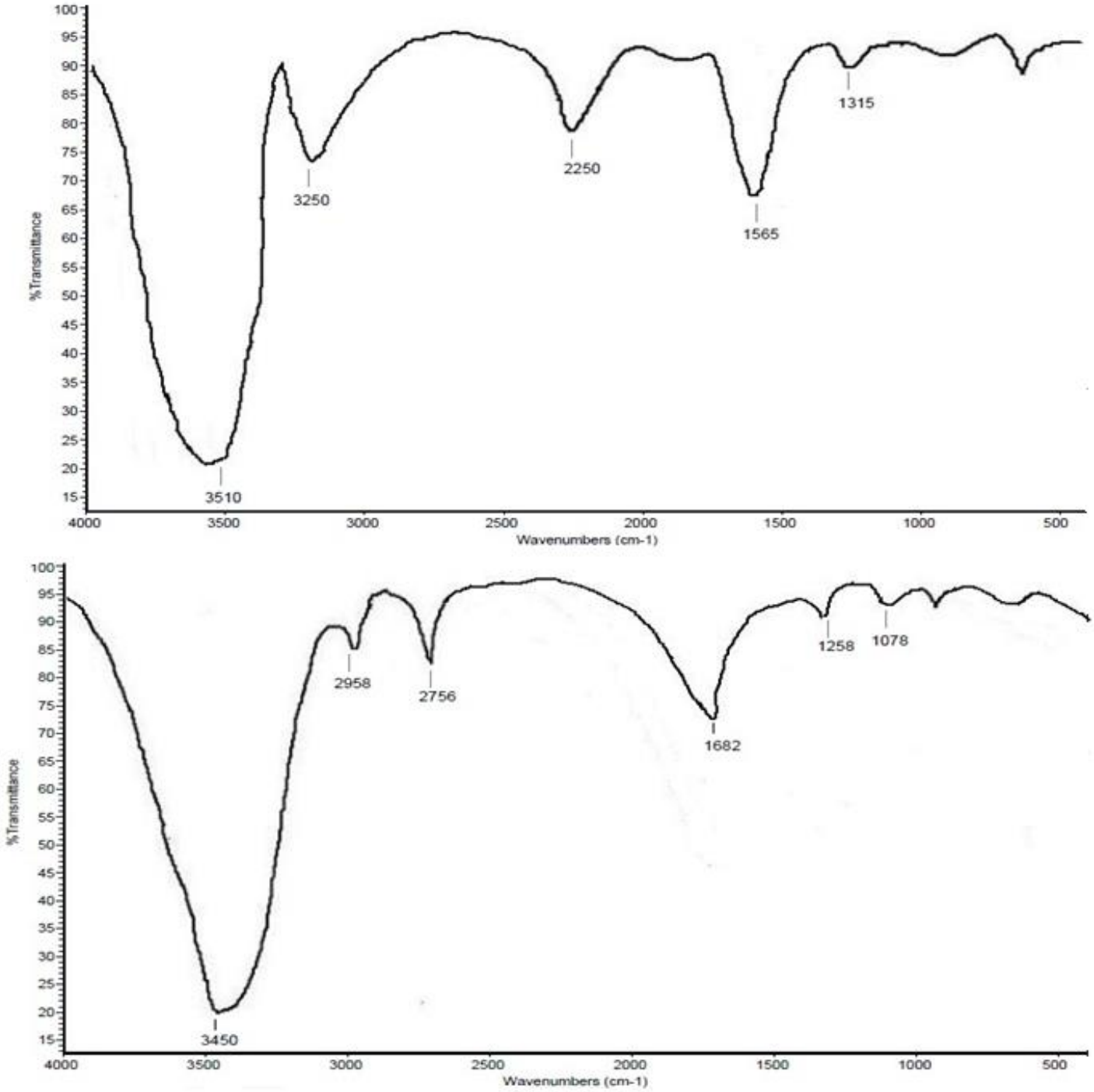

Figure 3. FT-IR spectra of raw and algal treated tannery wastewater.

wastewater will be studied in future.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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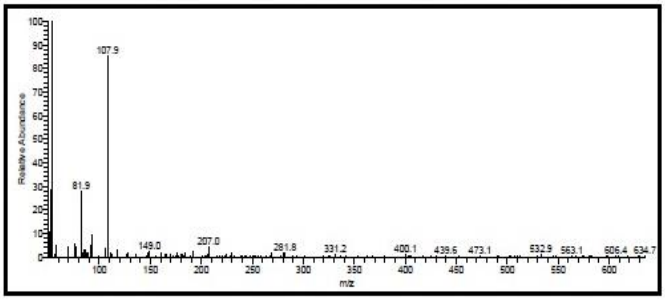
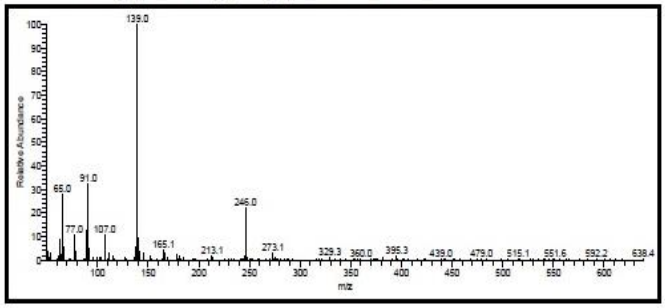
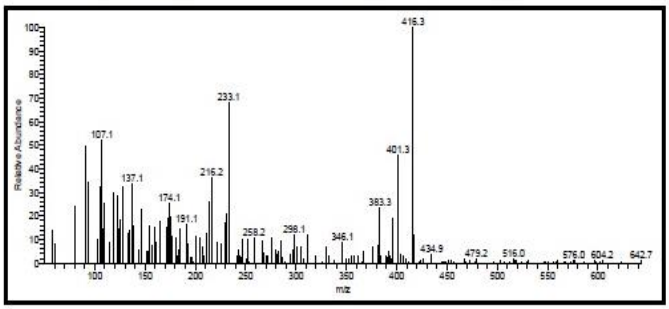
(a) Mass spectrum of 1,6-Dihydroimidazo[4,5-d] imidazole



(b) Mass spectrum of 1H-1,2,4-triazol-3-yl-N-[2-(3-methylphenoxy)ethyl]carboxamide

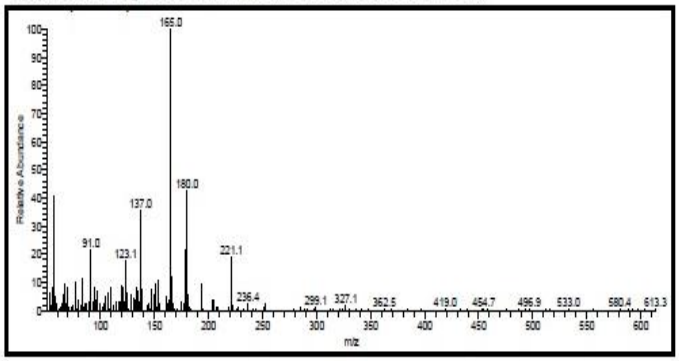


(C) Mass spectrum of 4-(4-(3,5-Dimethylphenyl)-2-[4-methyl sulfonyl)phenyl]-1,3-thiazol-5-yl}2,6-dimethyl pyridine

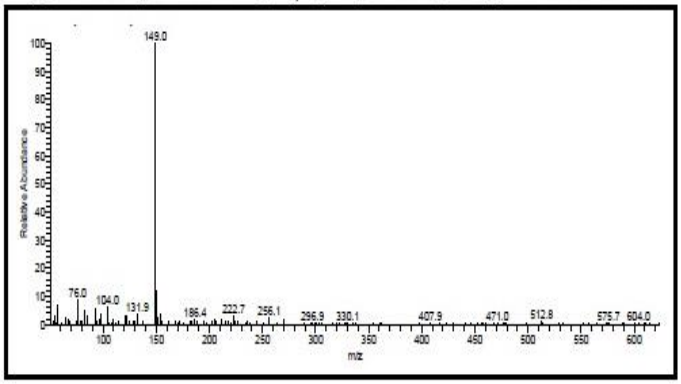


(I)

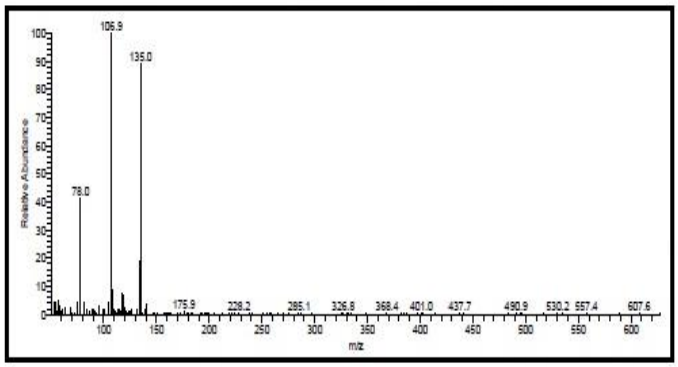
(a) Mass spectrum of 1,6-Methanofluorene



(b) Mass spectrum of,4-(2-propen-1-xyloxy) Benzeneamine



(c) Mass spectrum of 2,3,4,4a,5,6,- Hexahydroquinoline



(II)

Figure 4. GC-MS analysis of i) raw and ii) treated tannery wastewater.

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