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Phytoremediation of xenobiotics – A green approach for the treatment of domestic wastewater

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ABSTRACT

Remediation of xenobiotics from wastewater is highly essential due to its harmful impacts. Several chemical and physical processes are used to recover xenobiotics from wastewater, including ion exchange, reverse osmosis, electrodialysis, and ultrafiltration. However, the biological method of employing microalgae, has aroused the scientific community's interest due to its cheap operating costs and effectiveness in absorbing and/or removing organic and elemental contaminants from wastewater. *Chlorella vulgaris* was used as a biological absorbent, in the remediation of domestic wastewater (DWW). The results of this work indicated that *C. vulgaris*, effectively eliminated pollutants and improved the physicochemical characteristics of wastewater such as pH, DO, alkalinity. Simultaneously, it also decreased BOD, COD, suspended solids, heavy metals (HMs) such as iron (Fe), zinc (Zn), cadmium (Cd), mercury (Hg), copper (Cu), lead (Pb) and nutrient load (phosphate and nitrate) of DWW. Effective remediation of the pollutants was attained within 4h of *C. vulgaris* cultivation. The removal of toxic compounds such as 4-methoxy carbonyl benzo hydrazide, benzamine 4,4′ methylene bis, acetamide, and N-(2-methyl phenyl), was confirmed with GC-MS analysis. The removal of polysulfides and aliphatic bromo compounds was evident from the FTIR studies. Pearson's correlation analysis showed a positive correlation between physicochemical variables and algal biomass. Hence, the current study recommends biological wastewater treatment,by utilizing *C. vulgaris* as an effective and eco-friendly option, for removing pollutants and restoring the physicochemical properties of water.

Key words: *Chlorella vulgaris, Microalgae, Phytoremediation, Hheavy metals, Domestic wastewater.*

Introduction

The unprecedented growth of industries, fostered by the industrial revolution and increased demand for water, owing to rapid urbanization, are the major causes of water pollution (Karakurt *et al.*, 2019; Kim *et al.*, 2019). The presence of various organic, inorganic, and HMs in water bodies may be the result of natural processes including wind, precipitation, water run-offs, and anthropogenic pursuits (Gupta and Joia, 2016). These factors transferthe pollutants from the air and surface into the water environment (Warmate *et al.,* 2011). HMs are the most important contaminants, as they are non-biodegradable and cause deleterious effects on humans and ecology due to their persistent nature (Alqadami *et al*., 2018; Kwaansa-Ansah *et al*., 2019). Further, HMs in water limit the availability and accessibility of clean water (Dixit *et al*., 2015).

Removing the contaminants from DWW is highly essential before their discharge into water bodies. Several physical and chemical treatment processes such as electrolytic technologies, ion exchange, precipitation, chemical extraction, hydrolysis, polymer microencapsulation, and leaching, are employed for removing HMs from wastewater (Jais *et al.,* 2017). Since majority of these treatment solutions are ineffective and also expensive to implement on a large scale and need rigorous supervision and regular monitoring, bioremediation, using algae species (Phycoremediation), is considered an effective alternate and environmentally benign strategy, to annihilate HMs from polluted water. Phycoremediation has recently gained popularity as a method for absorbing nutrients and xenobiotics from wastewater (Ahmad, 2015; Babu *et al.,* 2013; Oyetibo *et al.,* 2017; Poo *et al.,* 2018). It is advantageous over other bioremediation and membrane separation processes because of the adaptability of microalgae to thrive in extreme habitats, its ability to rapidly eliminate HMs, without producing sludge or toxic substances as by-products (Brinza *et al.,* 2007). Microalgae function as useful sources of bio-absorbents in wastewater treatment and also promote the production of feedstock for biofuel, feed for animals, and fishes (Abdel-Raouf *et al.,* 2012). This green approach to utilize microalgae for treating wastewater has two-fold benefits: (1) cultivation of microalgae biomass, utilizing wastewater and (2) subsequent remediation of xenobiotics by microalgae.

A previous study showed that the biomass of microalgae, *Platymonas subcordiformis,* increased significantly (8.9 times) when cultivated in aquaculture wastewater, with 87-95% of nitrate and 98-99% of phosphate removal efficiency (Guo *et al.,* 2013). Thus, this green approach in wastewater treatment can be a cost-effective, highly efficient, and eco-friendly strategy(Gani *et al.,* 2016; Rawat *et al.,* 2016; Suresh Kumar *et al.,* 2015). The Chlorella species are reported for their effective role in absorbing NH_4-N, phosphorus, COD, (Wang *et al.,* 2010) ammonia (AlMomani and Örmeci, 2016), and their bioremediation potential of municipal, pulp, paper, and diary effluents (Chinnasamy *et al.,* 2010; Hiibel *et al.,* 2015; Kumar *et al.,* 2018; Sirin and Sillanpää, 2015; Usha *et al.,* 2016). Against this background, the current study employed the microalgae, *Chlorella vulgaris,* for the remediation of HMs, organic, inorganic and, nutrient load as well as in the restoration of physico-chemical variables of domestic wastewater. The percentage yield of microalgae and the removal of nutrients and heavy metals, as a function of microalgae cultivation time were measured.

Materials and Methods

Collection of wastewater samples

Domestic wastewater samples were collected from 25 different residential sites in and around Kumbakonam city (10.97 °N and 79.42 °E), Thanjavur District, Tamilnadu, India (Fig. 1). 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 °C.

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 °C 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,

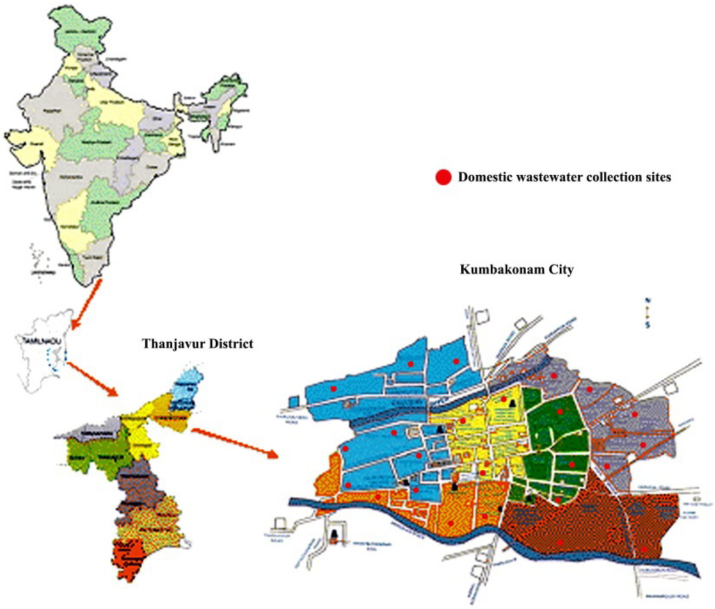


Fig. 1. Bird's eye view of domestic wastewater collection sites at Kumbakonam

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 (Palmer, 1977) and used for domestic wastewater treatment.

Experimental design

The experiments were carried out in a completely randomized design by Taiwo *et al*., (2016) with slight modifications. The cultivation of microalgae, *Chlorella vulgaris* using domestic wastewater, was carried out at room temperature (34±1 ^{o}C) and relative humidity (65%). 20 L of wastewater samples were taken in 35L clean and labeled bowls. To this wastewater 100 ml of ATCC medium and 0.15g of algae, *C. vulgaris* were 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 domestic wastewater samples were filtered, using a 0.45 µm pore-sized Whatman membrane filter, to remove suspended solids and microorganisms. The physico-chemical parameters of DWW such as pH, alkalinity, Dissolved Oxygen (DO), Electrical conductivity (EC), Total Dissolved Solids (TDS), Total Solids (TS), Total Suspended Solids (TSS), Biochemical 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 ofAPHA, (2012) manual. The nutrient content (phosphate and nitrate) of DWW 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 (Manivasakam, 2005). 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). 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).

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

Where,

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

FTIR and GC-MS analysis

The functional groups of pollutants in domestic 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}. 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) (Pandey and Dubey, 2012).

Statistical analysis

The descriptive means and standard deviations, Pearson's correlation analysis of physico-chemical 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

Bioremediation is the removal of contaminants through bioabsorption and indirect metabolic activities of algae, bacteria, molds, fungi, and yeasts. Three species of microalgae, including *Scenedesmus, Chlorella* and *Spirunila,* are commonly used for the bioremediation of pollutants in wastewater (Dwivedi, 2012). Rapid growth, simple adaptable nature, and tolerance to adverse conditions are some of the attributes that make *C. vulgaris* suitable for a variety of applications.

Properties of raw domestic wastewater

The variations in the physico-chemical parameters between the raw (untreated) and *C. vulgaris* - treated DWW, are tabulated in Table 1. Effective removal of HMs, organiccompounds, and nutrients was achieved between 360 to 480 h of MT. Table 2 presents the variations in physico-chemical parameters of DWW, during thephase of *C. vulgaris* treatment. *C.vulgaris* increased pH, DO, and alkalinity of treated wastewater. The slightly acidic pH (6.94) of the raw DWW was gradually transformed into alkaline (pH 8.98), with the progression of *C. vulgaris* cultivation days. An increase in pH (~9) and alkalinity was reported in large-scale algal ponds (Craggs *et al.*, 2012). The high rate of photosynthetic activity by microalgae drags dissolved CO_2 from wastewater, resulting in a high concentration of bicarbonate and carbonate ions. The increased level of carbonate and bicarbonate is expressed as alkalinity. Thus, the alkalinity of untreated DWW(315.7 mg/l) increased significantly (362.67 mg/l) after 480 h of MT. DO is

Table 1. Characteristics of raw (untreated) and microalgae treated domestic wastewater (DWW)

Parameters	Before treatment	After treatment
Physicochemical parameters		
pH	6.94±0.02	8.98±0.01
Alkalinity (mg/l)	315.72±1.28	362.67±2.49
DO (mg/l)	0.15±0	7.97±0.27
TDS (mg/l)	975.48±0.85	832±1.63
TS (mg/l)	1685.36±45.71	1446.37±4.82
TSS (mg/l)	709.88±43.95	614.37±3.84
EC (ms/cm)	2.15±0.01	0.64±0.03
BOD (mg/l)	125.05±0.09	81.49±0.22
COD (mg/l)	170.06±0.1	30.63±0.07
Heavy metals		
Zinc (mg/l)	12.03±0.01	4.08±0.02
Cadmium (mg/l)	.001±1.19	0±0.22
Copper (mg/l)	3.83±0.33	0.41±0.01
Iron (mg/l)	6.65±0.09	5.03±0.01
Chromium (mg/l)	0.04±0.85	0.01±3.86
Lead (mg/l)	0.26±0.01	0±0.0
Mercury (mg/l)	0.02±0.85	0±0.0
Cadmium (mg/l)	89.35±1.19	10.40±0.22
Organic and inorganic elements		
Nitrate (mg/l)	1.9±0.06	0.21±0.01
Phosphate (mg/l)	3.17±0.13	1±0.01
Calcium (mg/l)	110.48±0.85	7.33±1.25
Chloride (mg/l)	251.72±1.28	40±1.63

the measure of organic pollutants, their decomposition, and the self-purification of water bodies. The concentration of DO, in untreated DWW (0.15 mg/l), increased with MT and it was observed to be 7.9 mg/l after 480 h (Table 1). The increase in pH and DO was highly influential in the removal of nutrients, organic, and metals from DWW. The growth of microalgae increases the DO content of the growth medium (Moondra *et al.,* 2021a, 2020).

The measure of the quantity of oxygen, consumed by microorganisms in the process of decomposing organic matter, is referred to as BOD. Thus, the BOD content of wastewater represents the organic load, oxygen depletion (Awomeso *et al.,* 2019) and the presence of food and excretory materials (Mahapatra *et al.,* 2013). The BOD concentration of untreated DWW was 125.05 mg/l and after 480 h of MT, it was reduced to 81.49 mg/l (Table 2). The percentage of BOD removal was 34.83% after 480 h days of treatment (Fig. 2). Atoku *et al.*, (2021) reported that *C. vulgaris* was effective in reducing the BOD content by 81%, after 45 days of treatment. The oxygen equivalent of inorganic material, that can be oxidized chemically, is inferred as COD. In the present study, the initial concentration of COD in DWW (170.06 mg/l) was reduced to 30.63 mg/l with MT in 480 h (Table 2) and the percentage of removal was 81.99% (Fig. 2). The cultivation of microalgae, *C. vulgaris* in wastewater resulted in 50% removal of COD (Mahapatra *et al.,* 2013) agreed with the present study. The correlation between BOD and COD (r=0.984, $p<0.01$) indicated the presence of readily degradable organic matter to that of

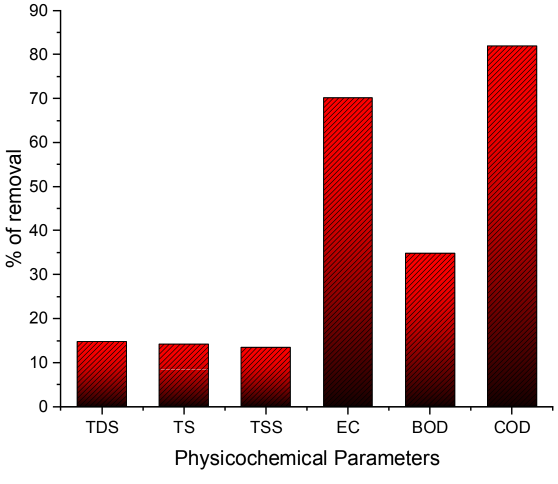


Fig. 2. Removal efficiency (%) of physico-chemical variables by microalgae, *C.vulgaris*

Table 2. Physico-chemical variables of domestic wastewater 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)	6.94 ± 0.02^{c}	315.72 ± 1.28^{d}	0.15 ± 0.00^{a}	975.48 ± 0.85^{a}	1685.36 ± 45.71^{a}	709.88 ± 43.95^{a}	2.15 ± 0.01^{a}	125.05 ± 0.09^{a}	170.06 ± 0.10^{a}
T2 (24 h)	8.04 ± 0.03^{b}	317.33 ± 1.25^{d}	0.52 ± 0.04^{b}	968.67 ± 579^{b}	$1659.67\pm8.65a^{b}$	691.00 ± 8.65^{b}	2.10 ± 0.07^{a}	123.77 ± 0.54^{a}	159.63 ± 4.00^{b}
T3 (48 h)	8.47 ± 0.28^{ab}	320.67 ± 4.11^{d}	0.88 ± 0.02^{c}	960.67 ± 2.49^{c}	$1630.99\pm4.99b^{c}$	670.32 ± 4.99^{c}	1.89 ± 0.04^{b}	119.41 ± 0.86^{b}	146.04 ± 0.12^{c}
T4 (72 h)	8.52 ± 0.34^{ab}	323.00 ± 6.98^{d}	1.23 ± 0.02^{d}	951.33 ± 2.87^{d}	$1592.58\pm7.20c^{d}$	641.25 ± 7.20^{d}	1.76 ± 0.08^{c}	115.14 ± 0.41^{c}	134.98 ± 0.02^{d}
T5 (96 h)	8.68 ± 0.35^{ab}	326.00 ± 6.68^{cd}	2.14 ± 0.01^{e}	945.55 ± 1.95^{d}	1572.20 ± 8.49^{d}	626.65 ± 8.49^{d}	$1.54\pm0.04bb^{c}$	111.69 ± 0.90^{d}	115.66 ± 0.47^{e}
T6 (120 h)	8.72 ± 0.15^{a}	327.33 ± 7.04^{cd}	3.30 ± 0.04^{f}	929.33 ± 4.19^{e}	1541.47 ± 8.16^{e}	612.14 ± 8.16^{e}	1.38 ± 0.03^{c}	108.24 ± 0.42^{e}	110.69 ± 2.49^{f}
T7 (240 h)	8.74 ± 0.05^{a}	342.67 ± 6.55^{bc}	4.32 ± 0.08^{g}	870.00 ± 0.82^{e}	1528.51 ± 1.25^{f}	658.51 ± 1.25^{e}	1.23 ± 0.08^{d}	102.65 ± 0.12^{f}	80.83 ± 5.14^{g}
T8 (360 h)	8.90 ± 0.04^{a}	354.33 ± 6.24^{ab}	6.19 ± 0.02^{h}	850.33 ± 6.94^{f}	1485.21 ± 8.16^{g}	635.21 ± 8.16^{f}	1.01 ± 0.02^{de}	96.27 ± 0.21^{g}	50.53 ± 0.15^{h}
T9 (480 h)	8.98 ± 0.01^{a}	362.67 ± 2.49^{a}	7.97 ± 0.27^{i}	832.00 ± 1.63^{g}	1446.37 ± 4.82^{h}	614.37 ± 4.82^{g}	0.64 ± 0.03^{e}	81.49 ± 0.22^{h}	30.63 ± 0.07^{i}

biodegradable suspended solids (Eckenfelder, 1994).

The concentration of TDS, TS, and TSS of untreated DWW was 975.48, 1685.36, and 709.88 mg/l respectively and they were reduced to 832, 1446.37, and 614.37 mg/l (Table 2). Moondra *et al*. (2021b) reported that TDS, TS, and TSS were effectively removed by *C. vulgaris.* The maximum removal of 14.71% (TDS) was achieved while a minimum removal efficiency of TSS (13.45%) and 14.18% (TS) was recorded with *C. vulgaris* treatment. The removal of suspended solids (TS, TSS, and TDS) was comparatively low to that of other physicochemical variables estimated, in the study (Table 2 and Fig. 2). The capacity of water to carry electric current is termed electrical conductivity and it is directly proportional to dissolved minerals in water (Dahaan *et al.,* 2016). The high EC of DWW may be due to the addition of salt in food substances, natural salt content in water, and due to the presence of other mineral discharges (Ma *et al.,* 2020). The EC of DWW before and after MT was 2.15 and 0.64ms/cm respectively. The percentage of reduction after 480 h of treatment was 67.01% (Fig. 2). The EC content of untreated DWW reported in the present study was in agreement with the observation of Moondra *et al.* (2020).

The removal of HMs by microalgae,atdifferent phases of microalgae treatment is presented in table (3). The initial concentration of calcium and chloride (110.48 and 251.92 mg/l respectively) was effectively remediated, with a removal efficiency of 93.37% and 84.11% respectively (Fig.4), with the treatment of *C. vulgaris*. Iron was removed by *C. vulgaris,* with a removal efficiency of 98.15%. The concentration of HMs such as zinc, cadmium, and copper in untreated DWW, was observed to be 12.03, 0.001, and 3.83 mg/l respectively and the percentage of removal of these HMs by *C. vulgaris* was 66.08%, 100%, and 89.30% respectively. The percentage removal of chromium was 100%, after 48 h. 100% remediation of lead and mercury was achieved in 48 h of treatment. Above 90% removal was achieved against calcium (93.37%), mercury (100%) cadmium (100%), chromium (100%) and lead (100%). Oyebamiji *et al*., (2019) agreed with the present work.*C.vulgaris* recorded a 40% removal of chromium from tannery wastewater (Subashini and Rajiv, 2018). But the present study recorded 100% of chromium removal from DWW, with 48 h of *C. vulgaris* treatment.

The nitrogen and phosphate load in DWW is consumed by microalgae for its growth. The present study witnessed a decrease in the concentration of nitrate and phosphate, concerning the treatment duration (Table 4). This removal or absorption of nitrate and phosphate from wastewater might be

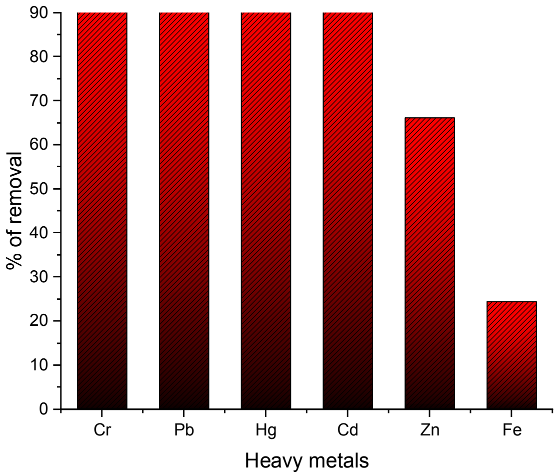


Fig. 3. Removal efficiency (%) of heavy metals by microalgae, *C.vulgaris*

Table 3. The heavy metal concentration of domestic wastewater at different phases of microalgae treatment

Duration of Treatment (h)	Heavy metals (mg/l)						
	Iron (Fe)	Zinc (Zn)	Cadmium (Ca)	Copper (Cu)	Chromium (Cr)	Lead (Pb)	Mercury (Hg)
T1 (0 h)	6.65 ± 0.09^{a}	12.03 ± 0.01^{a}	0.001 ± 1.19^{a}	3.83 ± 0.33^{a}	0.04 ± 0.85^{a}	0.26 ± 0.01^{a}	0.02 ± 0.85^{a}
T2 (24 h)	6.64 ± 0.07^{b}	11.99 ± 0.02^{ab}	-	3.64 ± 1.28^{a}	0.01 ± 3.86^{b}	0.24 ± 0.03^{a}	0.01 ± 1.58^{a}
T3 (48 h)	6.38 ± 0.03^{c}	11.94 ± 0.02^{b}	-	3.02 ± 0.25^{b}	-	0.19 ± 0.02^{b}	-
T4 (72 h)	6.00 ± 0.01^{d}	10.83 ± 0.02^{c}	-	2.81 ± 0.29^{b}	-	0.16 ± 0.01^{bc}	-
T5 (96 h)	5.92 ± 0.01^{e}	10.12 ± 0.02^{c}	-	2.50 ± 0.62^{c}	-	0.14 ± 0.01^{c}	-
T6 (120 h)	5.83 ± 0.01^{f}	8.70 ± 0.02^{d}	-	2.38 ± 0.82^{c}	-	0.09 ± 0.01^{d}	-
T7 (240 h)	5.23 ± 0.02^{g}	6.44 ± 0.02^{e}	-	1.48 ± 0.12^{d}	-	-	-
T8 (360 h)	5.10 ± 0.01^{h}	5.33 ± 0.01^{f}	-	0.95 ± 0.33^{e}	-	-	-
T9 (480 h)	5.03 ± 0.01^{i}	4.08 ± 0.02^{g}	-	0.41 ± 0.22^{f}	-	-	-

attributed to the photosynthetic activity of microalgae, *C. vulgaris*. The percentage of nitrate and phosphate removal was 88.95% and 68.45% respectively, with 480 h of algal treatment (Fig. 4). The high concentration of nutrients (nitrate and phosphate) is highly essential for the growth of microalgae (Ajala and Alexander, 2020). The initial concentration of nitrate and phosphate in DWW was 1.9 and 3.17 mg/l (Table 4) and with MT it was reduced to a concentration of 0.21 and 1 mg/l respectively over a period of 20 days. The decrease in the nutrient concentration of DWW with *C. vulgaris* treatment, was reported by Ali *et al*., 2021).

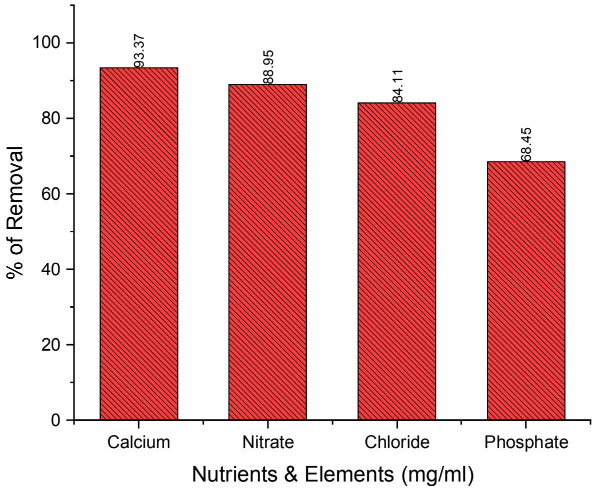
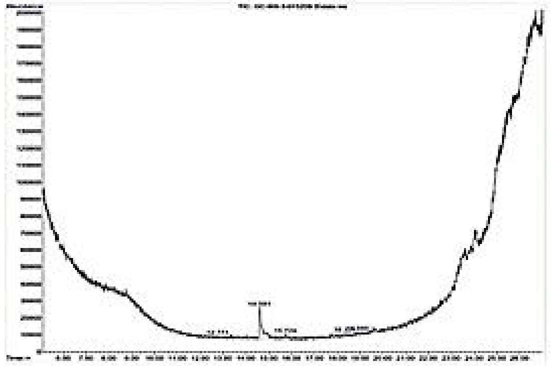


Fig. 4. Removal efficiency (%) of nutrients and elements by microalgae, *C.vulgaris*

GC-MS and FTIR analysis

The GC-MS analysis of DWW before treatment with *C. vulgaris* (Table 5a and Fig. 5a) showed the presence of toxic substances such as Benzene 1,4-dicarboxylic acid, monohydrazide, methyl ester, (2-oxo-3-bornyl) glyoxylic acid, Benzenamine,4,4'-methylene bis-, and Acetamide, N-(2-methyl phenyl)- and they were found to be effectively removed by *C. vulgaris* (Table 5b and Fig 5b). The removal of azo compounds from tannery effluent by *C. vulgaris* has been reported earlier (Subhashini and Rajiv *et al.*,

(a) Before treatment



(b) After treatment

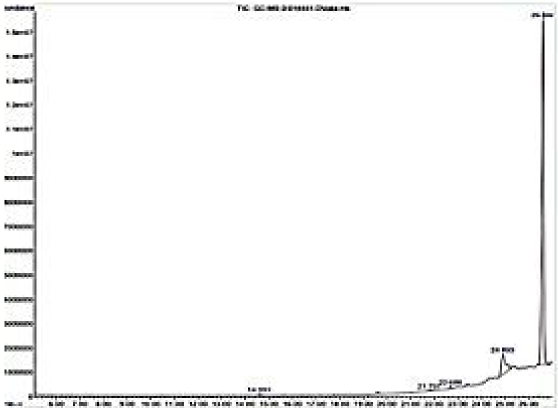


Fig. 5. GC-MS chromatogram of domestic wastewater

Table 4. Nutrient and element concentration of domestic wastewater 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)	$1.90+0.06^{a}$	3.17 ± 0.13^{a}	110.48 ± 0.85^{a}	251.72 ± 1.28^{a}
T2 (24 h)	1.79 ± 0.10^{ab}	3.04 ± 0.07^{a}	84.33 ± 1.25^{b}	235.33 ± 3.30^{b}
T3 (48 h)	1.71 ± 0.15^{abc}	2.87 ± 0.03^{b}	80.00 ± 0.82^{c}	217.00 ± 1.63^{c}
T4 (72 h)	1.61 ± 0.02^{bc}	2.67 ± 0.04^{c}	59.67 ± 1.25^{d}	192.33 ± 1.25^{d}
T5 (96 h)	1.55 ± 0.02^{c}	2.61 ± 0.02^{cd}	56.67 ± 1.25^{d}	177.00 ± 1.63^{e}
T6 (120 h)	1.51 ± 0.01^{c}	2.49 ± 0.08^{d}	45.67 ± 0.94^{e}	161.00 ± 1.63^{f}
T7 (240 h)	0.78 ± 0.02^{d}	1.57 ± 0.03^{e}	22.33 ± 0.94^{f}	123.33 ± 2.49^{g}
T8 (360 h)	0.32 ± 0.06^{e}	1.39 ± 0.01^{f}	18.33 ± 1.25^{g}	82.33 ± 1.70^{h}
T9 (480 h)	0.21 ± 0.01^{e}	1.00 ± 0.01^{g}	07.33 ± 1.25^{h}	40.00 ± 1.63^{i}

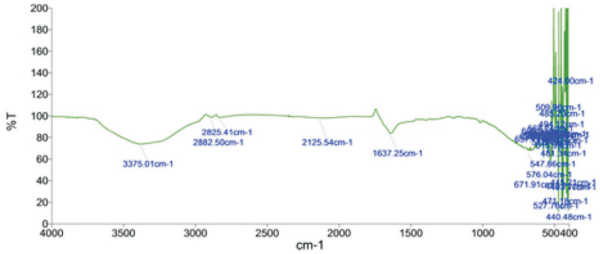
2018). The degradation of azo compounds was because of azo reductase in *C. vulgaris,* involved in the breakage of the N=N bond (Jinqi and Houtian, 1992).

The untreated DWW showed major stretching vibrations at 3375.01 cm^{-1}, 2885.50 cm^{-1}, 2825.54 cm^{-1} and 1637.25 cm^{-1} indicating the presence of hydroxyl group, H-bonded OH stretch, methylene C-H stretch, isocyanate (-N=C=O asymmetric stretch) and amide groups (Fig.6a). After treatment with microalgae, there was a slight shift in the wavelength with peaks appearing at 3360.25 cm^{-1}, 2840.81 cm^{-1}, 2160.06 cm^{-1} and 1646.43 cm^{-1} indicating the presence of OH group of phenol and alcohol, methyl C-H symmetric stretch, terminal alkyne (monosubstituted), alkenyl C=C stretch respectively. The untreated wastewater showed the presence of polysulfides (S-S stretch) with peaks corresponding to 424.00 cm^{-1}, 485.20 cm^{-1}, 494.12 cm^{-1}, 481.34 cm^{-1}, 415.21 cm^{-1}, 440.77 cm^{-1}, 471.18 cm^{-1}. The aliphatic iodo compounds were represented by peaks at 515.99 cm^{-1}, 547.86 cm^{-1}, 576.04 cm^{-1}, 576.04 cm^{-1}, 527.76 cm^{-1}, 568.24 cm^{-1} and 516.99 cm^{-1}. The comparison of the FTIR spectrum of treated and untreated wastewater indicated that polysulfides were removed to a significant level and aliphatic bromo compounds were removed completely with microalgae as the peaks at 671.91 cm^{-1}, 657.00 cm$^{-1,}$ and 609.00 cm^{-1} were not detected in treated wastewater (Fig. 6b). Similarly, the removal of aliphatic iodo compounds could also be observed, as peaks were reduced at 506.99 cm^{-1}, 516.78 cm^{-1}, 538.48 cm^{-1} and 546.10 cm^{-1} in treated samples. The presence of carboxyl, hydroxyl, amine, and sulphydryltogether constitute a net negative charge that confirms a high affinity for binding with HMs (Gupta and Rastogi, 2008). The carbonyl group has a high affinity for binding HMs followed by –OH, -SO_3, and –P_2O_3 groups (Gardea-Torresdey *et al.,* 1990). Subashini and Rajiv, (2018) confirmed the effective removal of azo compounds by *C. vulgaris* treatment with FTIR analysis.

Biomass productivity

To determine the productivity of algal biomass, a

(a) Before treatment



(b) After treatment

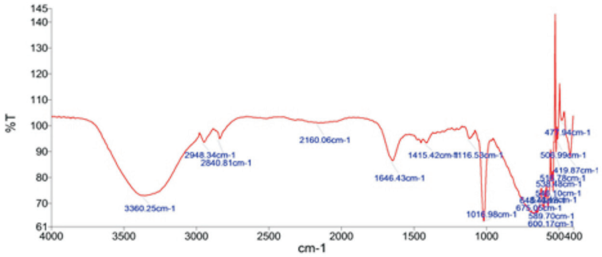


Fig. 6. FTIR spectrum of domestic wastewater

Table 5. Compounds identified from domestic wastewater using GC-MS

(a) Before treatment with microalgae, *Chlorella vulgaris*

S. No	R.time	Area %	Compound name
1	14.591	0.41	Benzene 1,4-dicarboxylic acid, monohydrazide, methyl ester
2	21.797	0.44	(2-Oxo-3-bornyl)glyoxylicacid
3	22.686	0.52	Benzenamine,4,4'-methylenebis-
4	24.893	10.75	Acetamide,N-(2-methyl phenyl)-
5	26.604	87.89	Decanedioic acid, bis(1,2,2,6,6-pentamethyl-4-piperidinyl)ester
		(b) After treatment with microalgae, *Chlorella vulgaris*	
1	12.771	4.79	Indolelactic acid TMS
2	14.591	76.77	1,4-Benzenedicarboxylicacid, dimethylester
3	15.724	4.63	1,2-Benzenedicarboxylic acid,monobutylester
4	18.324	6.74	9-Aza-bicyclo[4.2.1]nona-2,4-diene-9- carboxaldehyde
5	18.886	7.07	Nohitswereretrieved.

Table 6. Intraspecific relationship between microalgae biomass and physico-chemical, heavy metal, nutrient, and elemental variables

	Biomass	pH	Ala	DO	TDS	TS	TSS	EC	BOD	COD	N	P	Cl	Ca	Cr	Pb	Hg	Ca	Zn	Fe	Cu
Biomass	1																				
pH	.826**	1																			
Ala	.913**	0.664	1																		
DO	.937**	.694*	.986**	1																	
TDS	-.935**	-.674*	-.990**	-.978**	1																
TS	-.986**	-.830**	-.936**	-.964**	.937**	1															
TSS	-.798**	-.854**	-0.594	-.679*	0.581	.828**	1														
EC	-.973**	-.776*	-.958**	-.983**	.955**	.992**	.781*	1\													
BOD	-.938**	-.727*	-.973**	-.989**	.959**	.973**	.730*	.990**	1												
COD	-.966**	-.760*	-.984**	-.989**	.980**	.981**	.716*	.990**	.984**	1											
N	-.907**	-0.655	-.995**	-.970**	.993**	.918**	0.548	.937**	.948**	.974**	1										
P	-.942**	-.701*	-.989**	-.975**	.997**	.946**	0.606	.962**	.967**	.983**	.989**	1									
Cl	-.964**	-.761*	-.982**	-.991**	.977**	.985**	.729*	.992**	.992**	.998**	.968**	.982**	1								
Ca	-.987**	-.865**	-.917**	-.931**	.937**	.980**	.781*	.963**	.938**	.963**	.914**	.949**	.964**	1							
Cr	-.712*	-.961**	-0.498	-0.524	0.512	.703*	.818**	0.638	0.577	0.610	0.489	0.549	0.611	.743*	1						
Pb	-.989**	-.797*	-.916**	-.926**	.950**	.960**	.715*	.953**	.918**	.957**	.921**	.954**	.950**	.978**	.686*	1					
Hg	-.712*	-.961**	-0.498	-0.524	0.512	.703*	.818**	0.638	0.577	0.610	0.489	0.549	0.611	.743*	1.000**	.686*	1				
Ca	-0.532	-.899**	-0.364	-0.388	0.378	0.538	0.648	0.463	0.419	0.458	0.367	0.409	0.461	0.621	.884**	0.509	.884**	1			
Zn	-.956**	-.676*	-.979**	-.986**	.991**	.958**	0.645	.974**	.970**	.984**	.974**	.987**	.983**	.946**	0.511	.959**	0.511	0.363	1		
Fe	-.987**	-.774*	-.944**	-.943**	.963**	.971**	.719*	.967**	.943**	.975**	.944**	.970**	.971**	.977**	0.657	.988**	0.657	0.466	.970**	1	
Cu	-.970**	-.779*	-.979**	-.979**	.979**	.981**	.716*	.987**	.981**	.996**	.970**	.986**	.995**	.969**	0.647	.966**	0.647	0.478	.978**	.982**	1

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

standard graph was plotted between the absorbance read at 680 nm and the known concentration of algal biomass. The linear regression equation calculated wasy = 0.0548x + 0.0167 with R^2 value of 0.9829. Table 7shows an increase in the concentration of algal biomass concerning cultivation days. A similar trend in the increase in the concentration of biomass with cultivation days has been reported (Ali *et al.*, 2021).

Table 7. Microalgae biomass productivity concerning cultivation days

Cultivation Days	Absorbance at 680 nm	Biomass concentration (g/l)
0	0.05	1.000
1	0.07	1.500
2	0.10	2.000
3	0.13	2.700
4	0.16	3.000
5	0.18	3.600
10	0.21	4.200
15	0.23	4.500
20	0.24	4.700

Correlation analysis

Pearson's correlation analysis was done, to analyze the relationship between microalgal biomass and various parameters evaluated in the study (Table 6). A positive correlation of biomass with pH (r=.826), alkalinity (r=.913) andDO (r=.937) was obtained and it was significant with p-value<0.01. The organic, inorganic elements and HM variables showed a negative correlation. In the present study, the microalgal biomass was negatively correlated with organic, inorganic, and HMs variables suggesting that the algal biomass was not effective enough to remove the xenobiotics at high concentrations (Kurade *et al.*, 2016).

Conclusion

This study applied *Chlorella vulgaris*, to treat the domestic wastewater, collected from household discharges. Microalgae cultivation in domestic wastewater, helps conserve fresh water and mitigate CO_2 sequestration for alleviating climate change. The production of algal biomass in wastewater can be further used as a biofuel feedstock. The current study observed that *C. vulgaris* cultivation reduced the concentration of nutrients, organic and inorganic contaminants in wastewater. The maximum concentration of biomass was 8.4g/l with 480h (20 days) of cultivation in DWW. The microalgae were also effective in removing toxic chemicals and heavy metals. Hence, this study recommends the adoption of the microalgae, *C. vulgaris,* for the treatment of wastewater before discharge into the water bodies.

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