CHLORELLA VULGARIS-MEDIATED REMEDIATION OF XENOBIOTICS FROM DOMESTIC SEWAGE WATER

Bathmapriya. L@, Jeyaraj. M[1*], Udayakumar. R[2].

Abstract

The remediation of xenobiotics from wastewater is of utmost importance due to its detrimental effects. Various chemical and physical processes are employed for the retrieval of xenobiotics from wastewater, encompassing ion exchange, reverse osmosis, electrodialysis, and ultrafiltration. The utilisation of microalgae in the biological approach has garnered attention from the scientific community due to its cost-effectiveness and efficiency in the removal and absorption of organic and elemental pollutants from wastewater. *Chlorella vulgaris* was employed as a biological adsorbent in the process of remediating domestic wastewater (DW). The findings of this study demonstrated that the use of *C. vulgaris* led to the efficient removal of pollutants and the enhancement of various physicochemical properties of wastewater, including pH, dissolved oxygen (DO), and alkalinity. Concurrently, it also resulted in a reduction of biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, as well as heavy metals (HMs) including iron (Fe), zinc (Zn), cadmium (Cd), mercury (Hg), copper (Cu), lead (Pb), and nutrient load such as phosphate and nitrate in domestic wastewater (DW). The successful mitigation of pollutants was achieved within a time frame of 4 hours through the cultivation of *C. vulgaris*. The results of Pearson's correlation analysis revealed a positive correlation between the physicochemical variables and algal biomass. Therefore, the present study proposes the implementation of biological wastewater treatment, employing *C. vulgaris* as a viable and environmentally sustainable alternative, to effectively eliminate contaminants and reinstate the physicochemical characteristics of water.

Keywords: *Chlorella vulgaris*; microalgae; Phycoremediation; heavy metals; domestic wastewater.

[1*] Assistant Professor, Department of Biochemistry, Rajah Serfoji Government College (Autonomous), Thanjavur, (Affiliated to Bharathidasan University, Palkalaiperur, Thiruchirappalli– 24)
[2*] Assistant Professor, Department of Biochemistry, Government Arts College (Autonomous), Kumbakonam, (Affiliated to Bharathidasan University, Palkalaiperur, Thiruchirappalli– 24)
[@] Research Scholar, Department of Biochemistry, Government Arts College (Autonomous), Kumbakonam, (Affiliated to Bharathidasan University, Palkalaiperur, Thiruchirappalli– 24)

***Corresponding Author:** Dr. Jeyaraj. M
*Assistant Professor, Department of Biochemistry, Rajah Serfoji Government College (Autonomous), Thanjavur, (Affiliated to Bharathidasan University, Palkalaiperur, Thiruchirappalli– 24)
Email: jayarajmala@yahoo.com

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Introduction

The primary factors contributing to water pollution are the remarkable expansion of industries, which has been facilitated by the industrial revolution, and the escalating need for water due to rapid urbanisation (1-2). The occurrence of diverse organic, inorganic, and heavy metals (HMs) in aquatic environments can be attributed to natural phenomena such as wind, precipitation, water runoff, as well as human activities (3). The pollutants are transferred from the air and surface into the water environment due to various factors (4). Heavy metals (HMs) are considered to be of utmost significance in terms of contaminants due to their non-biodegradable nature and their ability to cause detrimental impacts on both human health and ecological systems, primarily attributed to their persistent characteristics (5-6). Moreover, the presence of harmful microorganisms in water restricts the availability and accessibility of potable water (7).

The removal of contaminants from domestic wastewater (DW) is of utmost importance prior to its release into aquatic ecosystems. Various physical and chemical treatment methods, including electrolytic technologies, ion exchange, precipitation, chemical extraction, hydrolysis, polymer microencapsulation, and leaching, have been utilised for the purpose of eliminating heavy metals (HMs) from wastewater (8). Due to their limited efficacy and high implementation costs, as well as the requirement for strict oversight and frequent monitoring, many of the available treatment methods for addressing heavy metal (HM) contamination in water are deemed ineffective and financially burdensome when implemented on a large scale. As a result, bioremediation, specifically the utilization of algae species through a process known as phycoremediation, is regarded as a viable and environmentally sustainable strategy for the eradication of HMs from polluted water sources. In recent years, phycoremediation has emerged as a prominent technique for the removal of nutrients and xenobiotics from wastewater, as evidenced by studies conducted by Ahmad (9), Babu et al. (10), Oyetibo et al. (11), and Poo et al. (12). The adaptability of microalgae to thrive in extreme habitats, along with its rapid elimination of heavy metals without generating sludge or toxic by-products, confers an advantage over other bioremediation and membrane separation processes (13). According to Abdel-Raouf et al. (14), microalgae serve as valuable bio-absorbents in the context of wastewater treatment. Additionally, they contribute to the generation of feedstock for biofuel production, as well as feed for animals and fish. The implementation of a green methodology involving the utilization of microalgae for the treatment of wastewater offers dual advantages. Firstly, it involves the cultivation of microalgae biomass through the utilization of wastewater. Secondly, it facilitates the subsequent remediation of xenobiotics by microalgae.

According to a study conducted by Guo et al. (15), it was observed that the biomass of *Platymonas subcordiformis*, a type of microalgae, exhibited a substantial increase (8.9 times) when cultivated in wastewater from aquaculture. Furthermore, the study found that this cultivation method demonstrated high efficiency in removing nitrate (87-95%) and phosphate (98-99%) from the wastewater. Therefore, the implementation of this environmentally conscious method in the treatment of wastewater has the potential to be both economically advantageous and environmentally sustainable (16-18). The *Chlorella* species have been documented for their ability to efficiently absorb NH_4-N, phosphorus, COD (19), ammonia (20), as well as their potential for bioremediation of municipal, pulp, paper, and dairy effluents (21-25). In light of the aforementioned context, the present study utilized *Chlorella vulgaris*, a type of microalgae, to address the issue of remediating heavy metals (HMs), organic and inorganic pollutants, nutrient overload, and the restoration of physicochemical parameters in domestic wastewater. The study quantified the percentage yield of microalgae and assessed the efficiency of nutrient and heavy metal removal over varying durations of microalgae cultivation.

Materials and Methods

Collection of domestic wastewater

The researchers obtained samples of domestic wastewater from a total of 25 residential locations situated in and around Kumbakonam city, which is located in the Thanjavur District of Tamilnadu, India. The geographical coordinates of Kumbakonam city are approximately 10.97°N latitude and 79.42°E longitude. The specimens were obtained in containers that had been pre-treated with acid, subsequently fixed with HNO_3, and then transported to the laboratory for storage at a temperature of 4^oC.

Microalgae cultivation

The microalgae species, *Chlorella vulgaris*, were acquired from the Microalgal Mass Cultivation Centre (MMCC) at the Department of Microbiology, Bharathidasan University, located in Tiruchirappalli, Tamilnadu, India. The microalgae were cultured and sustained in ATCC medium: 824 ASN-III media.

Chlorella vulgaris culture and its maintenance

The specimens of *C. vulgaris* were acquired (Bill No. 103) from the National Repository for Microalgae and Cyanobacteria (NRMC) located at Bharathidasan University in Tiruchirappalli, Tamil Nadu, India. The *C. vulgaris* samples were inoculated onto an ATCC agar medium. The plates that had been inoculated were kept at a temperature of 25ºC in a controlled environment, specifically a culture chamber. This chamber was equipped with a white fluorescent light source that operated on a 12-hour cycle of light and darkness. The growth was observed periodically. Following the harvest, the microalgae colonies were subsequently transferred to a liquid medium provided by the American Type Culture Collection (ATCC). Algal cultures from the university were generated through a process of repeated streaking, and the identification of the algae was conducted based on their morphological and cultural traits. This identification process involved referencing the book "Biology of the Algae" by Palmer (26). The resulting algal cultures were subsequently employed for the treatment of domestic wastewater.

Experimental setup

The experiment involved the cultivation of *Chlorella vulgaris* microalgae using domestic wastewater under controlled environmental conditions, specifically at a constant room temperature of 34±1ºC and a relative humidity of 65%. A total of 20 liters of wastewater samples were collected using clean and labeled bowls with a capacity of 35 liters. In this experimental setup, 100 ml of ATCC medium was combined with 0.15g of the algae species *C. vulgaris* in the wastewater. Control was sustained in the absence of growth media and microalgae. The experimental procedure and data analysis were conducted in triplicate. The growth of algae was maintained for a duration of 20 days, equivalent to 480 hours (27).

Physico-chemical evaluation of wastewater

The domestic wastewater samples were subjected to filtration using a Whatman membrane filter with a pore size of 0.45μm in order to eliminate suspended solids and microorganisms. The physicochemical characteristics of domestic wastewater (DW), including 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 examined both prior to and subsequent to treatment with the microalgae species Chlorella vulgaris. The analysis was conducted in accordance with the recommended protocol outlined in the APHA (28) manual. The spectrophotometric method was employed to determine the nutrient content, specifically phosphate and nitrate, in DW. The potentiometric method was employed to determine the pH and EC. The concentrations of Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and Dissolved Oxygen (DO) were quantified using volumetric analysis methods. The levels of heavy metals (HMs), including iron, cadmium, zinc, copper, chromium, mercury, and lead, were measured using a digital UV-spectrophotometer according to the analytical method described by Manivasakam (29). The analysis was conducted in triplicate.

Determination of microalgae biomass productivity

The determination of microalgae biomass productivity was conducted using a UV-Visible spectrophotometer at a wavelength of 680 nm, which served as an indicator of microalgae density. The experimental procedure outlined in Kumar et al. (30) was followed for this purpose. A graph was generated using established biomass concentration values (mg/ml). Various concentrations (ranging from 1 mg/ml to 10 mg/ml) of microalgae were prepared and utilised as standard solutions. The measurement of absorbance was conducted on the standard microalgae solution at a specific wavelength of 680 nm.

Xenobiotics removal efficiency

The calculation of the removal efficiency (RE) of pollutants by C. vulgaris was performed using the formula proposed by Taiwo et al. (27).

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

Where,

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

Statistical analysis

The descriptive statistics, including means and standard deviations, were calculated for the physicochemical variables, heavy metals (HMs), and elements. Pearson's correlation analysis was performed to examine the relationships between these variables. The IBM software SPSS (version 25) was utilised for these statistical analyses. The principal component analysis (PCA) was conducted using the PAST software. The observed correlation was deemed statistically significant at a significance level of $p < 0.01$.

Result and Discussion

Bioremediation refers to the process of eliminating pollutants by means of bioabsorption and the indirect metabolic actions of various microorganisms such as algae, bacteria, moulds, fungi, and yeasts. According to Dwivedi (31), the bioremediation of pollutants in wastewater often involves the utilization of three species of microalgae: *Scenedesmus*, *Chlorella*, and *Spirunila*. The suitability of *C. vulgaris* for various applications can be attributed to its rapid growth, adaptable nature, and tolerance to adverse conditions.

The differences in the physicochemical parameters between the untreated raw wastewater and the wastewater treated with *C. vulgaris* are presented in Table 1. The successful elimination of HMs, organic compounds, and nutrients was observed within the time frame of 360 to 480 hours of microbial treatment (MT). Table 2 displays the fluctuations in physicochemical parameters of domestic wastewater (DW) observed during the period of *Chlorella vulgaris* treatment. *Chlamydomonas vulgaris* exhibited a significant increase in the pH, dissolved oxygen (DO), and alkalinity levels of the treated wastewater. The pH of the raw DW, which initially measured 6.94, gradually shifted towards alkalinity (pH 8.98) as the cultivation days of *C. vulgaris* progressed. Craggs et al. (32) reported a rise in pH levels, approximately reaching 9, as well as an increase in alkalinity within the large-scale algal ponds. The elevated level of photosynthetic activity exhibited by microalgae facilitates the extraction of dissolved carbon dioxide from wastewater, leading to a notable accumulation of bicarbonate and carbonate ions. Alkalinity is the term used to describe the elevated concentration of carbonate and bicarbonate ions. Therefore, the alkalinity of the untreated domestic wastewater (DW) exhibited a significant increase from 315.7 mg/l to 362.67 mg/l after a period of 480 hours of microbial treatment (MT). Dissolved oxygen (DO) serves as a metric for assessing the presence of organic pollutants, the process of their decomposition, and the natural purification mechanisms within aquatic ecosystems. The dissolved oxygen (DO) concentration in untreated domestic wastewater (DW) was initially measured at 0.15 mg/l. However, with the addition of microbial treatment (MT), the DO concentration significantly increased to 7.9 mg/l after a duration of 480 hours, as shown in Table 1. The observed rise in pH and dissolved oxygen (DO) levels had a significant impact on the removal of nutrients, organic compounds, and metals from domestic wastewater (DW). The proliferation of microalgae leads to an elevation in the dissolved oxygen (DO) levels within the growth medium (33-34).

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

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 parameter biochemical oxygen demand (BOD) quantifies the amount of oxygen utilized by microorganisms during the decomposition of organic substances. Therefore, the measurement of biochemical oxygen demand (BOD) in wastewater serves as an indicator of the organic load, oxygen depletion (35), and the presence of food and excretory materials (36). The initial biochemical oxygen demand (BOD) concentration of the untreated domestic wastewater (DW) was measured to be 125.05 mg/l. After a period of 480 hours of microbial treatment (MT), the BOD concentration decreased to 81.49 mg/l, as shown in Table 2. The rate of biochemical oxygen demand (BOD) removal reached 34.83% after a treatment period of 480 hours. According to the findings of Atoku et al. (37), the application of *C. vulgaris* resulted in a significant reduction of 81% in the biochemical oxygen demand (BOD) content over a treatment period of 45 days. The chemical oxygen demand (COD) is a measure of the amount of oxygen required to chemically oxidize inorganic materials. The current study observed a decrease in the initial concentration of chemical oxygen demand (COD) in domestic wastewater (DW) from 170.06 mg/l to 30.63 mg/l over a period of 480 hours using a specific treatment method referred to as MT (Table 2). This reduction corresponds to a removal percentage of 81.99%. The present study aligns with the findings of Mahapatra et al. (36), which demonstrated a 50% reduction in chemical oxygen demand (COD) through the cultivation of *C. vulgaris* microalgae in wastewater. The study conducted by Eckenfelder (1994) revealed a strong positive correlation ($r=0.984$, $p < 0.01$) between biochemical oxygen demand (BOD) and chemical oxygen demand (COD), suggesting the presence of easily degradable organic substances in relation to biodegradable suspended solids.

The initial concentrations of total dissolved solids (TDS), total solids (TS), and total suspended solids (TSS) in the untreated domestic wastewater (DW) were measured to be 975.48, 1685.36, and 709.88 mg/l, respectively. After treatment, these concentrations were observed to decrease to 832, 1446.37, and 614.37 mg/l, as shown in Table 2. In a recent study conducted by Moondra et al. (38), it was observed that the removal of total dissolved solids (TDS), total solids (TS), and total suspended solids (TSS) was effectively achieved through the use of *Chlorella vulgaris*. The treatment involving *C. vulgaris* demonstrated a maximum removal efficiency of 14.71% for TDS. Conversely, the minimum removal efficiencies for TSS and TS were recorded as 13.45% and 14.18% respectively. The study found that the removal of suspended solids (TS, TSS, and TDS) was relatively lower compared to the removal of other physicochemical variables, as shown in Table 2. The phenomenon by which water is able to transmit electric current is referred to as electrical conductivity, and this property is positively correlated with the presence of dissolved minerals in water (39). The elevated electrical conductivity (EC) observed in DW can be attributed to several factors, including the inclusion of salt in food products, the inherent salt content present in water, and the discharge of other mineral substances (40).

Table 2. Physicochemical 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}

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}	-	-	-

The electrical conductivity (EC) of DW was measured to be 2.15 ms/cm before the implementation of microalgae treatment, and subsequently decreased to 0.64 ms/cm after the treatment. The results indicate that there was a reduction of 67.01% after 480 hours of treatment. The electrical conductivity (EC) content of the untreated domestic wastewater (DW) examined in this study aligns with the findings reported by Moondra et al. (34).

Table 3 presents the elimination of heavy metals (HMs) by microalgae at various stages of microalgae treatment. The initial concentrations of calcium and chloride, measured at 110.48 mg/l and 251.92 mg/l respectively, were successfully remediated through the treatment of *C. vulgaris*. The removal efficiency for calcium was determined to be 93.37%, while chloride exhibited a removal efficiency of 84.11% . The removal of iron was achieved by *C. vulgaris,* exhibiting a high efficiency of 98.15%. The study measured the levels of heavy metals (HMs), specifically zinc, cadmium, and copper, in untreated domestic wastewater (DW). The concentrations of these HMs were found to be 12.03 mg/l, 0.001 mg/l, and 3.83 mg/l, respectively. Additionally, the research observed the percentage of removal of these HMs by the species *Chlorella vulgaris*, which was determined to be 66.08% for zinc, 100% for cadmium, and 89.30% for copper. After a duration of 48 hours, the chromium exhibited complete removal, with a removal efficiency of 100%. Complete remediation of lead and mercury was successfully accomplished within a treatment period of 48 hours. A removal rate of over 90% was attained for calcium (93.37%), mercury (100%), cadmium (100%), chromium (100%), and lead (100%). Oyebamiji et al. (41) concurred with the findings of the current study. According to Subashini and Rajiv (42), *C.vulgaris* demonstrated a chromium removal efficiency of 40% when exposed to tannery wastewater. However, the current investigation observed a complete elimination of chromium from domestic wastewater (DW) through the application of *Chlorella vulgaris* treatment for duration of 48 hours.

Microalgae utilise the nitrogen and phosphate content present in domestic wastewater (DW) as a source of nutrients to support their growth. The current study observed a reduction in the levels of nitrate and phosphate with respect to the duration of treatment, as shown in Table 4. The observed reduction or uptake of nitrate and phosphate from wastewater can potentially be ascribed to the photosynthetic process carried out by the microalgae species, *Chlorella vulgaris*. The result indicates that the nitrate and phosphate removal percentages were 88.95% and 68.45% respectively after 480 hours of algal treatment. The growth of microalgae is heavily reliant on the presence of a substantial concentration of essential nutrients, such as nitrate and phosphate (43). According to Table 4, the initial concentrations of nitrate and phosphate in the diluted wastewater (DW) were measured to be 1.9 and 3.17 mg/l, respectively. After a duration of 20 days of microalgae treatment, reduction in the nitrate concentration to 0.21 mg/l and the phosphate concentration to 1 mg/l was observed. Ali et al. (44) reported a reduction in the nutrient concentration of DW following treatment with *C. vulgaris*.

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}

Algal biomass production

In order to assess the productivity of algal biomass, a conventional graph was constructed by correlating the absorbance measurements obtained at a wavelength of 680 nm with the predetermined concentrations of algal biomass. The calculated linear regression equation is represented as $y = 0.0548x + 0.0167$, and it is accompanied by an R^2 value of 0.9829. There is a noticeable rise in the concentration of algal biomass as the cultivation days progress (Table 5). Ali et al. (44) have reported a comparable pattern of elevated biomass concentration with the passage of cultivation days.

Correlation analysis

Pearson's correlation analysis was conducted to examine the association between microalgal biomass and the various parameters assessed in the study, as presented in Table 6. A statistically significant positive correlation was observed between biomass and pH (r = .826), alkalinity (r = .913), and dissolved oxygen (r = .937), with a p-value of less than 0.01. A negative correlation was observed between the organic, inorganic elements, and HM variables. The findings of this study indicate a negative correlation between microalgal biomass and variables related to organic, inorganic, and heavy metals (HMs). This suggests that the effectiveness of algal biomass in removing xenobiotics at high concentrations is limited (45).

Table 5 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

Table 6 Inter and Intra specific relationship between physicochemical variables and algal biomass

	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

Conclusion

The present study utilized *Chlorella vulgaris* as a means of treating domestic wastewater obtained from household discharges. The cultivation of microalgae in domestic wastewater serves as a means to conserve freshwater resources and mitigate the sequestration of carbon dioxide, thereby contributing to efforts aimed at alleviating the impacts of climate change. The utilization of algal biomass derived from wastewater holds potential as a viable feedstock for biofuel production. The present investigation examined the impact of *C. vulgaris* cultivation on the reduction of nutrient levels as well as organic and inorganic contaminants in wastewater. The highest recorded biomass concentration observed during the 480-hour (20-day) cultivation period in diluted wastewater (DW) was 8.4 g/l. The efficacy of microalgae in the removal of toxic chemicals and heavy metals was also observed. Therefore, this study proposes the utilization of the microalgae species, *Chlorella vulgaris*, as a viable option for the remediation of wastewater prior to its release into aquatic ecosystems.

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