In-situ and ex-situ Phycoremediation Competence of Innate Scenedesmus sp. on Polluted Thirumanimuthar River Water

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Abstract
This research was designed to investigate the ex-situ and in-situ phycoremediation efficacy of native Scenedesmus sp. in polluted Thirumanimuthar River water sample. Based on the macroscopic and microscopic analysis the predominant microalgae culture from polluted water was identified as Scenedesmus sp. Most of the physicochemical parameters (Electric Conductivity (EC): 3110.00 mhos cm⁻¹, turbidity: 17.10 mg L⁻¹, total hardness: 812.00 mg L⁻¹, Biological Oxygen Demand (BOD): 230 mg L⁻¹, and Chemical Oxygen Demand (COD): 352 mg L⁻¹) and some minerals such as Ca: 232.00 mg L⁻¹ and Sulfate: 532.00 mg L⁻¹ were crossing the permissible limits. Among three sets of treatments (I, II, & III), 3% in ex-situ (treatment III), and 30% in in-situ (treatment III) were shown a better reduction in physicochemical properties of polluted river water in 14 days of treatment. The in-situ study has shown better pollutants reduction than ex-situ as it reduced BOD & COD 27.83% and 23.30%, respectively. Further, the chloride, sulphate, phosphate, sodium, calcium, and magnesium were reasonably reduced up to 40.00, 43.61, 31.03, 18.75, 70.26, and 33.93%, respectively. The Fourier transform infrared (FTIR) analysis confirmed that the presence of pollutants absorbing functional groups in dried biomass of Scenedesmus sp. and the Scanning Electron Microscope (SEM) analysis image confirmed the absorption of pollutants by resulting in morphology changes of Scenedesmus sp.

The results concluded that a significant reduction was found in most tested physicochemical and minerals contents in the treated water through the in-situ study than the ex-situ approach. Through this sustainable phycoremediation strategy, the pollutant reducing Scenedesmus sp. could be used as feedstock for biofuel production.

Keywords: Phycoremediation, Polluted water, Scenedesmus sp., SEM, FTIR

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Introduction
Globally, most of the rivers and freshwater streams are primarily polluted by industrial wastes which are derive from different industries such as petrochemicals, fertilizers, oil refineries, paper, textiles, sugar mills, steel, tanneries, distilleries, and pharmaceuticals, etc. [1-5]. The untreated effluents released from textile industries contain different kinds of chemicals (acids, minerals, alkalis, and several dyes) that cause severe water pollution [6-11]. The more population-holding nations from Asian continents, the water pollution became a thoughtful issue since more population eruption leads to the discharge of more volume of untreated municipal wastewater to existing water reservoirs and create water pollution [10, 12-14]. Besides, in India, all types of pollutions were collectively accountable for 21% of demises from cerebrovascular disease, 26% of demises due to coronary artery disease, 23% by hemorrhagic stroke, 51% to respiratory infections, and 43% due to lung carcinoma [15-17]. In developing countries, environmental pollution has been accountable for numerous demises as AIDS and tuberculosis collectively made [14, 18-20]. Among the various pollution, water pollution is one of the significant causes of illness to humans and animals [20].

The rapid urbanization leads to releasing a huge volume of untreated sewage into the river water. According to the national green tribunal principal bench, New Delhi, India, the untreated effluent releasing is the solitary cause of

pollution of surface and groundwater [21, 22]. Further, about 60% of untreated urban sewage of India is directly disposed into the river and reduce the quality of water and made it unfit for human utilization (consumption) and cause diseases, demises, and critical destruction to water, earth, and air [23-25]. The Central Pollution Control Board (CPCB) of India, performed a regular portfolio of water resource, polluted water creation, and it reported that the disposal of around 33,000 million liters of polluted water created every day from Class–I and Class –II municipalities. These unprocessed discharges from houses pollute the surface and groundwater resources [25-27].

The modern industrialization process and inappropriate discharge of residues contained effluents into water bodies caused heavy metal pollution in the surrounding environment and produced serious issues [28, 29]. Hence, finding the most suitable eco-friendly technologies with potential pollutants removing or minimizing agents is significant now [30, 31]. Traditional methods such as physical (filtration, sedimentation, etc.), chemical (flocculation, precipitation, etc.), and biological (phytoremediation, bioremediation, etc.) treatment are reducing due to economic crisis, treatment time duration, and limitations in using a large scale [32-34]. The phycoremediation is an alternative, eco-friendly, efficient, and low cost technique to treat polluted water [35-37]. Since microalgae are one of the crucial parts of the polluted microbial niche, which act as a significant contributor in polluted water self-purification and also used for the elimination of pollutants such as excess nutrients, xenobiotics, and CO₂ which is low cost-effective, non-intrusive and safer technology [28, 38, 39]. Historically, microalgae have been employed to treat domestic polluted water through raceway ponds and photo-bioreactors [26, 27]. Besides that, they possess the efficiency to uptake heavy minerals from polluted water and serve as a significant role in heavy metal removal [35]. For the past few decades, the two common algal species like Chlorella and Scenedesmus sp. are majorly used to treat polluted water [34, 40].

Recently, many countries, namely, Australia, the USA, Thailand, Taiwan, and Mexico, developed algal and fungal technology to remediate polluted waters in the environment [23, 36]. The domestic sewage water in a warmer climate is the ideal habitats for the growth of Scenedesmus sp. and Chlorella sp. The various species of microalgae viz, Chlorella sp., Scenedesmus sp., Phormidium sp., Botryococcus sp., Chlamydomonas sp., and Spirulina sp. have been utilized for treating domestic polluted water [1, 22, 23]. As per the recent National Green Tribunal report, the physicochemical properties of Thirumanimuthar River were not meeting the India standards. With this brief background, the current study was focused on in-situ and ex-situ phycoremediation of contaminated water collected from highly polluted region of the “Thirumanimuthar” river (Salem, Tamil Nadu, India) using native microalgae species.

Materials and Methods

Brief profile about the study area

The study area Thirumanimuthar River located at the Poolavari village belongs to Salem District, Tamil Nadu, India (11° 36’40.8” N latitudes, and 78°06’16.9” E longitudes, Figure 1). This area received an average annual rainfall was 800 mm. The geography of the study area revealed that the presence of archaean crystalline rocks and surro

Collection of polluted water

The polluted water sample was collected from Thirumanimuthar River, at Poolavari village, Salem district of Tamil Nadu. The samples were collected in sterile polyethylene bottles that added a few drops of HNO₃ to prevent loss of elements and transported to the laboratory for further investigation [42].

Isolation, culturing, and identification of microalgae

Initially, 25 µm diameter sized phytoplankton net was used to filter the dust from the polluted river water. Later, 0.45 µm size Whatman No. 1 filter paper was used to filter the microalgae species from polluted water and immediately observed under a standard microscope and confirmed the presence of microalgae in filter paper. The filter paper was rinsed with sterile distilled water and 1 mL of microalgae suspended rinsed water was inoculated in petri plates containing 25 mL of semi-solid Bold's Basal Medium (BBM) with 200 µg mL⁻¹ of ampicillin (to avoid bacterial growth) and incubated for 3 weeks at 28±1 °C with day/night cycling illumination (40 µmol photons m⁻² s⁻¹; 15h light/ 9 h dark). The growth of green color colonies has appeared in the 2nd and 3rd weeks of the incubation period, and those
algae were identified as *Scenedesmus* sp. (through the study of macroscopically and microscopically: using a compound binocular microscope) that transferred into a sterilized BBM containing new petri dish. A single colony of *Scenedesmus* sp. was isolated and inoculated in liquid BBM media. The growth of algae (after two weeks of incubation) was measured using a UV-visible spectrophotometer at 680 nm [43].

**Figure 1** Study area for sample collection (encircled in red color)

**Ex-situ experimental setup for phycoremediation**

The *Scenedesmus* sp. culture was spun at 7000 rpm for 12 min and obtained pellet (biomass) was rinsed with sterile distilled water, and seed inoculum was prepared with three different density (5 × 10⁴ cells mL⁻¹, 7.5 × 10⁴ cells mL⁻¹, and 10 × 10⁴ cells mL⁻¹) using spectrophotometer (680 nm) and a hemocytometer. These three different cell densities (5 – 10 × 10⁴ cells mL⁻¹) of *Scenedesmus* sp. were chosen for ex-situ phycoremediation experiment study as per the methodology of Tripathi et al. [44] as follows in triplicates in a glass container.

- **Treatment I:** 5 × 10⁴ cells mL⁻¹ of *Scenedesmus* sp. in 3 L of polluted river water sample supplemented with 200 µg mL⁻¹ of ampicillin
- **Treatment II:** 7.5 × 10⁴ cells mL⁻¹ of *Scenedesmus* sp. in 3 L of polluted river water sample supplemented with 200 µg mL⁻¹ of ampicillin
- **Treatment III:** 10 × 10⁴ cells mL⁻¹ of *Scenedesmus* sp. in 3 L of polluted river water sample supplemented with 200 µg mL⁻¹ of ampicillin
- **Control:** 3 L of polluted river water sample supplemented with 200 µg mL⁻¹ of ampicillin without seed inoculum.

The treatment setups were incubated at 28±1 °C for 14 days under sterile laboratory conditions. Illumination was provided to cultures using casual white luminous lamps at 4000 Lux with a dark/light period of 16:8 h with periodical manual shaking [45].

**In-situ phycoremediation of polluted water**

**In-situ** phycoremediation study was performed near the bank of the Thirumanimutharu River at Poolavari village, Salem District, Tamil Nadu by setting up a waste stabilization pond system (5.75 x 2 x 2 m in size), and the treatment of polluted water was conducted for 14 days with (periodical manual blending) following experimental setups

- **Treatment I:** 5 × 10⁴ cells mL⁻¹ of *Scenedesmus* sp. in 100 L of polluted river water sample supplemented with 200 µg mL⁻¹ of ampicillin
- **Treatment II:** 7.5 × 10⁴ cells mL⁻¹ of *Scenedesmus* sp. in 100 L of polluted river water sample supplemented with 200 µg mL⁻¹ of ampicillin
Treatment III: $10 \times 10^4$ cells mL$^{-1}$ of *Scenedesmus* sp. in 100 L of polluted river water sample supplemented with 200 µg mL$^{-1}$ of ampicillin

Control: 100 L of polluted river water sample supplemented with 200 µg mL$^{-1}$ of ampicillin without culture

Triplicate setup was performed, and at the end of the 14$^{th}$ day, both the treated and control polluted river water sample was collected from the respective ponds and analyses the physicochemical properties of them and calculated the percentage of phycoremediation occurred by *Scenedesmus* sp. [46]. Before the sample was taken, the suspected amount of water evaporated during the treatment process was filled with elements free sterile distilled water.

**Growth kinetics of *Scenedesmus* sp. during in-situ and ex-situ phycoremediation process**

During the in-situ and ex-situ treatment, the pH of water and growth rate (density: number of cells mL$^{-1}$) of *Scenedesmus* sp. were measured at 48 h intervals up to 14 days of treatment [47], using a pH meter, hemocytometer, and spectrophotometer (at 680 nm). Later, the biomass of *Scenedesmus* sp. from the treated (in-situ and ex-situ) water samples were harvested and dried in an oven for further analyses such as Fourier Transform Infrared (FTIR) and Scanning Electron Microscope (SEM).

**Analysis of physicochemical properties of pre and post-treated river water sample**

The various physicochemical characteristics of pre and post-treatment of in-situ and ex-situ polluted water were carried out in terms of odor, turbidity, electrical conductivity, pH, Total Dissolved Solids (TDS) (mg L$^{-1}$), total alkalinity, total hardness, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), free ammonia, and nitrite along with the quantification of minerals (calcium, magnesium, sodium, iron, chloride, sulfate, and phosphate according to the standard protocols developed by American Public Health Association [48] using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES Agilent 5900 SVDV). All the analyses were carried out at District Water Testing Laboratory (TWAD), Govt. of Tamil Nadu, Salem District, Tamil Nadu, India.

**FTIR and SEM analysis**

The dry *Scenedesmus* sp. biomass was subjected to the FTIR spectroscopy and SEM analysis. The Infrared spectra were documented in the range of 4000 cm$^{-1}$ to 400 cm$^{-1}$ using an IR spectroscopy (IR-200, Nicolet, Thermo Fisher). The morphology of algae (0.71 to 1.0 mm in size dried) was observed under the Scanning Electron Microscope (SEM, JSM-5400, JEOL) [42]. The FTIR and SEM analyses of the samples were performed at Central Instrumentation Facility, St. Joseph’s College (Autonomous), Tiruchirappalli, Tamil Nadu, India.

**Results and Discussion**

**Isolation and characterization of microalgae**

The pure culture of microalgae grown in BBM was enumerated genomic identification by macroscopic and microscopic observations. Under the macroscopic observation, each colony morphological appearance seemed as massive with the flourishing growth in nature on BBM containing plates (Figure 2a). Since it contains an enormous quantity of plastids filled with chlorophylls, it showed flourishing green color colonies [47], which support the photosynthesis and support active metabolisms of overloaded pollutants in water [49]. Besides, that under the binocular microscopic observation, the cells appear as spherical to ellipsoidal in shape and single-celled or colonial forming 2 to 32 celled, usually 4 or 8 celled coenobia or colonies and arranged in either linear or alternating or in 2-3 rows and connecting with the adjacent walls (Figure 2b). Similarly, Gour et al. [50] reported that the *Scenedesmus quadricauda* and *Scenedesmus dimorphus* developed distinctive colonies comprising 4 or 8 cells while two cell formation and they noted the *Scenedesmus* sp. with single cells arrangement.

Similarly, Wang et al. [51] reported the microscopic view of the algal cells appeared as spherical to ellipsoidal, elongate, or fusiform, which supported the present study. The isolated colonies were characterized and identified as *Scenedesmus* sp. based on the morphology under the microscopic characteristics. Previously, some of the microalgae were isolated from polluted water and identified by using microscopic characteristics and the predominant species are belongs to Cyanobacteria, *S. obliquus* and *C. vulgaris* [52], *Chlorella* sp., *Scenedesmus* sp., and *Neochloris* sp. [53] that strengthen the present findings.

Growth rate of *Scenedesmus* sp.
Figure 2 Macroscopic and microscopic observation of microalgae. (a) Colony morphology (b) microscopic view of Scenedesmus sp.

The algal growth in the ex-situ and in-situ based phycoremediation process with various concentration (Figure 3a-d) of Scenedesmus sp. (Treatment I, II, & III) culture on the treatment of polluted river water was measured and counted by a spectrophotometer and hemocytometer. Out of three, the treatment III results showed that algal growth was increased gradually in both ex-situ and in-situ conditions on the 6th day onwards. Conversely, the algal growth in the lab-scale was lower than the in-situ trial. Further, significant pH changes (from 6.99 to 8.44) were observed in both ex-situ and in-situ study on the 12th day of treatment (Figure 4a and Figure 5a) and it was statistically significant at P < 0.005. The results showed that Scenedesmus sp. growth in polluted water continued up to the 14th day of treatment from 6th day onwards (Figure 4b and 5b). Entirely, the Scenedesmus sp. growth was considerably higher in in-situ treatment than ex-situ study, and it was statistically significant at P < 0.005. Since, in the in-situ study, high light intensity 25,000 lux in the morning, 75,000 lux in the noon, and 15,000 lux in the evening were recorded. The better aeration was observed in an entire day and, thus, might be a possible reason for the high growth of algae in in-situ than ex-situ study (Figure 4b and 5b) [47]. Besides that, light intensity the cycles of light and dark period was also the most significant factor in the growth of Scenedesmus sp. [54]. Nonetheless, it was limited in ex-situ study. Several researchers reported growth conditions for various microalgae species in polluted water treatment which including N. oleoabundans, Chlorella, Scenedesmus sp. A. microscopica, S. platenesis [55], Chlamydomonas sp.

Figure 3 Phycoremediation of polluted water using Scenedesmus sp. in ex-situ study. (a) Control (b) Treatment I (c) Treatment II (d) Treatment III

Physicochemical and metal analysis

The pretreated physicochemical properties of polluted Thirumanimuthar River water sample results showed that specific parameters (EC: 3110.00 micro mho cm⁻¹, turbidity: 17.10, total hardness: 812.00, BOD: 230, and COD: 352 mg L⁻¹) and some minerals (Ca: 232.00, Sulfate: 532.00 mg L⁻¹) were crossing the permissible limits of Indian
standards like Central Pollution Control Board (CPCB), Indian Council of Medical Research (ICMR), and Indian Standards Institution (ISI) of India. Further, a considerable amount of phosphate, nitrite, ammonia, sodium, magnesium, and chloride was also present in the river water (Table 1). The excess quantity of nutrients such as N₂ and P₄ enriched effluents can create eutrophication in the freshwater system [56]. These excess nitrogen and phosphorus nutrient load in water could enhance the growth of several species of microalgae such as Chlorella sp., Scenedesmus sp., Neochloris sp., etc. Hence these native green microalgae have been used for several years as an alternative biological treatment to eliminate organic pollutants (N₂ and P₄) from various polluted water [53].

Figure 4 The mentioned values are mean and standard error (±) of triplicates. **: Statistically significant at P < 0.005
(a) The changes in pH value of polluted water during the ex-situ treatment. (b) Growth kinetic of Scenedesmus sp.
during the phycoremediation process on river water: ex-situ analysis

Figure 5 The mentioned values are mean and standard error (±) of triplicates. **: Statistically significant at P < 0.005
(a) The changes in pH value of polluted water during the in-situ treatment (stabilization pond system). (b) Growth kinetic of Scenedesmus sp. during the phycoremediation process on river water: in-situ analysis

The major source of these pollutants in this river water might be received from untreated effluents of dyeing, bleaching industries, and municipal effluents [42]. From the past seven decades onwards, microalgae like Chlorella sp., Scenedesmus sp., Dundiella sp., etc. have been commercially used as suitable agents for polluted water treatment. The physicochemical and metal analyses of three sets (Treatment I, II, & III) of treated polluted water of both ex-situ and in-situ methods were presented in Table 1. The treatment III of ex-situ and in-situ results show better phycoremediation than I and II. Initially, both the ex-situ and in-situ (stabilization pond system) remediation of the polluted water appeared as small black (Figure 6a) in color with an odor which turned into green color and odorless at the end of treatment (Figure 6b).
### Table 1 Physicochemical properties of \textit{ex-situ} and \textit{in-situ} treated polluted water samples

<table>
<thead>
<tr>
<th>Physicochemical parameter</th>
<th>Pre-Treated</th>
<th>Post-Treated water</th>
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<tr>
<td></td>
<td>\textit{Ex-situ}</td>
<td>\textit{In-situ}</td>
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<tr>
<td></td>
<td>TI</td>
<td>TII</td>
</tr>
<tr>
<td>Odor</td>
<td>Odor</td>
<td>Odorless</td>
</tr>
<tr>
<td>Turbidity (mg/L)</td>
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<td>13.00±1.0</td>
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<tr>
<td>EC</td>
<td>3110.00±3.6</td>
<td>2950.00±2.9</td>
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<tr>
<td>pH</td>
<td>6.99±2.2</td>
<td>7.18±1.0</td>
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<tr>
<td>TDS (mg/L)</td>
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<td>1941.00±3.8</td>
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<tr>
<td>Total alkalinity</td>
<td>600±1.1</td>
<td>408.00±2.6</td>
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<tr>
<td>Total hardness</td>
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<tr>
<td>BOD</td>
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<tr>
<td>COD</td>
<td>352.00±2.8</td>
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</tr>
<tr>
<td>DO</td>
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<td>Free ammonia</td>
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<tr>
<td>Nitrate</td>
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<tr>
<td>Calcium</td>
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<tr>
<td>Phosphate</td>
<td>2.90±0.80</td>
<td>2.58±0.36</td>
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</table>

**Legend:** The mentioned values are mean and standard error (±) of triplicates. EC: Electric Conductivity, TDS: Total dissolved solids, BOD: Biological Oxygen Demand, COD: Chemical Oxygen Demand, DO: Dissolved Oxygen, CPCB: Central Pollution Control Board of India, ICMR: Indian Council of Medical Research of India, ISI: Indian Standards Institute of India.

![Figure 6](image-url) **Figure 6** Percentage of phycoremediation in polluted river water by \textit{ex-situ} and \textit{in-situ} analysis compared with control. The mentioned values are mean and standard error (±) of triplicates. **: Statistically significant at $P < 0.005$.
The BOD and COD values in the in-situ study were found as 166 (mg L\(^{-1}\)) and 270 (mg L\(^{-1}\)) while lab-scale 205 (mg L\(^{-1}\)) and 310 (mg L\(^{-1}\)), respectively. The BOD and COD level in the in-situ study was reduced to 27.83% and 23.30%, whereas in the lab-scale, 10.87% and 11.93% compared with the control respectively. Tang et al. [52] noted a similar amount of BOD and COD reduction in the treated municipal sewage polluted water used C. vulgaris and S. obliquus. This might be high growth of microalgae and photosynthesis mechanism. Gupta and Rastogi [57] explained microalgae role in polluted water treatment, which reduced the significant number of contaminants in various physicochemical parameters like COD, BOD, and turbidity. Biological oxygen demand is especially useful in evaluating the auto-purification capacity of streams, which serves as a measure to assess the quantity of polluted water, safely assimilated by a stream. Similarly, Goncalves et al. [53] reported that the Dundiella sp. has the highest removal of BOD, COD, and ammonium from polluted water. Significantly increased pH values (up to 8.44, 14% - 20%) were found in both ex-situ and in-situ phytoremediation processes (Figure 4a, 5a, and 7). The present results were agreed with Liu et al. [58]. They found that the effect of stirring on the high growth of algae and reported that the photosynthesis could induced higher pH values in ammonia polluted water during the phycoremediation by Scenedesmus obliquus. Norvill et al. [59] found that ammonia may be exposed off into the air and resulted in an increased level of pH in algal cultures. The TDS content of polluted river water in both ex-situ and in-situ studies was 32% decreased compared to the control that depends on utilizing various types of nutrients by the algae. Glibert et al. [56] demonstrated that the removal of >50% of TDS in 9 days of polluted water treatment was carried out by the Chlorella minutissima, supporting the present findings. We obtained the efficient removal of ammonia (100%) from polluted water by Scenedesmus sp. and it was supported by the outcome of Yang et al. [60], who reported the efficient removal of ammonia in polluted water and utilized the harvested algae for biodiesel production. The obtained result was quite comparable to the findings of Wang et al. [61], who reported the efficacy of C. vulgaris and S. dimorphus are similar to Phormidium bonheri in removing ammonia (95%).

Scenedesmus sp. shown a remarkable effect on the electrical conductivity value of polluted water which has been reduced to 32% (2110 mho cm\(^{-1}\)) in both ex-situ and in-situ. The EC value of water is quite linear with the volume of dissolved ions were reported by Mata et al. [62]. Mencio et al. [63] stated that the increased total hardness level in water was unsuitable for agricultural purposes. The present study observed the total hardness of in-situ and ex-situ analysis were reduced to 316 mg L\(^{-1}\) (61.08%) and 328 mg L\(^{-1}\) (59.61%) from the control value 812 mg L\(^{-1}\) by Scenedesmus sp., due to the efficient uptake of the nutrients, respectively.

The ex-situ phycoremediation study by Scenedesmus sp. on minerals removal in polluted river water was analyzed. The result states that the treatment III has effectively performed the phycoremediation I and II. In the III treated water sample, the presence (mg L\(^{-1}\)) of 252, 300, 2, 260, 69, and 37 of chloride, sulphate, phosphate, sodium, calcium, and magnesium respectively were occurred and that was equivalent to 40.00%, 43.61%, 31.03%, 18.75%, 70.26%, and 33.93% reduction (Figure 7) while compared to those found in control as 420, 532, 2.90, 320, 232, and 56 mg L\(^{-1}\), respectively. Whereas, 248, 295, 1.5, 260, 67, and 36 mg L\(^{-1}\), of chloride, sulfate, phosphate, sodium, calcium, and magnesium, were found in the in-situ study (in-situ treatment III) that expressed more mineral reduction potential of Scenedesmus sp. than the ex-situ. An increased level of potassium (33.33% - 55.56%) was noted in the treated river water, and it might be derived from as an output of metabolic activity of Scenedesmus sp.

![Figure 7](image-url)

**Figure 7 (a)** In-situ phycoremediation experimental set up (waste stabilization pond system) (b) Pre and post treated water samples from the in-situ treatment

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The amount of DO concentration was increased significantly from the control value 2.86, and 3.80 mg L\(^{-1}\) in the ex-situ and in-situ study after the treatment (III) that corresponds to 73.33\% and 130.30\% surge, respectively. Since the microalgae in polluted water treatment provide oxygen during the phycoremediation process, it could enhance the native bacterial breakdown of organic pollutants and minimize their toxicity. After treatment, 100\% nitrate (NO\(_3\)) removal was obtained in both ex-situ and in-situ trials. Ammonia (NH\(_3\)) concentration was drastically reduced from 3.5 to 0.00 mg L\(^{-1}\) (100\%) in ex-situ and in-situ treatment. In general, the total amount of minerals was remarkably reduced in the treated polluted water.

Cheah et al. [64] reported that the total hardness (due to Na, K, Ca, etc.) was reduced drastically (69\%) when the sewage water was treated with *Scenedesmus* sp. Pham and Bui [65] reported that the algal-based remediation of river water found a reduction in calcium (67.1 \%) and potassium (67.4\%) contents from polluted river water in a short duration of the treatment process. The sulfate content of treated effluent with *Scenedesmus* sp. was reduced to 43.9\%. Similarly, Liang et al. [66] reported the reduction of Sulfate in the sewage effluent by microalgae *Chlorella* sp. Lee et al. [67], reported that the alkalinity of effluent was reduced to 44\% by *Scenedesmus dimorphus* and noted high alkalinity, which harms aquatic organisms. In-situ phycoremediation mainly focused on treating the polluted water in field-based waste stabilization ponds system has proven to be for treating polluted water in terms of the reduced physicochemical level parameters than ex-situ phycoremediation. Similarly, Whitton et al. [68] studied aerobic, facultative, and anaerobic pond methods to remediate the polluted water by nutrient removal. The results indicate an efficient reduction of BOD, COD, and TSS. Live algae possess intracellular and extracellular polyphosphates that are involved in metal sequestration and metals binding process. The cell wall of microalgae consists of several functional groups such as carboxyl, hydroxyl, phosphate, amino, and sulphhydril moieties from polysaccharides, proteins, and lipids molecules. Functional groups bestow a net negative charge to the cell surface and are reported to have an outstanding metal binding nature [57].

**Fig. 8** FTIR spectrum of *Scenedesmus* sp. biomass compared with control (a) control (b) ex-situ treatment (c) in-situ treatment
FTIR and SEM analysis of Scenedesmus sp.

The FTIR analysis of microalgae is a real preference for tedious documentation of a functional group of dried microalgae [54]. In the present study, the FTIR spectra of control biomass (without treated) of *Scenedesmus* sp., and the polluted river water ex-situ and in-situ treated biomass are shown in Figure 8a-c respectively, and the characteristic functional groups were presented in Table 2. The prominent region between the 3200 cm$^{-1}$ - 3600 cm$^{-1}$ represented the stretching vibration of -OH groups. The peak value at 3311.42 cm$^{-1}$ was shown the -OH groups stretching vibration. The intensives peaks between 2800 cm$^{-1}$ - 3000 cm$^{-1}$ showed the presence of alkanes (CH) groups. The untreated biomass indicated the curve at 2922 cm$^{-1}$ of C-H unequal vibration of aliphatic (CH$_2$) efficient clusters. The curve at 1655 cm$^{-1}$ reflects a sharper due to the C=C stretching of carbonyl clusters. The IR region between 1600-1585 cm$^{-1}$ indicating the prominent peaks of C-C vibrational stretching of 1551 cm$^{-1}$ aromatic clusters. 1100-1058 cm$^{-1}$ were P=O or C-O stretching is maybe polysaccharides.

Table 2 FTIR analysis of Scenedesmus sp. biomass

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Control</th>
<th>Wavelength (cm$^{-1}$)</th>
<th>Types of Vibrations</th>
<th>Functional Groups</th>
<th>Wavelength (cm$^{-1}$)</th>
<th>Types of Vibrations</th>
<th>Functional Groups</th>
<th>In-situ study</th>
<th>Wavelength (cm$^{-1}$)</th>
<th>Types of Vibrations</th>
<th>Functional Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3311.42</td>
<td>O-H stretch</td>
<td>Alcohols</td>
<td>3920.42</td>
<td>O-H stretch</td>
<td>Alcohols</td>
<td>3920.47</td>
<td>O-H</td>
<td>3920.47</td>
<td>Alcohols</td>
<td>3920.47</td>
</tr>
<tr>
<td>2</td>
<td>2922.40</td>
<td>C-H stretch</td>
<td>Alkanes</td>
<td>2924.91</td>
<td>C-H stretch</td>
<td>Alkanes</td>
<td>2926.87</td>
<td>C-H</td>
<td>2926.87</td>
<td>Alkanes</td>
<td>2926.87</td>
</tr>
<tr>
<td>3</td>
<td>2853.17</td>
<td>CH$_2$ symmetric</td>
<td>Aliphatic compound</td>
<td>2855.77</td>
<td>-CH$_3$ stretch</td>
<td>Aliphatic compound</td>
<td>2861.31</td>
<td>Aliphatic</td>
<td>2861.31</td>
<td>Aliphatic compound</td>
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<tr>
<td>4</td>
<td>1655.96</td>
<td>-C=C stretch</td>
<td>Alkanes</td>
<td>1651.19</td>
<td>-C=C stretch</td>
<td>Alkanes</td>
<td>1652.88</td>
<td>Alkanes</td>
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<td>Alkanes</td>
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<tr>
<td>5</td>
<td>1449.86</td>
<td>C-H bend</td>
<td>Alkanes</td>
<td>1423.14</td>
<td>-C=C stretch</td>
<td>Aromatics</td>
<td>1452.41</td>
<td>N-O</td>
<td>1452.41</td>
<td>Nitro compound</td>
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<td>6</td>
<td>720.21</td>
<td>C-H rock</td>
<td>Alkanes</td>
<td>698.98</td>
<td>C-H bend</td>
<td>Alkynes</td>
<td>690.54</td>
<td>C-H</td>
<td>690.54</td>
<td>Alkynes</td>
<td>690.54</td>
</tr>
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</table>

FTIR spectra of untreated biomass curves were expressed at 720 cm$^{-1}$ show the occurrence of C-Cl stretching. The IR spectra of polluted water treated biomass represented an unstable of the peaks at 698 cm$^{-1}$ and 690 cm$^{-1}$. Similarly, Yu et al. [55] reported the FTIR transmittance of dried biomass of *Scenedesmus* sp. as an aliphatic character at 400–800, phenols, and alcoholic groups at 1,000–1,400, carboxyl group at 1,500–1,700 cm$^{-1}$, hydroxyl group: 3,200–3,400 cm$^{-1}$. These chemical groups could act as an agent to absorb the pollutants present in the polluted environment [54], and the spectrum of polluted effluent (Pb, Cr, Co, Ni, and Cu) treated biomass (*in-situ* and *ex-situ*) revealed broad peaks in frequency observed at 3,386 cm$^{-1}$ and 3,380 cm$^{-1}$, but these peaks were absent in control biomass. Few functional group changes were perceived in the spectra of treated biomass, which indicates that the -OH groups might be involved in the metal absorption mechanism of *Scenedesmus* sp. Similarly, Castrillo et al. [69] stated that the *Chlorella* sp. treated with minerals has interacted with COOH, -OH, and C=O clusters that have absorbed the pollutants effectively from the polluted water.

Fig. 9 SEM analysis of treated *Scenedesmus* sp. morphology compared with control (a) Control biomass (b) *ex-situ* treated biomass (c) *in-situ* treated biomass
The results of the SEM analysis of the present study showed that the untreated algal cells shown standard shape with a smooth, transparent external layer found in the outer cell surface (Figure 9a). After treating the water, the cells become slightly rough and crenelated textures, and some elements were found on the surface of the cell wall (Figure 9b and 9c). Chiu et al. [70] explained that surface protuberance on the algal cell surface due to the deposition of crystallized salts absorbed from polluted water. The approximate size of the Scenedesmus sp. was recorded as 6.04 to 8.01 μm (Magnification of 4000x). Similarly, Gour et al. [50] reported the size of Scenedesmus quadricauda and Scenedesmus dimorphus ranged from 6.21 to 8.3 μm and 8.28 to 8.74 μm, respectively.

Conclusions

Based on the macroscopic and microscopic study, the predominant native microalgae culture was identified as Scenedesmus sp. The physicochemical properties such as EC (3110.00 micro mho cm⁻¹), turbidity (17.10 mg L⁻¹), total hardness (812.00 mg L⁻¹), BOD (230 mg L⁻¹), and COD (352 mg L⁻¹) and some minerals such as Ca (232.00 mg L⁻¹). Sulfate (532.00 mg L⁻¹) were not under the acceptable limits of Indian standards. Three sets of treatments (I, II, & III) were performed with various percentages of the biomass of Scenedesmus sp. in ex-situ and in-situ conditions on polluted river water samples. The treatment III showed a better reduction in river water pollution load in 14 days of treatment in ex-situ and in-situ study. The FTIR and SEM analyses confirmed that the presence of active functional groups in biomass of Scenedesmus sp. and SEM image described the absorption of pollutants resulting from the changes in the morphology of Scenedesmus sp. under 4000x Magnification. The present study findings concluded that a significant reduction was found in most of the tested physicochemical and basic minerals in the treated polluted water using microalgae Scenedesmus sp. in the in-situ study than ex-situ approach. The cultivation of algae in polluted water could be offering duel benefits: 1. Environmental wares with the fabrication of algal biomass as phycoremediation. 2. Which serves as a raw material for the production of biofuel and provides a valuable solution for contaminated water with possible obtaining of valued added raw material for biofuel production.

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