Research Article

# *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 micro mho $cm^{-1}$, 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 $CO_2$ 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 surrounded by several hillocks in all directions, namely, Shevaroys and Nagaramalai (North), Jarugumalai Hills (South), Kanjamalai (West), and Goudamalai (East). This river has been polluted by releasing untreated dyeing and bleaching (155 unauthorized dyeing and bleaching units and 125 illegal manual dyeing units) industrial effluents, municipal and medical wastes from in and around the Salem district of Tamilnadu. Further, it polluted the surface and groundwater and made them unfit for drinking and irrigation [41]. The farmers depend entirely on this river for agriculture activities and drinking purposes due to insufficient annual rainfall.

### *Collection of polluted water*

The polluted water sample was collected from Thirumanimutharu River, at Poolavari village, Salem district of Tamil Nadu. The samples were collected in sterile polyethylene bottles that added a few drops of $HNO_3$ 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 $^oC$ 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 $2^{nd}$ and $3^{rd}$ 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].

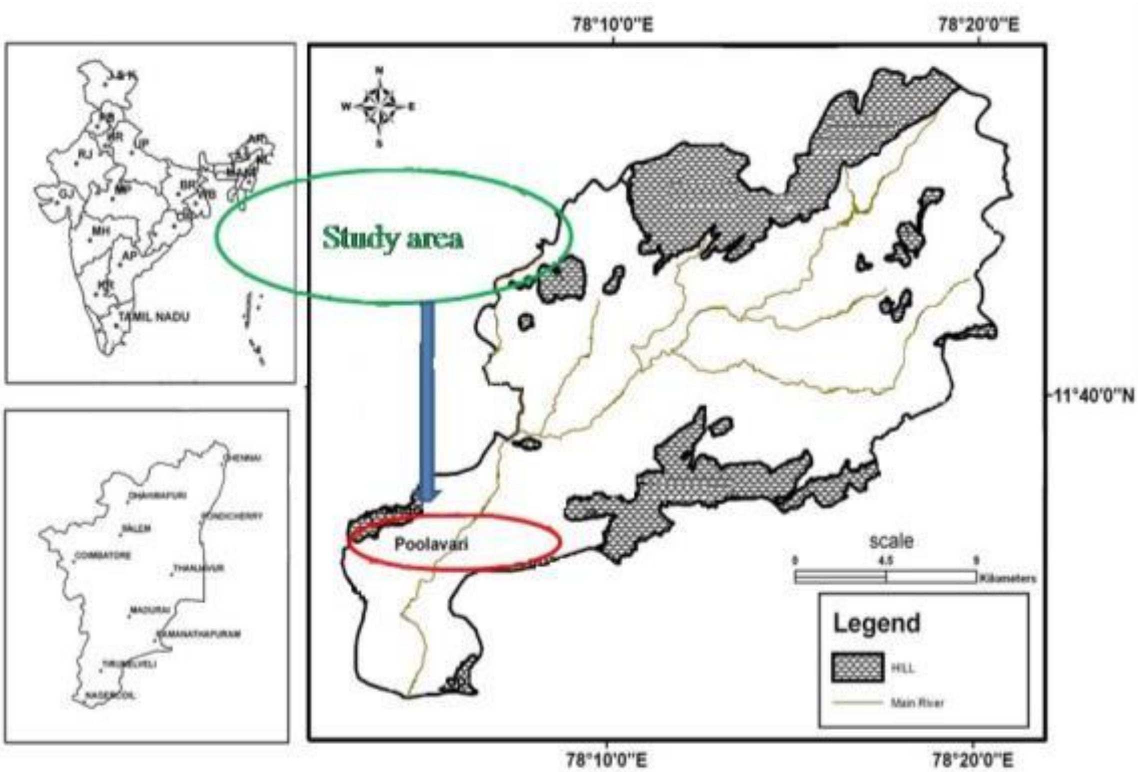


**Figure 1** Study area for sample collection (encircled in red color)

### *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 ($5 \times 10^4$ cells $mL^{-1}$, $7.5 \times 10^4$ cells $mL^{-1}$, and $10 \times 10^4$ cells $mL^{-1}$) using spectrophotometer (680 nm) and a hemocytometer. These three different cell densities ($5 - 10 \times 10^4$ cells $mL^{-1}$) of *Scenedesmus* sp. were chosen for e*x-situ* phycoremediation experiment study as per the methodology of Tripathi et al. [44] as follows in triplicates in a glass container.

Treatment I: $5 \times 10^4$ cells $mL^{-1}$ of *Scenedesmus* sp. in 3 L of polluted river water sample supplemented with 200 µg $mL^{-1}$ of ampicillin (to assure the pollutant reduction efficiency by test microalgae species)

Treatment II: $7.5 \times 10^4$ cells $mL^{-1}$of *Scenedesmus* sp. in 3 L of polluted river water sample supplemented with 200 µg $mL^{-1}$ of ampicillin

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

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

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.

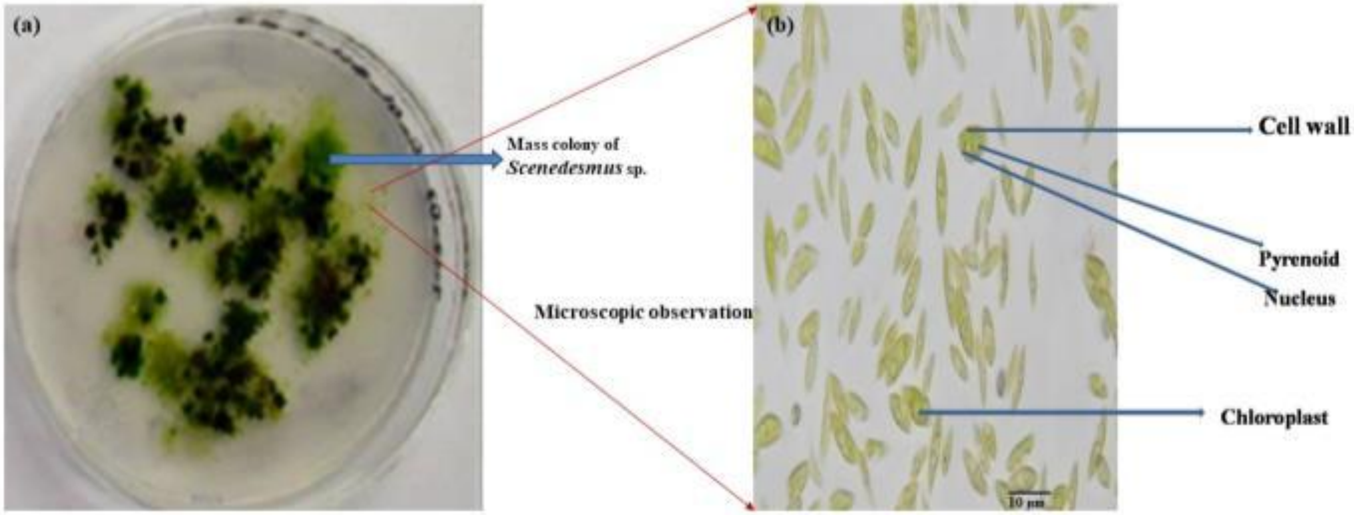


**Figure 2** Macroscopic and microscopic observation of microalgae**. (a)** Colony morphology **(b)** microscopic view of *Scenedesmus* sp.

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 6$^{th}$ 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 12$^{th}$ 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 14$^{th}$ day of treatment from 6$^{th}$ 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.

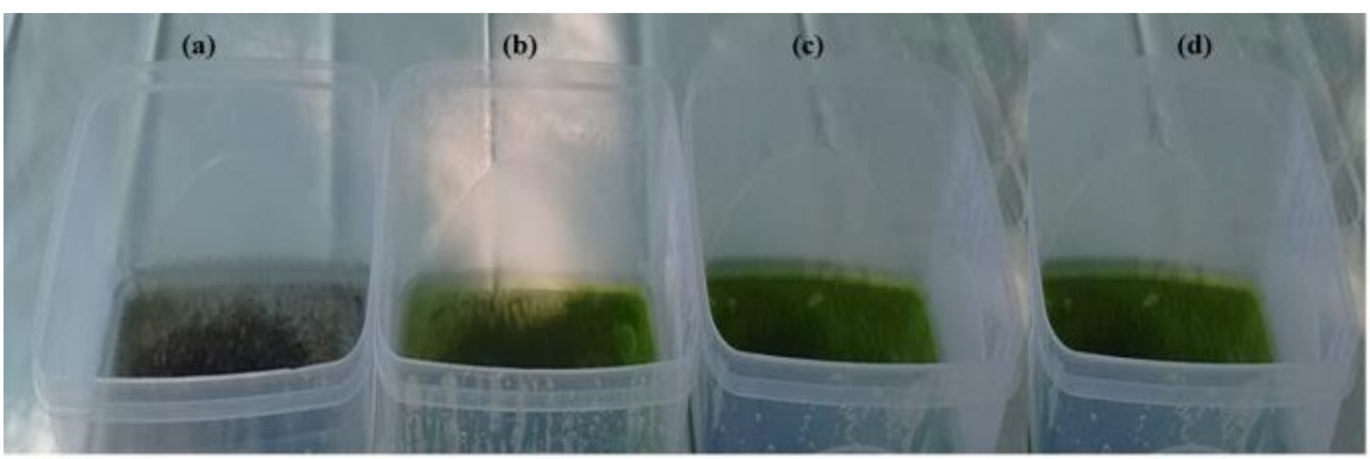


**Figure 3** Phycoremediation of polluted water using *Scenedesmus* sp. in *ex-situ* study. **(a)** Control **(b)** Treatment I **(c)** Treatment II **(d)** Treatment III

### *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 $N_2$ and $P_4$ 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 ($N_2$ and $P_4$) from various polluted water [53].

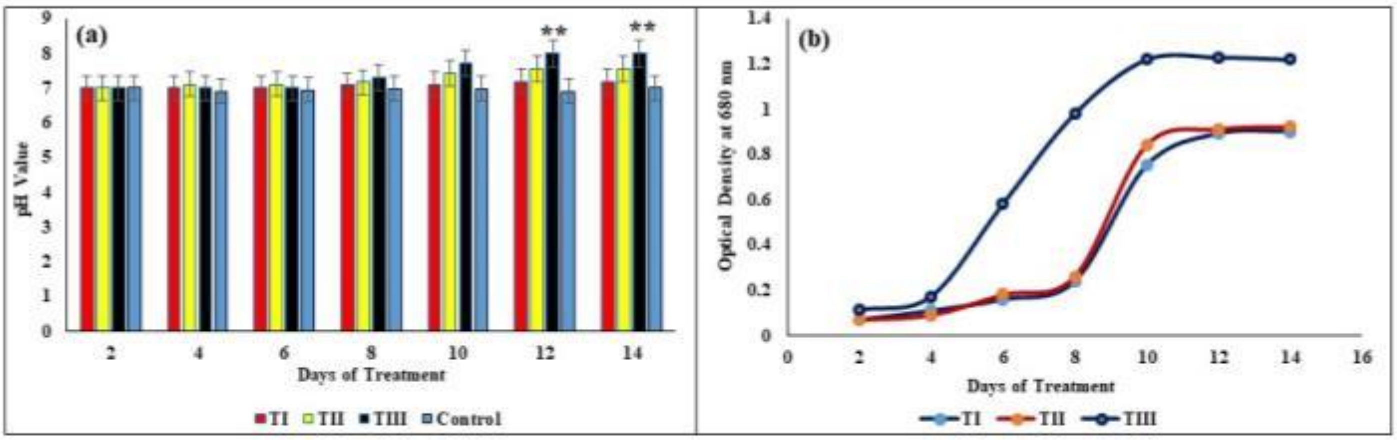


**Figure 4** The mentioned values are mean and standard error (±) of triplicates. **: Statistically significant at $P < 0.005$ **(a)** The changes in pH value of polluted water during the *ex-situ* treatment**. (b)** Growth kinetic of *Scenedesmus* sp. during the phycoremediation process on river water: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

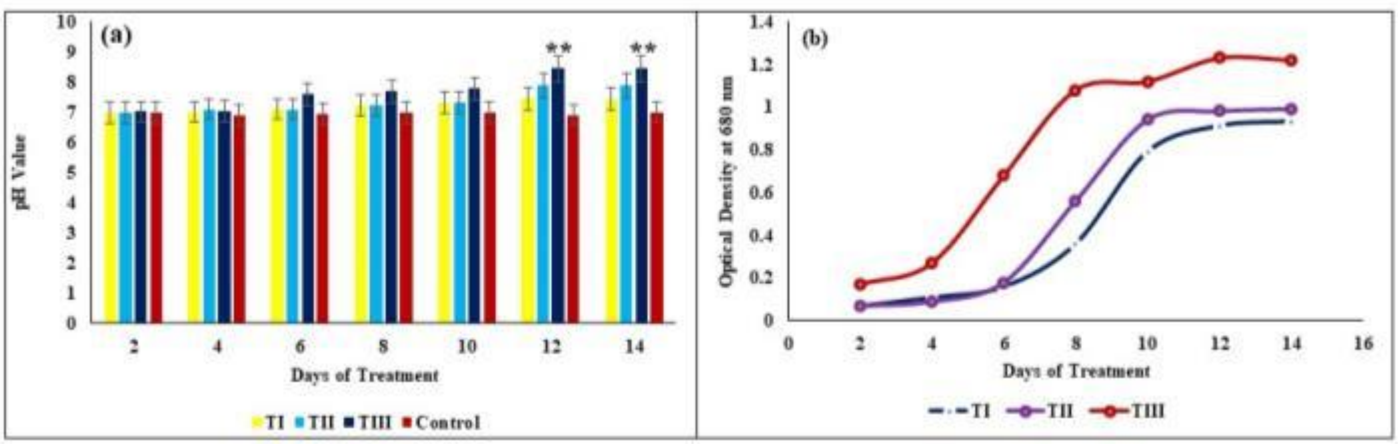


**Figure 5** The mentioned values are mean and standard error (±) of triplicates. **: Statistically significant at $P < 0.005$ **(a)** The changes in pH value of polluted water during the *in-situ* treatment (stabilization pond system). **(b)** Growth kinetic of *Scenedesmus* sp. during the phycoremediation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

| Physico chemical parameters (mg L$^{-1}$) | Indian standards permissible limits (mg L$^{-1}$) | | | Pre-Treated | Post-Treated water | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Ex-situ* | | | *In-situ* | | |
| | CPCB | ICMR | ISI | | TI | TII | TII | TI | TII | TII |
| Odor | - | - | - | Odor | Odorless | Odorless | Odorless | Odorless | Odorless | Odorless |
| Turbidity (mg/L) | 10 | 25 | 10 | 17.10±.03 | 13.00±.1 | 9.10±.2 | 4.00±.2 | 13.10±. | 2.00±. | 2.00±. |
| EC | 2000 | - | - | 3110.00±3.1 | 2950.00±3.6 | 2504.00±2.9 | 2100.00±3.2 | 2961.00±2.4 | 2308.00±3.8 | 2110.00±2.5 |
| pH | 6.5-8.5 | 6.5-9.2 | 6.5-8.5 | 6.99±.2 | 7.18±.1 | 7.54±.3 | 7.98±.3 | 7.44±.2 | 7.89±.3 | 8.44±.2 |
| TDS (mg/L) | - | - | - | 2177.00±4.3 | 1941.00±3.8 | 1817.00±2.7 | 1477.00±2.4 | 1924.20±2.6 | 1629.00±3.5 | 1470.00±3.1 |
| Total alkalinity | 600 | - | - | 432.00±1.1 | 408.00±2.1 | 312.00±2.5 | 288.00±2.4 | 401.00±2.4 | 327.00±1.9 | 272.00±3.2 |
| Total hardness | 600 | 600 | 300 | 812.00±2.4 | 734.00±3.6 | 617.00±3.7 | 328.00±4.1 | 523.00±3.6 | 409.00±4.2 | 316.00±4.2 |
| BOD | 30 | - | - | 230.00±1.9 | 221.00±2.5 | 218.00±1.6 | 205.00±2.8 | 206.00±1.9 | 184.00±3.1 | 166.00±2.6 |
| COD | 250 | - | - | 352.00±2.8 | 345.00±3.1 | 323.00±2.6 | 310.00±3.8 | 308.00±2.7 | 289.00±2.1 | 270.00±3.6 |
| DO | | | | 1.65±.41 | 1.86±.22 | 2.28±.75 | 2.86±.82 | 2.11±.47 | 2.71±.61 | 3.80±.83 |
| Free ammonia | - | - | - | 3.50±.53 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Nitrite | - | - | - | 1.20±.64 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Calcium | 200 | 200 | 75 | 232.00±3.4 | 219.00±2.6 | 126.00±1.7 | 69.00±.8 | 187.00±1.9 | 118.00±1.6 | 67.00±1.2 |
| Magnesium | 100 | - | 30 | 56.00±.89 | 51.00±.53 | 48.00±.42 | 37.00±.78 | 51.00±.34 | 48.00±.35 | 36.00±.75 |
| Sodium | - | - | - | 320.00±2.6 | 308.00±4.1 | 291.00±2.5 | 260.00±3.2 | 301.00±4.1 | 279.00±2.4 | 260.00±2.3 |
| Iron | 1.0 | 1.0 | 0.3 | 0.90±.0 | 0.71±.01 | 0.54±.0 | 0.10±.0 | 0.64±.0 | 0.48±.0 | 0.10±.0 |
| Chloride | 1000 | 1000 | 250 | 420.00±4.2 | 372.00±2.7 | 301.00±3.1 | 252.00±3.5 | 403.00±2.8 | 308.00±3.1 | 248.00±3.7 |
| Sulfate | 400 | 400 | 150 | 532.00±3.6 | 502.00±2.4 | 410.00±3.7 | 300.00±2.9 | 511.00±3.1 | 401.00±2.8 | 295.00±2.7 |
| Phosphate | - | - | - | 2.90±.80 | 2.58±.36 | 2.29±.27 | 2.00±.41 | 2.51±.18 | 2.05±.52 | 1.50±.11 |

**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.

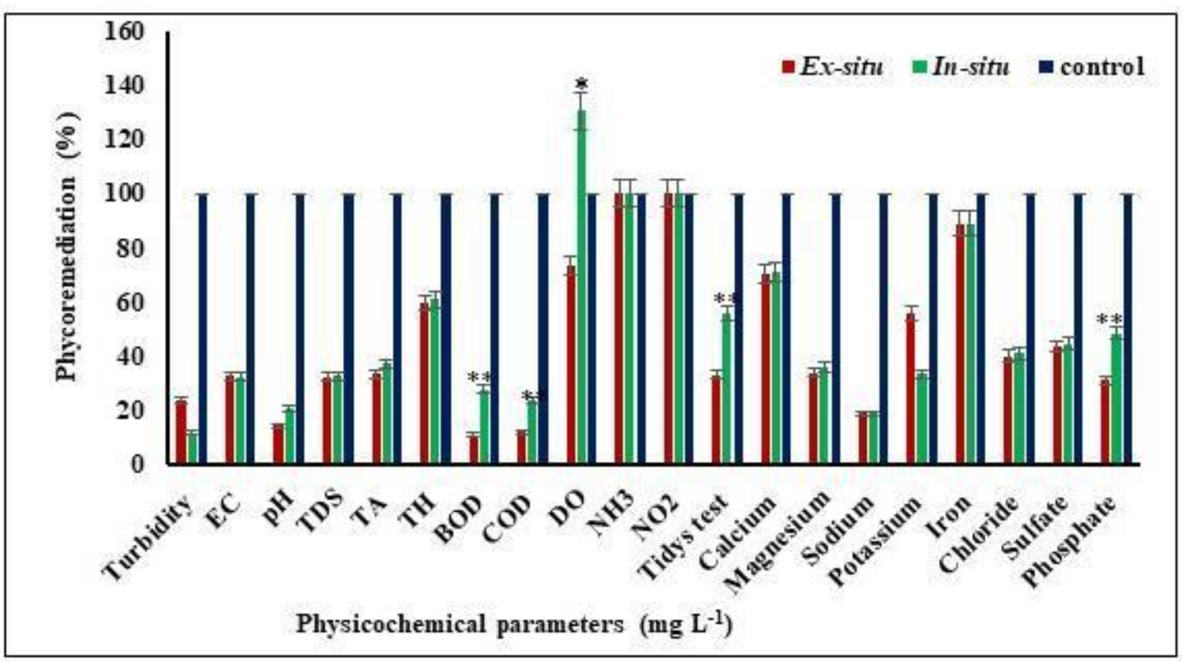


**Figure 6** Percentage of phycoremediation in polluted river water by *ex-situ* and *in-situ* analysis compared with control. The mentioned values are mean and standard error (±) of triplicates. **: Statistically significant at $P < 0.005$

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, Gonçalves 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 thevolume 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.

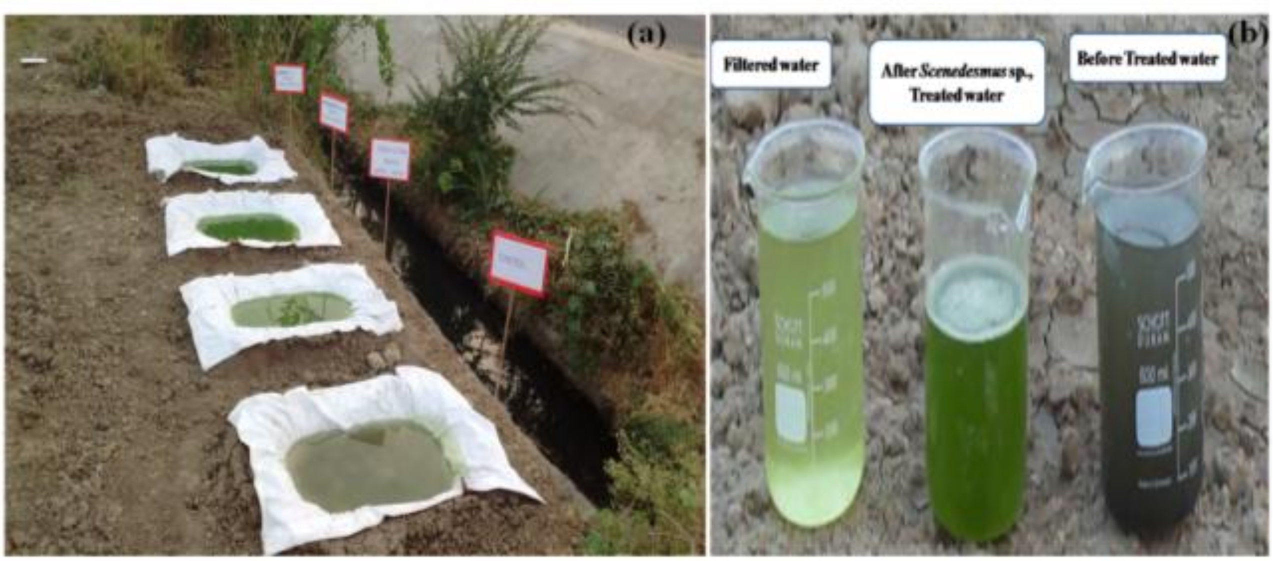


**Figure 7 (a)** *In-situ* phycoremediation experimental set up (waste stabilization pond system) **(b)** Pre and post treated water samples from the *in-situ* treatment

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 microalga *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 sulphydryl 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].

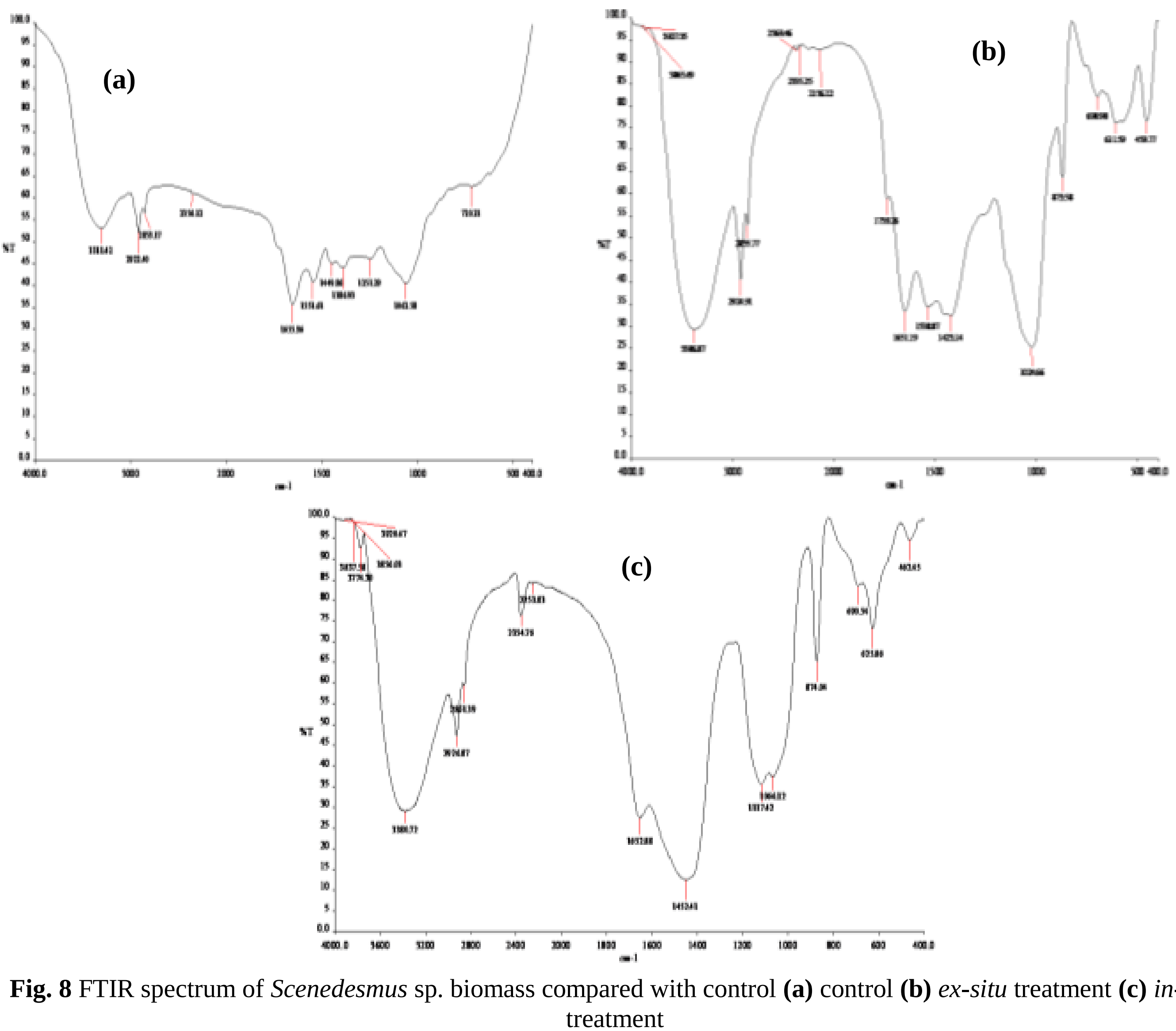


**Fig. 8** FTIR spectrum of *Scenedesmus* sp. biomass compared with control **(a)** control **(b)** *ex-situ* treatment **(c)** *in-situ* treatment

### *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 intensive 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

| S. No. | Control | | | *Ex-situ* study | | | *In-situ* study | | |
|---|---|---|---|---|---|---|---|---|---|
| | Wavelength ($cm^{-1}$) | Types of Vibrations | Functional Groups | Wavelength ($cm^{-1}$) | Types of Vibrations | Functional groups | Wavelength ($cm^{-1}$) | Types of Vibrations | Functional groups |
| 1. | 3311.42 | O-H stretch | Alcohols | 3920.42 | O-H Stretch | Alcohols | 3920.47 | O-H Stretch | alcohols |
| 2. | 2922.40 | C-H stretch | Alkanes | 2924.91 | C-H Stretch | Alkanes | 2926.87 | C-H Stretch | alkanes |
| 3. | 2853.17 | $CH_2$ symmetric Phosphines | -$CH_2$ in Aliphatic compound | 2855.77 | -$CH_3$ stretch | Aliphatic compound | 2861.31 | -$CH_3$ Stretch | Aliphatic compound |
| 4. | 1655.96 | -C=C stretch | Alkanes | 1651.19 | -C=C stretch | Alkanes | 1652.88 | -C=C stretch | Alkanes |
| 5. | 1449.86 | C-H bend | Alkanes | 1423.14 | -C=C stretch | Aromatics | 1452.41 | N-O asymmetric | Nitro compound |
| 6. | 720.21 | C-H rock | Alkanes | 698.98 | C-H bend | Alkynes | 690.54 | C-H bend | Alkynes |

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.

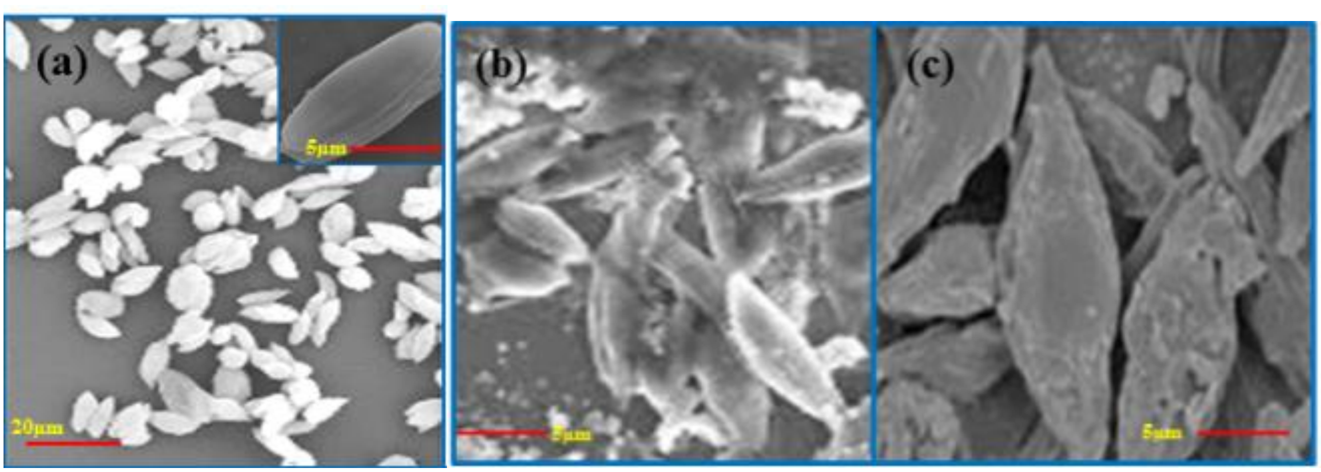


**Fig. 9** SEM analysis of treated *Scenedesmus* sp. morphology compared with control **(a)** Control biomass **(b)** *ex-situ* treated biomass **(c)** *in-situ* treated biomass

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 4000×). 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 4000× 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 welfares 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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