In-Situ and Ex-Situ Phycoremediation Competence of Innate Scenedesmus Sp. On Polluted River Water

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Abstract

The present study was aimed 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 (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 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 FTIR analysis confirmed that the presence of pollutants absorbing functional groups in dried biomass of *Scenedesmus* sp. and the 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.

1. 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. (Liu et al. 2019; Leng et al. 2020). The untreated effluents released from textile industries contain different kinds of chemicals (acids, minerals, alkalis, and several dyes) that cause severe water pollution (Sarayu and Sandhya 2012). 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 (Angelaalincy et al. 2017). 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. In developing countries, environmental pollution has been accountable for numerous demises as AIDS and tuberculosis collectively made (NGT 2019). Among the various pollution, water pollution is one of the significant causes of illness to humans and animals (Leng et al. 2020).

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 (NGT 2019), the untreated effluent releasing is the solitary cause of pollution of surface and groundwater. 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 (Murty 2011; Asselborn et al. 2015). 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 (CPCB Report 2009). These unprocessed discharges from houses pollute the surface and groundwater resources.

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 (Verma et al. 2020). Hence, finding the most suitable eco-friendly technologies with potential pollutants removing or minimizing agents is significant now. 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 (Li et al. 2013). The phycoremediation is an alternative, eco-friendly, efficient, and low cost (Gonçalves et al. 2017; Li et al. 2013) technique to treat polluted water. Since microalgae are one of the crucial parts of the polluted microbial niche, which act as a significant contributor in polluted water self-purification (Gonçalves et al. 2017) 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 (Ajayan et al. 2015). Historically, microalgae have been employed to treat domestic polluted water through raceway ponds and photo-bioreactors. Besides that, they possess the efficiency to uptake heavy minerals from polluted water and serve as a significant role in heavy metal removal (Kumar et al. 2015). For the past few decades, the two common algal species like *Chlorella* and *Scenedesmus* sp. are majorly used to treat polluted water (Krohn-Molt et al. 2017).

Recently, many countries, namely, Australia, the USA, Thailand, Taiwan, and Mexico, developed algal and fungal technology to remediate polluted waters in the environment (Liu et al. 2013). The domestic sewage water in a warmer climate is the ideal habitats for the growth of *Scenedesmus* sp. and *Chlorella* sp. (Tripathi et al. 2019). 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 (Wang et al. 2015; Gonçalves et al. 2017; Liu et al. 2019). As per the recent National Green Tribunal report (2019), 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.

2. Materials And Methods

2.1. 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, Fig. 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, Shevaroy 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 (NGT 2019). The farmers depend entirely on this river for agriculture activities and drinking purposes due to insufficient annual rainfall.

2.2. 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 (Ajayan et al. 2015).

2.3. 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 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 $\mu\text{g mL}^{-1}$ of ampicillin (to avoid bacterial growth) and incubated for 3 weeks at $28 \pm 1^\circ\text{C}$ with day/night cycling illumination (40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; 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 (Mehrabadi et al. 2015).

2.4 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 (5×10^4 cells mL^{-1} , 7.5×10^4 cells mL^{-1} , and 10×10^4 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 *ex-situ* phycoremediation experiment study as per the methodology of Tripathi et al. (2019) as follows in triplicates in a glass container.

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

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

Treatment III: 10×10^4 cells mL^{-1} of *Scenedesmus* sp. in 3 L of polluted river water sample supplemented with 200 $\mu\text{g mL}^{-1}$ of ampicillin

Control : 3 L of polluted river water sample supplemented with 200 $\mu\text{g mL}^{-1}$ of ampicillin without seed inoculum.

The treatment setups were incubated at $28 \pm 1^\circ\text{C}$ 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 (Verma et al. 2020).

2.5. 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: 5×10^4 cells mL^{-1} of *Scenedesmus* sp. in 100 L of polluted river water sample supplemented with 200 $\mu\text{g mL}^{-1}$ of ampicillin

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

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

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

Triplicate setup was performed, and at the end of the 14th 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. (Sutherland and Ralph 2019). Before the sample was taken, the suspected amount of water evaporated during the treatment process was filled with elements free sterile distilled water.

2.6 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 (Derakhshandeh and Tezcan Un 2019), 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 like FTIR and SEM.

2.7. 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 (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 (APHA 2005) 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.

2.8. Fourier Transforms Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (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) (Ajayan et al. 2015). The FTIR and SEM analyses of the samples were performed at Central Instrumentation Facility, St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India.

3. Results And Discussion

3.1. 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 (Fig. 2a). Since it contains an enormous quantity of plastids filled with chlorophylls, it showed flourishing green color colonies (Derakhshandeh and Tezcan 2019), which support the photosynthesis and support active metabolisms of overloaded pollutants in water (Wan et al. 2016). 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 (Fig. 2b). Similarly, Gour et al. (2016) 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. (2016) 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* (Tang et al. 2018), *Chlorella* sp., *Scenedesmus* sp., and *Neochloris* sp. (Gonçalves et al. 2017) that strengthen the present findings.

3.2. Growth rate of *Scenedesmus* sp.

The algal growth in the *ex-situ* and *in-situ* based phycoremediation process with various concentration (Fig. 3a, b, c, & 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

(Fig. 4a & Fig. 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 (Fig. 4b & 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 (Fig. 4b & 5b) (Derakhshandeh and Tezcan Un 2019). Besides that, light intensity the cycles of light and dark period was also the most significant factor in the growth of *Scenedesmus* sp. (Xiong et al. 2016). Nonetheless, it was limited in *ex-situ* study. Several researchers reported growth conditions for various microalgae species in polluted water treatment which including *N. oleabundans*, *Chlorella* (Anjos et al. 2013), *Scenedesmus* sp. A. *microscopica*, *S. platenesis* (Yu et al. 2019), *Chlamydomonas* sp. (Suresh Kumar et al. 2015).

3.3. 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 (Glibert et al. 2017). 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 (Zhao et al. 2015; Gonçalves et al. 2017).

The major source of these pollutants in this river water might be received from untreated effluents of dyeing, bleaching industries, and municipal effluents (Ajayan et al. 2015). From the past seven decades onwards, microalgae like *Chlorella* sp., *Scenedesmus* sp., *Dundia* 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 (Fig. 6a) in color with an odor which turned into green color and odorless at the end of treatment (Fig. 6b). 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. (2018) 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 (2008) 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 (Rahman and Hasegawa 2011). Similarly, Gonçalves et al. (2017) reported that the *Dundella* 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 (Fig. 4a, 5a, and 7). The present results were agreed with Liu et al. (2019). 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. (2016) found that ammonia may be exposed off into the air and resulted in an increased level of pH in algal cultures. The total dissolved solids (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 (Posadas et al. 2015). Glibert et al. (2017) 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. (2016), 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. (2015), 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. (2012). Mencia et al. (2016) 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 (Fig. 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.

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 (Cheah et al. 2018). After treatment, 100% nitrate (NO₃) removal was obtained in both *ex-situ* and *in-situ* trials. Ammonia (NH₃) 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. (2018) 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 (2020) 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. (2013) reported the reduction of Sulfate in the sewage effluent by microalga *Chlorella* sp. Lee et al. (2013), 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. (2016) 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 (Zhao et al. 2015). The cell wall of microalgae consists of several functional groups such as carboxyl, hydroxyl, phosphate, amino, and sulphhydryl 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 (Gupta and Rastogi, 2008).

3.4. 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 (Xiong et al. 2016). 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, b, & 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 (CH₂) 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.

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. (2019) 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 (Xiong et al. 2016), 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. (2013) 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.

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 (Fig. 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 (Fig. 9b and 9c). Chiu et al. (2015) 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. (2016) 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

4. Conclusion

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 dual 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.

Declarations

The authors declare the following consent

Funding

Not applicable

Competing interests

The authors declare that they have no competing interests.

Ethics approval

Not applicable

Availability of data and materials

The detailed methodology and analytical data of the present findings are available from the corresponding author on reasonable request.

Code availability

Not applicable

Authors' contributions

S.V., M.P., & P.A. planned the outline of the research work, carried out the research. M.N. prepared the manuscript draft, R.S., S.K., and B.P. support the manuscript writing and editing. D.N., project supervisor and administrator. The authors have read and approved the final manuscript.

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Tables

Table 1: Physicochemical properties of *ex-situ* and *in-situ* treated polluted water samples

Physicochemical parameters (mg L ⁻¹)	Indian standards permissible limits (mg L ⁻¹)			Pre-Treated	Post-Treated water					
					<i>In-vitro</i>			<i>In-situ</i>		
	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±0.3	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.

Table 2: FT-IR analysis of *Scenedesmus* sp. biomass

S. No.	Control			<i>Ex-situ</i> study			<i>In-situ</i> 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

Figures

Figure 1

Study area for sample collection (encircled in red color)

Figure 2

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

Figure 4

The mentioned values are mean and standard error ($\pm$) 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 ($\pm$) 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

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 ($\pm$) of triplicates. **: Statistically significant at $P < 0.005$

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

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