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# Naturally floating microalgal mat for in situ bioremediation and potential for biofuel production

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ABSTRACT

The objective of this study is to understand the fatty acid composition of a naturally floating microalgal mat with respect to seasonal variations in the water of a eutrophic lagoon on the west coast of India. The floating microalgal mat was explored for its bioaccumulation of metals and lipids for sustainable utilization in biofuel production. The effects of the nutrient profile and heavy metals in the water on the community composition of microalgal mat in different seasons were also studied and a principal component analysis was performed. It was observed that the unsaturated fatty acids such as C18:1*n*9c and C18:2*n*6c increased in low temperatures. The reverse effect was observed during the pre-monsoon season with augmented C16:0 fatty acid and an overall increment in saturated fatty acids. The Cyanophyceae and diatom community nurtured the growth during the pre-monsoon season by acquiring a higher bioaccumulation concentration factor (BCF) for Mn (3812.6 ± 680), As (90.47 ± 15.38), Ni (1620 ± 163.64), Cr (73.27 ± 21.23), and Cu (46.40 ± 11.44). The higher abundance of Cyanophyceae in the microalgal mat exhibited better metal endurance during the pre-monsoon season, which could be obviously tilized for the bioremediation of heavy metals. The biodiesel prepared from the microalgal mat met European biodiesel standards.



## 1. Introduction

Microalgae are primarily photoautotrophic unicellular or multicellular life forms which are abundant in both fresh water and marine environments. They exist individually, in chains, or in groups, and to grow they need light, $CO_2$, N, P, and K to produce carbohydrates, proteins and lipids in sufficient quantity over a short period of time [1]. It is estimated that about 40% of photosynthesis on earth is achieved by microalgae [2]. It has been reported that the increased microalgal growth is evident due to a concurrent enrichment in the nutrient load [3–5]. A high nutrient load in water bodies causes eutrophication, which leads to the formation of algal mats and blooms. Coastal marine ecosystems have been extensively affected by eutrophication [3,6]. Among the nutrients from anthropogenic sources, phosphorus (P) and nitrogen (N) are of major concern in eutrophication [7–10]. The environmental parameters of water and biomass productivity are correlated and have a regular seasonal pattern along coastal bays [11]. Some metals like Cu, Ni, Bo, Zn, Mg, Mn, Mo, Co, Fe, and Ca are important, which trigger the growth of microalgae up to a certain limit. Algae adopt different strategies for survival and growth when exposed to higher metal concentrations; a few of them are also heavy metal accumulators [12] and they play a key role in bioremediation in their native habitats [13]. Microalgae have molecular mechanisms for discrimination among non-essential and essential heavy metals for their growth [14]. The effects of heavy metal toxicity depend on the algal strains with respect to their characteristics, metal concentration, and environmental conditions [15,13]. Massive algal mats cause imbalances in ecosystem functions as they reduce the macro-faunal community (%), de-nitrification processes, the oxygen level in the bottom water and sediment, along with the mechanical prevention of larval immigration [16]. Microalgae are also a potential source of the lipids and fatty acids required for the food web and fuel [17]. The microalgal fatty acid composition changes with respect to environmental variables like temperature, light, heavy metals, and nitrogen and phosphorus concentrations [18,19]. During its growth, a microalgal mat remediates nutrients and pollutants from the aquatic body. The regeneration of a naturally occurring floating microalgal mat observed after two months of each

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harvesting provides an efficient vehicle for biomass generation for biofuel [20,21]. Furthermore, the bioremediation of the heavy metals in such aquatic systems can be well managed. The present study endeavors to study the effects of environmental variables on the fatty acid composition of a microalgal mat during different seasons and the microalgal mat's potential for the bioaccumulation of metals and lipid production. In previous research work, PCA (principal component analysis) was used to study microalgae such as diatoms in relation to abiotic stress with varying water quality [22] and a variation in intact polar lipids with respect to environmental variables [23]. Hence, PCA is used to understand complex relationships among environmental variables and the fatty acid composition of a floating microalgal mat.

## 2. Materials and methods

### 2.1. Study site and collection campaign

The study site (Supplementary Fig. A.1) was located on the west coast of India (N 20°42.87, E 70° 57.88), where the high tide water occasionally reaches and the lagoon is deposited with effluent from nearby plastic industries and ice factories. During the monsoon season, the site also receives urban sewage and agricultural runoff. This site is a marshy lagoon, dominated by a microalgal mat of *Microspora* sp. together with other strains of Cyanophyceae, Chlorophyceae, and Bacillariophyceae (Supplementary Figs. A.2; A.3). The samples were collected from three different transects depending on the abundance of the microalgal mat during the pre-monsoon (representing summer), monsoon (rainy), and post-monsoon (winter) seasons (June, August, and November 2011), respectively. These three environmental conditions are characteristically different due to variations in temperature, cloud formation, precipitation, light inclination, light intensity, light hrs, and air humidity.

### 2.2. Water and biomass analysis

The physicochemical parameters of water such as pH, temperature, salinity, total dissolved solids (TDS), dissolved oxygen (DO), and ammonia were analyzed using a pre-calibrated multi-parameter probe (ThermoFischer, USA) at the site. Water samples for dissolved inorganic phosphates and nitrates were collected in airtight, sterile, dried, and clean polypropylene bottles and preserved at 4 °C. For B.O.D., samples were collected in D.O. bottles and incubated at 20 °C in a B.O.D. incubator for five days. The biomass samples were collected in sterile vials and stored at 4 °C, and sample digestion was carried out within 48 h after collection. The water samples of dissolved nitrates, dissolved total inorganic phosphates, total organic carbon, total inorganic carbon, and heavy metals were analyzed after filtration through 0.45 μM pore size Whatman filter paper. The nitrates ($NO^{-3}$) and the dissolved inorganic phosphates ($PO_4^{-3}$) were determined spectrophotometrically and the biochemical oxygen demand (B.O.D.) was determined by iodometric titration using Winkler's method [24]. The dry biomass was weighed and kept for dry-ashing at 450 °C for 5 h and then digested with ultrapure nitric acid (Merck, Germany), followed by dilution with deionized Milli-Q water (Millipore, USA) for all the metals except for Hg, where dry ashing was not done, as described elsewhere [25]. The heavy metal analysis of the water samples was done using 5% ultrapure $HNO_3$ according to the methods described elsewhere [26]. The metals such as magnesium (Mg), iron (Fe), calcium (Ca), potassium (K), and heavy metals such as mercury (Hg), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), manganese (Mn), zinc (Zn), lead (Pb) and metalloid arsenic (As) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 2000 DU-Perkin Elmer) and multi-elemental standards used for the calibration and revalidation of the results. The total organic carbon and total inorganic carbon were analyzed using a TOC analyzer (elementar Liqui TOC/TN) after the filtration of the water with 0.45 μM Whatman filter paper. The %C, %H, and %N composition of the grounded dried biomass (105 °C for 24 h in an oven) was analyzed using a CHNS analyzer (elementar vario Micro). Approximately 2 mg of the sample was taken in a tin boat and folded. During analysis CHNS mode was chosen for the analysis and 1150 °C opted for the combustion. The method and operation of the instrument were followed as it is mentioned in Elementar condensed manual (vario Microcube). Sulphanilamide was used as a reference standard, which had a <0.02% variation as compared to the theoretical value of its C, H, N and S composition. The oxygen was calculated by deducting the sum of %CHNS from 100. The aerial biomass productivity was measured by dry weight estimated from the harvested biomass of the microalgal mat from transects (1 $m^2$) during all three seasons. The biomass productivity was calculated by the following formula:

$$\text{Average Aerial Biomass Productivity}\left(\text{g/m}^2\text{/day}\right) = \text{Weight(g) of dry biomass (average of three transects)} / \text{Area of transect}\left(\text{m}^2\right) / \text{Growth interval (days)}.$$

The bioaccumulation concentration factor (BCF) was calculated as a ratio of the concentration of heavy metals/metalloid in the biomass to the concentration of metals in the water [27]. The BCF was not calculated for the samples (water or biomass) where the concentration was not detected by ICP-OES analysis.

### 2.3. Scanning electron microscopy (SEM)

Scanning electron microscopy of the biomass was carried out (Supplementary Fig. A.2). The samples were fixed overnight in 2% (v/v) glutaraldehyde in a 0.1 M phosphate buffer, pH 7.5 at room temperature. The samples were subsequently washed with a 0.1 M phosphate buffer (pH 7.5) at room temperature for 1 h. Thereafter, the water was removed by a graded water–ethanol series: 25% ethanol, 15 min; 50% ethanol, 15 min; 75% ethanol, 30 min; 90% ethanol, 60 min; and absolute alcohol, 30 min. The specimens were coated with gold in a sputter coater (Polaron, SC7620) prior to microscopy. The material was examined in a Scanning Electron Microscope (LEO 1430 VP) at an accelerating voltage of 15 kV.

### 2.4. Lipid extraction and fatty acid profiling

The collected microalgal mat was thoroughly cleaned with water for the removal of sand and other particulate materials in it. The clean microalgal mat was frozen in liquid nitrogen and was lyophilized in a vacuum freeze dryer (Virtisbenchtop-K) at −86 °C until it reached a constant weight to confirm the complete water removal from the sample. The total lipid was extracted from the lyophilized samples by using chloroform:methanol (2:1) [28]. The total lipid content was calculated gravimetrically. Further, the extracted lipid samples were transesterified using methanolic KOH to their respective fatty acid-methyl esters (FAMEs) [17]. Subsequently, the eluted FAME samples were analyzed using a GC-2010 twinned with a GC–MS QP-2010 (Shimadzu, Japan) employed with an RTX-5 fused silica column. The pre-column pressure was 53.6 kPa with a linear velocity (36.3 cm/s) flow control mode. Helium gas (99.9% pure) was used as an inert carrier gas with a column flow rate of 1 ml/min. The initial column oven temperature was 50 °C with a holding time of 1 min, which was extended successively up to 200 °C with a rate of 25 °C/min, followed by 3 °C/min to reach a final temperature of up to 230 °C with a holding time of 18 min. The split ratio was 50. The starting and ending m/z of the mass spectrometer were 40 and 400, respectively. The ion source temperature and interface temperature were 200 and 240 °C, respectively. FAME peaks were recognized and assessed by comparing the respective retention time with their standard mixture (Sigma-Aldrich Supelco 37 component FAME mix) by GC–MS postrun analysis. Area normalization techniques were used for the