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Phycoremediation of hydrocarbon using two marine seaweeds from the Bay of Bengal coast of India

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ABSTRACT

Microalgae and seaweeds could accumulate pollutants in their tissues when grown in polluted water. Using algae as raw material, toxic pollutants can be removed from contaminated water which could be advantageous, as they are ubiquitous and have colonized almost all parts of the world. They can be grown easily with little and very simple growth requirements. The present study is aimed at revealing the phycoremediation potentials of two locally available seaweeds *Sargassum wightii* (Greville) and *Gracilara corticata* (J. Agardh) against boat engine oil (Servo Boat Engine Oil 20W- 40). Lipid accumulation in the thallus of seaweeds was observed both in the culture amended seaweeds.

Keywords: Phycoremediation; Physico-chemical analysis; Biochemical analysis; *Sargassum wightii; Gracilara corticata*

1. Introduction

Marine pollution constitutes a wide series of threats such as oil spills, dumping of land-based marine litters like heavy metals from mine tailings, acidification, radioactive substances, discharge of untreated sewage and persistent organic pollutants (POPs), heavy siltation, eutrophication, persistent growth of invasive and harmful species, overfishing and other destructive modes of coastal and marine habitats [1–3]. Discharge of cargo residues from bulk carriers can pollute Port waterways and oceans. In many instances vessels intentionally discharge illegal wastes, despite foreign and domestic regulations, thus prohibiting such actions. It has been estimated that container ships lose over 10,000 containers of oil into the sea usually during the storm [4]. Oil spills in aquatic environments originate due to biogenic, natural geologic and anthropogenic activities [5–9].

Ships can pollute waterways and oceans in many ways. Oil spills are one among them. They are toxic to marine life as they are polycyclic aromatic hydrocarbons (PAHs) which are the components in crude oil. They are found to be difficult to clean up and predominant constituents present in crude oil sediment and marine environment for a prolonged period [10]. Ecological effects of accidental oil spills is a challenging subject of both laboratory and field research [5].

Several study reports say that penetration of oil into the fur of mammals and plumage of birds results in the reduction of their insulating ability which leads them more vulnerable to temperature fluctuation, hypothermia and their buoyancy to water becomes diminished. Environmental effects of oil spills and its resulting toxicity in the sea have received the attention of public, student and

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scientific community [5]. Usually, animals find their offsprings babies using their body odor, which vanishes of, due to the strong aroma of the crude oil, in turn, becomes a root cause for the rejection of their offsprings leading to the condition of starving and die. Similarly, densely furred marine mammals and birds are affected due to oil coats. These living organisms which get poisoned by the oil undergoes, blindness, dehydration, changes in hormonal balance and luteinizing protein, irritation of digestive tract, change in liver function, kidney damage and may die. It is really a heartbreaking threat that the oil-soaked birds even after cleaning less than one percent of the birds can only survive. Crude oil is highly toxic which comprises of 10,000 different types of both aliphatic and aromatic hydrocarbons, which causes severe threats to the marine ecosystem whenever oil spills occur in the marine environment [5].

The process of recovery of oil depends on the location, the volume of the spill, the weather condition and the nature of the oil. If the oil reaches the shore, mechanical removal is possible on sandy areas but on rocky shores washing the oil back into the sea was attempted. Chemical adsorbents used on both floating oil and oil on rocky shore were found to be harmful to the environment. Physical and chemical methods are efficient but difficult to remove high molecular weight oil that may need a new strategy of phycoremediation. Algae have been reported to utilize various contaminants such as pesticides, cyanides, hydrocarbons and heavy metals as carbon and nitrogen sources [10].

India has a rich source of micro and macroalgae. Using algae as raw material, toxic pollutants can be removed from contaminated water which could be advantageous, since they are ubiquitous and have colonized almost all parts of the world. They can be grown easily and have very simple growth requirements. An advantage of using living organisms over dead biomass is that they have a fast growth rate and hence produce a regenerating supply of effluent – removal material. There is much evidence that algae could accumulate pollutants in their tissues when grown in polluted waters which includes macroalgae such as *Ulva rigida* [11], *Padina gymnospora* [12], *Gracilaria tenuistipitata* [13], *Undaria pinnatifida* [14], *Cladophora* sp. [15], and *Cladophora glomerata* [16]. Warshawsky et al. [17]; Kirso and Irha, [18]; Lei et al. [19] reported that some green algae and diatoms are capable of metabolising two and three-ring PAHs. The cell wall of the seaweeds contains some functional groups such as sulphate, amino, hydroxyl and caboxyl due to the presence of polysaccharides, proteins or lipid which functions as a binding site for the pollutants [20]. Seaweeds are unique because they have the potentiality of autotrophic photosynthesis by which it utilizes both organic and inorganic carbon substrate from polluted water and phycoremediate. Seaweeds are capable of bio-absorption of pollutants in wastewater because their cell wall is made up of carbohydrate structure [10].

Hence, the present study is aimed to evaluate the phycoremediation potentials of two locally available seaweeds *Sargassum wightii* (Greville) and *Gracilara corticata* (J. Agardh) procured from the Bay of Bengal coast of India against Boat Engine Oil (Servo Boat Engine Oil 20W-40).

2. Materials and methods

2.1. Collection of seaweeds and seawater

Chlorophycean seaweeds such as *Ulva fasciata* Delile [65], *Chaetomorpha antennina* (Bory de Saint-Vincent) Kützing [66], *Enteromorpha flexuosa* (Wulfen) J. Agardh [67] and rhodophycean species such as *Gracilaria corticata* (J. Agardh) J. Agardh [68] and *Grateloupia lithophila* Boergesen [69] were collected from Kovalam near Chennai. A phaeophycean seaweed *Sargassum wightii* Greville [70] used in the present study was procured from Krusadai Island, Gulf of Mannar, Tamilnadu, India (Figs. 1a–f).

Seawater from the Bharathi Dock – 1 (oil dock area, Chennai Harbour) was collected at a depth of 50–100 cm below the surface and transported to the laboratory in black polythene containers for physico-chemical analysis.

2.3. Physico-chemical analysis of seawater

The collected seawater sample was placed in an icebox and transported to the laboratory for immediate analysis of physico-chemical parameters [21] which includes temperature, pH, light intensity, humidity, salinity, turbidity, DO, NO_2, NO_3, NH_3, NH_4, PO_4, Ca, SiO_2 and Mg.

2.4. Cleaning and transportation of seaweeds

The macroscopic and other contaminants present in the collected seaweeds were carefully removed and washed in filtered seawater. The thallus of the seaweeds was wrapped in absorbent cotton moistened with seawater, placed in an ice box and transported to the laboratory within an hour. Further bioassays were carried out using this cleaned seaweeds and filtered seawater.

Healthy thallus was chosen selected and washed with filtered seawater under laminar air flow chamber and then treated with few drops of GeO_2 and maintained in stock culture for subsequent culture studies.

2.5. Identification of seaweeds

The algae were identified using "Key to the Taxonomic Identification of Green and Brown Marine Algae of India" [22] and "Key to the Identification of Indian Red Seaweeds" [23].

2.6. Maintenance of stock culture [24]

Composition of germanium dioxide and antibiotic mixture is as follows

Component		Quantity
Streptomycin sulphate	–	1 g
Penicillin-G	–	1 g
NaOH	–	4 g
GeO_2	–	250 mg

1 g streptomycin sulphate and Penicillin-G was mixed in 100 mL of distilled water is added to make the 1 mL antibiotic mixture. To prepare 1 mL GeO_2, 4 gm of NaOH and

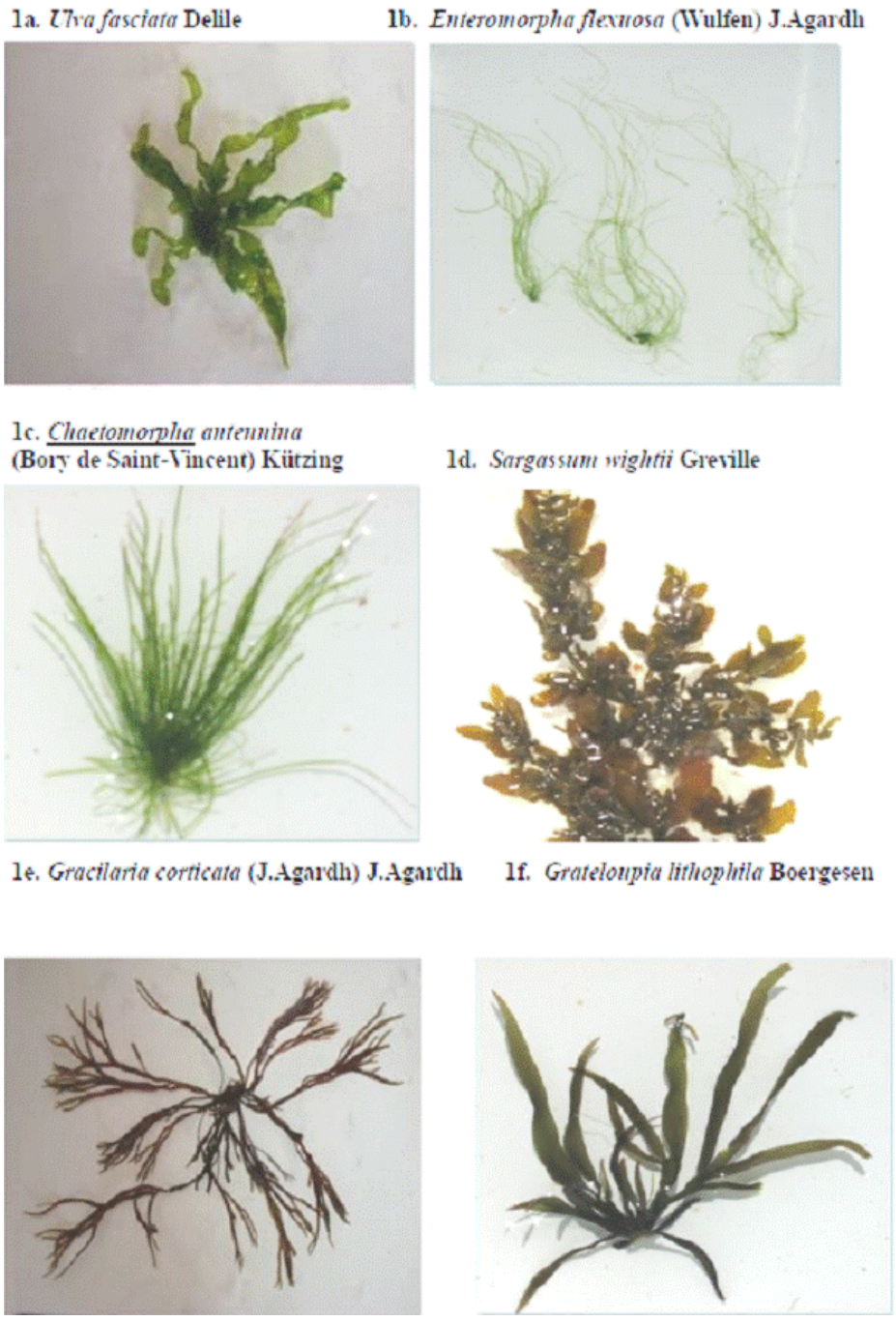


Fig. 1. Seaweeds of the present study.

250 mg of GeO_2 dissolved in 100 mL of distilled water later boiled for a few minutes.

2.7. Laboratory culture of seaweeds

Seaweeds used in the present study were cultured and maintained in double filtered autoclaved natural seawater containing CHU13 medium [25]. 5 g of fresh weight (FW) of biomass was suspended in 600 mL of medium in a sterile 1 L flask. Flasks were capped with foam stoppers to maintain sterility while allowing free exchange of carbon dioxide and oxygen. The cultures were kept at a light intensity of approximately 50 μE/m²/s on a 12 h light/12 h dark photo period. To promote gas transfer and to provide them with natural seashore wavy condition, the flasks were manually swirled for approximately 5 s each day and slightly in a shaker at a minimum rpm at every 5–10 min. To ensure sufficient nutrient availability, the medium was completely replaced and subcultured after 17 d.

After 30 d, *S. wightii* and *G. corticata* were survived in modified CHU13 medium while the other seaweeds got disintegrated. Hence, 5 g biomass of *S. wightii* and *G. corticata* were suspended in approximately 150 mL of fresh medium in sterile 250 mL flasks for further experimental studies.

2.8. The growth of algae in CHU13 medium amended with different concentrations of oil

5 g of biomass were inoculated in five different 1,000 mL conical flasks containing CHU13 medium with fine different concentrations (0, 25, 50, 75 and 100 ppm) of oil system Servo 20w-40 and the cultures were maintained at a light intensity of approximately 50 μE/m^2/s on a 12 h light/12 h dark photo period at a temperature of 24±1°C.

1 g of *G. corticata* were subcultured in six different Erlenmeyer flasks containing CHU13 medium. Crude oil system Servo 20W-40 (boat oil) was used. Different concentrations of oil such as 100, 200, 300, 400 and 500 ppm was added to the 5 respective flasks and the remaining one flask was kept without oil as a control. Similar kind of setup was made with *S. wightii*. After 3–4 d, it was observed that the of *S. wightii* was disintegrated at 100 ppm. Therefore, *S. wightii* was subjected to lower concentration such as 25, 50, 75 and 100 ppm and control was maintained without oil.

2.9. Solvent extractable oil from the medium

The solvent extractable amount of oil in the medium was analyzed at the end of 20, 40, 60 and 80 d by using [26].

2.10. Growth estimation

The study was conducted for a period of 80 d. The daily growth rate was calculated every 20 d interval. The daily growth rate (DGR) or specific growth rate (SGR) % was calculated using the following formula [27].

$$DGR\ \% = \frac{(W_f / W_o)}{t} \times 100$$

where W_f is the final fresh weight (g) at t day, W_o is the initial fresh weight (g), t is the number of culture days.

2.11. Bio-chemical studies

Bio-chemical parameters were observed on every 20th d i.e. on the 20th, 40th, 60th and 80th d of the experiment. Algal biomass was obtained and ground in mortar and pestle with distilled water and made up to 5 mL and centrifuged the supernatant was taken for the estimation of chlorophyll pigments, carbohydrates, amino acids and lipids.

Estimation of chlorophyll pigments [28]
Estimation of carbohydrates [29]
Estimation of protein [30]
Estimation of lipids [31]

3. Results and discussion

3.1. Physico-chemical properties of seawater

The surface water temperature was observed to be 29°C at the study site while collecting the water. The results in Table 1 show the physico-chemical properties of the seawater collected from Bharathi Dock-1 (oil dock area), Chennai Harbour. The silicate content was higher than that of the other nutrients (NO_3, NO_2 and PO_4) and the solvent extractable oil content was found to be 13 ppm which was assessed below the level of emission limit which was 15 ppm.

Table 1
Physico-chemical characteristics of Bharathi Dock-1 (oil dock area), Chennai Harbour

S.No.	Physical characteristics	Bharathi Dock-1 (oil dock area), Chennai Harbour
1	Temperature	29°C
2	Odour	None
3	Hydrogen-ion concentration (pH)	8.1
4	Turbidity NTU	5.1
5	Light intensity in lux	7600
6	Humidity in %	64
7	Salinity %	34.9
8	DO (mg/L)	5.84
9	Total hardness (as $CaCo_3$) mg/L	6500
10	Calcium (as Ca) mg/L	1560
11	Magnesium (as Mg) mg/L	624
12	Free Ammonia (as NH_3) mg/L	0.07
13	Nitrite (as NO_2) mg/L	4
14	Nitrate (as NO_3) mg/L	11.45
14	Phosphate (as PO_4) mg/L	8.01
15	Silica (as SiO_2)	18.64
16	Solvent extractable oil (ppm)	13

Physico-chemical properties of the ambient marine environment play a pivotal role in determining the parameters like the type of ecosystem, geomorphology, tidal amplitude and ecosystems promote a plethora of floral and faunal species with higher biological productivity [32,33]. The observed high value of temperature in the present study may be due to the intensity of solar radiation and evaporation freshwater influx and cooling and mix up with the ebb and flow from adjoining neritic water. The observed low value was due to strong land-sea breeze and precipitation [34,35]. The intensity of solar radiation, evaporation, freshwater influx and cooling influences the surface water temperature and mix up with the ebb and flow from adjoining neritic waters [36].

The silicate content was higher than that of the other nutrients (NO_3, NO_2 and PO_4) and this might be due to the fact that the low crude oil concentration stimulates the growth of oil-degrading diatoms [37,38], adsorption and co-precipitation of soluble silicate silicon with humic compounds and iron [39].

3.2. Studies on the growth of S. wightii and G. corticata in modified CHU13 medium

In the present study, exponential growth of *S. wightii* was observed as 3.78 mg/g in both 75 and 100 ppm on the 60th d and there after lag phase was noted as 3.64 mg/g and

3.67 mg/g at 75 and 100 ppm respectively on the 80th d. *G. corticata* has obtained a maximum growth of 5.83 mg/g at 400 ppm on 80th and in 500 ppm on 60th days. The lag phase was observed on the 80th d as 5.37 mg/g and 5.02 mg/g in 400 and 500 ppm respectively (Figs. 2a and 2b).

The algae, *S. obliquus* exhibited higher phycoremediation efficiency to aliphatic and aromatic hydrocarbons of crude oil [40]. The photosynthetic potentiality in turn triggers the growth of seaweeds. It is an interesting fact, that both the seaweeds *S. wightii* and *G. corticata* tested in this experiment belong to the phaeophycean and rhodophycean members, which grows sub-tidally and they are attached to coral, rocks or shells and moderately exposed to sheltered rocky or pebble areas. So, they can grow in diffused light, even though, the oily surface reduces the amount of light penetration. However, seaweed can efficiently phycorrmediate wastewater without the need of external sources such as light and carbon because they can sequester CO_2 environment through autographical growth [10].

3.3. The concentration of oil in the medium before and after phycoremediation using S. wightii and G. corticata

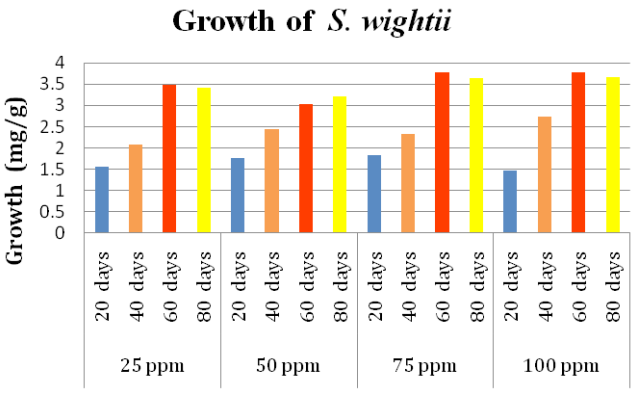
It was observed that *G. corticata* has the highest capability to phycoremediator up to 461 and 470 ppm on 60th and 80th d at 500 ppm concentration respectively. The study was analysed at the end of 20, 40, 60 and 80 d. Whereas, the maximum oil uptake capacity of *S. wightii* was recorded as 71.5 ppm of oil at 100 ppm concentration on the 80th d (Figs. 3a and b).

This shows that the seaweed could bioaccumulate oil pollutant, metabolize in their tissues as useful assimilates thus detoxifying it and further degrading it to smaller compounds, this could be easily activated by other microbes.

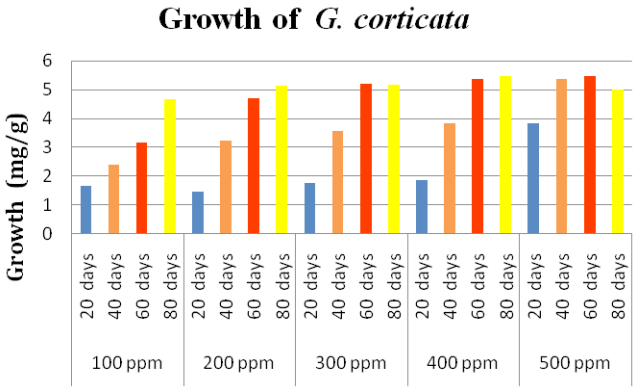
Several studies revealed that Crude oil constitute 4–26 number of atoms and it was a complex mixture of hydrocarbons. It encircles aromatic compounds and its constituents may be in straight branched or cyclic in their property. Crude oil is toxic to all marine organisms and their habitat. Crude oil contains aromatic and aliphatic compounds with varied molecular weight. It is stated that aromatic compounds are more poisonous than aliphatic compounds and middle molecular weight compounds is heavy toxic than high molecular weight compound. Low molecular weight compound are insignificant as they are volatile [41]. Both physical and chemical methods are efficient to mitigate and phycoremediate oil spills which are harmful to the environment. Even though high molecular weight oils will remain and need to be removed using some other method of biosorption.

Phycoremediation is the success scope to mitigate the effects of oil spills [42–46]. Limited studies are available on bioremediation which employs microbes to degrade oil in marine water in [47]. The oil contains carbon complexes with heavy metals such as lead etc.

Cheney et al. [48] has studied on green seaweed - *Acrosiphonia coalita* and a red seaweed - *Portieria hornemannii* and concluded the large capacity to uptake and metabolism organic pollutants 2,4,6-trinitrotoluene (TNT) and phenanthrene, and showed great efficiency as a phycoremediator in the biological system. Our results were also correlated



(a)



(b)

Fig. 2. Laboratory studies on the growth of *S. wightii* and *G. corticata* in CHU 13 medium at 24±1°C and 50 lux light intensity.

with the above studies and selected seaweeds showed great efficiency in phycoremediation oil spills and can be used to degrade oil pollutant.

Tang et al. [40] and Hong et al. [49] reported that *Skeletonema costatum* and *Nitzschia* species can phycoremediator and accumulate fluoranthene; Jacques and McMartin [50] has revealed that microalgae can phycoremediate light extractable petroleum hydrocarbons in petroleum-contaminated water. Subsequent studies inferred that *Laminaria japonica* can play an important role in removing polycyclic aromatic hydrocarbons (PAHs), it also phytoaccumulation 70–90% of phenanthrene and 65–90% of pyrene in their tissues Wang and Zhao [51].

Kathi and Khan [52] suggested that seaweeds can degrade PAHs and other organic pollutants from seawater, thus serving as an environmentally-friendly' phycoremediation system that protects ecological health and marine life.

3.4. Estimation of photosynthetic pigments

S. wightii showed a maximum amount of chlorophylls content as 3.136 µg at 75 ppm on 80th d whereas a sharp decline up to 0.254 µg was observed at 100 ppm on 80 d, thus 75 ppm was found to be optimum. Carotenoid content

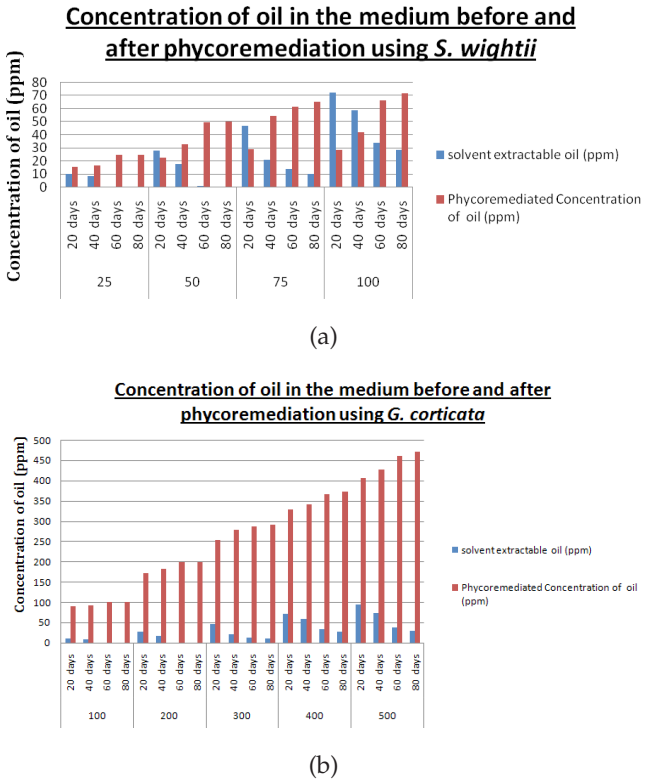


Fig. 3. Concentration of oil in the medium before and after phycoremediation using *S. wightii* and *G. corticata*.

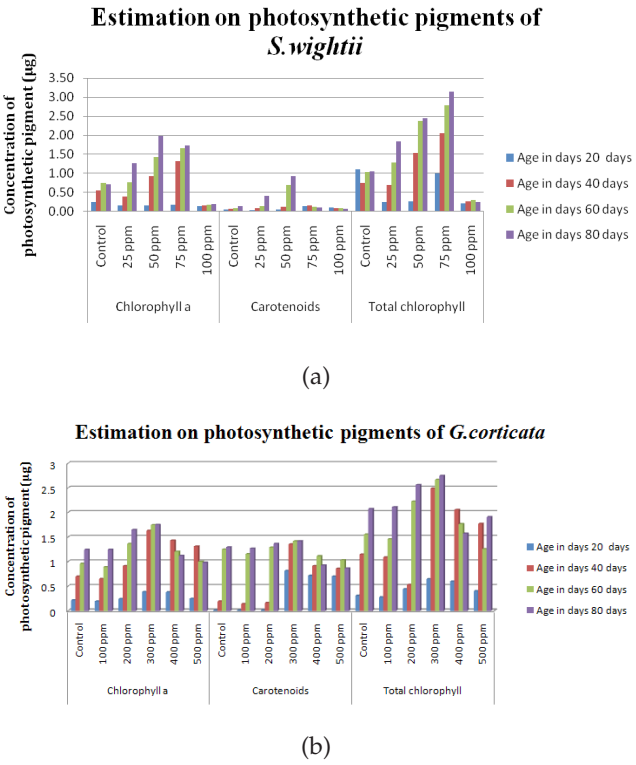


Fig. 4. Effect of various concentrations of oil on the photosynthetic pigment of *S. wightii* and G. corticata.

was also found to be high on the 80th d as 0.114 µg on the same day observation at 75 ppm and it was comparable to that of control as 0.119 µg. In *G. corticata* maximum amount of chlorophyll content was observed as 2.747 µg at 300 ppm on the 80th d and it is nearer to control as 2.564, meanwhile a sharp decline of 1.575 µg was observed in 400 ppm on the same day. Carotenoids were also found to be high as 1.419 µg on 80th d at 300 ppm which is similar to control as 1.365 µg with a decline in 400 ppm as 0.925 (Figs. 4a and b).

At the end on phycoremediation on the 80th d, the thallus exposed to oil showed bleaching of the apical cells which led to weight loss, significantly affected growth rate which in turn affects the chlorophyll content and accessory pigment. The presence of oil in the medium resulted in the increase of growth, chlorophyll and carotenoid content of *G. corticata* at 300 ppm and of *S. wighii* in 75 ppm. Further, observation showed a decreasing trend of both the photosynthetic pigments chlorophyll and carotenoids on the 80th d. In this study, higher concentration resulted in an inhibitory effect on growth and suppressed the photosynthetic pigment content. Our findings are similar to the reports of [53–55], dominant inhibition effect was observed on photosynthetic activity after exposure to petroleum hydrocarbons, due to lack of nitrogen content in the medium. On the other hand, stimulation effects were also observed on exposure to a lower concentration of oil pollutants. Likewise [56], stated that carotenoids have increased as a result of unfavourable condition.

Crude oil in the medium stimulated growth at all concentration in both the seaweeds. However, levels of photosynthetic pigments were affected at higher concentration.

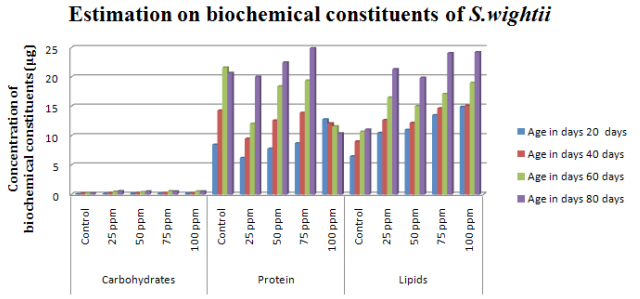
Atlas et al. [57] and Parsons and Waters [38] studied the stimulation of growth and photosynthetic pigments by microalgae exposed to lower concentrations of hydrocarbons. Wang and Zhao [51] reported that at high concentrations, the toxic nature of phenanthrene and pyrene on the growth of seaweed *Laminaria japonica* was significant. When an oil spill occurs in the sea, there are losses by evaporation and by dissolution, so that the toxic effect of the volatile constituents of crude oil decreases with respect to laboratory assays.

Sornalakshmi and Venkataraman Kumar [58] stated that seaweeds *Ulva lactuca, Caulerpa scalpelliformis, Padina tetrastromatica* and *Gracilaria corticata* at various concentrations of fly ash, the amount of chlorophyll a, chlorophyll c, carotenoids and protein similar to control.

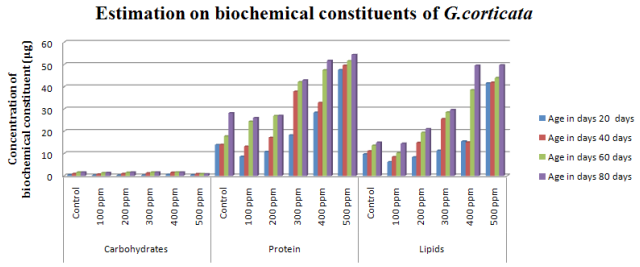
3.5. Estimation of biochemical constituents

In the present study carbohydrate content of *G. corticata* was found to be maximum as 1.610 µg in 400 ppm on 60 d but in *S. wightii* maximum content of 0.548 µg, 0.532 µg and 0.528 µg were recorded in 25, 50 and 75 ppm on 80 d (Figs. 5a and b). This might be probably due to a higher amount of nutrients and the increased light penetration enhanced the carbohydrate content leads to the growth and development of seaweed [71].

The present study exhibited high protein content of *S. wightii* was recorded as 22.50 µg at 50 ppm in 80th day which is comparably higher than control as 20.7 µg and in *G. corticata* as 42.98 µg, 51.76 µg and 54.40 µg on 300, 400 and 500 ppm respectively on 80th d (Figs. 5a and b) is proportional



(a)



(b)

Fig. 5. Effect of various concentrations of oil on the biochemical constituents of *S. wightii* and *G. corticata*.

to the concentration of oil in Bharathi Dock-1. Increase in concentration of pollutant in turn lead to an increase in protein level.

Lipid content of the seaweed *S. wightii* varied from 6.49 in control at 20 d with a maximum of 24.06 and 24.23 at 75 and 100 ppm in 80 d and in *G. corticata* from 9.75 in control at 20 d and maximum of 49.55 and 49.75 in 400 and 500 ppm at the end of 80 d (Figs. 5a and b). This may be due to the long-time exposure of the seaweeds in oil and the significant amount of oil that penetrates inside the seaweed. The long term uptake of lipids as oil pollutant and percolation of the same into the biomass, further metabolism had led to lipid accumulation which has reflected in the estimation of the same.

In the present study, maximum protein content of brown *S. wightii* was observed as 24.9 at 75 ppm in 80 d which is comparably higher than control 20.7 and *G. corticata* 42.98, 51.76 and 54.40 on 300, 400 and 500 ppm respectively on the 80th d is proportional to the concentration of oil.

Increase in concentration of protein or lipid present on the surface of the cell walls of seaweeds containing some functional groups such as amino, hydroxyl, carboxyl and sulphate, may act as binding sites for the oil molecules [20]. The increase in carbohydrate, protein and lipid content of both the seaweeds *S. wightii* and *G. corticata* on 80th d of the experiment is due to the presence of lipid, carbohydrate and protein in their cell wall, which absorbs the oil pollutant in the medium. Of the many types of biosorbents recently investigated for their ability to sequester heavy metals, microalgal biomass proved to be highly effective as well as reliable and predictable in the removal of heavy metals from aqueous solutions [59,60]. Microalgae was found to degrade petroleum hydrocarbons found in crude and motor oils. Interestingly, in the crude oil, 38–60% of the saturated aliphatic hydrocarbons and 12–41% of the aromatic compounds were degraded, whereas the discharge in motor oil showed 10–23% of the saturated aliphatic hydrocarbons and 10–26% of the aromatic compounds which were degraded. This suggested that the microalga were capable of degrading different oils to varying levels along with other microorganisms.

Lipid content of the seaweed *S. wightii* varied from 6.49 to 11.06 in control at 20th d and 80th d of the study with a maximum of 24.06 and 24.23 at 75 and 100 ppm in 80 d and in *G. corticata* from 9.75 and up to 4.96 ppm in control at 20th d and 80th d 20 d and maximum of 49.55 and 49.75 in 400 and 500 ppm at the end of 80 d. This may be due to the long time exposure of the seaweeds in oil and the significant amount of oil that penetrates inside the seaweed. The long term uptake of lipids as oil pollutant and percolation of the same into the biomass, further metabolism had lead to lipid accumulation which has reflected in the estimation of the same.

The cell surface of algae is a mosaic of metal-ion-binding sites made up of lipid, carbohydrate and protein this creates affinity and absorb nutrients over their entire surface. This enhanced the growth of both the seaweeds *S. wightii* and *G. corticata* [5]. The contaminant enters the algal cells either by active transport or endocytosis using chelating proteins and alters the physiological and biochemical processes of the algae. Oil contaminant binding has been found to be dependent on pH, temperature and presence of competing ions which with increasing concentration of oil reduces growth. Similarly, the thallus of the seaweeds *S. wightii* and *G. corticata* got bleached and disintegrated at 100 and 500 ppm respectively on the 80th d.

The investigation made by Praepilas and Pakawadee [61] revealed the potentials of utilizing industrial wastewater as a cheap nutrient for the growth and oil accumulation of two microalgae such as *Scenedesmus quadricauda* and *Chlorella* sp. The highest amount of lipid was estimated at 18.58% and 42.86% in cases of *S. quadricauda* and *S. obliquus* in culture condition. The increase in photosynthetic pigment and higher accumulation of oil by *S. wightii* and *G. corticata* is, due to excess photosynthesis algae can accumulate lipids and in some species, algae can accumulate lipids under environmental stress like salt stress or deficiency of nutrition or heterotrophy [62].

Seaweeds absorb light energy in the form of photons, which converts the inorganic compounds of CO_2 and water into sugars and oxygen. Thus produced sugar is further transformed as, complex carbohydrates, starches, proteins and lipids within the algal cells [63,64], *S. wightii* and *G. corticata* absorb the contaminants and biotransforms, thus leading to the concomitant propagation of biomass. In the present study, lipid accumulation in the thallus of seaweeds was observed in the culture amended with different oil concentrations which can be proved through further microscopic sections.

4. Conclusion

Six seaweeds collected from the Bay of Bengal coast of Tamil Nadu were cultured in CHU13 for laboratory acclima-

tization, of which *S. wightii* and *G. corticata* were survived after 30 days while others were disintegrated. Therefore the phycoremediation potential of the two survived seaweeds was tested against the degradation of various concentrations of boat engine oil (Servo Boat Engine Oil 20W-40). The present study revealed that *S. wightii* can phycoremediate up to 100 ppm after that its thallus got disintegrated whereas *G. corticata* has degraded up to a maximum of 500 ppm.

The photosynthetic pigment chlorophyll was recorded maximum as 1.987 µg in *S. wightii* and 1.921 µg in *G. corticata,* higher amount of accessory pigment carotenoids was as 1.925 µg in *G. corticata* and 0.924 µg in *S. wightii,* total chlorophyll content was recorded high as 3.136 µg in *S. wightii* and 2.975 µg in *G. corticata* at 75 and 400 ppm respectively on the 80th d.

Biochemical constituent Carbohydrates was observed high as 1.590 µg at 300 and 400 ppm in *G. corticata* and as 0.548 µg at 25 ppm in *S. wightii,* protein content was recorded maximum as 54.40 µg in *G. corticata* at 500 ppm and 24.9 µg in *S. wightii* at 75 ppm, lipid was high as 49.75 µg in *G. corticata* at 500 ppm and 24.23 µg in *S. wightii* at 100 ppm on 80th day of the experiment.

Both the seaweeds has potential to uptake and degrade oil contaminants of the seawater (Bharathi Dock-1 (oil dock area), Chennai Harbour). Utilization of *Sargassum wightii* (Greville) and *Gracilara corticata* (J. Agardh) against degradation of boat engine oil (Servo Boat Engine Oil 20W-40). As both these seaweeds are naturally available in the coastal areas of Bay of Bengal, this can be practiced as a cost-effective method. Phycoremediation of oil contamination using live seaweeds rapid and also an 'environmentally-friendly' method for the co-biota that exist in the marine ecosystem.

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