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BIOREMEDIATION AND RESOURCE RECOVERY FROM DOMESTIC AND INDUSTRIAL WASTEWATER WITH NEW TECHNOLOGY

Phycoremediation of crude oil polluted water from selected water sources in Ogoniland, Rivers State, Nigeria

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Abstract

Crude oil exploitation in the Niger Delta, particularly in Ogoniland, brought environmental devastation occasioned by petroleum pollution, as farmlands and water sources were destroyed. This study was designed to remediate crude oil contaminated water obtained from water sources in Ogoniland using two green algal species. Thirty water samples were collected from eight different water sources. The samples were analysed for total petroleum hydrocarbon (TPH) using gas chromatography/flame ionization detector (GC/FID). Algal samples were collected from Ogba River and at wetland in Military Hospital Benin, Edo State, Nigeria. The algal samples were identified, screened, optimized and grown in Bold basal medium. Results obtained from the determination of TPH showed that the infiltrated pond (Exc) sample site had the highest concentration among all the sites sampled with 198.8329 µg/L, R_2 with 134.1296 µg/L, R_1 with 108.9394 µg/L, R_3 with 105.8011 µg/L, R_4 with 98.442 8 µg/L, the hand-dug wells (Wll) had 9.6586 µg/L while the borehole (Bhl) had the lowest with 1.8310 µg/L. It was deduced that pollution of water sources was principally because of pollutants washed from the soil environment into the open surface water sources via run-off rather than through the seepage from the underground aquifers, incriminating illegal oil mining and artisanal refining. Results obtained from the analysis of algal growth medium indicated that the two algal species were able to absorb the hydrocarbon contaminants, albeit at different rates, corresponding with the algal growth rate. Analysis of algal biomass after 4 weeks of remediation showed that from the initial 10.27 µg/20 mL added to the growth medium, the highest TPH mean value of 0.490 µg/20 mL was extracted from *Ulothrix zonata* (F.Weber & Mohr) Kützing biomass grown in Exc compared to 0.344 µg/20 mL of TPH extracted from *Chlorella sorokiniana* Shihira & R.W.Krauss grown in the same sample site. Also, *Ulothrix zonata* had higher TPH yield 0.023 µg/20 mL in Bhl compared to *Chlorella sorokiniana* 0.021 µg/20 mL of TPH from the same water source. This result indicated *Ulothrix zonata* had superior TPH phycoremediation ability to *Chlorella sorokiniana*. While the present study calls for deployment of the algal species for field trial, it is strongly recommended that crude oil pollution should be discouraged.

Keywords Ogoniland · Phycoremediation · Hydrocarbon · *Ulothrix zonata* · *Chlorella sorokiniana*

Introduction

There has been a serious increase in the global demand for crude oil. This exponential demand is not unconnected with the rapid increase in global population and the consequent demand for wealth and comfort (Kadafa 2012; Utazi et al. 2022). According to the recent release from the Nigerian Bureau of Statistics, crude oil accounts for over 6.33% of the gross domestic product (GDP) (Sasu 2023) and contributes more than 90% of her foreign exchange (Utazi et al. 2022). It is a major source and form of energy in both residential and industrial centres for heating and cooking, a source of fuel for nearly every form of machine

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both in developed and developing countries (Manfred et al. 2018). Provision of electrical light also depends heavily on this all-important commodity (Das and Chandran 2011; Antwi-Akomea et al. 2018). This makes crude oil one of the most dependable sources of wealth among other natural resources, the most utilized source of energy and fuel and of course the most hunted of all the natural resources (Antwi-Akomea et al. 2018). As a result of these, there is particularly an increased global demand for crude oil as the entire world looks up to it as the principal source of energy and wealth (Elum et al. 2016). This almost complete dependence on petroleum comes with a great prize. The environment is particularly put at great risk due to the consequent crude oil contamination and the absolute disregard for the effect of the contamination to the health of the environment and of the people living therein (Bayat et al. 2015; Wu et al. 2017; Oyewole et al. 2019).

Anthropogenic activities, particularly but not limited to oil exploration and exploitation activities, and the natural processes are responsible for oil pollution of both the terrestrial and the aquatic environments (Genovese et al. 2014; Environment 2017). Scientific researches, both in the developed and developing nations, have produced numerous ways and systems through which the environments can be remediated of petroleum and petroleum wastes (Sui et al. 2021). Unfortunately, the results from these works, especially in third world nations like Nigeria, have not done much to salvage the environment, particularly the water sources from crude oil pollution. So, surface water bodies and underground water (wells and bore holes) are still heavily contaminated (Environment 2017). The deployment of chemical agents in the affected areas (Das and Chandran 2011), the use terrestrial plants to clean up pollutants, bacterial-based remediation systems (Yakimov et al. 2007) and the mechanical excavation/syphoning/controlled burning (Zhang et al. 2010; Utazi et al. 2022) have all been employed and have been found to have certain deficiencies.

Mechanical technologies are very expensive, highly laborious and sometimes difficult to execute (Rosalie et al. 2020). Syphoning can only be used at a small non-flowing water body and not prescribed for a large body of water (Khan et al. 2004). Besides, the dumpsite usually constitutes a pollution source (Jain et al. 2005). Burning the contaminants may convert it into a new more poisonous compound; the oil may become gaseous, resulting in greenhouse gases and air pollution. In addition, the resultant heat from the burning endangers soil/aquatic microbes (Zabbey et al. 2017). Chemical dispersant is not cost effective; spraying chemical on the water re-pollutes the water and the chemical may endanger aquatic lives (Okafor 2011).

In this study area particularly, several of these remediation strategies have been tried (Utazi et al. 2022), for instance, solidification of excavated contaminated soil for construction purpose was tried in Ejama-Ebubu in Eleme LGA (Oluremi and Osuolale 2014; Mmom and Igbuku 2015; Ergozhin et al. 2020). This strategy was abandoned because excavated ground became dumpsite for the contaminants as run-off brought back crude oil that was previously taken away (Amnesty International 2019). Remediation by enhanced natural attenuation (RENA) was also tried in some parts of Ogoniland (Ebuehi et al. 2005; Wegwu et al. 2010). It involves enriching the soil with organic or inorganic manure, mixing the nutrients by tilling the soil and allowing the indigenous microbe to spontaneously degrade the pollutants (Chen et al. 2011; Orji et al. 2012).

The most important drawbacks of the RENA strategy are that it can only be applied on the soil and mixing of the soil does not go deeper than 15–20 cm (Utazi et al. 2022). The biocell and biopile technologies tried in parts of Gokana local government area (Utazi et al. 2022) were judged to have been badly constructed and lacked the required monitoring (Amnesty International 2020). Having considered these obvious inadequacies of the traditional remediating protocols and the fact that the situation of the environment aggravates on daily basis, with the record of new spills and contamination of formerly uncontaminated sites (Utazi et al. 2022), the need for a new approach becomes inevitable.

Algae have been known to possess the ability to accumulate, degrade, immobilize or transform organic or inorganic environmental pollutants including crude oil (Perelo 2010). Some species have also demonstrated high competence in the heavy metals and xenobiotics hyper-accumulation and degradation (Abioye et al. 2020). The use of algae in the environmental remediation has drawn immense attention, not just because of the accumulation and degradation of the pollutants (Touliabah et al. 2022) but also for carbon dioxide sequestration (Indhumathi et al. 2013) and their essential role in dissolved oxygen (DO) generation (Rao and Velusamy 2016) which is needed by other members of the microbial community taking part in the environmental cleaning and repairs (Zhang et al. 2010). These therefore justify the change in the remediation technique and the application of phycoremediation of crude oil polluted water from selected water sources in Ogoniland, Rivers State, Nigeria.

Materials and methods

The study area

Ogoniland is one of the oil-producing communities in the Niger Delta of Nigeria (Fig. 1). Ogoniland is located in Rivers State and covers about 1000 km^2 (Genovese et al. 2014) and situated at latitude 4° 40′ 5″ N and 4° 43′ 19.5″ N and longitude 7° 22′ 53.7″ E and 7° 27′ 9.8″ E, south of Rivers State, Nigeria (Nkpaa et al. 2016; Nkpaa et al. 2017). Ogoni

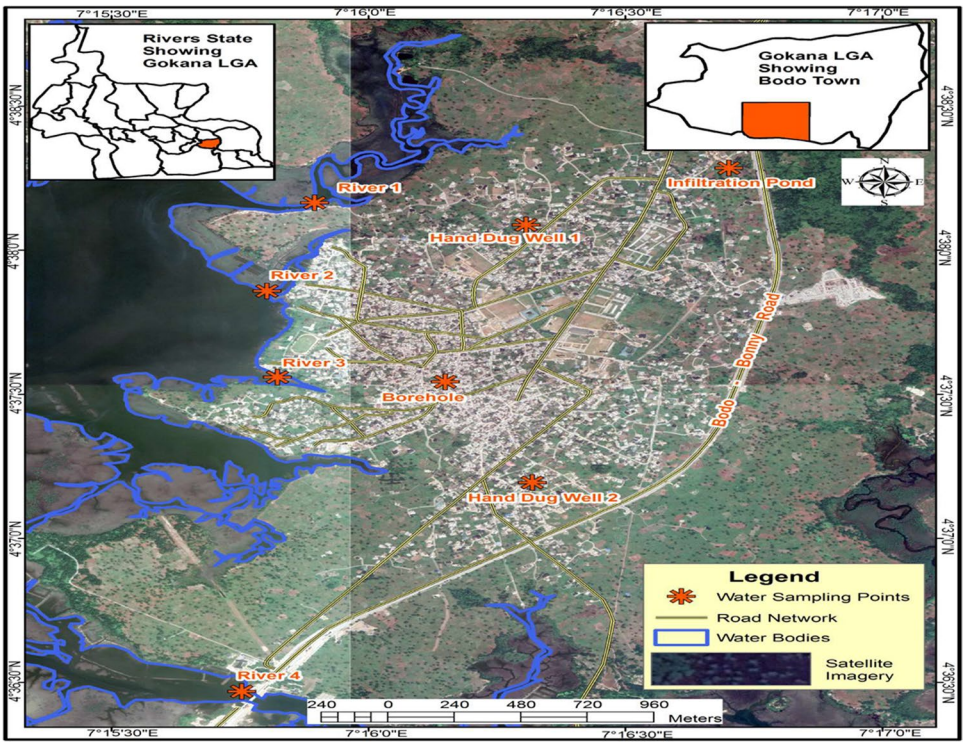

Fig. 1 Bodo Town showing water sampling points

has four local government areas, namely, Eleme, Gokana, Khana and Tai (Balouga 2009; Genovese et al. 2014). Bodo community is one of the oil-producing localities in Ogoniland, Niger Delta. It is located in Gokana LGA. The community is surrounded by large water bodies that are demarcated asymmetrically into smaller creeks by large interconnected vegetation, among which are the sample sites for the present study. Bodo is hosting the Anglo-Dutch oil company Shell Petroleum with 24 and 28 inches Trans-Niger pipeline located in Bodo (Genovese et al. 2014). The water in Ogoniland community is bedevilled with crude oil pollution.

Collection of samples

Thirty water samples were collected from eight different water sources. Four of these sources are flowing surface water bodies. The four water bodies are a big network of rivers demarcated from each other by thick riparian vegetation, extending to other communities before emptying into a larger sea. They are designated as R_1, R_2, R_3 and R_4. The R_1 is 200 m from Bodo community. It is heavily surrounded by thick riparian vegetation and the most visited by the locals for their domestic water needs. The R_2 is about 700 m away from R_1. Most fishing activities are done here on this site. The water looks clean from distance but dark underneath when closely approached. The R_3 is about 450 m away from R_2. It also looks clear and mostly used by farmers in the farm. The R4 is farthest and the biggest of the rest rivers around the community. There were also fishing activities going on in R4 as at the time of sampling. The water looks clear when viewed from a distance but darker underneath when closely approached. The infiltrated pond (Exc) is a shallow pit, about 4 ft deep dug at the outskirt of the community and about 1000 m away from the community centre. It has been taken over by contaminated water brought by run-off. The borehole (Bhl) is a community water source built by the NDDC while the wells are personal water sources for two individual families. The hand-dug wells (Wll) are situated about 500 m away from each other.

Six samples were collected from each of the four flowing surface water bodies at three different points labelled point A, B and C in duplicates (at the surface and at approximately 50 cm below the water surface). All the samples labelled 'A' were collected at the shorelines while 'B' and 'C' samples were collected at points randomly chosen away from the shorelines. The effect of hydrocarbon pollution was conspicuously noticed at the shorelines as the grasses at the shorelines were smouldered by the toxicity of the pollutant. Collection of samples from other randomly chosen sites was to compare the total petroleum hydrocarbon (TPH) concentration at sites away from the shorelines and confirm the action of water current in moving pollutants to the shorelines following spills. Three samples were collected from an infiltrated pond labelled as Exc. The samples were collected at the water surface and spread out at three different points named as point Exc_1, Exc_2 and Exc3. Two samples were collected from two different hand-dug wells, located at two different points in the community and labelled Wll_1 and

Wll_2. The samples were collected at the surface of the water. One sample was collected from a borehole tap and labelled borehole Bhl_1. So, twenty-four (24) samples were collected from the flowing water bodies, three (3) from the infiltrated pond and three (3) from underground sources (well water and borehole water), totalling thirty (30) samples.

Determination of total petroleum hydrocarbon concentration in the water samples

Sample separation

The step in the determination of individual component of crude oil in the water sample began with the physical splitting of the whole sample into two, where the pollutant was systematically removed from the water in a liquid-liquid separation. The apparatus used was separating funnel while the separating solvent was n-hexane. Ten millilitres (10 mL) of the whole sample was used while 6 mL of the solvent was used in a divided duplicate procedure. Ten millilitres (10 mL) of the whole sample was introduced into the separating funnel followed by 3 mL of the solvent. The mixture was vigorously shaken and allowed to set for 5 min. The analyte separates cleanly from the solvent and was collected from the bottom of the funnel when the tap was opened. This procedure was repeated using the same whole sample with the remaining 3 mL of the solvent. This was necessary to ensure complete extraction of all the analyte sought.

Chromatographic analysis

Column chromatography The stationary phase (adsorbent) used was finely divided silica gel. It is a polar solid, which is preferable as components sought are non-polar molecules. The mobile phase (the solvent) is *n*-hexane. The column is plugged with filter made of cotton to prevent escape of the powdered stationary phase. The stationary phase (silica gel) was packed down in the column, followed by a thin layer of sodium sulphate, which was added to the top of the fine particles of silica gel to ensure that any trace of water remaining with the sample after separation is removed. This was followed by the sample to be separated while the mobile phase (10 mL of n-hexane), which is the eluting solvent, was poured from the top of the column.

Gas chromatography (GC) Precisely 1 µL of hydrocarbon sample, dissolved in *n*-hexane was injected into a HP5890 PLUS II USA, gas chromatographic system via a HP7353 Autosampler with Agilent's chemstation software. The system is fitted with a flame ionization detector (FID) and a capillary column HP-5, 0.32 mm ID × 0.5 µm, 30 m with polysiloxane as the stationary phase. The carrier gas used was nitrogen (3.5 mL/min). The oven temperature was set at 50 °C, then ramped at a rate of 5 °C/min to 280 °C and held for 6 min. The injection temperature was 250 °C while the detector temperature was 350 °C; total run time was 52 min.

Collection of algae

Algal samples were collected from two different locations in Benin City, Nigeria. The first location was at the Ogba River. It is a fourth order (4°) stream, about 42 km long that flows behind the Ogba Zoo, cutting across Ekehuan road in Ogba community, Benin City in Edo State. It is located between latitude 6.20°N and longitude 5.34°E. The stream originates from Ekewan community and empties into the Atlantic Ocean via Ossiomo and Benin Rivers (Anyanwu 2012). Samples were also collected at various wet grounds in Military Hospital Benin (MHB). The hospital is a Nigerian Army owned health institution of tertiary status. It is located along airport road opposite the Benin City Airport.

Identification and screening of algae for crude oil bioaccumulation

Identification of the algae began during the sample collection. Certain behavioural characteristics like living in solitary, attachment to objects, floating on water surface/living at the benthic region and rates of growth of these organisms were taken into account. During the microscopic study, the major phenotypic characteristics that were considered in the classification and identification included the size, shape, arrangement of cells (morphology) and colour (Manoylov 2014). These steps were helpful in the classification and identification of the multiple algal samples collected (Wehr 2011).

Wet mount preparation was according to the method described by Francoeur et al. (2013): sterile tooth pick was used to pick a small piece from the algal sample in the sample container, placed on a standard glass microscopic slide and gently covered with a cover slip. The algal cells were carefully observed under light microscope (with total magnification of ×400) for their morphological features. These observed features were compared to the known standard morphological features of algae according to van Vuuren (2006) and Serediak (2011).

After identification of the algae, the resultant species were separated and labelled. They were planted in separate plastic bottles, using water obtained from the parent source and allowed to grow for 2 weeks. The water from the algal parent source was not used in the preparation of the algal growth medium. It was used to temporarily sustain the algae (creating a natural habitat in the artificial home) pending when the 'new environment' (growth medium) is prepared. Precisely 5 mL of clear light crude oil was introduced into each of the algal bottles and monitored for another 1 week. This process was repeated for another week to ensure a reliable result.

Two algal species (X and Y) emerged as candidates for the remediation as other species died with constant supply of crude oil. These two algal species (designated as X and Y) were then cultured in two different 1-L conical flasks containing the axenically prepared growth medium. To transfer into the conical flasks, a sterile inoculating needle was used to pick up a slice of algal species from the plastic bottle and inserted into the 1-L conical flask. The flasks were then monitored for another 2 weeks.

Molecular identification of isolates

DNA extraction

Total DNA was extracted using the modified CTAB extraction protocol. Briefly, approximately 100 mg algal tissue was ground in a sterile mortal and 1 mL CTAB extraction buffer (2% CTAB (cetyltrimethyl ammonium bromide) powder (w/v), 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl and 0.2% β-mercaptoethanol (v/v)) was added. Sap was poured into new sterile tube and vortexed briefly followed by an incubation at 60 °C for 10 min. Tubes were placed on ice and equal volume of phenol, chloroform and iso-amyl alcohol at 25:24:1 was added, followed by vortex and centrifuge at 12,000 g for 10 min. Then, 450 µL of the supernatant was collected into new sterile tube to which 300 µL of cold isopropanol was added and mixed gently. This was followed by incubation for 1 h at 20 °C. Samples were centrifuged at 12,000 g for 10 min to sediment the nucleic acid and the supernatant decanted gently to ensure that the pellets were not disturbed. The pellets were washed twice by adding 500 µL of 70% ethanol and centrifuge at 12,000 g for 5 min. Ethanol was then decanted and the DNA air dried at room temperature (28 °C ± 2). The pellets were finally suspended in 50 µL TE buffer.

Polymerase chain reaction (PCR)

The ITS universal primer set which flank the ITS1, 5.8S and ITS2 region were used. PCR sequencing preparation cocktail consisted of 10 µL of 5x GoTaq colourless reaction, 3 µL of 25 mM $MgCl_2$, 1 µL of 10 mM of dNTPs mix, 1 µL of 10 pmol each ITS 1: 5′ TCC GTA GGT GAA CCT GCG G 3′ and ITS 4: 5′ TCC TCC GCT TAT TGA TAT GC 3′ primers and 0.3 units of Taq DNA polymerase (Promega, USA) made up to 42 µL with sterile distilled water 8 µL DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystems Inc., USA). The PCR condition included a cycle of initial denaturation at 94 °C for 5 min. This was followed by 30 s of denaturation at 94 °C, 30 s of annealing of primers at 55 °C, 2 min of extension at 72 °C and a final extension for 7 min at 72 °C.

Agarose gel electrophoresis

The integrity of the amplified gene fragment was checked on a 1% agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 min. The molten agarose was allowed to cool to 60 °C and stained with 3 µL of 0.5 g/mL ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 min to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliters of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4 µL of each PCR product and loaded into the wells after the 100-bp DNA ladder was loaded into well one. The gel was electrophoresed at 120 V for 45 min visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100-bp (base pair) molecular weight ladder that was ran alongside experimental samples in the gel.

Purification of amplified products

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µL of Na acetate 3M and 240 µL of 95% ethanol were added to each about 40 µL PCR amplified products in a new sterile 1.5-µL Eppendorf tube, mix thoroughly by vortexing and kept at 20 °C for 30 min. Centrifugation for 10 min at 13,000 g and 4 °C was followed by removal of supernatant after which the pellet was washed by adding 150 µL of 70% ethanol and mixed well by centrifugation for 15 min at 7500 g. Supernatants were again removed and tube was inverted on paper tissue and allowed to dry in the fume hood at room temperature (28 °C ± 2) for 15 min. The purified fragment was checked on a 1.5% agarose gel ran on a voltage of 110 V for 1 h as previous, to confirm the presence of the purified products and quantified using a nanodrop 2000 (Thermoscientific, USA).

Sequencing of amplified products

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio Edit software and MEGA 6 were used for all genetic analysis.

Preparation of growth medium for algal cultivation

Bold basal medium (BBM) was used to grow the algae. The BBM was composed of stock solution and trace metal solution. The stock solution was dissolved in the trace metal solution according to Culture Collection of Algae and Protozoa Specification (Gachon et al. 2007; Abioye et al. 2020). Each 1 L of the BBM was prepared in distilled and autoclaved water at 121 °C for 15 min to ensure an axenic medium.

The algal growth medium was enhanced to accelerate the rate of algal growth and consequently optimized to improve the rate and efficiency of bio-accumulation and/or detoxification of the contaminant. The growing algae were copiously supplied with carbon dioxide, which was sourced from a local gas dealer. The gas was supplied to the algae through rubber ducts connected to a locally constructed gas chamber to the conical flasks (Fig. 2a). The growing algae were also supplied with 10 g of chicken dropping that was aseptically collected from a local poultry farmer, heat treated and blended into powdery form. In addition, 10 g of the chicken litter powder contained 720 mg of nitrate and 540 mg of phosphate. The chicken dropping was also plated in growth medium after the heat treatment to ensure axenic condition while the conical flasks were covered with locally made lids to avoid environmental re-contamination.

The set-up was behind the laboratory window that is spacious, devoid of disturbances and has exposure to sunlight particularly 'during the sunset' with a photoperiod of 5:19 h light:dark. The temperature was at the ambient (28 °C ± 2). There was a tree behind the laboratory, providing a shade for the setup.

Set-up for phycoremediation

The experimental design consisted of three batches of sixty-two 250-mL conical flasks, named batch 1, batch 2 and batch 3. Batches 1 and 2 had thirty 250-mL conical flasks each while batch 3 had only two 250-mL conical flasks, totalling sixty-two 250-mL conical flasks. All the conical flasks contained 200 mL of the axenically prepared algal growth medium (BBM), which was further enriched with the optimizing agent, 10 g powdered chicken manure. The thirty 250-mL conical flasks in batch 1 were designated as X batch, the thirty flasks in batch 2 were designated as Y batch while the two flasks in batch 3 were designated as control batch.

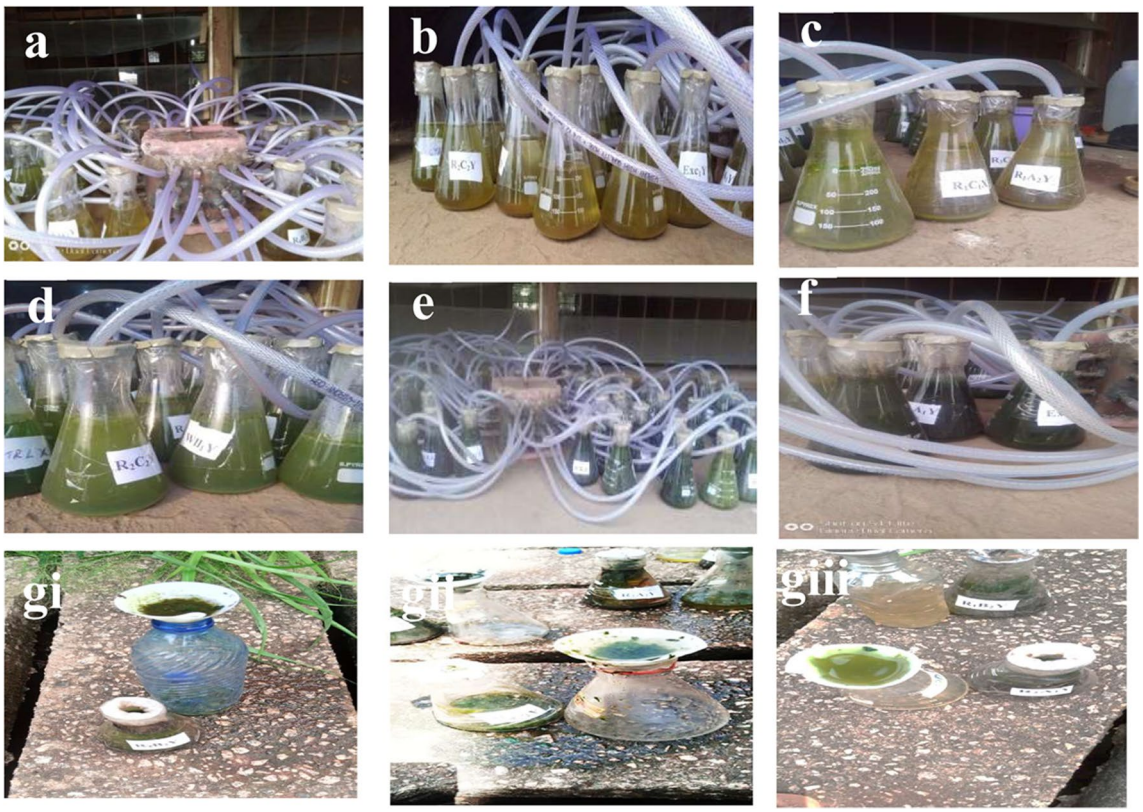

Fig. 2 Phycoremediation experiment. **a** Algal bottles connected to CO_2 gas distribution chamber, **b** planted algae on day 1, **c** planted algae on day 7, **d** planted algae on day 14, **e** planted algae on day 21 and **f** planted algae on day 28. **g**i–iii Harvesting of algae using Whatman filter paper size 4 after the experiment

The species of alga identified as X species was reproduced and grown in all the thirty 250-mL conical flasks in the X batch while species designated as Y was also reproduced and grown in the thirty 250-mL conical flasks in the Y batch. The third batch with two conical flasks also had the two different species (X and Y) grown in each of the conical flasks. The batches 1 and 2 were reproduced into additional thirty flasks each, which was used as experimental replicate. Each of the batches was inoculated with alga X and Y respectively and also exposed to the same optimal growth conditions.

Inoculation was by the use of 20-mL syringe and one syringe was used for each flask. Hence, 20 mL of the screened algal species from the 1-L conical flask was drawn and injected into all the flasks on day 1 (Fig. 2b). The set up was connected to the gas chamber via the rubber duct and monitored for algal proliferation. The algal growth rates, dissolved oxygen generation, nitrate and phosphate depletion and pH/temperature conditions were monitored on days 7, 14, 21 and 28 of the experiment following methods described by American Public Health Association American Public Health Association, APHA (2017).

Introduction of crude oil contaminated water into the algal flasks

The experiment involved a multi hydrocarbon treatment setup. The concentrations of individual hydrocarbon contaminants added into the algal flasks were mathematically worked out to ensure that the precise quantity was added to the growing algae. On this, 20 mL each of the contaminated water from all the sample containers was introduced into the sixty conical flasks in batches 1 and 2. Polluted water samples were not added to the batch 3 flasks (controls). The setup was monitored for another 28 days and the algal growth medium was analysed on days 7 and 28 for the presence or otherwise of the contaminants.

Analysis of algal growth medium for the concentration of TPH on days 7 and 28

Aliquot of the growth medium was taken from each of the algal conical flasks 7 days after the introduction of the contaminant. Twenty millilitres of the growth medium (now turned greenish) was taken from each of the flasks and poured into a cone-like funnel shaped filter paper of grade 602 h with pore size of 2 µm. It was allowed to stand for 5 h while water drained. The alga was peeled off from the filter paper and analysed. The mean TPH values obtained after the analysis were subtracted from the mean value of the TPH added into the algal conical flasks on day 1, resulting in the absorbed TPH concentration. This process was repeated after the fourth week. The mean values obtained after the analysis were subtracted from the mean value of the TPH remaining in the algal conical flasks on day 7, resulting in the absorbed TPH concentration after the fourth week.

Harvesting of algal biomass and extraction of TPH

The algal biomass was harvested after 4 weeks of the experiment. Whatman filter paper size 4 was used for the harvesting. The filter paper was made into a cone-like funnel shape where the alga and the growth medium were emptied and allowed to drain (Fig. 2)gi–iii.

The harvested biomasses were cleansed of debris such as sand and remnants of poultry litter that settled at the bottom of the conical flask. The biomasses were carefully separated from the debris using clean tooth picks and then rinsed with de-ionized water to remove any residual nutrients (NO_3 and PO_4) that may interfere with further analysis (Nurfarahanim and Amran 2017). The biomasses were air-dried under a tree to ensure complete removal of any residual moisture. The samples were scattered evenly on a tin foil paper to enhance homogenous drying. The adequately dried biomasses were wrapped in tin foil paper and securely stored in sealed containers prior to grinding. The algal biomass was weighed using a digital analytical weighing balance (Model KJ5003A, Germany). The weight of the alga from individual conical flask was determined after the biomass has been drained of water and debris of sand and poultry litter completely removed from the biomass.

The grinding was done using the bottom of a glass bottle. The air-dried biomass was wrapped in a two-fold tin foil paper and then gently pulverized with the bottom of the bottle. The traditional mortar-pestle method was not adopted because the biomasses were not plenty enough, hence using mortar and pestle may lead to losing the biomass to the mortar. Moreover, there were so many batches of the sample with different component of TPH potentially embedded in each of the samples. Moreover, using one mortar and pestle for all the grinding may give room for cross-contamination.

This procedure was necessary to extract the components of hydrocarbon absorbed in the alga. The solvent used for the extraction is *n*-hexane (n-C_6H_{14}). One hundred millilitres (100 mL) of the *n*-hexane was used for 5 g of the dried and pulverized algal biomass. The powdered algal biomass was weighed out and placed onto the Soxhlet thimble. The thimble is usually made of thick filter paper, which holds the solid materials but allows passage of liquid. One hundred millilitres (100 mL) of the extraction solvent *n*-hexane was poured into the 250-mL round-bottom flask and then the thimble, with the algal biomass placed into the extraction chamber. The round-bottom flask is placed on the heating plate, the bottom end of the Soxhlet extraction chamber that consists of the Soxhlet thimble, distillation path, syphon arm inlet and syphon arm outlet is mounted on the round bottom flask while the tubes for cooling water in and cooling

water out were connected to the ports on the reflux condenser and mounted on to the reduction adapter on the thimble. The boiling temperature of the extraction process was set as the boiling point of the extraction solvent, *n*-hexane, which is 69 °C. The extraction system was programmed for continuous solvent recycling and repeated extraction. The heating of the extraction solvent in the round bottom flask and opening of the cooling water supply to the condenser initiates the extraction process.

Each batch of extract was allowed to cool for 24 h before separation. Column chromatographic procedure was applied to separate the components from any residual water or particles of the algal biomass before the GC-FID analysis.

Data analysis

Two different algal species were used to remove crude oil from contaminated water. Individual algal species was examined under same conditions and at the same time to determine its preferences on different components of hydrocarbon. Hence, one-way ANOVA was used for analysis of the results.

Results

Results obtained from the analysis of the water samples via GC-FID

The result obtained from the GC-FID analysis of water samples showed that samples collected from the shoreline surface (R_1A_1) had more TPH than samples from other sampling points. Results also showed that hydrocarbon components encountered were majorly the alkane molecules C_8–C_{17} with occasional appearance of the heavier components (Table 1). Result showed almost equal distribution of contaminants among the sampling points. However, point at the shoreline still showed higher contaminants than points away from the shoreline. Hydrocarbon components encountered were majorly the lighter alkane molecules C_8–C_{17} with occasional appearance of the heavier components (Table 2). Sample collected from the shoreline surface (R_3A_1) had more TPH than samples from other sampling points. Hydrocarbon components encountered were majorly the alkane molecules C_8–C_{17} with occasional appearance of the heavier components (Table 3).

Sample collected from the shoreline surface (R_4A_1) had more TPH than samples from other sampling points. In fact, samples from R_4B_1, R_4B_2, R_4C_1 and R_4C_2 sampling points showed minimal contamination. Results also showed that hydrocarbon components encountered are majorly the lighter alkane molecules C_8–C_{17} with occasional appearance of the heavier components (Table 4). Results showed heavy contamination of the sample site. The three sampling points were almost equally contaminated. The Exc site was shallow; sampling was conducted almost through to the bottom of the pond; hence, some heavier components C_{28} and C_{29} were captured during sampling (Table 5). Results from these water sources showed minimal contamination with Wll B having higher value. However, the very lighter and heavier components were practically absent (Table 6).

Cumulative results of the total petroleum hydrocarbon (TPH)

The cumulative TPH results indicated that all the samples collected from the water surfaces R_1A_1, R_1B_1, R_1C_1, R_2A_1, R_2B_1, R_2C_1, R_3A_1 R_3B_1, R_3C_1, R_4A_1, R_4B_1 and R_4C_1 had higher cumulative TPH concentrations (43.2 µg/L, 22.1 µg/L, 23.5 µg/L, 45.7 µg/L, 32.4 µg/L, 26.6 µg/L, 41.7 µg/L, 19.9 µg/L, 21.8 µg/L, 35.1 µg/L, 16.2 µg/L and 21.8 µg/L) than the ones obtained from the sub-surfaces R_1A_2, R_1B_2, R_1C_2, R_2A_2, R_2B_2, R_2C_2, R_3A_2 R_3B_2, R_3C_2, R_4A_2, R_4B_2 and R_4C_2 (10.3 µg/L, 5.8 µg/L, 4.0 µg/L, 15.7 µg/L, 8.1 µg/L, 5.4 µg/L, 10.6 µg/L, 6.6 µg/L, 5.1 µg/L, 13.2 µg/L, 5.6 µg/L and 6.6 µg/L) (Table 7). However, the results also showed that all the samples collected at the surface of the shorelines R_1A_1, R_2A_1, R_3A_1 and R_4A_1 had higher cumulative TPH concentrations (43.2 µg/L, 45.7 µg/L, 41.7 µg/L and 35.1 µg/L) than the samples collected at surfaces of the randomly chosen points away from the shorelines R_1B_1, R_1C_1, R_2B_1, R_2C_1, R_3B_1, R_3C_1, R_4B_1 and R_4C_1 (22.1 µg/L, 23.5 µg/L, 32.4 µg/L, 26.6 µg/L, 19.9 µg/L, 21.8 µg/L, 16.2 µg/L and 21.8 µg/L) as shown in Table 7.

TPH cumulative for borehole, infiltrated pond and hand-dug well

Analysis of the TPH cumulative (µg/L) for borehole, infiltrated pond and hand-dug well showed that the infiltrated pond has the highest cumulative TPH (67.95 µg/L) while the borehole had the least (1.83 µg/L). There were significant differences ($p < 0.05$) between the cumulative TPH values for the three water sources sampled (Table 8).

Cumulative of TPH from the sample sites

Cumulative of TPH from the sample sites showing the level of the contaminant in each of the locations sampled is shown in Fig. 3. The R_2 had the highest TPH among the four rivers sampled with the total of 134.13 ± 3.91 µg/L. This is followed by R_1 with 108.94 ± 3.57 µg/L, R_3 with 105.80 ± 2.92 µg/L and R_4 with 94.44 ± 2.39 µg/L. The Wll had 9.66 ± 0.62 µg/L which is lower than the rivers while the Bhl sample had the lowest TPH yield among all the sites sampled with TPH value of 1.83 ± 0.05 µg/L. However, the

Table 1 GC-FID/R_1 result

S/n	TPH		Concentration (µg/L)					
			R_1A_1	R_1A_2	R_1B_1	R_1B_2	R_1C_1	R_1C_2
1	n-Octane	C_8H_{18}	2.7589	1.3524	2.4851	<0.01	4.3212	<0.01
2	n-Nonane	C_9H_{20}	4.2383	1.8934	2.9844	<0.01	1.9248	<0.01
3	n-Decane	$C_{10}H_{22}$	2.1892	1.2238	4.4820	<0.01	3.1820	1.9801
4	n-Undecane	$C_{11}H_{24}$	1.9890	0.8991	1.3825	<0.01	1.3415	<0.01
5	n-Dodecane	$C_{12}H_{26}$	1.0251	0.4383	0.3512	1.8212	2.0001	0.4241
6	n-Tridecane	$C_{13}H_{28}$	3.1205	0.9824	3.8235	1.7957	1.9831	<0.01
7	n-Tetradecane	$C_{14}H_{30}$	4.1746	1.3249	2.1022	2.0044	0.8241	<0.01
8	n-Pentadecane	$C_{14}H_{32}$	5.0124	1.5423	2.2512	0.4527	4.3218	1.3789
9	n-Hexadecane	$C_{16}H_{34}$	3.1749	0.4579	1.4333	0.1987	2.5312	<0.01
10	n-Heptadecane	$C_{17}H_{36}$	2.4301	<0.01	0.4103	<0.01	<0.01	<0.01
11	Pristane	*iso*-$C_{19}H_{40}$	3.8239	<0.01	0.2138	<0.01	<0.01	<0.01
12	n-Octadecane	$C_{18}H_{28}$	2.3498	<0.01	0.1141	<0.01	<0.01	<0.01
13	Phytane	*iso*-$C_{20}H_{42}$	2.1018	<0.01	<0.01	<0.01	<0.01	<0.01
14	n-Nonadecane	$C_{19}H_{40}$	0.5823	<0.01	<0.01	<0.01	<0.01	<0.01
15	n-Eicosane	$C_{20}H_{42}$	3.4819	<0.01	<0.01	<0.01	<0.01	<0.01
16	n-Heneicosane	$C_{21}H_{44}$	0.5289	<0.01	<0.01	<0.01	<0.01	<0.01
17	n-Docosane	$C_{22}H_{46}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
18	n-Tricosane	$C_{23}H_{48}$	<0.01	<0.01	<0.01	<0.01	0.2443	0.1240
19	n-Tetracosane	$C_{24}H_{50}$	0.2359	0.2003	0.1076	<0.01	0.3922	<0.01
20	n-Pentacosane	$C_{25}H_{52}$	<0.01	<0.01	<0.01	<0.01	0.5780	<0.01
21	n-Hexacosane	$C_{26}H_{54}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
22	n-Heptacosane	$C_{27}H_{56}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
23	n-Octacosane	$C_{28}H_{58}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
24	n-Nonacosane	$C_{29}H_{60}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
25	n-Triacontane	$C_{30}H_{62}$	<0.01	<0.01	<0.01	<0.01	0.1894	0.1121
26	n-Hentriacontane	$C_{31}H_{64}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
27	n-Dotriacontane	$C_{32}H_{66}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
28	n-Tritriacontane	$C_{33}H_{68}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
ΣTPH			43.2177	10.3148	22.1136	5.8191	23.4549	4.0193

Keys: R_1A_1, river one; point A (top); R_1A_2, river one; point A (≈50 cm below); R_1B_1, river one; point B (top); R_1B_2, river one; point B (≈50 cm below); R_1C_1, river one; point C (top); R_1C_2, river one; point C (≈50 cm below)

Exc had the highest TPH value of all the sites sampled with TPH value of 198.83 ± 5.55 µg/L as shown in Fig. 3.

Identification and screening of algae

Alga X (*Ulothrix zonata* (F.Weber & Mohr) Kützing) showed unbranched long green filaments under the microscope. The microscopic view also revealed multiple short cylinders that are entangled to one another. Numerous cells were seen attached end-to-end, forming a thin elongated filament called the holdfast, which is the specialized cell for attachment. Alga Y (*Chlorella sorokiniana* Shihira & R.W.Krauss) showed a single-celled, non-flagellated and non-motile green cell. The view also revealed that the cells are in form of sphere, like a plate with some having shallow hollow at the centre which makes it look like a cup. These cup-shaped cells are small, less than 10 µm in diameter and are scattered on the microscopic field in a solitary form, not really clustered or bunched in groups. The choice for the remediating algae was made having considered the number of days it took some species to start producing chlorophyll, which was noticed by the appearance of green colouration in the plastic bottles. Alga X (*Ulothrix zonata*) started growing from day 2 of seeding, alga Y (*Chlorella sorokiniana*) started growing from day 5; while some of the algae did not give any indication of growth.

Molecular identity of isolates

The molecular identity of the algae shows that the sample X was 97.94% identical to *Ulothrix zonata* while sample Y was 99.50% similar to *Chlorella sorokiniana* (Table 9). Both isolates had a size of 600 bp (Fig. 4).

Table 2 GC-FID/R_2 result

S/n	TPH		Concentration (µg/L)					
			R_2A_1	R_2A_2	R_2B_1	R_2B_2	R_2C_1	R_2C_2
1	n-Octane	C_8H_{18}	4.1990	2.5841	2.0118	1.6230	3.9238	1.5481
2	n-Nonane	C_9H_{20}	2.6585	1.512433	3.5822	0.9573	2.1348	0.9824
3	n-Decane	$C_{10}H_{22}$	1.2189	0.927310	1.4824	0.7273	1.3101	<0.01
4	n-Undecane	$C_{11}H_{24}$	3.0158	1.648019	4.3598	0.9442	3.0534	<0.01
5	n-Dodecane	$C_{12}H_{26}$	2.8793	0.872571	0.5390	1.2278	2.5319	1.2782
6	n-Tridecane	$C_{13}H_{28}$	10.3608	2.491021	7.1018	1.1176	5.2483	1.1934
7	n-Tetradecane	$C_{14}H_{30}$	8.9903	2.554460	3.0251	0.4223	0.5891	<0.01
8	n-Pentadecane	$C_{14}H_{32}$	4.1562	1.561290	2.5319	0.5102	1.5891	<0.01
9	n-Hexadecane	$C_{16}H_{34}$	2.3515	0.269841	1.5248	<0.01	1.3181	<0.01
10	n-Heptadecane	$C_{17}H_{36}$	2.2590	0.163442	0.5135	<0.01	0.5891	<0.01
11	Pristane	*iso*-$C_{19}H_{40}$	0.5680	0.099343	0.5241	<0.01	0.3282	<0.01
12	n-Octadecane	$C_{18}H_{28}$	1.0124	0.481134	1.2800	<0.01	2.1241	<0.01
13	Phytane	*iso*-$C_{20}H_{42}$	0.9253	0.297716	0.7582	<0.01	0.1225	<0.01
14	n-Nonadecane	$C_{19}H_{40}$	<0.01	<0.01	0.3892	0.2893	<0.01	<0.01
15	n-Eicosane	$C_{20}H_{42}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
16	n-Heneicosane	$C_{21}H_{44}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
17	n-Docosane	$C_{22}H_{46}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
18	n-Tricosane	$C_{23}H_{48}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
19	n-Tetracosane	$C_{24}H_{50}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
20	n-Pentacosane	$C_{25}H_{52}$	0.0297	0.240011	1.3895	0.1499	0.8345	<0.01
21	n-Hexacosane	$C_{26}H_{54}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
22	n-Heptacosane	$C_{27}H_{56}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
23	n-Octacosane	$C_{28}H_{58}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
24	n-Nonacosane	$C_{29}H_{60}$	0.9581	<0.01	1.3792	0.1241	0.8902	0.3489
25	n-Triacontane	$C_{30}H_{62}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
26	n-Hentriacontane	$C_{31}H_{64}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
27	n-Dotriacontane	$C_{32}H_{66}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
28	n-Tritriacontane	$C_{33}H_{68}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
ΣTPH			45.6635	15.7027	32.4028	8.0932	26.5874	5.3510

Keys: R_2A_1, river two; point A (top); R_2A_2, river two; point A (≈50 cm below); R_2B_1, river two; point B (top); R_2B_2, river two; point B (≈50 cm below); R_2C_1, river two; point C (top); R_2C_2, river two; point C (≈50 cm below)

Physicochemical parameters monitored during phycoremediation by algal species

During the experiment, there was an increase in the volume of the contents in some of the algal culture flasks particularly at days 14, 21 and 28 (Fig. 2d, e and f). It was also observed that the contaminant did not interfere with the normal algal growth. In comparison with the algae in the control flasks that were not fed with the pollutant, no effect was noticed; both the experimental and control algae showed same growth characteristic at the end of the experiment.

There was exponential generation of dissolved oxygen (DO) by alga X (*Ulothrix zonata*) from day 7 (3.82 mg/L), with increase on day 14 (5.95 mg/L), with day 21 (5.93 mg/L) being the peak of growth, which was accompanied by huge overgrowth and deep-green colouration. However, as the alga became aged at day 28, the concentration of DO reduced drastically to 1.15 mg/L and the deep-green was changed to dark-green. The alga Y (*Chlorella sorokiniana*) started growth rather very slow. This was observed in the DO level from day 7 (2.39 mg/L) and day 14 (3.27 mg/L), accompanied by poor growth as observed in the light greenish colouration. However, at the peak of the growth, alga Y (*Chlorella sorokiniana*) picked up growth and there was exponential generation of DO (2.86 mg/L) which stretched into the last week (Table 10).

Empirical analysis also showed depletion of the limiting nutrients NO_3 and PO_4. For the NO_3, after 7 days, alga X (*Ulothrix zonata*) was able to take up 141.2 mg/L of the nutrient while alga Y (*Chlorella sorokiniana*) was able to take up 102.7 mg/L of nitrate. At day 21, which marked the peak of growth, 165.7 mg/L of the nutrient was left on

Table 3 GC-FID/R_3 result

S/n	TPH		Concentration (µg/L)					
			R_3A_1	R_3A_2	R_3B_1	R_3B_2	R_3C_1	R_3C_2
1	n-Octane	C_8H_{18}	3.7009	1.3001	2.2490	1.0041	4.8549	1.3812
2	n-Nonane	C_9H_{20}	2.9983	1.2034	1.8251	0.4823	3.1104	0.1898
3	n-Decane	$C_{10}H_{22}$	3.0002	1.5232	2.0020	1.3589	2.8209	1.1482
4	n-Undecane	$C_{11}H_{24}$	1.2291	0.4377	0.9821	1.6645	1.4824	0.2490
5	n-Dodecane	$C_{12}H_{26}$	2.0234	0.2386	2.8205	1.4820	3.1121	<0.01
6	n-Tridecane	$C_{13}H_{28}$	2.6205	0.1324	0.8248	<0.01	0.2489	<0.01
7	n-Tetradecane	$C_{14}H_{30}$	3.1761	1.6949	1.0025	<0.01	2.3208	1.2096
8	n-Pentadecane	$C_{14}H_{32}$	4.0155	1.3423	3.9880	0.4513	2.4118	<0.01
9	n-Hexadecane	$C_{16}H_{34}$	4.1712	1.4574	1.1121	<0.01	0.1289	<0.01
10	n-Heptadecane	$C_{17}H_{36}$	1.4323	<0.01	<0.01	<0.01	0.1362	<0.01
11	Pristane	*iso*-$C_{19}H_{40}$	1.9000	<0.01	<0.01	<0.01	0.4898	<0.01
12	n-Octadecane	$C_{18}H_{28}$	4.3433	0.8723	1.4482	<0.01	0.0289	<0.01
13	Phytane	*iso*-$C_{20}H_{42}$	1.1032	<0.01	<0.01	<0.01	<0.01	<0.01
14	n-Nonadecane	$C_{19}H_{40}$	1.7723	<0.01	<0.01	<0.01	<0.01	<0.01
15	n-Eicosane	$C_{20}H_{42}$	1.1019	<0.01	<0.01	<0.01	<0.01	<0.01
16	n-Heneicosane	$C_{21}H_{44}$	0.9989	0.2335	<0.01	<0.01	0.2489	<0.01
17	n-Docosane	$C_{22}H_{46}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
18	n-Tricosane	$C_{23}H_{48}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
19	n-Tetracosane	$C_{24}H_{50}$	1.4159	<0.01	<0.01	<0.01	<0.01	<0.01
20	n-Pentacosane	$C_{25}H_{52}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
21	n-Hexacosane	$C_{26}H_{54}$	0.8220	<0.01	0.8201	<0.01	0.1128	0.8201
22	n-Heptacosane	$C_{27}H_{56}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
23	n-Octacosane	$C_{28}H_{58}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
24	n-Nonacosane	$C_{29}H_{60}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
25	n-Triacontane	$C_{30}H_{62}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
26	n-Hentriacontane	$C_{31}H_{64}$	<0.01	0.1824	0.8401	0.1590	0.2835	0.1432
27	n-Dotriacontane	$C_{32}H_{66}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
28	n-Tritriacontane	$C_{33}H_{68}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
ΣTPH			41.7335	10.6183	19.9147	6.60213	21.7914	5.1411

Keys: R_3A_1, river three; point A (top); R_3A_2, river three; point A (≈50 cm below); R_3B_1, river three; point B (top); R_3B_2, river three; point B (≈50 cm below); R_3C_1, river three; point C (top); R_3C_2, river three; point C (≈50 cm below)

the alga X (*Ulothrix zonata*) flasks while alga Y (*Chlorella sorokiniana*) flasks still had 235.5 mg/L of NO_3. For the PO_4, after 7 days, alga X (*Ulothrix zonata*) was able to take up 104.1 mg/L of the nutrient while alga Y (*Chlorella sorokiniana*) was able to take up 99.5 mg/L. At day 21, which marked the peak of growth, 77.3 mg of the nutrient was left on the alga X (*Ulothrix zonata*) flasks while alga Y (*Chlorella sorokiniana*) flasks still had 195.5 mg/L of PO_4 (Table 10).

The results obtained from the analysis of the algal growth medium on the first week of the experiment showed that the two algal species were able to absorb the hydrocarbon contaminants. However, it was seen that *Ulothrix zonata* by assumption absorbed more of the contaminants than the *Chlorella sorokiniana* (Fig. 5). By the second, third and fourth week, *Chlorella sorokiniana* was able to meet up with *Ulothrix zonata* and the rate of assumed absorption was almost the same (Fig. 6).

Results obtained from the analysis of the algal biomass for extracted TPH

Analysis of the algal biomass for extractable TPH showed that *Ulothrix zonata* had superior absorption ability than *Chlorella sorokiniana*. The result of the analysis of algal biomass showed that from the initial 10.27 µg/20 mL of TPH added to the growth medium, the highest TPH mean value of 0.490 µg/20 mL was extracted from *Ulothrix zonata* biomass grown in Exc compared to 0.344 µg/20 mL of TPH extracted from *Chlorella sorokiniana* grown in the same sample site. In all the samples (R1-Wll), *Ulothrix zonata* had a higher biomass for extracted TPH compared to *Chlorella*

Table 4 GC-FID/R_4 result

S/n	TPH		Concentration (µg/L)					
			R_4A_1	R_4A_2	R_4B_1	R_4B_2	R_4C_1	R_4C_2
1	n-Octane	C_8H_{18}	4.1180	2.5402	4.4792	1.3489	4.8289	2.5498
2	n-Nonane	C_9H_{20}	3.1280	1.4452	3.0410	1.2201	2.5448	1.2480
3	n-Decane	$C_{10}H_{22}$	2.1234	1.2310	1.8909	<0.01	1.2482	0.3211
4	n-Undecane	$C_{11}H_{24}$	5.4892	3.0248	3.1524	2.1846	4.0001	1.1480
5	n-Dodecane	$C_{12}H_{26}$	3.2489	0.9823	0.2187	<0.01	<0.01	<0.01
6	n-Tridecane	$C_{13}H_{28}$	0.5208	0.2765	0.1208	<0.01	<0.01	<0.01
7	n-Tetradecane	$C_{14}H_{30}$	4.5102	0.1935	<0.01	<0.01	4.2125	0.3451
8	n-Pentadecane	$C_{14}H_{32}$	1.2982	0.1700	<0.01	<0.01	0.3894	<0.01
9	n-Hexadecane	$C_{16}H_{34}$	0.4482	<0.01	0.1492	<0.01	<0.01	<0.01
10	n-Heptadecane	$C_{17}H_{36}$	0.9820	<0.01	0.4810	<0.01	<0.01	<0.01
11	Pristane	*iso*-$C_{19}H_{40}$	0.0480	0.3422	<0.01	<0.01	<0.01	<0.01
12	n-Octadecane	$C_{18}H_{28}$	4.1480	1.6521	1.4982	0.1287	3.3182	0.8430
13	Phytane	*iso*-$C_{20}H_{42}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
14	n-Nonadecane	$C_{19}H_{40}$	<0.01	<0.01	<0.01	<0.01	0.1811	<0.01
15	n-Eicosane	$C_{20}H_{42}$	1.8909	<0.01	<0.01	<0.01	0.2209	<0.01
16	n-Heneicosane	$C_{21}H_{44}$	<0.01	<0.01	<0.01	<0.01	0.1285	<0.01
17	n-Docosane	$C_{22}H_{46}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
18	n-Tricosane	$C_{23}H_{48}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
19	n-Tetracosane	$C_{24}H_{50}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
20	n-Pentacosane	$C_{25}H_{52}$	1.8040	0.2871	0.1805	<0.01	<0.01	<0.01
21	n-Hexacosane	$C_{26}H_{54}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
22	n-Heptacosane	$C_{27}H_{56}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
23	n-Octacosane	$C_{28}H_{58}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
24	n-Nonacosane	$C_{29}H_{60}$	0.9805	<0.01	<0.01	<0.01	0.2810	<0.01
25	n-Triacontane	$C_{30}H_{62}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
26	n-Hentriacontane	$C_{31}H_{64}$	0.2894	1.0245	0.9824	0.7005	0.4209	0.1202
27	n-Dotriacontane	$C_{32}H_{66}$	0.1181	<0.01	<0.01	<0.01	<0.01	<0.01
28	n-Tritriacontane	$C_{33}H_{68}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
ΣTPH			35.1461	13.1695	16.1945	5.5828	21.7746	6.5753

Keys: R_4A_1, river four; point A (top); R_4A_2, river four; point A (≈50 cm below); R_4B_1, river four; point B (top); R_4B_2, river four; point B (≈50 cm below); R_4C_1, river four; point C (top); R_4C_2, river four; point C (≈50 cm below)

sorokiniana, although there were no significant differences ($p > 0.05$) in the biomass for extracted TPH except in R4 and Wll (Table 11).

Discussion

The research investigated the phycoremediation of crude oil polluted water from selected water sources in Ogoniland, Rivers State, Nigeria. All the samples collected from the water surfaces had higher cumulative TPH concentrations than the samples obtained from the sub-surfaces. This was probably because the alkane molecules are non-polar and thus hydrophobic. They are insoluble in a polar solvent like water, explaining why oil cannot mix-up with water (Sunil et al. 2018). In addition, the densities of almost all the alkane molecules (gases not inclusive) are lesser than 1.0 g/mL, thus are not as dense as water with density of 1.0 g/mL. Hence, oil will always float on the water surface (Clark 2021). This agrees with the report of Yakubu (2017) which found extractable petroleum hydrocarbons (EPHs) in surface waters was up to 7420 µg/L, which are 900 times more than the recommended values set by WHO for drinking water (WHO 2003).

The result also showed that large percentage of the TPH encountered in the sample sites fell within the light hydrocarbon C_8–C_{16}. Following oil spill on a water body with temperature below the room temperature (28 °C ± 2), the components nonane through hexadecane (C_9–C_{16}) which are free-flowing liquids (at room temperature (28 °C ± 2) may lose their characteristic fluidity with consequent gradual increase in viscosity (Efimov et al. 2019) and

Table 5 GC-FID/Exc result

S/no	TPH		Concentration (µg/L)		
			Exc A	Exc B	Exc B
1	n-Octane	C_8H_{18}	<0.01	<0.01	<0.01
2	n-Nonane	C_9H_{20}	3.1825	4.7973	3.3592
3	n-Decane	$C_{10}H_{22}$	2.3246	3.4649	4.5369
4	n-Undecane	$C_{11}H_{24}$	2.1983	3.1962	4.3382
5	n-Dodecane	$C_{12}H_{26}$	8.1675	6.3472	5.9615
6	n-Tridecane	$C_{13}H_{28}$	6.1736	7.5218	4.7719
7	n-Tetradecane	$C_{14}H_{30}$	4.3582	6.9321	5.5547
8	n-Pentadecane	$C_{14}H_{32}$	7.3900	5.5201	7.6646
9	n-Hexadecane	$C_{16}H_{34}$	5.4482	3.3973	2.9220
10	n-Heptadecane	$C_{17}H_{36}$	5.9923	6.8332	4.6306
11	Pristane	*iso*-$C_{19}H_{40}$	1.1025	1.7459	1.4980
12	n-Octadecane	$C_{18}H_{28}$	2.4021	5.5925	4.9938
13	Phytane	*iso*-$C_{20}H_{42}$	0.7285	0.1526	1.5428
14	n-Nonadecane	$C_{19}H_{40}$	1.0235	0.3064	3.5175
15	n-Eicosane	$C_{20}H_{42}$	0.6105	2.7471	2.9039
16	n-Heneicosane	$C_{21}H_{44}$	1.3542	2.5781	2.0036
17	n-Docosane	$C_{22}H_{46}$	0.0821	1.0877	2.8222
18	n-Tricosane	$C_{23}H_{48}$	1.8100	2.1871	1.8155
19	n-Tetracosane	$C_{24}H_{50}$	2.3520	1.8986	0.7253
20	n-Pentacosane	$C_{25}H_{52}$	2.9205	1.9771	0.0459
21	n-Hexacosane	$C_{26}H_{54}$	0.8235	<0.01	1.2127
22	n-Heptacosane	$C_{27}H_{56}$	1.2345	<0.01	0.1129
23	n-Octacosane	$C_{28}H_{58}$	0.5239	<0.01	<0.01
24	n-Nonacosane	$C_{29}H_{60}$	<0.01	0.4591	1.9569
25	n-Triacontane	$C_{30}H_{62}$	<0.01	<0.01	0.0251
26	n-Hentriacontane	$C_{31}H_{64}$	<0.01	<0.01	<0.01
27	n-Dotriacontane	$C_{32}H_{66}$	<0.01	<0.01	<0.01
28	n-Tritriacontane	$C_{33}H_{68}$	<0.01	<0.01	<0.01
ΣTPH			62.9422	65.9751	69.9157

Keys: *Exc A*, excavated ground point A; *Exc B*, excavated ground point B; *Exc C*, excavated ground point C

Table 6 GC-FID/Wll result

S/no	TPH		Concentration (µg/L)	
			Well A	Well B
1	n-Octane	C_8H_{18}	<0.01	<0.01
2	n-Nonane	C_9H_{20}	<0.01	<0.01
3	n-Decane	$C_{10}H_{22}$	<0.01	<0.01
4	n-Undecane	$C_{11}H_{24}$	<0.01	<0.01
5	n-Dodecane	$C_{12}H_{26}$	<0.01	<0.01
6	n-Tridecane	$C_{13}H_{28}$	0.3205	<0.01
7	n-Tetradecane	$C_{14}H_{30}$	0.5289	0.9843
8	n-Pentadecane	$C_{14}H_{32}$	0.2434	0.3183
9	n-Hexadecane	$C_{16}H_{34}$	<0.01	1.3844
10	n-Heptadecane	$C_{17}H_{36}$	<0.01	0.4235
11	Pristane	*iso*-$C_{19}H_{40}$	1.0498	1.8205
12	n-Octadecane	$C_{18}H_{28}$	0.2811	0.1240
13	Phytane	*iso*-$C_{20}H_{42}$	1.5284	0.8948
14	n-Nonadecane	$C_{19}H_{40}$	<0.01	<0.01
15	n-Eicosane	$C_{20}H_{42}$	<0.01	<0.01
16	n-Heneicosane	$C_{21}H_{44}$	<0.01	<0.01
17	n-Docosane	$C_{22}H_{46}$	<0.01	<0.01
18	n-Tricosane	$C_{23}H_{48}$	<0.01	<0.01
19	n-Tetracosane	$C_{24}H_{50}$	<0.01	<0.01
20	n-Pentacosane	$C_{25}H_{52}$	<0.01	<0.01
21	n-Hexacosane	$C_{26}H_{54}$	<0.01	<0.01
22	n-Heptacosane	$C_{27}H_{56}$	<0.01	<0.01
23	n-Octacosane	$C_{28}H_{58}$	<0.01	<0.01
24	n-Nonacosane	$C_{29}H_{60}$	<0.01	<0.01
25	n-Triacontane	$C_{30}H_{62}$	<0.01	<0.01
26	n-Hentriacontane	$C_{31}H_{64}$	<0.01	<0.01
27	n-Dotriacontane	$C_{32}H_{66}$	<0.01	<0.01
28	n-Tritriacontane	$C_{33}H_{68}$	<0.01	<0.01
ΣTPH			3.70875	5.94985

Keys: *Well A*, hand-dug well point A; *Well B*, hand-dug well point B

hence are encountered on the water surface as oil slick. They may also be encountered few centimetres below the water surface depending on the water depth and mixing ability (Efimov et al. 2019). The temperature values of the four surface water bodies fell below the room temperature (28 °C ± 2); hence, the free-flowing components became less fluidly and congregate on the water surface. The heavier components heptadecane (C_{17} and above) which are thick viscous liquids to waxy solids became even thicker solids at such lower temperature range with increased specific gravity and density (Narve et al. 2001; Lee et al. 2015), thus became slightly denser than water and would submerge farther beneath the sampling region. They were however encountered occasionally during sampling depending on the depth of water source and the mixing power of the water.

The shorelines had more TPH than other points in the sampling sites. Following oil spill and weathering, thick oil slicks used to wash ashore and remain on the shoreline even long after the spill, coating aquatic vegetation and smothering aquatic lives (National Research Council 2005; Ewida 2014). This is in agreement with McGenity et al. (2012) who noted that spilled crude oil usually moved to the shorelines by the action of water current where it coats aquatic vegetation and debris. The infiltrated pond (Exc) had more contaminants when compared with the rivers and the underground water sources. The result is an indication that the pollutants are washed from the soil environment into the excavated ground via run-off (Genovese et al. 2014). By implication, the present hydrocarbon pollution of the water sources is not principally by seepage from the underground aquifers, which would have put the borehole and hand-dug well at severe condition, and by inference,

Table 7 TPH cumulative for river water in microgram/litre (µg/L)

River	A_1 (µg/L)	A_2 (µg/L)	B_1 (µg/L)	B_2 (µg/L)	C_1 (µg/L)	C_2 (µg/L)
R1	43.21 ± 0.01^{c}	10.16 ± 0.16^{a}	22.06 ± 0.05^{c}	5.81 ± 0.00^{b}	23.43 ± 0.02^{b}	4.02 ± 0.00^{a}
R2	45.16 ± 0.50^{d}	15.70 ± 0.00^{c}	32.30 ± 0.10^{d}	8.08 ± 0.05^{d}	26.50 ± 0.09^{c}	5.25 ± 0.10^{b}
R3	41.87 ± 0.13^{b}	10.52 ± 0.10^{a}	19.88 ± 0.03^{b}	6.66 ± 0.00^{c}	21.70 ± 0.20^{a}	5.14 ± 0.00^{b}
R4	35.15 ± 0.00^{a}	13.18 ± 0.01^{b}	16.19 ± 0.00^{a}	5.58 ± 0.37^{a}	21.77 ± 0.00^{a}	6.58 ± 0.00^{c}

Values are mean ± std. error of duplicate values. Means with dissimilar letter(s) differ significantly according to the Duncan's multiple range test (DMRT). *Significant at $p \leq 0.05$

NB: A_1, samples collected on the water surface at the shorelines; A_2, water collected from the subsurface at the shoreline; B_1, water samples collected on the water surface at 50 m away from the shoreline; B_2, sample collected at the subsurface from the 50 m away from the shoreline; C_1, sample collected on the water surface at 100 m point away from the shoreline; C_2, sample collected at the subsurface from the 100 m point away from the shoreline

Table 8 TPH cumulative for borehole, excavated ground and hand-dug well in microgram/litre (µg/L)

Water sources	TPH cumulative (µg/L)
Bhl	1.83 ± 0.00^{a}
Exc	67.95 ± 1.97^{c}
Wll	4.83 ± 1.12^{b}

Values are mean ± std. error of duplicate values. Means with dissimilar letter(s) differ significantly according to the least significant difference (LSD). *Significant at $p \leq 0.05$

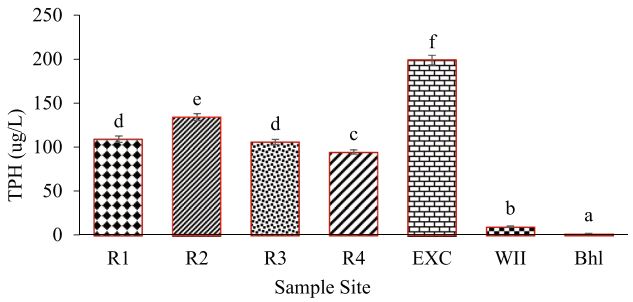

Fig. 3 Cumulative of TPH from the sample sites in microgram/litre (µg/L). Means with dissimilar letter(s) differ significantly according to the Duncan's multiple range test (DMRT). *Significant at $p \leq 0.05$

The hand-dug well had lower TPH concentrations than the rivers and the infiltrated pond. Since the hand-dug wells sampled have concrete-made apron walls with metallic lids cover/locks that protects the water sources from external contamination, hydrocarbon could have contaminated the water through the rope and bucket used for drawing water from the well as proposed by Lutterodt et al. (2018) which are usually left beside the well. The borehole had 1.8310 µg/L of TPH, the lowest among all the sites sampled. This scanty value could have resulted from the little underground seepage since the tap is not exposed to external contamination.

Noticeable increase in the volume of the content of some algal culture flasks as observed in algal cultures at days 14, 21 and 28 may be due to the increase in algal biomass, which resulted in increase in the volume of the medium. This is in agreement with Rahman et al. (2022) who noted that if the optimal growth parameters are met, a large volume of algal biomass is usually expected. This also agrees with Peimin (2015) who noted that under favourable conditions, algal growth and reproduction usually become rapid with consequent increase in volume of algae within its environment. Such spontaneous increase in the algal volume in natural aquatic environment is often referred to as algal blooms.

Table 9 NCBI blast revealing the identity of the edited sequences

Sample ID	Scientific name	Max score	Total score	Query cover	*E* value	Per. ident
Sample X	*Ulothrix zonata*	922	922	100%	0	97.94%
Sample Y	*Chlorella sorokiniana*	1096	1096	99%	0	99.50%

incriminating illegal oil mining and artisanal refining as proposed by Utazi et al. (2022). Also, river two which had the highest TPH cumulative among the four rivers sampled 134.1296 µg/L is located at a point where run-off washes contaminant from the soil environment into the river, hence the major recipient of contaminant from the soil environment.

The two algal species showed a noticeable progressive increase in biomass production as evidently seen in the rich chlorophyll generated in the bottle with consequent DO of 9.53 mg/L for *Ulothrix zonata* and 4.37 mg/L for *Chlorella sorokiniana* generation especially at the peak of algal growth. However, *Ulothrix zonata* generated

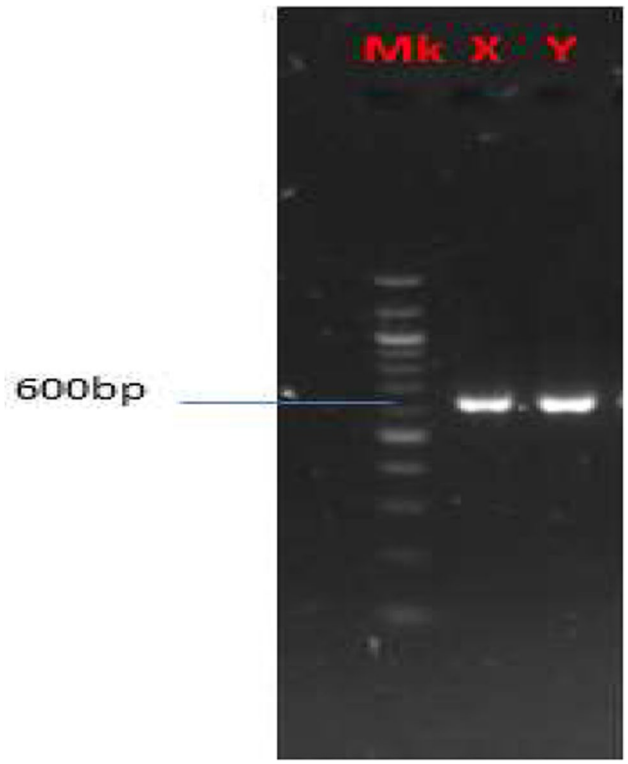

Fig. 4 Gel electrophoresis showing the positive amplification of the ITS regions of the selected algae samples, X—*Ulothrix zonata*, Y—*Chlorella sorokiniana*

more DO than *Chlorella sorokiniana* on days 7, 14 and 21 with 3.82 mg/L, 5.95 mg/L and 9.53 mg/L compared with *Chlorella sorokiniana* having DO of 2.39 mg/L, 3.27 mg/L and 4.37 mg/L, respectively. This was because the *Ulothrix zonata* had a faster growth and more biomass production than *Chlorella sorokiniana*. This is in agreement with Kulikova et al. (2018) who reported that the development of *Ulothrix zonata* begins almost immediately the cells are established in the immediate environment with an instant formation of continuous green cover of *Ulothrix zonata* overgrowths. The report also noted that increase in *Ulothrix zonata* overgrowths results in large biomass with copious saturation of dissolved oxygen. Also, *Ulothrix* can reproduce in three different ways; vegetative, asexual and sexual, which facilitate its rapid growth. Vegetatively, *Ulothrix* can reproduce even when there is fragmentation as a result of physical injury to the filament resulting in akinete formation. Asexually, *Ulothrix* can form zoospores, aplanospores, hypnospores, akinetes and palmella stage any of which can progress into zoosporangium. Sexual reproduction is via isogamy (fusion of physically identical gametes) and occurs by means of biflagellate gametes (Mani and Sahoo 2015). These enhanced the organism's quick exponential growth with consequent generation of dissolved oxygen.

Table 10 Monitored parameters during phycoremediation for the two algal species

Time	DO (mg/L)		NO_3 (mg/L)		PO_4 (mg/L)		pH		Temp (°C)	
	X	Y	X	Y	X	Y	X	Y	X	Y
7	3.82 ± 0.27^{b}	2.39 ± 0.23^{a}	578.80 ± 8.64^{a}	617.30 ± 7.92^{b}	435.90 ± 5.16^{a}	440.50 ± 2.83^{a}	5.80 ± 0.27^{a}	6.30 ± 0.21^{b}	17.40 ± 0.52^{a}	17.40 ± 0.39^{a}
14	5.95 ± 0.29^{b}	3.27 ± 0.19^{a}	439.70 ± 3.19^{a}	438.70 ± 1.36^{a}	272.80 ± 2.61^{a}	307.50 ± 5.78^{b}	6.50 ± 0.21^{a}	6.20 ± 0.15^{a}	16.10 ± 0.31^{a}	17.20 ± 1.12^{a}
21	9.53 ± 0.30^{b}	4.37 ± 0.23^{a}	165.70 ± 2.12^{a}	235.50 ± 4.88^{b}	77.30 ± 2.70^{a}	195.20 ± 4.85^{b}	6.10 ± 0.06^{a}	6.70 ± 0.32^{a}	16.20 ± 0.63^{a}	17.10 ± 0.34^{a}
28	1.15 ± 0.12^{a}	2.86 ± 0.12^{b}	51.50 ± 2.22^{a}	149.90 ± 4.07^{b}	16.50 ± 0.38^{a}	99.00 ± 3.31^{b}	5.10 ± 0.28^{a}	5.20 ± 0.31^{a}	19.30 ± 0.75^{a}	19.10 ± 1.07^{a}

NB: *X*, *Ulothrix zonata*; *Y*, *Chlorella sorokiniana*

Means with dissimilar letter(s) differ significantly according to the Duncan's multiple range test (DMRT). *Significant at $p \leq 0.05$

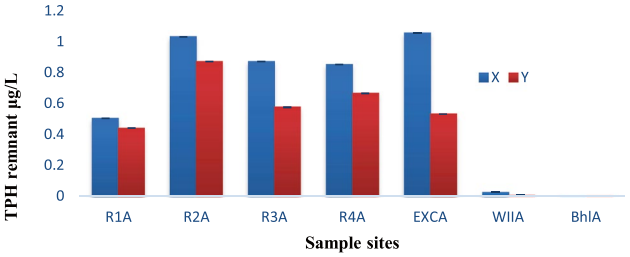

Fig. 5 Analysis of the algal growth medium for TPH at day 7. Alga X: *Ulothrix zonata* (in blue) absorbed more of the contaminant than alga Y: *Chlorella sorokiniana* (in orange)

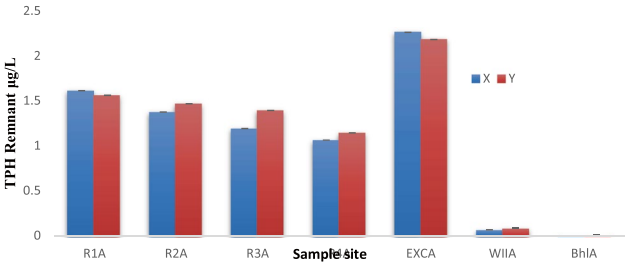

Fig. 6 Analysis of the algal growth medium for TPH at day 28. As the rate of *Chlorella sorokiniana* growth improved, the rate of absorption became almost even (alga X (*Ulothrix zonata*) in blue and alga Y (*Chlorella sorokiniana*) in orange)

Table 11 Mean values of algal biomass for extracted TPH in µg/20 mL

Samples	Alga X (µg/20 mL)	Alga Y (µg/20 mL)
R_1	$0.334^b \pm 0.033$	$0.201^a \pm 0.033$
R_2	$0.349^b \pm 0.026$	$0.203^a \pm 0.021$
R_3	$0.305^b \pm 0.035$	$0.218^a \pm 0.029$
R_4	$0.264^a \pm 0.044$	$0.200^a \pm 0.024$
Exc	$0.490^b \pm 0.029$	$0.344^a \pm 0.021$
Wll	$0.113^a \pm 0.024$	$0.079^a \pm 0.010$
Bhl	$0.023^a \pm 0.002$	$0.021^a \pm 0.002$

Means with dissimilar letter(s) differ significantly according to *t*-test. *Significant at $p \leq 0.05$

The results also showed that the *Ulothrix zonata* used more nutrients than the *Chlorella sorokiniana* on days 7, 14 and 21, which correlates with progressive increase in DO generation of 5.95 mg/L, 3.82 mg/L and 9.53 mg/L with consequent biomass overgrowth by *Ulothrix zonata* on the said days. This implies that as more nutrients were used, more biomass and dissolved oxygen were being generated. However, during the fourth week, there was a noticeable overall decline in nutrients usage and low dissolved oxygen generation, with *Chlorella sorokiniana*, which started with slow initial growth, generating more dissolved oxygen of 2.86 mg/L than the *Ulothrix zonata* that generated 1.15 mg/L of dissolved oxygen, as *Chlorella sorokiniana* had to complete its growth cycle later than *Ulothrix zonata*. This could be as a result of decline/death in the viable cells and no synthesis of new ones as the organism crosses its stationary phase (Willey et al. 2009). In this situation, age was beginning to tell on the alga resulting in poor viability, poor synthesis and change in colour.

It was also observed that there was an exponential growth of the algae which was accompanied by copious generation of dissolved oxygen on the third week (9.53 mg/L for *Ulothrix zonata* and 4.37 mg/L for *Chlorella sorokiniana*). During microbial growth, especially in a new environment and supply of different form of nutrient, growths are usually slow but progressive. Cellular constituents and by-products are usually synthesized in accordance with the rate of growth until a new balanced state (exponential phase) is reached. At this stage, there is always a marked rise in the rate of growth with consequent rise in the rate of synthesis (Willey et al. 2008). Hence, the third week seemed the exponential phase of the algae, which explains the boost in dissolved oxygen generation.

The biomass change of colour from the lively rich deep-green to dark-greenish colouration at week 4 may be as a result of the production of phlorotannins, which are often produced by several species of algae (Bocanegra et al. 2009; Farasat et al. 2014; Chakraborty et al. 2015). Due to importance attached to the potentials of these secondary metabolites of algal origin (Khan et al. 2022), studies have revealed that phlorotannins are not completely exclusive products of the brown algae as some red and green algae can also generate phlorotannins (Yang et al. 2010). However, the concentrations produced by these species were lesser than the ones produced by brown algae (Bocanegra et al. 2009; Farasat et al. 2014; Chakraborty et al. 2015; Khan et al. 2022) and the primary function of phlorotannins in the brown algae also differs significantly ($p<0.05$) in the red and green algal species (Bravo 1998; Kumar et al. 2022). While the brown algae generate phlorotannins for security reasons (Shibata et al. 2002), red and green algae produce phlorotannins in response to harsh environmental conditions such as high solar radiation, hypersalinity, pressure, temperature (Bravo 1998), as well as high biomass and ageing (Kumar et al. 2022). Hence, the dark-greenish colouration observed on the fourth week could be as a result of massive biomass generated and ageing of the algae (Kumar et al. 2022).

Also, the pH of the growth medium was generally slightly acidic. However, the pH value of the medium for *Ulothrix zonata* and *Chlorella sorokiniana* reduced to 5.1 and 5.2 from 5.8 and 6.3 for *Ulothrix zonata* and *Chlorella sorokiniana*, respectively. Carbon dioxide is dissolved during photoautotrophic growth of algae to form HCO_3^-, a weak acid which accumulates above the

microalgae's carbon concentrating mechanism called the thallomes (Mackinder et al. 2017) thereby facilitating the reduction in environmental pH (Mingcan et al. 2022). This decrease in pH may also probably results from concentrated exudates excreted from the algae during algal growth which made the medium more acidic and to some extent, aid dissolution of some contaminants (Mingcan et al. 2022). This is also in agreement with Mingcan et al. (2022) who noted that acidic metabolites secreted by algae during autotrophic growth induce pH decrease in the aquatic environment.

The temperature was also generally below the room temperature (28 °C ± 2) but noticeably, there was a slight increase in the temperature value at day 28. This may be due to toxic exudates and lower dissolved oxygen generation. This agrees with Phang et al. (2015) who noted that increase in toxicity can result in increase in temperature and vice versa.

The results obtained from the analysis of algal growth medium for the concentration of TPH on days 7 and 28 and the results obtained from the analysis of harvested algal biomass for TPH showed a decline in the concentration of TPH in the growth medium and the presence of extractable TPH from biomass, respectively. These showed that the two algal species in this study demonstrated excellent ability to remediate crude oil contaminated water. The green algae are notable for excellent binding capacity for both organic and inorganic contaminants. This feat is credited to the abundant secretion of mucilaginous substances on the cell wall surface, carbohydrate and hydrocarbon as components of the cell wall. These secretions have functional groups capable of luring and acting as attachment sites to contaminants. Although both algal species demonstrated hydrocarbon absorption abilities, *Ulothrix zonata* showed superior ability to absorb the TPH than the *Chlorella sorokiniana*. This was shown in the result obtained from the analysis of algal biomass for TPH as *Ulothrix zonata* had higher absorption ability in all the sample sources than *Chlorella sorokiniana*. This absorption superiority of *Ulothrix zonata* could be attributed to its fast exponential growth capabilities and generation of large algal biomass. For instance, the presence of extractable hydrocarbon components; $C_{14}H_{30}$ and $C_{17}H_{36}$ in *Ulothrix zonata* and *Chlorella sorokiniana* respectively positioned the species as good candidates for hydrocarbon degradation/absorption since the organisms naturally have the contaminants in them. This agrees with Abioye et al. (2020) who noted that green algae are able to seize contaminants by the formation of complexes between the ion of the contaminant and the functional groups on the surface of the algal cell wall.

Conclusion

Crude oil exploitation by legal and illegal means aided by run-offs has resulted in the pollution of Ogoni environments, particularly the aquatic environments. Algae are able to colonize crude oil polluted sites with their ability to generate massive overgrowths within the shortest period of time. These organisms are able to saturate the environment with dissolved oxygen, which is needed by man and aerobic microbes involved in environmental cleaning and repair. The results obtained from the present study showed that the algal species (*Ulothrix zonata* and *Chlorella sorokiniana*) were able to absorb total petroleum hydrocarbon (TPH) and can further be developed and deployed as an active bioremediating agent especially in the aquatic environment. However, the study also noticed that *Ulothrix zonata* had more affinity for the TPH as its absorption capacity was superior to that of *Chlorella sorokiniana*. It is therefore concluded that going by the results of the present study, phycoremediation using different species of algae such as *Ulothrix zonata* and *Chlorella sorokiniana* can completely remediate Ogoni environments, particularly the aquatic habitats of crude oil contaminants thereby availing a hygienic, healthy and productive environments for Ogoni people.

It is therefore recommended that these species of algae (*Ulothrix zonata* and *Chlorella sorokiniana*) should be deployed for field trial in crude oil polluted aquatic environments. While it is recommended that right measures are established to avert further oil spills and pollution of Ogoni environments, acts like illegal oil drilling and refining which usually results in massive crude oil pollution should be discouraged.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Basil Utazi Ezugwu. He also wrote the first draft of the manuscript. Jeremiah David Bala supervised the work; Olabisi Peter Abioye co-supervised the research and corrected the manuscript. Oluwafemi Adebayo Oyewole revised the manuscript. He was also a co-supervisor of the research. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability All data and materials that support the findings of this research comply with standards.

Declarations

Ethical approval Not applicable.

Consent to participate All authors participated in this research actively.

Consent for publication All authors agreed with the content of the article and all gave explicit consent to submit this article to ESPR.

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