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Biostimulation potential of some agro-wastes in the remediation of crude oil-polluted freshwater

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ABSTRACT

Crude oil pollution has become a significant problem, particularly because it disrupts the biotic and abiotic components of aquatic environments. This study investigated the bioremediation potential of certain agro-wastes in the remediation of crude oil-contaminated freshwater. A sample of crude oil-contaminated water was collected in a sterile container from Adobi community in Odagwa, Etche Local Government Area, Rivers State. Pineapple and watermelon peels, which were dried and ground into powder, served as organic supplements, while NPK fertilizer was used as the inorganic supplement. Physicochemical parameters were analyzed using standard methods, and the hydrocarbon-utilising and total heterotrophic bacterial loads were determined through standard plate counts. Isolated bacteria were identified phenotypically. The experiment involved eight treatments, including a control, in 1500 mL conical flasks. Pineapple, watermelon peels, and NPK fertilizer were used as supplements, and the experiment lasted for 70 days. The total heterotrophic bacterial count in the habitat water profile was 1.7×10^6 CFU/mL, while the hydrocarbon-utilising bacterial count was 1.5×10^5 CFU/mL. The hydrocarbon-utilising bacteria isolated from the sample included *Pseudomonas* sp., *Alcaligenes* sp., *Bacillus* sp., *Cedecea* sp., and *Flavobacterium* sp. Baseline physicochemical parameters included a pH of 5.49, temperature of 28.3°C, dissolved oxygen of 2.31 mg/L, turbidity of 165 NTU, total dissolved solids of 51 mg/L, electrical conductivity of 102.1 µS/cm, total suspended solids of 3.05 mg/L, biological oxygen demand (BOD) of 26.67 mg/L, total organic carbon of 2.66 mg/L, nitrate of 2.04 mg/L, and phosphate of 6.7 mg/L. The total petroleum hydrocarbon (TPH) was 354.87 mg/L. The changes in hydrocarbon-utilising bacteria (HUB) counts were as follows: Day 1 ($7.0 \times 10^3 – 3.9 \times 10^4$ CFU/mL), Day 14 ($2.7 \times 10^5 – 1.4 \times 10^6$ CFU/mL), Day 28 ($1.2 \times 10^4 – 1.2 \times 10^6$ CFU/mL), Day 42 ($6.5 \times 10^4 – 2.9 \times 10^5$ CFU/mL), Day 56 ($3.5 \times 10^4 – 2.4 \times 10^5$ CFU/mL), and Day 70 ($5.8 \times 10^5 – 2.9 \times 10^5$ CFU/mL). No significant differences ($P > 0.05$) were observed in the HUB and THB counts across treatments. During bioremediation, physicochemical parameters ranged as follows: pH (3.81–6.87), BOD (6.61–19.88 mg/L), nitrate (0.15–4.31 mg/L), and phosphate (0.30–1.44 mg/L). The concentrations of nitrate, phosphate, and potassium increased in the nutrient-supplemented samples on the first day but declined over the bioremediation period. The reduction and percentage reduction of total petroleum hydrocarbons were as follows: NPK (2032 ± 0.7 mg/L; 57.2%), Pineapple (2006.45 ± 0.1 mg/L; 53.3%), and Watermelon (2325.6 ± 0.1 mg/L; 40.8%). The individual supplements outperformed the consortiums. Using nutrients to stimulate indigenous bacteria is highly recommended due to its efficiency.

Keywords: Bioremediation, agro-wastes, crude oil, freshwater.

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INTRODUCTION

Crude oil, ranging from very light to heavy types, consists of a wide variety of hydrocarbons, with hydrocarbon fractions making up 50 to 98% of its composition (El-Din et al., 2018). Hydrocarbon contamination of land and water bodies is a significant global concern, especially in Nigeria (Onuoha et al., 2020). When oil spills into water, weathering processes such as evaporation, dissolution, oxidation, emulsification, sedimentation, spreading,

dispersion, and biodegradation occur (El-Din et al., 2018). These processes negatively impact aquatic life, necessitating rapid clean-up techniques to remove hydrocarbons from contaminated water bodies (Ataikiru and Ajuzieogu, 2023).

Various techniques (biological, mechanical, and physicochemical) are used to remove oil spills (Ataikiru and Ajuzieogu, 2023). These techniques can be applied individually or in combination (Sidiras et al., 2014). Chemical techniques include solidifiers, dispersion, and in-situ burning, while biological and mechanical methods involve booms, skimmers, and absorbents (Ortiz-Hernández et al., 2014; Sidiras et al., 2014; El-Din et al., 2018). However, mechanical and chemical methods have notable disadvantages, such as high costs and inefficiency in removing trace levels of oil (Sidiras et al., 2014). Moreover, mechanical methods, though effective for large-scale oil removal, are unsuitable for use in extreme weather conditions, such as strong winds and stormy seas (El-Din et al., 2018). These limitations have led to a preference for biological techniques, which are more cost-effective, environmentally friendly, adaptable, and capable of reducing the toxicity and concentration of a wide range of contaminants (Ataikiru and Ajuzieogu, 2023).

Biological techniques often involve the use of sorbents to clean oil spills from water. These sorbents are made from natural organic materials such as rice husk, banana trunk, garlic, and onion peels (El-Din et al., 2018). Sorbents absorb and convert liquids into semi-solid or solid phases, effectively removing oil from water without allowing it to drain out (El-Din et al., 2018). However, the limitations of existing sorbents have spurred interest in alternative materials, particularly agricultural wastes. These materials offer several advantages, including low cost, biodegradability, high oil sorption capacity, low water absorption, high buoyancy, and reusability (Kamaraj and Yamuna, 2016; Agarry, 2018; Yusuf and Yahaya, 2022; Ataikiru and Ajuzieogu, 2023). This study investigated the bioremediation potential of agro-wastes in the remediation of crude oil-polluted freshwater in Rivers State, Nigeria.

MATERIALS AND METHODS

Collection of water sample

A sample of crude oil-contaminated freshwater was collected in a sterile container from the Adobi community in Odagwa, Etche, Etche Local Government Area, Rivers State. The GPS coordinates of the collection site were 4.9801°N and 7.160622°E. The water sample was obtained from three points, which were combined to form a composite sample. This composite sample was placed in an ice-packed container and transported to the Microbiology Laboratory, Department of Microbiology, Rivers State University, for analysis.

Collection of organic and inorganic supplements

Organic supplements, including pineapple and watermelon peels, were sourced from fruit gardens in D-line, Port Harcourt, Rivers State. These supplements were sun-dried and ground into powdered form. The powdered supplements were placed in sterile 250 mL beakers and pasteurized in a water bath at 60°C for 15 minutes (Prescott et al., 2011) to eliminate or reduce contaminating microorganisms. The nitrogen-phosphate-potassium (NPK 15:15:15) fertilizer was obtained from the Agricultural Development Programme, Rumuodumanya, Obio-Akpor Local Government Area, Rivers State, Nigeria.

Enumeration and isolation of total heterotrophic bacteria

The total heterotrophic bacterial load of the water sample was enumerated using the spread plate method (Prescott et al., 2011). For this, an aliquot (0.1 mL) from a 10^{-4} dilution, prepared through a 10-fold serial dilution, was transferred to the center of freshly prepared, pre-dried nutrient agar (NA) plates in duplicates. The plates were evenly spread using a sterile bent glass rod and incubated at 37°C for 24–48 hours. After incubation, the plates were observed for bacterial growth. The number of colonies on the respective plates was recorded to determine the bacterial load, while distinct colonies were subcultured and purified by carefully streaking them onto freshly prepared NA plates.

The colony-forming unit (CFU) was calculated using the following formula:

$$\mathrm{CFU/ml} = \frac{\text{Number of Colonies}}{\text{Dilution used x Volume plated (0.1ml)}} \qquad \textit{Equation 1}$$

Enumeration and isolation of hydrocarbon utilising bacteria

The hydrocarbon-utilising bacteria in the water sample were enumerated using the spread plate method on pre-dried mineral salt agar. The composition of the mineral salt agar was as follows: agar-agar (15 g), K_2HPO_4 (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.3 g/L), NaCl (0.3 g/L), $MnSO_4 \cdot H_2O$ (0.2 g/L), $FeSO_4 \cdot 6H_2O$ (0.2 g/L), $NaNO_2$ (0.3 g/L), and $ZnCl_2$ (0.3 g/L). After preparation, the mineral salt agar was sterilized by autoclaving at 121°C for 15 minutes at 15 psi. The medium was then allowed to cool to 45°C before being supplemented with 1 mL of 250 mg/L Amphotericin B (Fungizone) (Owhonka and Obire, 2019). It was later dispensed into sterile disposable Petri dishes.

An aliquot (0.1 mL) from a 10^{-2} dilution was inoculated in duplicate on the surface of the prepared pre-dried mineral salt agar and spread evenly using a bent glass rod. The vapor-phase transfer method was employed, in

which sterile filter paper saturated with 2 mL of crude oil was placed inside the cover of the inverted inoculated Petri dishes. The plates were incubated at 37°C for 5 days (Sampson et al., 2016). After incubation, the colonies on the plates were counted, and the CFU was calculated using the formula in Equation 1. Discrete colonies were further subcultured on freshly prepared pre-dried nutrient agar plates and incubated at 37°C for 24 hours. The purified cultures were stored for identification.

Identification of the Isolates

The bacterial isolates were identified based on their colonial morphology (color, shape and texture), microscopy, motility, and a variety of biochemical tests, including Voges-Proskauer, Methyl Red, citrate utilisation, indole, and sugar (glucose, mannitol, lactose and sucrose) fermentation.

Physicochemical properties

Physicochemical properties, including biological oxygen demand (BOD), chemical oxygen demand (COD), turbidity, electrical conductivity (EC), temperature, nitrate, phosphate, and total petroleum hydrocarbon (TPH), were analyzed. The American Public Health Association (APHA, 2012) method was used to determine the physicochemical parameters of the water.

Hydrogen ion concentration (pH)

The measurement of hydrogen ion concentration (pH) was conducted as described by APHA (2012). The pH of the water samples was measured immediately upon arrival at the laboratory using a pH meter, model D46 (pH/MV/OC meter). The pH meter was calibrated using standard buffer solutions with pH values of 7, 4, and 10. Calibration was performed by pouring a small amount of pH 7 buffer into a clean beaker, inserting a magnetic stirrer bar, and placing the beaker on a magnetic stirrer to ensure a homogeneous mixture. The pH meter electrode was lowered into the beaker, allowing the tip to become immersed in the buffer solution, and the magnetic stirrer was started. The meter was adjusted to read the buffer. Afterward, the electrode was removed, rinsed with distilled water, and dried. The process was repeated using the pH 4 and pH 10 buffers. After calibration, the pH of the sample was measured using the same procedure, and the results were recorded.

Electrical conductivity

Conductivity is defined as the ability of an aqueous solution to conduct an electric current, which depends on the presence of ions, their mobility, total concentration, and temperature (APHA, 2012). To verify the conductivity results, a standard solution of potassium chloride with a known conductivity was used (0.01 N KCl, 745.6 mg in 1.0 L deionized water = 1413 µmhos/cm). The conductivity cell (electrode) was washed three times with the 0.01 N KCl solution, and the conductivity of the solution was measured. The conductivity cell was then immersed in the sample, and the conductivity was recorded (APHA, 2012).

Biological oxygen demand (BOD)

Airtight BOD bottles with a capacity of 300 mL were filled to the brim with the water samples. The initial dissolved oxygen (DO) in the samples was determined. The diluent was prepared by measuring 22.5 g/L $MgSO_4 \cdot 7H_2O$, 27.8 g/L $CaCl_2 \cdot 2H_2O$, and 0.25 g/L $FeCl_3 \cdot 6H_2O$. A phosphate buffer was also prepared using 8.5 g KH_2PO_4, 21.7 g $K_2HPO_4 \cdot 7H_2O$, 1.7 g NaCl, and a pH of 7.2, and the final volume was adjusted to 1 L with distilled water. The contents of the beaker were gently mixed by swirling and covered. The dilution water was first saturated with dissolved oxygen by shaking it in a partially filled bottle before being used to dilute the samples.

The BOD bottles were filled with the diluted samples, while two additional bottles with dilution water served as blanks. The bottles were carefully stoppered to prevent air from entering. The blank and one experimental BOD bottle were used to determine the initial dissolved oxygen (DO). The remaining two BOD bottles were sealed with water by filling the flared neck of the bottles with distilled water from a wash bottle. The caps provided with the BOD bottles were used to retain the water. The bottles were incubated at 20°C for 5 days. At the end of this period, the final DO was measured, and the BOD_5 (in mg/L) of the sample was calculated using the formula in Equation 2:

$$BOD = DI - D2/P \qquad \textit{Equation 2}$$

D1 represents the dissolved oxygen (mg/l) of the sample 15 minutes after preparation
D2 represents dissolved oxygen (mg/l) of sample 5 days after incubation at 20^oC
P represents the Decimal volumetric fraction of the sample used, APHA (2012).

Turbidity

Turbidity was determined using a standardized Hanna H198703 Turbidometer. Distilled water was used to calibrate the Nephelometer to 0.0 NTU. Hydrazine sulfate (1.0 g) was dissolved in 100 mL of distilled water to form Solution 1. Additionally, hexamethylenetetramine (10.0 g)

was dissolved in 100 mL of distilled water in a volumetric flask to prepare Solution 2. Then, 5 mL of Solutions 1 and 2 were mixed in a volumetric flask and kept for 24 hours at approximately 25°C. The mixture was then diluted to 1000 mL with distilled water to create a 400 NTU stock suspension. Afterward, 4 mL of the stock solution was diluted to 100 mL with distilled water to prepare a 40 NTU standard solution. Both solutions were thoroughly measured using the Nephelometric tube.

Turbidity (NTU) was calculated as:

Turbidity (NTU) = Nephelometer reading × Dilution factor

Equation 3

If the turbidity of the sample exceeded 40 NTU, the sample was diluted, and the dilution factor was incorporated into the final calculations (APHA, 2012).

Total suspended solids (TSS)

The water sample was used for the total suspended solids (TSS) test. A vacuum pump with distilled water was used to wash the membrane filter (pore size 0.45 µm), and suction was applied to remove excess water. The membrane filter was carefully separated, placed in a crucible, and dried in an oven at 103°C for 1 hour. During analysis, the dried filter paper was wetted with a small volume of distilled water and placed in the filtration unit. Fifty milliliters (50 mL) of a homogenously mixed sample were filtered through the membrane. The membrane filter was then carefully removed and transferred to the crucible. The content was dried in the oven to a constant weight at 103°C (APHA, 2012).

Total dissolved solids (TDS)

Total dissolved solids (TDS) were determined using the gravimetric method. A portion of the water was filtered, and 10 mL of the filtrate was measured into a pre-weighed evaporating dish. Following the procedure used for the determination of total solids, the TDS content of the water was calculated by subtracting the weight of the total suspended solids from the total solids.

$$Total\ Dissolved\ Solids\ \left(\frac{mg}{L}\right) = ((W1 - W2))\ x\ 1000ml\ \text{of the filterate used}$$

Equation 4

Where
W_1 = initial weight of evaporating dish
W_2 = Final weight of the dish (evaporating dish + residue).

Nitrate

Nitrate was determined using the phenol disulphonic acid method as described by Jackson (1973) and Trivedy and Goel (1984). Fifty milliliters (50 mL) of the water sample were evaporated over a hot plate until residues formed. The residues were dissolved in 3 mL of phenol disulphonic acid. The reaction was allowed to stand for 10 minutes, after which 15 mL of distilled water was added. Next, 7 mL of ammonia solution was added, and the final volume was adjusted to 50 mL. The intensity of yellow color transmission was measured at 410 nm. The concentration of NO_3-N (in mg/L) was obtained from the calibration curve and computed using the following formula:

$$Nitrate\ N = \frac{mg\ of\ Nitrate\ N}{ml\ of\ Sample}$$

Equation 5

Phosphate

Phosphorus was estimated as phosphate in the water sample in four forms: total ortho, acid hydrolyzable, total, and organic phosphate, following the APHA (2012) method. Phosphate determination was performed using the vanadomolybdo phosphoric acid method. In this method, ammonium molybdate reacts under acidic conditions in the presence of vanadium to form yellow vanadomolybdo phosphoric acid. The percentage transmission of the yellow color was measured at 490 nm. The phosphate value was determined using a calibration curve prepared from a standard solution. The amount of phosphorus per liter was calculated using the following formula:

Phosphate mg/l = mg P x 1000 ml of sample

Equation 6

Experimental Set-up

The crude oil-contaminated water was used to prepare the experimental setup. The setup consisted of eight treatments, including a control, in 1500 mL conical flasks. The three nutrient supplements used were pasteurized pineapple and watermelon peels (in powdered form) and NPK fertilizer. The details of the experimental setup are presented in Table 1.

Monitoring of the Bioremediation Process

The bioremediation process was monitored over a period of 3 months, at two-week intervals, from November 2023 to January 2024. The microbiological parameters measured included changes in the total heterotrophic bacterial (THB) and hydrocarbon-utilising bacterial (HUB) counts. Changes in the pH, BOD, turbidity, phosphate, nitrate, potassium, and total petroleum hydrocarbon (TPH) levels were also monitored throughout the bioremediation period.

Table 1. Experimental set-up.

Treatment	Volume of water (mL)	Type of supplement	Weight of supplement (g)	Final volume (mL)
Control	1000	None	0	1000
CCW+Pineapple	1000	Pineapple	30	1030
CCW+Watermelon	1000	Watermelon	30	1030
CCW+NPK	1000	NPK	30	1030
CCW+watermelon+Pineapple	1000	Pineapple + watermelon	15+15	1030
CCW+NPK+Pineapple	1000	Pineapple + NPK	15+15	1030
CCW+Watermelon+NPK	1000	Watermelon + NPK	15+15	1030
CCW+Watermelon+NPK+Pineapple	1000	Pineapple+Watermelon+NPK	10+10+10	1030

Keys: CCW = crude oil contaminated water; g = grams, mL = millilitre.

Determination of Total Petroleum Hydrocarbon (TPH)

Residual total petroleum hydrocarbon (TPH) was extracted from the samples and quantified using the Gas Chromatography-Flame Ionization Detector (GC-FID). The analysis was conducted using a Shimadzu GC-17A gas chromatograph with a flame ionization detector. Samples were extracted using liquid-solid and liquid-liquid extraction methods, respectively. A DB-I column was used with 30 m × 0.2 mm, 0.25 µm film thickness, and 0.32 i.d. Helium was the carrier gas at a flow rate of 1 ml/min. Analyses were carried out in split injection mode using a divided ratio 5:1. The injection port was set at 250^{o}C. The samples were automatically detected as they emerged from the column by the FID detector.

Percentage bioremediation

This was calculated as follows:

Step 1: The amount of pollutant remediated equals to Initial concentration of pollutant (Day 1) minus the Final concentration of the pollutant at the end of the experiment (Last day).

Step 2: Percentage (%) bioremediation equals to amount of pollutant remediated divided by Initial concentration of pollutant (Day 1) multiplied by 100.

BC = IC – FC Equation 7

Where
BC = Amount of pollutant remediated
IC = Initial concentration of pollutant (Day 0 or 1)
FC = Final concentration of a pollutant at end of the experiment (Last day)

$$\% \text{ Bioremediation} = \frac{BC \times 100}{IC}$$ (Nrior and Mene, 2017) Equation 8

RESULTS

Baseline data

The total heterotrophic bacteria load was 1.7±1.1×10^{6} CFU/mL, while the hydrocarbon-utilising bacterial count was 1.5±0.4×10^{5} CFU/mL (Table 2). Data also showed that there was no significant difference between the total heterotrophic bacterial and hydrocarbon-utilising bacterial counts.

Results of the bacterial isolates showed that *Staphylococcus* sp., *Serratia* sp., *Shigella* sp., *Bacillus* sp., *Micrococcus* sp., *Tatumella* sp. and *Proteus* sp. were the total heterotrophic bacteria isolated. In contrast, *Pseudomonas* sp., *Alcaligenes* sp., *Bacillus* sp., *Cedecea* sp. and *Flavobacterium* sp. were hydrocarbon-utilising bacteria isolated from the crude oil-polluted water.

The baseline physicochemical parameters of the water sample showed that the value of the parameters were pH (5.49), temperature (28.3°C), dissolved oxygen (2.31 mg/l), turbidity (165 NTU), total dissolved solids (51 mg/l), electrical conductivity (102.1 uS/cm), total suspended solids (3.05mg/l), biological oxygen demand (26.67), total organic carbon (2.66 mg/l), nitrate (2.04 mg/l), and phosphate (6.7 mg/l). The TPH was 354.87 mg/L (Table 3).

Table 2. Profile bacterial counts.

Sample	THB (×10^{6})	HUB (×10^{5})
Crude oil polluted water	1.7±1.1^{a}	1.5±0.4^{a}

*Means with similar superscript across the rows showed no significant difference (P>0.05).

Table 3. Physicochemical parameter profile of habitat water.

Parameter	Water sample	WHO (2011) limits
pH	5.49	6.5-8.5
Temperature	28.3	26-28
Dissolved Oxygen (DO) (mg/l)	2.31	7.5
Turbidity (NTU)	165	5
Total Dissolved Solids (TDS) (mg/l)	51	-
Electrical Conductivity (EC) uS/cm	102.1	1000
Total Suspended Solids (TSS) (mg/l)	3.05	-
Biological Oxygen Demand (BOD) (mg/l)	26.67	15
Total Organic Carbon (mg/l)	2.66	-
Nitrate (NO^{3-}) (mg/l)	2.04	10
Phosphate (PO_4^{3-}) (mg/l)	6.7	-
Total Petroleum Hydrocarbon (TPH) (mg/l)	354.87	40

Biodegradation of crude oil

Data on the changes in the total heterotrophic bacterial (THB) load of the set-up during the bioremediation showed that the THB ranges were day 1 (6.0×10^5-7.2×10^6cfu/ml), day 14 (3.4×10^7 – 4.8×10^8), day 28 (2.3×10^7 – 9.1×10^7), day 42 (3.5×10^6 – 3.4×10^7), day 56 (2.6×10^6 – 4.0×10^7) and day 70 (4.1×10^6 to 3.6×10^7 CFU/ml) (Table 4).

The changes in the hydrocarbon utilising bacteria (HUB) load of the samples during the bioremediation showed that the HUB ranges were day 1 (7.0×10^3 – 3.9×10^4), day 14 (2.7×10^5-1.4×10^6), day 28 (1.2×10^4 – 1.2×10^6), day 42 (6.5×10^4 – 2.9×10^5), day 56 (3.5×10^4 – 2.4×10^5), and day 70 (5.8×10^5 – 2.9×10^5 CFU/ml). Similar to the THB, there were no significant differences ($P>0.05$) observed in the HUB counts of the treatments on the respective days (Table 5).

Table 4. Change in total heterotrophic bacterial load (CFU/mL) during bioremediation of crude oil contaminated freshwater.

Treatment	Day 1 ($\times10^6$)	Day 14 ($\times10^8$)	Day 28 ($\times10^7$)	Day 42 ($\times10^7$)	Day 56 ($\times10^6$)	DAY 70 ($\times10^6$)
Control	7.2±0.8[a]	1.1±0.2[a]	2.5±0.4[a]	1.4±0.2[a]	2.6±0.3[a]	4.1±0.6[a]
CCW+Pineapple	5.7±0.7[a]	1.3±0.2[a]	4.1±0.5[a]	0.35±0.3[a]	9.1±0.1[a]	8.6±1.1[a]
CCW+Watermelon	5.1±0.6[a]	1.1±0.3[a]	6.1±0.8[a]	2.7±0.4[a]	15.2±2.1[a]	12.6±1.7[a]
CCW+NPK	2.1±0.3[a]	0.56±0.6[a]	9.1±1.2[a]	3.4±0.5[a]	5.1±0.7[a]	15.2±2.1[a]
CCW+Watermelon+Pineapple	6.6±0.9[a]	1.0±0.1[a]	5.1±0.6[a]	2.2±0.3[a]	6.1±0.8[a]	4.1±0.5[a]
CCW+NPK+Pineapple	0.60±0.5[a]	0.41±0.05[a]	2.6±0.3[a]	1.7±0.2[a]	5.6±0.7[a]	36.4±5.0[a]
CCW+Watermelon+NPK	2.1±0.3[a]	0.34±0.04[a]	4.5±0.6[a]	2.6±0.4[a]	40.6±0.6[a]	5.1±0.6[a]
CCW+Watermelon+NPK+Pineapple	9.6±1.3[a]	4.8±0.6[a]	2.3±0.3[a]	1.4±0.2[a]	25.4±3.4[a]	23.3±3.2[a]
P-value	0.916	0.977	0.969	0.974	0.784	0.843

*Means with similar superscript down the group showed no significant difference ($P>0.05$).
CCW = Crude oil-contaminated water.

Table 5. Change in hydrocarbon-utilising bacterial load (CFU/mL) during bioremediation of crude oil contaminated freshwater.

Treatment	DAY 1 (×10^4)	DAY 14 (×10^6)	DAY 28 (×10^5)	DAY 42 (×10^5)	DAY 56 (×10^5)	DAY 70 (×10^5)
Control	2.3±0.2 [a]	1.1±0.1 [a]	8.9±1.0 [a]	2.0±0.2 [a]	0.58±0.5 [a]	0.61±0.6 [a]
CCW+Pineapple	3.6±0.3 [a]	1.1±0.2 [a]	2.7±0.3 [a]	2.97±0.3 [a]	0.35±0.3 [a]	0.90±0.8 [a]
CCW+Watermelon	6.6±2.3 [a]	1.4±0.5 [a]	8.4±0.9 [a]	0.70±0.07 [a]	2.1±0.2 [a]	1.7±0.2 [a]
CCW+NPK	0.70±0.4 [a]	1.2±0.1 [a]	11.6±1.3 [a]	2.9±0.3 [a]	1.2±0.1 [a]	1.5±0.2 [a]
CCW+Watermelon+Pineapple	27.5±1.3 [a]	1.1±0.1 [a]	0.12±0.11 [a]	0.65±0.1 [a]	0.74±0.4 [a]	0.58±0.5 [a]
CCW+NPK+Pineapple	39.1±3.9 [a]	0.27±0.3 [a]	1.8±0.2 [a]	0.66±0.6 [a]	1.7±0.2 [a]	2.9±0.3 [a]
CCW+Watermelon+NPK	18.6±6.1 [a]	0.59±0.5 [a]	1.7±0.2 [a]	1.1±0.1 [a]	2.4±0.3 [a]	1.5±0.5 [a]
CCW+Watermelon+NPK+Pineapple	4.9±1.4 [a]	0.31±0.3 [a]	2.5±0.3 [a]	2.8±0.3 [a]	1.99±0.2 [a]	1.7±0.2 [a]
P-value	0.223	0.926	0.642	0.781	0.880	0.889

*Means with similar superscript down the group showed no significant difference (P>0.05).
Keys: A = Pineapple, B = Watermelon, C = NPK fertilizer, CCW = Crude oil-contaminated water.

Changes in physicochemical parameters and TPH

The results of the changes in pH during the bioremediation showed that the pH varied across the period of bioremediation as well as within the respective treatments. The pH ranged from acidic (3.81) to slightly acidic (6.87) and also, the pH increased from a more acidic nature from the initial day to a slightly acidic nature at the end of the bioremediation (Table 6). Change in biological oxygen demand (BOD) during the bioremediation showed that the BOD fluctuated and was highest in all the treatments on day 28 but decreased from days 42, 56 and 70, respectively (Figure 1).

Results of the total organic carbon during the bioremediation period showed that the TOC decreased across the period of the bioremediation in most of the treatments while in some of the treatments, there were slight fluctuations (Figure 2). Changes in nutrient concentration (nitrate, phosphate and potassium) of the setup during the period of bioremediation are presented in Figures 3 to 5, respectively. The results showed that all the nutrients decreased for the length of the bioremediation. Results further showed that phosphate had the lowest concentration on day 70 of the bioremediation compared to nitrate and potassium.

The chromatograph of the set-up for the period of bioremediation is presented in Figure 6 to 9.

Table 6. Change in pH concentration during the period of bioremediation of crude oil contaminated freshwater.

Treatment	DAYS					
	1	14	28	42	56	70
Control	5.41±0.0	6.82±0.0	6.75±0.0	6.55±0.0	6.84±0.0	6.87±0.0
CCW+PINEAPPLE	3.69±0.0	5.74±0.0	6.18±0.0	6.12±0.0	6.05±0.0	6.21±0.0
CCW+WATERMELON	4.05±0.0	6.53±0.0	6.49±0.0	6.7±0.0	6.5±0.0	6.8±0.0
CCW+NPK	4.69±0.0	5.71±0.0	6.19±0.0	5.8±0.0	6.03±0.0	6.24±0.0
CCW+WATERMELON+NPK	4.42±0.0	4.86±0.0	5.79±0.0	5.7±0.0	5.63±0.0	5.53±0.0
CCW+NPK+PINEAPPLE	3.81±0.0	4.97±0.0	5.81±0.0	5.4±0.0	5.04±0.0	5.61±0.0
CCW+WATERMELON+PINEAPPLE	3.82±0.0	4.91±0.0	6.14±0.0	6.1±0.0	5.63±0.0	5.76±0.0
CCW+WATERMELON+NPK+PINEAPPLE	4.15±0.0	6.31±0.0	6.73±0.0	6.4±0.0	6.42±0.0	6.85±0.0
P-value	No variance	No variance	No variance	No variance	No variance	No variance

Keys: CCW = Crude oil-contaminated water.

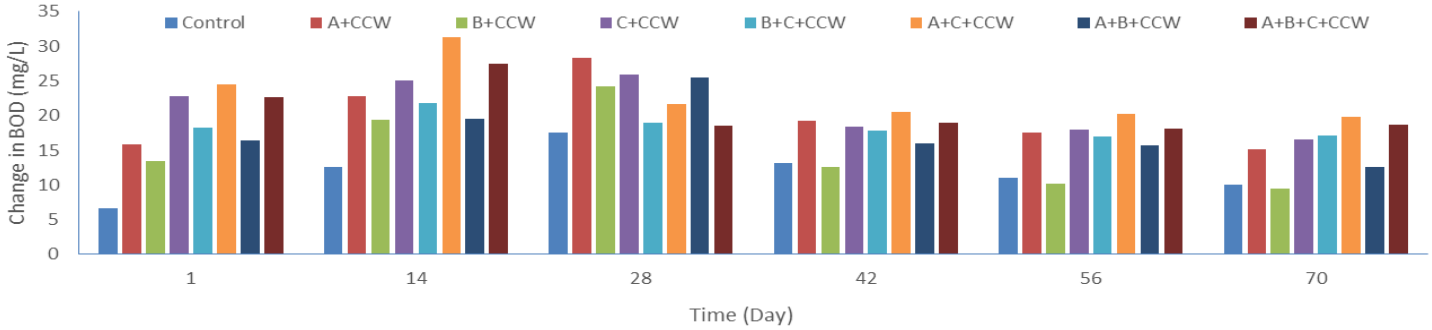

Figure 1. Changes in biological oxygen demand (BOD) (mg/l) during the period of bioremediation of crude oil contaminated freshwater.
Keys: A = Pineapple, B = Watermelon, C = NPK fertilizer, CCW = Crude oil-contaminated water.

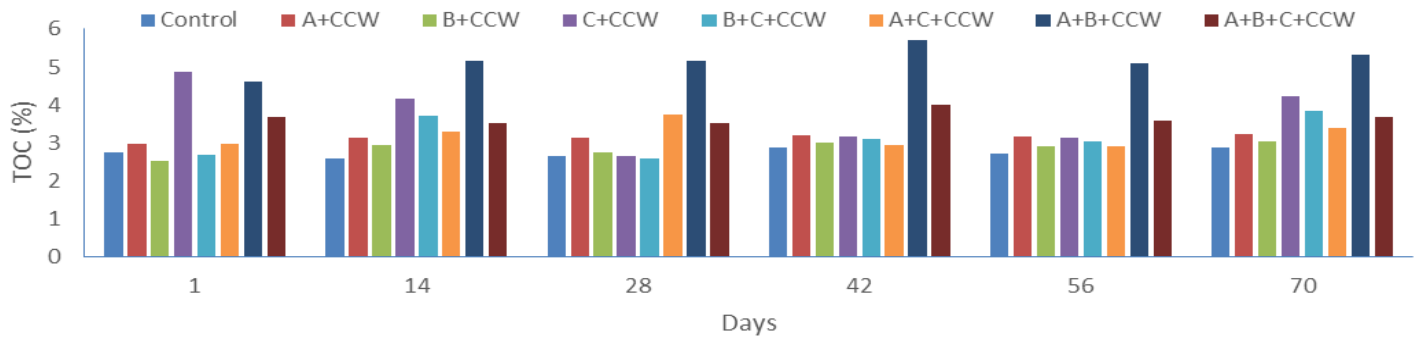

Figure 2. Changes in total organic carbon (TOC) (%) during the period of bioremediation of crude oil contaminated freshwater.

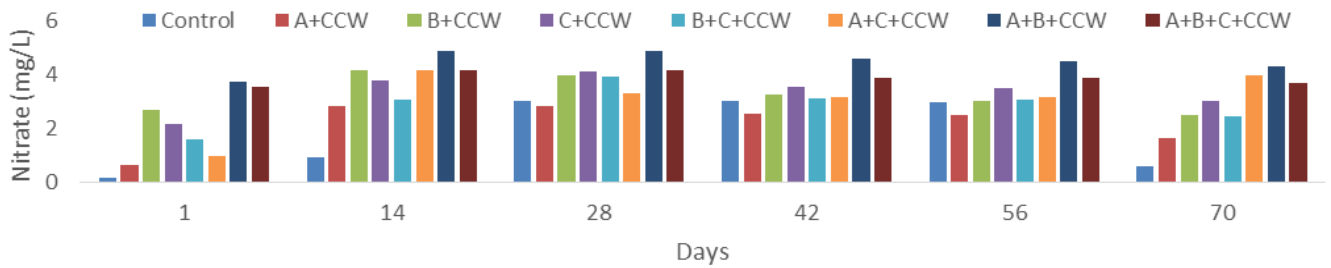

Figure 3. Changes in nitrate concentration (mg/l) during the bioremediation period of crude oil contaminated freshwater.
Keys: A = Pineapple, B = Watermelon, C = NPK fertilizer, CCW = Crude oil-contaminated water.

Figure 4. Change in phosphate concentration (mg/l) during the bioremediation period of crude oil contaminated freshwater.
Keys: A = Pineapple, B = Watermelon, C = NPK fertilizer, CCW = crude oil-contaminated water.

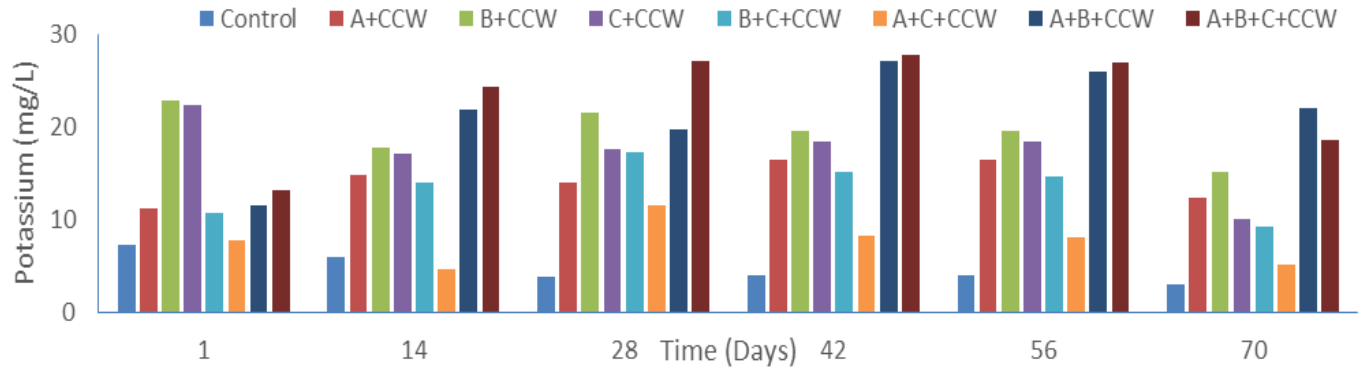

Figure 5. Changes in potassium concentration (mg/L) during the bioremediation period of crude oil contaminated freshwater.

Results of the percentage bioremediation of crude oil in the crude oil-polluted water are presented in Figure 10. Results showed that the setup CCW+NPK (NPK supplemented treatment) had the highest bioremediation potential of 57.2% followed by CCW+Pineapple (pineapple supplemented treatment) and the third highest bioremediation potential of 40.8% was observed in CCW+Watermelon (watermelon supplemented treatment). The consortium had the least bioremediation potential.

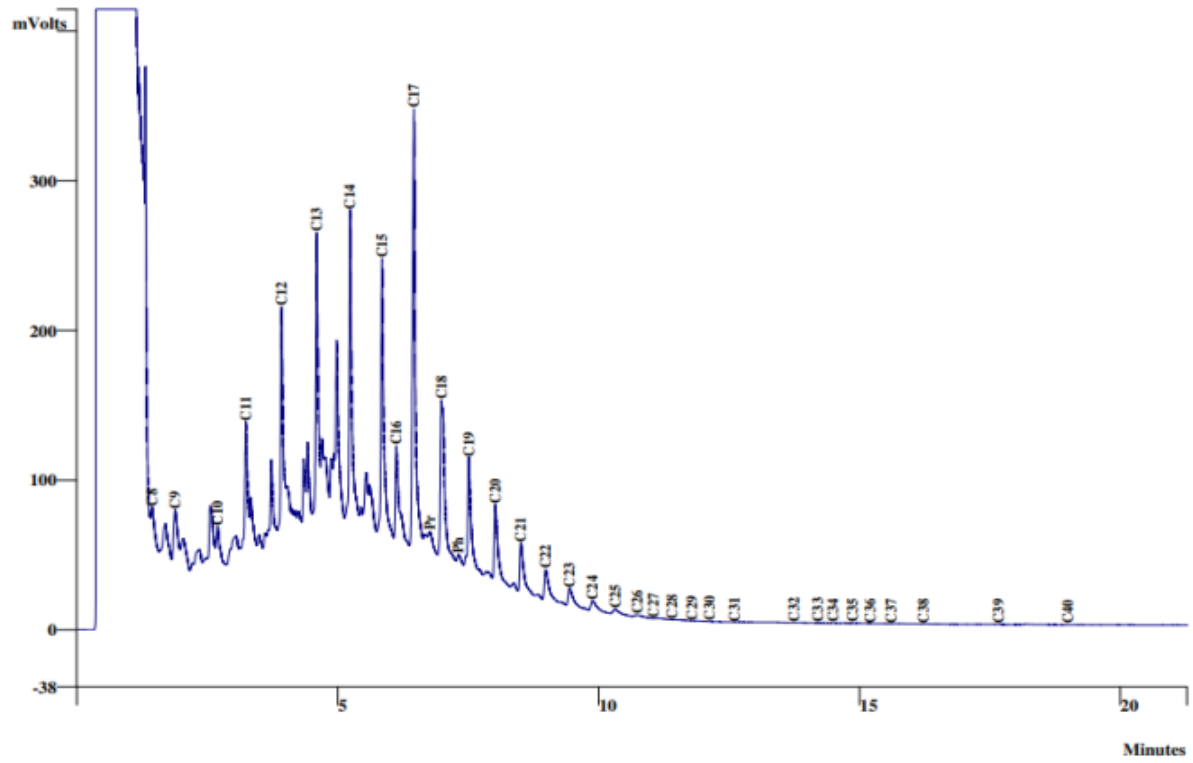

Figure 6a. Chromatogragh of the control in day 1.

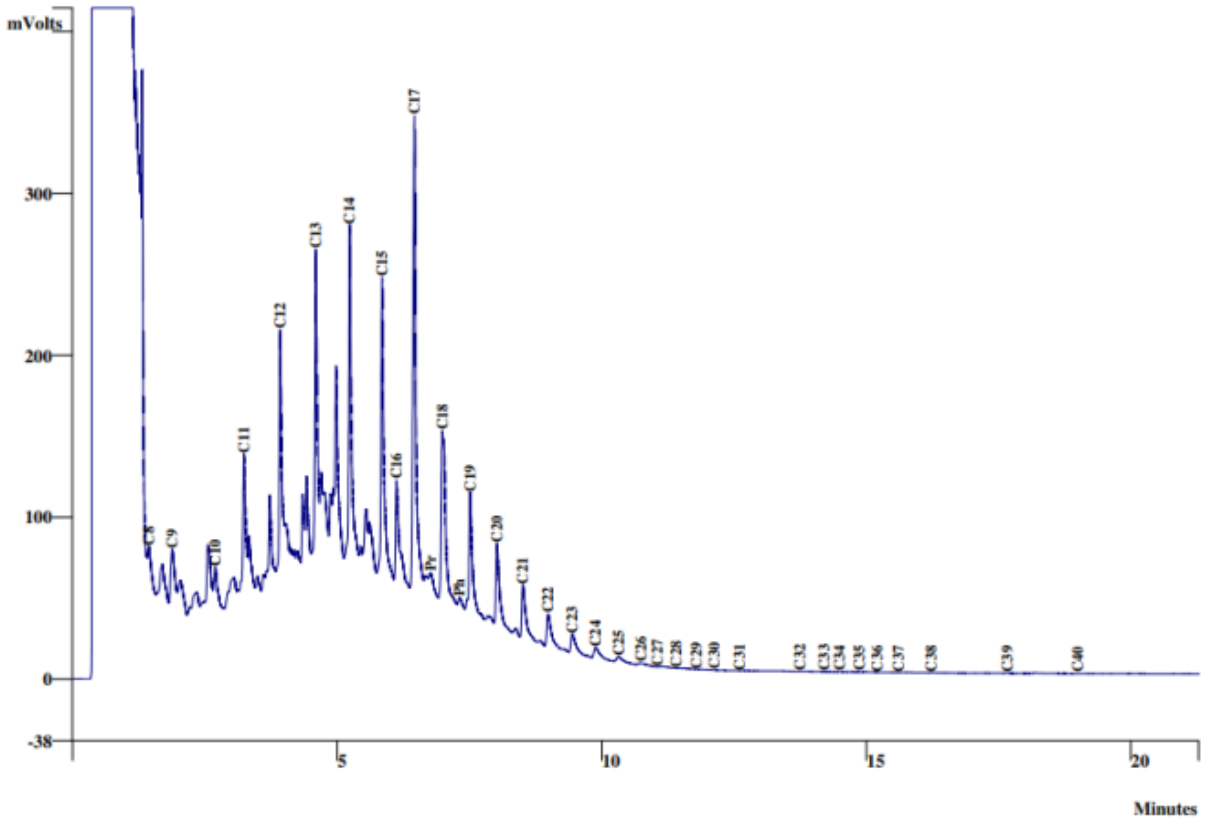

Figure 6b. Chromatograph of CCW+Pineapple treatment for day 1.

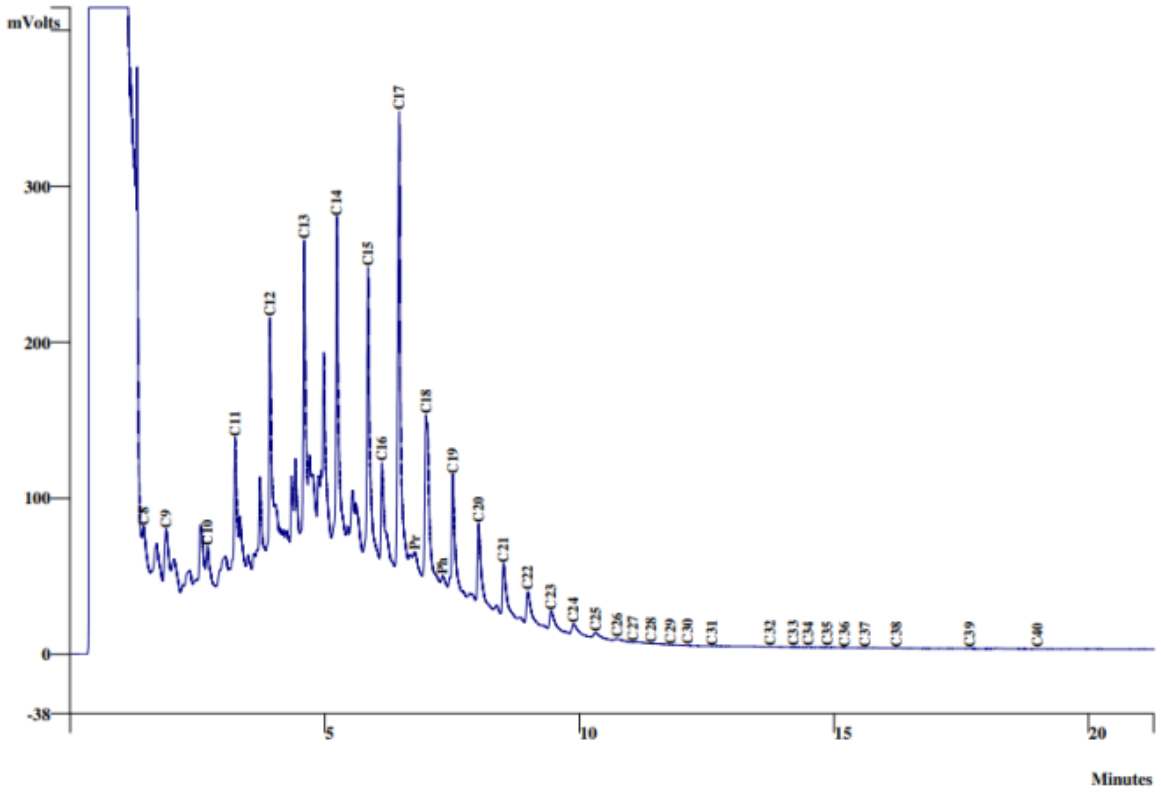

Figure 7a. Chromatograph of CCW+Watermelon treatment for day 1.

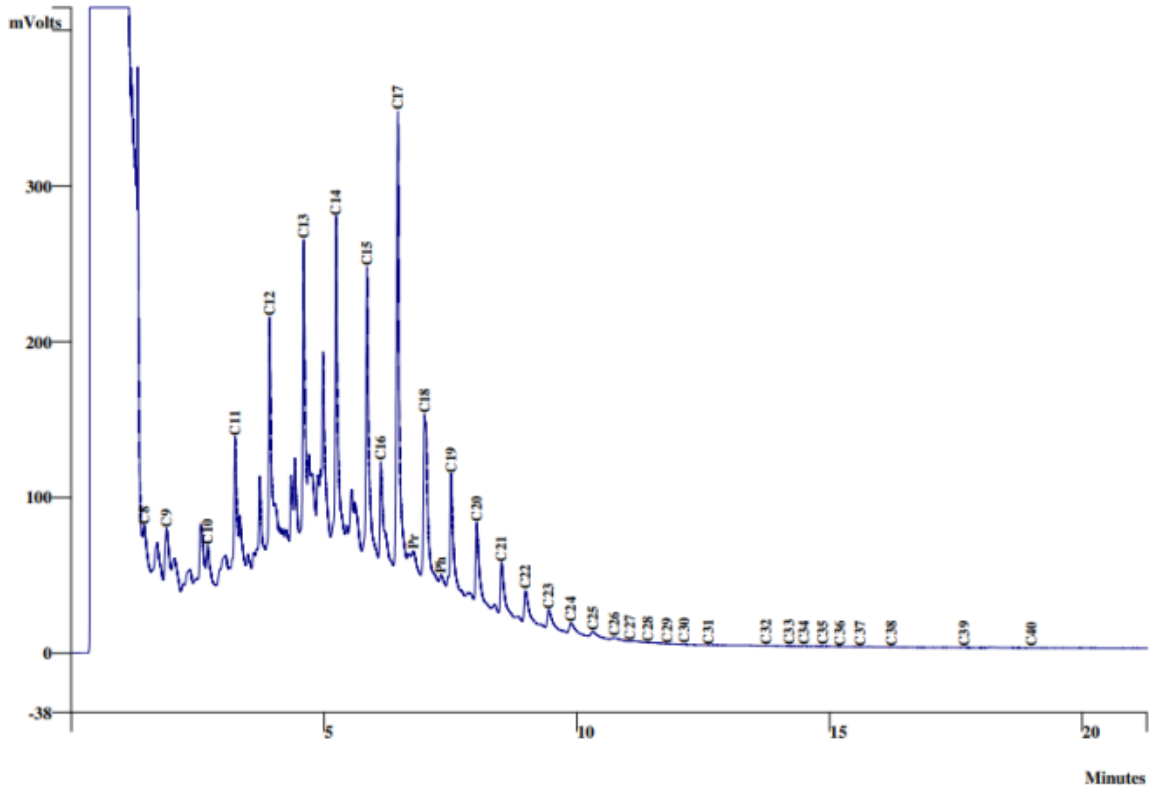

Figure 7b. Chromatograph of CCW+NPK treatment for day 1.

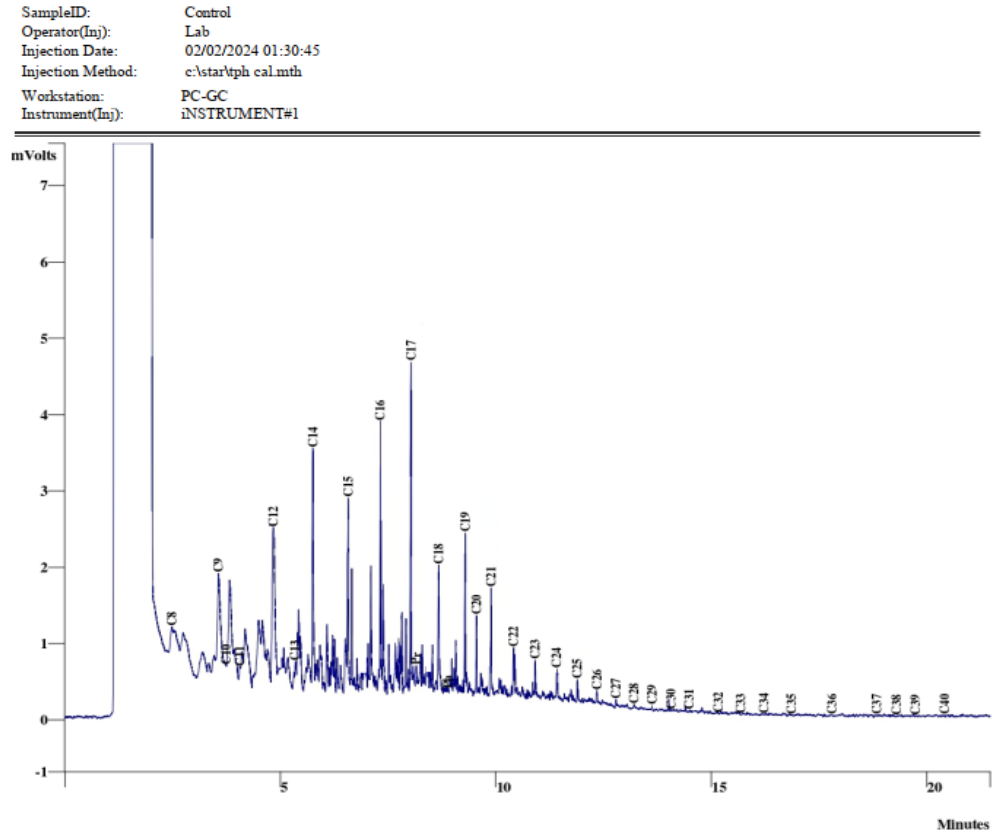

Figure 8a. Chromatograph of control in day 56.

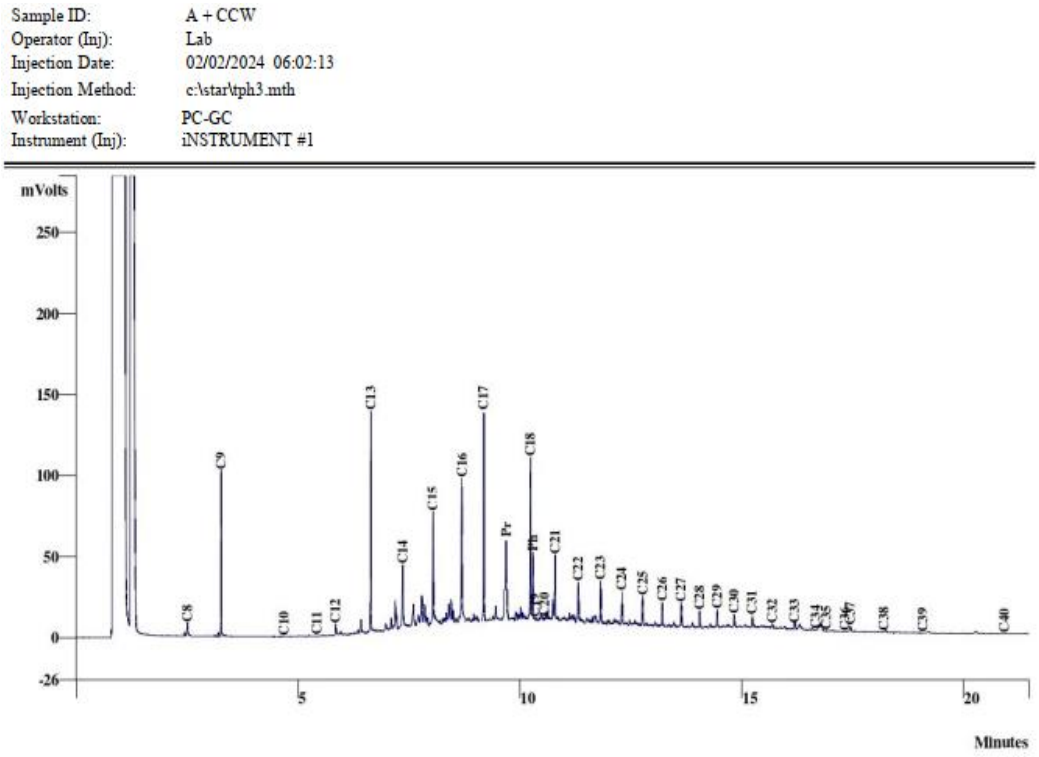

Figure 8b. Chromatograph of CCW+Pineapple in day 70.

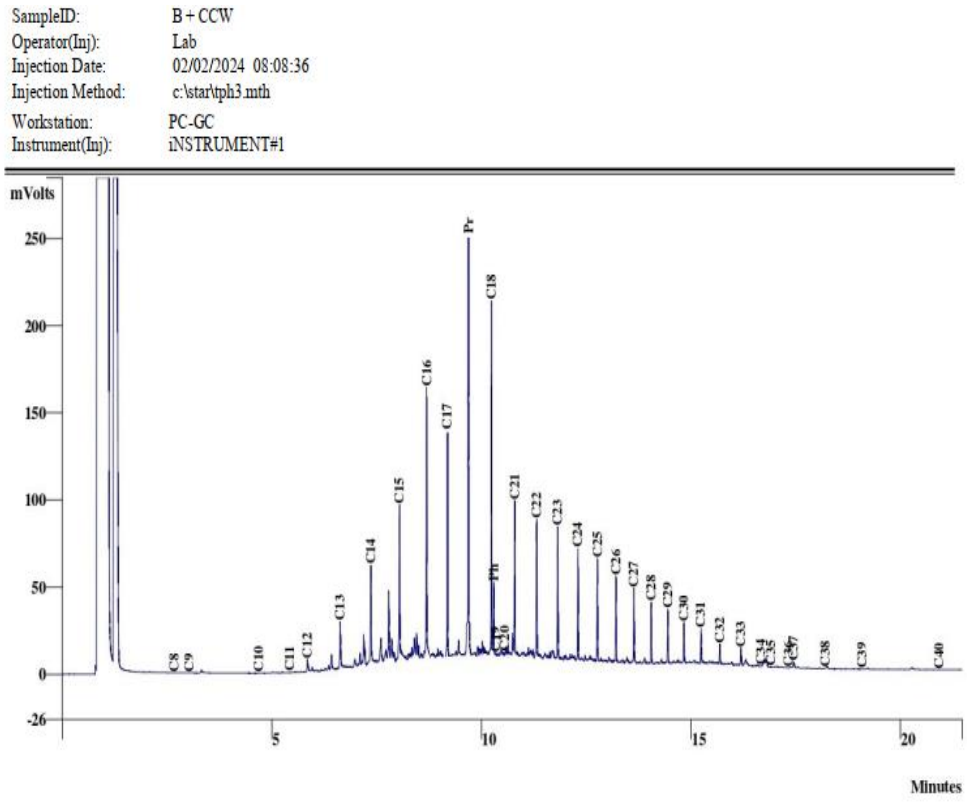

Figure 9a. Chromatograph of CCW+Watermelon in day 56.

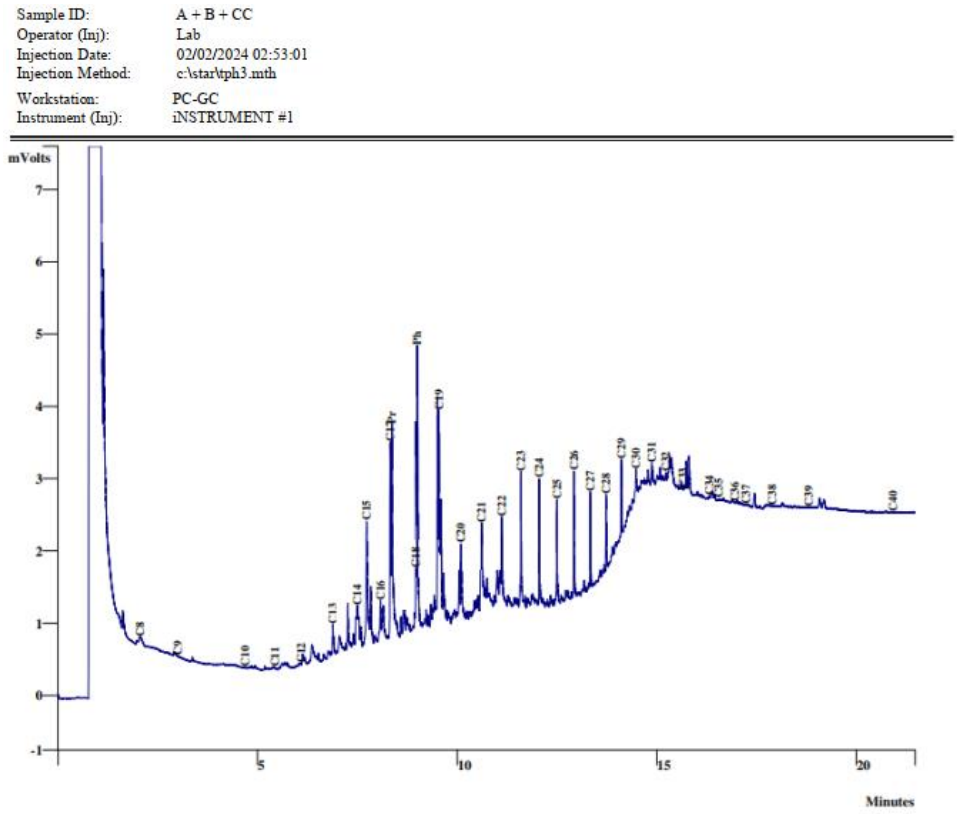

Figure 9b: Chromatograph of CCW+Watermelon+Pineapple in day 70.

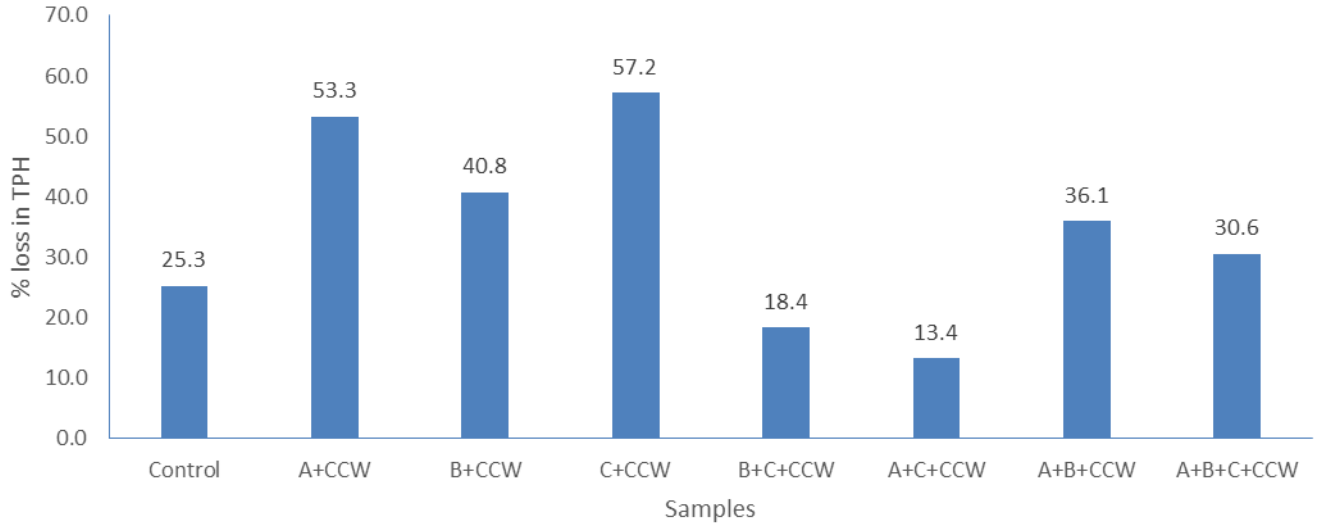

Figure 10. Percentage (%) loss of total petroleum hydrocarbon (TPH) after the final day of remediation.
Keys: A = Pineapple, B = Watermelon, C = NPK fertilizer, CCW = Crude oil-contaminated water.

DISCUSSION

Baseline study

The hydrocarbon-utilising bacteria and the total heterotrophic bacterial load obtained from the polluted water showed that despite the pollution, there were still indigenous bacteria present, and these bacteria could either be transient organisms or utilisers of the crude oil components.

The bacteria genera reported during the profile analysis of the crude oil-polluted freshwater could be the viable bacterial counts recorded. These bacterial genera, as earlier posited, could possess certain enzymes or strains that could adapt and utilise the crude oil.

The physicochemical parameters and total petroleum hydrocarbon (TPH) varied, and most of them were not within the recommended limits. For instance, the TPH value was higher than the 40 mg/L permissible limit. Thus, the need for bioremediation, especially as this value indicates pollution of the freshwater body with crude oil.

Bioremediation of crude oil-polluted water

Change in bacterial load

The disparity observed in the total heterotrophic bacteria and hydrocarbon-utilising bacteria load of the different treatments across the bioremediation period could be explained by their capacity to use the components of crude oil, available nutrients, or degree of exposure to hydrocarbon content. This was in line with the findings of Kawo and Bacha (2016), who held a similar opinion and explained that the high total heterotrophic populations and low percentage of bacteria that used crude oil were caused by the possibility that the environment from which the samples were taken had not previously experienced significant and repeated pollution from crude oil. Generally, the bacterial counts increased exponentially from day 14 in all treatments, with a slight decrease in Day 56 and Day 70. More so, there were no significant differences ($P > 0.05$) between the treatments and the control on the respective days of the hydrocarbon utilising bacteria and the total heterotrophic bacterial counts. On day 1, the control had the highest bacterial load ($7.2\pm0.8\times10^6$) compared to the pineapple, watermelon and NPK treatments. More so, findings showed that the consortium CCW+NPK+Pineapple, CCW+Watermelon +Pineapple and CCW+Watermelon+NPK had the highest HUB counts on day 1 compared to the experimental setups with single treatments and control, while for days 14, 28, and 42, the setup CCW+NPK had the highest HUB counts ($2.9\pm0.3\times10^5$). The increase in bacterial load could be attributed to the availability of nutrients (nitrate, phosphate and potassium), which were readily made available by the organic and inorganic nutrients added. Thus, as the nutrients depleted, the counts reduced. Sang-Haw et al. (2007) reported similar results, concluding that the population of hydrocarbon-degrading bacteria increased rapidly during the first 30 days of their bioremediation study. They proposed that the feasibility of bioremediation in an oil-polluted environment could be assessed using this discovery as a benchmark. However, over time, as a result of oil-resistant components with high chains and in the presence of fewer nutrients, bacterial growth and oil degradation declined (Schaefer and Juliane, 2007). This corroborates the present study, which also showed a decline in the bacterial load on day 70.

Changes in physicochemical parameters

It was observed that as the bioremediation time increased, the pH of the samples stimulated with nutrients slightly increased. The fluctuations in the pH, especially on the samples could suggest bioremediation (Edward et al., 2019). Thus, the crude oil pollution in the water was broken down into less toxic and acidic byproducts, as evidenced by the rise in pH values. For the bioremediation of crude oil-polluted water, Amenaghawon et al. (2014) reported similar outcomes. Changes in pH resulting to fluctuations in pH values were also reported in a previous study (Sampson et al., 2016). Furthermore, the pH of the crude oil-polluted freshwater samples, including the experimental setups, was below the DPR and WHO pH limits of 6.5-8.5 for water. According to Edoris and Iyama (2017), pH has a significant impact on the types of organisms found in the environment, the availability of nutrients to plants, and the solubility of metals (Muyoma et al., 2018). The observed low pH of the crude oil-polluted water samples could indicate that the crude oil made the samples more acidic. This supports a previous study of Nweze and Aniebonam (2009), who reported that a decrease in the pH of polluted samples is suggestive of the fact that petroleum pollutants cause habitats to become more acidic, which may alter biodiversity.

The decrease in the BOD in the crude oil-polluted water supplemented with various nutrients as well as the control could be attributed to the microbial activities of both the indigenous and biostimulated microbial consortium present in the crude oil-polluted water, which breaks down crude oil into less toxic substances like CO_2, H_2O and numerous intermediates like organic acids, lipids, esters, complex alcohols, and microbial proteins in the form of enzymes (Obahiagbon et al., 2014). BOD serves as a gauge for the amount of oxygen required by microorganisms during the biodegradation of organic materials. According to Amenaghawon et al. (2013), a decrease in BOD therefore indicates a decrease in the organic matter in the contaminated crude oil water. The decrease was more significant with polluted water samples stimulated with watermelon waste (CCW+Watermelon). A similar trend of results on BOD reduction on crude oil contaminated water stimulated with nitrates was reported by Amenaghawon et al. (2014); Satyawali and Balakrishnan (2008).

The total organic carbon (TOC) is an indicator used in measuring the extent of organic pollution in a water body or the environment (Owhonka and Obire, 2019, 2020). The high total heterotrophic bacterial load observed during the bioremediation period may have contributed to the reduction of the TOC, especially in the nutrient supplemented crude oil-polluted water. It is well known that heterotrophic microbes are in charge of using organic carbon, releasing it for use by various food webs (Owhonka and Obire, 2020). The present study contradicts Albert and Anyanwu (2012), who reported constant TOC in their study.

The decrease in the phosphate, potassium and nitrate concentrations of the experimental setups could be attributed to the depletion of the nutrients by the indigenous and biostimulated microorganisms in the crude oil-polluted water as they utilise or bioremediate the crude oil pollutant. More so, the crude oil-polluted water supplemented with nutrients had higher nitrate, phosphate and potassium than the control, which was void of any nutrient supplement. The pineapple supplemented crude oil-polluted water had a higher nitrate concentration, followed by the consortium of pineapple and watermelon (CCW+Watermelon+ Pineapple), while phosphate and potassium were higher in NPK supplemented crude oil-polluted water. Albert and Anyanwu (2012) reported that phosphate and nitrate depleted during the periods of their bioremediation study. In agreement with the present study is a study by Muhammad et al. (2015) and Sampson et al. (2016), who also reported fluctuation and nutrient depletion during bioremediation. More so, the low concentration of nutrients (nitrate and phosphates) in the control from the first day of bioremediation could be attributed to the effect of crude oil pollutant. This agreed with Hamoudi-Belarbi et al. (2018), who reported that the low phosphate concentration in a crude oil-polluted environment was most likely brought on by the high contamination/pollution index (C/P) ratio as a consequence of the crude oil spill.

The reduction in the total petroleum hydrocarbon from the initial value in all the treatments, including the control, showed that the indigenous microorganisms were utilising the components of the crude oil as carbon sources. Although the reduction in TPH was rapid, especially with the nutrient supplemented crude oil-polluted water, the value of the TPH at the end of the bioremediation was still higher than the 40 mg/L Department of Petroleum Resources (DPR) permissible limits (EGASPIN, 2018). More so, the NPK supplemented was more efficient in TPH reduction as it reduced the TPH from 4753.2±0.07 mg/L to 2032±0.7 mg/L with a percentage reduction of 57.2%, while the pineapple peel supplemented crude oil-polluted water had a percentage TPH reduction of 53.3% and reduced the TPH from 4297.87±0.01 to 2006.45±0.01 mg/L. The watermelon supplemented crude oil-polluted water had the third % TPH reduction of 40.8% and reduced the TPH from 3928.86±0.001 to 2325.6±0.1 mg/L at the end of the 70 day bioremediation period. The respective consortium of nutrients was also effective in reducing the TPH of the crude oil-polluted water body. Thus, the TPH value of the Pineapple supplemented crude oil-polluted water was significantly ($P<0.05$) lower than the TPH values of other treatments. The utilisation of crude oil components by the indigenous microorganisms especially the experimental setups supplemented with nutrient showed that the nutrients increased the growth of the microorganisms which in turn attached properly to the crude oil components thereby reducing it to lesser value. Das and

Chandran (2011) reported that petroleum degradation amongst other factors is mediated by attachment of the microbial cells to the substrates. Onuoha et al. (2020) reported the highest loss of Hydrocarbon in the 84th day treatment with percentage reduction of 89% using pineapple peel as supplement in the bioremediation of crude oil-polluted water and attributed the significant difference to the presence of the available nutrient elements in pineapple. More so, the use of pineapple peel extract in bioremediation has resulted in 40% loss of TPH (Nzenwa et al., 2021). The effective of pineapple waste as one of the major sources of biostimulant in the bioremediation of crude oil polluted environment was also reported by (Mordi et al., 2023).

The use of watermelon peel in the bioremediation of crude oil-polluted environments has been reported. Yusuf and Yahaya (2022) reported a reduction in TPH of 8409.55±15.87 mg/g to 646.15±78.84 mg/g using 20g of watermelon peel and suggested that the impact of the watermelon peels on the reduction of TPH could be attributed to the bioavailability of the nutrients in the organic wastes to bacterial species in the oil-polluted environment. Victor et al. (2015) reported that the biodegradation rate constants of oil-contaminated soil samples amended with agro-waste increased compared to the unamended (control) soil samples. Generally, the importance of NPK and the agro-wastes used in the present study could be seen to be the major players in the stimulation of the indigenous microorganisms to degrade the crude oil components.

CONCLUSION

The freshwater profile study of the physicochemical parameters showed that the pH, turbidity, BOD, COD, nitrate, phosphate, potassium, and TPH were very high and exceeded permissible limits. The NPK fertilizer was the most efficient nutrient during the bioremediation of crude oil, followed by the pineapple peel and the watermelon peel. The % reduction of TPH was highest in the NPK, followed by the pineapple peels. Thus, the nutrients stimulated the reduction of TPH to a lesser value. Similarly, the heavy metals were reduced as a result of the effect of the stimulants. Pineapple peel was one of the most efficient agro-waste in the present study; thus, it is recommended for further study, especially in exploring the concentrations of the agro-waste that have high remediation efficiency.

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