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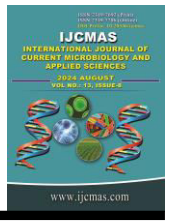

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Bioremediation of Crude Oil-Polluted Environment using Organic Amendments

Ibiene Rogers Longjohn[1]*, Victor Kasi Robinson[2] and Raymond Awortu[2]

[1]*Department of Science Laboratory Technology, Federal Polytechnic of Oil and Gas Bonny, Rivers State, Nigeria*
[2]*Department of Microbiology, Rivers State University Npkolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria*

**Corresponding author*

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A B S T R A C T

The contamination of water surfaces brought about by crude oil pollution has intensified the need for bioremediation. The enhanced bioremediation of crude oil-polluted water using organic amendments was carried out. The water sample was collected in a sterile container from Bonny River. The nutrients used included rhamnolipids and cow dung. Four experimental setup such as control, P+CCW, Q+CCW and P+Q+CCW was designed. The fungi and hydrocarbon-utilising fungi were monitored using standard microbiological techniques. The physicochemical parameters of the samples were determined using the American Public Health Association Standard methods for the examination of water and wastewater. The total petroleum hydrocarbon was determined using the Gas chromatography. Baseline pH of the control and polluted water was 8.4 and 5.6, respectively. The TPH of the control was 47.0mg/l while crude oil polluted water was 5838.8 mg/l. *Rhizopus* sp, *Aspergillus* sp, *Rhodotorula* sp, *Penicillium* sp, *Geotrichum* sp and *Mucor* sp were the hydrocarbon utilizing fungal isolates. The pH range for Days 1, 28 and 56 was 3.69-5.41, 5.62-6.21 and 5.81-6.84, respectively. The change in phosphate concentration for Days 1, 28 and 56 was 0.31-2.74 mg/l, 0.24-1.77 mg/l and 0.19-1.83 mg/l, respectively. The nitrate range for Days 1, 28 and 56 was 0.61-4.2 mg/ml, 0.94-3.82 mg/l and 0.15-2.55 mg/l. The % loss in TPH ranged from 21.6-52.6%. The Cow dung had the highest percentage loss followed by the rhamnolipids amendment. Both amendments could be used in bioremediation of crude oil polluted water. More so, it is recommended to use them singly rather than in consortium since they had better results when used alone.

Introduction

The quality of the water greatly impacts the health of people. The World Health Organisation estimates that inadequate water quality results in 829,000 deaths annually (WHO, 2022). A number of pollutants, including pesticides, fertilisers, dyes, hydrocarbons, heavy metals, and medications cause water pollution. In

recent years, there has been an apparent spike in concerns regarding the presence and contamination of hydrocarbon components and heavy metals in our water resources (Briffa *et al.,* 2020; Masindi and Muedi, 2018; Hazen *et al.,* 2016). Elements like cadmium (Cd) and mercury (Hg) are toxic to humans at relatively low concentrations. Humans are also affected by silver (Ag), chromium (Cr), lead (Pb), copper (Cu), and zinc (Zn), albeit at much higher concentrations than those needed to cause toxicity from cadmium or mercury (Murugesan *et al.,* 2006). More so, crude oil which is an extremely complex mixture of aliphatic and aromatic hydrocarbons has been found to cause chronic sub-acute toxicological effects, alter population dynamics and disrupt trophic interactions and the structure of natural communities within ecosystems (Bejarano and Michel, 2010). In locations like Bonny, Nigeria where seawater is a source of livelihood, removing harmful contaminants from aquatic environments is imperative. Since toxic metals have been a major source of contamination in aqueous systems due to human activity, there is need for effective and affordable ways to remove them. For centuries, metals have been removed from aqueous streams using physico-chemical methods such as adsorption, ion exchange, and chemical precipitation. These methods require the use of a large number of chemicals at high cost, even with the risk of incomplete removal, particularly when the concentrations of contaminants are between 10 and 100 mg/L (Adewoye *et al.,* 2021). In addition, they take a lot of time and produce more waste (Moosavi *et al.,* 2020; Qasem *et al.,* 2021). Due to their ability to adsorb toxic metals from aqueous systems, agro-wastes and other plant wastes have recently attracted attention as sorbents for toxic metals in aqueous systems (Ahmad and Zaidi, 2020; Çelebi *et al.,* 2020). More so, fungi have been shown to play significant roles in the bioremediation of contaminants including hydrocarbon components and pesticides (Prasad, 2017). The present study investigated the effect of organic amendments in the removal of total petroleum hydrocarbon from contaminated aquatic body.

Materials and Methods

Source of Water Sample

The water sample was collected in a sterile container from a part of the Bonny River known to have high anthropogenic activity including oil spillage. The GPS coordinates of the location was 4º26'55.00428''N, 7º10'19.60932'E, Bonny Local Government Area, Rivers State, Nigeria. The water sample was collected from three points to make a composite of one sample from the location. This was placed in an iced-packed container and transported to the Microbiology Laboratory, for analysis.

Organic Supplements

Rhamnolipid was bought from Jochemicals Nigeria Limited, Choba, Rivers State, Nigeria. The cow dung was obtained from the Rivers State University School farm. The organic amendments were transferred to the Microbiology Laboratory, Rivers State University.

Baseline Analysis of Sample

The baseline analysis of the pH, Temperature (℃), Electrical conductivity (µS/cm), Turbidity, nitrate, phosphate, dissolved oxygen (mg/l), biological oxygen demand (mg/l), and total hydrocarbon content (mg/l) was determined using the (APHA, 2012). The fungal counts and hydrocarbon-utilizing fungi were also determined using the standard plate count (Prescott *et al.,* 2011).

Enumeration and Isolation of Fungal Counts

The total fungal counts of the water sample were enumerated using the spread plate method (Prescott *et al.,* 2011). In this method, ten-fold serial dilutions of the water sample were carried out by transferring 1ml of the sample with the aid of a sterile 1ml pipette and transferred to a sterile 9 mL normal saline to make a dilution of 10^{-1}. Subsequent tenfold serial dilutions were carried out until 10^{-3} was achieved. Aliquots from the 10^{-1} and 10^{-2} dilutions were inoculated on the surface of freshly prepared Sabouraud Dextrose agar plates in duplicates and spread evenly using a sterile bent glass rod before incubating at 25°C for 5 days. After incubation, plates were observed and counts were recorded for estimation of the spore-forming units (SFU).

Enumeration and Isolation of Hydrocarbon Utilizing Fungi

The hydrocarbon utilizing fungi of the water sample was enumerated using the spread plate method on prepared pre-dried mineral salt agar. The composition of the mineral salt agar was: agar-agar (15g), K_2HPO_4 (0.5 g/L), $MgSO_47H_20$ (0.3 g/L), NaCl (0.3 g/L), $MnSO_4H_2O$ (0.2] g/L), $FeSO_46H_2O$ (0.2 g/L), $NaNO_2$ (0.3 g/L), and $ZnCl_2$ (0.3g/L). The mineral salt agar after preparation was supplemented with tetracycline to inhibit bacterial

growth. An aliquot (0.1 ml) from 10^{-1} and 10^{-2} dilutions was inoculated in duplicate on the surface of the prepared, mineral salt agar and spread evenly using a bent glass rod. After inoculation, sterile filter paper was inserted inside the lid of the petri dish and 1mL crude oil was transferred onto the filter paper. Plates were counted and the SFU was calculated after 3-7 days of incubation.

Characterization of fungal Isolates

The fungal isolates were identified based on morphological features (colony colour, shape, texture and size of colony) and microscopic examination (conidial shape, arrangement of hyphae and type of spore) of their wet mounts prepared with lactophenol cotton blue and reference made to fungal identification manual (Sarah *et al.,* 2016).

Experimental Design

The water sample was polluted with 5% crude oil (Bonny light crude) and allowed to stand for 21 days (Ogbonna *et al.,* 2019). The crude oil-contaminated water was used in preparing the experimental set-up. The experimental setup consisted of four pots which includes the control in 500 mL conical flasks and three treatments. The heterotrophic fungal and hydrocarbon utilising fungal counts were monitored during the bioremediation period likewise the phosphate, nitrate and total petroleum hydrocarbon. The study was carried out for 3 months. The experimental set-up is presented in Table 1.

Determination of pH

The pH of soil sample was determined by APHA Standard Methods (APHA, 2012). The meter was switched on and allowed for some time. It was then calibrated with buffer solutions of high pH range between 8 and 9 as well as a lower pH range between 1 and 6 by dipping the electrode into the buffer solutions. Fifty millilitres (50 ml) of the water sample were transferred in to a beaker and the pH meter was immersed into it and the pH values for each sample/ treatment were recorded accordingly.

Determination of Phosphate (PO_4^{3-}) (Phosphomolybdenum blue (PMB) method)

Nitrate was determined by Phenol Di-Sulphonic acid method. Fifty milliliters (50 ml) of the water sample was taken and evaporated over a hot plate till residues were formed, which was dissolved in three milliliters (3 ml) of phenol Di-Sulphonic acid. The reaction was allowed to stand for 10 minutes and then fifteen milliliters (15 ml) of distilled water was added. Seven milliliters (7 ml) of ammonia solution was added and the final volume was made to be fifty milliliters (50 ml). The intensity of yellow color transmission percentage was measured at 410 nm. The Values of NO_3-N as mg/l was obtained in reference to the calibration curve and value was computed in the following formula: -

$$\text{Mg/l Nitrate N} = \frac{\text{mg Nitrate N}}{\text{ml of sample}} \quad \ldots \text{Eqn. 1}$$

Phosphate

Phosphorous was estimated as phosphate in the water sample (APHA, 2012). The determination was made by Vanadomolybdo phosphoric acid method. In this method, ammonium molybdate was reacted under acidic condition in presence of vanadium which formed a yellow Vanadomolybdo phosphoric acid. The percentage (%) Transmission of yellow color was measured at 490 nm. Phosphate value was determined with the help of calibration curve prepared from standard solution. The amount of phosphorus/L was calculated by using following formula: -

Phosphate mg/l = mg P x 1000 ml of sample …Eqn. 2

Total Petroleum Hydrocarbon (TPH)

Residual total petroleum hydrocarbon (TPH) was extracted from the samples and quantified using Gas Chromatography- Flame Ionization Detector (GC-FID). The analysis was carried out using a Schimadzu GC-17A Gas Chromatography equipped with flame ionization detector. Samples were extracted using liquid-solid and liquid-liquid extraction methods respectively. A DB-I column was used with the following dimensions 30 m × 0.2 mm; 0.25 µm film thickness; 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 split ratio 5:1. The injection port was set at 250 ^{o} C. The oven temperature was programmed from 40^0 C for 10 minutes, the 20^0 C per min to 330^0 C, holding this temperature for 10 minutes. Separation occurs as the vapour constituent

partition between the gas and the liquid phases. The samples were automatically detected as it emerges from the column by the FID detector.

Percentage Biodegradation

Percentage biodegradation was calculated as follows:

$$\% \text{ bioremediation} = \frac{BC \times 100}{IC} \quad \ldots \text{ Eqn. 3}$$

BC =IC – FC…Eqn. 4

Where;

BC = Amount of pollutant remediated

IC = Initial concentration of pollutant (Day 0 or 1)

FC = Final concentration of pollutant at end of experiment (Last day)

Statistical Analysis

The mean and standard deviation of the fungal counts, the physicochemical parameters and TPH of the soil samples were determined using the statistical package for social science (SPSS version 27). ANOVA was carried out to check significant differences in the fungal counts and the Duncan Multiple range test was adopted in separation of means at 95% confidence interval.

Results and Discussion

Baseline Results

The baseline parameters of the sample showed that the fungal counts of the unpolluted was higher than that of the polluted water while the hydrocarbon utilizing fungal counts of the polluted water was higher than that of the control (Table 2). More so, the pH of the control and unpolluted water was 8.4 and 5.6, respectively. The total petroleum hydrocarbon content of the control was low (47.0 mg/l) compared to that of the crude oil polluted water (5838.8 mg/l) (Table 2).

The tentative identities of the fungal isolates showed that they were *Rhizopus* sp, *Aspergillus* sp, *Rhodotorula* sp, *Gliocladium* sp, *Cunninghamella* sp, *Candida* sp, *Blastomyces* sp, *Penicillium* sp, *Scopulariopsis* sp, *Geotrichum* sp and *Mucor* sp (Table 3). Amongst these isolates, *Rhizopus* sp, *Aspergillus* sp, *Rhodotorula* sp, *Penicillium* sp, *Geotrichum* sp and *Mucor* sp were isolated in the hydrocarbon polluted water (Table 4).

Bioremediation Analysis

The change in the heterotrophic fungal and hydrocarbon utilizing fungal counts in Tables 5 and 6 showed that the fungal population in the respective treatments varied across the treatments and the period of the bioremediation. More so, the fungal counts of the rhamnolipids supplemented crude-oil polluted water was significantly higher ($P<0.05$) than other treatments in Day 1 and Day 28, respectively while in Day 56, the consortium of rhamnolipids and Cow dung was significantly ($P<0.05$) higher than other counts (Table 5).

The hydrocarbon utilizing fungal counts showed that despite the observed changes in the treatments across the period of bioremediation, there was no significant differences in all the treatments including the control for Days 1 and 28, respectively while in Day 56, the cow dung supplemented crude-oil polluted water was significantly ($P<0.05$) higher than the hydrocarbon utilizing fungal counts in other treatments. Also, the hydrocarbon utilizing fungal counts in the control was the least recorded counts (Table 6).

The pH of the treatments in Day 1 ranged from 3.69-5.41. The least pH was recorded in the rhamnolipids supplemented crude-oil polluted water while the highest pH was recorded in the control. In Day 28, the pH ranged from 5.62-6.21 while in Day 56, the pH ranged from 5.81-6.84. The pH of the treatment was acidic (Fig. 1).

The phosphate concentration varied across the period of bioremediation as well as the treatments (Fig. 2). The phosphate ranged from 0.31 of the control to 2.74 mg/l of the cow dung supplemented crude oil-polluted water (Day 1). In Day 28, the phosphate ranged from 0.24 of the control to 1.77 mg/l of the cow dung supplemented crude-oil polluted water while in Day 56, the phosphate ranged from 0.19 of the control to 1.83 mg/l of the cow dung supplemented crude-oil polluted water.

The change in nitrate concentration also varied across the treatments (Fig. 3). The nitrate ranged from 0.61 in the control to 4.2 mg/ml of the consortium in Day 1. The nitrate range in Day 28 was from 0.94 to 3.82 mg/l in the

cow dung supplemented crude-oil polluted water while in Day 56, the nitrate ranged from 0.15 in the control to 2.55 mg/l in the consortium.

The percentage loss of TPH is presented in Fig. 4. The percentage loss in TPH ranged from 21.6-52.6%. The Cow dung had the highest percentage loss followed by the rhamnolipids amendment while the control had the least (21.6%) % TPH loss.

Naturally occurring attenuation causes bioremediation to proceed more slowly. Thus, a faster rate of degradation will result from the addition of nutrient sources (Sampson *et al.,* 2016). The enhanced bioremediation of crude-oil polluted water using organic amendments was investigated.

The physicochemical parameters including the total petroleum hydrocarbon (TPH) of the crude oil polluted water were not within the recommended limits as shown in the baseline results. The recommended limit for TPH in soil 40 mg/kg based on the Department of Petroleum Resources (DPR) guidelines (EGASPIN, 2018).

Although permissible limit for water is not determined but adopting this limit for water, the water is contaminated. Thus, the need for bioremediation. The fungal counts varied and isolates identified has been reported in a previous study (Okoye *et al.,* 2019).

The dynamics in the hydrocarbon utilizing fungal counts during the period of bioremediation could be a reflection of the nutrient concentration and the pH concentration of the treatments. The findings showed that the hydrocarbon utilizing fungal isolates increased exponentially from Day 1 to Day 28 with a slight decrease in Day 56. The high hydrocarbon utilizing fungal counts recorded in the treated samples, especially in Days 1 and 28 over the control could be attributed to the effect of the nitrate and phosphate nutrients in the amendments. This agreed with Sampson *et al.,* (2016) who reported similar observations and noted that poor counts observed in the control were due to the reliance on the crude oil which served as the only carbon source, unlike the nutrient-supplemented treatments. In addition, the findings showed that the application of rhamnolipids waste and cow dung stimulated the increase in the fungal counts than the control. Thus, suggesting the potential in ameliorating the polluted water with a corresponding increment in the population of indigenous oil-degrading microbiota (Mordi *et al.,* 2023).

The pH during the bioremediation study varied from acidic to slightly acidic concentration. The pH significantly influences the type of microorganisms found in an environment (Prescott *et al.,* 2011). It could be possible that the crude oil increased the acidity of the water samples because of the low pH of the contaminated samples. This backs up Nweze and Aniebonam (2009), who found that a drop in the pH of polluted samples points to the possibility that habitats become more acidic due to petroleum pollution, potentially changing biodiversity. Furthermore, the changes in the pH from acidic to slightly acidic implied bioremediation. This agreed with Edward *et al.,* (2019) who reported that fluctuations in pH during bioremediation implied bioremediation of the contaminants.

The study also showed that the nitrate and phosphate in the treatments depleted during the bioremediation. This nutrient depletion could imply that the fungal isolates utilised them for growth while degrading the crude oil components. This is reflected in the fungal counts which increased exponentially. The nutrient-supplemented crude oil-polluted water had higher nitrate and phosphate levels than the control, which lacked any nutrient supplementation. The cow dung-supplemented crude oil-polluted water had a higher nitrate concentration followed by the combination of rhamnolipids and cow dung, whereas the cow dung-supplemented crude oil-polluted water had the highest phosphate concentration. Decrease in nutrients (phosphate and nitrate) during bioremediation has been reported in previous studies (Albert and Anyanwu, 2012; Muhammad *et al.,* 2015; Sampson *et al.,* 2016).

The efficiency in the loss or reduction in total petroleum hydrocarbon showed that the cow dung-supplemented crude-oil-polluted water had the highest percentage reduction of 52.6% followed by the Rhamnolipids-supplemented (52.0%) crude-oil-polluted water.

This reduction implied that the nutrient supplemented crude-oil polluted water enhanced the bioremediation of the crude oil components as compared with the natural (control) attenuation which had the least (21.6%) TPH removal. The cow dung enhanced the reduction of the TPH from 4234.11 mg/L to 20006.43 mg/L with a percentage reduction of 52.6% while the rhamnolipids enhanced the reduction of TPH from 4234.11 to 2032 mg/L. The combination of rhamnolipids and cow dung reduced the TPH from 4234.11 to 2694.13 with a percentage reduction of 36.4%.

Table.1 Experimental Set-up

Treatment	Volume of water (mL)	Type of supplement	Weight of supplement (g)	Final volume (mL)
Control	500	None	0	500
P+CCW	500	Rhamnolipids	15	515
Q+CCW	500	Cow Dung	15	515
P+Q+CCW	500	Rhamnolipid + Cow dung	7.5+7.5	515

Keys: CCW = crude oil contaminated water; g = grams; mL = millilitre; P = Rhamnolipids; Q = Cow dung

Table.2 Baseline of the water sample

Parameter	Control	Crude oil-polluted water	WHO limits
Fungal count (SFU/ml)	$31\pm0.3\times10^{5}$	$8.0\pm0.2\times10^{3}$	-
Hydrocarbon utilizing fungi (SFU/mL)	$3.0\pm0.2\times10^{3}$	$1.5\pm0.2\times10^{4}$	-
pH	8.4	5.6	6.5-8.5
Temperature(℃)	27.0	28.8	26-28
Electrical conductivity (µS/cm)	1220	104.3	1000
Turbidity (NTU)	1.45	168	5
Nitrate (mg/l)	0.3	1.06	10
Phosphate (mg/l)	0.59	0.8	-
Dissolved Oxygen (mg/l)	1.05	2.31	7.5
Biological Oxygen Demand (mg/l)	3.40	26.68	15
Total Petroleum Hydrocarbon (mg/l)	47.0	5838.8	40

Table.3 Phenotypic Characteristics of the Fungal Isolates

Isolate code	Macroscopy	Microscopy	Probable identity
A.	White cottony, brownish grey to black-grey coloration, brown reverse	Smooth walled and non-septate branched sporangiophores, presence of rhizoids	*Rhizopus* sp
B.	White periphery, dense black spores, dark brown reverse	Hyaline conidiophore phialides borne on vesicles, chains of conidia with septate hyphae	*Aspergillus* sp
C.	Pink-red smooth colonies	Ovoid, elongate budding cells	*Rhodotorula* sp
D.	Dark green slimy suede-like conidia	Erect conidiophores with phialides bearing one-celled hyaline conidia	*Gliocladium* sp
E.	White cottony colonies, pale white reverse	Aseptate hyphae with round head sporangiophores bearing spine-like structures	*Cunninghamella* sp
F.	Cream to shiny round colonies	Large oval cells with budding cells	*Candida* sp
G.	Glabrous, tan, nonsporulating colonies	Hyaline, one-celled, smooth-walled conidia	*Blastomyces sp*
H.	Green powdery surface surrounded by white lawn, brown reverse	Septate hyphae with septate conidiophores bearing conidia	*Penicillium sp*
I.	Buff to brown like growth	chains of single-celled conidia produced in basipetal succession	*Scopulariopsis* sp
J.	Flat white dry sued-like colonies, white reverse	Cylindrical arthroconidia	*Geotrichum* sp
K.	Fluffy white cottony, white reverse	Aseptate hyphae bearing round sporangiospores	*Mucor* sp

Table.4 Heterotrophic and hydrocarbon utilizing fungal isolates

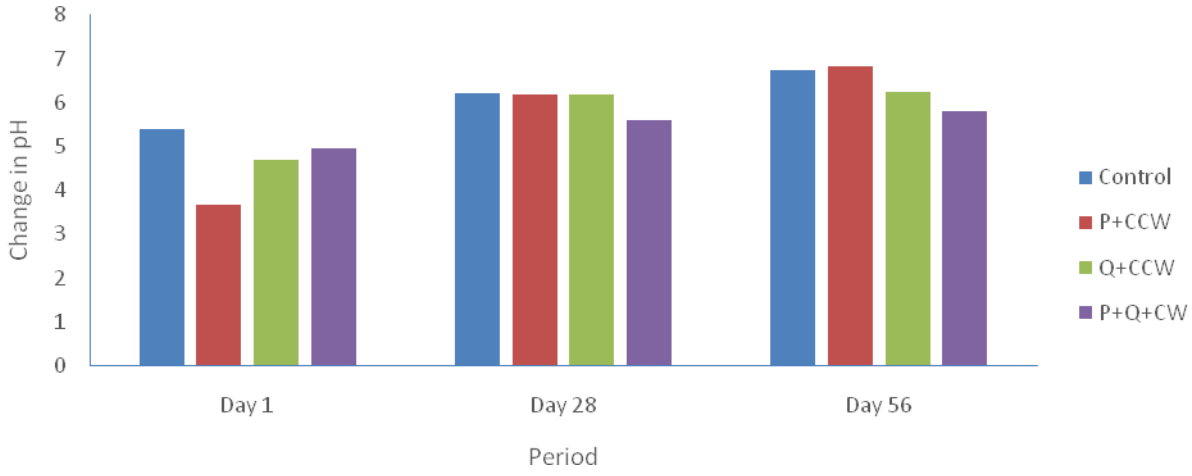
Isolates	Heterotrophic fungi	Hydrocarbon utilizing fungi
***Rhizopus* sp**	+	+
***Aspergillus* sp**	+	+
***Rhodotorula* sp**	+	+
***Gliocladium* sp**	+	-
***Cunninghamella* sp**	+	-
***Candida* sp**	+	-
Blastomyces sp	+	-
Penicillium sp	+	+
***Scopulariopsis* sp**	+	-
***Geotrichum* sp**	+	+
***Mucor* sp**	+	+

Table.5 Total Heterotrophic Fungal counts during the Bioremediation period

Treatment	Day 1 ($\times10^5$)	Day 28 ($\times10^5$)	Day 56 ($\times10^4$)
control	0.60±0.56 [a]	4.0±0.4 [a]	2.8±0.3 [a]
P+CC	3.5±0.4[b]	8.4±0.9[b]	1.2±1.1 [a]
Q+CC	1.0±1.12 [a]	1.2±1.3 [a]	2.4±2.3 [a]
P+Q+CC	2.4±2.3 [ab]	9.5±1.2[b]	4.5±0.5[b]

*Means with similar superscript down the group showed no significant difference (P>0.05)
Keys: CCW = crude oil contaminated water; g = grams; mL = millilitre; P = Rhamnolipids; Q = Cow dung

Figure.1 Change in pH during the Bioremediation Study



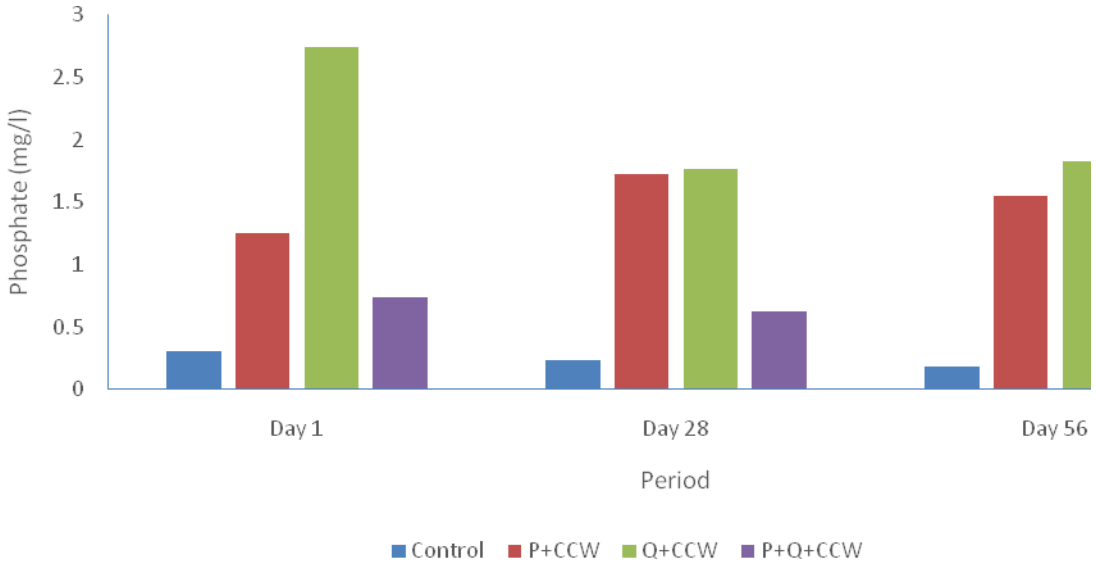
Keys: CCW = crude oil contaminated water; g = grams; mL = millilitre; P = Rhamnolipids; Q = Cow dung

Table.6 Total Hydrocarbon Fungal counts during the Bioremediation period

Treatment	Day 1 ($\times 10^4$)	Day 28 ($\times 10^4$)	Day 56 ($\times 10^3$)
Control	0.55±0.6[a]	2.8±0.3 [a]	7.5±8.6[b]
P+CC	1.2±0.1 [a]	4.5±0.5 [a]	4.6±1.6 [a]
P+Q+CC	1.6±0.2 [a]	2.3±0.2 [a]	8.5±9.2[b]
Q+CC	0.75±0.3 [a]	1.9±02 [a]	17.0±8.4[c]

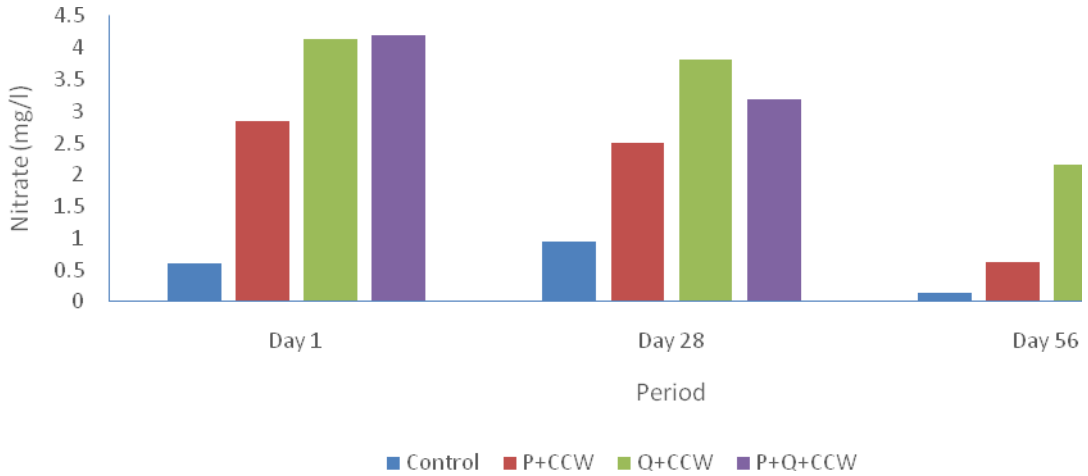
*Means with similar superscript down the group showed no significant difference (P>0.05)
Keys: CCW = crude oil contaminated water; g = grams; mL = millilitre; P = Rhamnolipids; Q = Cow dung

Figure.2 Change in Phosphate concentration



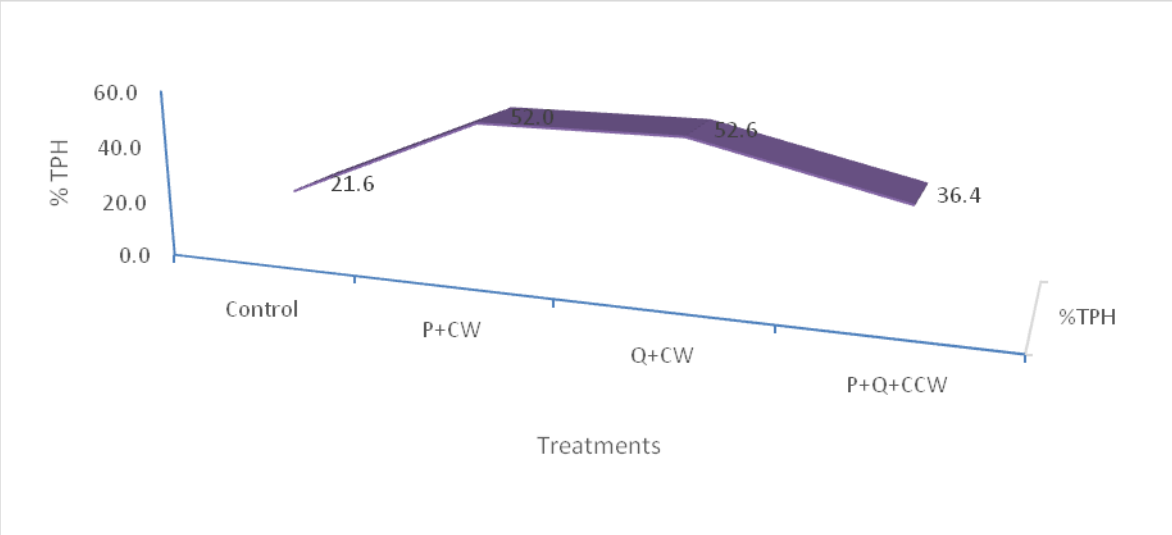
Keys: CCW = crude oil contaminated water; g = grams; mL = millilitre; P = Rhamnolipids; Q = Cow dung

Figure.3 Change in Nitrate (mg/l) Concentration During the study



Keys: CCW = crude oil contaminated water; g = grams; mL = millilitre; P = Rhamnolipids; Q = Cow dung

Figure.4 Percentage (%) Reduction of Total Petroleum hydrocarbon (TPH)



Keys: CCW = crude oil contaminated water; g = grams; mL = millilitre; P = Rhamnolipids; Q = Cow dung

There was no significant difference (P>0.05) in the percentage reduction of TPH between the cow dung and rhamnolipids supplement while the % reduction in both supplements was more significant (P<0.05) than the control. The bioremediation efficiency of cow dung and rhamnolipids on crude oil components is well documented. In a previous study, cow dung was reported to enhance the growth of microorganisms as well as influencing the physicochemical parameters of the environment to enhance bioremediation of a crude oil polluted environment (Yadav, 2019). Onos (2020) reported the reduction of TPH from 0.44 to 0.14mg/g. Furthermore, previous studies have shown that the biosurfactant rhamnolipids enhanced the bioremediation of crude oil in a crude oil polluted environment by enhancing microbial colonization and activities (Bao *et al.,* 2022; Liu *et al.,* 2021; Tahseen *et al.,* 2016).

In this study, the nutrient supplemented crude oil polluted water had better reduction of total petroleum hydrocarbon than the natural attenuation. The Cow dung had the best TPH reduction efficiency than the rhamnolipids but since the TPH reduction efficiency of both amendments had no significant difference either could be used. The consortium showed less TPH reduction efficiency implying that these amendments could function better when used individually than in consortium.

Author Contributions

I. R. Longjohn: Investigation, formal analysis, writing—original draft. V. K. Robinson: Validation, methodology, writing—reviewing. R. Awortu:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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