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Development of a Novel Path for the Bioremediation of Crude Oil-Impacted Non-Turbulent Water Bodies under Isothermal Conditions

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ABSTRACT

Crude oil spill has become a common phenomenon during oil production, transportation, and refining operations. It can occur in both land and water. While bioremediation and the use of cow dung for oil spill treatment have been established as an effective and environmentally friendly approach in the management of crude oil spill in land, little has been reported as it concerns water and most importantly, non-turbulent water bodies. The possibility of this application is of interest because non-turbulent water bodies have been reported to support the transport of petroleum hydrocarbon components down the water column, making the containment and treatment of polluted top surface insufficient. This paper reports on effort to treat an entire non-turbulent water body polluted with crude oil and with petroleum hydrocarbons diffusing through different depth of the water column. This was achieved by designing balls of cow dung with densities corresponding to that of the different depth strata of the water column, and then introduced into the polluted water. The balls then dissociate after a while, and filling the entire water volume with the cow dung particles, thus providing the necessary nutrients for microbial growth and activities across the entire water body. Samples of the water after treatment were then collected within a four (4) weeks post-treatment period and analysed using a GC-FID. At the end of the first week of treatment, the result showed a reduction of aliphatic hydrocarbons from 171.827mg/l, 98.641mg/l, 31.075mg/l and 18.675mg/l to 95.991mg/l, 33.914mg/l, 4.128mg/l, and 0.42mg/l and 1.12mg/l, 0.075mg/l, 0mg/l, and 0mg/l in the fourth week and at depths of 0.25m, 0.5m, 0.75m and 1.0m respectively. In the same vein, the aromatic hydrocarbons at the same depths, and after the first week of pre-treatment were 23.816mg/l, 12.942mg/l, 3.336mg/l and 0.962mg/l. At the end of the fourth week of post-treatment, negligible amount in all depths were recorded. These results show that the approach can be adopted in the treatment of oil spills in non-turbulent mangrove water bodies.

1. INTRODUCTION

Over the years, oil spill has become a very serious challenge arising from either mal-handling, failure or sabotage of equipment and facilities dedicated for oil production, processing, transportation and storage. Oil spill can occur in both land and water environments. The challenges associated with managing oil spill have been observed to be more in water than in land because of

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the ease of spread occasioned by different transport phenomena ranging from advection, spreading, diffusion and dissolution. However, it becomes even more challenging when the spill occurs in a non-turbulent water body of a mangrove swamp. This is because in the absence of wave action, the petroleum hydrocarbon components have the capacity to dissolve and then diffuse through the water column down to the very bed of the water depending of the volume and residence time of the originally spilled oil. These phenomena lead to the accumulation of dissolved hydrocarbon components at different depth of the water column (Odisu, 2020; Odisu et al. 2020). But water bodies with flowing capabilities enhanced by waves and tides will only support more of simple spreading with little or no vertical transport of whole oil, beside their self-cleansing capabilities (Rohrs et al., 2019; Rohrs et al., 2018).

As a result of the calmness and stillness of water bodies in mangrove swamps (Figure 1a and 1b), the residence time of eventually spilled oil is relatively higher, thereby making the transport pattern to be based on partitioning rather than particulate as obtained in high flowing or turbulent waters where the oil slick is broken into whole- oil particles, occasioned by bulk velocity. Thus, the high retention time in non-turbulent water bodies allows for gradual dissolution and diffusion of water-soluble petroleum hydrocarbon components vertically and into the water column; a phenomenon which should make efficient and total hydrocarbon recovery extremely difficult in time of clean up.

Several approaches have been established for the management of crude oil spill in water. These include the application of booms or floating barriers designed to contain oil against further spreading, skimmers, adsorbents, in-situ burning, dispersants, emulsifiers and bioremediation ((Hoang, 2018). Application of cow dung for bioremediation have been carried out on land in addressing the presence of PAHs and heavy metals (Oludele et al, 2021; Wokem and Madufuro, 2020; Lei, 2020; Obinna and Akinmulewo, 2019; Shweta, 2019) by spreading the bioremediating agent over the entire surface of the spill and allow to remediate the top surface. Farag et al, (2017), applied bacterial consortium immobilized on environment-friendly biocarriers in the management of oil spill in the Nile River, which is a flowing river while Iwuozor et al, 2022 studied the removal of pollutants (dyes and heavy metals) from aqueous media using cow dung-based adsorbents. Khalid et al, (2021), conducted a study on the bioremediation of TPH by bioaugmentation and bio-stimulation in water with floating oil spill containment booms as bioreactor basin. In all of these, little or no research have been carried out on the application of cow dung as a bioremediating agent in non-flowing water with attention to the dissolved and diffusing petroleum hydrocarbon components in the water column as earlier highlighted. This work is therefore focused on pathway to the design and application of cow dung based-bioremediating solution that focuses on the mineralization of dissolving, diffusing and adsorbed hydrocarbon components across different depth strata of the water column.

Figures 1a and 1b: Showing typical non-turbulent, fresh mangrove and crude oil-impacted swamps

2. MATERIALS AND METHODS

2.1.Spill Site Preparation

A spill site was simulated using a 200-litre volume, 1metre deep plastic drum. Water from a swamp in Ugboromro community near Warri, Delta State, Nigeria with high suspended sediment load arising from decaying organic matter and vegetation (Figure 2a) was collected and loaded into the drum. The simulation is typical of parts of Nigerian Niger Delta swamps because of its non- flowing nature, presence of suspended sediments and vegetation in and around the water body. 2 litres crude oil obtained from a crude oil production and flow station near Warri was then spilled on it to pollute the simulated site (Figure 2b). This volume was used to ensure that the oil sleek covers the entire surface with a height of between 0.1-0.2cm at least, and then allowed to stand for four (4) weeks before introducing the treatment agent and then, monitoring and post treatment sample collection for another four (4) weeks.

Figures 2a and 2b: Simulated spill site before and after impacting with crude oil

2.2.Design of Bioremediation Agent

The cow dung used was collected from a cow -slaughter in Ugbomoro, near Warri, Nigeria. To enable the suitability of the cow dung, the densities of the water at each depth (0.25m, 0.5m, 0.75 and 1m) were determined. Next, the cow dung was moulded into balls, sun dried with careful monitoring, to a weight/density corresponding to the target water- depth density, with each ball floating at a specific water depth (each specific depth has differing density). The floating dung were allowed to stay in these different depths until they start to dissociate, and the dissociating particles distributed all around the water column. Figure 3 shows a sample of the cow dung floating at a specified density and depth of the water.

Figure 3: Sample of Cow dung designed to float at specific depth and water density

2.3.Samples collection and Analysis

Pre and post treatment samples of the polluted water were collected in line with standard procedure at different depths for the following microbial test and petroleum hydrocarbon analysis.

i. *Determination of Hydrocarbon Utilizing Bacteria (HUB) in the simulated swamp*
1g of cow dung was diluted with 9ml of sterilized distilled water in sterilized test tube to make a serial dilution of 10^-1 to 10^-7. 1ml of water was diluted with 9ml of sterilized distilled water in sterilized tubes to make serial dilutions of 10^-1 to 10^-7. 1ml aliquot from one of the serial dilutions was poured on already prepared mineral salt agar. Plates with anti-fungal were added to suppress fungi growth. A filter paper was placed ascetically saturated with sterile crude oil on the inside of the inverted petri dish of the cultured plate. Thereafter, the cow dung was incubated at 37 degrees Celsius for a period of 4 days. The colonies were then counted with a colony counter.

ii. Bacterial Identification
The presumptive identification of the isolates using phenotypic characteristics was based on the various tests carried out using Bergey's Manual of systemic Bacteriology (Sneath et al. 1986)

a) *Gram's staining:* Thin smear of bacterial culture was made on a clean glass slide, air dried and heat fixed. The smear was covered with crystal violet for 30 seconds. Thereafter, the slide was washed with distilled water. The smear was covered with iodine solution for 60 seconds. The slide was washed with 95% ethyl alcohol and also with distilled water. Again, the smear was covered with safranin for 30 seconds, washed with distilled water and blot dried. It was air dried and observed under the microscope.

b) *Mobility Test:* Sterilized wire loop was used to make a drop of the cow dung on a clean slide. Three drops of peptone were added and was covered with a cover slip and examined microscopically under 45x objective

c) *Catalase Test:* Nutrient agar medium was prepared. This medium was poured into culture tube and flask. The medium was sterilized by autoclaving at 151b pressure for 15 minutes. The nutrient agar slants were inoculated with test organism. An inoculated nutrient agar slant was kept as control. The cultures were incubated at 35℃ and 3-4 drops of hydrogen peroxide was added on the growth of each slant culture. The culture was observed for the appearance or absence of gas bubbles. Active bubbling shows positive catalase test and no bubbles shows negative catalase test.

d) *Oxidase Test:* A piece of paper was divided into three equal section and labelled with the name of the organism (cow dung). A lop full of culture was rubbed on the moistened filter paper using a sterile loop. The color of the smear was checked exactly 15-30 seconds after rubbing the cell on the reagent moistening filter paper. A deep blue color indicates positive reaction. Light violet or purple colour developed with 10 seconds is recorded as negative.

e) *Indole Production Test:* 1% tryptone broth was prepared and sterilized using autoclave at 151b for 15 minutes. The tryptone broth was inoculated with test organism and an uninoculated tube was kept as control. The tubes were incubated at 35℃ for 48hours; 1ml of kovacs reagent was added 48hours of incubation. The tubes were shaken gently after intervals of 10 to 15 minutes. The tubes were observed for cherry red layers in the top layer. Red surface layer indicates

positive indole test and no red surface layer indicates indole negative test.

f) *Methyl-Red Test:* MRVP broth was prepared and sterilized using autoclave. 5ml of the broth was poured into each tube. The tubes were inoculated with test organism. All the tubes were incubated at 25℃ for 48 hours. 5 drops of methyl red indicator were added to the tubes of each set. The change in color of methyl red was observed for methyl red test

g) *Citrate Utilization Test:* Simmon's citrate agar media was prepared and sterilized using autoclave. 5ml of media was poured into the culture tubes and slants were prepared. Simmon's citrate agar slant was inoculated with test organism. The uninoculated tubes were kept as control. The tubes were incubated at 37℃ for 48 hours. Slant culture was observed for growth and coloration of the medium.

h) *Starch Hydrolysis Test:* SIM agar medium was prepared and sterilized using autoclave. SIM agar tube was labeled with the name of the organism to inoculate. Each organism was inoculated into its appropriately labeled tubes by means of stab inoculation.

i) *Hydrogen Sulphide Production Test:* SIM agar medium was prepared and sterilized using autoclave. SIM agar tube was labeled with the name of the organism to inoculate. Each organism was inoculated into its appropriately labeled tubes by means of stab inoculation.

j) *Coagulate Test:* A drop of distilled water was placed two separate slides. A colony of the test organism was emulsified in each of the drops to make two thick suspensions. A loopful of plasma was added to one of the suspensions and mixed gently. This was observed for clumping of the organisms within 10 sec. No plasma was added to the second suspension, this was used to differentiate any granular appearance of the organism for true coagulase. Clumping of the organism within 10 sec is an indication of a positive reaction, while no clumping indicates negative reaction.

Figure 4. Autoclave: sterilization of agar nutrient to kill microorganisms

iii. To investigate the potential of the bacteria to utilize crude oil, samples were collected from the simulated swamp and analysed. Samples of the remediating agent were also collected and analysed. The samples were analysed by gas chromatography. Liquid samples were taken from the tank at depths of 0.25m, 0.5m, 0.75m, 1.0m below the water surface. Total viable count (TVC) was performed with a plating count method. The number of hydrocarbon degrading bacteria (HDB) was detected using an MSM agar plate. The concentrations of dissolved phosphate, ammonium, nitrate and nitrite were determined using the photometry method. The concentration of dissolved petroleum hydrocarbon was tested by UV spectrophotometry.

iv. Petroleum Hydrocarbon Analysis: The Petroleum hydrocarbon analysis was carried out using a Gas Chromatography (GC-FID), in line with the Modified US EPA 8240 Method, 2007.

The analytical procedure used in this work for the determination of the extractable total petroleum aliphatic hydrocarbon (ETPALH) and extractable total aromatic hydrocarbon (ETPARH) is in line with that of the American Society for Testing and Materials (ASTM) Method D 3328-78 (American Society for Testing and Materials (ASTM), 1982) and Method 8270 of the US Environmental Protection Agency (EPA) (US Environmental Protection Agency, 2004).

Water samples collected were separated into sediments and water fractions by filtration. 200 ml of water fraction samples for

ETPALH analysis was measured using a 250ml volumetric beaker (Pyrex) and transferred to a 2-liter separating funnel. 30 ml dichloromethane (DCM) (all chemicals were obtained from local chemical vendor in Warri who imports laboratory chemicals from Europe) was then added to the sample in the separating funnel (Pyrex). The separating funnel was then sealed and shaken vigorously for at least three (3) minutes with periodic venting to release excess pressure. The organic layer observed was then allowed to separate from the water phase for a minimum of 5 minutes. The solvent extract was collected in an Erlenmeyer flask. A rotary evaporator (ST 15 OSA, serial no RE 1916) apparatus along with hot water bath (80-90°C) was assembled such that the concentrator tube was partially immersed in the hot water and the entire lower rounded surface of the flask bathed with hot vapor. The vertical position of the apparatus and the water temperature were adjusted to complete the concentration in 10 minutes. additional 30 ml portions of solvent. The three solvent extracts were then combined in a 250-ml Erlenmeyer flask and then dried over sodium sulfate thereafter solvent exchanged into hexane, and then concentrated using the rotary evaporator. The extract was then dried by passing it through a column housed by a syringe containing anhydrous sodium sulfate, silica gel and glass wool. Then, DCM was exchanged with hexane by adding 50 ml of hexane to the concentrate and the aliphatic extract collected over the column was concentrated to less than 10 ml. The 10-ml concentrate was then placed onto an air blow-down apparatus where the extract volume was further reduced to 1 ml under a gentle stream of air. This was repeated for the extraction of ETPALH of the sediment fraction. The same procedure was followed for the extraction of the ETPARH but for the use of DCM in place of hexane for both the water and sediment fractions. The extracts (ETPALP and ETPARH) from both water and sediment were then analyzed using a GC-FID (HP 5890 series II) in a laboratory in Warri.

All materials and equipment as well as their specifications are highlighted in Table 1.

Table 1: Summary of equipment and apparatus applied

S/N	Materials and Equipment	Make/Source	Application
2	Crude oil s	Oil production facility near Warri, Delta State	For pollution of the simulated pound
3	Cow dung	From a cow slaughter in Warri	For bioremediation of the crude oil imparted water
4	Pipette	Universal glass graduated pipet	Measurement of small volumes of liqui
5	Retort stand		To hold glassware
6	Conical Flask	Pyrex glass	For preparation and storage of solution
7	Magnetic Stirrer		To stir the solution of the water contaminated sample
8	Weigh Balance		Measurement of specific weights of all used samples
9	Separator	Pyrex Glass	For liquid-liquid separation of contaminated water sample
10	Hot Air Oven		Used for dry heat sterilization
11	Stopwatch		To measure time at required interval
12	Incubator		To grow and maintain microbiological or cell culture
13	Colony counter		To estimate liquid culture's density of the microorganisms
14	Gas Chromatography (GC/FID)		To check for the Total Hydrocarbon Content (TPH) present

3. RESULT AND DISCUSSION

This section reports the results that were obtained from the bacterial identification, pre-treated water samples, design of the bioremediation agent (treatment agent), and the post-treated samples of the crude-oil impacted water at the different depth considered.

The microorganisms identified from the cow dung samples were Proteus vulgaris, Escherichis coli, staphylococcus *aureus, and* Enterococcus *faecalis*. While the water sample gave Bacillus spp. They were responsible for remediation of oil under favorable conditions.

Densities of Water and Bio-Remediation Agent

Table 1.0 shows the resultant densities of the dried cow dung designed to suit the densities of the water at the specific depths and to float at specified water depth.

Table 1: Showing the densities of the cow dung and water at specific depth.

S/N	Depth (M)	Density Water Sample (G/Ml)	Density Of Cow Dung (G/Ml)
1	0.25	0.950	0.947
2	0.50	0.955	0.957
3	0.75	0.970	0.965
4	1.0	0.980	0.975

The cow dung which was designed to float at specified water depth, were so developed to ensure that each (3 balls per depth) float at their corresponding water depth. Once they are introduced into the system, they then start to break into small particles as water is absorbed into them. From Table 2, the densities of the water at the different levels are 0.950g/ml, 0.955g/ml, 0.970g/ml, and 0.980g/ml. The variation in the densities is as a result of the differences in the water quality arising from the presence of suspended sediments from decaying plants and other aquatic lives while the densities of the cow dung meant to be used for the corresponding water depths are 0.947g/ml, 0.957 g/ml, 0.965 g/ml, and 0.975 g/ml respectively. In a short time, the cow dung and the de-mineralizing bacterial saturate the entire volume of the water. This was evident from samples of treated water monitored and collected at various depth of the water after specific time intervals. To evaluate the bioremediation potentials of the microbes, a control set-up (un-treated) was used to monitor the concentration of the aliphatic and aromatic hydrocarbons after the initial four (4) weeks that allowed for adequate dissolution and diffusion of petroleum hydrocarbon components and another four (4) weeks of every week monitoring. The samples were collected at the different depths using GC-FID, with the results shown in Figures 5 and 6. This is to enable a reasonable conclusion on the efficiency of the remediation process.

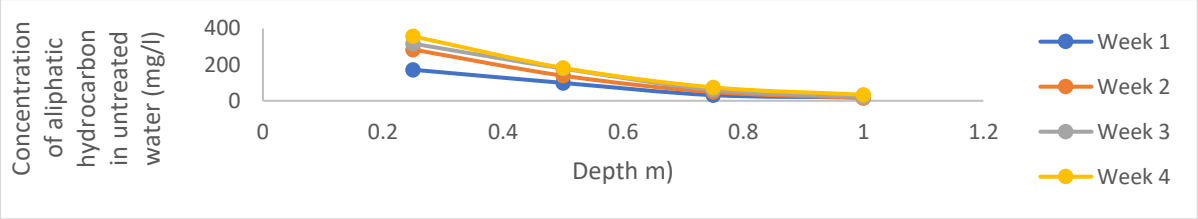

Figure 5: Concentration of total aliphatic hydrocarbon in water at different depth after four weeks without treatment.

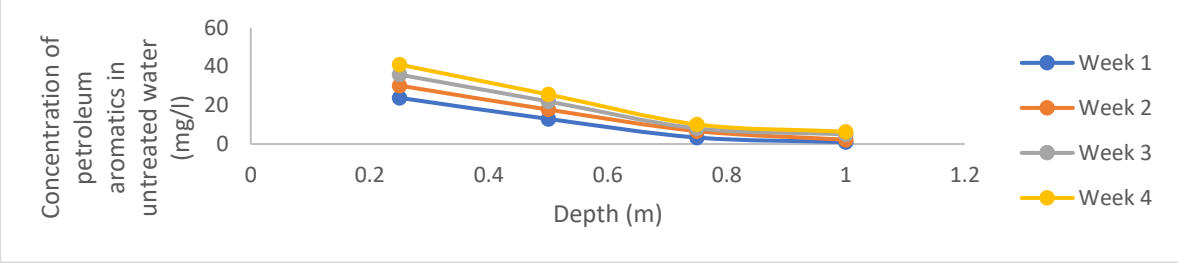

Figure 6: Concentration of total aromatic hydrocarbon in water at different depth after four weeks without treatment.

Figures 5 and 6 show the concentration of petroleum aliphatic and aromatic hydrocarbons in water after the first four weeks that allowed for dissolution and diffusion of the petroleum hydrocarbons and another four weeks where samples were collected per week for four weeks. The result shows that at the end of the initial four weeks, the concentration of the petroleum aliphatic hydrocarbons at the 0.25m, 0.5m, 0.75 and 1.0m depths were 171.827mg/l, 98.641mg/l, 31.075mg/l and 18.675mg/l. After the last four weeks, we now had 356.574mg/l, 182.171mg/l, 74.214mg/l, and 33.515mg/l. In the same vein, the aromatic hydrocarbons at the same depths, and after the first four weeks, 23.816mg/l, 12.942mg/l, 3.336mg/l and 0.962mg/l were registered, whereas after the second four weeks, 40.955mg/l, 25.561mg/l, 10.112mg/l, and 6.337mg/l were obtained. The distribution across the different depth of the water column suggest that petroleum aliphatic and aromatic hydrocarbons present in the spilled crude oil have the capacity to transport or migrate from the top to down the depth of the water column (Odisu et al, 2022). Another concern is that these concentrations of either aliphatic or aromatic hydrocarbons are well above the allowable limit of 300µg/L of petroleum hydrocarbons in river and basin water (EUEPA, 2009). This also suggest that attempt to recover the spilled crude oil and its traces from the water surface will not ensure complete cleaning of the water and thus its return to normalcy and fitness for aquatic life (Odisu and Okieimen, 2023). This realization therefore makes further action extremely necessary.

Figure 7 shows the concentration of total petroleum aliphatic hydrocarbons present at the different depth for each week in the four weeks post-treatment period.

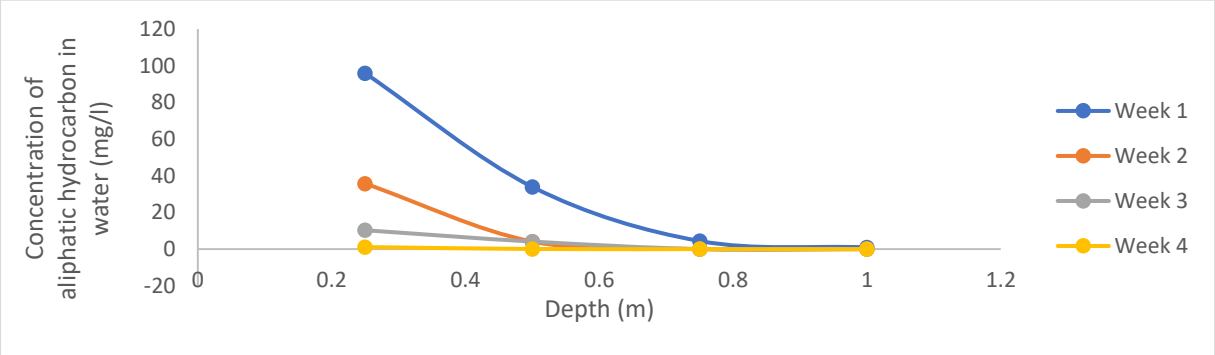

Figure 7: Concentration of total aliphatic hydrocarbon in water at different depth after four weeks of treatment.

Figure 7, highlights the concentration of total aliphatic hydrocarbons at different depths of the water within four (4) weeks post-treatment. Comparing the pre-treatment result recorded after the first one week (171.827mg/l, 98.641mg/l, 31.075mg/l and 18.67 at depths of 0.25m, 0.5m, 0.75m and 1.0m respectively) to that of the first one week of post-treatment (95.991mg/l, 33.914mg/l, 4.128mg/l, and 0.42mg/l being the values recorded for depths of 0.25m, 0.5m, 0.75m and 1.0m respectively), shows a remarkable decrease. Further comparing the result of the fourth week of pre-treatment (356.474mg/l, 182.171mg/l, 74.214, and 33.515mg/l at depths of 0.25m, 0.5m, 0.75m and 1.0m respectively) with the fourth week of post-treatment (1.12mg/l, 0.075mg/l, 0mg/l, and 0mg/l at depths of 0.25m, 0.5m, 0.75m and 1.0m respectively) confirms that the application of the cow dung that was deliberately designed to have its particles spread over the water column to stimulate

microbial growth at the different depth helped in the speedy reduction of the aliphatic hydrocarbon components across the depths of the water to an appreciable and safe level in line with globally acceptable standards. Inyang et al. 2018, reported that in some communities in Nigeria's Niger Delta where they focused their research was, the total petroleum hydrocarbon (TPH) of water samples collected were in significant concentration. For ground water samples, 1352-12,110µg/L was reported by Alimor et al., 2014, while Adewuyi et al., 2011 reported that surface water from Ubeji, a community in the Niger Delta, contained 73,500µg/L. These reports suggest that the approach used in this present research imparted positively on the environment and can be adopted in the management of oil spill cases in non-turbulent water areas.

Figures 8 shows the concentration of the total petroleum aromatic hydrocarbons present at the different depth for each week in the four weeks period when the samples were collected.

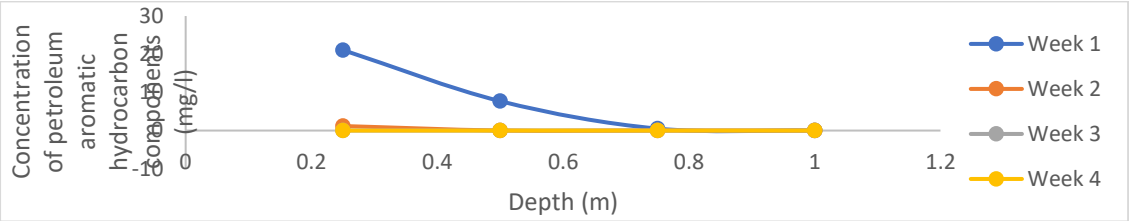

Figure 8: Concentration of total petroleum aromatic hydrocarbons in water at different depth for four weeks.

Figure 8 shows the concentration of petroleum aromatic hydrocarbons found in the water at the different depths in the water column after treatment with the cow dung for a period of four (4) weeks. As observed for the case of the aliphatics, there was a gradual build-up of the aromatics down the column. It was observed that at depths of 0.25m, 0.50m, 0.75m, and 1.0m of the water after the first week, 21.092mg/l, 7.712mg/l, 0.518mg/l and 0.0035mg/l respectively were recorded. While for the fourth (4) week, very negligible amounts were recorded in all depths. Comparing these with the result recorded in the pretreatment stage suggest that the remediating microbes were able to reduce all the diffused petroleum aromatic hydrocarbons available in the water column. Hernando et al., 2010 reported that microbes have shown preference for aromatics during bioremediation. This and the relative availability support the result in this research on the almost complete degradation of the available petroleum aromatic hydrocarbons initially present.

Figure 9 shows Pristane concentration in water present at the different depth for each week in the four weeks post treatment period when the samples were collected.

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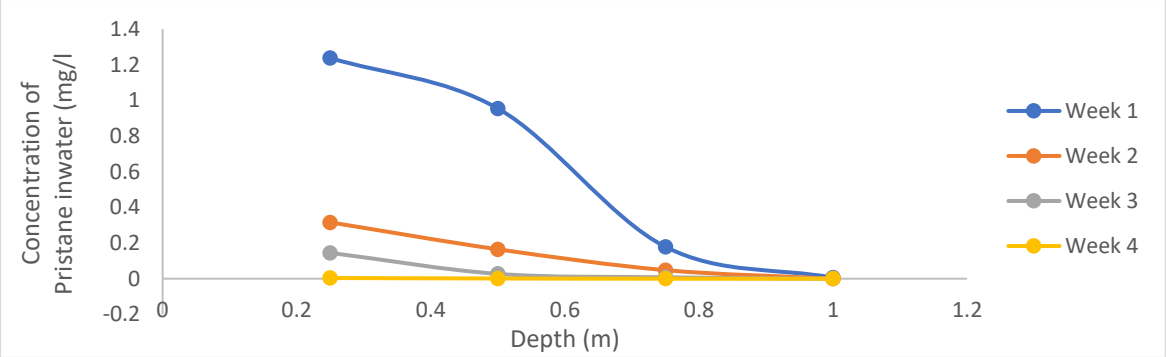

Figure 9: Concentration of pristane in water at different depth for four weeks.

The availability of pristane- an aliphatic hydrocarbon and isoalkane with 19 carbon atoms- was also considered. It was observed that at the fourth week before the treatment, the concentrations of pristane distributed across the water column at depths of 0.25m, 0.5m, 0.75m, and 1.0m were 8.182mg/l, 5.139mg/l, 1.615mg/l, and 0.432mg/l respectively. However, at the end of the first week of post-treatment, pristane concentration depleted to 1.238mg/l, 1.133mg/l, 0.179mg/l, and 0.0013mg/l respectively while at the fourth (4^{th}) week, samples collected and analysed showed that the treatment has reduced the pristane concentration better with the result showing 0.0038mg/l for 0.25m and 0mg/l in the other depths. This is a clear indication that the microbes were able to and effectively reduce the concentration of pristane to a tolerable level just like the case of the general alkanes considered earlier. This isnotable so as to sustain the aquatic community and its attendant value chain.

Figure 10 shows Pristane concentration in water present at the different depth for each week in the four weeks period when the samples were collected

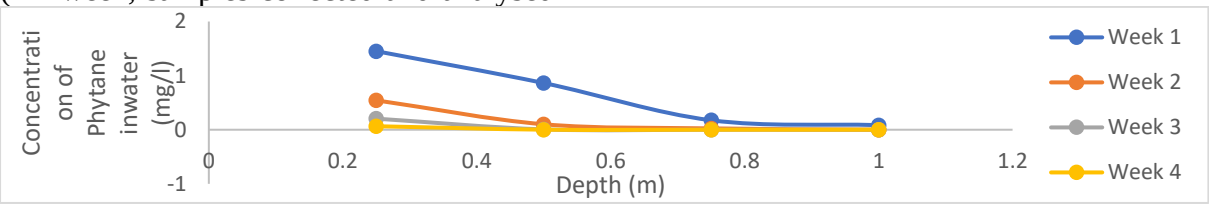

Figure 10: Concentration of total phytane in water at different depth for four weeks.

Figure 10 highlights the concentration of Phytane, another specific petroleum aliphatic hydrocarbon in the different depth of the water at 0.25m, 0.5m, 0.75m, and 1.0m. As was done in the other cases, the concentration of phytane transported across the water column before treatment were 7.164mg/l, 2.597mg/l, 0.192mg/l and 0.0057mg/l respectively. However, after the 1^{st} week of treatment, 1.452mg/l, 0.863mg/l, 0.175mg/l, and 0.085mg/l respectively were registered. After the 4^{th} week, it was observed that like the other cases considered, the concentration of phytane across the water column and at the specific depths has also been depleted to a tolerable limit by the microbes thus making the process feasible in oil spill management particularly for non-turbulent water bodies.

Figure 11 shows anthracene concentration in water present at the different depth for each week in the four weeks period when the samples were collected

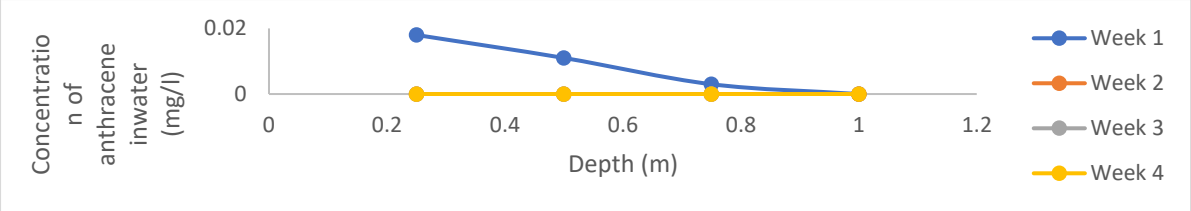

Figure 11: Concentration of total anthracene in water at different depth for four weeks.

Figure 11 shows the concentration of anthracene across the water column and specifically at the specific depths of 0.25m, 0.5m, 0,75m, and 1.0m. The laboratory result shows that the microbes were effective in reducing the concentration of anthracenes present before treatment to a tolerable limit. This is true because prior to treatment, the concentrations were 0.986mg/l, 0.665mg/l, 0.155mg/l, and 0.0067mg/l respectively. After 1st week of treatment, the concentrations reduced to 0.018mg/l, 0.011mg/l, 0.003mg/l, and 0mg/l respectively while after the 4th week, the concentrations became 0mg for all the depth sampled. This reduction is a clear indication that the microbes are able to reduce anthracene concentrations to environmentally acceptable level.

Figure 12 shows fluorathene concentration in water present at the different depth for each week in the four weeks period when the samples were collected

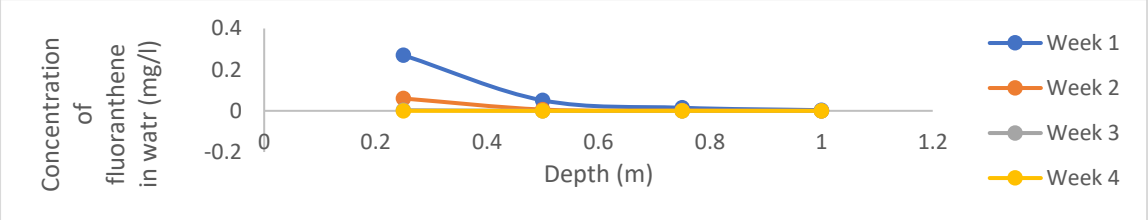

Figure 12: Concentration of total fluorathene in water at different depth for four weeks.

Figure 12 shows the concentration of fluoranthene available in water for every week within the four weeks period when the bioremediation was monitored. The pre-treated samples collected at depths of 0.25m, 0.5m, 0.75m, and 1.0m recorded 1.097mg/l, 0.732mg/l, 0.119mg/l, and 0.0435mg/l respectively. After the first one week of treatment, analyzed samples at these designated depths gave 0.27mg/l, 0.051mg/l, 0.015mg/l, and 0.003mg/l of fluorathene respectively and at the end of the fourth week of treatment, the concentrations of fluorathene were omg/l at all depths. The total absence of fluorathene in the water shows that the bioremediation agent applied effectively reduced the fluorathenes in the water.

4. CONCLUSION

The relatively low research attention has been accorded to the bioremediation of polluted stagnant water bodies of the mangrove swamps particularly efforts towards mineralizing the dissolving and diffusing petroleum hydrocarbon components in water. This have led to poor clean-up efforts of oil spill cases in non-turbulent water bodies of mangrove swamps. This gap, led to the development cow dung balls with densities corresponding to that of the different depths of the water. This will ensure that the cow dung is evenly distributed across the different depth of the water and able to

stimulate microbial action across the water body. The product developed were applied and the result obtained after four weeks of monitoring show that the product and approach are efficient because they (aided the mineralization of available petroleum hydrocarbons (aliphatics and aromatics) from initial concentrations that were well above the allowable limits to almost negligible in most of the cases.

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