



Phytoremediation, Vermiremediation and Efficiency Assessments of Total Petroleum Hydrocarbons in Contaminated Surface Water from Okpoka Creek, Niger-Delta Nigeria using *Cyperus odoratus*, *Colocasia esculenta*, *Phoenix roebelenii* and *Eisenia fetida*

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Authors' contributions

This work was carried out in collaboration among all authors. Author CF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SJS and DAD managed the analyses of the study. Author ACH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The liquid-liquid extraction of Petroleum contaminated surface water samples were conducted in accordance with standard procedure of US EPA. Identification and quantification of TPHs was performed by Gas chromatograph with Mass spectrometric detection (GC/MSD) in accordance with standard analytical method of US EPA 8270;625. The TPHs raw sample showed an elevated value of 104762.42 mg/L above the DPR/EGASPIN maximum contamination limit (MCL). At week 5, TPHs phytoremediations in the monoculture reactors removal efficiency was (31.28 mg/L) 99.97% and mixedculture indicated (19.72 mg/L) 99.98%; their concentrations were above DPR/EGASPIN MCL while polyculture indicated (8.91 mg/L) 99.99% value was below DPR/EGASPIN MCL. The

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combination of phytoremediation and vermiremediation techniques in polyculture reactors showed better and spectacular results as the biotas demonstrated good potentiality of hyperbioaccumulation to serve as hydrocarbon sinks from the ecotoxics of TPHs.

Keywords: *Phytoremediation; Vermiremediation; efficiency; assessments; Total-Petroleum-Hydrocarbons (TPHs); Petroleum-Contaminated; surfacewater; Niger-Delta.*

1. INTRODUCTION

Three-quarter of the earth's surface is covered by water, it is not surprising that water has the ultimate sink for most anthropogenic chemicals. Generally, surface water are contaminated by point or non-point sources. An effluent from an industrial plant or a sewage treatment plant is an example of a point source; a field from which pesticides and fertilizers are carried by rainwater into a river is an example of a non point source. Industrial wastes probably constitute the greatest single pollution problem in water. One of these contaminants is petroleum and its derivatives. The usefulness of petroleum cannot be over emphasised but its exploration and exploitation have brought about horrendous health and environmental concerns to the world. Basically, pollution from petroleum compound has been a major concern in oil producing region in Nigeria (Niger-Delta). Nigeria being the sixth largest oil producer in the world has been confronted with a couple of sapping challenges for 6 decades. The continuous challenges relate to the prevalence of untreated and indiscriminate discharge into the water bodies from refineries, illegal artisanal refineries, pipeline theft/bunkering, storages and vandals. In deed the incidence of oil pipeline vandalism has been on the rise. According to 2013 annual report of the Nigerian Extractive Industry Transparency Initiative (NEITI), Nigeria lost a total of 10.9 billion US Dollars to oil theft between 2009 and 2011 [1] and [2].

Recent pipeline explosions in Nigeria from 2009 to 2013 had exposed Nigerians to different kinds of health and environmental risks. In 2009 Ogun State (Arepo Local Government Area) experienced loss of lives and environmental pollution, in 2010 Delta State (Amukpe, near Sapele LGA) experienced destruction of public property (NNPC facilities), in 2010 Lagos State (Idu, near Ijegan LGA) experienced loss of lives and environmental degradation, 2011 Delta State (Oko Owore-Amukpe) witnessed loss of lives, environmental damage and population displacement and in 2011 Bayelsa State (Okoroma Clan Nembe LGA) witnessed population displacement and family dislocation [3] and [4].

As approximated by the US. Environment Protection Agency (US.EPA) 500 gallons of fresh water is used to produce a barrel of crude oil; this implies 20,000 litres of fresh water is required for 1 barrel of crude oil. Thus, 8 trillion, 45 billion and 4 million litres of fresh water is required to produce 2.53 million barrels of crude oil per day in Nigeria. However, 98% of the fresh water for crude oil exploration and production turned out to be produced water with no process of revamping for reuse and reinjection. As a result, average of 7 trillion, 884 billion and 492 million litres of petroleum-contaminated waste water are indiscriminately discharged and untreated into environment per day in Nigeria with the exception of contaminations from illegal oil operations pipelines; bunkering, artisanal refineries, leakages, equipment failures, spillages and storages, sabotage and vandalism. These have undoubtedly be chief contributor to the environmental devastation; destruction of large tracts of the mangrove forests, soil, crops and aquaculture resulting to increase in hunger and poverty. The numerous hydrocarbons and chemicals present in oil had affected the drinking water or portable water posing enormous health risks of humans and animals even sudden deaths [5] and [6].

Therefore, the aim of this study was to combine the potentials of three species of plants (*Cyperus odoratus*, *Colocasia esculenta* and *Phoenix roebelenii*) and earthworm (*E. fetida*) to decontaminate the Total Petroleum Hydrocarbons (TPHs) in petroleum contaminated surface water for healthy reuse and to assess the efficacies of their biological matrix in artificial wetland system.

2. LITERATURE REVIEW

Since the late 1980s, after the physical and chemical approaches of lands, water bodies and thermal treatment (incineration) of hazardous wastes proved economically and environmentally unsustainable, focus shifted towards the biological methods which are cost effective as well as environmentally sustainable and socially acceptable also have proven to be superlative as compared to the physical and chemical

processes [7], [8] and [9]. Vermiremediation is earthworm based technology that has the potentials of converting inorganic or organic contaminants to improve soil nutrients for healthy growth plants [10]. The plants are often used in combination with other traditional technologies for cleaning up contaminated sites because of the phytoremediation's limitations. Zooremediation (vermiremediation) mostly involves biostimulation process; the actions improve the environmental conditions of the site and thereafter influence the activities of microorganisms. This is a less researched area; however some investigations involving earthworms and other invertebrates indicates that animals play a role in enhancing the activities of microorganism and hence could be exploited for bioremediation [10] and [11]. However, it has been suggested that ideal plants for phytoremediation should possess properties such as fast growing, high biomass, and deep roots, easy to harvest and should tolerate and accumulate a range of toxic pollutants in their aerial and harvestable parts [12] and [13].

Hyperaccumulator plants are able to tolerate high concentrations of contaminants by converting them to non toxic products and preventing encroachment on sensitive plant tissues that may kill the plant [5].

In the natural setting, several plants have been identified which have the potential to uptake toxic pollutants. At least 45 families have been identified to hyperaccumulate heavy metal, some of the families are *Brassicaceae*, *Fabaceae*, *Euphorbiaceae*, *Asteraceae*, *Lamiaceae* and *Scrophulariaceae*. Although there are some problems associated with edible crops for phytoremediation of toxic pollutants. Ecologically, use of edible crops for phytoremediation is not viable because the contaminants enter into food chain either consumption by human or animals [14] and [15]. Recently, a safe, economically feasible and eco-friendly approach for phytoremediation using non edible aromatic plants have been proposed. The contaminants do not easily enter the food chain through phytoremediation by aromatic plants.

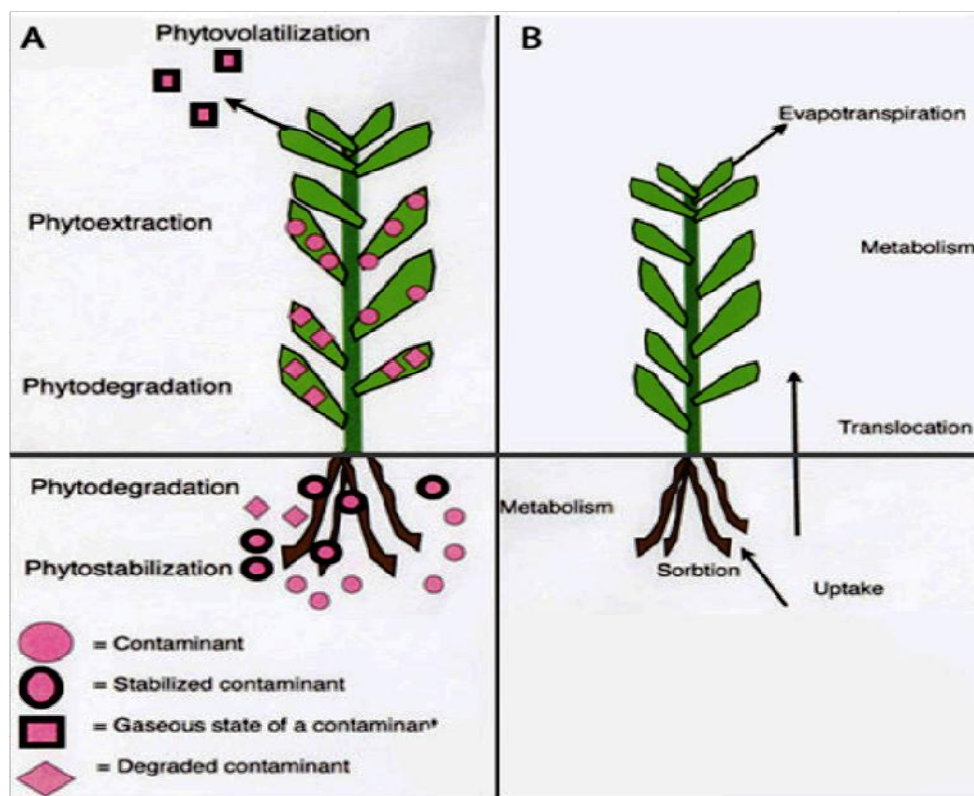


Fig. 1. (A) Schematic Model of Different Phytoremediation Technologies Involving Removal and containment of contaminants; (B) physiological processes that take place in plants during phytoremediation (<http://www.nature.com>)

The wild animals do not eat the aromatic crops due to its essence. In fact, aromatic plant resources are very abundant, and they can be used on large scale. These plants offer a novel option for their use in phytoremediation of contaminated sites [16]. Aromatic plants like vetiver (*Vetiveria zizanioides*), palmarosa (*Cymbopogon martinii*), lemon grass (*Cymbopogon flexuosus*), citronell (*Cymbopogon winterianus*), geranium mint (*Mentha* sp.), tulsi (*Ocimum basilicum*) are ecologically feasible and viable. Some aromatic grasses like, lemon grass, palmarosa, *citronella*, vetiver, and among other, are stress tolerant and perennial in nature [16], [14] and [15].

During phytoremediation, the roots of established plants absorb contaminants from the soil and translocate them to the above-ground shoots where they accumulate. However, remediative plants must have mechanisms to detoxify or tolerate high contaminant(s) concentrations accumulated in their shoots (US EPA, 2000). After sufficient plant growth and contaminants accumulation, the aboveground portions of the plant are harvested and removed, resulting in the permanent removal of contaminants from the site [17] and [18].

3. MATERIALS AND METHODS

3.1 Study Site

The petroleum-contaminated wastewater sample was obtained from Okopoka creek, (Port Harcourt, River State) Niger-Delta. Okpoka creek is one of the adjoining creeks off the upper Bonny River estuary in the Niger-Delta. Bonny River is a major shipping route for crude oil and other cargoes that leads to the Port Harcourt quays, Federal ocean terminals, Onne, Port Harcourt Refinery Company Terminal Jetty and Okirika. Port Harcourt is the chief oil refining city in Nigeria. Okpoka creek is about six kilometres long and continues to Abuloma town of Rivers State where it finally joins the Bonny River before fading into the Atlantic Ocean. There are many communities along the main course namely; Oginigba, Azubiae, WOji, Okujagu, Okuru-ama, Abuloma, Ojimba, George-ama, oba-ama, Kalio-ama, Marine base and Okirika. The petrogenic activities going on around the creek, oil slick floating on the surface water and some dead aquatic animals gave evidences of petroleum contamination. Collection of samples were obtained with the location coordinates of N04⁰

46' 38" E007⁰ 03' 55" and 4.94 km from the heart of the Pipelines and Product Marketing Company (PPMC) Oil Company, (Fig. 2). The pilot experimental studies were performed in the green house of Federal College of Forestry, Jos Nigeria with location coordinates N09⁰50'31" E008⁰53'55". Consequently, samples analytical preparations were executed in the Chemistry Postgraduate Research Laboratory, University of Jos, Nigeria with the location coordinates N09⁰53'46.2" E008⁰53'58.5".

3.2 Construction of Wetland Reactors

The artificial wetland system was constructed for the pilot scale experiments to decontaminate TPHs contaminant. The wetland reactors consisted of 8 mesocosms (36cm height and 29 cm of internal diameter), hydraulically equipped and placed in the greenhouse (properly sheltered from the rain) of Federal College of Forestry, Jos. The reactors made of plastics had components such as top-soil, cow-dung (manure), sharp-sand and gravels were layered as shown in Fig. 3a, were operated according to the vertical subsurface-flow principle (VSF). Incubation period of 90 days was observed before introducing 4 litres of contaminated wastewater sample into the remediation reactor after 1 week of dehydration.

3.3 Soils Collections

The US EPA Environmental Response Team Soil (ERTS) sampling standard operating procedures on sample preservation, containers, handling and storage were optimally adopted [19]. Guidelines for quality management in plant and soil laboratories were put into practice in accordance with the United Nations – Food and Agriculture Organization [20].

3.4 Plants Collections

Fresh butches of plant species (*Cyperus odoratus*, *Colocasia esculenta* and *Phoenix roebelenii*) were identified specifically for their tolerance and extensive fibrous root systems giving them a competitive advantage to survive under unfavourable soil condition. They were carefully uprooted from Eden Hotels and Suites Garden, Lamigo Road, Jos Nigeria (N090 53' 59.45" E008⁰ 54' 33.07") and transplanted into the different wetland reactors at the greenhouse.



Fig. 2. Study Site Okpoka Creek is 4.94km from the Heart of PPMC Oil Company

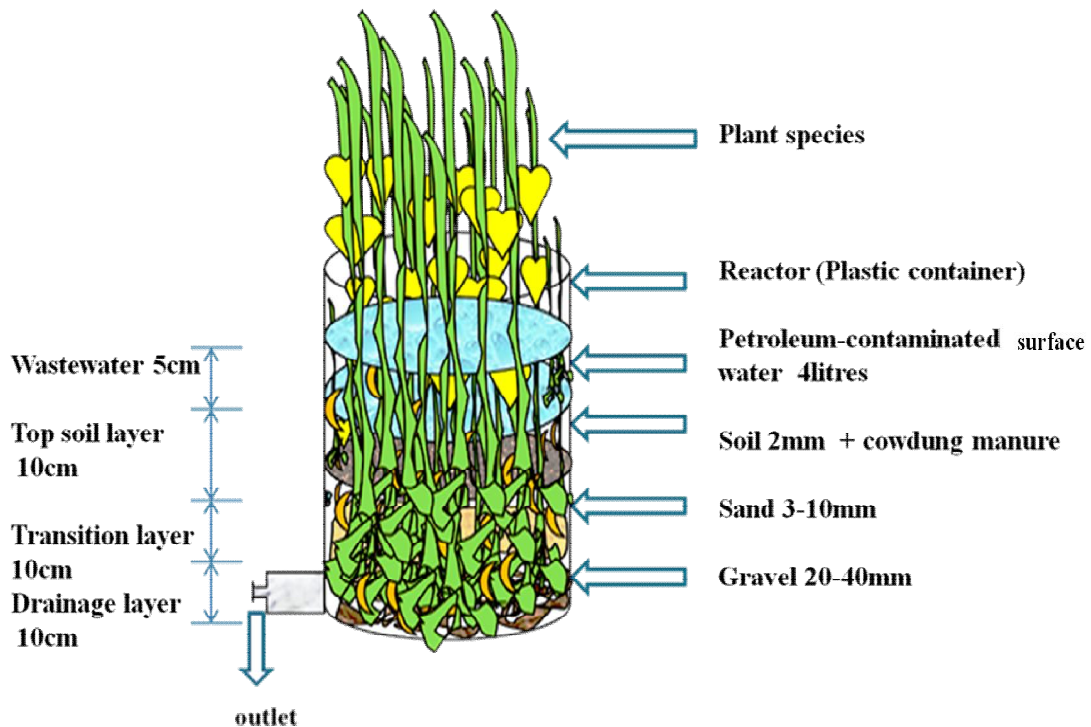


Fig. 3a. Schematic of the Constructed Wetland Reactor

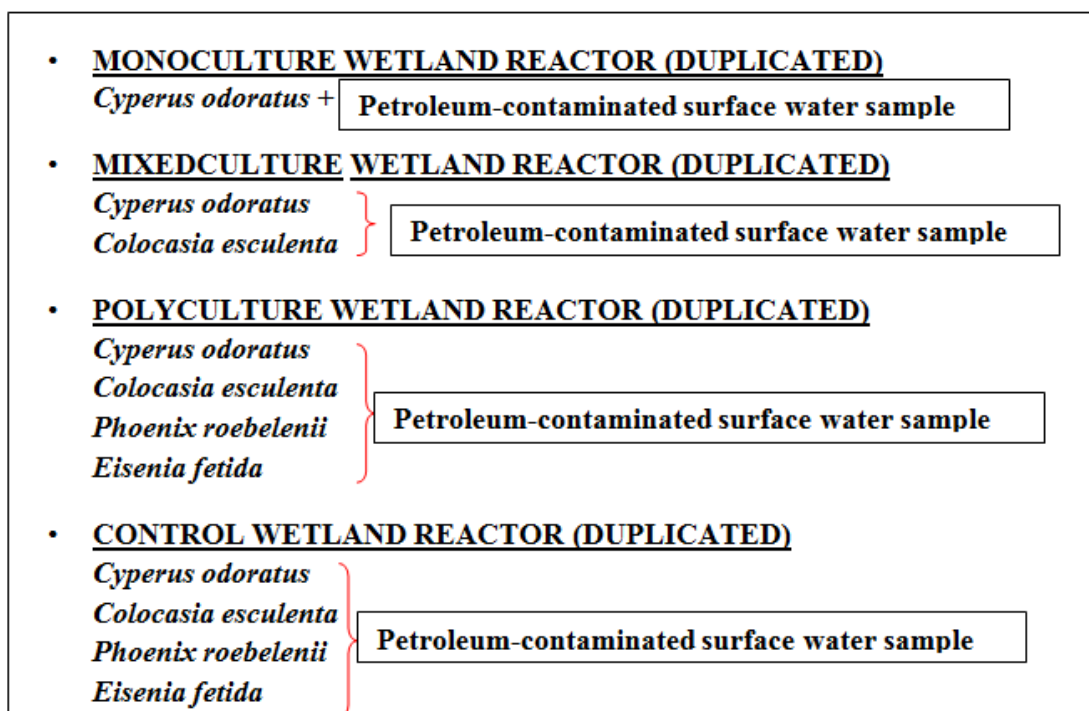


Fig. 3b. Outline of the constructed wetland reactor

3.5 Earthworms Collections

Eisenia fetida squirms were identified and collected from vegetation of the Federal College of Forestry, Jos Nigeria. The adult and juvenile earthworm squirms were incubated, acclimatized and multiplied in the wetland mesocosm for vermiremediation processes for 90 days, as adopted the guideline of OECD 207 (OECD, 1984).

3.6 Petroleum-contaminated Wastewater Sampling

Petroleum-contaminated surface water samples were collected from the study site using the grab samples method. Discrete grab samples were taken using a glass cup and amber bottles with teflon lined caps previously washed and dried in a laboratory oven at 105°C. Collections of samples were obtained at different depths (2, 5 and 10cm) of a selected location at the study site. However, collected samples were pooled together to obtain 60,000cm³ of amalgamated sample. The homogenised and amalgamated sample was filtered and preserved (acidified) on the site with 120 cm³ concentrated H₂SO₄ to bring the pH to ≤ 2. The sample was refrigerated

at ≤ 4°C and extracted within 14days of collection for the analyses of TPHs.

In the wetland system 7 days dehydration period was observed, subsequently, 5000 cm³ from the 60,000 cm³ of preserved petroleum-contaminated surface water sample were poured into each of the 8 wetland reactors after the incubation period of 90 days. Samples of 500 cm³ were collected from each of the wetland reactors weekly for the analysis of TPHs, for 5weeks detention period.

3.7 Extraction of Surface Water Samples

The liquid-liquid extraction of wastewater samples of raw and from different wetland reactors were performed in accordance with standard procedures of U.S EPA 3510; 1664. The filtered wastewater samples were subjected to different separatory funnels extraction procedure. 500cm³ of each surface water sample were individually extracted in 1000cm³ glass separatory funnels fitted with glass stoppers. 125cm³ of acetone and n-hexane (1:1, v/v) were added to each sample and were shaken on a reciprocating mechanical shaker at 120 oscillations per minute for 4hours. The mixtures

of each sample were poured into different separatory funnels and allowed to stand for a couple of minutes for the organic layer to separate clearly from the aqueous phase. The organic (extract) layers were collected and stored in different amber bottles with Teflon-lined caps and refrigerated at 4°C.

3.8 TPHs Analysis

The extracted samples of petroleum-contaminated surface water and the decontaminated samples were toxicologically identified and quantified using Gas chromatograph with Mass spectrometric detection (GC/MSD) Agilent Technologies 7890A in adherence to the standard analytical method of U.S EPA 8270;625. The TPHs was classified into surrogated fractions (C₈ – C₁₀, C₁₁ – C₂₈ and C₂₉ – C₄₀) characterised by similar adverse health effects, toxicologic information, chemical and physical properties; in accordance to the guidelines developed by the Agency for Toxic Substances and Disease Registry [21], Environmental Protection Agency [22], TPH Criteria Working Group [23], US Department of Environmental Quality [24] and American Petroleum Institute [25]; for ease of quantification and interpretation.

3.9 Data Analysis

Sample results were analytically pooled for statistical data analysis using Microsoft Excel 2007, version 12.0. The processed and interpreted data were compared with maximum permissible limits or maximum concentration limits of relevant international standards and regulatory agencies.

4. RESULTS AND DISCUSSION

4.1 Results

Table 1 represents the values of Total Petroleum Hydrocarbons (TPHs) in the raw surface water sample. Table 2 represents (monoculture) the values of phytoremediation process of *C. odoratus* to decontaminate TPHs in the surface water sample. Table 2 represents (mixed culture) the value of phytoremediation processes of *C. odoratus* and *C. esculenta* to decontaminate TPHs in the surface water sample. Also Table 4 represents (polyculture) values of the combination of phytoremediation and vermiremediation processes of *C. odoratus*, *C.*

esculenta, *P. roebelenii* and *E. fetida* to decontaminate TPHs in the surface water sample. Hence, the experiment was carried out for 5 weeks (35 days).

The assessment of TPHs value provided information about the petroleum contamination of the surface water as depicted in Table 1. TPH is a kind of chemical mixture that is also used to determine the hydrocarbon contamination in an environment. The raw surface water revealed TPHs elevated concentration of 1.05E+05 mg/L ranged C₈ – C₄₀ as categorized and measured in accordance to the guidelines developed by ATSDR, TPHCWG, API and DEQ. Thus, result was considerably higher than the recommended limit values for surface water by DPR/EGASPIN (10 mg/L). Surrogated fraction of the TPHs of gas range organics (GRO); the volatile aromatic and aliphatic hydrocarbons (C₈ – C₁₀) was 1397.53 mg/L. Diesel range organics (DRO) of aliphatic and aromatic hydrocarbons was 102305.63 mg/L. Extended diesel range organics (EDRO) of lube oil fraction depicted 1059.25 mg/L. The elevated value in this study suggested TPH (mg/L) may affect the human health in different ways. Some of the TPH compounds, particularly the smaller compounds at the range of (C₈ – C₁₀), can affect the human central nervous system. If exposures are high enough, death can occur. Breathing the compound at concentrations greater than 100 parts per million (100 ppm) for more than several hours can cause fatigue, headache, nausea, and drowsiness. When exposure is stopped, the symptoms will go away [26]. However, if someone is exposed for a long time, permanent damage to the central nervous system can occur. One TPH compound in the range of (C₈ – C₁₀) can affect the central nervous system in a different way, causing a nerve disorder called peripheral neuropathy characterized by numbness in the feet and legs and, in severe cases, paralysis [27]. Swallowing some petroleum or petroleum contaminated media may cause irritation of the throat and stomach, central nervous system depression, difficulty breathing, and pneumonia from breathing liquid into the lungs. The compounds in some TPH fractions can also affect the blood, immune system, liver, spleen, kidneys, developing fetus, and lungs [21] and [6].

The surrogated fraction of TPHs diesel range organics (DRO) (C₁₁ – C₂₈) of aliphatic and aromatic hydrocarbons was 102305.63 mg/L. The recommended non-cancer oral and

inhalation toxicity reference values for C₁₁-C₂₈ aliphatic hydrocarbons are 0.1 mg/kg-day, and 0.3 mg/m³ (0.09 mg/kg-day) respectively. The oral reference value was based on the results of several subchronic studies in rodents of various petroleum streams covering the aliphatic range [23]. Change in liver weight was the most common critical effect in developing a toxicity criterion for each study. The reference value of 0.1 mg/kg-day for oral exposure was based on two studies with a no-observed-adverse-effect-level (NOAEL) of 100 mg/kg-day an uncertainty factor of 1000, and a study with a lowest-observed-adverse-effect-level (LOAEL) of 500 mg/kg/day and uncertainty factor of 5000. Both Total Petroleum Hydrocarbon Criteria Working Group and Commonwealth of Massachusetts, Department of Environmental Protection recommended this value for this hydrocarbon range (MADEP, 2003; [23]. The recommended non-cancer oral and inhalation toxicity reference values for unspecified aromatics of carbon number C₁₁-C₂₈ are 0.004 mg/kg-day, and 0.05 mg/m³ respectively [23]. The reference value for oral exposure was based primarily on the US EPA oral RfD for 2-methylnaphthalene because methylnaphthalenes may comprise a significant portion of this hydrocarbon range [28]. There were at least eight other aromatics in this hydrocarbon range for which RfDs have been derived (isopropylbenzene, naphthalene, pyrene, fluoranthene, fluorene, acenaphthene, anthracene, and biphenyl). The range of RfDs for the nine aromatics was 0.004 to 0.3 mg/kg-day. Most of these have an oral RfD of 0.04 mg/kg-day or higher. The only aromatics in this range

with a lower RfD were pyrene (0.03 mg/kg-day), naphthalene (0.02 mg/kg-day), and 2-methylnaphthalene (0.004 mg/kg-day). For sites at which naphthalene and the methylnaphthalenes are evaluated individually the MADEP RfD of 0.03 mg/kg-day can be used [22].

The surrogated fraction of TPHs extended diesel range organics (EDRO) (C₂₉ – C₄₀) of lube oil fraction depicted 1059.25 mg/L from the present study (Table 1). The recommended non-cancer oral toxicity reference value for aliphatics of carbon number range (C₂₉ – C₄₀) is 2.0 mg/kg-day [23]; MADEP 2003). No toxicity reference value has been developed for inhalation. For the oral toxicity criterion both TPHWG and MADEP relied on a study of several white mineral oils conducted in 1996 by the British Industrial Biological Research Association (BIBRA) [29] and [23]. The non-cancer oral toxicity reference value for aromatics of carbon chain length C₂₉ – C₄₀ is 0.03 mg/kg-day. No toxicity reference value has been developed for inhalation. Due to a lack of appropriate studies of this carbon range TPHCWG or MADEP selected a USEPA oral RfD for a surrogate to represent this group. Pyrene was chosen as the surrogate because it was the closest compound to this carbon length for which an RfD was available from the USEPA. The oral RfD for pyrene is 0.03 mg/kg-day (IRIS, 2008). Thus, in the present study elevated petroleum hydrocarbon content (TPH) from the Okpoka creek have provided evidence of severe crude oil contamination of the surface water.

Table 1. Total Petroleum Hydrocarbons (TPHs) Concentrations for Raw Petroleum-contaminated Surface Water

Components	Categories	Concentrations (mg/L)
	¹ TPHCWG	
	² USEPA/ ³ DEQ/ ⁴ API	
C ₈ - C ₁₀	+ GRO	1397.53
C ₁₁ - C ₂₈	++ DRO	102305.63
C ₂₉ - C ₄₀	+++EDRO	1059.25
C ₈ - C ₄₀	TPH	104762.42
*DPR/EGASPIN (MCL)	TPH	10.00

MCL Maximum Contamination Limit (2002) on Surface water

¹Total Petroleum Hydrocarbons Criteria Working Group, US (1998)

²United States Environmental Protection Agency (2003)

³Department of Environmental Quality, US (2003)

⁴American Petroleum Institute (2000)

+ Gasoline Range Organics

++ Diesel Range Organics

+++ Extended Diesel Range Organics

* Department of Petroleum Resources / Environmental Guidelines and Standards for the Petroleum Industry in Nigeria

Table 2. Decontamination Processes of TPHs in Petroleum-contaminated Wastewater via Monoculture (*Cyperus odoratus*) Reactors

Raw Petroleum Contaminated ww (Inlet) (mg/L)	Week I (outlet) mg/L	Week II (outlet) mg/L	Week III (outlet) (mg/L)	Week IV (outlet) (mg/L)	Week V (outlet) (mg/L)	Removal Efficiency Week I %	Removal Efficiency Week II (%)	Removal Efficiency Week III (%)	Removal Efficiency Week IV (%)	Removal Efficiency Week V (%)
1.05E+05	976.34	132.54	57.37	34.86	31.28	99.07	99.87	99.95	99.97	99.97

ww =wastewater, RF = Removal Efficiency

Table 3. Decontamination Processes of Toxic TPHs of Petroleum-contaminated Wastewater via Mixedculture (*Cyperus odoratus* and *Colocasia esculenta*) Reactors

Raw Petroleum Contaminated ww (Inlet) mg/L	Week I (outlet) mg/L	Week II (outlet) mg/L	Week III (outlet) mg/L	Week IV (outlet) mg/L	Week V (outlet) mg/L	Removal Efficiency % Week I	Removal Efficiency % Week II	Removal Efficiency % Week III	Removal Efficiency % Week IV	Removal Efficiency % Week V
1.05E+05	340.57	120.26	53.26	33.65	19.72	99.67	99.89	99.95	99.97	99.98

ww =wastewater, RF = Removal Efficiency

Table 4. Decontamination of Toxic TPHs of Petroleum-contaminated Wastewater from Polyculture (*Cyperus odoratus*, *Colocasia esculenta*, *Phoenix roebelenii* and *Eisenia fetida*) Wetland Reactors

Raw Petroleum Contaminated ww (Inlet) mg/L	Week I (outlet) mg/L	Week II (outlet) mg/L	Week III (outlet) mg/L	Week IV (outlet) mg/L	Week V (outlet) mg/L	RF (%) Week I	RF (%) Week II	RF (%) Week III	RF (%) Week IV	RF (%) Week V
1.05E+05	140.68	57.06	39.12	30.22	*8.91	99.87	99.95	99.96	99.97	99.99

ww =wastewater, RF = Removal Efficiency, * Below the maximum concentration limit (MCL) on surface water of 10 mg/L DPR /EGASPIN (2002)

The phytoremediation and vermiremediation of toxic TPHs present in the surface water were performed employing different wetland reactors of monoculture (*Cyperus odoratus*), mixedculture (*Cyperus odoratus* and *Colocasia esculenta*) and polyculture (*Cyperus odoratus*, *Colocasia esculenta*, *Phoenix roebelenii* and *Eisenia fetida*).

The phytoremediation that occurred in the monoculture reactor (Table 2) depicted clear decontamination processes of TPHs present in the raw sample of the surface water after complying with the 5 weeks detention period of the experimental setup. Week I began the degradation processes of TPHs from 1.05E+05 mg/L (raw sample) to 976.34 mg/L, with performance efficiency 99.07%. The degradation mechanism continued at Weeks II (132.54 mg/L), III (57.37 mg/L), IV (34.86 mg/L) and V (31.28 mg/L) with performance efficiency of 99.87%, 99.95%, 99.97% and 99.97% respectively. The Table 2 revealed remarkable results but were all above the maximum contamination limit of Department of Petroleum Resources/Environmental Guidelines and Standard for the Petroleum Industry in Nigeria (DPR/EGASPIN) 2002 for surface water. The decontamination processes of toxic TPHs in the monoculture reactor transpired through the scientific principle and mechanism of phytoremediation. However, the *C. odoratus* in the monoculture had the potential to tolerate and survive the environmental hardship of the TPHs contamination until harvest of 5 weeks detention period.

The combined plant species of *C. odoratus* and *C. esculenta* from the mixedculture reactor further exhibited credible potentials of degradation of phytoremediation. Their biological environment for mechanism of phytoremediation proved better than the monoculture reactor. The output sample from the mixedculture reactor (Table 3) after observing the 5 weeks detention period; week I portrayed better phyto-degradation process of the initial TPHs value of 1.05E+05 mg/L (raw sample) to a drastic reduction of 340.57 mg/L with performance efficiency of 99.67%. Weeks II, III, IV and V (120.26, 53.26, 33.65 and 19.72 mg/L) respectively sustained the degradation and decontamination processes with performances efficiencies of 99.89%, 99.95%, 99.97% and 99.98% respectively.

Table 4 further presented remarkable values in decontaminating toxic TPHs within the 5 weeks detention period. Polyculture reactor was a demonstration of the mutualistic and synergistic biological relationship among the biotas (*C. odoratus*, *C. esculenta* and *P. roebelenii*) that was enough to detoxify TPHs elevated value of 1.05E + 05 mg/L (raw sample). In addition, the inoculation of *E. fetida* also indicated natural ability to crush, digest and absorb TPHs contaminants, besides, expedite the processes of TPHs decontamination by stimulating microbial growth and activity in soil. This bioactivity is referred to vermiremediation. Earthworms were found to bioaccumulate TPHs in the fatty deposits of their bodies [29]. In the present study, week I outlet depicted 140.68 mg/L with removal or performance efficiencies of 99.87%, week II depicted 57.06 mg/L (99.95%), week III depicted 39.12 mg/L (99.96%), week IV depicted 30.22 mg/L (99.97%) and week V depicted 8.91 mg/L (99.99%). The final detoxification was recorded at week V with the result that was lower than the regulatory agency of DPR/EGASPIN (2002). This result (8.91 mg/L) could have been possible at week V because of the vermiremediation technology present in the polyculture reactor. *E. fetida* squirms were exceptionally resistant to toxic TPHs and their biological combination with *C. odoratus*, *C. esculenta*, *P. roebelenii* were exceedingly incredible and sustainable as shown in Table 4. However, the polyculture wetland scientifically demonstrated the combined potentials of *C. odoratus*, *C. esculenta*, *P. roebelenii* and *E. fetida* bioaccumulation of toxic TPHs as fair candidate of speedy remediation processes.

5. CONCLUSION

The biota of polyculture reactor gave better advantage of bioremediation (phytoremediation and vermiremediation) and removal efficiency of the total petroleum hydrocarbons (TPHs) in the contaminated surface water sample over monoculture and mixedculture reactors; more homogeneity of biological activities, richer in plant species, more population of degrader microbes within the system, improved soil nutrients for plants survival amidst the ecotoxic TPHs. Stimulation of microbial activities by *E. fetida* squirms introduced easy biodegradation of TPHs, also indicated good efficiency of contaminants removal. The soil becomes lighter and porous, rich in biological activities and productivity. However, earthworms have the mechanism to improve the fertility of soil by

adding their excreta – the vermicast (as vermicompost) which contains rich nutrients (NPK and micronutrients), enzymes, growth hormones, beneficial nitrogen-fixing and decomposer microbes. Hence *C. odoratus*, *C. esculenta*, *P. roebelenii* and *E. fetida* are good candidates of bioremediation while there combination improves the efficiency of decontamination.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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