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Assessing Earthworm Influence on Remediating Potentials of Soil Micro-Organisms, and Bioavailable Hydrocarbon Pollutant in the Niger Delta, Nigeria

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Abstract

In the Niger Delta region of Nigeria, oil explorations and exploitations abound, causing environmental pollution with serious consequences on soil ecosystem and its biodiversity. In spite of the relationship between microbes and fauna in soil ecosystem, such that both organisms can metabolize certain range of petroleum hydrocarbon substrates with the fauna influencing the remediation potentials of bacteria, yet soil fauna is still not fully considered in bioremediation. The influence of earthworm; *Lumbricus terrestris* on the remediating potentials of soil bacteria in petroleum hydrocarbon contaminated soils was investigated. Eighteen pots were filled with 700 g of soil each, with nine treated with mixture of 3 levels crude oil and remediated with earthworm, while the other nine had no earthworm. The total petroleum hydrocarbon (TPH), soil physical, nutrient compositions, and TPH degrading bacteria biodiversity were determined before contamination or commencement of study and thirty days after. The results showed a decrease in TPH concentration of 55.58%, 62.57% and 67.07% in 1 ml, 2 ml and 3 ml crude oil contaminated soil, respectively. Species richness and abundance of bacteria organisms increased with high relative abundance in soils remediated with earthworms, hydrocarbon utilizing bacteria increased from less than 0.1 cfu/g to 0.4 cfu/g, and total heterotrophic bacteria 1.6 cfu/g at the end of the study. Earthworms increased rate of remediation potentials of bacteria, such that within 30 days post remediation treatment, 34.14% of reduced concentration was achieved over soil samples without earthworms at 3 ml, and 25.14% at 2 ml concentration. Reduction in pH levels in remediated soils was between

6.39 to 6.17 and 6.74 to 6.72 in unremediated soils, while moisture content was 6.73% to 6.77% unremediated and 5.85% to 6.62% in earthworm remediated soils. Total organic carbon, nitrates in soils inoculated with earthworms were lower in concentration than those without earthworms. Reverse was the case with potassium, phosphate and phosphorous concentrations which were above those without earthworms. Results indicate statistically, significant difference between reduction in TPH in earthworm remediated soils and unremediated soils, pointing out that earthworm is a good candidate for facilitation of bacteria remediation-petroleum hydrocarbon contamination.

Keywords

Petroleum Hydrocarbon Contamination, Bacteria Biodiversity, Soil Fauna, Total Organic Carbon, Nitrates

1. Introduction

Soil micro-organism comprising bacteria and fungi directly initiate chemical decomposition process of organic matter, however the processes cannot be achieved without the facilitating activities of soil fauna biodiversity such as micro arthropods, mainly meso fauna and macro fauna. Soil micro-organisms, particularly bacteria have the potentials to bio-remediate soil ecosystem polluted with hydrocarbon contaminants. The presence of micro-organisms with the appropriate metabolic capabilities is the most important requirement for petroleum oil spill bio-remediation (Venosa et al., 2002). Atlas (1995) stated that microbial communities adapted to hydrocarbon contaminated ecosystem can respond to the presence of hydrocarbon pollutants within hours, and exhibit higher biodegradation rates than ecosystem with no history of hydrocarbon contamination (Leahy and Colwell, 1990). Consequently, the ability to isolate high numbers of certain oil degrading micro-organisms from an environment is commonly taken as evidence that those micro-organisms are the most active oil degraders of that environment (Atlas, 1995), and can be used in bio-remediation of petroleum polluted ecosystem. Micro-organism that has remediating potentials requires a mixture of different bacterial groups or consortia functioning to degrade a wider range of hydrocarbons. This combined effort is efficient for biodegradation of petroleum hydrocarbon because crude oil is made of a mixture of compounds, and individual micro-organisms metabolize only a limited range of hydrocarbon substrates (Al-Saleh et al., 2009; Bordenave et al., 2007).

Hydrocarbon contaminated ecosystem is an ecosystem that contain intolerable concentration of Total Petroleum Hydrocarbon (TPH). Total Petroleum Hydrocarbon can be defined as the measurable amount of petroleum based hydrocarbon in an environment media (ATSDR, 1999). TPH is dependent on analysis of the medium such as soil in which it is found (Gustafson, 1997).

Micro-organism that occur in large numbers in oil polluted than uncontaminated ecosystem according to Walworth and Reynolds (1995); Okoh (2003) includes hydrocarbon-degrading species, *Pseudomanas*, *Vibrio*, *Corynebacterium*, *Arthrobacter*, *Brevibacterium*, *Flavobacterium*, *Sporobolomyces*, *Achromobacter*, *Bacillus*, *Aeromonas*, *Thiobacillus*, *Lactobacter*, *Staphylococcus*, Penicillium, and Articulosporium. These hydrocarbon degraders take advantage of cytotoxic immediate metabolites and flourish in contaminated ecosystem while those that are sensitive to the contaminant decline in richness and phylogenetic diversity due to disruption of the nitrogen cycle, with species and functional genes involved in nitrification being significantly reduced (van Dorst et al., 2014).

Bioremediation potentials of micro-organisms is not efficient enough to solely remediate contaminated ecosystem as it will take a long time, particularly without the participation of other soil fauna that inhabit the soil ecosystem. Furthermore, availability of nutrients in the soil ecosystem is paramount for efficiency of micro-organisms.

Earthworm is a principal soil fauna that influence bio-remediating activities of soil micro-organism in degrading hydrocarbon contamination, as they are capable of inhabiting such ecosystem (Eijsackers, 2010). There are three groups of soil-dwelling earthworms, the epigeic, a red colour type with 0.5 to 5 cm in size, have poor burrowing capacity and are good tolerant to low pH values; the endogeic, are non-pigmented with body size of 1 to 8 cm, good borrowers and restricts to pH below 5. They make horizontal tunnels. The third group is the reddish-brown type with sizes above 5 cm called Anecic. They are excellent burrowers, and make vertical tunnels. While the epigeic occur at superficial layers of soil, endogeic inhabit the first few centimeters of mineral soils, and anecic occur in several meters soil depth and tolerates soil with low moisture (Menta, 2012).

The dominance of earthworm in an oil polluted ecosystem has been reported. Zavala-Cruz et al. (2013) noted that earthworm's population in a soil contaminated with 12,000 mg TPH kg^{-1} of weathered crude oil over a 20 years period was dominated by *Pontosclex corethrurus Gossodrillus sp.* and *Dichogaster saliens*. *Pontoscolex corethrurus* an earthworm was 75% the most predominant species depicting about 88% of the total biomass in an environment polluted with benzo (a) pyrene (Bap) of 39 $mg \cdot kg^{-1}$ (Hernández-Castellanos et al., 2013).

Earthworms are highly tolerant to different varieties of organic and inorganic contaminants; insecticides, Polycyclic Aromatic Hydrocarbons (PAH), crude oil and heavy metals found in soil (Shahmansouri et al., 2005; Patnaik and Reddy, 2011; Dada, 2015). These faunal organisms increase their interactions with contaminants and soil micro-organisms due to their activities in the soil (Hickman and Reid, 2008). For instance, earthworms accelerate the biodegradation of PAH through the biostimulation of microbial growth and activity in the soil thereby eliminating them by passive absorption and bioaccumulation (Eijsackers et al., 2001; Tang et al., 2002). Similarly, Contreras-Ramos et al. (2006) reported that earthworm accelerate biodegradation of PAH by bioaccumulating PAHs in their fatty deposits. For example, 3 - 4 ring PAHs compound are bio-accumulated from contaminated soils by earthworms (Parish et al., 2006).

The involvement of earthworms on the reduction of concentration of hydrocarbon contaminant in petroleum impacted soils has been reported by several studies; Sinha et al. (2010); Ekperusi and Aigbodion (2015), and Njoku et al. (2016). The earthworm *Eudrilus eugeniae* has been reported as a viable candidate for vermi-assisted remediation of petroleum hydrocarbon-contaminated mechanic workshop soils (Ameh et al., 2013). The authors reported that earthworms introduced to hydrocarbon contaminated soil caused a higher decrease in TPH concentration after a 35 days period, while the control group recorded a minimal decrease. The presence of earthworms in hydrocarbon contaminated soil showed after 28 days a loss in TPH concentration from 17% - 42%, and also an improvement in soil respiration and microbial biomass (Schaefer et al., 2005; Schaefer and Juliane, 2007).

Reduction in hydrocarbon concentration in contaminated soils is enhanced by combining earthworms with compost which showed a higher decrease of about 65% (Hickman and Reid, 2008). *Lumbricus terrestris* followed by *Eisenia fetida* and *Allolobophora chlorotica* have higher efficiency in the reduction of the concentration of hydrocarbon contaminant in soils (Schaefer et al., 2005). Accordingly, *L. terrestis* reduced 30% - 42%, *E. fetida* 31% - 37% and *A. chloroica* 17% - 18% of 10,000 $mg \cdot kg^{-1}$ TPH.

Earthworms introduced to hydrocarbon contaminated soils increased the rate of biodegradation and hydrocarbon concentration reduction. Ma et al. (1995) showed a fast rate reduction of PAHs concentration in soils treated with earthworms than soil without earthworms.

Earthworms naturally helps in maintenance of available nutrients in soil ecosystem through its burrowing and production of vermicasts, however, in hydrocarbon contaminated soil, reduction in nitrogen and phosphate levels occurs and caused slow pace of biodegradation of oil constituents. The soil pH level is also affected in hydrocarbon contaminated soils. Studies on role of earthworms in the improvement of nutrients and pH in hydrocarbon contaminated soils are scantily reported in literature. Presence of earthworms has been reported to cause accumulation of organic acids produced in the degradation of petroleum hydrocarbon, leading to a reduction in pH levels (Alexander, 1999; Eweis et al., 1998). Manyuchi et al. (2013) reported increase in potassium content in hydrocarbon contaminated soil with earthworm after 25 days period.

In the Niger Delta region of Nigeria, where oil exploration and associated activities are predominant causing environmental pollution with serious consequences on soil ecosystem and its biodiversity, remediation attempts are yet to involve the role of soil fauna in its strategy. The role of earthworms in remediation programs is underrated and left in the hands of natural attenuation. Combined efforts of the relationship between soil fauna (earthworms) and micro-organisms (bacteria) in bioremediation of hydrocarbon contaminated soil ecosystem is yet to be investigated and exploited in the region.

A serious gap exists in available literature on the impact of earthworms on the soil nutrients, pH in hydrocarbon contaminated ecosystem and this has to be investigated.

It is the concern of this present study to breach up these gaps and provide necessary information, particularly on the way earthworms influence bacteria in the bioremediation of hydrocarbon contaminated soil ecosystem. This study is aimed at evaluating the influence of earthworm *Lumbricus terrestris* on remediating potentials of soil micro-organisms in hydrocarbon contaminated soil ecosystem, with objectives to determine:

1) Influence on Bioavailable Hydrocarbon concentration.
2) Influence on Soil Bacteria Biodiversity Dynamics, and
3) Influence on Maintenance of Physicochemical Parameters.

2. Materials and Methods

2.1. The Niger Delta Region

This region in Nigeria is the region of oil exploration and exploitation that accounts for the main source of income of the country. Soil pollution triggered by oil activities is predominant in the region (**Figure 1**).

2.2. Experimental Design

The soil used for this study where obtained from the University of Port Harcourt farm land with the aid of a soil auger. A total of 18 experiment pots made from plywood measuring about 20 cm in length, 10 cm in width and 15 cm in height were packed filled with 700 g of loamy soil. These soils were measured with the aid of an electronic measuring scale, afterwards polluted with crude oil respectively. Each set was divided into three subsets representing 1 ml, 2 ml and 3 ml of crude oil respectively. Every subset was replicated thrice. The earthworm (*Lumbricus terrestris*) were inoculated alive into one of the two sets however the second set had no earthworms but then it was polluted with same volume of crude oil as the first set. The inoculations of earthworms (10) into contaminated soils were carried out two days after pollution. The experimental pots were always kept moist by the sprinkling of 200 ml of water after every two days. In quality assurance, foreign objects such as sticks, leaf litter and rubbish were picked out of the soil; these soils were subsequently sieved using a 2 mm mesh sized sieve to achieved better-quality grains. Approximately 25 kg of soil was weighed with an electric weighing scale for sample preparation. Soil samples were taken before contamination (initial) and thirty days after contamination (initial) and thirty days after contamination (final) for laboratory analysis of Total Petroleum Hydrocarbon (TPH) content, physicochemical parameter, soil nutrients and microbial flora. Below are a diagrammatic illustration of the experimental design and an image of the experiment pots (**Figure 2**).

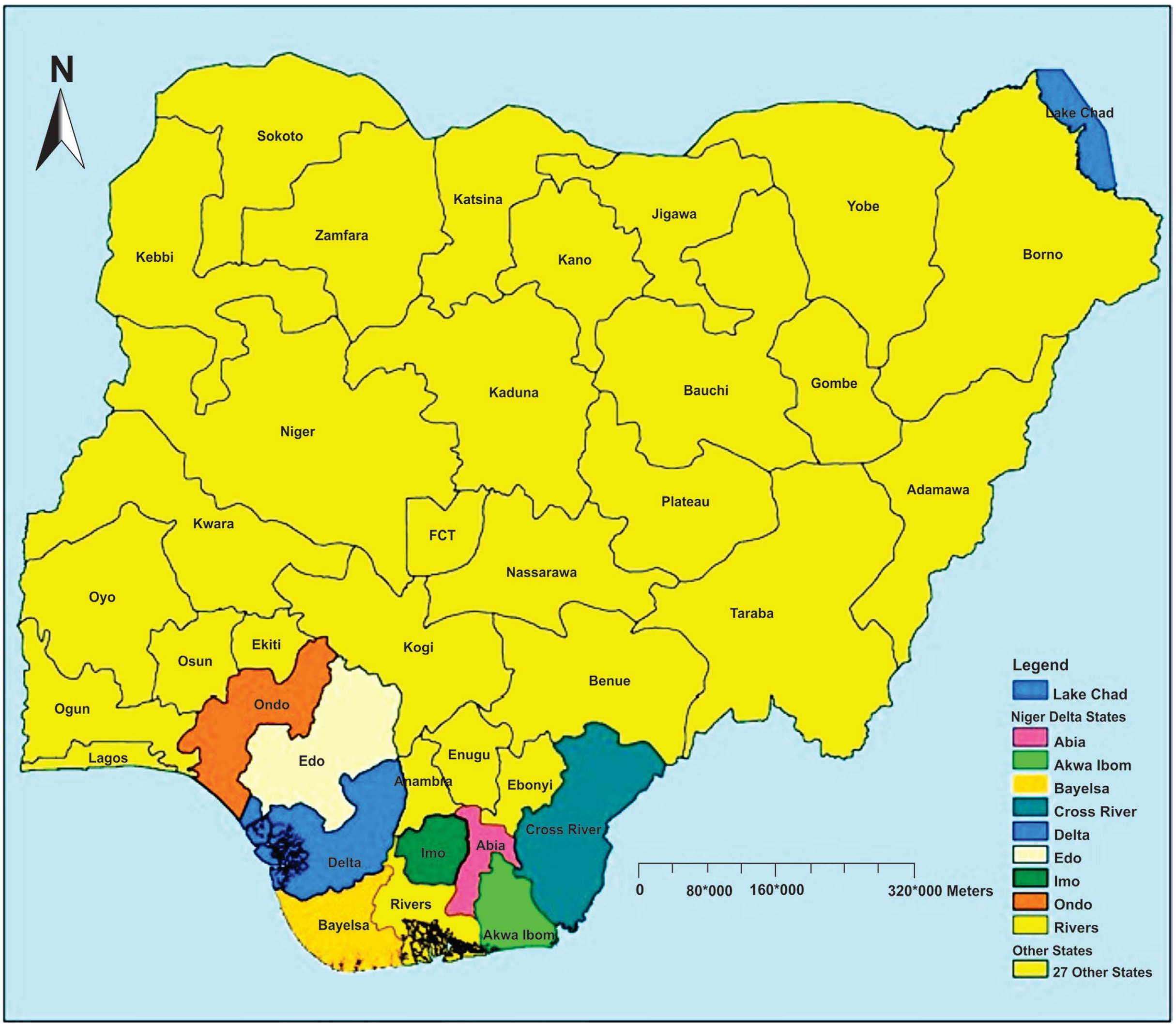

Figure 1. Map of Nigeria showing the Niger Delta region. (Source: Aniefiok et al., 2013).

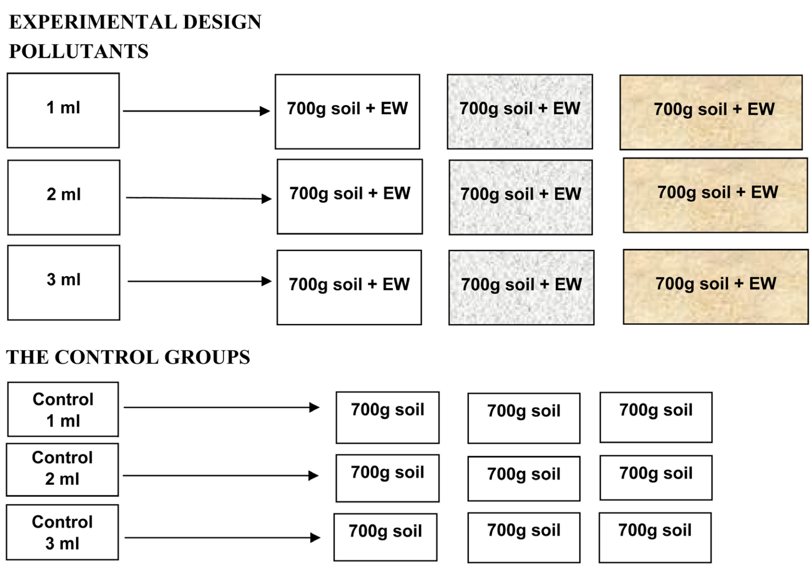

Figure 2. Schematic experimental design. Legend: EW = Earthworm.

2.3. Breeding of Earthworms *Lumbricus terrestris*

The earthworm employed for this study was the *Lumbricus terrestris*. It was bred for a period of six months under favourable environmental conditions. The motive for breeding was to ensure that the earthworms were free from inorganic substances in their body. Earthworms were bred in a wooden frame box measuring about 50 cm in length, 45 cm in width and 70 cm in height. The frame boxes were filled with about 20 kg of soil. The wood frame was perforated at the base and on every side to ensure proper aeration and to drain away excess water. Poultry manure measuring about 5 kg were introduced as feeds to the earthworms during the breeding period. The soil samples were always kept moist by sprinkling of water on the soil surface in other to give way for easy burrowing.

2.4. Soil Contamination with Petroleum Hydrocarbon

The crude oil used for this experiment was obtained from a Shell Petroleum Development Company (SPDC) located at Obelle, SPDC location, in Emohua Local Government Area of Rivers State. The volume of crude oil used was measured with a measuring cylinder into 1 ml, 2 ml and 3 ml concentrations.

Soil contaminated was achieved through the heterogeneous mixture of 700 g virgin soil and crude oil. Afterwards, the contaminated soils were introduced into a total of 18 experimental pots.

2.5. Determination of Earthworm Influence on Soil Physico-Chemical Parameters

2.5.1. Soil Analysis and Moisture Content

The moisture content of the soil was determined by weighing 100 g of soil sample in an analytical weighing device to obtain the wet weight. The samples were kept in a flat bowl and then placed in an oven for a period of 9 hours at a constant temperature of 90. Afterwards the sample were removed from the oven, and weighed again to obtain the dry weight. Moisture content was calculated using formula similar to Gbarakoro et al. (2010).

Moisture content (%) = Loss in weight (g)/initial weight (g) × 100

Loss in weight = Fresh weight − Dry weight

2.5.2. Analysis of Soil pH

During this process, 20 g of sample was collected into a 50 ml beaker and properly mixed with 20 ml of distilled water. The mixture was left for 30 minutes in order to achieve proper equilibration. Occasionally, the mixture was stirred using a glass rod and thereafter allowed to settle. The electrode in the pH meter was carefully inserted into the suspension hence the readings. This process was done in accordance to Bates (1954) procedure.

2.5.3. Analysis of Soil Nutrients

Concentration of nitrates, phosphorus and potassium contained in each soil sample were determined using standard methods with spectrophotometer at a wavelength of 660 nm. Determination of total organic carbon was carried out using wet oxidation method described by Williams (1969) and Oceanography International Crop (1970).

2.6. Determination of Earthworm Influence on Bacteria Biodiversity Count

A direct count method was employed for the microbial count, while the power plate method was used for the inoculation. This method is similar to the method used by Mbanaso et al. (2013). Following the APHA method 9215, the inoculation by the pour plate method was done. The Hydrocarbon utilizing bacteria and Total Heterotrophic bacteria encountered in each sample were identified at the Research Laboratory of Microbiology, University of Port Harcourt.

2.7. Determination of Earthworm Influence on Bioavailable Hydrocarbon Concentration

10 g of each soil sample was collected from contaminated, non-contaminated and mixed or combined contaminated and earthworm treatment and transferred into a soxhlet thimble. The thimble was then put into the soxhlet apparatus, connected to a 250 ml boiling flask for extraction. The determination was carried out at Giolee Global Resources Laboratory, Port Harcourt, Nigeria in accordance with the Environmental Proctection Agency (EPA) method 9071B. The method employed the utilization of a soxhlet extractor similar to Okop and Ekop (2012), which uses hexane as the solvent, and a rotary evaporator, which evaporates the hexane and the nickel extracts in the oil after extraction.

At the end of the extraction, the available Total Hydrocarbon level contained in each of the soil samples, including those treated with earthworm was recorded. This is because the amount of TPH found in the soil sample after is remediation, is useful as a general indicator of impact of remediation on the petroleum contamination of that soil. Total Petroleum Hydrocarbon (TPH) is the measurable amount of petroleum-based hydrocarbon considered in our study, because it describes compounds contained in crude oil (ATSDR, 1999).

2.8. Statistical Analysis

The data obtained from the experiment were subjected to statistical analysis using Analysis of Variance (ANOVA) to determine the difference, if any between treated samples and the control.

3. Results and Discussion

Earthworms generally influenced both the biodiversity of soil bacteria and their remediating potentials for the remediation of hydrocarbon contaminants in soil ecosystem.

In our study, the biodiversity of bacteria increased in both treated and untreated soil samples, with higher increase in treated samples (**Figure 3** and **Table 1**).

Table 1. Percentage of hydrocarbon utilizing bacteria and total heterotrophic bacteria in Treated and untreated hydrocarbon contaminated soil samples.

TPH degrading bacteria	Samples 1 MIL + EW	Samples 1 MIL + soil	Samples 2 MIL + EW	Samples 2 MIL + soil	Samples 3 MIL + EW	Samples 3 MIL + soil
hydrocarbon Utilizing Bacteria	50.14%	49.86%	66.79%	33.21%	22.22%	77.78%
Total heterotrophic Bacteria count × 10	47.35%	52.7%	54.9%	45.1%	68.2%	31.8%

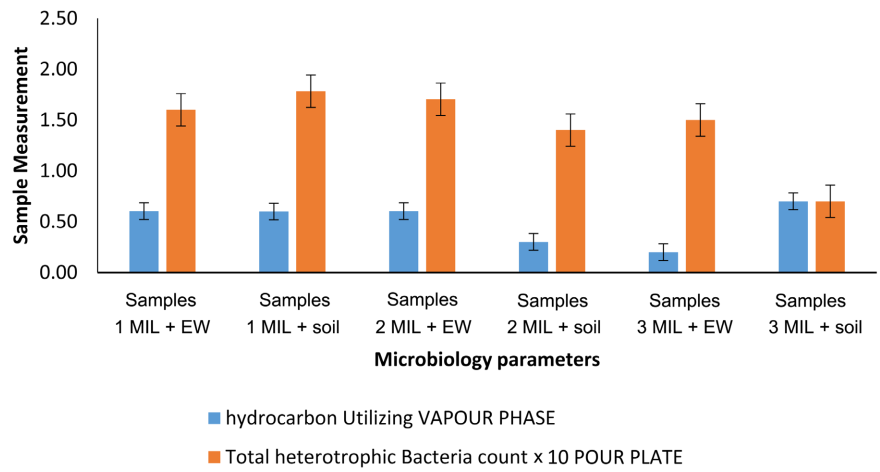

Figure 3. Hydrocarbon utilizing bacteria and total heterotrophic bacteria abundance in treated and untreated soils.

Hydrocarbon utilizing bacteria organisms (HUB) increased from less than 0.1 cfu/g at the commencement of the study to 0.4 cfu/g at the end of 30 days post treatment. A similar increased was recorded with the total heterotrophic bacteria which increased to 1.6 cfu/g at the end of the study. Earthworm treated hydrocarbon soil samples influenced the bacteria remediating potentials by increasing the biodiversity of bacteria which was three species of HUB and four species of total hetrotrophic bacteria (THB). These species occurred in both earthworm treated and untreated soil hydrocarbon contaminated samples, however, their species richness was higher in treated than the untreated. The relative abundance of hydrocarbon utilizing bacteria influence by earthworm increased the rate of remediation potentials of bacteria. It was two times higher than soil samples where earthworm was absent, such that within 30 day-post remediation treatment, a 34.14% of reduced concentration was achieved over the soil samples without earthworm treatments at 3 ml concentration, and 25.14% at 2 ml concentration (**Table 2**). Earthworm in this study increased the number of participating bacteria species, created more working spaces in the soil and increased the rate of remediation potentials. The increase in microbial community increases the level of hydrocarbon degradation enhancing microbial remediation potentials. Our report on creation of working spaces agrees with Domínguez et al. (2002) that earthworm through its activities increase the superficial area for microbial activity, thereby increasing remediation. Furthermore, the increased

Table 2. Impact of earthworm on Bioavailable hydrocarbon concentration in 30 day-post treatment soil samples.

Total petroleum hydrocarbon	Initial concentrations (day 0)	Final concentrations (day 30) with Earth Worm	Final concentrations (day 30) without Earth Worm
3 ml	3405 mg/kg	1121.27 mg/kg (67.07%)	2283.73 mg/kg (32.93%)
2 ml	2825 mg/kg	1057.40 mg/kg (62.57%)	1767.6 mg/kg (37.43%)
1 ml	1412.5 mg/kg	627.43 mg/kg (55.58%)	785.35 mg/kg (44.42%)

Legend: TPH; Total Petroleum Hydrocarbon.

microbial community ensures proficiency of microbes in changing the functions of soil bacteria in degrading contaminants (Azedah and Zarabi, 2015), and our study agrees with this submission. Working spaces also stimulates accessibility to contaminants by microbes (Njoku et al., 2016).

Another way, earthworm in our study, showed influence on remediating potentials of bacteria in the remediation of hydrocarbon contaminated soils is the participatory role played by earthworms. Earthworms, particularly epigeics are involved in biodegradation, and excrete worm casts which contain micro-organisms (Pavithra et al., 2017). These micro-organisms are large quantity of non-digested bacteria which proliferate easily (Zanella et al., 2001). The bacteria excreted in the worm casts increases the microbial community and consequently enhanced the remediating potentials of the hydrocarbon utilizing bacteria encountered. The bacteria contained in the worm casts was not counted separately, however, increased microbial community recorded included them and was influenced by the earthworm treatments which accounted for their higher numbers in treated samples.

The concentration of hydrocarbon is reduced during feeding and production process of worm casts in the digestive tract of earthworms. According to Rodriguez-Campos et al. (2014), microbes prevailing in the digestive tract of earthworm caused a reduction in petroleum hydrocarbon concentration. The high percent reduction in hydrocarbon contaminants in our study do not only support the report, but also agree with the fact that earthworms are good cleaners of contaminated soils (Ekperusi and Aigbodion, 2015; Sinha et al., 2010). Our study indicates that total petroleum hydrocarbon level (TPH) decreased in all the treatments, including the control in 30 day-post remediation treatment. The decreased was higher in soils inoculated with earthworm than untreated soil samples (**Table 2**). The percent or degradation contaminant loss was two times higher in treated soil samples (67.07% 3 ml, 62.57% 2 ml) than untreated (32.93% 3 ml, 37.43% 2 ml), respectively.

In our study, the earthworm; *Lumbricus terrestris* had great influence on petroleum remediating potentials of bacteria by reducing the TPH from initial concentration of 3405 mg/kg to 1121.27 mg/kg (67.07%) against 2283.73 mg/kg (32.93%) in untreated samples within 30 days-post treatment (**Table 2**). This agrees with the report that earthworm has the ability to metabolize TPH to waste products (Lansdell and McConnell, 2003), and reduce the level of TPH. In 3 ml hydrocarbon contaminated soil samples for instance, both hydrocarbon utilizing and total heterotrophic bacteria increased in similar rate, but in earthworm treated soil samples, THB increased far above HUB as it occurred in all the treatments (**Figure 3**), indicating that *L. terrestris* improved the growth of heterotrophic bacteria in hydrocarbon contaminated soils than hydrocarbon utilizing bacteria. Metabolize TPH to waste products (Lansdell and McConnell, 2003), and thus reduce the level of TPH.

Alexander (1999) and Eweis et al. (1998) stated that hydrocarbon contaminated soil that contain earthworms caused a reduction in soil pH levels, due to the release of acidic intermediates and accumulation of organic acids produced in the degradation of petroleum hydrocarbon in the guts of earthworm. Our study agrees with the above submission, as the pH level of untreated soil samples show a reduction range between 6.74 and 6.72 while earthworm treated contaminated soils ranges between 6.39 and 6.17 all within the same 30 days study (**Table 3**). The reduction in pH values was 0.55 and 0.35 in earthworm treated soils that had a TPH reduction of 67.07% and 62.57%, respectively. This is another evidence to show that earthworms participated in the degradation of petroleum hydrocarbon contamination which led to organic acids accumulation and release of intermediates that enhanced pH reduction. The pH values obtained in the study were all above the soil pH value at the commencement of the study, however, with the influence of the earthworm treatment, a reduction in the pH values was obtained but still above the initial pH. The reduction in pH value is striving towards the initial value which may be attained when remediation is almost complete. Our study therefore, indicates that earthworm application is ideal for the restoration of pH values in hydrocarbon contaminated soil ecosystem.

The moisture content was lower than the initial moisture in all the treatments (**Table 3**). It ranges between 6.73% and 6.77% (untreated) and 5.85% to 6.62% (earthworm treated) samples, while initial moisture was 8.77%.

Restoration of physico-chemical parameters of the soil ecosystem is another way earthworm influence the remediation potentials of bacteria. At the end of

Table 3. Influence of earthworm on physico-chemical parameters of hydrocarbon contaminated soil samples at the end of study.

Sample parameters	1 ML + EW	1 ML + SOIL	2 ML + EW	2 ML + SOIL	3 ML + EW	3 ML + SOIL
PH	6.39 ± 0.01^a	6.74 ± 0.01^b	6.36 ± 0.01^a	6.66 ± 0.01^b	6.17 ± 0.01^a	6.72 ± 0.01^b
Temperature	25 ± 1.00^a	25 ± 1.00^a	25 ± 1.00^a	25 ± 1.00^c	25 ± 1.00^a	25 ± 1.00^a
Moisture	6.62 ± 0.03^a	6.77 ± 0.02^b	6.57 ± 0.04^a	6.76 ± 0.03^b	5.85 ± 0.08^a	6.73 ± 0.03^b

($p < 0.05$). a, b, c superscripts indicate whether there is significant difference or not. With the same superscript there is no significant difference. With different superscript there is a significant difference.

the study, the levels of total organic carbon, phosphates, potassium, phosphorus, nitrates were compared with those collected at commencement of study called initial values. Increase in levels above initial values was observed in total organic carbon, phosphates, and nitrates, while potassium and phosphorus levels showed decrease below the initial values. These were observed in both untreated and treated earthworm soil samples. Gbarakoro and Ozonma (2019) reported a low level of phosphorus in petroleum hydrocarbon polluted soil and stated that the increase in mineral elements, proteins and fats levels in polluted soils over those of non-polluted was abnormal and hydrocarbon-induced. Therefore, the increased level of nutrients above initial values in our study is in agreement with the stated reports. However, the increase in nutrients was lower in earthworm-remediated soil than unremediated soil samples, indicating enhancement of the bacteria remediation potentials. The total organic carbon, nitrates for instance in soils inoculated with earthworms were lower than those without any earthworm (**Table 4**).

The reduction in TPH is not only because of the ability of the earthworm to metabolize the petroleum hydrocarbon but also the fact that earthworm and bacterial utilize petroleum hydrocarbon as energy source. The utilization of TPH as energy source was higher in the earthworm remediation soil because both earthworm and bacteria organisms are involved than in unremediated soil sample where only bacteria is involved. This gives credence to the fact that earthworms' presence and activity influenced remediation potentials of bacteria in decreasing TPH faster. Our findings align with Schaefer et al. (2005) position, that the concentration of TPH declines considerably in soils with *Lumbricus terrestris*, while intake of large quantities of nitrogen reduces nitrate levels (Iordache and Borza, 2012). The earthworm remediated soil samples showed increase in potassium, phosphate and phosphorus concentrations above those without earthworms at the end of the study indicating the influence of earthworm on the remediation potentials of bacteria particularly in restoring those soil parameters to normalcy (**Table 4**) Similarly increase in potassium level in hydrocarbon contaminated soil with earthworm has been reported (Manyuchi et al., 2013; Zavala-Cruz et al., 2013).

Table 4. Impact of earthworm treatment on soil nutrients in hydrocarbon contaminated soil samples at the end of study.

Sample parameters	1 ML + EW	1 ML + SOIL	2 ML + EW	2 ML + SOIL	3 ML + EW	3 ML + SOIL
Potassium	0.386 ± 0.001^a	0.403 ± 0.001^b	0.423 ± 0.001^a	0.348 ± 0.001^b	0.404 ± 0.001^a	0.300 ± 0.001^b
Nitrate	29.87 ± 0.06^a	31.40 ± 0.10^b	26.40 ± 0.10^a	36.13 ± 0.06^b	40.80 ± 0.10^a	40.77 ± 0.06^a
Phosphate	62.40 ± 0.10^c	28.90 ± 0.10^a	38.60 ± 0.10^a	28.60 ± 0.02^b	48.20 ± 0.10^a	28.23 ± 0.12^b
Phosphorus	20.80 ± 0.10^b	9.65 ± 0.01^a	12.90 ± 0.05^a	9.54 ± 0.01^b	16.10 ± 0.01^a	9.44 ± 0.01^b
TPH	109.8 ± 0.058^a	137.3 ± 0.12^b	223.8 ± 0.10^a	374.1 ± 0.01^b	287.5 ± 0.01^a	585.5 ± 0.01^b
Total organic carbon	2.769 ± 0.00^b	3.392 ± 0.002^a	3.452 ± 0.001^a	3.822 ± 0.00^b	3.686 ± 0.00^a	3.867 ± 0.05^b

($p < 0.05$). a, b, c superscripts indicate whether there is significant difference or not. With the same superscript there is no significant difference. With different superscript there is a significant difference.

This is in conformity to the findings of Azizi et al., 2013; Ameh et al., 2013, that a loss in total organic carbon was due to the usage of organic carbon by earthworms and micro-organism as source of energy. Statistical evidence revealed that the reduction in TPH concentrations was significantly different between earthworm remediated soils and un-remediated soils. The reduction in TPH concentrations was significantly lower between earthworm remediated and un-remediated soil samples after 30 days study ($p < 0.05$). Soil samples without any earthworm had the lowest percentage loss while the highest percentage loss in TPH concentration was observed in the soil samples with earthworms (**Table 4**). Similarly, there was significant difference between earthworm remediated soils and un-remediated soils in the physico-chemical parameters.

4. Conclusion

The results showed that micro-organisms that possess petroleum hydrocarbon remediating potentials requires a mixture of different bacterial group and a combined effort of soil fauna such as earthworms to remediate polluted soils. Earthworms influenced the remediation potentials of such micro-organisms (bacteria) through increase in hydrocarbon utilizing and total heterotrophic bacteria biodiversity, reduction in petroleum hydrocarbon-induced carbon, and increased hydrocarbon-induced reduced potassium and phosphorus levels. The influence of earthworms on the available nutrients is to normalize their respective contents in the soil ecosystem.

The application of earthworms as bioremediation agent in influencing the remediation potentials of bacteria in a petroleum hydrocarbon contaminated soil ecosystem is encouraged.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

Agency for Toxic Substances Disease Registry (ATSDR) (1999). *Toxicological Profile for Total Petroleum Hydrocarbons (TPH).* U.S. Department of Health and Human Services. https://www.atsdr.cdc.gov/Toxprofiles/tp%20123-p.pdf

Alexander, M. (1999). *Biodegradation and Bioremediation* (2nd Ed.). Academic Press. http://microbeonline.com/pour-platemethod-principle-procedure-uses-dis-advantages

Al-Saleh, E., Drobiova, H., & Obuekwe, C. (2009). Predominant Culturable Crude Oil-Degrading Bacteria in the Coast of Kuwait. *International Biodeterioration & Biodegradation, 63,* 400-406. https://doi.org/10.1016/j.ibiod.2008.11.004

Ameh, A. O., Mohammed-Dabo, I. A., Ibrahim, S, & Ameh, J. B. (2013). Earthworm-Assisted Bioremediation of Petroleum Hydrocarbon Contaminated Soil from Mechanic Workshop. *African Journal of Environmental Science and Technology, 7,* 531-539. https://doi.org/10.5897/AJEST2013.1506

Aniefiok, E., Udo, J., Margret, U., & Sunday, W. (2013). Petroleum Exploration and Production: Past and Present Environmental Issues in the Nigeria"s Niger Delta. *Journal of Envi-*

ronmental Protection, 1, 78-90. https://doi.org/10.12691/env-1-4-2

Atlas, R. M. (1995) Bioremediation of Petroleum Pollutants. *International Biodeterioration & Biodegradation, 35,* 317-327. https://doi.org/10.1016/0964-8305(95)00030-9

Azedah, F., & Zarabi, M. (2015). Combining Vermiremediation with Different Approaches for Effective Bioremediation of Crude Oil and Its Derivatives. In *Proceedings of International Conference on "Global Issues" in Multidisciplinary Academy Research* (pp. 1-12).

Azizi, A. B., Liew, K. Y., Noor, Z. M., & Abdullah, N. (2013). Vermiremediation and Mycoremediation of Polycyclic Aromatic Hydrocarbons in Soil and Sewage Sludge Mixture: A Comparative Study. *International Journal of Environmental Science and Development, 4,* 565-568. https://doi.org/10.7763/IJESD.2013.V4.414

Bates, R. A. (1954). *Electrometric Determination.* John Wiley and Sons, Inc.

Bordenave, S., Goñi-Urriza, M. S., Caumetta, P., & Duran, R. (2007). Effects of Heavy Fuel Oil on the Bacterial Community Structure of a Pristine Microbial Mat. *Applied and Environmental Microbiology, 73,* 6089-6097. https://doi.org/10.1128/AEM.01352-07

Contreras-Ramos, S. M., Álvarez-Bernal, D., & Dendooven, L. (2006). *Eisenia fetida* Increased Removal of Polycyclic Aromatic Hydrocarbons from Soil. *Environmental Pollution, 141,* 340-396. https://doi.org/10.1016/j.envpol.2005.08.057

Dada, E. O. (2015). *Heavy Metal Remediation Potential of a Tropical Wetland Earthworm Species, Libyodrilus violaceus* (192 p). Ph.D. Thesis, University of Lagos.

Domínguez, J., Parmelee, R. W., & Clive, A. E. (2002). Interactions between *Eisenia andrei* (Oligochaeta) and Nematode Populations during Vermicomposting. *Pedobiologia, 47,* 53-60. https://doi.org/10.1078/0031-4056-00169

Eijsackers, H. (2010). Earthworms as Colonisers: Primary Colonisation of Contaminated Land, and Sediment and Soil Waste Deposits. *Science of the Total Environment, 408,* 1759-1769. https://doi.org/10.1016/j.scitotenv.2009.12.046

Eijsackers, H., van Gestel, C. A. M., De Jonge, S., Muijs, B., & Slijkerman, D. (2001). Polycyclic Aromatic Hydrocarbon-Polluted Dredged Peat Sediments and Earthworms: A Mutual Interference. *Ecotoxicology, 10,* 35-50. https://doi.org/10.1023/A:1008954706150

Ekperusi, O. A., & Aigbodion, F. I. (2015). Bioremediation of Petroleum Hydrocarbons from Crude Oil-Contaminated Soil with the Earthworm: *Hyperiodrilus africanus*. *Journal of Biotechnology, 5,* 957-965. https://doi.org/10.1007/s13205-015-0298-1

Eweis, J. B., Ergas, S. J., Chang, D. P., & Schoroeder, E. D. (1998). *Bioremediation Principles.* Mc-Graw Hill.

Gbarakoro, T. N., & Ozonma, O. U. (2019). Determination of Petroleum Hydrocarbon Contamination Tolerance Limit by Food Insect (*Brachytrepes membranaceus*) in Bodo Community, Niger Delta, Nigeria. *Journal of Health and Environmental Research, 5, 8-13.* https://doi.org/10.11648/j.jher.20190501.12

Gbarakoro, T. N., Okiwelu, S. N., Badejo, M. A., & Umeozor, O. C. (2010). Soil Microarthropods in a Secondary Rainforest in Rivers State Nigeria: I-Seasonal Variations in Species Richness, Vertical Distribution and Density in an Undisturbed Habitat. *Scientia Africana, 9,* 48-56.

Gustafson, J. (1997). *Using TPH in Risk-Based Corrective Action.* United States Environmental Protection Agency. http://www.epa.gov/swerustl/rbdm/tphrbca.htm

Hernández-Castellanos, B., Ortíz-Ceballos, A. I., Martínez-Hernández, S., NoaCarrazana, J. C., Luna-Guido, M., Dendooven, L., & Contreras-Ramos, S. M. (2013). Removal of

Benzo (a) Pyrene from Soil Using an Endogenic Earthworm *Pontoscolex corethrurus* (Müller 1857). *Applied Soil Ecology, 70,* 62-69. https://doi.org/10.1016/j.apsoil.2013.04.009

Hickman, Z. A., & Reid, B. J. (2008). Earthworm Assisted Bioremediation of Organic Contaminants. *Environment International, 34,* 1072-1081. https://doi.org/10.1016/j.envint.2008.02.013

Iordache, M., & Borza, I. (2012). Earthworms Response (*Oligochaeta*: *Lumbricidae*) to the Physical Properties of Soil under Condition of Organic Fertilization. *Food, Agriculture and Environment, 10,* 1051-1055.

Lansdell, S., & McConnell, S. (2003). Ecological Considerations in Setting Soil Criteria for Total Petroleum Hydrocarbons (<C15) and Napthalene. In A Langley, M Gilbey, & B. Kennedy (Eds.), *Proceedings of the Fifth National Workshop on the Assessment of Site Contamination.* National Environment Protection Council.

Leahy, J. G., & Colwell, R. R. (1990). Microbial Degradation of Hydrocarbons in the Environment. *Microbiology and Molecular Biology Reviews, 53,* 305-315. https://doi.org/10.1128/mr.54.3.305-315.1990

Ma, W. C., Immerzeel, J., & Bodt, J. (1995). Earthworm and Food Interactions on Bioaccumulation and Disappearance in Soil of Polycyclic Aromatic Hydrocarbons: Studies on Phenanthrene and Fluoranthene. *Ecotoxicology and Environmental Safety, 32,* 226-232. https://doi.org/10.1006/eesa.1995.1108

Manyuchi, M., Chitambwe, T., Phiri, A., Muredzi, P., & Kanhukamwe, Q. (2013). Effect of Vermicompost, Vermiwash and Application Time on Soil Physicochemical Properties. *International Journal of Chemical and Environmental Engineering, 4,* 216-220.

Mbanaso, F. U., Coupe, S. J., Charlesworth, S. M., & Nnadi, E. O. (2013). Laboratory-Based Experiments to Investigate the Impact of Glyphosate-Containing Herbicide on Pollution Attenuation and Biodegradation in a Model Pervious Paving System. *Chemosphere, 90*, 737-746. https://doi.org/10.1016/j.chemosphere.2012.09.058

Menta, C. (2012). Soil Fauna Diversity-Function, Soil Degradation, Biological Indices, Soil Restoration. In G. A. Lameed (Ed.), *Biodiversity Conservation and Utilization in a Diverse World.* IntechOpen. https://doi.org/10.5772/51091

Njoku, K. L., Akinola, M. O., & Anigbogu, C. C. (2016). Vermiremediation of Soils Contaminated with Petroleum Products Using *Eisenia fetida*. *Journal of Applied Science and Environmental Management, 20,* 771-779. https://doi.org/10.4314/jasem.v20i3.31

Oceanography International Crop (1970). *The Total Carbon System Operating Manual.* UK Government.

Okoh, A. I. (2003). Biodegradation of Bonny Light Crude Oil in Soil Microcosm by Some Bacterial Strains Isolated from Crude Oil Flow Stations Saver Pits in Nigeria. *African Journal Biotechnology, 2,* 104-108. https://doi.org/10.5897/AJB2003.000-1021

Okop, I. J., & Ekpo, S. C. (2012). Determination of Total Hydrocarbon Content in Soil after Petroleum Spillage. In *Proceedings of the World Congress on Engineering 2012* (pp. 1722-1726). International Association of Engineers.

Parish, Z. D. White, J. C., Asleyan, M., Gent, M. P. N., Lannucci-Berger, W., Eitzer, B. D., Kelsey, J. W., & Mattina, M. I. (2006). Accumulation of Weathered Polycyclic Aromatic Hydrocarbons (PAHs) by Plant and Earthworm Species. *Chemosphere, 64,* 609-618. https://doi.org/10.1016/j.chemosphere.2005.11.003

Patnaik, S., & Reddy, M. V. (2011). Heavy Metals Remediation from Urban Wastes Using Three Species of Earthworm (*Eudrilus euginiae, Eisenia fetida* and *Perionyx excavates*). *Journal of Environmental Chemistry and Ecotoxicology, 3,* 345-356. https://doi.org/10.5897/JECE11.036

Pavithra, M, Yerva, A. R., & Anila, M. A. (2017). Recent Techniques in Bio-Compositing of Solid Wastes. In P. B. Kumar, & A. Chauhan (Eds.), *Land Reclammation, Soil Quality and Agriculture.* Discovery Publishing House Pvt. Ltd.

Rodriguez-Campos, J., Dendooven, L., Alvarez-Bernal, D., & Contreras-Ramos, S. M. (2014). Potential of Earthworms to Accelerate Removal of Organic Contaminants from Soil: A Review. *Applied Soil Ecology, 79,* 10-25. https://doi.org/10.1016/j.apsoil.2014.02.010

Schaefer, M., & Juliane, F. (2007). The Influence of Earthworms and Organic Additives on the Biodegradation of Oil Contaminated Soil. *Applied Soil Ecology, 36,* 53-62. https://doi.org/10.1016/j.apsoil.2006.11.002

Schaefer, M., Petersen, S. O., & Filser, F. (2005). Effects of *Lumbricus terrestris, Allolobophora chlorotica* and *Eisenia fetida* on Microbial Community Dynamics in Oil-Contaminated Soil. *Soil Biology and Biochemistry, 37,* 2065-2076. https://doi.org/10.1016/j.soilbio.2005.03.010

Shahmansouri, M. R., Pourmoghadas, H., Parvaresh, A. R., & Alidadi, H. (2005). Heavy Metals Bioaccumulation by Iranian and Australian Earthworms (*Eisenia fetida*) in the Sewage Sludge Vermicomposting. *Iranian Journal of Environmental Health Science and Engineering, 2,* 28-32.

Sinha, R. K., Chauhan, K., Valani, D., Chandran, V., Soni, B. K., & Patel, V. (2010). Earthworms: Charles Darwin's 'Unheralded Soldiers of Mankind': Protective & Productive for Man & Environment. *Journal of Environmental Protection, 1,* 251-260. https://doi.org/10.4236/jep.2010.13030

Tang, J., Liste, H., & Alexander, M (2002). Chemical Assays of Availability to Earthworms of Polycyclic Aromatic Hydrocarbons in Soil. *Chemosphere, 48,* 35-42. https://doi.org/10.1016/S0045-6535(02)00046-2

van Dorst, J., Siciliano, S. D., Winsely, T., Snape, I., & Ferrari, B. C. (2014). Bacterial Targets as Potential Indicators of Diesel Fuel Toxicity in Subantarctic Soils. *Applied and Environmental Microbiology, 80,* 4021-4033. https://doi.org/10.1128/AEM.03939-13

Venosa, A. D., Lee, K., Suidan, M. T., Garcia-Blanco, S., Cobanil, S., Moteleb, M., Haines, J. R., Tremblay, G., & Hazelwood, M. (2002). Bioremediation and Biorestoration of a Crube Oil-Contaminated Freshwater Wetland on the St. Lawrence River. *Bioremediation Journal, 6,* 261-281. https://doi.org/10.1080/10889860290777602

Walworth, J. L., & Reynolds, C. M. (1995). Bioremediation of a Petroleum-Contaminated Cryic Soil: Effects of Phosphorus, Nitrogen, and Temperature. *Journal of Soil Contamination, 4,* 299-310. https://doi.org/10.1080/15320389509383499

Williams, P. M. (1969). The Determination of Dissolved Organic Carbon in Seawater: A Comparison of Two Methods. *Limnology and Oceanography, 14,* 297-298.

Zanella, A., Tomasi, M., De Siena, C., Frizzera, L., Jabiol, B., Nicotimi, G. et al. (2001). *Humus forestali: Manuale di ecologia per il riconoscimento e l'interpretazione: Applicazione alle faggete.* Centro di Ecologia Alpina.

Zavala-Cruz, J., Trujillo-Capistrán, F., Ortíz-Ceballos, G. C., & Ortiz-Ceballos, A. I. (2013). Tropical Endogeic Earthworm Population in a Pollution Gradient with Weathered Crude Oil. *Research Journal of Environmental Sciences, 7,* 15-26. https://doi.org/10.3923/rjes.2013.15.26