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Effects of heavy metals in well water on biodegradation of petroleum hydrocarbon by consortia of bacteria and fungi

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This paper reports a study that measured the effect of some heavy metals at different concentrations (Lead 0.86 ppm, Copper <0.001 ppm, Manganese 0.05 ppm, and Nickel <0.001 ppm) in well water contaminated with petroleum hydrocarbon on biodegradation by a consortium of hydrocarbon utilizing bacteria and fungi, using distilled water amended with 5% bonny light crude oil and varying concentrations of the salts of the heavy metals described earlier, either singly or combined. Also the effects of these heavy metals on the physicochemical characteristics of the treatments were analysed after 14 days. The control (no heavy metal addition) had the highest percentage of total petroleum hydrocarbon reduction (81.66%). There was no significant difference in total petroleum hydrocarbon reduction in Copper, Nickel, Lead and the combination of the heavy metals Copper, Nickel, Manganese and Lead at P≥0.05, while percentage of total petroleum hydrocarbon degraded of well water and Manganese was 35.29 and 59.67%, respectively and showed significant difference (P≤0.05) from the control. Electrical conductivity, salinity, temperature, total dissolved solid, dissolved oxygen and pH in various treatments showed no significant difference (P≥0.05), but Biological Oxygen Demand of Lead was significantly different (P≤0.05) from the control. These results suggest that the heavy metals present interfere with the extent of biodegradation of petroleum hydrocarbons in water.

Key words: Well water, petroleum hydrocarbons, heavy metals, bacteria and fungi consortia, biodegradation.

INTRODUCTION

Crude oil or petroleum is a naturally occurring heterogeneous composition of hydrocarbons and nonhydrocarbon compounds (Obire, 2018a). The hydrocarbon compounds include aliphatic hydrocarbon, cycloaliphatic hydrocarbon and aromatic hydrocarbons, while the non-hydrocarbon components are the nitrogen, sulphur and oxygen (NSO) compounds and heavy metals (Thakur, 2012a), such as Beryllium, Cobalt, Manganese,

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Mercury, Molybdenum, Nickel, Vanadium, etc. The concentrations of these metals are generally low (Stigter et al., 2000) but can inhibit various cellular processes and the effect are often concentration dependent and vary in their individual toxicity (Talley, 2006).

Transportation of petroleum products is rampant in Rivers State. In the process of transporting these petroleum products (diesel, kerosene, petrol), spills may occur in water bodies and on land. Subsequently, hydrocarbons from these spills may find their way into water wells, which are in close proximity either in storm waters, floods or by seepage through the soil. Heavy metal constituents of the oil may be complemented by naturally occurring heavy metals in soil that may reach the water through seepage (Akoto and Adiyiah, 2007). Thus the resulting well water may be a cocktail of hydrocarbons, heavy metals and other organic and inorganic components. Oil spill is not only the source through which heavy metals gain entry into well water, others sources includes solid waste disposal, industrial and domestic effluent and some heavy metals constitute a natural components of the earth crust, although the general increase of heavy metals in the environment (soil and water) has been largely caused by crude oil spill (Anoliefo and Vwioko, 1995). The cleanup of petroleum hydrocarbon in well water is usually very slow because oxygen which is the preferred electron acceptor for rapid degradation of petroleum hydrocarbon by obligate aerobes is limited (Alexander, 1994), hence oxygen is provided for by injecting pure oxygen with sparging or hydrogen peroxide which decomposes to liberate O_2 and water (Obire, 2018b; Kosaric, 2001).

Biodegradation is a biologically catalyzed reduction of complex chemical compounds. In the case of crude oil biodegradation, according to Bennet et al. (2002) there are three strategies for bioremediation of petroleum hydrocarbon by hydrocarbon utilizing microbes and these include:

(1) The petroleum hydrocarbon is used as a source of carbon and energy by microorganisms, this strategy results in the complete breakdown of petroleum hydrocarbon into water, Co_2 or other inorganic end product, this process is known as mineralization.
(2) The petroleum hydrocarbon is enzymatically biotransformed by microorganisms, but these organisms are unable to use petroleum hydrocarbon as a source of carbon and energy to support microbial growth. This process is known as cometabolism or cooxidation metabolism.
(2) The petroleum hydrocarbon is not metabolized but is taken up and concentrated within cell biomass. This process is known as bioaccumulation.

Crude oil is a liquid that is immiscible with water because the hydrocarbon components are hydrophobic and may persists in an environment, because they are not readily bioavailable for microbial attack. Often, surfactants (surface active agents), which are chemical compounds with both hydrophilic and hydrophobic moieties (Muthusamy et al., 2008) are used to reduce surface and interfacial tension leading to solubilisation of crude oil in water (Deleu and Paquot, 2004; Gautam and Tyagi, 2006; Nitschke and Costa, 2007). Although some hydrocarbon degraders synthesize biosurfactant which has both hydrophobic and hydrophilic domains capable of reducing surface tension of petroleum, the economic production efficiency and high cost of biosurfactants hinder its use in applications such as oil recovery, which requires large amount of biosurfactant (Cameotra and Makkar, 1998). According to Rahman and Gakpe (2008), biosurfactant super-producing species are rare and known species are not capable of producing high surfactant yields.

In circumstance in which the period for destruction or reduction of petroleum hydrocarbon is so important, microorganisms that exhibit degradative capacities are inoculated into the environment in order to enhance decomposition of petroleum hydrocarbon, such inoculation is known as bioaugmentation. Since petroleum hydrocarbon is a complex mixture, it requires mixed microbial communities with degradative potential to biodegrade petroleum hydrocarbon (Thakur, 2012b). Petroleum biodegradation has been reported to be mostly enhanced in the presence of a consortium of bacteria species compared to monospecies activities (Ghazali et al., 2004). Mixed microbial communities have the most powerful biodegradative potential because the genetic information of more than one organism is necessary to degrade complex mixtures (Joutey et al., 2013).

Although numerous studies have shown that a wide range of concentrations of Ni, Cu, Zn and Pb have inhibitory effects on the degradation of organic compound (Mittal and Ratra, 2000; Amor et al., 2001; Sokhn et al., 2001). According to Nam et al. (2015), there is a need to investigate the effect of heavy metals on the degradation of organic pollutant in order to develop the inorganic detoxification reactor successfully in future study. Thus, the objective of this study was to determine the impact of the heavy metals present in petroleum hydrocarbon-contaminated well water on biodegradation of the contaminant oil in well water and distilled water amended with 5% Bonny Light crude oil by consortia of hydrocarbon utilizing bacteria and fungi.

METHODOLOGY

Site description

The petroleum hydrocarbon-contaminated well water use for the study was obtained from Ogbema water front in Ogbema Community of Abua/Odual Local Government Area, Rivers State, Nigeria. Due to transportation of petroleum products to other interior villages, the river is contaminated with petroleum hydrocarbons and domestic effluents which have resulted in the contamination of the well water close to the river. The well water was contaminated with petroleum hydrocarbon and heavy metals due to seepage of petroleum hydrocarbons and heavy metals from the polluted river. The global positioning system (GPS) location of the well, 32N 0234154, UTM 0532380 was obtained using Etrex manufactured by Garmin Ltd 2000 - 2010.

Sample collection

The well water contaminated with petroleum hydrocarbon was collected using a sterile polyethylene bottle with a piece of concrete fastened to it by means of a rope which was plunged into the well to collect the sample and gently pulled out. The water sample was poured into sterile polyethylene bottles and securely sealed.

Physicochemical analysis

The parameters analysed were temperature, pH, electrical conductivity, total dissolved solid, dissolved oxygen and salinity using Extech multimeter manufactured by FLIR Company, Model DO700, while the BOD was measured after 5 days using the same device.

The petroleum hydrocarbon-contaminated well water was analyzed in the laboratory for heavy metals present by using Atomic Absorption Spectrophotometer (AAS) Model 201 VGP (Buck Scientific). The obtained concentrations of the heavy metals in the well water were (Pb 0.86 ppm; Ni <0.001 ppm; Mn 0.05 ppm; and Cu <0.001 ppm). Similar concentrations were prepared using stock solution of the various heavy metals salt dissolved in distilled water. The effect of these heavy metals on biodegradation of oil in the 5% Bonny Light crude oil amended treatments was analyzed by measuring the residual concentrations of oil in the treatments.

The determination of total petroleum hydrocarbons (TPH) in the petroleum hydrocarbon-contaminated well water was done by Gas Chromatography coupled with flame ionization detector (GC-FID). Residual concentrations of hydrocarbons in the well water and the amended distilled water were measured after 14 days of incubation by using the same instrument.

Microbial cultures

Microorganisms used in the biodegradation studies were *Alcaligenes feacalis*, *Bacillius cereus, Aspergillus aculeatus* and *Penicillum citrinum* harvested from an aged oil impacted soil (Sokolo et al., 2018). Stock culture of each isolate was prepared in nutrient broth and Sabouraud dextrose broth and 2 ml of each isolate were inoculated unto the bioreactor. Hence, consortia of these organisms were used in each conical flask.

Experimental design

Seven experimental treatments, which were in duplicates, were used in the biodegradation studies.

Treatment 1 contained the well water contaminated with 20.49 ppm of petroleum hydrocarbons and 2 ml each of the consortium of bacteria and fungi.

Treatment 2 contained a mixture of 5 ml of each heavy metals salt in varying concentrations (Pb 0.86 ppm+Ni <0.001 ppm+MN 0.05 ppm+Cu <0.001 ppm), 5 ml of 5% Bonny light crude oil containing 92.17 ppm of petroleum hydrocarbon and 2 ml each of the consortium of bacteria and fungi in a broth and in distilled water.

Treatment 3 contained <0.001 ppm of Cu, 5 ml of 5% Bonny light crude oil containing 92.17 ppm of petroleum hydrocarbon, 2 ml each of the consortium of bacteria and fungi in a broth and in distilled water.

Treatment 4 contained 0.05 ppm of Mn, 5 ml of 5% Bonny light crude oil containing 92.17 ppm of petroleum hydrocarbon, 2 ml each of the consortium of bacteria and fungi in a broth and in distilled water.

Treatment 5 contained <0.001 ppm of Ni, 5 ml of 5% Bonny light crude oil containing 92.17 ppm of petroleum hydrocarbon, 2 ml each of the consortium of bacteria and fungi in a broth and in distilled water.

Treatment 6 contained 0.86 ppm of Pb, 5 ml of 5% Bonny light crude containing 92.17 ppm of petroleum hydrocarbon, 2 ml each of the consortium of bacteria and fungi in a broth and in distilled water.

Treatment 7 contained 5 ml of 5% Bonny light crude oil containing 92.17 ppm of petroleum hydrocarbon, 2 ml each of the consortium of bacteria and fungi in a broth and in distilled water and no heavy metals. These bioreactors served as the control.

These treatments were carried out in opened bioreactors or conical flasks kept on a mechanical shaker at 150 rpm for 14 days.

Quantification of total petroleum hydrocarbons degraded

The amount of total hydrocarbon degraded was calculated with the formula:

TPH amount degraded = Initial TPH amount – Mean of final TPH amount of each treatments (1)

Percentage of total petroleum hydrocarbon degraded was calculated with formula:

$$\text{\% of TPH degraded} = \frac{\text{TPH amount degraded}}{\text{initial TPH amount}} \times 100 \quad (2)$$

Statistical analysis

SPSS version 22 was used to perform Analysis of Variance (ANOVA) to determine the differences in the levels of degradation in each treatment and the level of significance was performed estimated by Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Physicochemical constituents of the petroleum hydrocarbon-contaminated-Ogbema Well Water

Table 1 shows the baseline physicochemical constituents of the well water contaminated with petroleum hydrocarbon (TPH). The TPH present was 20.49215 ppm, the heavy metals (Pb, Ni, Cu, and Mn) concentrations were Pb 0.86 ppm, Mn 0.05 ppm, Cu <0.001 ppm and Ni <0.001 ppm. The pH in the hydrocarbon contaminated well water on site was 5.22. pH affects metal toxicity according to Pennanen et al. (1996), a decrease in pH causes stress on microbial communities.

The biological oxygen demand (BOD) of the well water was 5.27 and the dissolved oxygen was 4.16. This is an

Table 1. Physicochemical charateristics of the Well Water from Ogbema.

Physicochemical parameter	Concentrations
Dissolved oxygen (mg/L)	4.16
Temperature (°C)	25.0
pH	5.22
Total dissolved solid (mg/L)	60.8
Salinity (PPt)	0.04
Electrical conductivity (µ∫)	93.1
Depth of the well water (m)	402
Water table of the well water (m)	329
BOD_5 (mg/L)	5.27
Copper (Cu) (ppm)	<0.001
Nickel (Ni) (ppm)	<0.001
Manganese (Mn) (ppm)	0.05
Lead (Pb) (ppm)	0.86
Total Petroleum Hydrocarbon (ppm)	20.49

Table 2. Changes in total petroleum hydrocarbon of well water and the other treatments during the period of experimentation.

Treatment	TPH on 1st day	TPH on day 14	TPH degraded	% TPH degraded
1	20.49 ± 0.00^{b}	13.26 ± 1.23^{a}	7.23 ± 1.23^{b}	35.29 ± 6.0^{b}
2	92.17 ± 0.00^{a}	24.43 ± 6.62^{a}	67.74 ± 6.62^{a}	73.49 ± 7.18^{a}
3	92.17 ± 0.00^{a}	22.95 ± 6.44^{a}	69.23 ± 6.44^{a}	75.11 ± 6.99^{a}
4	92.17 ± 0.00^{a}	37.17 ± 18.2^{a}	55.00 ± 18.29^{a}	59.67 ± 19.84^{ab}
5	92.17 ± 0.00^{a}	25.04 ± 4.28^{a}	67.13 ± 4.28^{a}	72.84 ± 4.64^{a}
6	92.17 ± 0.00^{a}	18.48 ± 0.72^{a}	73.69 ± 0.72^{a}	79.95 ± 0.78^{a}
7 (control)	92.17 ± 0.00^{a}	16.90 ± 3.28^{a}	75.27 ± 3.28^{a}	81.66 ± 3.56^{a}

Means with the same superscript along the columns are not significantly different ($P \geq 0.05$).

indication that oxygen is being depleted and aerobic microorganisms present in the well water may be experiencing oxygen stress and may die.

Total petroleum degraded from various treatments

Table 2 shows analysis of variance (ANOVA) of total petroleum hydrocarbon (TPH) degradation in the different treatments. On the first day, Pb, Ni, Cu, Mn and the combined heavy metal (Pb+Cu+Mn+Ni) in their different concentrations were not significantly different ($P \geq 0.05$) from the control, because they all contain 5% Bonny light crude oil. The TPH of the well water was significantly different ($P \leq 0.05$) from the control, because it is the TPH present in the contaminated well water on site.

Total petroleum hydrocarbon on the 14th day indicates that there was no significant difference ($P \geq 0.05$) in the means of different treatments. The means of percentage TPH degraded in the treatment 2 containing the mixture of heavy metals (Pb+Cu+Mn+Ni) and those containing Pb, Ni, and Cu (Treatments 6, 5 and 3) separately show no significant difference ($P \geq 0.05$) compared to the control (Treatment 7), while degradation in the treatment 1 containing the contaminated well water was significantly different from the control ($P \leq 0.05$) and treatment 4 that contains Mn was significantly different ($P \leq 0.05$) from the control (treatment 7).

In Treatment 1 which contains the well water and inoculated with the treatment organisms, percentage of total petroleum hydrocarbons degraded was 35.29%. This is because most of the petroleum hydrocarbons are in the Non-aqueous Phase Liquids (NAPLs). The NAPLs is a barrier for microorganisms to get access to more of the petroleum hydrocarbon. The petroleum hydrocarbons degraded are those in the liquid phase, which is less than the hydrocarbons in the NAPLs. NAPLs are composed of molecules that have low water solubility and high solubility in solvent such as diethyl ether and the concentrations in the water phase are quite low. It is assumed that the portion of the hydrocarbons that are soluble in water is easily utilized by microorganisms. Solubility of petroleum hydrocarbon is essential, in general, biodegradation rate of aliphatic hydrocarbons reduces as the number of carbon in the molecules increases due to decline in solubility in water (Venosa and Holder, 2007; Coates et al., 1985). However, there are instances where high molecular weight hydrocarbons (C_{44} H_{90}) that are less soluble in water are mineralized by microorganisms (Haines and Alexander, 1974).

In Treatment 2, which contains distilled water, 5% bonny light crude oil, a mix of the heavy metals (Pb+Cu+Mn+Ni) and inoculated with the treatment organisms, the percentage of total petroleum hydrocarbons degraded was 73.49% which was less toxic when compared with Mn which was 59.67% during biodegradation of bonny light crude oil. This result is in accordance to those of Benka-Coker and Ekundayo (1998) that investigated the impact of a combination of Zinc, Lead, Copper and Manganese on crude oil biodegraded by *Micrococcus* and *Pseudomonas* species and discovered that a combination these metals were less toxic when compared with some single metals.

In treatment 4, which contained distilled water, 5% bonny light crude oil, 0.05 ppm of Mn and inoculated hydrocarbon degrading microbes, total petroleum hydrocarbon degraded was 59.67%. This treatment was more inhibitory to biodegradation of bonny light crude oil when compared with treatments 3, 5, and 6, which contained other single metals. According to Babatunde et al. (2017), The concentrations of Manganese was not detected in bonny light crude oil while the concentrations of Nickel, Copper and Lead in bonny light crude oil were 3.18, 0.08 and 0.1 ppm, respectively. The bottom line is Mn at a concentration of 0.05 ppm reduces the extent of biodegradation of petroleum hydrocarbon in bonny light crude oil.

Treatment 7 (control) which comprises distilled water, 5% bonny light crude oil and inoculated hydrocarbon degrading microbes (no heavy metals addition) has 81.66% of total petroleum hydrocarbons degraded. There was high total petroleum hydrocarbon degraded when compared with other treatments. This result is in line with earlier reports by Atagana (2011) that indicated that heavy metals interfered with biodegradation of total petroleum hydrocarbons in soil.

The percentage of total petroleum hydrocarbon degraded from treatments 2 to 7 are 73.49% (Pb+Cu+Mn+Ni), 75.11% (Cu), 59.67% (Mn), 72.84% (Ni), 79.95% (Pb) and 81.66% (Control), respectively which were higher compared to treatment 1 that has 35.29% (well water), because the 5% bonny light crude oil was solubilize with diethyl ether solvent. Diethyl ether is good solvent for a wide range of polar and nonpolar organic compound. Nonpolar compound are generally more soluble in diethyl ether because ether does not have a hydrogen bonding network that must be broken up to dissolve the solute. Also, because diethyl ether has a moderate dipole moment, polar substances dissolves readily in it. These facts make diethyl ether suitable solvent in solubilizing the hydrophobic hydrocarbon compound in petroleum, hence causing these hydrocarbons compounds bioavailable for hydrocarbon degrader to utilize.

Physicochemical characteristics of different treatments

As shown in Table 3, the dissolved oxygen in all the treatments after 14 days was higher than the biological oxygen demand (BOD_5) indicating that there was sufficient dissolved oxygen for hydrocarbon utilizing microbes to successfully carry out biodegradation of petroleum hydrocarbon. This is as a result of the opened bioreactors which were on a mechanical shaker; agitation of the bioreactor attracts more atmospheric oxygen into this microenvironment.

The pH in all the treatments after 14 days ranges from 7.43 to 7.97, which is within the pH range of 6 to 9 stipulated for successful biodegradation of petroleum hydrocarbon (Balschandran et al., 2012; Das and Chandran, 2011). As the pH increases, heavy metals become less soluble and subsequently become less biodegradable (Hahne and Kroontje, 1973).

The BOD_5 of lead (Treatment 6) at 0.86 ppm was 4.13 mg/l which is significantly different ($P \leq 0.05$) from the control (Treatment 7) that is 3.19 mg/l, because lead plays no biological role and is potentially harmful to microorganisms (Sobolev and Begonia, 2008). But since pH of the treatments is high, it reduces lead toxicity due to precipitation of the metal from the aqueous phase.

Conclusion

Manganese at a concentration 0.05 ppm reduces the extent of biodegradation of petroleum hydrocarbons in water. Inoculation of aged hydrocarbon degraders (fungi and bacteria) and the addition of diethyl ether solvent enhances biodegradation of petroleum hydrocarbons and reduces some heavy metals bioavailability in water in an aerobic condition. The non-aqueous phase liquids (NAPLs) of petroleum can be utilized by microorganisms when the petroleum is solubilized with diethyl ether solvent which increases the extent of biodegradation of petroleum hydrocarbons in water. Although lead at a concentration of 0.86 ppm increases the biological oxygen demand but not above the dissolved oxygen indicating that successful biodegradation of petroleum

Table 3. Physicochemical characteristics of the well water and the different treatments after 14 days of experimentation.

Treatment	Temp. (0^c)	pH	Total dissolved solid	Salinity (PPt)	Electrical conductivity (µS)	BOD_5 (mg/L)	Dissolve oxygen (DO) (mg/L)	Total petroleum hydrocarbon (ppm)
1	30.50±0.28[a]	7.94±0.04[a]	381.50±19.09[a]	0.30±0.06[a]	644.50±127.99[a]	2.66±0.18[ab]	5.30±0.68[a]	13.27±1.23[a]
2	30.70±0.00[a]	7.69±0.36[a]	307.50±17.68[a]	0.22±0.01[a]	455.00±22.63[a]	3.29±0.02[ab]	5.24±0.23[a]	24.43±6.62[a]
3	30.75±0.07[a]	7.72±0.35[a]	347.00±12.73[a]	0.25±0.01[a]	514.00±12.73[a]	3.89±0.60[a]	5.08±0.13[a]	22.94±6.44[a]
4	30.80±0.28[a]	7.66±0.17[a]	323.00±32.53[a]	0.24±0.02[a]	479.00±49.50[a]	3.61±0.06[a]	4.71±0.40[a]	37.23±18.22[a]
5	30.60±0.28[a]	7.63±0.27[a]	320.00±16.97[a]	0.23±0.01[a]	472.50±24.75[a]	3.40±0.02[a]	5.04±0.44[a]	25.04±4.28[a]
6	30.70±0.00[a]	7.67±0.38[a]	310.50±20.51[a]	0.22±0.01[a]	458.50±30.42[a]	4.13±0.56[b]	5.17±0.51[a]	20.50±2.14[a]
7 (control)	30.65±0.07[a]	7.68±0.28[a]	300.00±53.74[a]	0.22±0.04[a]	455.00±57.98[a]	3.19±0.15[a]	5.28±0.07[a]	16.90±3.28[a]

Means with the same superscript along the columns are not significantly different ($P \geq 0.05$).

hydrocarbons is possible when all environmental conditions such as pH, oxygen and temperature are suitable. Synergistic activities of hydrocarbon utilizing fungi and bacteria in petroleum hydrocarbon catabolism, enhance ecological recovery of petroleum contaminated water.

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COMPETING INTERESTS

All authors have not declared any conflict of interests.

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