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Vermiremediation of Soils Contaminated with Mixture of Petroleum Products using *Eisenia fetida*

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ABSTRACT: In this paper, vermiremediation, a biological technique was utilized in order to clean-up soil contaminated with gasoline, diesel and spent engine oil using an earthworm - *Eisenia fetida*. The contaminated soils were analyzed for the total petroleum hydrocarbon (TPH) level every 24 hours over a period of 120 hours using gas chromatography. It was observed that at each sampling time, the soils samples without the earthworm had more quantity of TPH than the corresponding samples with the earthworms. Pentadecane, 2,6,10, trimethyl had 100% reduction after 120 hours followed by octadecane with 67.30 % reduction and tetracosane with 50.28% reduction. In all sampling time, the initial octadecane level was significantly higher than the final octadecane level in soil with *E. fetida* ($P<0.05$). Also, the initial hexadecanoic acid methyl ester level was significantly higher than the level of the hydrocarbon in the soils with *E. fetida* after 96 hour incubation and soil without the earthworm after 72 hours incubation ($P<0.05$). After the 24 hours incubation the octadecane level in soil with *E. fetida* was significantly lower than the initial level and the level in soil without the earthworm ($P<0.01$).The results showed that *E. fetida* enhances the degradation and reduction of TPH levels in soils and therefore can be used for cleaning up of soils contaminated with mixture of petroleum products. This is useful in reclaiming mechanic workshop soils for agricultural purposes hence increase in food production. © JASEM

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KEYWORDS: Vermiremediation, petroleum, contamination, earthworm, *Eisenia fetida*

There is a high demand of petroleum and petroleum products globally as fuel and as lubricants for proper functioning of machines. Due to this high demand, petroleum products are widely transported from one location to another. This may lead spill of the products into the soil or water with subsequent adverse effects. The need to reclaim such contaminated soils and water has led to evaluation of several remediation techniques. Hitherto, mechanical, chemical and physical methods have been employed in cleaning up site contaminated with petroleum products. However, recent studies have shown that microbes, fungi, algae, plants and earthworms have the ability to facilitate the remediation of petroleum polluted sites

Earthworms are burrowing animals and form tunnels by literally eating their way through the soil. The distribution of earthworms in soil depends on factors like soil moisture, availability of organic matter and pH of the soil. They occur in diverse habitats specially those which are dark and moist. Earthworms perform various functions in soil. They improve the physical, chemical and biological properties of the soil to enhance its fertility. According to Dabke (2013), earthworms stimulate and accelerate microbial activity by creating favorable conditions for bacteria and improving soil aeration. According to Hickman and Reid (2008) earthworms can be directly employed within bioremediation strategies to promote biodegradation of organic contaminants. Also, earthworms have been shown to aerate and bioturbate soils and improve their nutritional status and fertility, which are variables known to limit bioremediation (Hickman and Reid 2008).

According to Sinha *et al.,* (2008), vermiremediation may prove a very cost-effective and environmentally sustainable way to treat polluted soils and sites contaminated with hydrocarbons in just few weeks to months. Vermiremediation leads to significant improvement in the quality of soil and land where they inhabit (Sinha *et al*., 2008). Dabke (2013) reported a reduction in chromium level in the soil and survival of introduced earthworms, which reproduced after soil treatment, indicating improved conditions. According to Azizi *et al.,* (2013) vermiremediation utilising *Lumbricus rubellus* has proved its potential to degrade polycyclic aromatic hydrocarbons

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(phenanthrene, anthracene and benzo(a)pyrene (BaP)) in 30 days of incubation. From the work of Azaripa *et al.,* (2013) it has been observed that microelements like sodium ion and magnesium ion as well as salts like nitrate, phosphate and can be brought to their lowest level in sheep manure and garden soil in presence of *Eudrillus eugeniae.* Azaripa *et al.,* (2013) reported a significant reduction (50-80 %) in trace elements and soluble salts in sheep manure and garden soil in presence of *Eudrilus eugeniae.* The Rajiv *et al.,* (2013) reported that 30-35% of organic carbon and 32-48% of phenol contents were reduced during vermicomposting after 45 days of *Eudrilus eugeniae's* activity. In addition, Dada *et al*. (2015) showed that earthworms can be used to in cleaning up polluted soils.

In most developing countries such as Nigeria, mixtures of petroleum products are indiscriminately spilled in soils of mechanic workshops where different forms of petroleum products can migrate into same soil at same time or at different time. Vermiremediation data may contribute in recovering such soils which may have been abandoned or degraded due to contamination from petroleum products. Therefore, the aim of this investigation was to employ vermiremediation technique to determine the effectiveness of *Eisenia fetida* to clean up petroleum products contaminated soils within a short period.

MATERIALS AND METHODS

Sources of Materials: The *Eisenia fetida* used for the study was obtained from the Zoological garden of the University of Lagos, Akoka Lagos, Nigeria. Adult earthworms were used in the experiment and each had an average weight of 0.6±0.15g. The gasoline and diesel fuels were purchased from a petrol station in Akoka Lagos and the spent engine oil was obtained from a mechanic workshop in Latunde Street, Okota Lagos Nigeria. The gasoline, diesel and spent engine oil were mixed in equal (1:1:1) proportion to obtain a homogeneous petroleum product mixture.

Experimental design: One hundred grams of fine sieved previously identified loam soil were added to each of twenty 500ml beakers followed by the addition of 10ml of the petroleum mixture into each beaker. The soil-petroleum mixture was properly stirred following the procedure outlined by Mohan *et al* (2011). The beakers were divided into five groups based on the sampling times (24, 48, 72, 96 and 120 hours). For each group of the beakers, two were with earthworm and two were without earthworm. The control experiment had no earthworm. For the beakers with earthworm, the mixture was allowed to stay 48 hours before ten earthworms were weighted and added on top of each soil in each beaker (Barkley *et al*, 2011). The experiment and the control experiments were incubated at a room at room temperature for 120 hours. The beakers were covered with a gauze lid and placed at room temperature

Soil Sample Collection and TPH Analyses: Soil samples were obtained every 24 hours for 120 hours from each of the beakers. The total petroleum hydrocarbon content of the soil was extracted using modified method of the protocols outlined by Contrerus-Ramos *et al* (2006). A sample of 1.5g of soil was weighed into 15ml pyrex tube and 10ml mixture of 70% dichloromethane and 30% n-hexane. The extract was filtered using filter paper. The filtrate was purified by the concentrate through a pasture pipette packed with anhydrous sodium sulphate. The types and levels of total petroleum hydrocarbon in the soil samples were determined using an Agilent Technologies Interface Detector Model 5975C gas chromatography. These were done according to the manufacturer's description.

Statistical Analyses: Data from the TPH analyses of the soil samples at the different sampling times were statistically analysed using Graph Pad 5.0 software. Two ANOVA was performed followed with Bonferroni posttest analysis.

RESULTS AND DISCUSSION

The types and amount of petroleum hydrocarbon present in the soils with and without *E. fetida* after different incubation times are shown in figures 1 - 5. Figures 6-9 show the gas chromatogram of the total petroleum hydrocarbons present in soils with and without *E. fetida* incubated for the different study periods. There was a general reduction of the levels of the different hydrocarbons in the soil with *E. fetida* compared with the ones in the soils without earthworm. Pentadecane, 2,6,10 -trimethy and dodecane 2,6,11-trimethyl were totally removed from the soil with *E. fetida* after 24 hours incubation (figure 1). The initial octadecane and hexadecanoic methyl ester levels were significantly lower than the level of the hydrocarbons ($P < 0.001$ and $P < 0.05$ respectively) after 24 hours incubation with *E. fetida*. There was 100% loss of dodecane, 5,8-diethyl and tricosane after 48 hour incubation (figure 2). After 48 hours incubation, the octadecane and hexadecanoic acid methyl ester levels were significantly reduced ($P<0.001$ and $P<0.05$ respectively) compared with the initial levels). There was also a 100% removal of dodecane, 5,8-diethyl from the soil with *E. fetida* as compared with soil without *E. fetida* after 72 hours incubation (figure 3). The octadecane in the soil incubated for 72 hours with *E. fetida* was significantly reduced ($P<0.05$) compared to the initial level of the hydrocarbon. In the soils incubated for 96 hours (figure 4), there was 100% loss of tridecane, tetracosane and pentadecane, 2,6,10- trimethyl in soil with *E. fetida* compared with level in soil without *E. fetida*. In addition, the octadecane level significantly reduced ($P<0.05$) after 96hours compared to the initial level. Also, the hexadecanoic acid methyl ester in soil incubated for 96 hours with *E. fetida* was significantly ($P<0.001$) reduced compared to the initial level. In the case of soils incubated for 120 hours (figure 5), many by products were observed in soil with *E. fetida*. Also, there was 100% loss of pentadecane, 2,6,10- trimethyl and Bacchotricuneatin c/tetradecane from the soil with *E. fetida* after 120 hours incubation. There was a significant reduction of octadecane ($P<0.001$) after 120 hours of incubation with *E. fetida*.

The total loss of some of the petroleum hydrocarbons from the soil with earthworm as compared to the soils without the earthworm indicates that ability of the earthworm to remove such from soil. The reduction of the levels of the other petroleum hydrocarbons in the soils with earthworm as compared with the soils without earthworm shows the ability of the earthworm to reduce the levels of such hydrocarbons in soil. The reduction or removal of the petroleum hydrocarbon from the soils could be attributed to the mineralization of the petroleum products by the earthworm. This is similar to the findings of Contreras-Ramos *et al* (2008), Tejadas and Masciandaro (2011), Azizi *et al* (2013) among others. Rajiv *et al*. (2013) also reported similar trend of result as obtained in this study with reduction of 30–35% of organic carbon and 32–48% of phenol contents in soil during vermicomposting, which was achieved after 45 days of earthworm's activity.

Earlier studies have suggested possible mechanisms used by earthworms to clean up polluted or contaminated sites. The observation of Rodriguez-Campos *et al* (2014) that accelerated removal of contaminants from soil by earthworms can be due to improvement of soil as a result of activities of earthworm and the microorganisms in their digestive track can be used to explain the possible mechanism used by *E. fetida* to remove the hydrocarbons in this study. The stimulation and increase in the activities of microorganisms which Dabke (2013) had stated as one of the functions of earthworm may account for the removal or reduction of the petroleum hydrocarbons as we observed in this study.

Another possible mechanisms by *E feitda* in the cleaning of the petroleum contaminated soil as observed in this study could be one of those outlined in the earlier studies. For instance, the earthworms could have triggered the bioremediation of oil contaminated (Schaefer and Juliane, 2007) by enhancing the activities of the microbes which degrade petroleum in the soil. According to Sun *et al* (2011), the presence of earthworms improves contaminant bioavailability and microbial activities. Such improvement could have in turn triggered the cleaning up the soil remediation noticed in this study. Studies have also shown some earthworms (eg E.fetida) possess cytochrome-P450 enzymes which is capable of degrading benzo(a)pyrene (Azedah and Zarabi, 2015). Possession of such enzymes could have facilitated *E. fetida* cleaning up of the soil as cytochrome-P450 has also been suggested to enhance petroleum remediation.

Earthworms enhance oil degradation by three mechanisms – enhancing oxidation process by aerating of soil, enhancing microbial activity and increasing microbial availability of hydrocarbons (Schaefer and Juliane, 2007). Earthworms combine mechanical activity upon soil through abiotic system and biotic processes (burrowing, ingestion, grinding digestion and subsequent promotion of microorganism benefit remediation processes (Azedah and Zarabi, 2015). Earthworms excrete secretions containing mucoproteinaceous and products of nitrogen metabolism; thereby stimulate microorganisms and plants activity and growth (Contreras -Ramos *et al*., 2009). Such will in turn increase the remediation activities of soil microbe hence enhancing remediation. The *E fetida* used in this study could have also facilitated the removal of petroleum hydrocarbon from the soil is through its burrowing activities as was described by Rajiv *et al* (2009). Such can serve as input points for nutrient and oxygen which are known to enhance the activities of aerobic microbial petroleum degraders. In addition, the burrowing activities help to increase the surface area of the soil and thereby enhance the activities petroleum degraders.

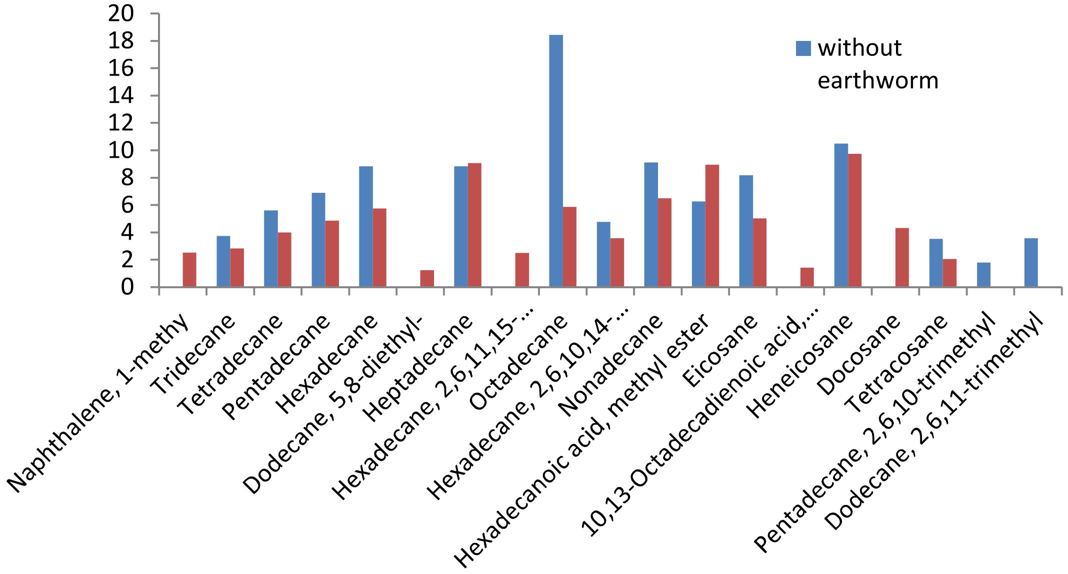

Fig. 1: The types and Levels (%) of Petroleum Hydrocarbon in soil samples with and without *E. fetida* after 24 hours incubation

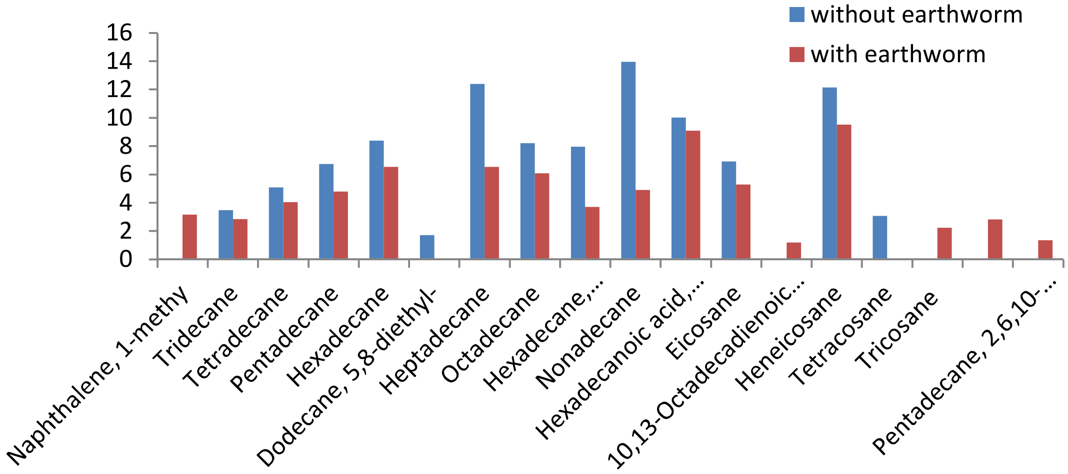

Fig. 2: The types and Levels (%) of Petroleum Hydrocarbon in soil samples with and without *E. fetida* after 48 hours incubation

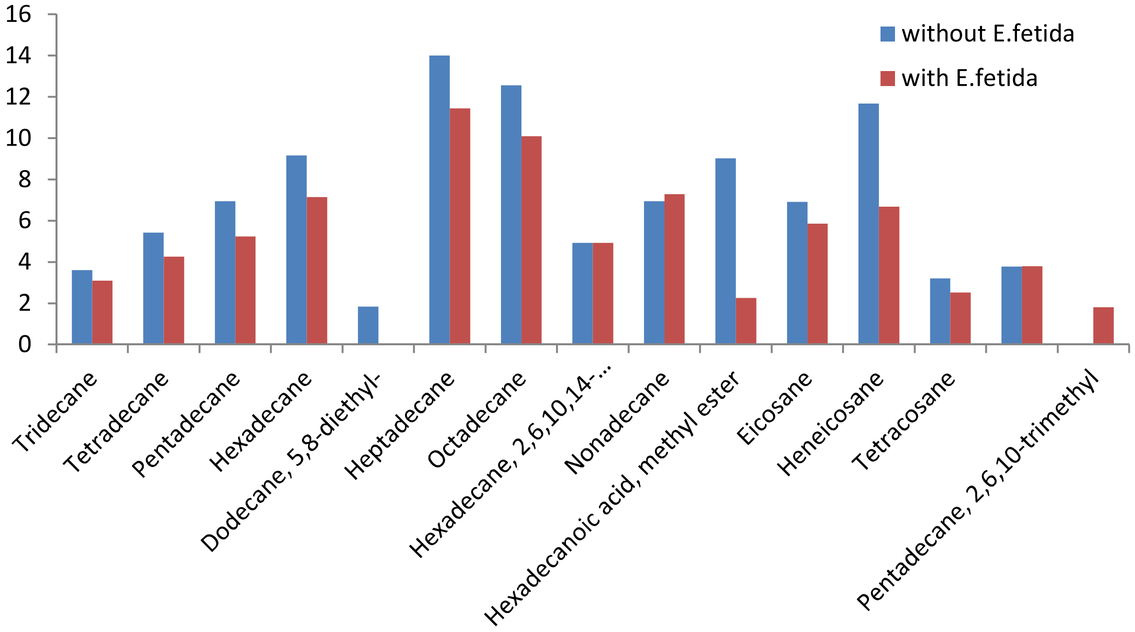

Fig. 3: The types and Levels (%) of Petroleum Hydrocarbon in soil samples with and without *E. fetida* after72 hours incubation

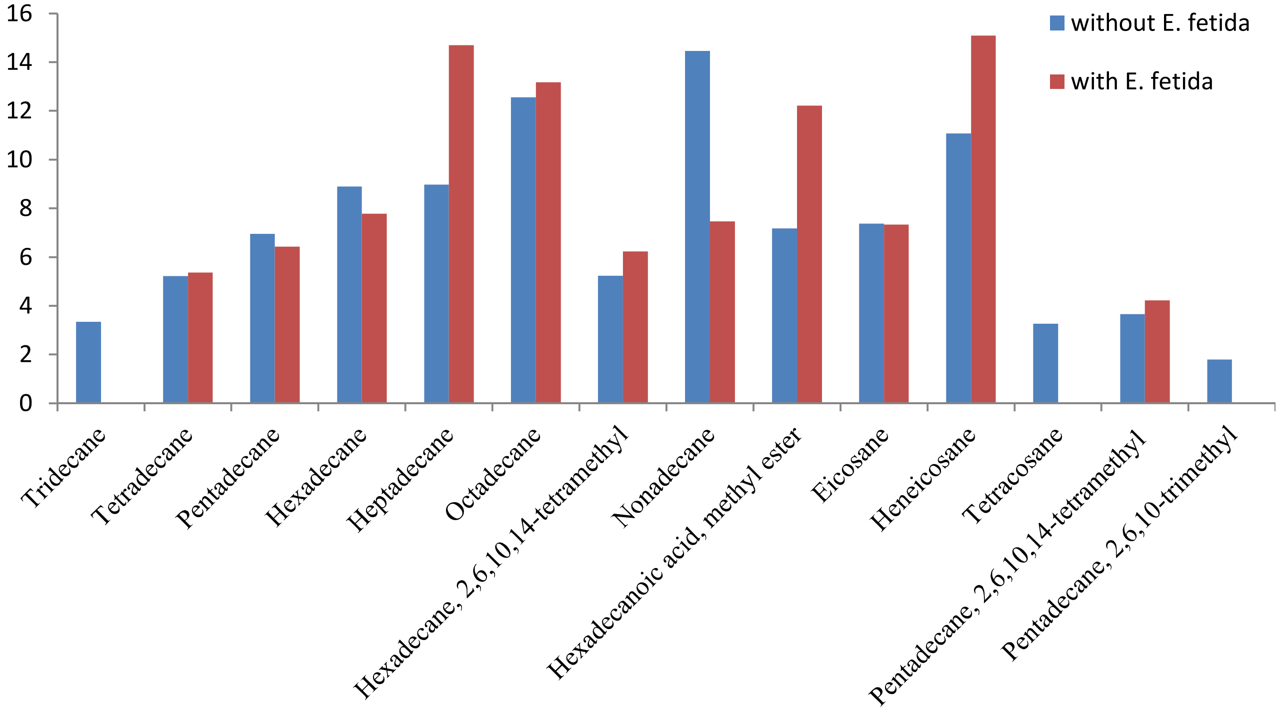

Fig. 4: The types and Levels (%) of Petroleum Hydrocarbon in soil samples with and without *E. fetida* after 96 hours incubation

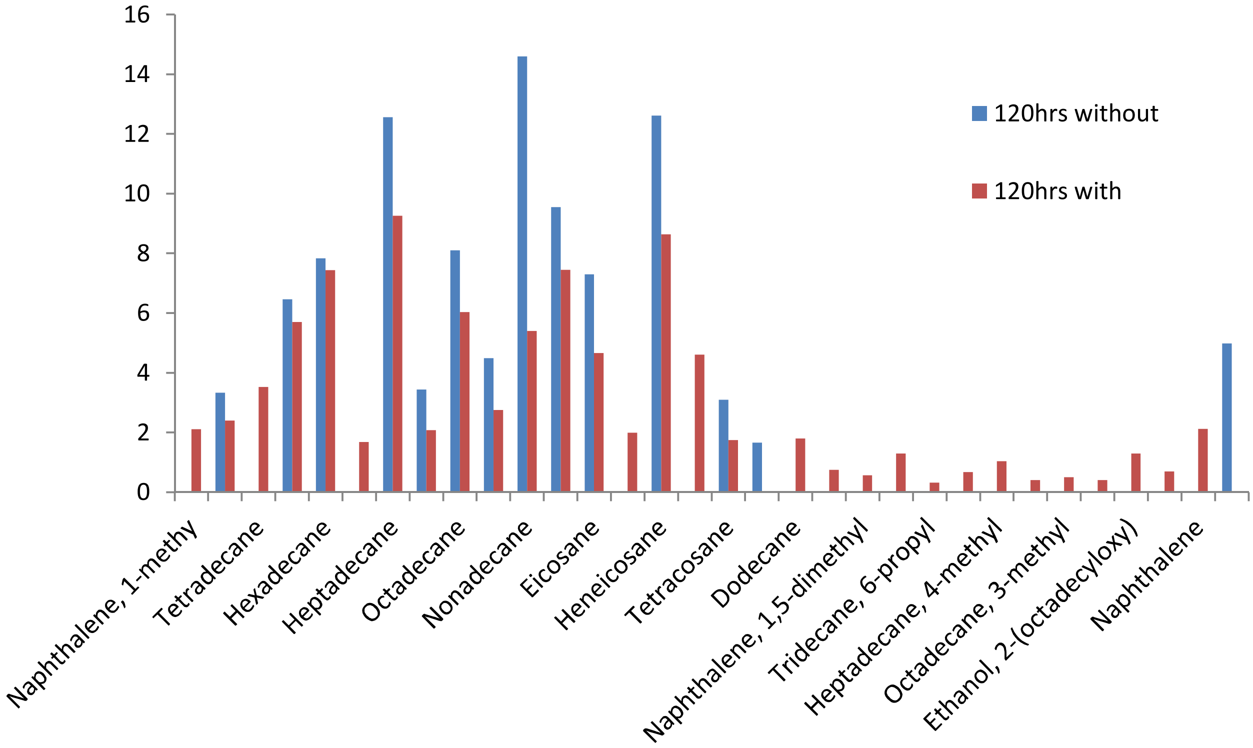

Fig 5: The types and levels (%) of Petroleum Hydrocarbon in soil samples with and without *E. fetida* after 120 hours incubation

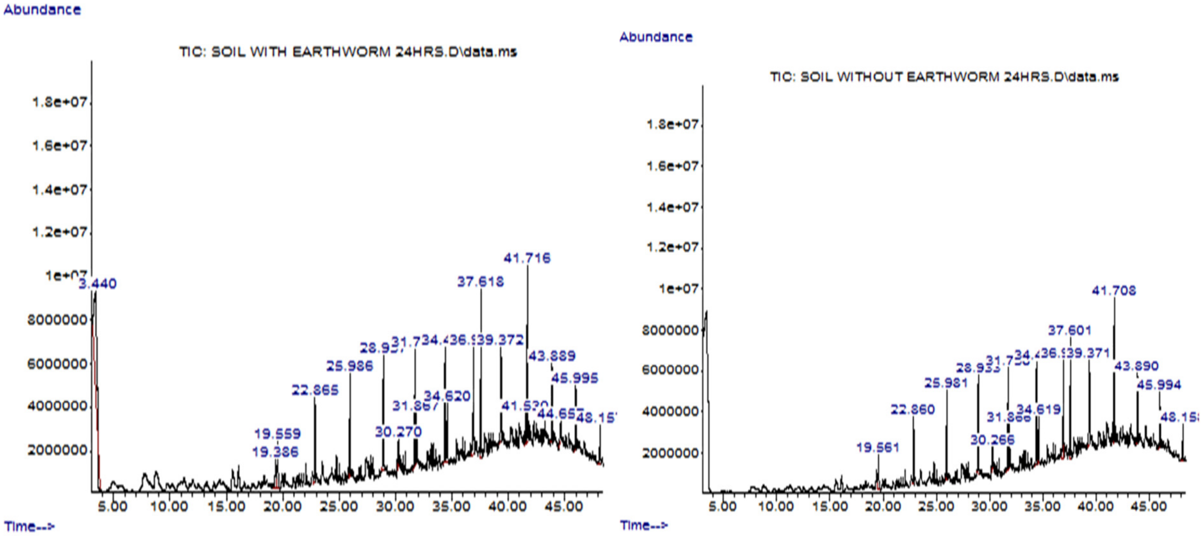

Fig. 6: The gas chromatogram of the total petroleum hydrocarbons present in soils with and without *E. fetida* incubated for 24 hours

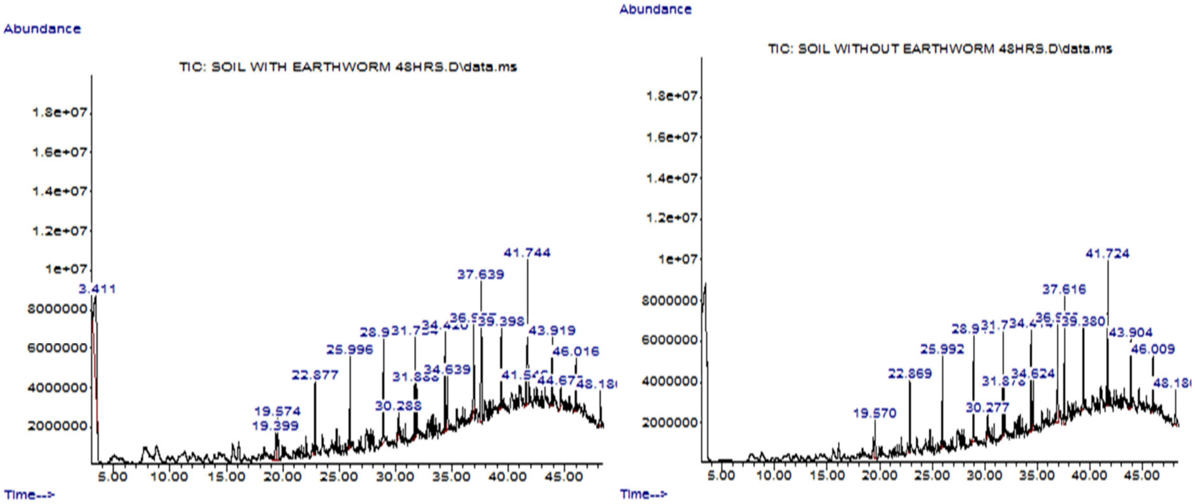

Fig.7: The gas chromatogram of the total petroleum hydrocarbons present in soils with and without *E. fetida* incubated for 48 hours

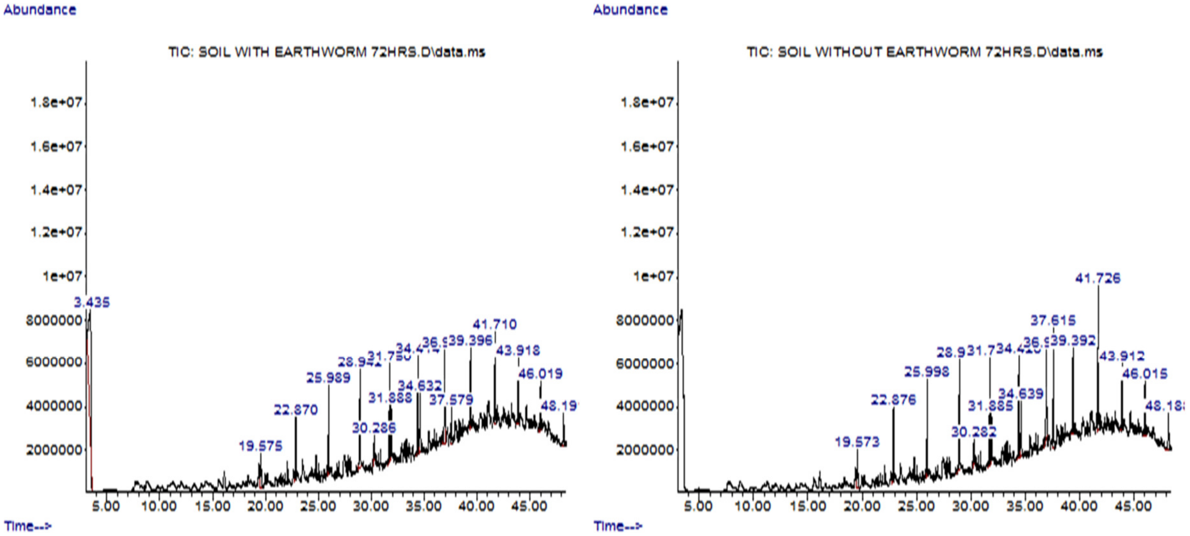

Fig. 8: The gas chromatogram of the total petroleum hydrocarbons present in soils with and without *E. fetida* incubated for 72 hours

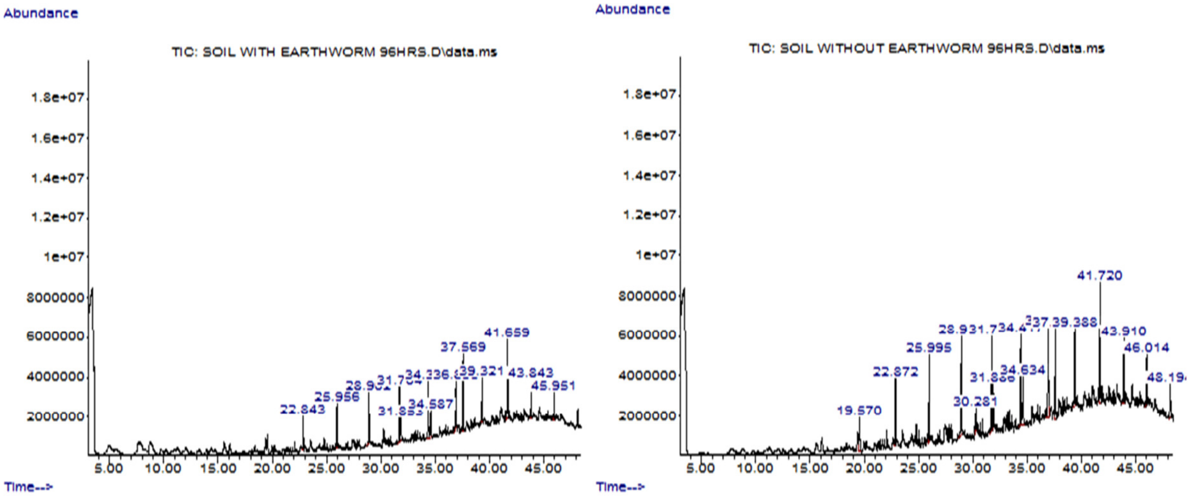

Fig. 9: The gas chromatogram of the total petroleum hydrocarbons present in soils with and without *E. fetida* incubated for 96 hours

Table 1 shows the levels of the petroleum hydrocarbons in the soil incubated for 24 hours without earthworm and in soil incubated for 120 hours with *E. fetida*. Tridecane, tetradecane, pentadecane, octadecane, hexadecane, 2,6,10,14-tetramethyl, eicosane, heneicosane, tetracosane, and dodecane, 2, 6, 10- trimethyl were significantly reduced after 120 hours incubation with *E. fetida***.**

Table 1: Percentage levels of some petroleum hydrocarbons in soils incubated for 24 hours without earthworm and 120 hours with earthworm

Petroleum Hydrocarbon	24 hours without earthworm	120 hours with earthworm	% reduction (remediation level)
Tridecane	3.73	2.40	35.66
Tetradecane	5.61	3.52	37.25
Pentadecane	6.89	5.70	17.27
Octadecane	18.44	6.03	67.30
Hexadecane, 2,6,10, tetradecane	4.77	2.75	42.34
Eicosane	8.17	4.66	42.96
Heneicosane	10.48	8.64	17.56
Tetracosane	3.52	1.75	50.28
Pentadecane, 2,6,10, trimethyl	1.80	0	100
Dodecane, 2,6,10, trimethyl	3.57	0.75	78.99

The presence of some petroleum hydrocarbon in the polluted soils incubated with the earthworm against their absence in soils incubated without the earthworm could mean that such products could have resulted from the degradation of petroleum. It was observed in the study that soil incubated for 120 hours with *E fetida* had 28 petroleum hydrocarbons while that incubated for same period without the earthworm had 14 petroleum hydrocarbons. This may imply that vermiremediation of petroleum by *E. fetida* occurs through degradation (vermidegradation). That implies that *E. fetida* enhances the degradation of petroleum in soil and those hydrocarbons are possible degradation products of petroleum. The more by-products of degradation noticed in soil incubated with earworm for 120days signifies that the longer the period the more the degradation. It was also observed that only 13 petroleum hydrocarbons were observed initially in the soil some which were completely lost during the study

Conclusion: The results of this study have shown that *E. fetida* has the ability to enhance the removal of petroleum hydrocarbons from soil. Going by the result obtained from this study, *E fetida* can be said to have the potential to facilitate the cleaning up petroleum contaminated soil. To achieve a higher level of remediation, such can be extended to a longer period as 120 hours would not be enough period achieve full remediation.

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