J. BIOL. ENVIRON. SCI.,
2017, 11(31), 43-50

Original Research Article

Vermiremediation of Crude Oil Contaminated Soil Using *Eudrillus euginae* and *Lumbricus terrestris*

Kelechi Longinus Njoku[*], Emmanuella Uche Nomba and Akinola Modupe Olatunde
Environmental Biology Research Unit, Cell Biology and Genetics Department, University of Lagos, Akoka, Lagos, NIGERIA

Received: 16.02.2017; Accepted: 22.04.2017; Published Online: 16.05.2017

ABSTRACT

The negative and cost implications of remediation of petroleum contaminated sites using physical and chemical techniques have necessitated the use of biological techniques like vermiremediation. In this study the individual and synergistic abilities of two earthworms – *Eudrillus euginae* and *Lumbricus terrestris* to clean up crude oil contaminated soil were evaluated. It involved experimentally contaminating the soils with various quantities of crude oil. The total petroleum hydrocarbon (TPH), pH and nutrient contents of the soils were determined immediately after contamination and after thirty days of earthworm activities. Data obtained were statistically analysed using graphpad prisms 6.0 and SPSS 20.0 softwares. Activities of *E. euginae* led to 88.50% TPH loss, *L. terrestris* led to 76.42% loss while combined activities of the two earthworms led to 73.06% loss of TPH from the soil contaminated with 3ml crude oil after 30days. In soil without any earthworm but contaminated with same amount of crude oil there was only 21.19% loss of TPH for same period of time. Similar trends were observed in soils contaminated with 1ml and 2ml of crude oil for same period of study. The pH, sulphate, phosphate, nitrate and organic matter contents of the contaminated soils were reduced after 30 days and the reduction was more in soils with the earthworms than in the soils without the earthworms. The findings of this study show that the *E. euginae* and *L. terrestris* can help in the remediation crude oil contamination soil and that they are more effective individually than in a combined form. Also *E. euginae* had more impact on the contaminated soil than *L. terrestris.*

Keywords: Vermiremediation, Earthworms, *E. euginae*, *L. terrestris*, Crude oil, Contamimation

INTRODUCTION

Bioremediation is one of the nature's ways of purifying contaminated environment (Dada *et al.* 2015). It includes all those processes and actions that take place in order to transform an environment by the use of living organisms to remove or detoxify contaminants within the environment (Gupta *et al.*, 2003; Rahimi *et al.*, 2012). Bioremediation involves the transformation of complex or simple chemical compounds into non-hazardous forms by biological agents. It is a relatively cheap and effective means of cleaning the environment and involves the application of organisms and nutrients such as inorganic or organic phosphate and nitrogen to the contaminated soil (Atlas and Bartha, 1995). Bioremediation techniques include microbial remediation/microremediation, phytoremediation, phycoremediation, mycoremediation, zooremediation, and vermiremediation (Garbisu and Alkorta, 2003; Gifford *et al*., 2006; Sinha *et al.*, 2009; Rahimi *et al*., 2012).

Vermiremediation is the use of earthworms to clean up contaminants from the soil environment. Research on the potential utilisation of earthworms has shown an ability to manage polluted land and even sewage sludge. It has been discovered that earthworms are tolerant to, and can remove, or aid the removal of a wide range of organic and inorganic contaminants such as pesticides, polycyclic aromatic hydrocarbons (PAH), crude oil and heavy metals from the soil (Shahmansouri *et al*., 2005; Pattnaik and Reddy, 2011; Dada, 2015). Hickman and Reid (2008) highlighted possible approaches for vermiremediation to include: (1) by direct application of earthworms to contaminated soils; (2) co-application of earthworms to contaminated soils with another organic media such as compost; (3) application of contaminated media to earthworms as part of a feeding regime; (4) indirect use of earthworms through the application of vermidigested material (vermicompost).

Significantly, earthworms lead to improvement in the quality of soil and land where they inhabit. They swallow large amount of soil every day, grind them in their gizzard and digest them in their intestine with the aid of enzymes. Only about 5- 10% of the material ingested is absorbed into the body and the rest is excreted out in the form of fine mucus-coated granular aggregates called vermicast (Chaoui *et al*., 2003). The organic matter in the soil undergoes humification in the worm intestine in which the large organic particles are converted to a

[*] Corresponding author: kecynjoku@gmail.com

complex amorphous colloid containing "phenolic" materials (Contreras-Ramos *et al*., 2006). About one-fourth of the organic matter is changed to humus which acts as a slow release fertilizer in the soil (Butt, 1999). In its basic form, earthworms can be used in the processing or treatment of organic wastes (Azizi *et al*., 2013).

According to Njoku *et al.* (2016), vermiremediation is very cost effective and environmentally sustainable to alleviate polluted soils and sites contaminated with hydrocarbons in just few weeks to months. Earthworms are considered ecosystem engineers because they affect the physicochemical and biological properties of the soils they inhabit through their activities such as casting and burrowing (LaVelle *et al*., 1997). 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).

Several researches have established the potentials of earthworms to bioremediate crude oil and other petrochemicals from laboratory and field trials polluted soil. Earthworms accelerate the removal of hydrocarbons as they burrow through soil by rendering contaminants available for microbial degradation, by feeding on the organic matter that harbour contaminants, and by improving soil structure and aeration. Ma *et al.* (1995) studied the influence of earthworm species *Lumbricus rubellus* on the disappearance of spiked PAHs, phenanthrene and fluoranthene (100 μg/kg of soil), and found that the losses of both polycyclic aromatic hydrocarbons (PAHs) occurred at a faster rate in soils with earthworms than the soil without worms. Ameh *et al*. (2013) investigated the use of earthworms (*Eudrilus eugeniae*) for vermi-assisted bioremediation of petroleum hydrocarbon-contaminated mechanic workshop soils. After 35 days of treatment, earthworm inoculation affected a higher drop in total petroleum hydrocarbon contents as compared to the samples without worms, indicating that earthworms may be used as biocatalysts in the bioremediation process.

The goal of this study was to compare the abilities of two species of earthworm in remediating crude oil contaminated soil and to determine the efficacy of combining the two earthworms in crude oil remediation. None of the information available to us as at the time of this research shows that such comparison had been shown in previous studies. Information obtained will help in solving the problem of crude oil pollution and enrich the database of biological agents of crude oil remediation

MATERIALS AND METHODS

The crude oil used for this study was obtained from the Well Head of Energia Ltd, Kwale, Delta State and Platform Petroleum and Gas Ltd, Kwale, Delta State. Two earthworm species (*Lumbricus terrestris* and *Eudrilus eugeniae*) as well as the soil were obtained from the University of Lagos Zoological/Botanical Garden, Akoka Lagos, Nigeria. The earthworms were acclimatized for 48 hours in moist soil before introducing them into the crude oil polluted soil.

A total of thirty six containers were filled with 400g of sun-dried soil and divided into four groups with each having nine containers. Each group was divided into three subgroups representing the treatments (1ml, 2ml and 3ml crude oil respectively). Each subgroup had three replicates. Earthworms were introduced into three of the four groups (A for *E. eugeniae* only, B for *L. terrestris* only and C for a combination of *E. eugeniae* and *L. terrestris*) while the fourth group was without any earthworm but was contaminated with same amount of crude oil as the others. The earthworms were introduced two days after contamination and ten earthworms were introduced into each container. The containers were regularly kept wet with 30ml of water added every to two days.

Soil samples from each container were collected at the surface and 10cm depth from each container on the day of contamination (initial) and thirty days after contamination (final). The soils from each container at each sampling time were thoroughly mixed to obtain a homogenous mixture and were sieved through 2mm mesh. The samples were then analysed for their total petroleum hydrocarbon (TPH) content, pH, moisture, and nutrient contents

The TPH contents of the soil samples were determined using the method described by using gas chromatography and mass spectrophotometer (GC-MS) technique. The protocol followed for the determination of the total petroleum hydrocarbon was that outlined by La Dreau *et al*. (1997). Ten (10g) of soil sample was weighed into an amber bottle and 20ml of dichloromethane was added to it, and shaken vigorously for 30minutes using a mechanical shaker, after which it was filtered into a glass beaker. One (1ml) of the filtrate was transferred into a sample vial and stored in a refrigerator awaiting analysis. One (1µl) of pure crude oil was first injected into the GC-MS machine to obtain a standard chromatogram and peak area and to calibrate the GC-MS machine. Then 1µl of the sample was injected into the GC-MS machine as well as to obtain equivalent chromatogram and peak area. The concentration of the sample was calculated as follows:

$$\text{Sample concentration} = \frac{\text{Sum of peak area of sample} \times \text{Concentration of standard}}{\text{Sum of peak area of standard}} \div 10$$

Where: Peak area of sample = Total peak area – Solvent peak area in the chromatogram,
Standard concentration (S_c) = 10,000mg/kg
Standard peak area (S_{PA}) = 210670.99
Dilution factor = 10

The pH of the soil samples was determined following standard methods described by Eneje and Ebomotei (2011) while the total organic matter content was determined using Walkey-Black method described by Skoog (2008). The sulphate, phosphate and nitrate levels in the soil samples were determined following the procedure described by Ben-Mussa *et al*. (2009).

Statistical Analysis

The results of from the laboratory analysis were subjected to descriptive statistics (mean and standard error of mean) and analysis of variance (two-way ANOVA) at 95% confidence level using Graph pad prism 6.0 and Statistical Package for Social Sciences (SPSS) version 20.0

RESULTS

Impacts of *E. eugunae* and *L. terrestris* on the TPH content of Crude oil Contaminated Soil

The amounts of total petroleum hydrocarbon present in the soils samples on the initial day and final day of this study are shown in Table 1. The TPH content decreased after thirty days of study (final day). In soil without earthworm but decreased more in the contaminated soils inoculated with the *E. eugeniae* and *L. terrestris*. Of the soils with earthworms, there was lowest amount of TPH in soils with *E. eugeniae* alone followed by the soils with combination of *E. eugeniae* and *L. terrestris* while the soil with *L terrestris* alone had the highest amount of TPH. The amounts of TPH in the soils with earthworm were significantly lower than the amount of TPH in the soil without earthworm on day 30 ($p<0.001$).Soil samples without any earthworm had the lowest percentage loss while the highest percentage loss was observed in the soil samples with *E. eugeniae*.

Table 1. Total petroleum hydrocarbon contents of soil contaminated with crude oil (percentage values are in bracket).

Amount of crude oil added	Initial(day 0)	Final(day 30) without earthworm	Final for *L. terrestris*	Final for *E. eugeniae*	Final for *L. terrestris + E. eugeniae*
3ml	2187.43mg/kg	1723.98mg/kg (21.19%)	515.80mg/kg (76.42%)	251.55mg/kg (88.50%)	589.26mg/kg (73.06%)
2ml	1667.19mg/kg	1192.94mg/kg (28.44%)	472.95mg/kg (71.63%)	304.19mg/kg (81.75%)	565.52mg/kg (66.08%)
1ml	1367.12mg/kg	1023.94mg/kg (25.10%)	410.65mg/kg (69.96%)	311.27mg/kg (77.23%)	474.17mg/kg (65.32%)

Impact of *E. euginae* and *L terrestris* on the pH of crude oil contaminated soil

The pH of the soil samples contaminated with crude oil is shown in Table 2. The soil samples on day 0 (initial pH) had the highest pH for each treatment while on the final day, the lowest pH for soil with 1ml crude oil (5.91) and soil with 3ml crude oil (5.59) each treatment was observed in the soil with *E eugeniae* alone. However, for the soil contaminated with 2ml crude oil, the soil inoculated with *L. terrestri*s had the least pH (5.84). Generally, the pH level decreased with increase in the amount of crude oil added into the soil. Statistical analysis indicated that there was no significant difference ($p>0.05$) between the pH for the control (soil without earthworm) and the treatments (soils with earthworms) at various concentrations

Table 2. Impact of earthworm activity on the pH of the soil.

Amount of crude oil added	Initial (day 0) pH	Final (day 30) without earthworm	Final pH for soil with *L. terrestris*	Final pH for soil with *E. eugeniae*	Final pH for soil with *L. terrestris* + *E. eugeniae*
1ml	6.35 ± 0.05	6.28 ± 0.04	5.93 ± 0.07	5.91 ± 0.05	6.05 ± 0.04
2ml	6.22 ± 0.02	6.18 ± 0.03	5.84 ±0.03	5.88 ±0.10	5.94 ± 0.05
3ml	6.14 ± 0.01	6.09 ± 0.03	5.80 ± 0.02	5.59 ± 0.03	5.88 ± 0.02

Impact of *E. euginae* and *L. terrestris* on the Nutrient Content of crude oil contaminated soil

The nutrient (sulphate, phosphate, nitrate and total organic matter) contents of the contaminated soils are shown in Table 3. The initial nutrient contents were generally higher than the final nutrient contents (in soils with earthworm and soils without earthworms). The sulphate contents of the soil generally decreased with increase with the crude oil content. Soil contaminated with 1ml crude oil had sulphate values of 54.01±0.76mg/kg and 44.96±0.33mg/kg for day 0 and day 30 respectively while the soils containing the earthworms had sulphate values of 38.86±0.28mg/kg, 37.07±0.26mg/kg and 41.34±0.42mg/kg for *L. terrestris*, *E. eugeniae* and combination of *L. terrestris* and *E. eugeniae* respectively at day 30. Soil contaminated with 2ml crude oil but without earthworm had sulphate values of 54.96±0.11mg/kg and 44.70±0.38mg/kg for day 0 and day 30 respectively, while the soils containing the earthworms had sulphate values of 38.00±0.18mg/kg, 34.99±0.11mg/kg and 41.22±0.24mg/kg for *L. terrestris, E. eugeniae* and combination of *L. terrestris* and *E. eugeniae* respectively on day 30. Soil polluted with 3ml crude oil had sulphate values of 54.87±0.16mg/kg for day 0 and 42.68±0.31mg/kg for day 30 while the soils containing the earthworms had sulphate values of 35.20±0.23mg/kg for soil with *L. terrestris,* 30.43±0.52mg/kg for soil with *E. eugeniae* and 37.73±0.34mg/kg for soil with a combination of *L. terrestris* and *E. eugeniae* on day 30. There was a significant difference ($p<0.05$) of the sulphate content of soil with 2ml of crude oil at day 30 (without earthworm), 3ml crude oil at day 30 (without earthworm) and the combination of *L. terrestris* and *E. eugeniae* treatments at all concentrations.

The initial phosphate level of the soil samples decreased with increase in the amount of crude oil added into the soil. The phosphate content of the soil samples decreased on day 30 (final level) compared to the initial levels. There was more reduction of the phosphate level in the soil samples with earthworm than in the soil without any earthworm. For the soils with earthworm, greatest reduction was in the soil with *E. eugeniae* and least reduction was observed in the soil with *L. terrestris*. Statistical analysis indicated a significant difference ($p<0.05$) for the 2ml of crude oil at day 30, 3ml crude oil at day 30 and the combination of L. *terrestris* and *E. eugeniae* treatments at all concentrations. There was also a significant difference ($p<0.05$) between 1ml, 2ml and 3ml concentrations for soils with *E. eugeniae* and the combination of *L. terrestris* and *E. eugeniae* between 1ml and 3ml concentrations.

On the final day, the soil samples without any earthworm had more nitrate content than the soil samples from pots with earthworms. For the samples with earthworms, the least nitrate levels were observed in the samples with *E .eugeniae* and highest in the samples inoculated with both earthworms (*E.eugeniae* and *L. terrestris*). Statistical analyses indicated a significant difference ($p<0.05$) between the nitrate contents of soil with 2ml and 3ml of crude oil for both *L. terrestris* and *E. eugeniae*, and the combination of *L. terrestris* and *E. eugeniae* at 3ml of crude oil.

The initial organic matter content of the crude oil contaminated soils decreased as the amount of crude oil added into the soil increased. The amount of nitrates in the soil samples generally decreased with increase in the amount of crude oil in the soil. Also, the final organic matter content of the soils increased with the amount of crude oil added to the soils. Generally there was lower organic matter in the soils at the end of the study (day 30) than at the beginning of the study (day 0). The soils that were inoculated with the earthworms had lower organic matter content than those without any earthworm. Activities of *E. eugeniae* led to greatest reduction of the organic matter content while the combined activities of the *E. eugenia* and *L. terrestris* led to the least reduction of organic matter in the soils with earthworm. There was a significant difference ($p<0.05$) between the organic matter contents of soils with 2ml and 3ml crude oil that had *E. eugeniae*, and that with the combination of *L. terrestris* and *E. eugeniae* for 3ml crude oil contamination

Table 4. Impact of earthworm activity on the Nutrient Content (mg/kg) in the soil (* Significant at p<0.05).

Amount of crude oil added to soil	Nutrient	Initial (day 0) nutrient level	Final nutrient level for soil without earthworm	Final nutrient level for soil with *L. terrestris*	Final nutrient level for soil with *E. eugeniae*	Final nutrient levelfor soil with *L. terrestris + E. eugeniae*
1ml	Sulphate	54.01 ± 0.76	44.96 ± 0.33	38.86 ± 0.28	37.07 ± 0.26	41.34 ± 0.42*
	phosphate	42.67±0.37	34.04±0.08	24.07±0.09	17.96±0.06*	26.69±0.21*
	nitrate	65.79±0.24	50.77±0.15	35.29±0.11	31.14±0.13	37.09±0.19
	Organic matter	86.26±0.13	75.17±0.11	48.14±0.12	44.08±0.09	51.54±0.06
2mL	sulphate	54.96 ± 0.11	44.70 ± 0.38*	38.00 ± 0.18	34.99 ± 0.11	41.22 ± 0.24*
	phosphate	42.60 ± 0.39	33.59 ± 0.11	23.40 ± 0.09	17.29 ± 0.18*	26.15 ± 0.11
	nitrate	65.96±0.06	50.41±0.09	33.33±0.09*	30.29±0.15*	36.27±0.21
	Organic matter	86.57±0.39	75.05±0.09	47.26±0.25	43.74±0.13*	50.30±0.41
3mL	sulphate	54.87 ± 0.16	42.68 ± 0.31*	35.20 ± 0.23	30.43 ± 0.52	37.73 ± 0.34*
	phosphate	42.26 ± 0.52	31.46 ± 0.05	21.81 ± 0.26*	15.64 ± 0.07*	22.98 ± 0.24*
	nitrate	65.76±0.13	49.64±0.38	32.10±0.02*	28.67±0.33*	32.13 ±0.06*
	Organic matter	86.14±0.06	72.93±0.07	45.07±0.10	40.85±0.10*	46.93±0.12*

DISCUSSION

The total loss of some of the petroleum hydrocarbons from the soil with earthworms as compared to the soils without the earthworms conforms to the findings of Sinha *et al*. (2010); Ekperusi and Aigbodion (2015) and Njoku *et al*. (2016) that the earthworms can be used to clean up contaminated soil. The reduction of the levels of the petroleum hydrocarbon from the soil was possibly caused by the ability of the earthworms to mineralize these petroleum products as was suggested by Contreras-Ramos *et al.* (2008) and Azizi *et al*., (2013). Earlier studies have suggested possible mechanisms used by earthworms during remediation. For instance, Rodriguez-Campos *et al* (2014) observed that the loss of petroleum hydrocarbon in contaminated soil was related to the activities of earthworm and the microorganisms found in their digestive tract. Dabke (2013) stated that stimulation and increase in the activities of micro organisms in the gut of earthworms may account for the removal of the petroleum hydrocarbons as observed in this study. Another possible mechanism used by *E. eugeniae* and *L. terrestris* in the cleaning up of the crude oil contaminated soil as observed in this study could be one of those outlined by Zhang *et al*., (2000); Schaefer and Juliane, (2007); Njoku *et al*., (2016) is the enhancement of the activities of the microbes in the soil due to earthworm activities. According to Njoku *et al* (2016), the presence of earthworms improves contaminant bioavailability and microbial activities, leading to an improvement in the cleaning up of contaminated soil hence the less amounts of TPH in the contaminated soils with the earthworms as we observed in this study

There are some other possible mechanisms that earthworms can use to enhance remediation of contaminated soil. For instance, Albanell *et al*. (1988) and Azedah and Zarabi, (2015) showed that earthworms (such as *Eisenia fetida*, *Eudrilus eugeniae* and *Lumbricus terrestris*) can modify physiology of soil microbial community and trigger enzyme activities like cytochrome P450 enzyme which is capable of degrading benzo(a)pyrene. Cytochrome P450 has also been implicated to enhance petroleum hydrocarbon remediation.

Earthworms are mainly responsible for fragmentation and conditioning of the substrate increasing surface area for microbial activity hence increased remediation of such substrates (Dominguez *et a*l., 2002). With such views of Albanell (1988), Sun *et al* (2011), Azedah and Zarabi (2015), and Njoku *et al* (2016), it can be suggested that the improved TPH loss recorded in the soils with earthworms in this study may be attributed to any or a combination of such factors (improved bioavailability, modification of microbial physiology and the triggering of enzyme activities). In addition, as was stated by Schaefer and Juliane, (2007), earthworms enhance oil degradation by three mechanisms – enhancing oxidation process by aerating of soil, enhancing microbial activity and increasing microbial availability of hydrocarbons.

The higher loss of petroleum from the contaminated soils by either individual earthworm species or combination of *L. terrestris* and *E. eugeniae* than those soils without earthworms suggests that earthworms are capable of degradation of petroleum hydrocarbons more than natural attenuation. *E. eugeniae* in this study showed to be the better remediating agent compared with *L. terrestris* and even degraded better than when combined with *L. terrestris*. This suggests that *E. eugeniae* and *L. terrestris* may not be compatible together in remediating crude oil contaminated soils but can be better individually. This result is similar to the findings of Sinha *et al* (2010) who reported *E. eugeniae* as a better and more effective biodegrader of crude oil.

For their growth and sustainability, earthworms ingest soil and through digestion and assimilation take up nutrient in the soil. Such can lead to lower nutrient contents of soils with earthworms than in soils without earthworms as was observed in this study. The extent and the level nutrient taken up by earthworms can vary amongst species and soil their influence on remediation and thus the variation of the abilities of the two earthworm species to clean crude oil contaminated soil in this study. Our observations in this shows that there is a correlation between the nutrient content of the soils and their TPH contents and this can lead to infer that the more nutrient consumed by earthworms, the more impact they will have on their contaminant content and thus more remediation. Also we observed the soil samples with low pH values had lower nutrient values and lower TPH value. The reduction in the pH may due to the production of some acidic byproducts during the degradation of the crude oil by the earthworms.

Various experimental studies suggest that earthworms have potentially negative consequences on fertilizer-N retention studies (Postma-Blaauw, *et al* 2006). Hence the lower nitrate levels in the soils with earthworms compared the ones with the earthworms that we observed in this study could be due to such negative consequences. This contradicts the reports of Brown *et al* (1998), Cortez and Hemeed, (2001) and Brown *et al (*2004) who stated that earthworms can also increase nutrient availability in systems with reduced human influence and low nutrient status, that is, no tillage, reduced mineral fertilizer use, and low organic matter content.

CONCLUSIONS

The findings of this study have shown that earthworms (*E. eugeniae* and *L. terrestris)* are good agents for remediating crude oil contaminated soils, most especially the more active *E. eugeniae*. Form the results of the performance of the earthworms individually and when combined, we can suggest than it is better to use *E. euginae* and *L. terrestris* individually than combining them when cleaning up of crude oil contaminated soils. It is recommended that the mechanisms used by the earthworms and ways of improving their remediation potentials should be study to enhance their efficacy in remediation of crude oil polluted media.

ACKNOWLEDGEMENT

We thank the managements of Energia Ltd, Kwale, Delta State, Platform Petroleum and Gas Ltd, Kwale, Delta State and Department of Petroleum Resources, Nigeria for their assistance in providing the crude oil used for this study.

REFERENCES

Albanell, E., Plaixats, J. and Cabrero, T. (1988). Chemical changes during vermicomposting of sheep manure mixed with cotton industrial wastes. *Biological Fertility of Soils* **6**: 266-269

Ameh A.O, Mohammed-Dabo, I.A., Ibrahim S, and Ameh, J.B. (2013) Earthworm-assisted bioremediation of petroleum hydrocarbon contaminated soil from mechanic workshop. *African Journal of Environmental Science and Technology*.**7**(6):531–539.

Azedah, F. and Zarabi, M. (2015). Combining vermiremediation with different approaches for effective bioremediation of crude oil and its derivatives. *Proceedings of International Conference on "Global Issues" in Multidisciplinary Academy Research* 1: 1-12.

Azizi, A. B. Liew, K. Y. Noor, Z. M. and 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** (5), 565- 568 DOI: 10.7763/IJESD.2013.V4.414

Ben-Mussa, S. A., Elferjani, H. S., Haroun, F. A. and Abdelnabi, F. F. (2009). Determination of Nitrate, Phosphate and Sulphate in Soil Samples. *International Journal of PharmTech Research* 1(3): 598-604.

Brown, G. G. Edwards, C. A. and Brussaard, L. (2004) "How earthworms affect plant growth: burrowing into the mechanisms," In: *Earthworm Ecology*, C. A. Edwards, Ed., CRC Press, Boca Raton, Fla, USA, 2nd edition, pp. 13–49,

Brown, G. G. Hendrix, P. F. and Beare, M. H. (1998) "Earthworms (Lumbricus rubellus) and the fate of 15N in surface-applied sorghum residues," *Soil Biology & Biochemistry*,. 30, (13), 1701–1705.

Contreras-Ramos, S. M., Alvarez-Bernal, D. and Dendooven, L. (2006). *Eisenia fetida* increased removal of polycyclic aromatic hydrocarbons (PAHs) from soil. *Environmental Pollution* **141**: 396-401.

Cortez, J. and Hameed, R. H. (2001) "Simultaneous effects of plants and earthworms on mineralisation of 15N-labelled organic compounds adsorbed onto soil size fractions," *Biology and Fertility of Soils*, **33**, (3), 218–225,

Dabke, S. V. (2013). Vermiremediation of Heavy Metal-Contaminated Soil. *Blacksmith Institute Journal of Health Pollution* 3(4): 4-10

Dada, E.O. (2015). Heavy metal remediation potential of a tropical wetland earthworm species, *Libyodrilus violaceus*. Ph.D. Thesis, Department of Cell Biology and Genetics, University of Lagos, Lagos, Nigeria, 192 pp.

Dada, E.O., Njoku, K.L., Osuntoki, A.A. and Akinola, M.O (2015) A review of current techniques of in situ physico-chemical and biological remediation of heavy metals polluted soil. *Ethiopian Journal of Environmental Studies and Management* **8**(5): 606 – 615

Dominguez, J., Robert, W., Parmelee, R. W. and Clive, A. E. (2002). Interactions between *Eisenia andrei* (Oligochaeta) and nematode populations during vermicomposting. *Pedobiologist* 47: 53-60

Ekperusi, O. A. and Aigbodion, F. I. (2015) Bioremediation of petroleum hydrocarbons from crude oil-contaminated soil with the earthworm: *Hyperiodrilus africanus Biotechnology*. **5**(6): 957–965.

Eneje, R. C. and Ebomotei, E. (2011). Effect of crude oil pollution on soil physiochemical properties and germination percent of *Amaranthus hybridus*. *Nigerian Journal of Soil and Environment Research* 9: 97-103.

Garbisu, C. and Alkorta, I. (2003). Basic concepts on heavy metal soil bioremediation. The European *Journal of Mineral Processing and Environment Protection* **3**(1): 58-66.

Gifford, S., Dunstan, R.H., O'Connor, W., Koller, C.E. and MacFarlane, G.R. (2006). Aquatic zooremediation: deploying animals to remediate contaminated aquatic environments. *Trends in Biotechnology* **25**(2): 60-65.

Gupta, A.K., Yunus, M., and Pandey, P.K. (2003). Bioremediation: ecotechnolgoy for the present century. Enviro New, *Bulletin of the International Society of Environmental Botanists* 9(2)

Hickman, Z.A. and Reid, B.J. (2008). Earthworm assisted bioremediation of organic contaminants. *Environment International* **34**(7): 1072-1081.

Jayasree S., Deepa, S. Bincy, B. Krishnapriya, M. Remya, P.K. Anusha, C. Deepthi, V. Gayathri, V., and Vincent , E. (2013) Bioremediation studies of petroleum contaminated soil by vermicompost for sustainable agriculture in *Hibiscus esculentus* (L) Moench. *Acta Biologica Indica*, **2**(1):300-309

Lavelle, P. and Martin, A. (1992) "Small-scale and large-scale effects of endogeic earthworms on soil organic matter dynamics in soils of the humid tropics," *Soil Biology and Biochemistry*, **24** (12), 1491–1498

Ma W.C, and Imerzeel B.J. 1995Earthworm and food interactions on bioaccumulation and disappearance in soil of polycyclic aromatic hydrocarbons: studies on phenathrene and fluoranthene. *Ecotoxicol Environ Safety*; **32**: 226–232. doi: 10.1006/eesa.1995.1108.

Njoku, K. L., Akinola, M. O. and Anigbogu, C. C. (2016). Vermiremediation of Soils Contaminated with Petroleum Products using *Eisenia fetida*. *Journal of Applied Science and Environmental Management*. 20(3): 771-779.

Pattnaik, S. and 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**(14): 345-356.

Postma-Blaauw, M. B. Bloem, J. Faber, J. H. van Groenigen, J. W. de Goede, R. G. M. and Brussaard, L. (2006) "Earthworm species composition affects the soil bacterial community and net nitrogen mineralization," *Pedobiologia*, **50 (**3), 243–256,

Rahimi, M., Farhardi, R. and Yusef, P.H. (2012). Phytoremediation of Arsenic. *International Journal of Agronomy and Plant Production* **3**(7): 230-233.

Rodriguez-Campos, J., Dendooven, L., Alvarez-Bernal, D. and Contreras-Ramos, S. B. (2014). Potentials of earthworms to accelerate the removal of organic contaminants from soil: A Review. *Applied Soil Ecology* 79: 10-25.

Schaefer, M. and Juliane, F. (2007). The influence of earthworms and organic additives on the biodegradation of contaminated soil. *Applied Soil Ecology* **36**: 53-62.

Shahmansouri, M.R., Pourmoghadas, H., Parvaresh, A.R. and Alidadi, H. (2005). Heavy metals bioaccumulation by Iranian and Australian earthworms (*Eisenia fetida*) in the sewage sludge vermicomposting. *Iranian Journal of Environmental Health, Science, Engineering* **2**(1): 28-32.

Sinha, R.K., Chauhan, K., Valani, D., Chandran, V., Soni, B.K., and Patel, V. (2010). Earthworms: Charles Darwin's 'Unheralded Soldiers of Mankind': Protective and productive for man and environment. *Journal of Environmental Protection* **1**: 251-260.

Skoog, E. (2008). *Fundamentals of Analytical Chemistry*. 8th edition, BabaBarkha Nath Printers, Hayana. 868pp.

Sun, H., Li, J. , Wang, C., Wang, L., and Wang, Y (2011) Enhanced Microbial Removal of Pyrene in Soils in the Presence of Earthworm. *Soil and Sediment Contamination: An International Journal* **20** (6) 617 - 630

Zhang, B. G., Li, G. T., Shen, T. S., Wang, J. K. and Sun, Z. (2000). Changes in microbial biomass C, N and P and enzyme activities in soil incubated with the earthworms *Metaphire guillelmi* or *Eisenia fetida*. *Soil Biology and Biochemistry* 32: 2055-2062.