Research Article

Vermiremediation strategy for remediation of Kuwaiti oil contaminated soil

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Abstract
The role of vermiremediation technique has been accepted all over the world for many years to reduce the concentrations of pollutant in the contaminated soil to acceptable levels. However, limited study has been found in the case of Kuwaiti oil contaminated sand using earthworms. This paper address the potential of decomposing petroleum hydrocarbons in terms of total petroleum hydrocarbon (TPH) present in Kuwaiti oil contaminated sand ranging from 5000 to 25,000 mg/kg using earthworms (*Eisenia fetida*). It was observed that the TPH of < 5000 mg/kg was not harmful for the survival of earthworms, nevertheless TPH of 10,000 mg/kg reduced their survival to 50%. Moreover, the results indicate that that vermiremediation could effectively reduce the TPH of about 5000 mg/kg every 5 weeks. This study suggested that the crude oil could be consider as one of the main challenging for the survival of earthworms, therefore several factors such as type of soil, moisture content, temperature, organic matter content and pH need to maintain and enhance during the remediation process to support the earthworm's activity.

Keywords Vermiremediation · TPH · Oil contaminated sand · *Eisenia fetida* · *Lumbricus terrestris* · Organic wastes

1 Introduction

Vermiremediations is the term used to describe a process based on biological technology which uses earthworms in improving degradation as well as eliminating contaminants in contaminated sites throughout the globe. Generally, earthworms as such especially *Eisenia fetida* have good resistance against pollutants including heavy metals and organic contaminants soil. It has been described that this approach involves the use of earthworms to bioaccumulate the contaminants within their bodies and biodegrade or bio-transform them into safe products with the assistance of enzymes. Chachina et al. [1] reported that the worms host microbes that are able to biodegrade chemicals within their gut. They concluded that in petroleum contaminated soil with petroleum concentration of 20–60 g/kg, 99% of hydrocarbons content decreased after 22 weeks in the presence of *Eisenia fetida*. However, in diesel-contaminated soil with concentration of 40 g/kg in the presence of same earthworms, 30% of the worms died after 14 days in front of the toxic impact.

A study by Martin-Gel [2] on the efficiency of composting and vermicomposting in degrading fuel oil in water emulsions from oil spills. Three trays for vermicompost processes of $0.40 \times 0.50 \times 0.12$ m^3 in volume and 0.20 m^2 in surface area, the density of *Eisenia fetida* earthworms were 300 g-earthworms/m^2. The experiment was conducted through 6 months, while the moisture was maintained between 60 and 80%. After short period, it was found that the asphaltens become as a source of microorganisms to generate carbon and energy. Further factors play significant role such as potato peelings and cow bed, which allow to breakdown the complex compound into smaller compound, therefore further degradations using earthworms are necessary.

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Some composting processes that are helpful to remove or degrade volatile aspheltens and hydrocarbons into H_2O and CO_2 are by dissolution, evaporation and/or photo-oxidation. So these composting techniques and followed by other techniques such as vermicomposting, increases the effectiveness to degrade, destroy or remove complex organic matters in polluted soil such as 'resins and asphaltens'. A laboratory study was conducted by Safawat et al. [3] to ascertain the highest concentration level of crude in soil that worms are able to withstand. The study showed that within 7 days, the worms were only able to successfully withstand 0.5% of crude oil. On the other hand, when the soil is contaminated with 1.5% oil, the worms' survival was reduced to lower than 40%. However with Verimremedition, earthworms are able to withstand 1.2% of crude oil in soil for as long as even 10 days. The result also showed that the worm's vermicast contained 0.2% TPH when the soil contaminated by oil is despoiled by earthworms. Another study was conducted by Ma et al. [4], which focuses on an earthworm species *L. rubellus* and its influences on the disappearance of spiked PAHs fluoranthene and phananthrene (100 µg/kg of soil). And the study showed that compared to bare soils, the soils inhibited by worms resulted faster disappearance of both PAHs. And after 8 weeks (56 days), 86% of phenanthrene was removed. A different study by Contreras-Ramos et al. [5], emphasized on altering the level of concentrations of three PAHs named as anthracene, benzo(s)pyrene and phanantherene by *Eisenia fetida*. The experiments were conducted for 11 weeks and within that time frame, concentrations of PAHs in the earthworms' tissues and in the soil were measured. Compared against the bare soil, the concentration of anthracene dropped to 23% from 51% which is more than double. As for benzo(a)pyrene, the soil without earthworms showed a decrease of 13% while the soil with earthworms showed a reduction between 1 and 4 times with average of 47%. As for Phenantherene, in soil absent of earthworms, the microbes present in the soil were able to remove up to 77% of Phenanthrene whilst in soil with earthworms were able to achieve 100% removal of Phenanthrene in soil with 100 mg/kg of chemical content. Schaefer and Juliane [6] conducted a study of treatment in soil contaminated by crude oil within a period of 56 days, which showed a decrease of 91% (1074 mg/kg of soil to 96 mg/kg) due to the presence of *Eisenia fetida* which had caused for an increased catabolic activity for the microbes. Additionally, they conducted another investigation for 28 days, focusing on degradation by three different species of earthworms (*Allolobophora chlorotica*, *Eisenia fetida* and *Lumbricus terrestris*) in soil contaminated with oil. As such, the concentration of TPH showed a decrease of 17–18%, 30–42% and 31–31% respectively. Nonetheless, in samples without earthworms showed a slight decrease of 9–17% in TPH concentrations compared to the TPH initial concentrations. Earthworms are an effective tool as an in-situ bioremediation for oil-contaminated soil with concentration of TPH are less than 4000 mg/kg. One of the advantages also includes that earthworms generally have a high resistance to more than a few of chemical pollutants that are present in the soil. Earthworms not only allows for polluted land to be cleansed but also improve the quality of the soil in terms of its biological, chemical and physical attributes, and hence plays a huge, important role environmentally and economically.

2 Methodology

A bench-scale investigation conducted to study the degradation rates of oil-contaminated soil under a combination of various remediation techniques over a period of time. The laboratory work was focused on vermiremediation. Four bench-scale experiments were conducted, each tray contained 5 kg of contaminated soil. The first bench-scale experimental is consider as an control to confirm the test results, this experiment was conducted without using microbial activity. The second bench-scale designed to simulate the combination system of various remediation methods. Bench-sale testing aims to reduce the concentrations of hydrocarbon to acceptable levels (regulatory objective of 2000 mg/kg TPH) within a short period, at a low cost and with lower environmental effects. It is recommended that the following scheme be established in order to deal with Kuwait environmental issue. The earthworm activity is dependent on a number of critical factors, including density, feeding, pH and moisture content, while distilled water was used to maintain the moisture within content ranges of 50–80%. These factors were observed weekly. All experiments were run in the Glass house located in University of Portsmouth, the ambient temperature was maintained between 20 and 21 °C.

2.1 Sample preparation

A physical separation technique decreased the volume of contaminated soil by reduction of the concentration of contaminated soil, according to the size of the particles. In this case, a samples were sieved using a 2 mm screen to isolate material finer than coarse sand and a 20 mm screen was used to separate out the gravel material. The bioremediation process requires the soil to be homogeneous before the start of the treatment. Generally, the screening process is able to separate oversized fractions, as long as they are not contaminated, such as with debris, metals or rocks.

2.2 Earthworms

Vermiremediation of weathered oil-contaminated soil was conducted usng two different type of earthworms *Eisenia fetida* and *Lumbricus terrestris*. The adult earthworms were selected during this experiment. These earthworms were purchased from the Al Yarmok farm (Abdaly, Kuwait). The average weight of the used earthworm was around 0.6 g.

2.3 Vermiremediation

The vermiremediation method is not always successful in destroying or removing residual, heavy and hydrocarbons in contaminated soil. However, there is some evidence that this remediation is able to degrade such oil [1]. In this paper, the available compost reactor has been constructed from a plastic box, the length, width and height of which are 0.49 m, 0.30 m and 0.35 m, respectively. This gives the box a total surface area of 0.147 m^2, see Fig. 1. The reactor was drilled with 1.5 mm ventilation and drainage holes around the base, upper edge and lid. 5 kg of oil-contaminated soil was transported from the sample tray to the compost reactor, when adding 3 kg of *Eisenia fetida*. As a result, the stocking density of the compost reactor was around 20.41 kg/m^2.

The earthworm activity is dependent on a number of critical factors, including temperature, density, feeding, pH and moisture content, while distilled water was used to maintain the moisture within content ranges of 50–80%. These factors were observed weekly.

2.4 Data analysis

The treatment processes were replicated three times in each stage of the combining remediation system in order to confirm data obtained, while the "Statistical Package for Social Science" (SPSS Window) Version 12.0 was used to analyse the collected data. The samples were coded before being entered into the computer. Median, means, standard deviation and multivariate statistics was used to detect differential expressions. Means were selected for normal distribution graphs and medians for abnormal distribution graphs. Obtaining the same value in each replicate process could support the data obtained.

Fig. 1 The compost reactor for vermiremediation process

2.5 Extraction of oil from sand

A number of researchers have developed and utilised methods for the extraction of oil from soil, sand or sediment. The following procedures were suggested to measure TPH using acetone: hexane (1:1 mix) for a period of 5 min. The contaminated sand and casts were analyzed for TPH according to EPA method 9071B using a Gravimetric Method.

3 Result

Three experiments were conducted using plastic containers, the length, width and height of which are 0.49 m, 0.30 m and 0.35 m, respectively. This gives the box a total surface area of 0.147 m^2, approximately 3 kg of soil was added to each container and the container was divided centrally across the width with a thin rigid plastic barrier. As a result, the stocking density of the compost reactor should be 20.41 kg/m^2. The soil on one half was treated with un-weathered oil to contain 0.0, 0.5, 1.0, 1.5 and 2.0% oil on a soil dry weight basis. Water-content was adjusted to 25% on a soil dry weight basis on both sides of the barrier. Water content was maintained during incubation by making additions as determined from reweighing containers. The jars were kept in an incubator at 20 °C as recommended and the number of viable earthworms was determined after 5, 7, 10 and 15 days as shown in Table 1. Although the containers were quite large only 200 earthworms were added so that it would be easier to monitor movement. Once each day the earthworms were observed to determine if they were in contaminated or uncontaminated soil. Because of space requirements the containers were incubated in a room maintained between 20 and 22 °C.

The experimental design was randomized with three replicates of each treatment. Data were compared by analysis of variance. Twenty-four hours after the addition of the earthworms to the containers with soil treated with 0.5% oil, samples of soil and earthworm casts were collected from the container. They were carefully picked from the surface of the soil of each replication with a spatula to minimize contamination with soil. The casts were collected to determine if the digestive systems of the earthworms were being exposed to oil. In the first experiment, *Eisenia fetida* was used in this investigation because it

Table 1 Survival of earthworms in soil treated with different concentrations of weathered and unweathered crude oil

Day	*Eisenia fetida*				*Lumbricus terrestris*				
	Survival of earthworms				Survival of earthworms				
	0.5%	1.0%	1.5%	2. 0%	0.5%	1.0%	1.5%	2.0%	2.5%
5	100	100	100	80	100	100	100	80	40
7	100	90	40	40	100	100	20	20	20
10	100	80	20	0	100	60	0	0	0
15	90	70	10	0	40	40	0	0	0

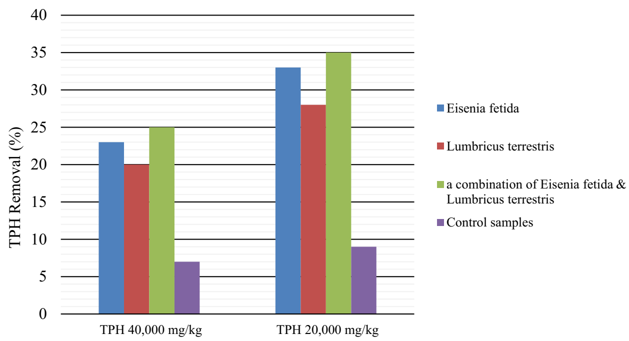

Fig. 2 TPH removal for oil contaminated soil using *Eisenia fetida*, *Lumbricus terrestris*

is recommended by Rorat et al. [7] for laboratory toxicity tests. The experimental design was completely randomized, with a factorial arrangement of treatments and three replications. The second experiment was similar to the first except that only unweathered crude oil was used and the oil concentrations in the soil were 0, 0.5, 1.0 and 1.5%. *Lumbricus terrestris*was used in this investigation because it is a deep burrowing earthworm and would be likely to have higher exposure to oil in soil than *Eisenia fetida* which normally feeds on litter at the soil surface [8], because these earthworms were much larger than Eisenia fetid. The third experiment was designed to determine survival of earthworms when they had opportunity to move away from oil-contaminated soil into uncontaminated soil.

Another three trials were conducted using 40.0 wt% of soil amendment with manual mixing each day to simulate burrowing activity. The main aim of this experiment is to improve the biodegradation of weathered oil contamination in soil (average TPH 40,000 mg/kg) using *Eisenia fetida*, *Lumbricus terrestris* and a combination of *Eisenia fetida* and *Lumbricus terrestris*, the results shown significantly ($p < 0.05$) reduction in TPH concentration after 60 d incubation, whereas minor reduction was observed in controls without soil amendment (Fig. 2). The TPH concentration decreased by 19–25% in samples with *Eisenia fetida*, by 18–23% in samples with *Lumbricus terrestris*, and by 23–28% in samples with a combination of *Eisenia fetida* amd *Lumbricus terrestris*. Average TPH concentrations in the control samples without soil amendment were 5–9%. Further experiments were conducted using oil contaminated soil of 20,000 mg/kg with *Eisenia fetida*, *Lumbricus terrestris* and a combination of *Eisenia fetida* amd *Lumbricus terrestris*. Also, the results shown significant reduction in TPH concentration with minor reduction in controls without soil amendment. The average reduction TPH concentration were 28.0%, 33.0% and 35.0% for *Lumbricus terrestris*, *Eisenia fetida* and a combination of *Eisenia fetida* and *Lumbricus terrestris*, respectively. Average TPH concentrations in the control samples without soil amendment were 9.0%.

TPH degradation of oil contaminated soil mixed with soil amendment were thus higher than observed without soil amendment. Interestingly, this correlated with a difference in behavior; whereas Hubalek et al. [9] clarified that the degradation and reproduction rate of *Eisenia fetida* could be effected by hydrocarbone contamination in soils. This point was confirmed in this study, where the degradation and reproduction rate of *Eisenia fetida* or *Lumbricus terrestris* were significantly decreased in soils contaminated with oil. Moreover, this research suggested that the reproduction rate of *Eisenia fetida* or *Lumbricus terrestris* in the soils restored by bioremediation might be due to the high parentage of soil amendment used during the treatment process which leads to an increase the degradation and reproduction of earthworm. Winding et al. [10] described the relation between degradation and earthworm reproduction.

4 Discussion

Vermiremediations is a newly developed technology which centres upon principles of strong environmental engineering. The present research identify that the earthworms are capable to degrade low concentration of hydrocodone compounds in contaminated soil, also they could collaborate with microorganism and encourage their activity and growth during their interactions with contaminants [11]. In the present research, three laboratory scale experiments were conducted to determine the survivability of earthworms in the presence of crude- oil-contaminated soil. The experimental design was completely randomized with three replicates of each oil concentration. It can be suggested that this paper is working with worms and utilising there potential in decomposing petroleum hydrocarbons in term of total petroleum hydrocarbon (TPH) in the Kuwaiti oil contaminated sand. First experiments were carried out to assess the efficiency of using earthworms (*Eisenia fetida*) to degrade TPH of 5.0–20.0%. It was found that the TPH of < 5.0% was not harmful to survival of earthworms, similar results were obtained by Rodriguez-campose et al. [12], they identified that the vermicomposting earthworm species *E. andrei, Eisenia* fetida and *L. rubellus* show high survival rates at TPH of 3.5%. Conversely, TPHs of 10.0% in the oil-contaminated soil examined in this research are toxic and able to cause a significant body weight loss, decrease of reproduction and reduced the survival rate to 50%. Furthermore, exposure of *Eisenia fetida* to a mixture of hydrocarbons compound for 70 days is able to cause a complete inhibition of reproduction [13]. In accordance with Sforzini et al. [14], mixture of hydrocarbon compounds are able to cause oxidative damage which can effects on reproduction and survival levels. This paper indicated that the Vermiremediation was able to reduce the mean values of TPH 5000 mg/kg after 5 weeks. This point suggested that the earthworms was in hard position in front of crude oils therefore, the Vermiremediation method needs further enhancement to support the earthworm's activity.

Eisenia fetida earthworms were used in a second experiment and evaluated if they were capable enough to either reduce or completely remove hydrocarbon contamination from soil as seen in Table 2. This experiment was conducted for a total of 150 days with 1 kg of soil and roughly 200 *Eisenia fetida* earthworms. The water-content of the total dry weight of the soil was then adjusted to 25%. By using reweighing containers, water can be added to maintain the moisture level of the soil. The temperature of the incubator were kept constant at 20 °C.

Results of the third experiment indicate that earthworms do play significant role, both, directly (through ingestion and enzymatic degradation) and indirectly (by stimulating microbial action) in removal of contamination of hydrocarbon from soil. Degradation of 42% or more of crude oil within 150 days is realistic even with TPH concentrations exceeding 30,000 mg/kg. This level of TPH degradation in a native soil from a remediation site is strong evidence that vermiremediation is a potentially viable treatment technology for crude oil contaminated soils. This experiment found that the TPH concentration decreased significantly in samples with *Eisenia fetida* within 150 days compared to the treatments without worms. The results of these experiments suggested that earthworms, particularly *Eisenia fetida*, can be used to enhance bioremediation and accelerate crude oil TPH degradation. Even though it is not a universally applicable and acceptable solution, is has greatly developed into achieving pedagogical maturity which elevates its status to be one of the more desired options in addressing environmental problems. The bio mechanism by which earthworms improve the degradation of TPH is limited. Oil-contaminated soil with soil amended 40.0% with various type of earthworms showed increases in microbial activity and biomass, which allow to increase the availability of metabolizable soil organic compound. It is notable that both metabolizable material and burrowing activity play significant role by accelerating TPH degradation in earthworm-amended soil. This study concluded that daily mixing, soil amendment, a combination of earthworms were able to increase and enhance TPH degradation.

Table 2 Reduction of various level of hydrocarbons using Earthworms *E. fetida*

Hydrocarbon	Soil with earth worm		Soil without earth worm
	Amount decrease	% Removal	% Removal
TPH	2-Fold	42	11
SVOC	1.3-Fold	52	9
VOC	Complete	100	62

5 Conclusion

Land and soil that are populated by worms, also known as vermiremidiation, shows a total overall quality improvement. The worms, aided by enzymes in their intestine consume a certain quantity of soil and digest and absorb them. Bench scale experiment implemented in this study allowed for an evaluation of the mutual contact of selected contaminant, namely TPH and *Eisenia fetida* earthworms. In this study, selected earthworms have completed the process of remediated polluted by reducing significantly the TPH for contamination soil of < 5.0% of TPH. The combination of *Eisenia fetida*, *Lumbricus terrestris* had higher removal efficiency for TPH (2.0–30.0%), this study concluded that the TPH of < 5.0% was not harmful to survival of earthworms. Consequently, *Eisenia fetida* worms unlikely to survive in the hostile environment of TPH around 10.0%.. Furthermore, the use of *Eisenia fetida* removed around 42% of TPH from oil contaminated soil of 30,000 mg/kg after 150 days. This level of TPH degradation is strong evidence that vermiremediation is a potentially viable treatment technology for oil contaminated soils.

However, pilot project is essential to scale up and implement the treatment method to non-controlled conditions where the interactions between earthworms could be more effective. This approach is economical, practical and further offering high potential in dealing with a range of environmental problems in addition to soils contaminated with hydrocarbons and other hazardous chemicals.

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Compliance with ethical standards

Conflict of interest The author declare that he has no conflict of interest.

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