LenteraBio
LenteraBio, 2025; Volume 14, Issue 2: 229-235
p-ISSN: 2252-3979 e-ISSN: 2685-7871
https://journal.unesa.ac.id/index.php/lenterabio/index

Effectiveness of Earthworms (*Eisenia fetida*) and Vermicompost Combination in Bioremediation of Oil-Contaminated Soil

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Article History:
Received: 17-April-2025
Revised: 28-May-2025
Available online: 31-May-2025
Published regularly: 31-May-2025

Abstract
Used oil waste contains toxic substances such as PCBs and PAHs, which are mutagenic and carcinogenic. Illegal disposal into soil without treatment can kill soil microorganisms. This study aims to evaluate the effectiveness of using earthworms (*Eisenia fetida*) and vermicompost in lowering Total Petroleum Hydrocarbon (TPH) concentrations and enhancing pH, temperature, and moisture conditions in oil-contaminated soil. The research was conducted through an experimental approach employing a Completely Randomized Design (CRD) with a single treatment factor: The experiment included five replicates in 25 treatment units. Parameters measured included TPH levels, pH, temperature, and moisture on day 0, day 18, and day 35. The data were subjected to one-way ANOVA, followed by Duncan's multiple range test for further comparison. The results showed that all three treatment types effectively reduced TPH levels and optimized pH, temperature, and moisture levels. However, the most effective treatment was the combination of earthworms (*Eisenia fetida*) and vermicompost, which achieved a TPH reduction of 86.91%, pH ranging from 6.8 to 7.8, temperature ranging from 26.1 to 27.9ºC, and moisture levels between 65.4% to 79.1%. It was concluded that the combination of earthworms (*Eisenia fetida*) and vermicompost effectively improves the soil quality.

Keywords: Bioremediation; Oil waste; Earthworms (*Eisenia fetida*); Vermicompost

How to Cite: Permatasari D, Fitrihidajati H, 2025. Effectiveness of Earthworms (*Eisenia fetida*) and Vermicompost Combination in Bioremediation of Oil-Contaminated Soil. LenteraBio; 14(2): 229-235.

DOI: https://doi.org/10.26740/lenterabio.v14n2.p239-235

INTRODUCTION

Currently, the automotive industry continues to grow through various sectors that enhance production capacity and drive innovation (Javier & Trihadiningrum, 2024). Based on data from the Badan Pusat Statistik (2022), the number of motor vehicles in Indonesia reached 125,305,332 units, with motorcycles being the most dominant type, while the population stood at 275,8 million. According to Priyambodo (2018), on average the motor vehicle count in East Java grows by 5 to 10 percent per year. The continuous growth in the number of vehicles impacts the waste generated due to irregular maintenance by the owner, such as regular oil changes and repainting. These vehicles are usually serviced at official workshops or small repair shops. These service processes generate various types of waste, one oil (Indrawati & Surtikanti, 2024).

Oil is a lubricant fluid composed of base oil and various additives that reduce friction, prevent corrosion, and assist in engine cooling (Zhang *et al*., 2021). Despite playing a crucial role in mechanical systems, oil contains various components that can harm human health and the environment. According to research by Wolfe et al. (2020), used oil contains hazardous heavy metals such as lead, chromium, cadmium, and arsenic that accumulate during use, as well as *Polycyclic Aromatic Hydrocarbons* (PAHs), which are formed through thermal degradation and oxidation and are potentially carcinogenic. This creates used oil waste a problem to ecosystems and people's wellbeing (Buana & Sulastri, 2021).

Soil is a significant natural wealth for people, animals and even plants for certain reasons. A crops elemental makes up is highly influenced by the features of the elements within the soil in which they grow. The disposal of used oil waste into the soil and retention by the soil particles can impede the water retention capacity of the soil which is detrimental for land. Oil contaminated soil has its pore spaces filled, which further reduces porosity and therefore permeability. This makes the soil denser, prevents free water movement into the soil, retains water and considerably hinders water release causing excessive water accumulation at the surface (Buana & Sulastri, 2021).

Addressing oil waste soil contamination requires a remediation method that has the four E's: effective, efficient, economical, and environmentally sustainable. One treatment technique that meets these criteria is known as bioremediation. This technique uses living organisms, usually microorganisms, to destroy or reduce harmful waste in the environment. Bioremediation can also be conducted through composting methods, including earthworm usage in the process called vermicomposting (Rodríguez-Rodríguez *et al*., 2020). Diesel and petroleum derivatives are changed during the process to high-quality vermicompost (Bhat *et al*., 2017).

During bioremediation processes, worms help enhance the public engagement, concentration of primary resources, and fertility of the soils. These soil-dwelling creatures are also capable of transforming organic materials within the fresh to semi-decomposed phase into compounds that aid in soil fertility. In the case of oil-contaminated soil, earthworms may function as bioremediation agents so long as the concentration of Total Petroleum Hydrocarbon (TPH) does not exceed 4000 mg/kg, since they thrive in soils with up to 3500 mg/kg of oil contamination (Chachina *et al*., 2015).

During this phase, Indonesia is starting to focus on the biological remediation of oil-contaminated soils. Surendrakumar et al. (2022) found that earthworms (*Eisenia fetida*) and zinc oxide (ZnO) nanoparticles combined with cow dung can reduce hydrocarbon content in diesel-contaminated soil by up to 50% within 70 days. Based on the above, this study aims to Utilize earthworms (*Eisenia fetida*) and vermicompost in the process of bioremediating soil polluted by used oil.

MATERIALS AND METODS

The study was carried out through an experimental approach using a Randomized Block Design (RBD) with a single treatment factor, namely the type of treatment, which includes earthworms (*Eisenia fetida*) (PI), vermicompost (PII), and a combination of both (PIII). The experiment was conducted with five replications, resulting in 25 experimental units. The RBD method was chosen due to the non-homogeneous nature of the research environment, allowing for treatments to be randomly assigned and enabling statistical analysis to be carried out in a more straightforward and more accurate manner.

The study was conducted for three months, from November 2024 to January 2025. The bioremediation research was conducted at the Greenhouse of Building C10, Biology Cluster, Faculty of Mathematics and Natural Sciences, State University of Surabaya. TPH levels, pH, temperature, and humidity were analyzed at the Ecology Laboratory, Building C14, Biology Cluster, Faculty of Mathematics and Natural Sciences, State University of Surabaya. The parameters measured in this study included the reduction of TPH levels, as well as the optimization of pH, temperature, and humidity.

The research was carried out in two phases: the bioremediation phase and the TPH measurement phase. During the bioremediation phase, 300 grams of soil were placed into each container. The soil was treated with 13.5 mL of used oil in all three treatments, which included earthworms (*Eisenia fetida*), vermicompost, and their combination, then left in containers for 35 days for soil contamination. All treatments were covered with gauze to prevent the entry of other animals while allowing air circulation. Aeration was carried out every two days by adding 25 mL of water to each experimental unit.

The next stage measure TPH levels, pH, temperature, and humidity on day 0, day 18, and day 35. TPH levels were measured using the gravimetric method. For each treatment, 2 grams of earthworm samples were taken and homogenized, while 10 grams of soil samples were collected. The samples were then dissolved in 25 mL of *n*-hexane and filtered to collect the filtrate. The filtrate was placed into a pre-weighed beaker glass to determine the initial weight. It was then heated on a hot plate at 70°C until all the *n*-hexane evaporated, resulting in a final weight (Buana & Sulastri, 2021). The TPH level was then calculated using the following formula (Hendrasari & Eka, 2011 as cited in Buana & Sulastri, 2021).

$$\text{TPH concentration} = \frac{\text{B} - \text{A}}{\text{Sampel weight}} \times 100\%$$

A = Initial vial weight (before extraction) (grams)
B = Final vial weight (with extracted oil) (grams)
TPH concentration = Degraded oil content

To determine the % of the degradation, calculations can be performed using the following formula :

$$\% \text{ Degradation} = \frac{TPH0-TPHn}{TPH0} \times 100\%$$

TPH_0 = TPH control week 0
TPH_n = TPH control week n

In the bioremediation stage, data were obtained from initial and final TPH levels and the percentage reduction in TPH. Additionally, pH, temperature, and humidity levels were recorded at three different measurement intervals. TPH levels, pH, temperature, and humidity were statistically analysed using one-way ANOVA with Duncan's Multiple Range Test (DMRT) as post hoc test to assess the effectiveness of the percentage reduction at 0.05 level of significance. Subsequently, the results were compared against the quality standards established by the Decree of the Minister of Environment No. 128 of 2003.

RESULTS

Based on the studied data, several results including mean percentage and initial and final TPH levels, percentage reduction in TPH levels and percentage values for pH, temperature, and moisture were recorded. The results from these parameters were subsequently analyzed against the Decree of the Minister of Environment No 128 of 2003. Table 1 provides the measurements of TPH levels.

Table 1. Analysis results of total petroleum hydrocarbons (TPH) levels in soil polluted with waste oil using earthworms (*Eisenia fetida*) and vermicompost.

Treatment	Initial TPH Concentration (%)	Final TPH Concentration (%)		Environmental Quality Standart
		Day 18th	Day 35th	
PI (Earthworms (*Eisenia fetida*))	5,66	2,86 ± 0,75[b]	1,52 ± 0,79[b]	<1%
PII (Vermicompost)		4,18 ± 0,46[a]	3,62 ± 0,52[a]	
PIII (Combination)		1,22 ± 0,22[c]	0,71 ± 0,30[c]	

Note: Based on Duncan's test, different notations signify statistically significant differences among the treatments at the 95% confidence level.

There was a difference in the average initial and final TPH levels (Table 1). The initial TPH level in soil contaminated with used oil was 5.66%, exceeding the maximum TPH limit of <1% as stated in the Decree of the Minister of Environment No. 128 of 2003. After 35 days of bioremediation, TPH levels decreased in all three treatments by 0.71%. Among the three results, the highest TPH level was found in treatment PIII, which involved the combination of earthworms (*Eisenia fetida*) and vermicompost at 0.71%. Meanwhile, the lowest TPH level was found in treatment PII, which involved vermicompost alone, at 3.62%.

The percentage decrease in TPH levels was determined using the TPH concentration formula and subsequently analyzed statistically with one-way ANOVA, followed by Duncan's test. Subsequently, the %degradation of TPH was calculated. This analysis aimed to determine the impact of the combination of earthworms (*Eisenia fetida*) and vermicompost on the percentage reduction in TPH levels, as shown in Table 2.

Table 2. Results of the percentage degradation of TPH levels in used oil-contaminated soil treated with earthworms (*Eisenia fetida*) and vermicompost.

Treatment	Initial TPH Concentration (%)	Decrease in TPH Concentration (%)	
		Day 18th	Day 35th
PI (Earthworms (*Eisenia fetida*))	5,66	42,08 ± 17,3[b]	72,48 ± 15,6[b]
PII (Vermicompost)		25,60 ± 9,63[a]	33,46 ± 7,9[a]
PIII (Combination)		78,08 ± 5,45[c]	86,91 ± 5,2[c]

Note: Based on Duncan's test, different notations signify statistically significant differences among the treatments at the 95% confidence level.

Table 2 shows that the percentage of TPH degradation increased across all treatments. The three treatments each showed a reduction in TPH levels over 35 consecutive days of 72.48%, 33.46%, and 86.91%, respectively. The greatest reduction in TPH levels, at 86.91%, was recorded in treatment PIII, which combined earthworms (*Eisenia fetida*) and vermicompost. In comparison, the lowest reduction was found in treatment PII (vermicompost) at 33.46%.

The initial and final pH values in soil contaminated with used oil differed. After a bioremediation process lasting 35 days, the pH of the contaminated soil showed a slight increase in all treatments, with respective values of 7.3, 7.5, and 7.8. This pH level occurred because, on day 0, it was already within the optimal range of 6.62–7.1. These pH levels comply with the Decree of the Minister of Environment No. 128 of 2003, which sets the acceptable range at 6–9.

The temperature levels shown in Table 3 experienced a significant decrease. Before the bioremediation process, the temperatures in the three treatments—PI (Earthworms (*Eisenia fetida*)), PII (Vermicompost), and PIII (Combination of (*Eisenia fetida*) and vermicompost)—were 28.7°C, 28.3°C, and 27.9°C, respectively. However, on day 35, the temperatures had decreased to 26.9°C, 26.7°C, and 26.3°C, respectively. These temperature levels follow the quality standards set by Larasati & Mulyana (2016), which range between 20–30°C.

Table 3. pH level, temperature, and moisture in soil contaminated with used oil waste after the bioremediation process.

Treatment	Concentration on day 35 (%)		
	pH	**Temperature (°C)**	**Moisture (%)**
KI (Earthworms (*Eisenia fetida*))	7,3 ± 0,57b	26,9 ± 0,65a	79 ± 1,58a
KII (Vermicompost)	7,5 ±0,79c	26,7 ± 0,67b	79,1 ± 7,35b
KIII (Combination)	7,8 ±0,83a	26,3 ± 0,83c	84 ± 4,94c

Note: Based on Duncan's test, different notations (a, b, c) signify statistically significant differences among the treatments at the 95% confidence level.

An increase in moisture content was noted comparing pre to post bioremediation measurement. For the three treatments, the moisture levels before bioremediation were 64.2%, 65.4%, and 69% percent respectively. Post 35-day bioremediation process, moisture content was recorded at 79%, 79.1%, and 84% respectively. As noted by Zhang et al. (2020), these values meet quality standards which fall within the interval of 60–85%.

DISCUSSION

Results showed that earthworms (*Eisenia fetida*) and vermicomposting had positively changed the oil-contaminated soil by assisting in waste decomposition and soil remediation. The most significant reduction in TPH (Total Petroleum Hydrocarbon) levels was noted in combination treatments (PIII). A combination of earthworms (*Eisenia fetida*) and vermicompost yielded the best results regarding TPH concentrations in oil contaminated soil, achieving over 86.91% reduction. This value is high due to the earthworm's ability to improve soil's oil-exposed structure and circulation because of pore formation, bioturbation activity, and hydrocarbon degradation. Vermicompost was added to provide nutritious support to earthworms and significantly enhanced the soil remediation process through effective bioremediation (Indriani *et al*., 2024).

The decrease of TPH levels happens as a result of the activities of earthworms (*Eisenia fetida*) and vermicomposting within oil-polluted soil which enhances the enzymes synthesis and nutrient decomposition within the microorganisms. Carbocycle breakdown in oil pollutants is achieved through a sequence of biochemical transformation of microorganisms. Hydrocarbonoclastic bacteria synthesizes certain other types of inductive enzymes, like monooxygenase and dioxygenase, which cleave the intricate structure of hydrocarbon chains. Degradation starts with terminal oxidation, where monooxygenase catalyzes the ascertained oxidation of the alkane moiety, which transforms into a primary alcohol, and into an oxygenated hydrocarbon compound. Subsequently, this is later transformed alcohol dehydrogenase, which converts the given compound above to an aldehyde and then subsequently into fatty acid by dehydrogenase. The fatty acids thus formed are further oxidized within the microbial cell, forming acetyl-CoA which may enter the citric acid cycle to yield energy, CO_2 and H_2O as end products (Nwankwegu *et al.*, 2016). With regards to aromatic hydrocarbon functionalities, ring cleavage-gaining addition of two oxygen atoms to open dioxygenase is a capability of other enzymes forming degradation intermediates simpler forms (Varjani & Upasani, 2017).

The action of microorganisms on breaking down hydrocarbons is governed by factors such as pH, temperature, and moisture content (Sayara & Sanchez, 2020). Among these factors, pH is the dominant one for determining the state of oil contaminated soil. In this case, the ranges of pH values for all treatments during the 35th day interval were between 7.3-7.8. These values are neutral and correspond with the Decree of the Minister of Environment No. 128 of 2003, which stipulated a pH range of 6-9. Furthermore, the rings for pedagogical practice of 6A and 6B at the University of Bucharest indicate that the activity of earthworm (*Eisenia fetida*) in the soil leads to the formation of castings enriched with microorganisms and digestive enzymes, which serve to raise the pH of soil that, due to oil contamination, was acidic. The vermicompost added serves as a natural buffer which enhances soil pH towards neutral. The increase in pH is caused by microbial ammonification of nitrogen leading to the formation of ammonium, combined with the action of earthworm enzymes which release Calcium Carbonate ($CaCO_3$) into the soil (Sharma & Singh, 2019).

Temperature control is critical in every ecosystem. From all treatments by day 35, the average soil temperature was between 26.3-26.9 degree Celsius on table 3. The soil temperature varied during bioremediation using earthworm (*Eisenia fetida*), as well as during the growth of earthworm castings (vermicomposting). The temperature tends to increase because the metabolic processes of the earthworms and the microorganisms, responsible for degrading the hydrocarbons, generate a considerable amount of heat. But the vermicompost serves as a temperature stabilizing material due to its porous soil structure and airspace which improves circulation. Following Larasati & Mulyana (2016), the range of these values is in the bracket of 20°C to 30°C. Hydrocarbon structures are effectively disintegrated biochemically during this state (Li *et al*., 2018).

The degree of humidity also has an impact on the quality of the environment. The soil moisture content for all treatments on day 35, according to Table 3, was between 79 - 84%. Based on Zhang et al. (2020), optimal soil moisture level is between 60% and 85%. This level of moisture provides earthworms the necessary conditions for active decomposition of organic matter and hydrocarbons. Earthworm (*Eisenia fetida*) mucous secretion increases micro-moisture level emplacement for earthworm activity and vermicomposting creates moisture control functions as an aquifer for the water depletion system. Within such an aquifer the organic content in the vermicompost is slowly decomposed at a water level appropriate to sustain bioremediation (Zhang *et al*., 2020).

The combination of earthworms (*Eisenia fetida*) and the processes of vermicomposting the reactors increased the rate of hydrocarbon degradation in contaminated soils. Based on the results, TPH reduction of PIII at 86.91% was higher compared to PI of 72.48% and PII at 33.46%. This result illustrated the combined effect of earthworms and vermicompost bioremediation technologies for oil wastes. Both treatments are acknowledged for their complementary abilities to degrade a broad spectrum of organic and inorganic pollutants more efficiently than individually (Oktaviani *et al*., 2020).

Vermicomposting is one of the processes of decomposition which provide nutrients and organic matter needed to maintain soil structure, aeration, and housing for soil dwelling organisms. Furthermore, bioturbation done by earthworms (*Eisenia fetida*) creates channels in soil which improves its porosity, circulation of air, and aerobic decomposition. These worms also assist in digestion by excreting hydrocarbon biting enzymes and microorganisms. The castings produced are nutrient-rich and benefit the microorganisms within the vermicompost. It creates an ideal environment in which the vermicompost microorganisms, along with earthworm digestive systems, decompose complex hydrocarbon structures into simpler compounds or into the process of "collaboration" (Rodriguez-Campos *et al*., 2019).

This oil waste treatment explanation suggests that bioremediation through the use of earthworms (*Eisenia fetida*) and vermicompost improves waste quality parameters. Table 1 indicates that the TPH value of soil contaminated by oil and treated using earthworms (*Eisenia fetida*) and vermicompost had reduced to 0.71%, which is compliant with the limits set by the Decree of the Minister of Environment No. 128 of 2003 stipulates that for biological treatment of petroleum waste and polluted soil, the limit is less than 1%. The soil after treatment was found to have higher concentration of some essential nutrients like phosphorus, nitrogen, and potassium which were added by the vermicompost along with the pH and cation exchange capacity, and soil factors supporting plant growth (Sharma & Singh, 2019).

CONCLUSION

According to the study, the combined treatment of earthworms (*Eisenia fetida*) and vermicompost termed PIII, was most effective in reducing TPH levels in oil contaminated soil with an oil reduction rate of 86.91%. Earthworms (*Eisenia fetida*) and vermicompost create a favorable setting where vermicompost microorganisms and the earthworm's digestive tract break down complex hydrocarbons into simpler forms, or undergo "collaboration." All three treatments had optimal pH of

6.8 to 7.8, temperature of 26.1 to 27.9°C, and moisture content of 65.4% to 79.1% in compliance with the standards set by the Decree of the Minister of Environment No. 128 of 2003. This is the benchmarks for the environmental quality requirements.

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude for the reviewers and editors because all of them were the ones who, in one way or another, contributed significantly to the improvement of this document.

CONFLICT OF INTEREST

There is no conflict of interest.

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