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Research Article

Bioremediation of Polycyclic Aromatic Hydrocarbons in Contaminated Soils Using Vermicompost

Fazel Mohammadi-Moghadam[1] **Ramazan Khodadadi**[1] **Morteza Sedehi**[2] **and Mohsen Arbabi**[1,3]

[1]*Department of Environmental Health Engineering, School of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran*
[2]*Department of Biostatistics, School of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran*
[3]*Social Determinants of Health Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran*

Correspondence should be addressed to Mohsen Arbabi; m.arbabi@skums.ac.ir

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Bioremediation of polycyclic aromatic hydrocarbons (PAHs) in contaminated soils are reported in many literatures. Composting, in addition to bioremediation, can simultaneously increase soil organic matter content and soil fertility and is thus regarded as one of the most cost-effective methods of soil remediation. In this study, biodegradation of phenanthrene (PHE) and pyrene (PYR) is reported by microbial consortia enriched by vermicompost. After soil samples preparation and grinding, the samples were contaminated with 100, 200, and 300 mg/kg of PHE and PYR concentrations and inoculated with three concentrations (2, 4, and 6 wt.%) of vermicompost. PHE and PYR concentrations were analyzed by HPLC during bioremediation. After 70 days, two highly capable microbial consortia were used to remove the pollutants in bioaugmentation conditions. Analysis of their microbial composition revealed that the consortia contain several Proteobacteria phylum bacterial species, and the most common genera were *Pseudomonas* and *Citrobacter*. Decontamination rates for PHE and PYR were estimated to be 89% and 83% over 45 days, respectively. Biodegradation kinetics revealed that microbial degradation followed a first-order kinetics. This study provides clear evidence on the biodegradation of PHE and PYR, paving the way for the development of bioremediation technologies for the recovery of polluted ecosystems.

1. Introduction

The great interest in the study of bioremediation of polycyclic aromatic hydrocarbons (PAHs) as well as other recalcitrant contaminants is concerning bioavailability [1]. Biodegradation of PAHs in soils is complex and not well understood completely due to the fact that soils and sediments typically contain a variety of different microorganisms with different metabolic pathways and substrate ranges [2, 3]. PAHs are hydrophobic compounds with two or more condensed benzene rings in their molecular structure [4, 5]. PAHs are one of the most common environmental pollutants, some of which, such as phenanthrene (PHE) and pyrene (PYR), have toxic, mutagenic, and carcinogenic properties, thus posing a serious threat to the health of humans, organisms, and living ecosystems, and so far, the treatment of these compounds from soil and water environments have often not been successful [6, 7]. PHE and PYR are used as model compounds for 3-ring and 4-ring PAHs, respectively, in many biodegradation studies [8]. Degradation of one type of low-molecular-weight (LMW) PAHs may affect the degradation of high-molecular-weight (HMW) PAHs and vice versa due to the induction of enzyme activities by related degradation intermediates or competitive inhibition [9]. During the last two decades, many investigations have been performed to determine the possible role of the indigenous microorganisms on the degradation rate of PAHs and the persistence of these contaminants in different natural environments [10–14].

Oil-contaminated soils can be improved by stimulating indigenous microorganisms, increasing nutrients and oxygen to soil, or inoculating soil-rich microbial consortium [4, 15]. Some microorganisms are able to use oil compounds as a source of carbon and energy and thus reduce or eliminate petroleum compounds [16, 17]. Chemical and physical technologies for soil remediation are either incompetent or too costly. Composting or compost addition can increase soil organic matter content and soil fertility besides bioremediation and thus is believed to be one of the most cost-effective methods for soil remediation [7]. Composting, the major process of stabilizing agricultural solid waste and municipal solid waste (MSW) through the degradation of biodegradable components by microbial communities, has been adopted as one of the most cost-effective technologies for soil bioremediation [7, 18, 19]. Vermicompost has a wide range of microorganisms, including vermi (earthworm) and compost (fertilizer) and has a significant impact on the physical, chemical, and biological properties of soil [20, 21].

The application of biological methods has been known for 80 years and was briefly described by Davis for the first time [22, 23]. Sinha et al., in a case study of PAHs removedfrom soil using vermiremediation, stated that the worms are highly tolerant of many pollutants. They also found that worms such as *Eisenia fetida* can absorb PAH compounds in their body and thus remove them from contaminated soils [24]. In 2012, Wang et al., in their study of eliminating the simultaneous contamination of PAHs and cadmium compounds using pig manure vermicompost (PMVC) and a plant species called *Sedum alfredii*, found that most PAHs were removed when the plant and PMVC are used at the same time. They also stated that the use of PMVC with other biological elimination methods would promote the removal of pollutants [21].

Iran was the fifth-largest crude oil producer in OPEC in 2020 and the third-largest natural gas producer in the world in 2019 [25]. Considering the special position of the oil industry in this country, the presence of oil pollutants in its environment is more evident, so it is necessary to pay attention to the problem of pollution elimination and reduction of its adverse environmental impacts. In view of this, the present research aimed to investigate the effect of vermicompost on the bioremediation of contaminated soils with emphasis on removal of PHE and PYR.

2. Materials and Methods

2.1. Chemical Products. Hydrocarbons were obtained from Sigma-Aldrich (USA) with purity >96% for PHE and 99% for PYR. All other compounds used in this study were prepared from MERCK Co. with purity greater than 97%.

2.2. Experimental Setup. In this experimental study, agricultural soil was obtained at the vicinity of the Shahrekord University of Medical Sciences. Soil samples were transferred to the laboratory and dried at room temperature. All samples sieved with sieve #80 for homogenization. Then, for the preparation of contaminated soil samples, three concentrations of PHE and PYR (100, 200, and 300 mg/kg) were added. Also, in order to produce biological inoculum, different concentrations of vermicompost (2, 4, and 6%), which already have qualitative parameters and microbial strains had been identified and specified were added to each sample individually.

The moisture content in the samples was at the standard level of biological removal methods (40 to 50% of water holding capacity) and soil pH was monitored at sampling times. Soil grinding analysis was done based on the American Society for Testing and Materials (ASTM-D-2487) [26]. In addition, analysis of the number of bacterial colonies, moisture, and pH as well as testing the qualitative characteristics of vermicompost were conducted using the standard methods [27]. The bioremediation process was carried out for 70 days by daily control of humidity, temperature, and homogeneous mixing of samples. Furthermore, tests on concentration of PHE and PYR and also microorganisms (CFU) were performed once a week.

2.3. Bacteria Analysis. In order to separate and determine the microbial population of the soil, the plate counting method of heterotrophic bacteria (HPC) was used. A colony counter machine (RTC Model 1002, China) was used to count the bacterial colonies formed on Petri dishes after the incubation period. The number of bacteria that were capable to use cyclic aromatic hydrocarbons was calculated and expressed as CFU/g of soil [28]. Macroscopic and microscopic features were examined to detect bacteria. Finally, after Gram staining and observing bacteria by using an optical microscope, top strains were identified by a variety of biochemical tests such as oxidase, catalase, MRVP, motility, hydrogen sulfide production, and culture in phenylalanine deaminase (PAV).

2.4. PAH Analysis. The measurement of PAHs (PHE and PYR) from soil was carried out by HPLC/UV manufactured by the Agilent Company (Model 11000) in accordance with the US Environmental Protection Agency (USEPA) [29] as follows:

Two grams of soil was carefully weighed, homogenized, and passed through a 20-mesh standard sieve. A cleaner ultrasound device (Model CD-4820) was used for the extraction of PHE and PYR. At this stage, samples after 15 minutes of sedimentation of coarse particles, and to clean up the samples in order to inject into the HPLC, they were centrifuged at 3000 rpm for 15 minutes. Then, these samples were filtered using the PTFE filter of 0.2 μm pore size. Finally, 2 ml of solution was injected to HPLC.

2.5. Statistical and Data Analysis. In this study, sample size estimation was carried out using the full factorial method. In order to increase the precision and accuracy of the tests, sampling and analysis were performed in triplicate. SPSS 16 software and Excel 2010 were used to analyze the data and draw the charts, respectively.

3. Results

3.1. Soil and Vermicompost Analysis. The results of the physicochemical analysis of the vermicompost sample used in this study are presented in Table 1. Also, the soil samples are composed of of 75% clay and 25% sand.

3.2. Chemical Analysis of PHE Samples. The concentrations of PHE at different sampling times during the bioremediation process by various volume percent of vermicompost (2, 4, and 6%) are presented in Figure 1. As seen, all the curves show the decreasing trend of the PHE, and also, lower decreasing is observed in high concentrations of PHE. In addition, Figure 1 shows that the increase in the amount of vermicompost from 2 to 6 percent improves the bioremediation process and reduces the concentrations of PHE significantly.

3.3. Chemical Analysis of PYR Samples. Figure 2 shows the concentrations of PYR at different sampling times during the bioremediation process by various concentrations of vermicompost (2, 4, and 6%). As seen, all graphs show the decreasing trend of PYR.

3.4. Microbial Analysis. The microbial population trends at different times of biological treatment in samples containing PHE and PYR are shown in Figures 3 and 4. It was observed that, at first, due to the toxicity of the environment, the bacterial population decreased significantly, but over time, the microorganisms adapted to the conditions and began to decompose the compounds. It should be noted that the trend of microbial population changes in samples containing PYR was more irregular than samples containing PHE, meaning that microorganisms needed more time to adapt to the environment.

After 9 weeks, when the process of PYR and PHE eliminating had decreased and become nearly uniform, it was possible to identify and isolate bacteria in samples with greater growth and a better ability to decompose these compounds. By performing the usual tests of microbiology and examining the results of biochemical tests, it was found that *Citrobacter* and *Pseudomonas* bacteria were more effective in eliminating both of these oil compounds (Table 2).

4. Discussion

In the present study, we applied the effect of vermicompost on the bioremediation of contaminated soils with emphasis on removal of PHE and PYR. Oil compounds provide a good source of carbon and energy for microorganisms, and therefore, the concentration of these compounds will play a significant role in improving the biological process.

The results showed that by increasing the oil compounds, the bioremediation process was improved. This could be due to an increase in the microbial population. Moreover, the concentration of polycyclic compounds also decreased significantly during the process (Figure 1). However, at higher concentrations of PHE and PYR, lower efficiencies were observed in the bioremediation process. In fact, at higher concentrations, the toxicity of the polycyclic compound increases and more time is required to decompose these compounds. Moreover, with the increasing in the bioremediation time, the highest removal efficiency of PHE and PYR was observed at 200 mg/kg concentration (Figures S1 and S2). These findings are in line with the results of Arbabi et al.'s study, so it was found that there was a significant relationship between the concentration and type of microbial consortium with the removal efficiency over time. Also, they indicated that bioremediation at low concentrations of petroleum compounds requires a shorter time than the higher concentrations [13].

In this study, the highest decrease in the concentrations of PHE and PYR were observed in the first 45 days of process, and with the increase in vermicompost, the PHE and PYR concentrations also decreased. The average removal rates of PHE and PYR in the same period were 88% and 83%, respectively (2% vermicompost) (p value <0.05). The reason for this can be explained by the fact that the nature of petroleum compounds are different and the consumption of some of them is easier for bacteria, as with the increase in the number of benzene rings, the resistance of this material to decomposition also increases. The results of the present study were consistent with the Shafiee et al.'s studies [30, 31]. In this study, with the increase in the concentration of vermicompost, the removal efficiency also increased. Indeed, vermicompost addition has a significant impact on the successful biodegradation of hydrocarbons in soil. It is hypothesized that the addition of vermicompost increases hydrocarbon degradation by increasing the amount and activity of microbial biomass in the soil and improving acclimatization and microbial adaptation. Overall, the results indicate that the reduction process for hydrocarbons is evident in all samples that have undergone bioremediation. With the application of vermicompost and subsequent inoculation of the bacteria into the contaminated soil, the decomposition rate of the petroleum compounds increased [32, 33]. Dores-Silva et al.'s results corroborate the data presented in this study [18, 34].

It is demonstrated that at the onset of process due to the environment toxicity, the population of bacteria has been significantly reduced, but over time, microorganisms adapted to conditions and began to function and decompose compounds (Figure 3). Also, the microbial population trend in PYR-containing samples has more variations over the duration of the experiments due to more toxicity of the four-ring compounds. It should be noted that the process of population changes in samples containing PYR is more unusual than PHE-containing samples, and microorganisms require more time to adapt to the environment (Figure 4). At the end of 9 weeks, when the process of eliminating the PYR and PHE compounds were reduced and nearly uniformed, bacterial isolation in samples with the highest percentage of removal was done. According to the results of bacterial isolation experiments, in the removal of both PYR and PHE compounds, mainly *Acinetobacter* and *Pseudomonas* bacteria were involved. As it can be seen, the highest percentage of removal of the compounds is related to the initial hours

Table 1: The physicochemical analysis of the vermicompost.

No.	Parameter	Standard limit		Results
		Class 1	Class 2	
1	Organic matter	Min. 35%	Min. 25%	39%
2	Organic carbon	Min. 20%	Min. 10%	23%
3	Organic nitrogen	Min. 1%	Min. 0.5%	1.5%
4	C/N ratio	15–25	10–15	15.1
5	Potassium	Min. 1%	Min. 0.5%	2.3%
6	Electrical conductivity	Max. 8 ds/m	Max. 10 ds/m	2.35 ds/m
7	pH	6.5–8.5	6.5–8.5	7.78
8	Moisture	20–30	20–30	39
9	Ash	Max. 50%	Max. 50%	63
10	Sodium	—	—	1.2%
11	Saturation percentage	—	—	86
12	Percentage of neutralizing agents	—	—	19

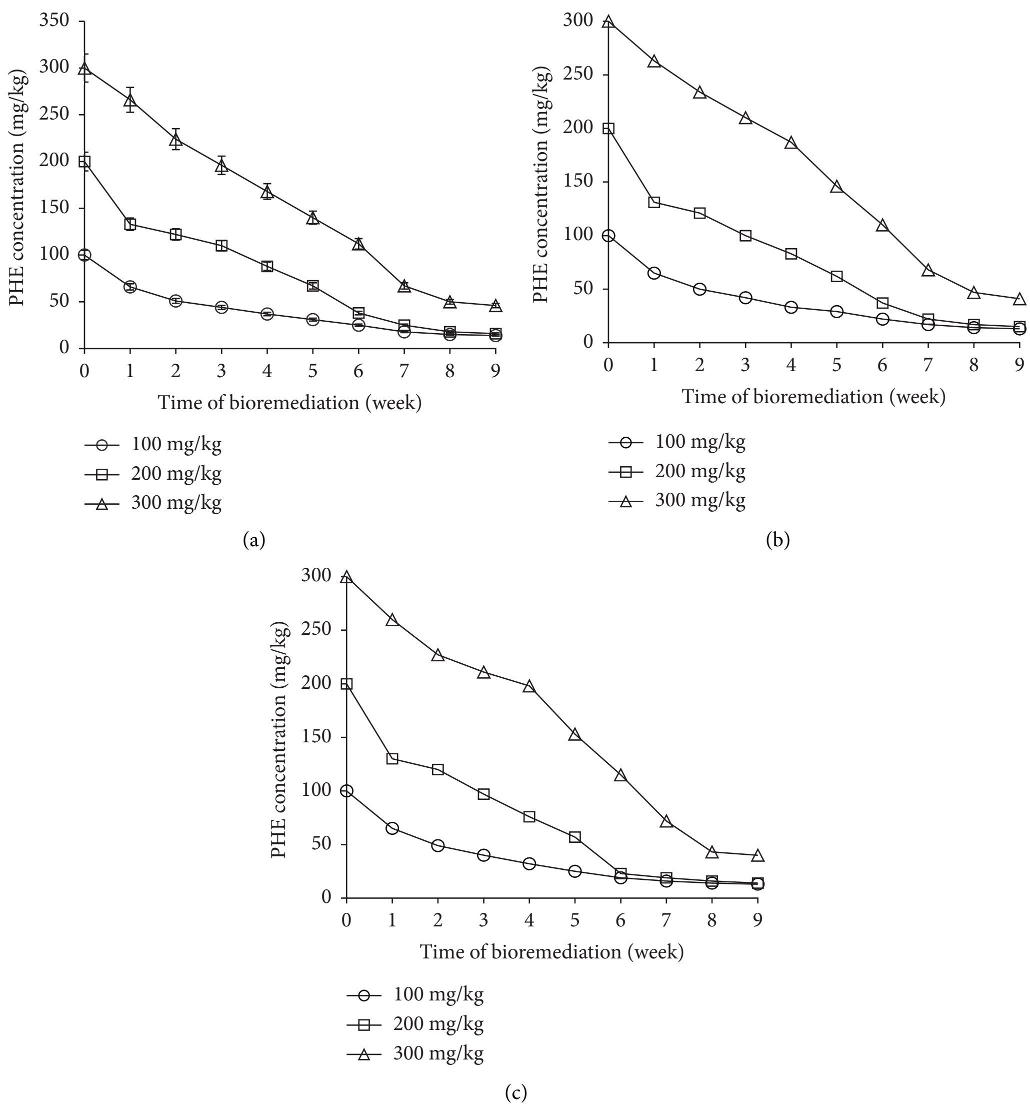

Figure 1: PHE concentrations in vermicompost of (a) 2%, (b) 4%, and (c) 6%.

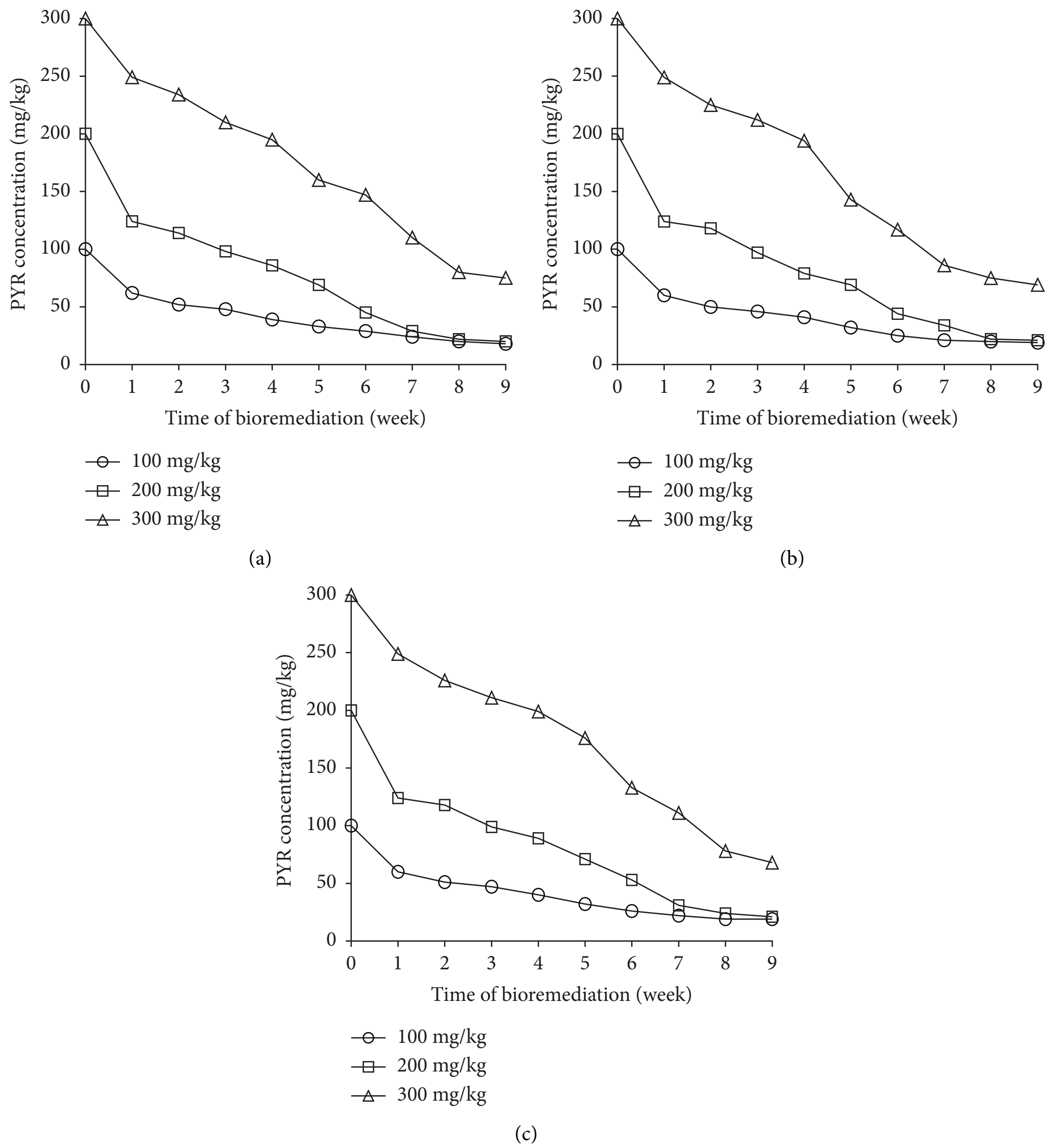

Figure 2: PYR concentrations in vermicompost of (a) 2%, (b) 4%, and (c) 6%.

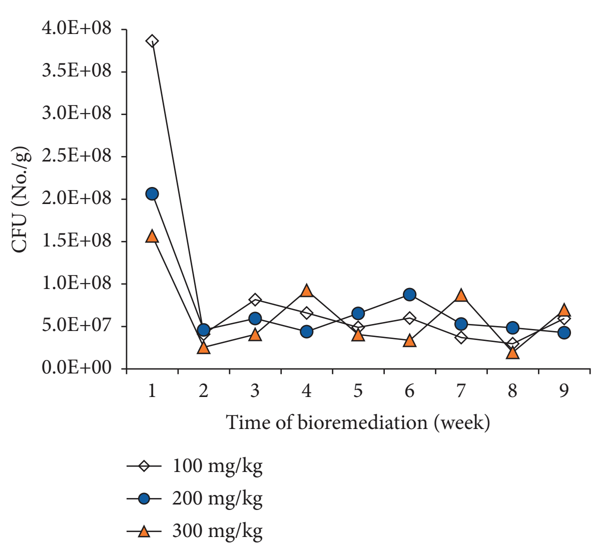

Figure 3: Microbial population trends in samples containing PHE.

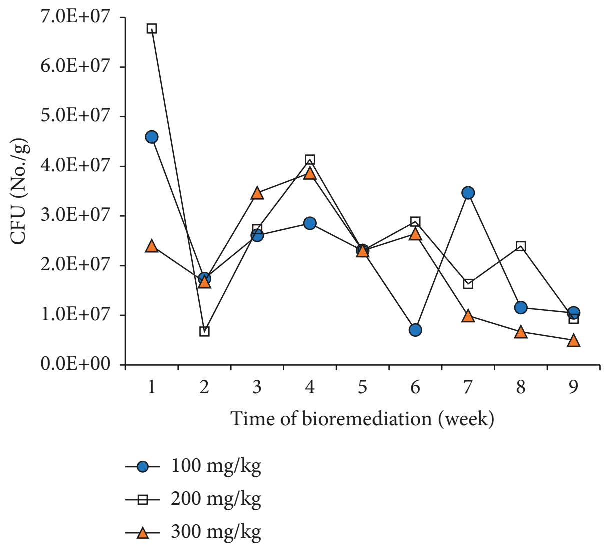

Figure 4: Microbial population trends in samples containing PYR.

Table 2: Morphological and biochemical characteristics of two selected bacterial species.

Test	*Pseudomonas*	*Citrobacter*
Gram staining	−	−
Cell	Bacillus	Bacillus
Oxidaze	+	−
Oxygen of fermentation	+	−
H_2S	−	+
MR	+	+
VP	−	−
Iodine test (phenylalanine dehydrogenase)	−	−
Catalase	+	+
Citrate	+	+
Movement	+	−
Urease	−	−

after inoculation, after which the reduction of oil compounds from the soil became almost constant.

In the present study, according to the findings of biochemical tests, there are many reports about the potential of the genus *Pseudomonas* in the decomposition of petroleum compounds, which (2012) highlight the efficacy of this strain in the elimination of petroleum compounds such as PHE [35]. Also, Onifade and Abubakar conducted studies in 2007 on the characteristics of microorganisms isolated from raw oil-contaminated soils with hydrocarbon decomposition ability which were able to isolate and identify five bacterial species (*Bacillus* spp., *Arthrobacter, Lactobacter, Micrococcus*, and *Pseudomonas*) [36]. The study of Arbabi et al. showed that contaminated soils contain more microbial populations; in fact, these microorganisms in contaminated soils could degrade the oil and use these compounds as a source of carbon and energy, and thus, the growth of their microbial population rose [13, 37]. Finally, the result of this study showed that the initial concentration of the pollutant, the microbial population and also bacterial inoculation, the conditions of activity of microorganisms, including temperature and pH, are the most important criteria for biodegradation [38].

5. Conclusion

The control of oil pollution is very difficult, and the presence of these pollutants causes ecotoxicity. These problems are more serious in oil-producing areas such as the Middle East. According to the results of this study, the use of vermicompost is one of the most cost-effective and environment-friendly methods for cleaning up contaminated soils. The main isolated strains were *Pseudomonas* and *Acinetobacter* bacteria which reveals that these potent bacteria can use oil compounds as a source of energy and carbon. So this characteristic makes this strain the perfect choice for bioremediation of the oil-contaminated area. The findings of this study suggest that the use of vermicompost could be a suitable technology for PAH (PHE and PYR) bioremediation.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interests.

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Supplementary Materials

Removal efficiency (%) of PHE and PYR at 2% (a), 4% (b), and 6% (c) vermicompost were provided in Figures S1 and S2. (*Supplementary Materials*)

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