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Bioremediation of Petroleum-Contaminated Soil Using Earthworms: A Study on Hydrocarbon Reduction in Southern Iraq

Buthainah Mahdi Younus [1,], Majida Sabah Alenazi [2,] and Manal Mohammed Akbar [*,3,]

[1,2,3] Department of Biology ,College of Education for Pure Sciences, University of Basrah, Iraq

[*] buthainah.younus@uobasrah.edu.iq
Orcid: https://orcid.org/0000-0002-1994-0508

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Abstract:

The current laboratory study was conducted to verify the possibility of biological treatment (Vermiremediation) of the earthworm Lumbricus terrestris in soil contaminated with crude oil, four concentrations (1%-2%-5%-8%) were prepared from soil contaminated with crude oil that was brought from the Shuaiba area near the south refineries in Basrah Province, southern Iraq, after growing them with earthworms and examining them every 10 days for 40 days, the statistical results showed that the earthworms significantly reduced total petroleum hydrocarbons (TPH).Mean concentration of total petroleum hydrocarbons for the 1% pollution concentration before treatment was 16.636 µg/g dry weight, and after 40 days of treatment it reached 5.330 µg/g dry weight, while at a contamination concentration of 2% it reached 33.426 µg/g dry weight before treatment and at the end of treatment the mean concentration reached 5.730 µg/g dry weight, as for contamination concentration of 5%, it reached 51.370 µg/g dry weight before treatment and decreased after 40 days of treatment to 7.340 µg/g dry weight. Pollution concentration 8% it was 70.540 µg/g dry weight before treatment, and after the end of treatment it decreased to 11.350 µg/g dry weight. The statistical results also showed that there is a direct correlation between (TPH) and pH and an inverse relationship with soil temperature.

Keywords: *Lumbricus terrestris*- petroleum hydrocarbon -Soil Contaminated- Vermiremediation .

1-Introduction

Environmental pollution has become the most important challenge threatening the fate of humanity and life worldwide, and one of the biggest problems of environmental pollution is the leakage of crude oil and its derivatives into the environment. The continuous development of the global economy and the exploration of oil, which plays a prominent role in the lives of people, has led to the pollution of the ecosystem with petroleum hydrocarbon compounds, which has become a source of global concern (1). The soil in Basrah Province suffers from severe pollution with petroleum hydrocarbons, as the soil pollution classification system indicates that it is severely polluted with polycyclic aromatic hydrocarbons, including oil areas and roads. (2) Soil is a critical environment because several pollutants come from different human activities, such as mining, transportation, agriculture, and industry, which can contaminate the soil with many serious pollutants (3), these toxic compounds greatly affect soil, plants, animals, and human health. Soil contamination with crude oil may contaminate groundwater beneath this soil and create many environmental problems (4) the toxicity equivalents of polycyclic aromatic hydrocarbons in the soil of oil regions showed highest level and in general, all sites in Basrah Province were significantly contaminated with aromatic hydrocarbons and their health risks due to soil exposure to them along Basrah Province (5). Bioremediation has become an important method for restoring oil-polluted environments to their former state (6). Earthworms have been found to be useful in removing pollutants from soil, known as organic pollutant removal (7), an important aspect of worm technology that has received relatively less attention is vermiremediation, a term used to describe the process by which earthworms clean up soil pollutants. Worms use biotic and abiotic interactions, their life cycle, and burrowing and feeding behavior to transform, decompose, or remove pollutants from the soil environment (8). Vermiremediation offers a number of advantages, in addition to removing contaminants from the soil, unlike physical and chemical remediation techniques that typically involve digging up the soil or treating it with chemicals, vermiremediation is not environmentally destructive, but is environmentally friendly and potentially sustainable. Vermiremediation is a cost-effective remediation technique when compared to some physical and chemical remediation methods (9, 10, 11) bioremediation is a promising technology used to restore and clean polluted ecosystems (12) it can be one of the best methods used in the analysis of toxic petroleum hydrocarbon pollutants, it is a simple process that can be applied to large areas it is a profitable and environmentally beneficial alternative and can be a source of economic profit (13). Vermiremediation uses earthworms to remove soil pollutants or help decompose them (14), the most commonly used earthworms in vermiremediation of organic and inorganic compounds are A. chlorotica, Eisenia, E. eugeniae, D. veneta, Lumbricus, P. excavates(15). The efficiency of bioremediation is affected by several factors, including pH and temperature (16).(17) also conducted a laboratory study on bioremediation of diesel contaminated soil using earthworm Eudrilus eugeniae. The study showed that E. eugeniae can be used as a potential bioremediation agent in diesel contaminated soil. in a previous study by (18) that illustrates the potential and limitations of earthworms of the species Eisenia fetida, which is one of the types of earthworms used in the remediation of oil-contaminated soil, (19)study also showed that the earthworm P. corethrurus and palm oil work together synergistically to achieve more effective removal of total petroleum hydrocarbons from soil, these results demonstrate the potential of using P. corethrurus to remove crude oil from soil, either directly or indirectly. The term vermiremediation has recently been used to refer to use of earthworms to remove pollutants from soil (9). Earthworms also help in the decomposition of compounds that are not recyclable (20). During research studies, the positive effect of earthworms on the removal of pollutants, such as oil, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides and heavy metals has been demonstrated by several authors (21, 22,23, 24, 25)

The aim of the research: Isolating earthworms (Lumbricus terrestris) that reside in the soil and using them in the laboratory to treat soils contaminated with petroleum hydrocarbons using vermiremediation technology, which is considered simple, cheap and environmentally friendly.

2-Materials and methods

2.1.Study area and Sample collection

The study site is the Shuaiba area near the south refineries in Basrah Province, southern Iraq, the oil spill that occurred at the sample collection site takes a form similar to a waterway as a result of the increase in oil discharges from the refinery, soil samples contaminated with crude oil were collected from three sites along the contaminated stream, as shown in figure 1.The soil was dug to a depth of (0-30) cm using a field shovel. As for the earthworms Lumbricus terrestris, they were prepared by collecting some of them from the orchards and gardens in Basrah Province and purchasing another group from the earthworm breeding fields in Baghdad Province the worms were acclimatized for two weeks and used for the experiment, where the adults were isolated from them (depending on the presence or absence of the clitellum) and their lengths ranged between 7-10 cm.

2.2. Experiment container design

16glass basins were created, their dimensions were (20-25-25) cm, after which the soil samples contaminated with crude oil and taken from the three study sites were mixed well, they were placed in glass tanks and after conducting the LC50 toxicity test for earthworms in this mixture of soil contaminated with crude oil. Four concentrations of this mixture were prepared (1%, 2%, 5%, 8%) after diluting it with agricultural soil brought from agricultural nurseries, three replicates of these concentrations were made, each glass tank contained 3 kg of oil-contaminated soil with the addition of 30 adult L. terrestris earthworms to each tank, as in figure 2. The last four tanks it was considered a control group without worms, the containers were moistened daily with 30 mL of distilled water, then soil samples were taken from the basins at different time periods, including time zero (the start time of the experiment), then after 10 days, after 20 days, after 30 days, and finally after 40 days from the start of the experiment to observe the changes occurring in the mean concentrations of total petroleum hydrocarbons (TPH), pH values, and soil temperature, for each concentration and for each period.

2.3.Soil temperature measurement

The soil temperature was measured with a Hygro-thermometer clock device after placing the device's electrode at a depth of 5 cm in the soil for 5 minutes, and it was measured in the Celsius unit of measurement (°C).

2.4.pH measurement

Take 50 g of dry soil in a 100 mL glass beaker, add 50 mL of distilled water, mix well, then leave the mixture for 30 minutes, stirring continuously every 10 minutes during this period. Leave the solution to settle for one hour and calibrate the pH meter. Place the electrode in the solution at a distance of about (3 cm), then take the reading after 30 seconds(26).

2.5.Extraction of petroleum hydrocarbons

Petroleum hydrocarbons were extracted from soil according to the method mentioned in (27), where soil samples were air-dried at room temperature, then ground in a stainless steel electric mortar and sieved using a metal sieve with a hole diameter of 63 µm, and 50 g of dried, ground and sieved soil was weighed in the extraction tool and extracted using intermittent extraction with saxolite. The concentrations of total petroleum hydrocarbons extracted from the soil were measured using a spectrofluorometer.

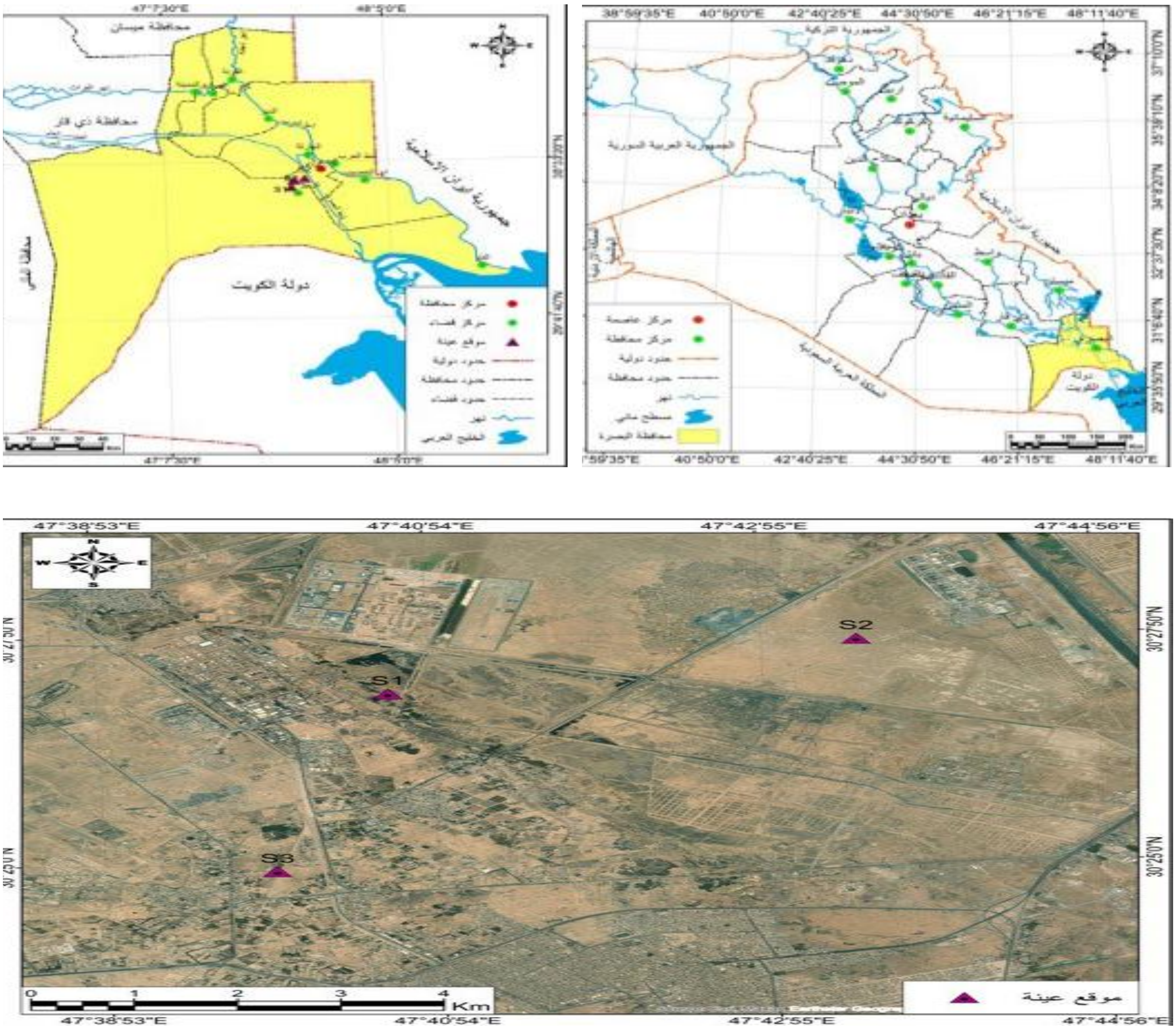

Figure 1. Map showing the collection of the three samples.

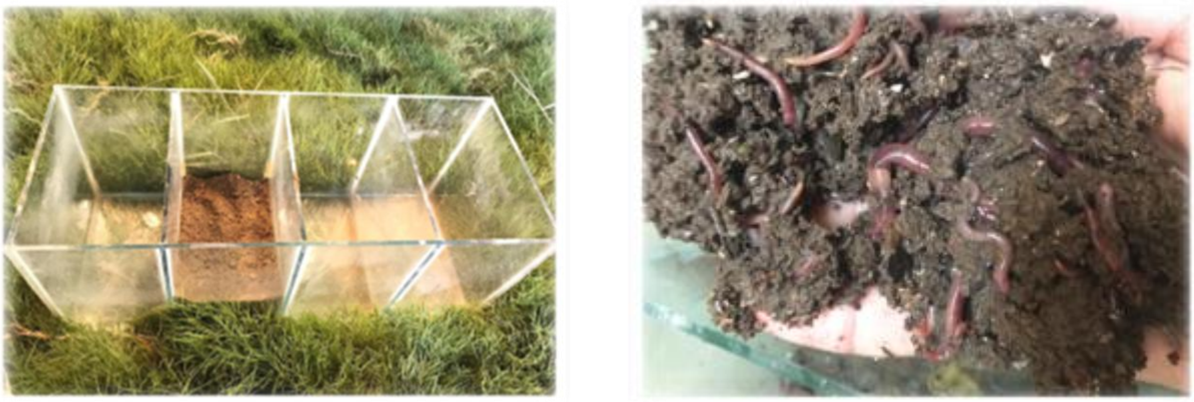

Figure 2. Earthworms *Lumbricus terrestris* and glass containers.

2.6.Statistical Analysis

The data were analyzed using the statistical program Statistical package for social sciences (SPSS) version 26 in the statistical analysis, and the differences were tested using the modified least significant difference test (RLSD) at a significance level of P ≥0.05.

3- Results

3.1. Total petroleum hydrocarbons

Table 1, shows the mean concentration of total petroleum hydrocarbons (TPH) for the sample mixture of Shuaiba, which reached 780.62 µg/g dry weight, while mean concentration of total petroleum hydrocarbons for agricultural soil was 26.73 µg/g dry weight. The results of the statistical analysis showed a significant difference between them at a significance level of P≥ 0.05 .The results of the statistical analysis of the current study showed that the mean concentration of total petroleum hydrocarbons was 16.63 µg/g dry weight for a 1% pollution concentration before treatment, and after 40 days of treatment it reached 5.33 µg/g dry weight, while with 2% pollution concentration before treatment it reached 33.42 µg/g dry weight at the end of treatment, the mean concentration reached 5.73 µg/g dry weight as for the pollution concentration of 5%, it reached 51.37 µg/g dry weight before treatment and decreased after 40 days of treatment to 7.34 µg/g dry weight, as for the pollution concentration of 8% it was 70.54 µg/g dry weight before treatment and decreased to 11.35 µg/g dry weight after end of treatment. Statistical analysis showed significant differences between control samples and pollution concentrations at a probability level of P≥ 0.05 as well as between the four pollution concentrations (1%- 2%- 5%- 8%) and treatment periods, as in Table 2.

Table 1: Mean concentrations of total petroleum hydrocarbons for of the Shuaiba sample and agricultural soil before starting the experiment µg/g dry weight

The sample	TPH concentration mean	standard deviation
Shuaiba soil	780.62	±20.86
Agricultural soil	26.73	±6.51

Table 2: Mean concentrations of total petroleum hydrocarbons µg/g dry weight.

Concentration mean and standard deviation	Treatment period 0 days	Control	Treatment period 10days	Control	Treatment period 20days	Control	Treatment period 30days	Control	Treatment period 40days	Control	total
Pollution 1%	16.63	16.59	13.37	16.56	10.76	16.45	7.83	16.36	5.33	15.70	13.56
	0.39±	0.09±	0.33±	0.06±	0.22±	0.05±	0.18±	0.08±	1.58±	0.65±	4.05±
pollution 2%	33.42	33.75	19.54	33.69	9.36	32.99	8.53	32.66	5.73	32.07	24.17
	0.5±	0.0±6	0.51±	0.09±	1.08±	0.48±	1.03±	0.59±	0.87±	0.04±	11.6±
Pollution 5%	51.37	51.67	27.43	50.99	13.52	50.51	9.45	49.99	7.34	49.87	36.21
	0.5±	0.54±	0.6±	0.22±	0.58±	0.35±	0.74±	0.13±	0.95±	0.94±	18.7±
Pollution 8%	70.54	71.02	54.2	70.52	32.27	70.39	25.69	69.87	11.35	69.59	54.5
	0.62±	0.09±	7.4±	0.41±	4.46±	0.44±	4.84±	0.14±	1.6±	0.27±	22.1±
Total	42.99	43.25	54.2	42.94	16.48	42.59	12.88	42.22	7.43	41.81	32.12
	20.9±	21.1±	7.4±	20.9±	9.84±	20.9±	8.04±	20.78±	2.7±	20.98±	21.8±

$LSD_{P<0.05}$=3.6 For(periods), $LSD_{P<0.05}$=10.618 For(concentrations)

3.2.pH

The results of Table 3, showed significant differences in the mean pH values for pollution concentrations (1%- 2%- 5%- 8%) and between the treatment time periods (10- 20- 30- 40) days at a probability level of $P\geq 0.05$, there were also significant differences between concentrations of pollution and the control samples, except for the period before treatment (0-10) days after treatment, there were no significant differences between them and control group , statistical results showed a direct relationship between TPH and pH value (r = 0.627) and the pH value decreased for all pollution concentrations during the treatment period, the highest mean pH was 8.6 in the period before treatment at a pollution concentration of 8% and the lowest pH value was 7.2 after 40 days of treatment at a pollution concentration of 1%.

Table 3: Mean value of pH in the soil.

Concentration mean and standard deviation	Treatment period 0 days	Control	Treatment period 10days	Control	Treatment period 20days	Control	Treatment period 30days	Control	Treatment period 40days	Control	total
Pollution 1%	8.16	8.3	7.76	8.23	7.53	8.2	7.3	8.13	7.2	8.1	7.59
	0.05±	0.2±	0.05±	0.15±	0.05±	0.11±	0.1±	0.11±	0.1±	0.15±	0.41±
pollution 2%	8.2	8.1	8.03	8	7.9	8.03	7.83	7.93	7.63	7.93	7.91
	0.1±	0.1±	0.05±	0.1±	0.1±	0.05±	0.05±	0.05±	0.2±	0.11±	0.17±
Pollution 5%	8.16	8.1	8.06	8.03	7.93	8.06	7.9	8.03	7.66	7.93	7.94

	0.11±	0.1±	0.05±	0.05±	0.11±	0.15±	0.1±	0.05±	0.15±	0.05±	0.16±
Pollution 8%	8.6	8.4	8.43	8.33	8.3	8.3	8.1	8.3	7.86	8.16	8..28
	0.1±	0.1±	0.05±	0.05±	0.1±	0.1±	0.1±	0.1±	0.05±	0.05±	0.2±
Total	8.28	8.22	8.07	8.15	7.91	8.15	7.78	8.1	7.59	8.03	8.03
	0.2	0.17	0.25±	0.16±	0.29±	0.15±	0.31±	0.15±	0.28±	0.13±	0.29±

$LSD_{P<0.05}$=0.083 For(periods), $LSD_{P<0.05}$=0.063 For(concentrations)

3.3. Soil temperature

Table 4, shows significant differences between the mean temperatures of pollution concentrations (1%, 2%, 5%, 8%) and the periods (10, 20, 30, 40) days, the statistical results showed significant differences between the mean soil temperatures for pollution concentrations and the control group at a probability level of $P \geq 0.05$, the highest mean soil temperature was 18.63 degrees Celsius at a pollution concentration of 8% after 40 days of treatment, and the lowest mean soil temperature was 17.13 degrees Celsius at a pollution concentration of 1% before treatment ,as the results indicated, there is an inverse correlation between soil temperature and TPH (r=0.286) .

Table 4: Mean soil temperature in degrees Celsius.

Concentration mean and standard deviation	Treatment period 0 days	Control	Treatment period 10 days	Control	Treatment period 20days	Control	Treatment period 30days	Control	Treatment period 40 days	Control	total
Pollution 1%	17.13	16.63	17.63	16.83	17.93	16.13	17.93	17.06	18.03	17.46	17.28
	0.47±	0.15±	0.1 5±	0. 5±	0.15±	0.05±	0.11±	0.15±	0.05±	0.05±	0.64±
pollution 2%	17.20	16.83	17.63	17.43	17.9	17.60	17.96	17.50	18.06	17.56	17.75
	0.1±	0.2±	0.05±	0.1±	0.1±	0.17±	0.15±	0.1±	0.05±	0.05±	0.37±
Pollution 5%	17.6	16.80	17.9	16.86	18.06	17.06	18.13	17.23	18.4	17.53	18.01
	0.3±	±0.2±	0.1±	0.2±	0.11±	0.11±	0.05±	011±	0.1±	0.05±	0.55±
Pollution 8%	17.76	16.83	17.96	16.96	18,33	17.46	18.33	17.7	18.63	17.76	18.2
	0.05±	0.2±	0.05±	0.05±	0.05±	0.05±	0.15±	0.17±	0.15±	0.15±	0.55±

Total	17.44 0.38±	16.77 0.18±	17.78 0.18±	17.02 0.16±	18.15 0.36±	17.06 0.6±	18.09 0.19±	17.37 0.28±	18.28 0.27±	17.58 0.14±	17.94 0.55±

$LSD_{P<0.05}$=0.192 For(periods), $LSD_{P<0.05}$=0.286 For(concentrations)

5- Discussion

Total petroleum hydrocarbons.

The results shown above for total petroleum hydrocarbons showed an increase in the Shuaiba sample and a decrease in the agricultural soil sample, this may be due to the contamination of the Shuaiba soil with crude oil and petroleum derivatives resulting from the discharge, spillage and leakage of the south refineries as well as from the transportation lines. After humans began to consume non-renewable resources, especially crude oil and its derivatives, environmental pollution problems began, as crude oil and hydrocarbon products can reach the soil as a result of the production, refining, import and export process or because of the use of petroleum products as a main source of energy, as well as the exploitation of crude oil and petroleum products, or because of accidents during the daily activities of the oil and its derivatives industry (28). Table 2, also shows a decrease in the mean total petroleum hydrocarbons during the treatment periods, and this may be a result of this is because earthworms secrete enzymes such as (Amylase, Cellulase, Lipase, Chitinase, etc.) which break down complex biomolecules into simple compounds that can be used by symbiotic intestinal bacteria with the help of its digestive enzymes, which work complex organic together with the enzymes of the worms, it increases the process of chemical decomposition pollutants into simpler ones (29) therefore, earthworms are important organisms in decomposing organic matter present in the soil and converting it into vermicompost (30). Treatment depends on the worms' sensitivity to pollutants as well as their metabolic ability to break them down (31). Earthworms are usually described as most important ecosystem engineers, as they are considered to be organisms that may modify the characteristics of their habitat and soil and thus affect the availability of resources for other species (32).

pH

The results are shown in table 3, that earthworms can tolerate a wide range of pH (from 4.2 - 8.0) but are unable to grow at lower concentrations (33) Because the activities of worms work to equalize the pH, for example, alkaline urine and excrete waste, thus it can be concluded that earthworms tend to adjust the pH level in the vermicompost (34), the intestine of earthworms has a pH of 7, which is why vermicompost has a neutral pH (35) earthworms increase the acidity of the soil in which they are found due to their various activities such as digesting organic matter and producing vermicompost (earthworm waste) the secretions of the intestinal contents of earthworms contain substances that work to reduce the pH, which is consistent with what(36) and (37) concluded.

Soil temperature

Earthworms alter the physical and chemical properties of soil by mixing it with organic matter and by burrowing they improve aeration and make pollutants available to microorganisms. The presence of earthworms in contaminated soil indicates their ability to survive with a wide range of different organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs), crude oil and polychlorinated biphenyls (PCBs), and can contribute to soil improvement due to the activity of microorganisms present in their digestive tract and in accelerating the removal of pollutants from the soil (15).Currently, the removal of pollutants from oil soil using worms has been gradually recognized as an expanding technology, and many studies on the decontamination of contaminated soil using worms have been conducted in the past decade(8), although worm-based organic decontamination is an ideal mechanism for cleaning up diluted soil, it is like other successful and environmentally friendly biological decontamination methods, which come with a number of challenges and not allowed limitations, this is because it does not work to remove pollution significantly, or it can only be applied to lightly or moderately polluted soil and in a limited area (38) based on these studies, we tried through this study to prove the ability of these worms to treat soils contaminated with crude oil and to demonstrate their effectiveness. They are also considered environmentally friendly and useful in improving and rehabilitating the soil, they are also low-cost and economical .

6- Conclusion

In light of the above results of the current study, it was concluded that vermiremediation using earthworms of the *L. terrestris* type was very effective in improving soil contaminated with crude oil and worked to reduce the level of contamination with total petroleum hydrocarbons, and it also led to soil neutralization by reducing the pH.

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