Asian Journal of Green Chemistry 8 (2024) 595-609

Asian Journal of Green Chemistry

Original Research Article
View Article Online | View Journal

Efficiency of Vermiremediation in Reducing Level of Heavy Element in Pollution Soils Containing Crude Oil

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ARTICLE INFORMATION

Submitted: 2024-07-30
Revised: 2024-08-03
Accepted: 2024-08-13
Published: 2024-08-18
Manuscript ID: AJGC-2407-1541
DOI: 10.48309/AJGC.2024.470681.1541

KEYWORDS

Heavy elements
Crude oil
Vermiremedation
Soil pollution
Lumbricus terrestris

ABSTRACT

This study aims to treat heavy elements pollution in soils polluted with crude oil near the southern refineries in the Shuaiba area in Basrah Governorate in an environmentally friendly way by using the earthworm *Lumbricus terrestris* and testing its efficiency in treating heavy elements pollution, this process is known as bioremediation (Vermiemediation*)*, four different concentrations of pollution were made (1%, 2%, 5%, and 8%). The worms were raised in these different concentrations of pollution for 40 days, where some heavy elements such as lead, nickel, cadmium, and copper were measured and examined every ten days. The results showed that the highest treatment efficiency for lead was 93% after 40 days of treatment at a pollution concentration of 1%, and the lowest treatment efficiency was 9% after 10 days of treatment at a pollution concentration of 8%. The nickel treatment efficiency was highest at 99% after 40 days of treatment at a pollution concentration of 1%-2%, respectively, and the lowest efficiency was 8.5% after 10 days of treatment at a pollution concentration of 5%, while the highest treatment efficiency for cadmium was 100% after 30-40 days of treatment at a pollution concentration of 1-2%, respectively, and the lowest efficiency was 51% after 10 days of treatment at a pollution concentration of 8% **,** the highest efficiency for treating copper was 99.9% after 40 days of treatment at a pollution concentration of 1%-2%, respectively, and the lowest treatment efficiency was 21% after 10 days of treatment at a pollution concentration of 8%. The results showed the high efficiency of the earthworm *Lumbricus terrestris* in treating Soils polluted with heavy elements.

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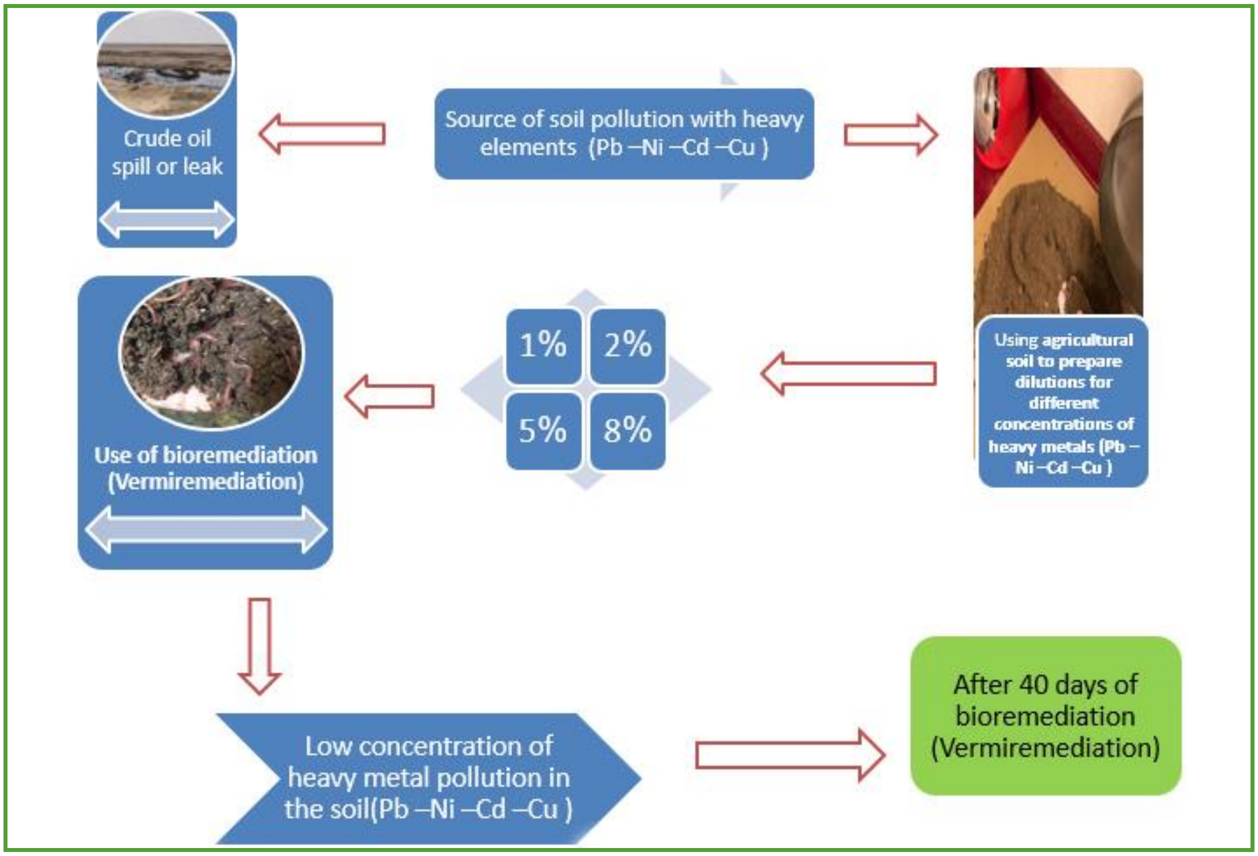
Graphical Abstract



Introduction

Heavy elements are considered natural components of the Earth's crust, and they are the elements whose density increases (5 g/cm³) [1-3]. These elements are often called trace elements because they are found in low concentrations in the Earth's crust, around 0.1% [4,5]. The two main sources of heavy elements in the soil are the natural chemical background of the earth and pollution resulting from various human activities. Crude oil extracted from oil fields may contain a large amount of heavy elements as part of the impurities present, and this depends to a large extent on the rocks carrying these elements in which the crude oil is formed. These impurities are removed to some extent only during crushing and refining the oil into different products [6], Oil areas contribute to add heavy elements in varying concentrations to polluted soils, as presented in [7]. The problem of oil pollution is widespread in many countries, as it leads to the accumulation of harmful waste resulting from the incinerators of oil industries, such as fumes, smoke, and oil leakage [8]. Pollution with toxic heavy elements in soil is one of the most important problems facing humans in the modern era. It has become increasingly serious in recent decades due to industrial discharges, mining, irrigation of polluted water, and the misuse of pesticides and chemical fertilizers [9], because of their nature, they remain bound to the soil for a long time ,and can accumulate in soil organisms and seep into groundwater [10], chemical and physical remediation approaches for heavy elements are widely applied, traditional chemical and physical methods are more expensive more difficult to apply and affect soil properties [11]. Biotechnologies are inexpensive, feasible, and environmentally friendly options [12]. Earthworms have been used in the environmental rehabilitation of degraded soils [13,14] besides the worms' treatment of polymetallic contaminants [15,16]. Phytoremediation and vermitreatment are the most environmentally friendly and effective ways to mitigate soil pollution with heavy elements [17-21]. Some environmental studies have demonstrated, as a study [22] confirmed that earthworms reduced the concentration of chromium, lead, copper, and zinc in worm sludge to below the limits set by the US Environmental Protection Agency after 60 days. While Ameh *et al*. [23] showed a decrease in the concentration of zinc, copper, nickel, and chromium exposed to soil polluted with motor oil using *Eudrilus Eugenia*, and Lui [24] showed that there were clear differences in the absorption of earthworms and the elimination movement of zinc and cadmium in the physiological variation between two species of earthworms (*Lumbricus terrestris*) and (*Eisenia foetida*) reared in three contaminated soils in the field after 30 days. In [25], it was shown that the *Namalycastis indica* worm was able to accumulate some heavy elements, such as, copper, nickel, cobalt, and cadmium in its tissues in a varying manner after growing it in food media containing different concentrations of these elements. Ekperusi *et al.* [22] studied the evaluation levels of heavy elements, present in soil polluted with crude oil and the application of the earthworm *Hyperiodrilus africanus*, with interest in bioremediation of elements in polluted soil after 90 days under conditions laboratory. There was a significant decreasing trend in the percentage of heavy elements found in the soil after inoculation with earthworms. The results showed that earthworms *H. africanus* can be effectively used in the bioremediation of heavy elements in soil polluted with crude oil. There is very little research on the bioremediation of soil polluted with crude oil with earthworms. Hence, there was a need to conduct a study to determine the effect of crude oil pollution on the soil content of some heavy elements and to explore the potential of the earthworm *Lumbricus terrestris* in the bioremediation of heavy elements in soil polluted with crude oil, and its efficiency.

Experimental

Sample collection

The oil spill that occurred at the sample collection site takes a shape similar to the watercourse. Soil samples polluted with crude oil were collected from three sites along the polluted stream close to the southern refineries in the Shuaiba area in Basrah Governorate on February, 2023 (Figure 1).

The soil was dug to a depth of 0-30 cm using a field shovel, and *Lumbricus terrestris* earthworms were prepared by collecting some of them from orchards and gardens in Basrah Governorate and purchasing a group of earthworm breeding fields in Baghdad Governorate. The worms were acclimated for two weeks and prepared for the experiment. Adults were isolated depending on the presence or absence of a saddle and their lengths ranged between 7 and 10 cm.

Experiment container design

16 glass containers were created; their dimensions were (20, 25, and 25) cm in width, length, and height, respectively. After mixing soil samples contaminated with crude oil from the three sites together, and after conducting an LC50 toxicity test for earthworms in this mixture of soil polluted with crude oil, four

were prepared. Concentrations of this mixture (1%, 2%, 5%, and 8%) after diluting it with agricultural soil, and three replicates of these concentrations were made. Each container contains 3 kg of soil, and 30 of adult *Lumbricus terrestris* earthworms were added to each container, and the last four containers were considered as a control group without worms. The containers were moistened daily with 30 mL of distilled water. After that, samples were taken from the soil of the containers at different periods of time, including times at time zero (the time the experiment began), 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 values of heavy metal concentrations in the soil and the extent of the efficiency of earthworms *Lumbricus terrestris* in treating each concentration and each period using the methods described in ROPME [26] in preparing and measuring heavy elements (Pb, Ni, Cd, and Cu) using the Thermo Fisher Scientific ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer).

Statistical analysis

The data were analyzed using the statistical program SPSS (Social Science Package Statistical, version 26) for statistical analysis, and differences were tested using the modified least significant difference test (R.L.S.D) with a significance level of $P \leq 0.05$.

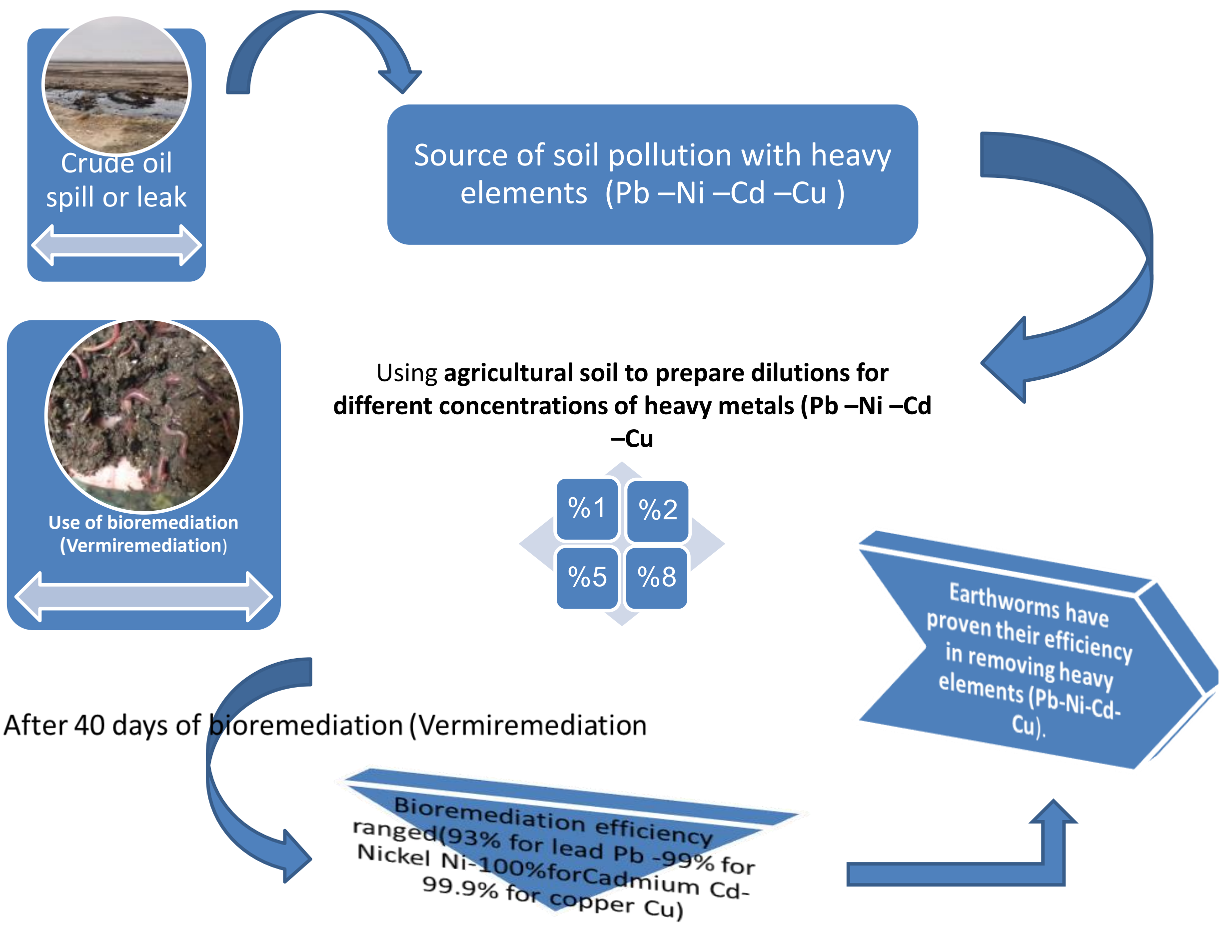


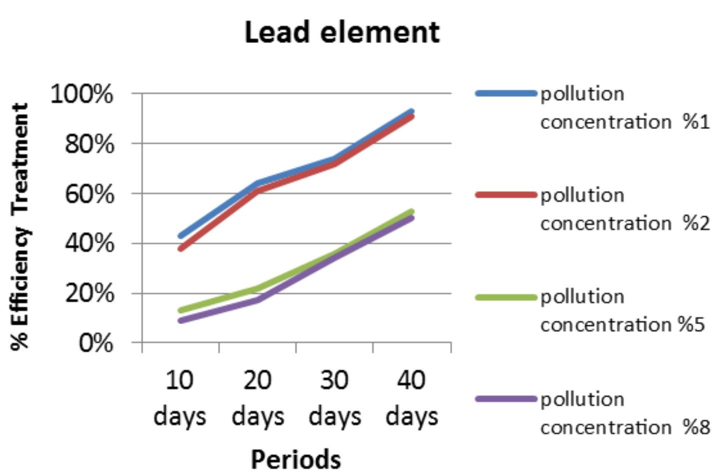
Figure 1. Sample collection sites for a study

Results and Discussion

The results of the statistical analysis showed an increase in the values of the mean concentrations of heavy elements in the mixture of the Shuaiba sample polluted with crude oil, and the presence of a significant difference between it and the agricultural soil sample at the probability level of $P \leq 0.05$, as listed in Table 1. Crude oil contains many heavy elements ions. Therefore, soil polluted with crude oil is accompanied by a high content of these heavy elements, the most important of which are (lead, nickel, cadmium, copper, iron, zinc, manganese, vanadium, and chromium). Crude oil contains various heavy elements accompanying it from the extraction well. When the crude oil is exposed at the beginning of refining to water washing in the desalination unit, the oil gets rid of part of the minerals accompanying it, which is the part that is in the form of salts dissolved in it, while the part that is in complex form (organic mineral compounds) that remains in the crude oil emerging from it [27,28] the current study agreed with the study of Khaddour *et al.* [8].

Table 1. Mean concentrations of heavy elements, for the mixture of Shuaiba sample and agricultural soil, µg/g dry weight before starting the experiment

Heavy metals (HM)	Polluted sample concentration mean	Agricultural soil concentration mean
Pb	150.50±9.7	4.5±1.5
Ni	97.00±9.6	9.05±1.33
Cd	13.48±1.5	0.01±0.002
Cu	33.80±2.44	8.84±0.15



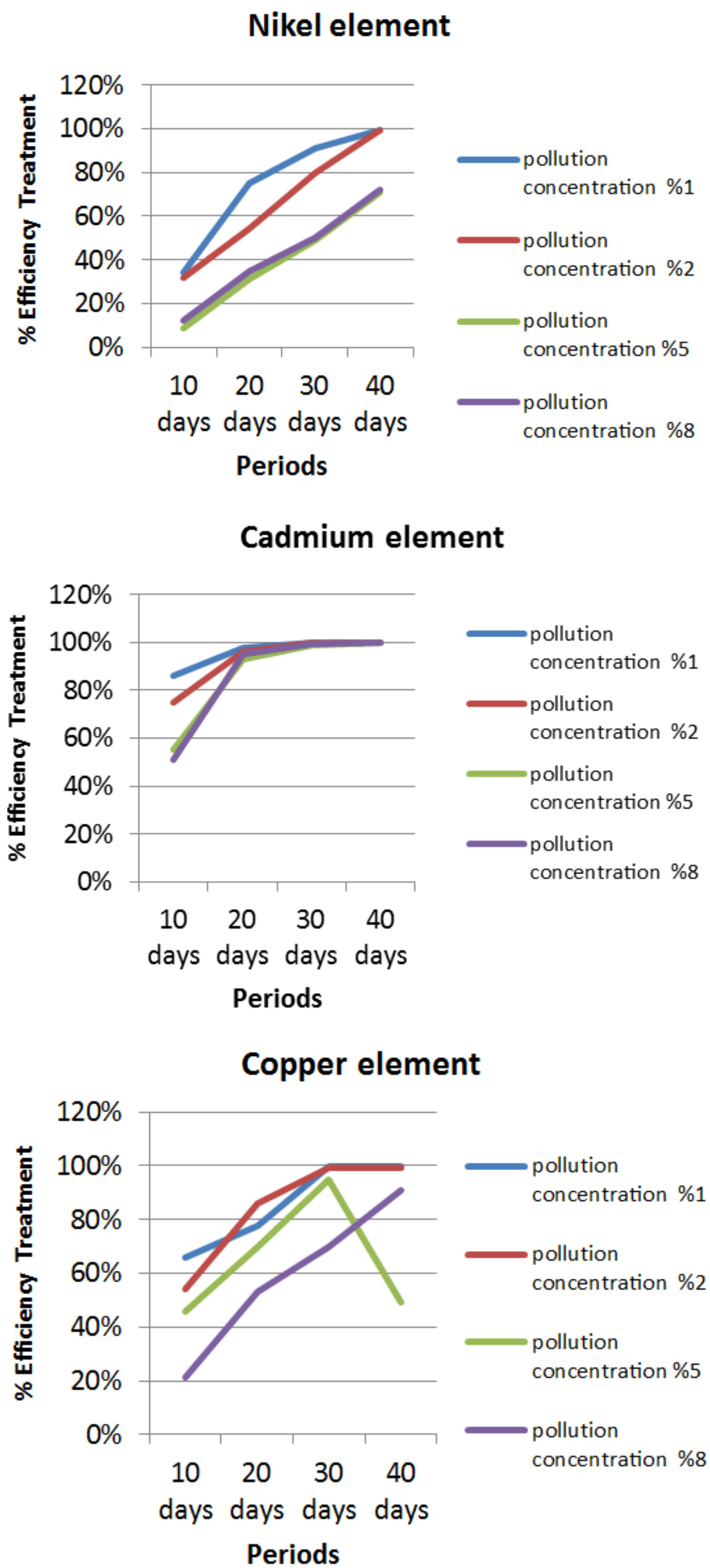


Figure 2. Efficiency of Vermiremediation (*Lumbricus terrestris)* of heavy elements lead, nickel, cadmium, and copper

The results of the current study indicate a decrease in the mean values of lead concentrations, as the results of the statistical analysis showed that there are significant differences at the probability level of P ≤0.05 between the mean concentrations of the four pollution levels (1%, 2%, 5%, and 8%) and the periods (10 days, 20 days, 30 days, and 40 days). There are significant differences between the treatment samples and the control group at the probability level P ≤ 0.05, except for the pre-treatment period (time 0) there were no significant differences between the treatment samples and the control group (Table 2). The treatment efficiency for lead in soil polluted with crude oil according to the pollution concentration reached (1%, 2%, 5%, and 8%) after ten days of follow-up (43%, 38%, 13%, and 9%) and after 20 days (64%, 61%, 22%, and 17%), after 30 days (74%, 72%, 36%, and 34%) and after 40 days (93%, 91%, 53%, and 50%), respectively (Figure 2).

The results of the statistical analysis showed a decrease in the mean concentrations of the nickel element, and there were significant differences at the probability level of P ≤0.05 between the pollution concentrations (1%, 2%, 5%, and 8%) and the periods (10 days, 20 days, 30 days, and 40 days). There are also significant differences between the treatment samples and the control group at the probability level P ≤0.05, with the exception of the pre-treatment period (Table 3). The treatment efficiency for pollution concentrations was (1%, 2%, 5%, and 8%) after ten days of Follow-up (34%, 32%, 8.5%, and 12%) while the treatment efficiency after 20 days was (75%, 54%, 31%, and 35%) and the treatment efficiency after 30 days was (91% - 80% - 49% -50 %) and treatment efficiency after 40 days (99%, 99%, 71%, and 72%), respectively (Figure 2).

The results of cadmium showed a decrease in the mean concentrations, as it was revealed through statistical analysis that there were significant differences at the probability level of P ≤0.05 between the mean concentrations of the four pollution levels (1%, 2%, 5%, and 8%) and the periods (10 days, 20 days, 30 days, and 40 days). There are significant differences between the treatment samples and the control group at the probability level P ≤0.05, except for the period (0 and 10) days of follow-up, as there were no significant differences between the treatment samples and the control group (Table 4). The treatment efficiency for pollution concentrations was (1%, 2%, 5%, and 8%) after ten days of follow-up (86%, 75%, 55%, and 51%), while the treatment efficiency after 20 days was (98%, 96%, 93%, and 95%) and treatment efficiency after 30 days (100%, 100%, 99%, and 99.5%) and treatment efficiency after 40 days (100%, 100%, 99.9%, and 99.7%), respectively, Figure 2.

Table 5 and Figure 2 show a decrease in the mean of nickel concentrations. The statistical results showed that there were significant differences at the probability level of P ≤0.05 between the mean pollution concentrations (1%, 2%, 5%, and 8%) and the periods (10 days, 20 days, 30 days, and 40 days). It was also found that there are significant differences between the treatment samples and the control group at the probability level P ≤0.05, with the exception of the pre-treatment period. The treatment efficiency for pollution concentrations was (1%, 2%, 5%, and 8%) after ten days of follow-up (34%, 32%, 8.5%, and 12%), while the treatment efficiency after 20 days was (75%, 54%, 31%, and 35%) and treatment efficiency after 30 days (91%, 80%, 49%, and 50%) and treatment efficiency after 40 days (99%, 99%, 71%, and 72%), respectively.

Table 2. Mean concentrations of lead, μg/g dry weight

Concentration mean	Treatment period 0 days	Control	Treatment period (10 days)	Control	Treatment period (20 days)	Control	Treatment period (30 days)	Control	Treatment period (40 days)	Control	Total
Pollution 1% and standard deviation	1.964±0.034	1.938±0.065	1.105±0.007	1.934±0.060	0.696±0.130	1.937±0.066	0.509±0.156	1.932±0.064	0.137±0.053	1.928±0.061	1.408±0.701
pollution 2% and standard deviation	2.934±0.179	2.914±0.027	1.810±0.345	2.914±0.025	1.119±0.161	2.910±0.019	0.792±0.122	2.912±0.032	0.240±0.054	2.909±0.031	2.145±1.033
Pollution 5% and standard deviation	4.838±1.113	4.840±0.051	4.208±1.207	4.802±0.198	3.767±1.125	4.842±0.042	3.072±0.919	4.843±0.079	2.268±0.795	4.843±0.076	4.232±1.079
Pollution 8% and standard deviation	11.755±0.781	11.779±0.034	10.650±0.034	11.775±0.807	9.752±1.055	11.769±0.199	6.665±1.135	11.525±0.469	5.795±0.318	11.523±0.462	10.299±2.252
Total and standard deviation	5.368±4.017	5.368±4.017	4.443±3.989	5.356±4.033	3.833±3.832	5.364±4.014	2.759±2.649	5.303±3.913	2.110±2.420	5.301±3.914	4.521±3.768

$LSD_{P<0.05}$=0.609 for periods and $LSD_{P<0.05}$=0.737 for concentrations.

Table 3. Mean nickel concentrations μg/g dry weight

Concentration mean	Treatment period (0 day)	Control	Treatment period (10 days)	Control	Treatment period (20 days)	Control	Treatment period (30 days)	Control	Treatment period (40 days)	Control	Total
Pollution 1% and standard deviation	1.041 ±0.042	1.014 ±0.005	0.678 ±0.097	1.015 ±0.014	0.258 ±0.084	1.013 ±0.002	0.084 ±0.014	1.009 ±0.003	0.008 ±0.003	1.007 ±.006	0.713 ±0.415
pollution 2% and standard deviation	2.049 ±0.286	1.685 ±0.616	1.375 ±0.546	1.683 ±0.613	0.925 ±0.617	1.703 ±0.638	0.390 ±0.543	1.690 ±0.598	0.005 ±0.002	1.673 ±0.588	1.318 ±0.781
Pollution 5% and standard deviation	5.229 ±0.152	5.153 ±0.049	4.783 ±0.219	5.229 ±0.501	3.560 ±0.450	5.225 ±0.659	2.656 ±0.313	5.220 ±0.393	1.499 ±0.497	5.123 ±0.097	4.368 ±1.329
Pollution 8% and standard deviation	8.012 ±0.010	8.013 ±0.077	7.051 ±0.013	8.011 ±0.001	5.145 ±0.284	8.006 ±0.006	3.979 ±0.099	8.005 ±0.005	2.207 ±0.230	7.999 ±0.002	6.643 ±2.045
Total and standard deviation	4.083 ±2.870	3.966 ±2.952	3.472 ±2.711	3.984 ±2.967	2.472 ±2.093	3.987 ±2.968	1.777 ±1.706	3.918 ±2.961	0.930 ±1.026	3.950 ±2.947	3.260 ±2.724

$LSD_{P<0.05}$=0.479 for periods and $LSD_{P<0.05}$=0.605 for concentrations.

Table 4. Mean cadmium concentrations µg/g dry weight

Concentration mean	Treatment period (0 day)	Control	Treatment period (10 days)	Control	Treatment period (20 days)	Control	Treatment period (30 days)	Control	Treatment period (40 days)	Control	Total
Pollution 1% and standard deviation	0.153 ±0.009	0.142 ±0.015	0.020 ±0.007	0.138 ±0.121	0.003 0.002	0.494 ±0.442	0±0	0.605 0.181	0±0	0.605 ±0.181	0.216 ±0.278
pollution 2% and standard deviation	0.295 ±0.010	0.283 ±0.015	0.071 ±0.052	0.275 ±0.013	0.010 ±0.005	0.275 ±0.013	0±0	0.272 ±0.016	0±0	0.269 ±0.012	0.175 ±0.131
Pollution 5% and standard deviation	0.821 ±0.074	0.819 ±0.016	0.364 ±0.557	0.839 ±0043	0.050 ±0.023	0.839 ±0.043	0.007 ±0.007	0.806 ±0.011	0±0	0.808 ±0.013	0.533 ±0.369
Pollution 8% and standard deviation	1.118 ±0.016	1.116 ±0.002	0.538 ±0.226	0.117 ±0.004	0.051 ±0.047	0.117 ±0.004	0.005 ±0.007	0.119 ±0.010	0.002 ±0.004	0.120 ±0.011	0.330 ±0.426
Total and standard deviation	0.597 ±0.409	0.590 ±0.413	0.248 ±0.224	0.431 ±0.340	0.028 ±0.032	0.431 ±0.340	0.003 ±0.005	0.450 ±0.292	0.006 ±0.002	0.456 ±0.009	0.313 ±0.346

$LSD_{P<0.05}$=0.140 for periods and $LSD_{P<0.05}$=0.115 for concentrations.

Table 5. Mean copper concentrations in μg/g dry weight

Concentration mean	Treatment period (0 day)	Control	Treatment period (10 days)	Control	Treatment period (20 days)	Control	Treatment period (30 days)	Control	Treatment period (40 days)	Control	Total
Pollution 1% and standard deviation	0.377 ±0.298	0.383 ±0.035	0.126 ±0.022	0.377 ±0.006	0.846 ±0.100	0.376 ±0.007	0.006 ±0	0.373 ±0.005	0±0	0.372 ±0.004	0.247 ±0.167
pollution 2% and standard deviation	0.167 ±0.024	0.818 ±0.017	0.372 ±0.034	0.820 ±0.025	0.114 ±0.008	0.815 ±0.170	0.008 ±0.002	0.810 ±0.170	0.0001 ±0.0001	0.805 ±0.014	0.538 ±0.358
Pollution 5% and standard deviation	1.909 ±0.091	1.888 ±0.020	1.024 ±0.210	1.882 ±0.017	0.564 ±0.043	1.880 ±0.160	0.079 ±0.021	1.877 ±0.017	0.006± 0.005	1.871 ±0.018	1.298 ±0.777
Pollution 8% and standard deviation	2.600 ±0.510	2.613 ±0.628	2.049 ±0.020	2.945 ±0.047	1.202 ±0.008	2.938 ±0.510	0.769 ±0.201	2.937 ±0.049	0.208± 0.176	2.931 ±0.051	2.119 ±1.015
Total and standard deviation	1.426 ±0.942	1.425 ±0.954	0.893 ±0.777	1.505 ±1.039	0.491 ±0.474	1.426 ±0.942	0.214 ±0.347	1.500 ±1.038	0.053 ±0.119	1.495 ±1.036	1.050 ±0.984

$LSD_{P<0.05}$=0.277 for periods and $LSD_{P<0.05}$=0.292 for concentrations.

The statistical results showed a decrease in the mean of copper concentrations Table 5, as there were significant differences at the probability level of P ≤0.05 between the mean pollution concentrations (1%, 2%, 5%, and 8%) and the periods (10 days, 20 days, 30 days, and 40 days), as shown. There are significant differences between the treatment samples and the control group at the probability level P ≤0.05, except for the pre-treatment period. The treatment efficiency for pollution concentrations was (1%, 2%, 5%, and 8%) after ten days of follow-up (66%, 54%, 46%, and 21%), while the treatment efficiency after 20 days was (77.5%, 86%, 70%, and 53%) and treatment efficiency after 30 days (99.8%, 99%, 95%, and 70%) and treatment efficiency after 40 days (99%, 99%, 99.6%, and 91%), respectively (Figure 2).

Earthworms have the ability to accumulate and biodegrade a wide range of pollutants including heavy elements [29] earthworms can bio-accumulate high concentrations of heavy elements such as cadmium, mercury, lead, copper, manganese, calcium, iron and zinc in their tissues without affecting their physiological functions [30]. All heavy metals in the current study recorded a highly significant reduction in the polluted soil containing *Lumbricus terrestris,* this means that heavy elements were biologically available to be easily assimilated by earthworms after soil bioremediation. Many factors may be responsible for the uptake of elements from polluted soil such as soil properties, elements form, species, and origin of elements in the soil. The chemical form in which the elements is present in the ecosystem may make it more bioavailable this is consistent with a study by D'Amore *et al.* [31]. Perhaps one of the most difficult bioremediation processes is treating pollutants present in a mixture, as there is rarely any (animal, plant, or microorganism) capable of treating the soil of all pollutants at the same time or in an equal proportion [32].

Conclusion

The current study showed the efficiency of the earthworm *Lumbricus terrestris* in the vermiremedation of heavy elements (lead, nickel, cadmium, and copper) in polluted soil with crude oil. The percentage of treatment efficiency varied between levels of pollution and between periods. The lowest efficiency of vermiremediation was in the first ten days of follow-up. At a pollution level of 5% for nickel, it reached 58%, and the highest efficiency treatment was after 30 days of follow-up, and at a pollution level of (1%-2%) for cadmium, it reached 100%. Even if the process of getting rid of pollutants or treating them using biological methods is the proposed solution, the basic solution that we have and can implement is to control the quantity and quality of pollutants emitted from human activity, as well as reducing the size because prevention is better than treatment.

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the article and agreed to be responsible for all the aspects of this work.

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How to cite this manuscript: B.M. Younus, M.M. Akbar, M.S. Alenazi. Efficiency of Vermiremediation in Reducing Level of Heavy Element in Pollution Soils Containing Crude Oil. *Asian Journal of Green Chemistry*, 8(5) 2024, 595-609.

DOI: 10.48309/AJGC.2024.470681.1541