Iranian Reasearch Organization for Science and Technology (IROST)

Advances in Environmental Technology 3 (2021) 137-151

Advances in Environmental Technology

Journal home page: https://aet.irost.ir/

Toxic hydrocarbon removal of contaminated soil using Eisenia fetida with response surface methodology

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ARTICLE INFO

Article history:
Received 8 August 2021
Received in revised form 12 October 2021
Accepted 13 October 2021

Keywords:
Oily sludge
Earthworm Eisenia fetida
Vermiremediation
Response surface methodology

ABSTRACT

Petroleum sludge is typically caused by petroleum contaminants, effluents, and wastes from various stages of hydrocarbon separation. In this study, samples of oily sludge were collected from heavy fuel storage tanks in Sirjan Petrochemical Company in order to investigate the bioremediation of oily sludge by Eisenia fetida earthworms. The effects of oily sludge content, soil ratio, and sawdust weight percentage on total petroleum hydrocarbon (TPH) removal and reproduction of earthworms were evaluated. According to the design of the experiment (DOE), 17 samples with different combinations of petroleum sludge, soil, cow manure, and sawdust were selected to be tested. Also, to determine the effectiveness of the bioremediation process, some properties of samples including pH, total organic carbon (TOC), total Kjeldahl nitrogen (TKN), carbon to nitrogen ratio (C/N), and electrical conductivity (EC) were measured. The results showed that all properties, except for the electrical conductivity, decreased. Besides, in the presence of worms, the TPH could reduce by 66% after 90 days for samples containing up to 40 g oily sludge. Moreover, a statistical model was proposed using the response surface methodology (RSM) to predict the TPH removal and earthworm population as the targeted responses. Keywords: oily sludge; earthworm Eisenia fetida; Vermiremediation; response surface methodology.

1. Introduction

Petroleum sludge, which is a type of solid emulsified waste, is typically composed of solid particulate matters, water and crude oil generated from different stages of hydrocarbon separation processes. Regarding the vast diversity of crude oil and separation processes, different complex compounds can possibly be produced, containing heavy metals, various hydrocarbons (aromatic and aliphatic) [1-5], nitrogen, sulfur and oxygen (NSO), asphaltene and solid particles [5-9]. Some of these compounds are extremely hazardous to environment and human health, particularly if they penetrate into water resources owing to their mobility in soil. These dangerous impacts of oily sludge, which are compounded increasingly as a global concern, have urged researchers to develop different treatment methods to address the

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DOI: 10.22104/AET.2021.5128.1390

associated environmental issues [10]. In recent years, various methods have been proposed for the treatment of oily sludge such as land farming, incineration, stabilization / solidification, solvent extraction, ultrasonic, pyrolysis, chemical treatment and bioremediation [2,11,12-18]. However, due to the nature of petroleum sludge and high concentration of petroleum hydrocarbons, a small number of these technologies can economically be used in compliance with environmental laws and regulations [10]. Currently, many efforts have been focused on identifying the best ways for removing, converting or isolating these pollutants through a variety of physical, chemical and biological processes [19]. One of the most common methods is the use of biological processes [20-23], which is comparatively more cost-effective and efficient than other methods. Specifically, earthworm purification can be exemplified as one of the most attractive biological methods in recent years for the treatment of contaminated soils [14,18]. This biotechnology uses earthworms as natural bioreactors to decompose organic substances or degrade non-recyclable chemicals [24]. In fact, earthworms penetrate through soil and affect the soil microbial processes through microbial communities inhabiting in the structures they build or their digestive track, which generates a hotspot of microbial activity. By modifying the physical and chemical properties of soil and increasing the contact surface area between contaminants and soil microorganisms, the content of contaminants reduces in oily sludge or oil-contaminated soils. Since earthworms can survive in harsh chemical conditions, they can also be used to purify aromatic contaminants with high concentrations of hydrocarbons [25,26]. During vermicomposting, various bulking agents are used as amendment materials such as Cow dung which is commonly used owing to its effective contribution to worm growth [27].The application of vermi remediation and vermicomposting has been frequently reported in the literature [28-30]. The normal activities of earthworms can aerate and mix the composting media, increasing the exposure of contaminants to microbial community. As the composting materials pass through their gut, the secreted enzymes may increase the degradation rate of targeted pollutants. Earthworms also increase the biomass and enzymatic activities of microbial population, improving the degradation efficiency (petroleum hydrocarbon (PHC) degradation) [29-30]. Multiple reports have indicated that the combination of two or more bioremediation technologies can improve PHCs removal [32]. In this regard, addition of bacterial strains (bio augmentation) increases the both survivability and reproduction rate of earthworms [31]. Although the potential of vermi remediation for improving degradation of PHCs has been well approved, still several variables have remained to be evaluated for further enhancement of the process [29-30]. Thus, in this paper, the bioremediation of a contaminated soil sample with oily sludge has been studied using Eisenia fetida earthworm as the natural bioreactor. Eisenia fetida has been selected as the earthworm due to its high fertility rate, production of a large number of cocoons, rapid growth of infants into adult worms, and consumption of various organic wastes. Moreover, Eisenia fetida can survive in varying environmental conditions such as humidity, pH and temperature. Besides, Response Surface Method (RSM) has been employed to predict the effects of three main factors involving the amount of oily sludge, soil percentage, and sawdust percentage over TPH removal and earthworm population through design of experiments (DOE). Finally, the key properties of soil samples as an important factor influencing the activities of earthworms after the bioremediation process have been measured using EC, pH, TOC, TKN, C/N ratio analyses.

2. Materials and methods

2.1. Materials

Dichloromethane was purchased from Kian Kaveh Azma Chemical and Pharmaceutical Industries Complex, hydrochloric acid and nitric acid from Merck Germany. The earthworm (Eisenia fetida) was provided from Misagh Vermicomposting company in Kerman. Cow manure was gathered from a farm around Kerman and washed with water for 10 days to remove the harmful materials. The soil sample was collected from the fields of Kerman University of Medical Sciences from a depth of 25 cm. The soil sample was dried and passed through a 2 mm sieve, and then its physicochemical properties were measured. Soil texture was made by hydrometric method containing 92% sand, 2% clay, and 6% slit. Petroleum sludge was provided from Sirjan Petroleum Products Distribution and Refining Company.

2.2. Experimental design

Experimental design was performed using DOE software and response surface methodology (RSM). In this regard, the amount of oily sludge, soil percentage in the soil / Cow manure mixture, and sawdust percentage, as the key parameters affecting the oily sludge removal and earthworm population, were selected to be evaluated. The high and low levels of each parameter are listed in Table 1. The Oily sludge removal was determined by measuring the total petroleum hydrocarbon (TPH) in the soil.

Table 1. Levels of variable parameters in the Experimental design.

Parameters	Unit	Low	High
Oily sludge	g	0	40
Soil/Cow manure	Percent	0	100
Sawdust	Percent	0	15

2.3. Sample preparation

Plastic containers with the size of 14 × 12 × 20 cm were used as test units. After preparing the raw materials, the samples were made based on dry weight, giving 17 different combinations of cow manure, soil, petroleum sludge and sawdust, as listed in Table 2. The total amount of soil and cow manure in all samples was set to 500 g. After adjusting the temperature in the range of 14-25 °C and humidity in its optimal level (60-90%), 25 yarns of Eisenia fetida adult worms were added to each sample and the performance of the worms in each container was studied for 90 days. In order to provide the suitable environmental conditions for the activity of earthworms, the temperature and humidity of the beds were monitored daily, and water was added to the sample when the humidity was lower than its optimum level.

2.4. Sampling for experiments

Every 30 days, in order to measure EC, pH, TOC, TKN, TPH removal percent, and earthworm population, a 15 g sample was taken and stored in the refrigerator at 0-5 ˚C (to prevent microbial activities).

2.5. Total petroleum hydrocarbon (TPH) measurements

For this purpose, 2 g of the dried sample was taken and added to Erlenmeyer flask, followed by the addition of 10 ml of dichloromethane. Then, the sample was placed in a shaker for 30 min at 150 rpm. After 30 min, another 10 ml dichloromethane was added to the sample and again shaken for 30 min, this process was repeated 3 times. Afterward, the sample was centrifuged, and the extract was poured into a weighed flask. The residue was then weighed after drying in an oven (to evaporate the solvent) at 45 °C for 24 hrs. The total amount of petroleum hydrocarbons was calculated according to equation (1).

$$\mathrm{TPH}\left({}^{\mathrm{mg}}/_{\mathrm{kg\ dry\ sludge}}\right) = \frac{\text{weight of residue(mg)}}{\text{dry sample(kg)}} \times 1000 \quad (1)$$

Table 2. Experimental parameters and results based on the central composite design.

Samples	Oily sludge(g)	Soil (g)	Cow manure(g)	Saw dust(g)
1	20	0	500	37.5
2	7.7	100	400	60
3	20	250	250	37.5
4	20	250	250	75
5	20	250	250	37.5
6	0	250	250	37.5
7	40	250	250	37.5
8	7.7	400	100	14.5
9	32.3	400	100	14.5
10	32.3	400	100	60
11	32.3	100	400	60
12	20	250	250	0
13	20	500	0	37.5
14	20	250	250	37.5
15	32.3	100	400	14.5
16	7.7	100	400	14.5
17	7.7	400	100	60

2.6. Electrical conductivity (EC) and acidity (pH) measurements

In this respect, 5g of the dry sample was added to 25 ml of distilled water to obtain the appropriate ratio (water/sample 5:1) for the extraction of material. The resulting mixture was placed in a shaker for 30 minutes and then filtered through a filter paper. Then, pH and EC of the filtrate samples were determined using pH meter and EC meter.

3. Results and discussion

3.1. TPH removal for different samples

TPH changes of samples contaminated with different weight of oily sludge) 0-40 g) are compared for three time intervals 30-days, as shown in Figure.1. For all tests, the temperature is controlled in the range of 19-23 °C, and humidity in the range of 80-90% during 90 days. Various studies reported that earthworms have the capability for adaptation in the presence of certain amounts of TPHs [33]. In a study conducted by Chachina et al [34] an amount of 97–99% removal of TPHs (20 g/kg concentration) was achieved in soil samples with 10 worms after 4 months. Martinkosky et al [35] reported a decrease of 80% of TPHs (C16–C35) with Eisenia fetida in soil contaminated with 30 g/kg crude oil. It can generally be observed that for all samples, the TPH declines with the passage of time, revealing that earthworms can effectively reduce the toxic hydrocarbon content of oily sludge. To illustrate, for sample 1, initially the TPH content is 17895 (mg/kg dry sludge), and this decreases to 15887(mg/kg dry sludge), 9961(mg/kg dry sludge), and 7873 (mg/kg dry sludge), after 30, 60 and 90 days, respectively. The best TPH removal is related to sample 2, which is obtained to be around 66% (Table 3). As perceived in this study, the TPH decreases significantly with increasing the number of earthworms in the samples. The highest TPH removal is related to samples 16, 6, 2 and 1, which had the highest number of earthworms. TPH reduction of the samples that has no earthworm survivors is due to the activity of native microorganisms and aeration by overturning the samples to provide oxygen because oxygen increases microbial activity. According to the TPH of sample 6 (control sample), at the beginning of the process, it can be concluded that all samples except samples 10, 9 and 7 are completely modified, and therefore their dangerous effects over the environment all substantially reduced. For samples 9 and 10, the TPH of the samples remains nearly constant owing to the absence of earthworms. In addition, the amount of total removal of petroleum hydrocarbons has increased over time [33] (Figure 1).

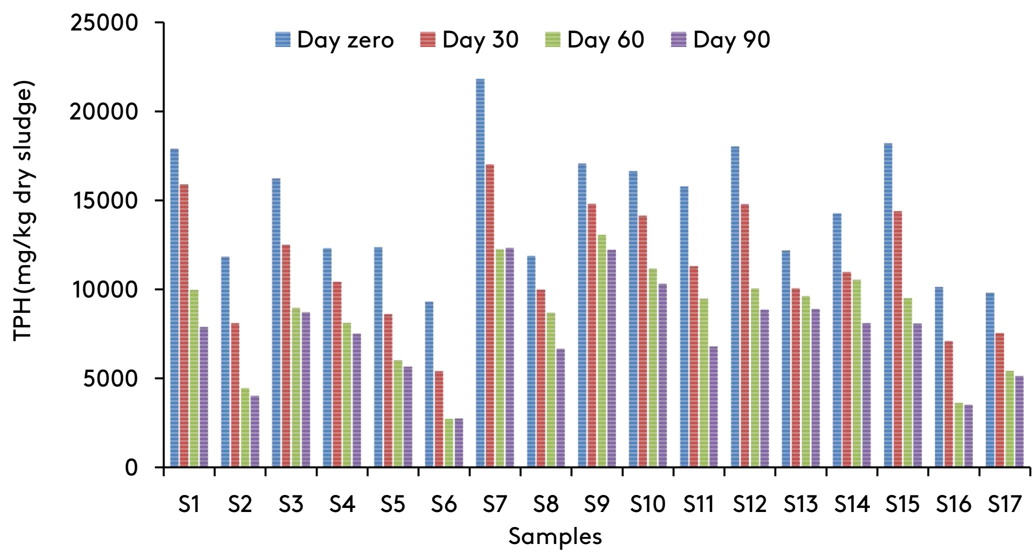


Fig. 1*.* The trend of TPHs removal changes in for different samples and time intervals.

Chromatographic testing is performed to confirm the validity of laboratory data on TPH levels obtained by extraction with dichloromethane. Since sample 2 was observed ton be the best one in terms of total worm population and TPH removal percentage, typically sample 2 is selected to be analyzed with respect to hydrocarbon removal through gas chromatography test at the beginning and end of the process (see Figure 2). It is observed that the area under the curve decreased by 66% at the end of 90 days, which verifies the accuracy of results obtained from the extraction with dichloromethane.

Table 3 .Percentage of removal during the process.

sample	TPH Removal Percent of each sample		
	Time(day)		
	30	60	90
1	11	44	56
2	32	62	66
3	23	45	46
4	15	34	39
5	30	51	54
6	42	71	72
7	22	43	44
8	16	27	44
9	13	23	28
10	15	33	38
11	28	40	57
12	18	44	51
13	17	21	27
14	23	26	43
15	21	48	56
16	30	64	65
17	23	45	48

3.2. Earthworm population optimization

The results obtained from the experiments were analyzed by statistical methods. In the first step, it is necessary to choose a suitable model that can accurately predict the results. For this purpose, a quadratic model that is also suggested by software was used. The proposed model for the system includes 3 terms for single component effects (A, B, C), 3 terms for interaction (AB, AC, BC) and 3 terms for curvature (A^2, B^2, C^2). A, B, and C represent the amount of oily sludge (gr), soil weight percent (wt%), and sawdust weight percent (wt%), respectively. Since all model terms may not affect the system response, the model can be simplified by eliminating ineffective parameters [36]. In this regard, the p-value of each parameter was calculated and reported in Table 4. P-values greater than 0.05 is suggestive for the strong effect of the term over the response. Thus, B, A, C^2, AB are the most influential ones in this model.

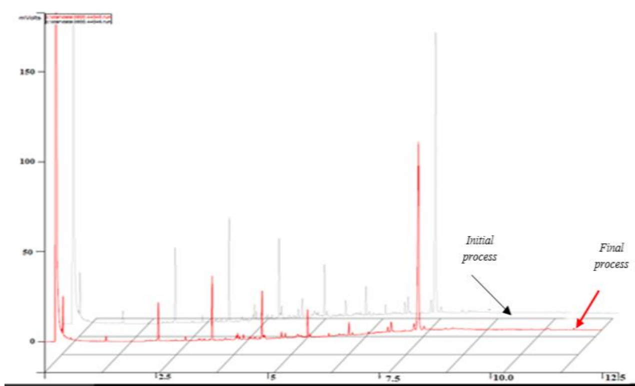


Fig .2. GC of sample 2 at the initial and end process.

Table 4. P-value of the parameters.

Factor	A	B	C	AB	AC	BC	A^2	B^2	C^2
p-value	0.0005	0.0001	0.9929	0.0137	0.8304	0.7678	0.9428	0.4640	0.0746

Finally, a model is developed to predict the total population of worms, which has a regression constant of 0.9383, confirming the reliability of the model. In fact, this statistical quantity ensures how reliable the model is for the prediction of the response, which is derived from the ratio of defined changes to total changes. The adjusted coefficient of determination, however, is only derived from the actual effect of the variables, and thus it is more reliable. The adjusted coefficient here is 91.02 %, indicating that the model can cover more than 91 % of the data. As a result, the selected model analyzes and predicts the results correctly.

Table 5. Values R^2 for earthworm population data.

R^2	Adj-R^2
0.9373	0.9102

The proposed model for the population of earthworms in terms of coded factors is given below as equation (2). In this model, single effects, interactions and curvature effects of parameters are considered, and ineffective factors are eliminated According to the coefficients of factors, the importance of each factor on the total population of worms can be realized.

$$\text{Earth worm population} = 224.48 + (-111.967) \times A + (-148.418) * B + (-0.168246) * C + 77.25 * AB + (-39.6704) * C^2 \quad (2)$$

According to equation (2), it can be deduced that the sawdust percentage has a positive effect and the others (amount of oily sludge and soil percentage) have negative effects on the total population of worms. Figure 3 shows the changes in the factors affecting the system at one point in the test space (amount of oily sludge: 20 g, soil percentage 50, sawdust percentage 7.5 Also, it can be seen that the three parameters have a curvature effect. Another observation is that the amount of oily sludge and soil percentage have a linear decreasing trend; i.e., with increasing either the amount of oily sludge or soil percentage, the number of worms decreases. The effect of the sawdust percentage is also well evident; initially, the population of earthworms in the samples increases due to the aeration improvement of soil structure, but for soil content of more than 7.5 %, the number of earthworms decreases.

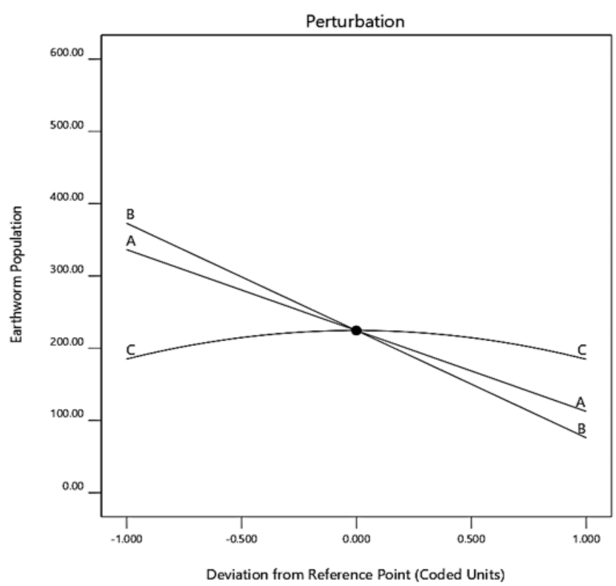


Fig. 3. purtubation plot of earthworm population.

3.3. *Statistical tests of earthworm population*

In the first step, it is necessary to ensure the validity of the data before performing any data analysis. Failure to assess the accuracy of the data makes the results obtained from statistical and non-statistical analysis of experimental data unreliable; therefore, the accuracy of the data must be scientifically verified. In this study, in order to ensure that there is no significant difference between the laboratory and model data, statistical tests are conducted. The first test to be examined is the normal function plot of the residuals. This diagram shows how errors are scattered. If most of laboratory data points are around the straight line, there is no abnormal term in the proposed system, and conversely, if points are scattered and nonlinear, there can be abnormal terms in the system. Accordingly, Figure 4 the linear pattern of the data in Figure 4 shows a good agreement between the experimental and model data, confirms the reliability of the proposed model.

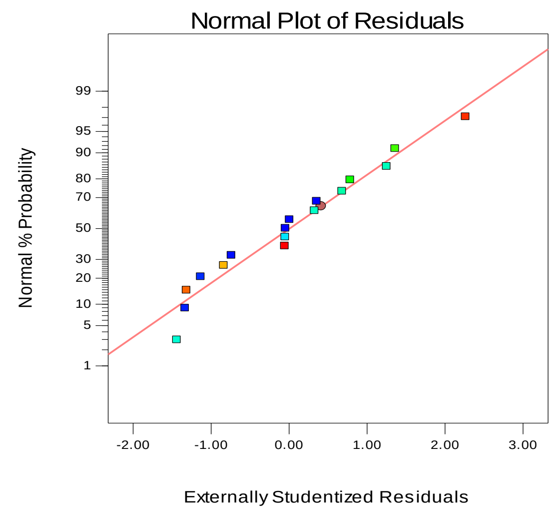


Fig. 4. Normal plot of residuals.

In the next test which shows the quality of the model, the predicted points are plotted using the model and based on the laboratory data (see Figure 5). In a suitable model, the points obtained from this diagram are placed around the 45-degree line. This indicates a good fit between the model and laboratory data. Thus, it can be concluded that the proposed model in this study predicts the data (Figure 5A). The diagram of the error values in terms of the values predicted by the model, which shows the hidden errors that affect the response, is shown in Figure 5B, ideally, the points are supposed to follow a random pattern, and thus a specific pattern indicates the time dependence of the response. Therefore, the third test justifies the model proposed for the number of earthworms justifies the third statistical test well, because the pattern of behavioral points is scattered and none of the predicted points are out of range.

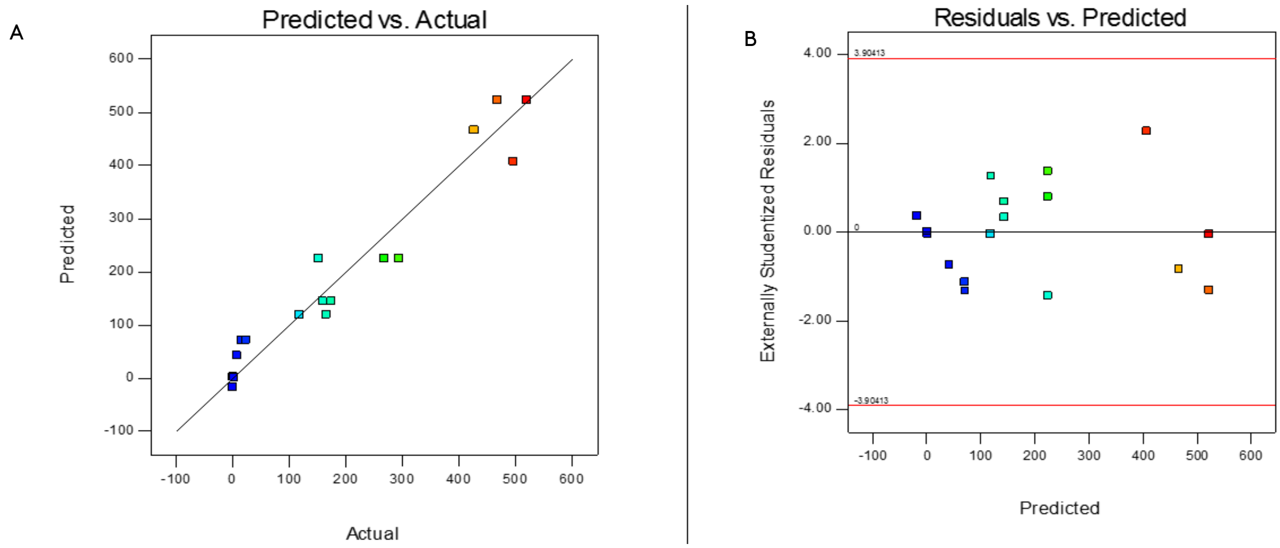
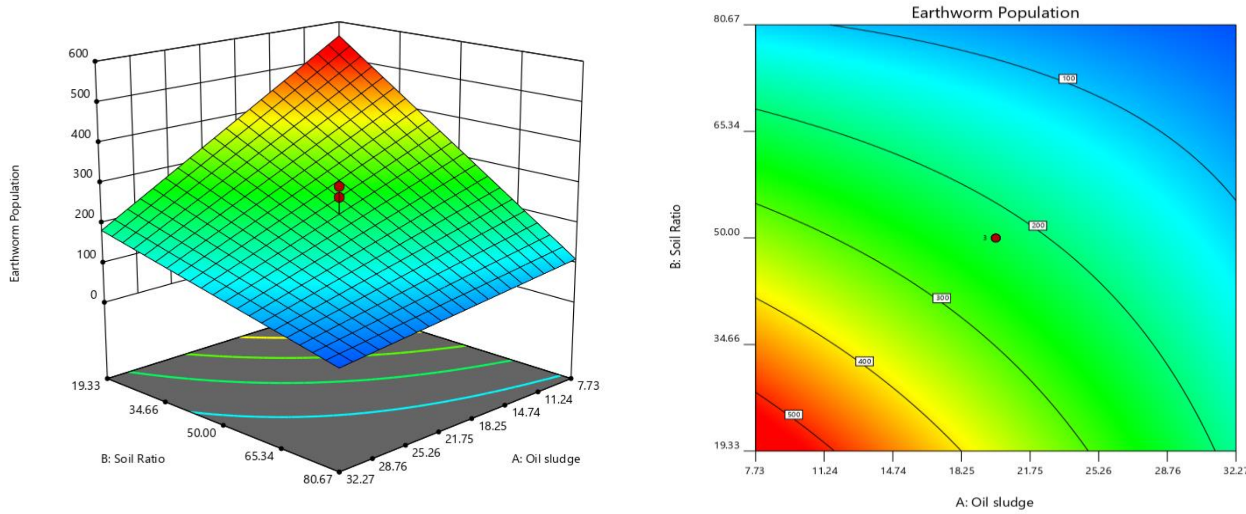


Fig .5 A. predicted response vs actual and **Fig .5 B.** Plot of residual vs predicted response for earthworm population**.**

3.4. Investigation of interaction effects of parameters on the population of earthworms

After determining the single effects of parameters on the system response, the interactions or dual effects are investigated as well. The best way to express interactions is to use 3-dimensional charts (Figure 6). In these graphs, the variation of two parameters is represented while the other parameter is kept constant. The interaction between the amount of oily sludge and soil percentage on the population of worms is shown in a 3-D diagram (Figure 6-A), and the corresponding contour is illustrated in Figure 6B. It can be observed that increasing the weight of oily sludge in all samples contributes to the reduction of earthworm population, and consequently lowers the reproductive rate. This is mainly due to the increase in toxicity of petroleum sludge at higher sludge concentrations. Also, with decreasing soil to manure ratio, the number of earthworms in the samples increases. These results show that sandy soils are not desirable choices for the growth and reproduction of earthworms due to the unfavorable soil properties involving low water holding capacity, soil fertility and low nitrogen content (or high C / N ratio (above 30)). Figure 7 compares the initial and final number of earthworms for 17 different samples. It can be seen that in samples 9 and 13, owing to the high percentage of soil, all worms are eliminated, while in sample 17, the majority of worms are survived due to the higher percentage of sawdust. Also, in sample 7, despite the appropriate C/N ratio, the high toxicity of petroleum sludge causes the elimination of a large number of earthworms. Generally, according to results of this study, for oily sludge of 40 g and more, the high toxicity, and subsequent poisoning of samples leads to the death of Eisenia fetida earthworms. The bioremediation was more effective after 60–90 days of the study indicating that the worms needed sufficient time to adapt to the contaminated environment [31].



A B

Fig. 6. (A) 3-dimentional plot of the earthworm population**. (B)** contour of the 3-D plot.

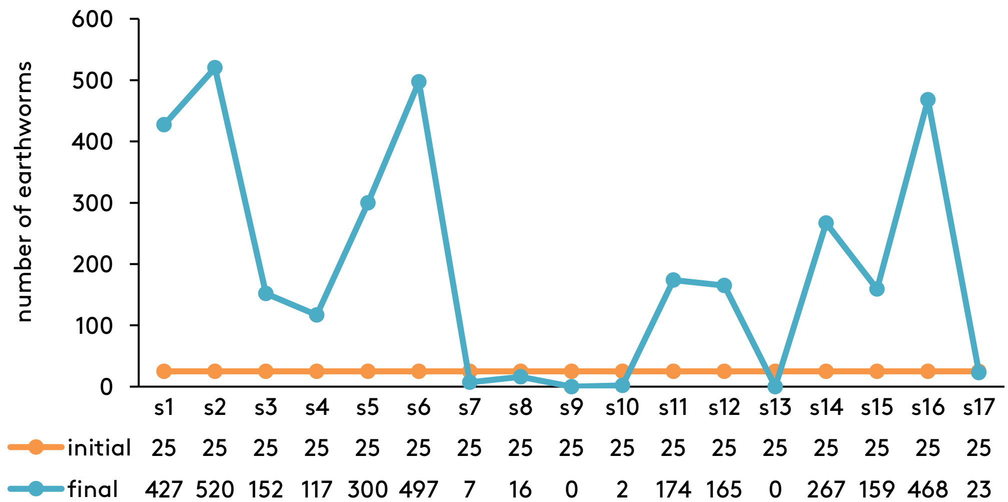


Fig. 7. Comparison of the initial and final number of earthworms.

Table 6. p-value of evaluting parameters for TPH Removal percentag.

factors	A	B	C	AB	AC	BC	A^2	B^2	C^2
p-value	0.0065	0.0004	0.9908	0.9021	0.8655	0.6982	0.1013	0.4564	0.9308

3.5. Statistical model of TPH removal percentage

Similar to worm population, a model is also proposed for the prediction of TPH removal percentage as the response. Table 6 shows the P-values corresponding to different terms, revealing the significance and effectiveness of each term as well as their interactions. Terms with P-values of less than 0.05 have significant effects on response, thus here A, B and A^2 are the most influential factors, meaning that the amount of oily sludge and soil percentage can affect the removal percentage significantly, and the amount of oily sludge has a curvature effect.

Also, the P-value for the all interactions including AB, AC, and BC is more than 0.05, which indicates that the interaction does not affect the removal percentage. The results of analysis of variance of experimental design data on TPH removal percentage are shown in Table 7.

Table 7. Values R^2 for TPH removal data.

R^2	Adj-R^2
0.8814	0.854

The results also show that the accuracy of the model is equal to 85.40% with a regression constant of 0.8842. The proposed model is finally given in the form of equation (3) to predict TPH removal.

$$\text{TPH removal} = 45.9056 + -6.3258 * A + -10.3206 * B + 3.86863 * A^2 \quad (3)$$

Figure 8 shows perturbation diagram of changes in the factors affecting the system at one point in the test space (amount of oily sludge: 20 g and soil percentage: 50). The TPH removal percentage decreases almost linearly versus soil percentage, and the curvature effect of the amount of oily sludge is also well evident. According to equation (3) and Figure 8, it can be concluded that increasing either the amount of petroleum sludge or soil percentage reduces the TPH removal. However, the former is the most important inhibitory factor for the removal of TPH, and thus its negative effect is more prominent than the latter.

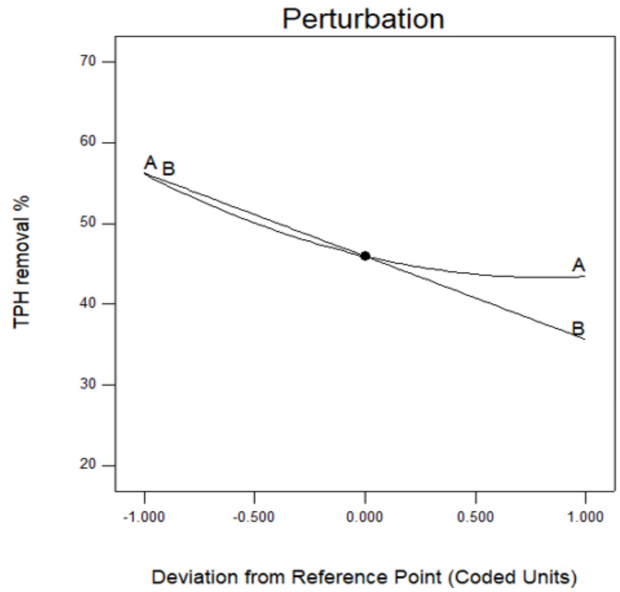


Fig. 8. Perturbation plot of TPH removal percentage.

Figure 9 shows the effect of AB interaction on TPH removal percentage. As can be seen at different soil ratios, the removal percentage varies linearly versus soil ratio However, increasing the amount of oily sludge have a curvature effect over the removal percentage. Considering the percentage of TPH removal within 90 days, it can be concluded that earthworms, in cooperation with native microorganisms, increase the removal of hazardous organic compounds. In addition, microbial interactions increase as a result of earthworm activity.

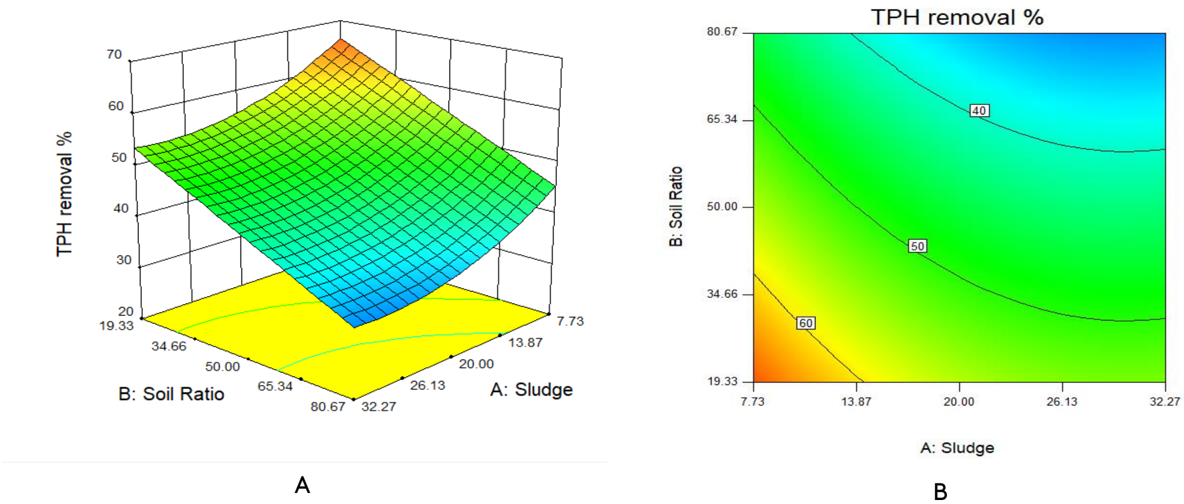


Fig. 9. **(A)** 3-dimentional plot of TPH removal percentage. **(B).** contour of the 3-D plot.

3.6. Investigation of changes in electrical conductivity (EC)

Electrical conductivity is an important parameter in salinity assessment. High concentrations of salinity may cause substrate poisoning, thus electrical conductivity is a good indicator of the quality of compost (in our case vermicompost). Due to the activity of earthworms, decomposition and mineralization of organic matter, the electrical conductivity of substrates changes during the bioremediation process. The increase in electrical conductivity can be attributed to the reduction of substrate organic matter, as well as the release of various mineral salts such as phosphate, ammonium and nitrate during the decomposition process of organic compounds[27]. Fig.10 shows that for all samples, the electrical conductivity of the substrates increases after the bioremediation process. The joint activity of earthworms and microorganisms causes the release of elements into available mineral forms, thus their concentrations increase gradually, leading to a higher electrical conductivity. The highest and lowest increase in electrical conductivity compared to the initial level are related to sample 16 and 9, correspondingly.

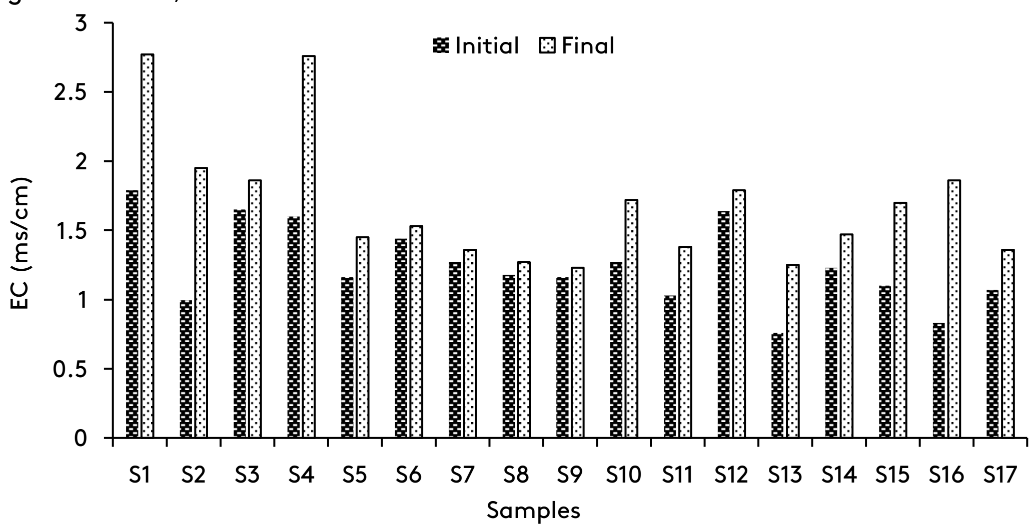


Fig. 10. Change EC initial and final process.

3.7. Investigation of pH changes

Evaluation of pH is of paramount importance since the activity of earthworms can be affected by the variation of soil pH. The presence of elements such as silica and large percentage of sand lowers the pH. Also, decomposition of nitrogenous organic matters leads to the production of ammonium ions and humic acids, which can exert contrasting effects on the pH level. Indeed, the presence of phenolic and carboxylic groups in humic acids results in a decrease in pH, while ammonium ions increase the pH, and thereby regulating the acidity. In this study, the pH of all samples from the beginning to the end of the process is reduced due to the stabilization of organic compounds by microorganisms and the formation of weak acids. In general, the decrease in pH of samples can arise from the production of carbon dioxide gas which is produced from the metabolic activities of earthworms, soil microorganisms and the production of organic acids. Similar results have also been reported by other researchers. Accordingly, the decrease in pH was ascribed to the release of carbon dioxide and mineral acids by microorganisms and the production of ammonia, which was attributed to nitrate during the mineralization process [27]. In addition, biodegradation of raw materials to intermediates including organic acids and mineralization of nitrogen and phosphorus and their conversion to nitrate, nitrite and orthophosphorus led to a decrease in pH [37]. In this study, pH values ranges from 7 to 7.8 during the bioremediation process. Figure 11 shows that the pH was within the tolerable range for earthworm growth and survival. The highest and lowest decreases in pH compared to the initial value are related to sample 12 with 9.1% and sample 3 with 0.9%, respectively.

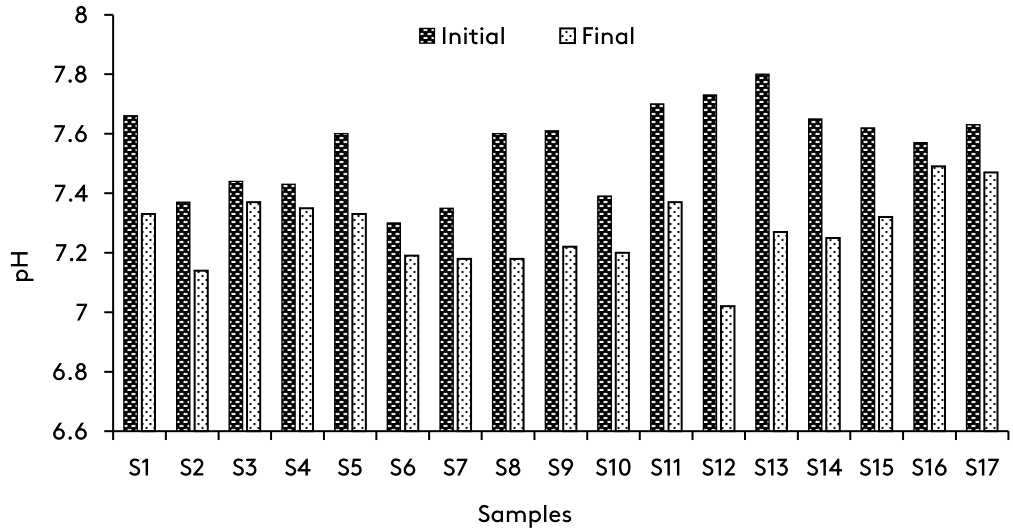


Fig.11. Change pH initial and final process.

3.8. Investigation of total organic carbon (TOC) changes

Earthworms use carbon as an energy source for the decomposition of organic matters. The activity of earthworms contributes to the oxidation of organic matters, and thereby converting carbon to carbon dioxide, which eventually lowers the amount of organic carbon significantly during the process. The reduction of organic carbon at the end of the process is due to the mineralization of organic matter and its decomposition by earthworms, microorganisms, and the release of organic carbon as carbon dioxide gas [27]. As shown in Figure 12 the amount of total organic carbon reduces for all samples with the passage of time. The highest decrease (64.2%) in organic carbon content compared to the initial content is related to sample 2, whereas the lowest one (2.5 %) is associated with sample 13.

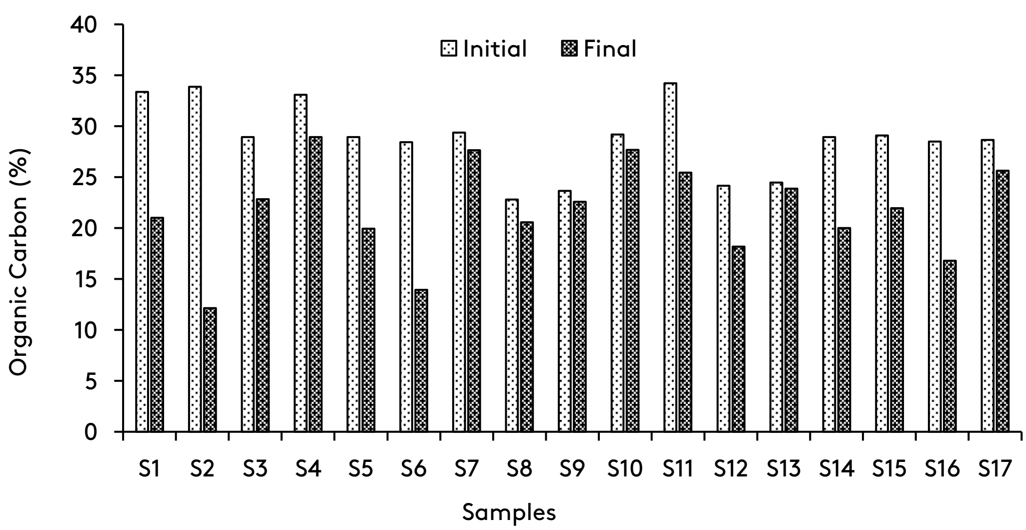


Fig.12.change TOC initial and final process.

3.9. Investigation of total nitrogen changes

Nitrogen is one of the most essential elements for the growth of earthworms, and thus the study of nitrogen changes is of particular importance. It can be observed that the amount of total nitrogen at the end of the process increase for all samples (Figure 13). Degradation of organic matter by earthworms and microorganisms leads to the conversion of organic carbon to carbon dioxide. To explain, earthworms increase the total nitrogen content by excreting nitrogenous substances and secreting hormones and enzymes during the digestion of organic matters [37]. In this study, the highest increase in total nitrogen compared to the initial level is around 46.0% and related to sample 2, while the lowest one is around 5.8 % and corresponds to sample 9.

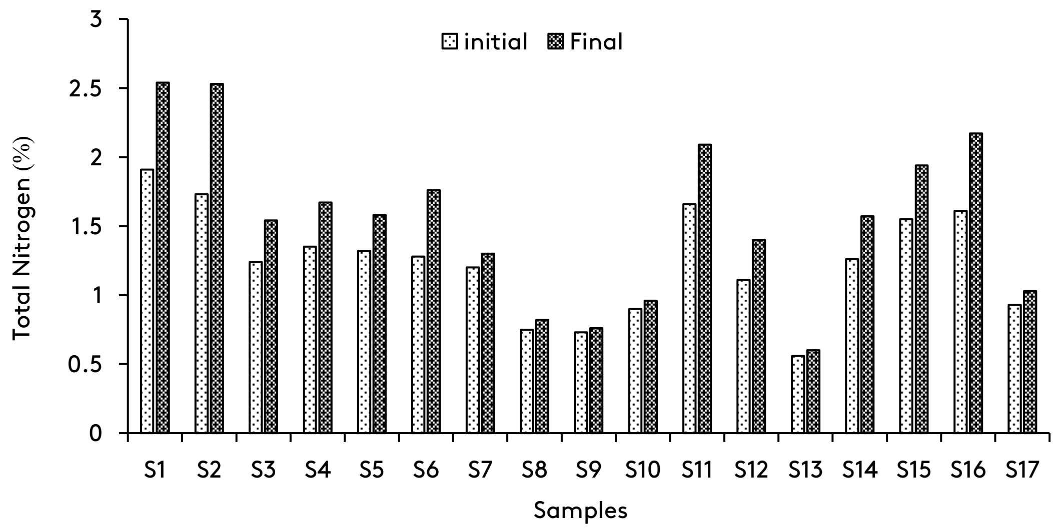


Fig .13. Change TKN initial and final process.

3.10. Investigation of changes in carbon to nitrogen ratio

The C/N ratio indicates the decomposition of organic compounds by earthworms and microorganisms. Results show that the C/N ratio decreases for all samples (see Figure 14). The reduction of C/N is most likely due to the decomposition of organic matter by worms and microorganisms, followed by the release of a proportion of the organic carbon in the form of carbon dioxide gas to the environment. Besides, the decomposition of organic matter and the addition of mucus, growth hormones and enzymes that are nitrogen-rich to the samples increase the C/N ratio [37]. The results indicate that initially the C/N is in the range of 17.4 to 43.2, while this decreases to 4.8 to 39.6 after 90 days of bioremediation process. The highest C/N at the beginning of the process was 43.21 and related to sample13. The highest decrease in C/N compared to the initial level of 75.5% is related to sample 2 and the lowest one compared to the initial level of 8.3% is connected to sample 9 (Figure 14). Further reduction in this parameter illustrates the decomposition of organic matter because of the presence of earthworms and the favorable conditions of these samples.

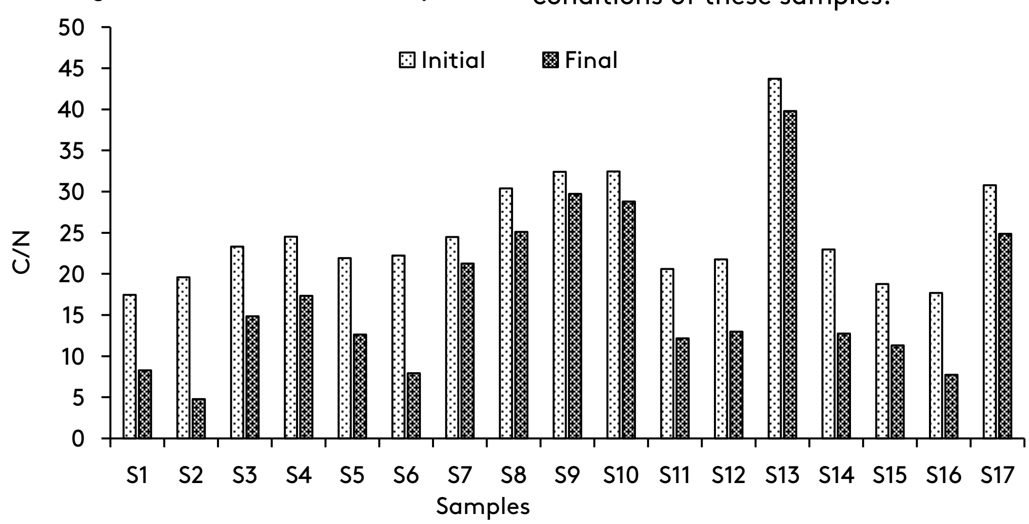


Fig. 14. Change C/N initial and final process.

Conclusions

In this research, the bioremediation of oil-contaminated soil through earthworms has been evaluated with respect to the TPH removal and improvement of soil properties both experimentally and statistically. In this regard, different amount of oily sludge, soil to cow manure ratio, and sawdust percentage were considered to make 17 samples. In bioremediation studies, it is of great importance to notice the fate of TPHs so as to reach a clear understanding of the bioremediation pathway. The results demonstrated that the Eisenia fetida earth worms have a great potential for the bioremediation of petroleum hydrocarbons from contaminated soils. It was observed that acidity level (pH), total organic carbon (TOC), and carbon to nitrogen ratio (C/N) reduced, while electrical conductivity (EC), total nitrogen content (TKN), increased with the passage of time during the process. The decline in TOC and increase in TKN, and following that the reduction in C/N were suggestive for the degradation of organic matters through earthworms and microorganisms. Besides, regarding the death of earthworms in samples with C/N ratio of higher than 30, it could be understood that this ratio had a strong effect over the survival and reproduction of earthworms. It was also revealed that earthworms could not only contribute to TPH removal, but also improve the soil properties.

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