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Remediation Of Agricultural Soils Contaminated With Total Petroleum Hydrocarbons Through The Use Of Compost And Earthworms

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Abstract.

Contamination by poor management of total petroleum hydrocarbons (TPH) causes negative impacts on the soil affecting agricultural areas. Therefore, it is a priority to investigate technological alternatives for its solution. The objectives were to determine the efficiency of treatments that include compost and earthworms (Eisenia foetida) in the remediation of soils contaminated with TPH in three types of substrates, as well as to establish the variation of substrate properties and their relationship with TPH removal. The study was conducted "ex situ", where three treatments were evaluated combining soil contaminated with TPH (SCTPH) and compost with earthworms (CCLT) in the following proportions: T1) 25% SCHTP and 75% CCLT; T2) 50% SCHTP and 50% CCLT and T3) 75% SCTPH and 25% CCLT. The results showed that all treatments were able to reduce the initial TPH content of the contaminated soil in the different fractions (F1, F2 and F3); however, T1 was more efficient in the removal of TPH, achieving remediation below the national EQS for agricultural soils. Regarding substrate properties, all treatments were able to increase organic matter, pH and cation exchange capacity; they attenuated the C/N ratio and stabilized electrical conductivity at non-saline levels. The linear correlation coefficients reveal that the C/N ratio; salinity and CEC at the end of the remediation process presented significant association with respect to the TPH removed; the highest correlation coefficient was between TPH and CEC with an inverse relationship between both.

Key words: bioremediation; soil contamination; Eisenia foetida; removal.

INTRODUCTION

The problems of environmental contamination by petroleum products are constant and evident [1,2] due to the fact that most industrial activities use inputs with hydrocarbons that after their use are not disposed of according to environmental management standards, causing risks of soil contamination [3,4]. Cases of soil contamination by total petroleum hydrocarbons (TPH) are frequently reported as environmental incidents [1,5] causing alterations in production processes as well as in habitat and natural landscapes [2,6].

Petroleum products such as automotive oil, diesel, gasoline and lubricants are hazardous materials that could cause soil contamination processes [7] because they contain toxic and carcinogenic hydrocarbons [8], affecting its quality with negative impacts on its properties [9]. In severe cases, they cause soil degradation and desertification, affecting populations that depend on this resource [10].

Currently, there is great interest in evaluating the impacts of TPH contamination on soil quality and health [2,4]. In this context, it is a priority to investigate technological alternatives for remediation of soils contaminated with TPH. In this regard, there are several technologies for remediation of contaminated soils [11] aimed at the containment, immobilization, and cleaning of impacted soils based on physical, chemical, and biological processes [12,13,14].

In this sense, the use of compost and earthworms is an option for the remediation of soils contaminated with TPH [15,16], being considered as a low-cost alternative that reduces concentrations of contaminating elements to non-toxic levels [8,17,18]. Worms can assimilate organic pollutants through their digestive tract and stimulate the activity of microorganisms such as bacteria and fungi to favor their degradation [19,20]. However, the adequate concentration of compost and worms to achieve an effective removal of TPH in agricultural soils is unknown. The objective of this research was to determine the efficiency of treatments that include compost and earthworms (Eisenia foetida) in the remediation of TPH-contaminated soils in three types of substrates, as well as to establish the variation of substrate properties and their relationship with TPH removal.

MATERIALS AND METHODS

Site and treatments evaluated

The research was developed in a greenhouse in the district of Characato, Arequipa, Peru; geographically located between UTM coordinates: 8176978 N and 236458 E. The following treatments were considered: T1 (25% soil contaminated with TPH and 75% compost with earthworms); T2 (50% soil contaminated with TPH and 50% compost with earthworms) and T3 (75% soil contaminated with TPH and 25% compost with earthworms). Each treatment (substrate) was placed in a rectangular plastic tray 60 cm long by 40 cm wide and 30 cm deep; the total weight of each substrate was 20 kg so that the treatments were composed as follows: T1) 5 kg of contaminated soil, 15 kg of compost and 50 earthworms; T2) 10 kg of contaminated soil, 10 kg of compost and 50 earthworms and T3) 15 kg of contaminated soil, 5 kg of compost and 50 earthworms. The treatments were arranged in a completely randomized experimental design with three replicates for each one, for a total of nine experimental units.

The research process

The samples of soil contaminated with TPH were obtained from land with spills due to poor disposal of residual oils from the machinery and vehicle maintenance service station of the MajesSiguas irrigation project located in the Arequipa Region, Peru. A representative sample of contaminated soil was taken for the corresponding analysis; the sampling was executed by identifying eight random points, in each one 1 kg of soil was obtained at a depth of 15 cm to then form a composite mixture from which 1 kg was separated for analysis [21]. The determinations of TPH fractions, pH and electrical conductivity of the soil were analyzed at the TYPSA-Peru Laboratory accredited by the Peruvian Accreditation Body of INACAL (National Institute of Quality) with registration No.LE-099. Complementary analytical determinations (organic matter, C/N ratio, cation exchange capacity and texture) were also carried out at the Soil, Water and Foliar Analysis Laboratory of the INIA (National Institute for Agrarian Innovation) Experimental Station, Arequipa. Due to the photophobic behavior of the earthworms, the trays of the treatments were covered with plastic and placed under shade in order to maintain an average temperature between 25oC and 28oC [22], permanent irrigation was applied to maintain the humidity at approximately 80%, with pH between 6.0 and 7.0 [23,24]. The substrates were mixed and stirred carefully every five days to favor an effective action of the earthworms. The remediation process lasted three months, then the soil was sieved to separate the earthworms and record their length and weight for each treatment; later, samples were taken from each treatment for laboratory analysis.

Evaluations carried out prior to the remediation process

Analysis of contaminated soil. Total petroleum hydrocarbon content (mg kg^{-1}) was determined in the following fractions: F1 (C6-C10); F2 (> C10 - C28) and F3 (> C28 - C40) by the EPA Nonhalogenated organics by gas chromatography method using the GC (gas chromatograph) technique; pH: by EPA method SW846 method 9045D soil and waste; electrical conductivity (dSm^{-1}): by EPA method 841 B-97-003; these analyses were performed at the TYPSA Laboratory. The following were also determined: organic matter (%) by the Walkley-Black method; C/N ratio (Walkley-Black for C; micro-Kjeldahl method for N) and cation exchange capacity (CEC) by the saturation method with sodium acetate (cmol kg^{-1}), at the INIA - Arequipa Experimental Station Laboratory. Regarding hydrocarbon fractions, the Peruvian regulations on environmental quality standards (ECA) published by the Ministry of the Environment specify that the F1 hydrocarbon fraction or light fraction refers to a mixture of hydrocarbons whose molecules contain between six and 10 carbon atoms (C6 to C10). F2 or medium fraction is the mixture of hydrocarbons whose molecules contain more than 10 and up to 28 carbon atoms (> C10 to C28) and F3 or heavy fraction is the mixture of hydrocarbons whose molecules contain more than 28 and up to 40 carbon atoms (> C28 to C40). These fractions must be analyzed in products and mixtures derived from petroleum.

Compost analysis. Itwas carried out in the laboratory of the INIA experimental station, with the following determinations: organic matter (%); C/N ratio; cation exchange capacity (cmol kg^{-1}); pH; electrical conductivity (dS m^{-1}) to evaluate salinity, by means of analytical procedures described for the preliminary analysis of the soil.

Characteristics of the earthworm population. This determination was made in the field by recording the length (cm) and weight (g) of 10 worms chosen at random to obtain an average value, respectively.

Evaluations carried out at the end of the remediation process

Total petroleum hydrocarbons. A 1 kg sample was collected for each experimental unit and sent to the TYPSA Laboratory to evaluate TPH determinations in the different fractions: F1(C6 - C10); F2 (> C10 - C28) and F3 (> C28 - C40) expressed in mg kg^{-1} using the method for prior analysis of contaminated soil, indicated above.

Substrate properties. In a complementary way, samples from each experimental unit were sent to the INIA, Arequipa Laboratory where these determinations made were: organic matter, C/N ratio, pH, electrical conductivity and CEC through the methodologies mentioned for the previous analysis of the soil.

Worm characteristics. The average length (cm) and average weight (g) were evaluated based on the recording of both in 10 worms chosen at random for each treatment.

Effective TPH remediation. It was calculated using the following formula proposed in this research:

$$RE = 100 - \left(\frac{CF \times 100}{CI} \right)$$

Where

RF is effective remediation for each TPH fraction (%); CF is the final content of each TPH fraction (mg kg^{-1}); and CI is the initial content of each TPH fraction (mg kg^{-1}).

Statistical analysis

The results were systematized and analyzed using SPSS version 21 software; the analysis of variance was performed to determine statistical differences, as well as Tukey's statistical significance test ($p \leq 0.05$) to establish significant statistical differences between treatments. A linear correlation test was also performed to establish associations between the dependent and independent variable by means of the coefficients of variability, regression, intersection and determination.

RESULTS AND DISCUSSION

Characterization of contaminated soil, compost and earthworms prior to the remediation process.

The results in Table 1 show that the TPH levels exceeded the environmental quality standards (EQS) for agricultural soils corresponding to hydrocarbon fractions F1; F2 and F3, which according to Peruvian regulations is 200 mg kg^{-1} for fraction F1; 1200 mg kg^{-1} for fraction F2 and 3000 mg kg^{-1} for fraction F3. The analysis detected evident contamination of agricultural soils with TPH.

Table 1. Characterization of contaminated soil subjected to treatment.

Parameters	Unit	Value
HTP: F1 (C6 - C10)	mg kg^{-1}	1866.50
HTP: F2 (> C10 - C28)	mg kg^{-1}	11669.0
HTP: F3 (> C28 - C40)	mg kg^{-1}	13770.0
pH	-	7.36
Electrical conductivity	dS m^{-1}	0.469
Organic matter	%	1.06
C/N ratio	-	98.50
Cation exchange capacity	cmol kg^{-1}	4.22
Texture	-	Sandy loam

It is important to evaluate the physical and chemical characteristics of soils contaminated with hydrocarbons in order to design the most relevant remediation technology [25]. In this context, the analysis report of the contaminated soil showed a pH close to neutrality [26], with a very limited level of salts [27], deficient organic matter content and high carbon-nitrogen ratio [25]. Considering that matter is one of the important indicators of soil quality, it was detected that the contaminated soil contains a deficient level of organic matter, affecting its capacity for natural self-purification against contaminants and also limiting its functions, especially from the productive point of view [12,26]. Table 2 shows the results of the analysis of the compost used as a substrate to make the TPH remediation process viable. The results establish a C/N nitrogen ratio adequate to improve the decomposition of the substrate and facilitate the work of the earthworms [22,28]. It presented an important content of organic matter to allow the remediation process of the soil contaminated with TPH [29,30].

Table 2. Characterization of the compost used as substrate.

Parameters	Unit	Value
C/N ratio	-	9.73
Organic matter	%	14.08
Cation exchange capacity	cmol kg^{-1}	27.19
pH	-	8.50
Electrical conductivity	dS m^{-1}	3.02

The cation exchange capacity was high and very determinant to optimize the TPH removal process [31]; the pH was alkaline; and a slight level of salinity of the compost was detected, which due to the humidity of the substrate should be attenuated during the remediation process. Therefore, the attributes of the compost were very convenient to achieve an effective removal of TPH contaminated soils. Initial characterization of the earthworm (Eisenia foetida) population reported an average length of 10.5 cm and 1.2 g average weight. The size and weight of the earthworms depend on the conditions where they develop [23].

Total petroleum hydrocarbons at the end of the remediation process

The TPH content was determined at the end of the remediation period. The results shown in Table 3 demonstrated that all treatments were able to decrease the initial TPH content of the contaminated soil corresponding to hydrocarbon fractions F1, F2 and F3 with significant statistical differences among them. This behavior allows inferring that all treatments had an important effect on the remediation of TPH-contaminated soils. However, considering as a reference the national RCTs for agricultural soils, the results revealed that only the T1 treatment managed to remediate the contaminated soil below the RCTs for the different hydrocarbon fractions [32]. Treatments T2 and T3 failed to remove TPH fractions below the national ECAs. Regarding the effectiveness of the TPH fraction remediation process, it was found that treatment T1 achieved the highest efficiency for fractions F1, F2 and F3. The results show that treatment T1 had the highest response in the TPH remediation process because it contains a higher percentage of substrate (75% compost with earthworms) compared to treatments T2 and T3. This condition would allow a higher performance of earthworms since the characteristics such as organic matter, C/N ratio, pH, salinity and cation exchange capacity of the substrate show favorable values for the development of earthworms

[22,23]. The compost used in the research, by offering an appropriate level of organic matter and CEC, would have boosted the work of earthworms [24] in benefit of the removal of TPH from the soil in the different fractions evaluated.

Table 3. Results of TPH determinations (F1, F2 and F3) at the beginning and end of the remediation process considering the environmental quality standard (EQS) for soils and percentage of effective remediation (ER) for each treatment.

Parameters		Treatments		
		T1	T2	T3
F1	Initial (mg kg-1)	1866.50	1866.50	1866.50
(C6 - C10)	Final (mg kg-1)	189.30 a[a]	498.60 b	1293.00 c
	RCT (mg kg-1)	200.00	200.00	200.00
	RE (%)	89.86 a[a]	78.29 b	30.73 c
F2	Initial (mg kg-1)	11669.0	11669.0	11669.0
(> C10 - C28)	Final (mg kg-1)	1034.90 a[a]	2917.25 b	5885.40 c
	RCT (mg kg-1)	1200.00	1200.00	1200.00
	RE (%)	91.13 c[a]	75.00 b	49.56 a
F3	Initial (mg kg-1)	13770.0	13770.0	13770.0
(> C28 - C40)	Final (mg kg-1)	2745.30 c[a]	5874.20 b	9724.17 a
	RCT (mg kg-1)	3000.00	3000.00	3000.00
	RE (%)	80.06 a[a]	57.34 b	29.38 c

[a]Differentlettersin each row refer that there is a significant statistical difference between them according to Tukey's test ($p \leq 0.05$).

Earthworms are efficient organisms in the biodegradation of contaminants. During the process, they change the physical and chemical properties of soils as well as their microbial activity [33], triggering important degradation processes in the remediation of soils contaminated with TPH [29]. Earthworms use microorganisms from the substrate and subject them to conditions in their gut to achieve greater activity that accelerates the remediation of oil-derived organic pollutants [34]. This process is potentiated by the joint application of organic sources such as compost [35]. Similar results were reported by [6], who published an investigation on the biodegradation process of a heavy oil contaminated soil in which treatments with Eisenia foetidaand horse manure offered high percentage of saturated hydrocarbon removal. Also, [33] investigated remediation processes of sludge from oil refinery using a combination of microorganisms, enzymes, compost and Eisenia foetidaachieving a great effect of pollutant degradation due to the action of earthworms.

Organic matter, C/N ratio, pH, salinity, CEC of substrates and characterization of earthworms at the end of the remediation process.

Table 4 shows the effect of treatments on organic matter (OM) content, C/N ratio, pH, electrical conductivity (EC) and cation exchange capacity (CEC). The average results submitted to Tukey's test ($p \leq 0.05$) indicate significant statistical differences between treatments for all evaluations with results that offer a coefficient of variability in acceptable ranges and granting reliability to the data recorded in the research. Considering the initial levels of contaminated soil, the trend of results showed that all treatments increased the OM, pH and CEC; decreased the C/N ratio, while the EC remained at non-saline levels. Treatment T1 presented the best response in the recovery of the complementary properties of the contaminated soil, which could be attributed to the higher percentage of compost and earthworms with which the contaminated soil was treated with respect to T2 and T3; being more effective in favor of the final organic matter content of the substrate. This action is evidenced by the decrease in the C/N ratio and is complemented by the increase in the CEC, both simultaneously favored the removal of TPH fractions.

Table 4. Results of evaluations of organic matter (OM), C/N ratio, pH, electrical conductivity (EC) and cation exchange capacity (CEC) at the end of the remediation process.

Treatments	Evaluations				
	OM (%)	C/N	pH	CE (dS m-1)	CIC (cmol kg-1)
T1	16.43 a[a]	12.54 a[a]	7.81 a[a]	0.68 a[a]	16.32 a[a]
T2	11.07 b	14.27 b	7.74 b	0.39 b	11.14 b
T3	11.74 c	20.03 c	8.09 c	0.38 b	8.86 c
CV	2.40%	0.85%	1.20%	13.41%	1.10%

[a]Differentlettersin each column mean that there is a significant statistical difference between them according to Tukey's test ($p \leq 0.05$). CV: Coefficient of variability.

The remediation process was able to decrease the C/N ratio of the substrate due to the viability in the decomposition of the compost with increased organic matter; according to [17,36, 37]; a C/N ratio below 25 facilitates the decomposition of organic matter sources such as compost. The accumulation of organic matter in the substrate was also mediated by earthworm action, which according to [38,39] is carried out in two phases. In the first phase, the physical properties of the substrate are modified and in the second phase the endosymbiont microorganisms of the earthworm intestine produce substrate degradation.

The pH of the substrate is a determining factor for the activity of the earthworms in the decomposition of organic matter of contaminated soils [6], being an acceptable range between 7.5 and 8.0 although it can tolerate between 5.0 and 8.5 [22,40]. The salinity of the remediated soil is an important indicator to assess its quality, so the EC values shown in Table 4 refer that the substrate at the end of the remediation process did not present salinity problems. Therefore, its restoration with respect to this property will not prevent the installation of plant species of agricultural value [27]. The CEC is a determining factor in decision making for the remediation and rehabilitation of contaminated soils. On this subject, [26,31] explain that the CEC makes the remediation of contaminated soils viable and its value depends mainly on the content of organic matter, type and amount of clay. In the present study, a marked direct association between the organic matter content and the CEC of the remediated substrate is evident, which is congruent with publications by various authors who conclude that soil organic matter is responsible for 25% to 90% of the CEC [3,9,36].

Regarding the effect of the treatments on the average length and weight of Eisenia foetida, Table 5 indicates that there are no significant statistical differences in the results, although earthworms evaluated in treatment T1 present a length and weight greater than those recorded prior to the remediation process. According to [23], the growth, weight and reproduction of the earthworm Eisenia foetidadepend directly on the type of substrate in which they develop.

Table 5. Tukey's test results for earthworm (Eisenia foetida) average length and weight evaluations at the end of the remediation process.

Treatments	Description	Length (cm)	Weight (g)
T1	25% soil contaminated with TPH + 75% compost with earthworms.	11.8 a[a]	1.6 a[a]
T2	50% soil contaminated with TPH + 50% compost with earthworms.	11.2 a	1.4 a
T3	75% TPH contaminated soil + 25% earthworm compost.	10.4 a	1.0 a
CV		5.87 %	9.84 %

[a]Similarlettersin each column mean that there is no significant statistical difference between them according to Tukey's test ($p \leq 0.05$). CV: Coefficient of variability.

Correlations

Table 6 provides results of the linear correlation test between the amount of TPH removed from the substrate and complementary characteristics, the correlation coefficients (r) exposed to the statistical significance test reveal that the C/N ratio, salinity and CEC present a significant association with respect to TPH values removed. On the other hand, the relationship between TPH and OM and pH did not present a statistically significant association. The highest correlation coefficient was achieved between TPH content (dependent variable) and CEC (independent variable) with an inverse association between both and demonstrating that as the CEC of the substrate increases the concentration of TPH decreases because there is a greater removal of TPH. The coefficient of determination establishes that the CEC affects 32.23 % in the variation of the TPH results. The behavior is similar for the case of substrate salinity, as the removal of TPH increases, salinity decreases.

Table 6. Results of linear correlation between TPH removal values and other evaluations in the substrate subjected to the remediation process.

TPH (mg kg-1)	r	r2	a	b	r (0.01)	Significance
OM (%)	-0.407	0.165	10031.31	-510.70	0.590	NS
C/N	0.556	0.309	-4815.46	523.07	0.590	**
pH	0.157	0.025	-18211.87	2736.45	0.590	NS
EC (dS m-1)	-0.500	0.250	8239.93	-10114.30	0.590	**
CEC (cmol kg-1)	-0.568	0.323	9989.40	-548.30	0.590	**

Dependent variable: HTP; independent variables: MO (organic matter); C/N; pH; EC (electrical conductivity); CEC (cation exchange capacity); worm length and weight; r: correlation coefficient; r2: coefficient of determination; a: intercept coefficient; b: regression coefficient. With statistical significance (1%). NS: not significant.

CONCLUSIONS

All treatments were able to decrease the initial content of the TPH fractions of the contaminated soil; however, the T1 treatment composed of 25% of soil contaminated with TPH and 75% of compost and earthworms, was the most efficient in the removal of TPH in the different fractions (F1, F2 and F3), achieving remediation below the national ECA for agricultural soils. Also, it was demonstrated that all treatments increased the OM, pH, CEC; attenuated the C/N ratio and the EC was stabilized at non-saline levels with respect to initial levels. Treatment T1 had the best performance in the recovery of contaminated soil characteristics. The simple linear correlation coefficients show that the C/N ratio, salinity and CEC measured in the substrate at the end of the remediation process presented a significant association with respect to the TPH values removed; the highest correlation coefficient was achieved between TPH content and CEC with an inverse association between the two because the increase in CEC made the TPH fraction removal process viable. Consequently, the use of compost and earthworms (Eisenia foetida) should be considered as a viable and innovative alternative for the remediation of agricultural soils contaminated with TPH.

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