Evaluation of the effectiveness of the technique of bioaumentation in sediments contaminated with hydrocarbons of a combustibles service station of the municipality of San Francisco Putumayo Colombia

Evaluación de la efectividad de la técnica de bioaumentación en sedimentos contaminados con hidrocarburos de una estación de servicio de combustibles del municipio de San Francisco Putumayo Colombia

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Received July 28, 2018; Accepted November 20, 2018

Abstract

The present investigation evaluated the effect of the technique of biostimulation of sediments contaminated with hydrocarbons in a service station of San Francisco Putumayo, these sediments are product of maintenance and cleaning of the operation site or island, as it is, the grease trap, channels perimeter of the fuel distribution and sale zone, perimeter channels of the filling area of fuel storage tanks and vehicle washing desander. For the development of this research, in the technique of bioaugmentation was added to the sediments of the service station, solid organic fertilizers (vermicompost and bocashi) and liquid (pantothenate), the objective of the investigation was the evaluation of degradation of total hydrocarbons (TPH), contained in sediments of grease traps, through the technique of bioaugmentation. The experimental design used was an unrestricted randomization (DIA), with three treatments and three repetitions of each, the T1 treatment was natural attenuation, the T2 was applied organic manure humus worm, the T3 treatment was applied organic fertilizer bocashi plus pantothenate to T2 and T3

Biostimulation, Hydrocarbons, Fertilizers

Resumen

La presente investigación evaluó el efecto de la técnica de bioestimulación de sedimentos contaminados con hidrocarburos en una estación de servicios de San Francisco Putumayo, estos sedimentos son producto del mantenimiento y limpieza del sitio de operación o isla, como es, la trampa de grasa, canales perimetrales de la zona de distribución y/o venta del combustible, canales perimetrales de la zona de llenado de tanques de almacenamiento de combustible y desarenador del lavado de vehículos. Para el desarrollo de esta investigación, en la técnica de bioaumentación se adicionó a los sedimentos, abonos orgánicos de tipo sólido (lombricompost y bocashi) y líquido (pantotenato), el objetivo fue evaluar la degradación de hidrocarburos totales (TPH), contenidos en sedimentos de las trampas de grasa, a través de la técnica de bioaumentación. El diseño experimental usado fue un irrestrictamente al azar (DIA), con tres tratamientos y tres repeticiones de cada uno, el tratamiento T1 fue de atenuación natural, el T2 se le aplicó abono orgánico humus de lombriz, el tratamiento T3 se le aplicó abono orgánico bocashi más pantotenato a T2 y T3

Bioamentación, Hidrocarburos, Abonos

Citation: GUERRA-ACOSTA, Adriana del Socorro, RODRIGUEZ-MONTENEGRO, Luis Carlos, AREVALO-LOPEZ, Judy Johana and ORTIZ-GOMEZ, Yeison Andres. Evaluation of the effectiveness of the technique of bioaumentation in sediments contaminated with hydrocarbons of a combustibles service station of the municipality of San Francisco Putumayo Colombia. ECORFAN Journal-Republic of Nicaragua 2018, 4-7: 1-12.

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Introduction

The inadequate disposal of sludge contaminated with residues of oils, lubricants, compounds by total petroleum hydrocarbons (TPH), polychlorinated biphenyls (PCB), polycyclic aromatics (PAH), metals and other pollutants cause a deterioration in the environment and human health due to their carcinogenic, toxic and poisonous effects, they are considered substances of difficult biodegradation and are classified as hazardous waste by the regulations established in the Basel Convention (Arroyo, Quesada, & Quesada, 2010).

The research was based on the use of the bioremediation technique of hydrocarbons in a service station, treating the sediments that are contaminated with their derivatives ACPM and gasoline, with organic fertilizers containing microorganisms which play an essential role to prevent and control the damages that can cause the spills or leaks of these pollutants.

Bioaugmentation was evaluated, which consisted in the application of ex situ treatments based on solid fertilizers (vermicompost) and (bocashi) and liquid (pantothenate) applied to sediments where decontamination was evidenced on a material contaminated with hydrocarbons.

The experimental design used was unrestrictedly randomized (DIA), with three treatments, the first was natural attenuation compared to two others where solid and liquid organic fertilizers were used for the decontamination of hydrocarbons, each treatment with three repetitions, for a total of nine experimental units, at the beginning of the investigation and after two months of carrying out the project, total hydrocarbon values (TPH) were taken, the other contol variables such as humidity, temperature, pH, apparent density, real density, porosity and color, were evaluated weekly.

The analysis of the total hydrocarbon values and for the control variables was carried out through an analysis of variance (Andeva) to determine if there are significant statistical differences between the three treatments evaluated and tests of significance of Tukey where ($P< 0.05$).

Finally it was determined that the technique of bioaugmentation allows the degradation of sediments contaminated by hydrocarbons and improve the physical properties evaluated in the sediments.

Objectives

General objective

To evaluate the degradation of total hydrocarbons (TPH), contained in sediments of grease traps, through the technique of bioaugmentation, in a service station of the municipality of San Francisco Putumayo.

Specific objectives

- Quantify the amount of sediment generated at the fuel service station every 15 days, both wet and dry.
- Determine the initial content of total petroleum hydrocarbons (TPH) in the sediments of the grease traps.
- Determine the percentages of total hydrocarbon degradation (TPH) and the behavior of control variables.

Methodology

The procedure was ex situ in nature, for this reason the sediment material was extracted from the grease trap and transported to a room house located in the Pablo VI neighborhood in front of the station.

The treatments evaluated were:

- T1: Contaminated sediment: 100% (25 Kg) of contaminated sediments from the service station.
- T2: 60% (15 Kg) sediments contaminated with hydrocarbons from the service station, 40% (10 Kg) of solid organic fertilizer type earthworm humus + (2L pantothenate + 4L of water).
- T3: 60% (15 Kg) sediments contaminated with hydrocarbons from the service station, 40% (10 Kg) of solid organic fertilizer type Bocashi + (2L pantothenate + 4L of water).
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For the assembly of the experimental units that are the sites where the different treatments of the research were located, the following materials were required (Figure 1).

- Contaminated sediment from the service station.
- Solid organic compost humus worm and bocashi and Pantotenato liquid.
- 9 containers of high density polyethylene, this material is chosen for its low permeability and minimal absorption of hydrocarbons (Vallejo, Salgado, & Roldan, 2012).
- Garden shovels to mix the sediments.

Figure 1 Contaminated sediments and polyethylene containers
Source: This research

For the assembly of the experimental units the following steps were taken, taking as reference (Ñustez Cuartas, 2012), in their thesis "Bioremediation for the degradation of total hydrocarbons present in the sediments of a fuel service station", which pose:

1. The sediments of the grease traps were collected (Figure 2).

Figure 2 Extraction of sediments from the grease trap
Source: This research

2. The sediments were deposited in a drying bed, to lower the humidity levels, for a week (Figure 3).

Figure 3 Contaminated sediments in the drying bed
Source: This research

After this time, samples were collected according to the technical standard (ICONTEC 3656, 1994) according to Table 1 of the follow-up of variables and evaluation method.

Variable	Méthod	Frequency
TemperaturE (ºC)	Thermometer	Three times a week in triplicate in each experimental unit
Humidity	Gravimetric	Once a week
TPH (total hydrocarbons)	Extraction by soxhlet-gravimetric method	At the beginning and end of the treatment
pH (Units)	Potentiometric	At the beginning and end of the treatment
Apparent density (g / cm3)	With graduated cylinder	Once a week
Real density (g / cm3)	Pycnometer	Once a week
Porosity (%)	1 - (Apparent density / Actual density) x 100	Once a week
Color	Table Munsell	Once every two weeks

Table 1 Seguimiento de variables y método utilizado
Source: This research

3. To the experimental units (Figure 4), water is added, once a week, in order to maintain the necessary humidity for the degradation process.

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Figure 4 Assembly of experimental units
Source: This research

4. Manual turning was done three times a week to increase the oxygen content and application of water for a correct moisture content.

5. After two months, after this procedure, samples were collected and sent to the laboratory for taking new values of the response variables that was total hydrocarbons (TPH) (ICONTEC 3656, 1994).

Results and Discussion

Diagnosis on weekly sediment production at the San Francisco service station

The sediments were extracted during 7 weeks and they were thought in humid to do so a weekly estimate, the average production was to 9.5 Kg in humid and 5,8 Kg in dry (Table 2).

Week	Weight sediments in wet (39% humidity) (Kg)	Weight of dry sediments (0% humidity) (Kg)
1	8,5	5,185
2	9	5,49
3	10,5	6,4
4	11,3	6,9
5	8,4	5,12
6	8,1	4,9
7	10,5	6,4
Total	66,3	40,443
Average	9,5	5,8

Table 2 Weight of wet sediments
Source: This research

Initial characterization of sediments

The initial values of the control and response variables were determined (Table 3), according to the certified laboratory the extracted sediments gave an initial concentration of 0.39% w / w, that is to say that for every kilogram of extracted sediment there was 3, 9 grams corresponding to total hydrocarbons and 6.74 units for pH.

Variable or parameter	Unit of measurement or concentration	Value
Temperature	°C	20,5
Humidity	Porcentaje %	39%
Apparent density	g/cm^3	2,6
Real density	g/cm^3	2,7
Porosity	%	5,0
pH	Unidades	6,74
Total hydrocarbons	Porcentaje % p/p	0,39

Table 3 Initial values for the control and response variables
Source: This research

According to these parameters, the sediments of each experimental unit have the following characteristics: the temperature is within the optimal range of 18 and 30 ° C, which allows enzymatic activity and cell exchange (Gómez Romero, et al., 2008), the pH with a value of 6.74, is between the optimum range values between 6.0 and 8.0 units, this interval is suitable for the growth of bacteria (Ríos, 2005), humidity with 39% is in the advisable range that goes from 20% - 75% humidity, which is important because it acts as a means of transporting nutrients and oxygen to the cell (Gómez, *et al*, 2008).

Analysis of the response and control variable

Total hydrocarbons (TPH)

Source	Sum of squares	Gl	Middle Square	Reason-F	Value-P
Between groups	406904,0	2	203452,	37,22	0,0004
Intra groups	32793,3	6	5465,56		
Total (Corr.)	439698,0	8			

Table 4 Anova for concentration of total hydrocarbons by treatment
Source: This research

ISSN-On line: 2414-8830

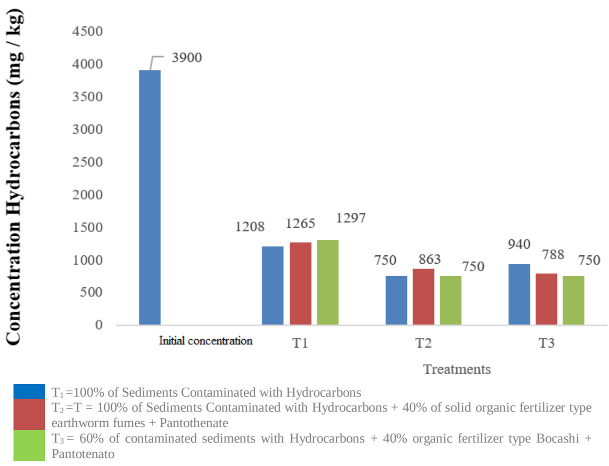
The analysis of variance in Table 4 shows highly significant statistical differences with respect to total hydrocarbons in the three evaluated treatments (P<0,05).

Treatment	Cases	Average	Homogeneous Groups
T2	3	787,667	A
T3	3	826,0	A
T1	3	1256,67	B

Stocks with a letter in common are not significantly different (p>0,05)

Table 5 Tukey tests for total hydrocarbon concentration by treatment
Source: This research

According to the Tukey test (Table 5), there is significant statistical difference between T2 and T3 with respect to T1, but between T2 and T3 there is no statistically significant difference because this test considers it as homogeneous groups.



Graphic 1 Concentration of total hydrocarbons in each experimental unit after the bioaugmentation process
Source: This research

The response variable of this investigation was total hydrocarbons. The initial analysis made in the laboratory showed that the sediments initially had a concentration of 3900 mg / kg, on the other hand it has that if the results are lower than 750 mg / kg the total hydrocarbons become undetectable.

For the T1 treatment that was the control, it obtained the highest results, as expected to happen according to the alternative hypothesis proposed at the beginning of the investigation, thus giving a maximum concentration of 1297 mg / kg and an average concentration per treatment of 1256.6 mg / kg.

T2 differed greatly from the control since the highest result corresponds to 863 mg / kg and the other repetitions gave levels below 750 mg / kg.

The T3 results indicate that the highest value for this treatment was 940 mg / kg, on the other hand one of the replicates yielded a result of 750 mg / kg.

In all three treatments there was a decrease in total hydrocarbons compared to the initial analysis, due to factors that the three treatments share in common, such as humidity and aeration management, which directly influence bioremediation, according to Roldan & Iturbe (2002). Another control variable was the temperature that played an important role since the metabolism of the microorganisms, the enzymatic and cellular activity depend on the temperature, likewise this influences the structural and chemical changes of the oil and its derivatives (Ñustez Cuartas, 2012), for which favorable conditions were maintained for the microorganisms added by the solid organic fertilizer and the liquid as pantothenate at the beginning of the process and at the same time the bacteria have a very rapid growth and a greater capacity to adapt to contaminated media, which increases the probability of success in the biodegradation of hydrocarbons (Velasco, 2004).

Temperature (ºC)

The analysis of variance of tables 6, where this variable is analyzed at the end of the process, indicates that significant differences were present (P <0.05), when observing the tests of significance of Tukey table 7, a difference was found statistic significant between T1 with average value of 20.90 ° C, with respect to T2 and T3 with averages of 20.10 ° C and 20.27 ° C respectively.

	Sum of squares	GL	Middle Square	Reason - F	Value - P
Model	1,07	2	0,53	17,18	0,0033
Treatment	1,07	2	0,53	17,18	0,033
Error	0,19	6	0,03		
Total	1,26	8			

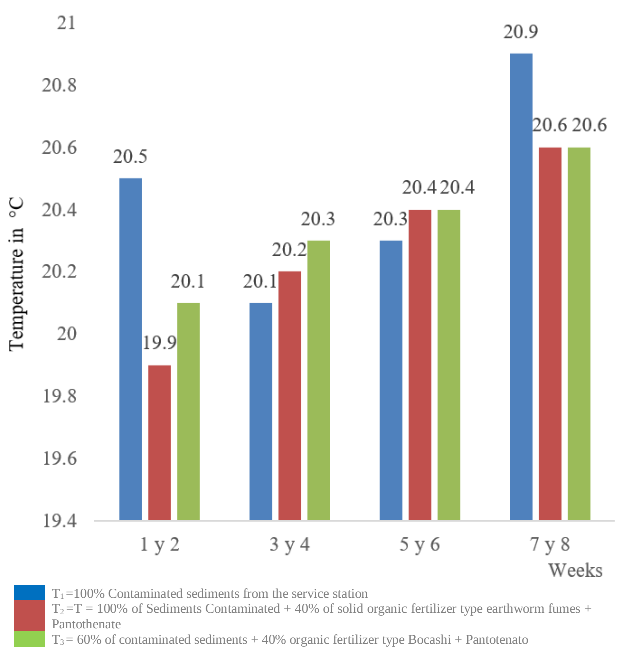
Table 6 Anova for final temperatures
Source: This research

Treatment	Average	Cases	Homogeneous groups	
T1	20,90	3	A	
T3	20,27	3		B
T2	20,10	3		B

Averages with a letter in comasún are not significantly different (p>0,05)

Table 7 Tukey test for final temperatures per treatment
Source: This research

Figure 2 shows that the average temperature for the first treatment ranges from 20.1ºC - 20 91ºC, presenting the highest values in the evaluation of this parameter., For the second and third treatment fluctuate between 19.9ºC - 20, 6ºC and 20.1ºC - 20.6ºC respectively in the course of two months, this is caused by the environmental conditions of the site.



Graphic 2 Average temperatures every two weeks in each treatment
Source: This research

The hydrocarbons in the soil cause changes both in the physical properties and in the chemical properties that, depending on the type and amount of oil discharged, as well as the temperature, soil texture and humidity make the processes more or less slow leads to a higher degree of toxicity (De Mesa., Quintero, Guevara, & Garcia, 2006).

In this regard (Gómez, et al., 2008) state that temperature directly influences the increase in volatility (separation) and rate of degradation of pollutants (Volke & Velasco, 2005), low values of temperature will increase the viscosity of the hydrocarbon and consequently it would increase the remediation time.

According to Maroto & Rogel (2008), who affirm that the optimum temperature for the increase of the metabolism of mesophilic microorganisms varies between 15 and 30 ° C and decreases the remediation in temperatures above 40 ° C and below 0 ° C.

Apparent density (g/cm^3)

For this variable, there was a statistically significant difference at the end of the process (p <0.05), according to tables 8 and 9, T2 and T3 with averages of 1.73 g / cm^3 and 1.57 g / cm^3, differentiated significantly from T1 that obtained an apparent density average of 2.13 g / cm^3.

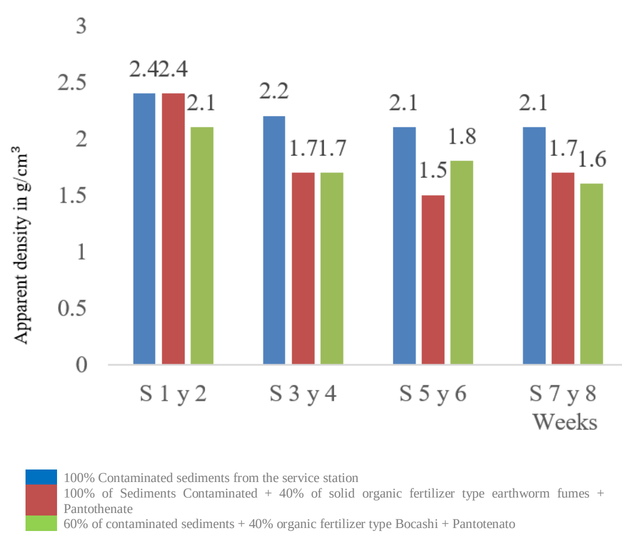
Source	Sum of squares	GL	Middle Square	Reason - F	Value – P
Model	0,51	2	0,25	25,44	0,0012
Treatment	0,51	2	0,25	25,44	0,0012
Error	0,06	6	0,01		
Total	0,57	8			

Table 8 Anova for apparent density at the end of the process
Source: This research

Treatment	Averages	Cases	Homogeneous groups	
T1	2,13	3	A	
T2	1,73	3		B
T3	1,57	3		B

Stocks with a letter in common are not significantly different (p>0,05)

Table 9 Tukey test for apparent density at the end of the process
Source: This research



Graphic 3 Average apparent density per two weeks in each treatment
Source: This research

Figure 3 shows that T1 starts with an apparent density of 2.4 g / cm^3, and for reasons of biotic control (adding water for good humidity and manual turning for aeration) for the last two weeks to reach 2, 1 g / cm^3.

Figure 3 shows that T1 starts with an apparent density of 2.4g/cm^3, and for reasons of biotic control (adding water for good humidity and manual turning for aeration) for the last two weeks to reach 2,1g/cm^3 On the other hand, T2 starts with a value equal to T1 of 2.4g/cm^3 and having the same behavior of decreasing up to 1.7g/cm^3 there was a maturity of the process.

The treatment T3 had a behavior with the same tendency to lower the density values for the final weeks reached 1.6 g / cm^3.

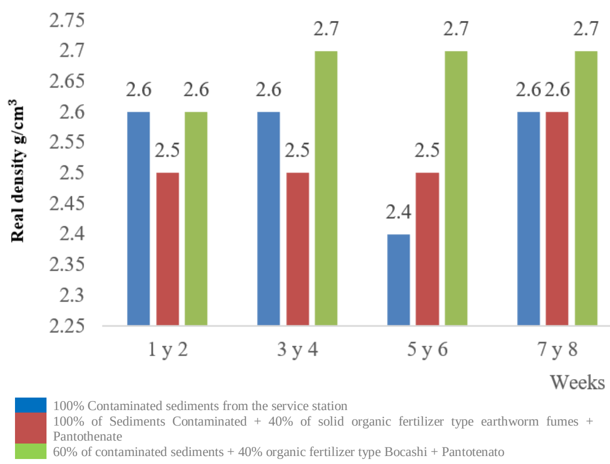
The values that the bulk density can take depend on many factors, including the texture, structure and content of organic matter, as well as its management. In contrast to the actual density, which is more or less constant, the bulk density is highly variable due to variations in the quantity / quality of the porous space (Rubio, 2010).

The changes in the apparent density reflect changes in the structure of the substrate, due to the existing relationship between bulk density and total porosity (Ingaramo, Paz Gonzales, & Dugo Paton, 2003).

In addition, the apparent density is affected by the solid particles and by the porous space, which in turn is determined mainly by the organic matter of the soil, as the organic matter and the porous space increase, the apparent density decreases and vice versa (Salamanca Jimenez & Sadeghian Khalajabadi, 2005).

The fertilizers had great direct influence, decreasing the values of apparent density, that is to say that positively affect other properties by increasing the porous spaces and improving the relationship between air and air.

Real density (g/cm^3)



Graphic 4 Average real density for every two weeks in each treatment
Source: This research

Figure 4, indicates the average values of real density of each treatment where the T1 control maintained its value of real stable density with a value of 2.6 g / cm^3 during almost all the time that the process lasted. The treatment T2 remained stable with a value of 2.5 g / cm^3 on average over the weeks 1, 2, 3, 4, 5 and 6 and finally in weeks 7 and 8 up to 2.6 g / cm^3.

The treatment T3 during weeks 1, 2, remained with a value around 2.6 g/cm^3, for already weeks 7 and 8 go up to 2.7 g/cm^3.

According to Unigarro et al. (2009), this property refers to the weight of the soil where only the solid particles of the soil are considered, this density is always greater than the apparent one. The treatments in this variable of real density remained constant since there were no major variations in it, as it is a property that does not change with the management.

Porosity (%)

According to the analysis of variance and Tukey's significance test (tables 10 and 11), there are statistically significant differences ($p < 0.05$), where T1 with an average of 17.97% porosity, differs from T2 and T3 who obtained averages of 33.67 and 42.73% respectively.

Source	Sum of squares	GL	Middle Square	Reason – F	Value – P
Model	942,08	2	471,04	13,04	0,0065
Treatment	942,08	2	471,04	13,04	0,0065
Error	216,78	6	36,13		
Total	1158,86	8			

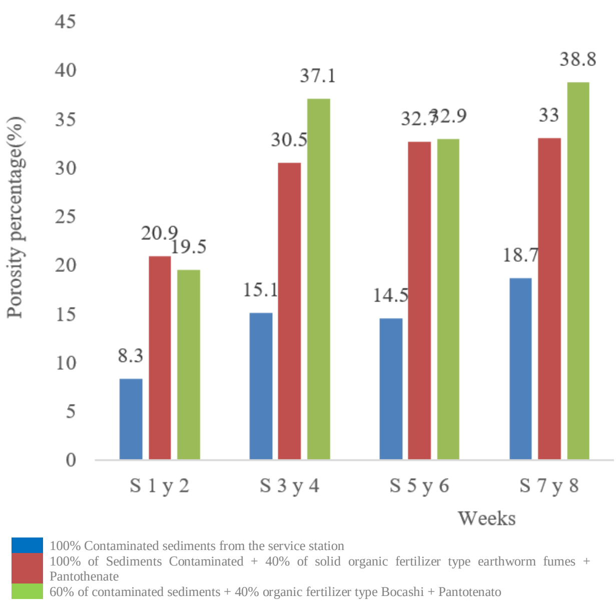
Table 10 Anova for percentage of porosity at the end of the process
Source: This research

Treatment	Average	Cases	Homogeneous groups	
T3	42,73	3	A	
T2	33,67	3	A	
T1	17,97	3		B

Stocks with a letter in common are not significantly different (p>0,05)

Table 11 Tukey test for percentage of porosity at the end of the process
Source: This research

Figure 6 shows that there is a significant difference of treatment 1 with respect to treatments 2 and 3 because these two treatments represent an increase in the percentage of porosity, this is due to the addition of organic fertilizers such as humus. of worm and bocashi, and a pantotenato liquid fertilizer for which these solid and liquid fertilizers, allow them to improve the physical and chemical properties of the sediment.



Graphic 5 Average percentage of porosity every two weeks for each treatment
Source: This research

Treatment 1 showed an increase in the percentage of porosity after the weeks of experimentation, since a percentage of porosity of 8.3% was obtained between week 1 and 2 and between weeks 7 and 8 a percentage of porosity of 18, 7%

When evaluating treatments 2 and 3, there was a significant increase in the percentage of porosity after the weeks of experimentation, since a percentage of porosity of 20.9% for treatment 2 and 19.5 was obtained between week 1 and 2. % for treatment 3, and between weeks 7 and 8 a percentage of porosity of 33% for treatment 2 and 38.8% for treatment 3, which allows establishing that if there was an understandable increase in porosity thanks to fertilizers present in these two treatments therefore these sediments maintained a good system of microorganisms.

The porosity is closely related to the real density and the apparent density, so that, if there are changes in them, there is a change in the percentage of porosity (Perez, Iturbe , & Flores, 2006). The porosity diminishes notably by the occupation of the hydrocarbon in the porous spaces, as the porosity is determined from the data of real density and apparent density, if some of these data increase or decrease (depending on the concentration and type of hydrocarbon, as well as by the type of soil), will be reflected directly in the porosity (Lopez & Martinez, 2001).

pH (Units)

Considering the analysis of variance (Table 12), where this variable is analyzed at the end of the investigation, it indicates that there were significant differences (P <0.05), in the Tukey test of significance (Table 13), a difference was found significant statistic among the three treatments, T1 with an average value of 6.63 pH units, T2 with an average of 7.33 pH units and T3 with 7.7 pH units.

Sorce	Sum of squares	Gl	Middle Square	Reason-F	Value-P
Between groups	1,96222	2	0,981111	49,06	0,0002
Intra groups	0,12	6	0,02		
Total (Corr.)	2,08222	8			

Table 12 Anova for pH at the end of the process
Source: This research

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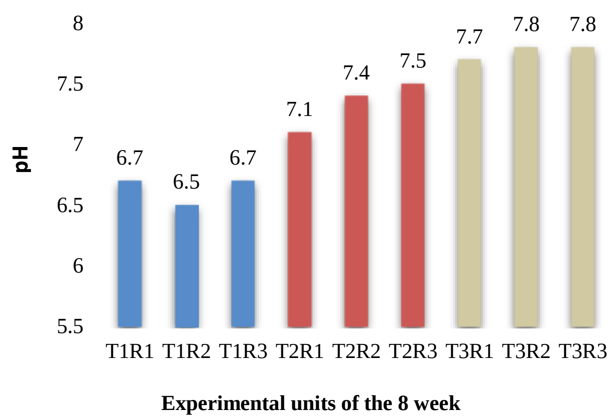
Treatment	Cases	Average	Homogeneous Groups
T1	3	6,63333	A
T2	3	7,33333	B
T3	3	7,76667	C

Averages with a letter in common are not significantly different (p>0,05)

Table 13 Tukey test for pH at the end of the process
Source: This research.

Figure 6 shows that treatment 1 with its three replicates has a pH of 6.7 - 6.5 - 6.7 units, while treatment 2 has a pH of 7.1 - 7.4 - 7.5 units in its three repetitions unlike treatment 3 contains pH of 7.7 - 7.8 and 7.8 units in relation to the last week of the process that was carried out.



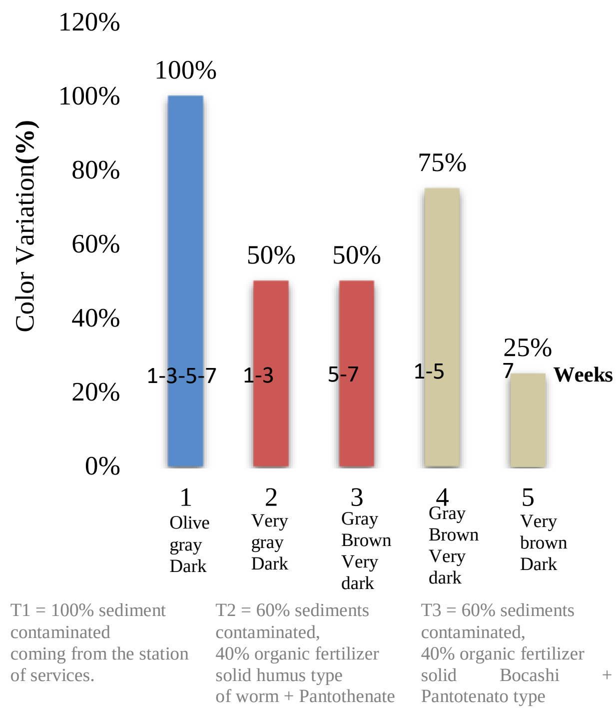
Graphic 6 PH behavior in the experimental units
Source: This research

According to (Maier, Pepper, & Gerba, 2008) they affirm that the pH influences the microbial activity of the soil. Bacteria develop best at neutral pH and filamentous fungi at acidic pH; thus, the degradation of hydrocarbons is better under conditions of neutral and alkaline pH than in acidic pH.

The pH is an important chemical factor that influences the recovery of soils contaminated by hydrocarbons, since it can mainly affect the populations of microorganisms and the bioavailability of carbon and energy sources. This factor constitutes one of the indicators of the bioremediation process and has a certain range of tolerance (Hambrick, DeLaune, & Patrick, 2008). Corroborating what was found in the research where solid organic fertilizers were used, the pH tended toward neutrality. At extremely alkaline or extremely acid pH, biodegradation becomes slow (Huertos & Romero, 2008). Generally, soils contaminated by hydrocarbons tend to be acidic, which limits growth and microbial activity.

The optimum range for biodegradation is between 6-8 pH (Alexander M., 1994). However, to maintain a better degrading capacity, for prolonged periods of time, the pH must be neutral, between 7.4-7.8, avoiding fluctuations as much as possible (Leahy & Colwell, 1990).

Color



Graphic 7 PH behavior in the experimental units at the end of the process
Source: This research

Graph 7 shows there are colorimetric differences where treatment 1 remained constant after the experimentation weeks a single color 5Y 3/2 (dark olive gray) which represents 100%, as for treatment 2 have been detonated two types of colors for which in the first weeks (1-3) a 2.5Y 3/2 color was presented (very dark gray) representing 50% and in the last two weeks (5-7) a color 10YR 3 / 2 (very dark gray brown) representing the other 50%, in terms of treatment 3 two types of colors were observed which for the weeks (1-3-5) I present a color 10YR 3/2 (very dark gray brown) which represents 75%, and for the last week (7) I present a color 10YR 2.5 / 2 (very dark brown) which represents the remaining 25%. This is due to the fact that the treatments contain solid organic fertilizers such as earthworm humus, bocashi and a liquid pantothenate type fertilizer.

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Which makes it possible to establish that they are totally different sediments with respect to treatment 1 of treatments 2 and 3 are combined that allow having other physical and chemical characteristics regarding color. According to (Burbano, 1989), it states that the typical dark color is due to the contents of organic matter, its intensity influenced by the moisture content, the dark colors are an indication of high contents of organic matter.

Conclusions

- The total hydrocarbons as response variables had positive results that allude to the alternative hypothesis since the treatments T2 and T3 have significant differences compared to the T1 evidenced in the laboratory results, which yielded the following data: an average concentration of T1 of 1256 mg / Kg, T2 and T3 can not be averaged since some results were below 750 mg / Kg which is the limit of quantification.

- According to the results of the response variables, the most effective treatment was T2 containing 60% of sediments and 40% organic fertilizer solid earthworm humus plus liquid organic fertilizer pantothenate, because it showed results lower than 750 mg / kg, surpassing the levels of decontamination of T1 and T3, however, T3 was very effective since it remains outside the decontamination of T2.

- The control variables (pH, humidity, temperature, bulk density, porosity) had statistically significant differences except for the real density, because the behavior they had was due to the control of the biotic factors to which this procedure was subjected.

- Comparing the treatment T1 with T2 and T3, the behavior of the real density was not due to each treatment because the p value is greater than 0.05 which shows that there was no statistically significant difference both at the beginning and at the end of the treatment. treatment, real density is a variable that is constant and very difficult to change.

- As a result of the investigation, it was demonstrated that the alternative hypothesis proposed at the beginning of the process was the one that was evidenced in each variable, both of control and of response, having significant differences, for which it is concluded that the treatments did have a positive response in comparison to the witness. The deposition of the sediments corresponds to an open space, where there is contamination of the soil and superficial and underground waters, with the results of this research an alternative of environmentally sustainable solution was proposed for the decontamination of the sediments that the service station produces.

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