BIOREMEDIATION OF CONTAMINATED SOIL CONTAINING CRUDE OIL

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ABSTRACT

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Bioremediation of contaminated soil containing crude oil is a technique process whereby biological systems are harnessed to affect the clean-up of environmental pollutants. Microbial systems are most widely employed in bioremediation programs, generally in the treatment of soil and water contaminants with organic pollutants. This thesis reports the experiment of treating the soil without use of any chemicals. Four treatments were used for this experiment. All of the treatments were containing bio-product to remediate the contaminated soil with crude oil. The bio-product in this case was sawdust and water. The experiment was done in the laboratory under the hood (incubation) the conditions were monitored with lamps to generate heat.

This bioremediation technique resulted in significant removal of hydrocarbons.. The total amount of hydrocarbons removed at best was 65.14% in 22 days of the experiment. This treatment the composition of 100g of contaminated soil, 50g of sawdust and 300ml of water that resulted in 80% humidity. Those results were achieved under the temperature of 22^0C to 28^0C.

This result shows that mixing the contaminated soil with sawdust and water under well monitored conditions 22^0C to 28^0C can result in reduction of total hydrocarbons on the soil. Thus there are indications thatwith a known proportion of water and sawdust the treatment might be suitable tool for the remediation of contaminated soil with crude oil.

Keywords: Bioremediation, hydrocarbons, contaminated soil

CONTENTS

ABBREVIATION AND TERMS

ATSDR	Agency for Toxic Substances and Disease Registry
CDC	Center for Disease Control and Prevention
CFU	Colony forming units
CO_2	Carbon Dioxide
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
H_2O	Water
HC	Hydro carbon
IARC	International Agency for Research on Cancer
K	Potassium
N	Nitrogen
NIOSH	National Institution for Occupational Safety and Health
nm	nanometer
NRC	Nuclear Regulation Commission
OBM	Oil Based Mud
OSHA	Occupational Safety and Health Administration
P	Phosphorus
pH	Logarithmic H^+ concentration
ppm	Parts per million (mg/l)
Soil H_20 %	Humidity
t	Time (min)
T	Temperature (^{0} C)
TPH	Total Petroleum Hydrocarbons
V	Volume (ml)

1 INTRODUCTION

The oil industry contributes significantly to the economy of countries that have the oil underground, which is why the exploration, production, refining, transportation and consumption of petroleum products are increased each day. The poor management practices of hydrocarbons, accidents during production, transportation fuels and other processed products, and the bunkering have brought environmental problems due to which it has become apparent contamination of large areas of surface soil and the allocation of water bodies. (Fingas 1978, 1-7).

Bioremediation with bio product can have an impact on the industries that use chemical product to remediate contaminated sites. The contaminated soil treatment with sawdust is based on the use of microorganisms that are able to transform organic pollutants into chemically simpler compounds such as carbon dioxide. (Singh and Ward. 2004, 3-13)

The problem of contaminated soil and contaminated water is how to recover or how to finally deposit it. In recent years the soil has been recovered through many techniques that remediate the soil with chemical products and then after the treatment the soil could be reused. Remediation with chemicals can cause great danger to human health and the environment. (ATSDR 1999).

The aim of this preliminary experiment is to test remediating the contaminated soil containing crude oil with bio product. The reason for this experiment was to find the treatment that had more reduction in TPH in the end of the 22 days under monitored conditions. Those monitored condition include microbial community, bacteria and fungi which are the key for this treatment. The treatment with more reduction in TPH can have the potential to remediate not only contaminated soil containing crude oil, but remediate spills of grease, petrol, diesel, engine oil and etc. This work was done in the laboratory of the Tampere University of Applied Sciences (TAMK). This work is a starting point in developing a method for oil contaminated soil remediation.

2 Material and methods

The material used for the experiment was oil based mud (OBM) it is contaminated soil with crude oil, the soil samples come from Cabinda; Angola (See Appendix 2). The treatment method was done in a simple way. The test set up was done in four separated treatments. A mass of 100g of contaminated soil was added to all four treatments with different proportion of water and sawdust was implemented. Table 1 presents the experiment setup. Temperature, humidity and pH were measured daily.

The experiment was done in 22 days.

2.1. Sawdust

The sawdust for the treatment come from a local company in Tampere, the sawdust was mixed with different types of wood sawdust and it was dry and thin. The reason behind this is that it would decompose easier. According to Udosen et al. (2001), bio remediating of contaminated soil containing petroleum was treated with sawdust and water that resulted in 81 % decrease in oil content after 6 months of treatment. This is leading information for this bioremediation experiment to happen. According to Godoy (2008), non-contaminated soils and sawdust have been used as cheap readily available sorbent materials for fuel oil spills. These materials consist of decomposing complex mixtures of aliphatic and aromatic hydrocarbons.

Incubating wet sawdust at 30°C has resulted in gradual increase of fungal and bacterial counts; to reach after 6 months between 5×10^{6} and 7×10^{6} CFUg^{-11}, and the appearance of hydrocarbon-utilizing bacteria in numbers between 8×10^{4} and 3×10^{5} cells per gram. (Ali and Eliyas 2001)). This reveals that the biological process of the bioremediation is starting to take place, with the help of bacteria that emerged after incubation.

2.2. Water

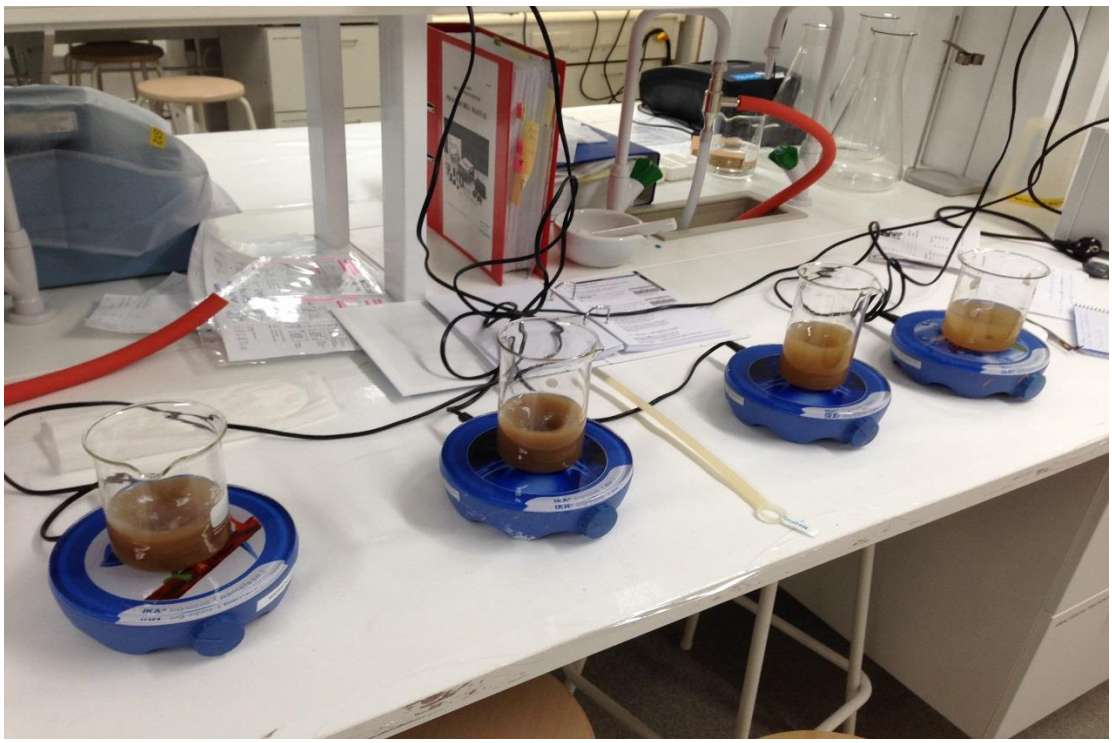
The water used for this experiment was fresh tap water from Tampere University of Applied Sciences (TAMK) laboratory. Tampere city water company has high standards according to Health Protection Act (763/1994) (See Appendix 1). The reason is that water carries nutrients, which will feed the biological process trough bacterial reproduction. The water was measured in different volume for each proportion according to the setup of the experiment in the table 1

2.3. PetroFLAG®

The PetroFLAG® method is developed for total hydrocarbon analysis and it responds to the chemical properties of the broad class of hydrocarbon compounds. The PetroFLAG® hydrocarbon analysis system is designed as abroad spectrum screening tool suitable for analyzing any tape of hydrocarbon contamination (Dexsil corporation 1997)

2.4. pH

The pH is an important parameter in this experiment, in order to remediate the soil the pH needs to be in range of 7.0 - 7.5. (Milton and Rachakonda, 2005, 181) pH electrode (Mettler Toledo FE20) was calibrated before, using a standard calibration solution of pH 4 then to the other solution of pH 7. After that, the sample experiment was mixed with distilled water according to a 1:15 ratio. The stirring magnet was added to the solution and placed on top of the magnet stirrer for 30 min. Next the pH electrode was introduced to the sample while it has being mixed, and pH value was taken.



Picture 1. The pH level measurement of the 4 setups (Rodolfo Casimiro 2014)

2.5. Thermometer

The thermometer was used to measure the temperature of the soil, the temperature is also one of important parameters for this experiment in order to find out is the experiment is working, the temperature have to be in the range of 20-30^{o} C. (Milton and Rachakonda 2005, 179)

2.6. Infrared moisture content measurer IR

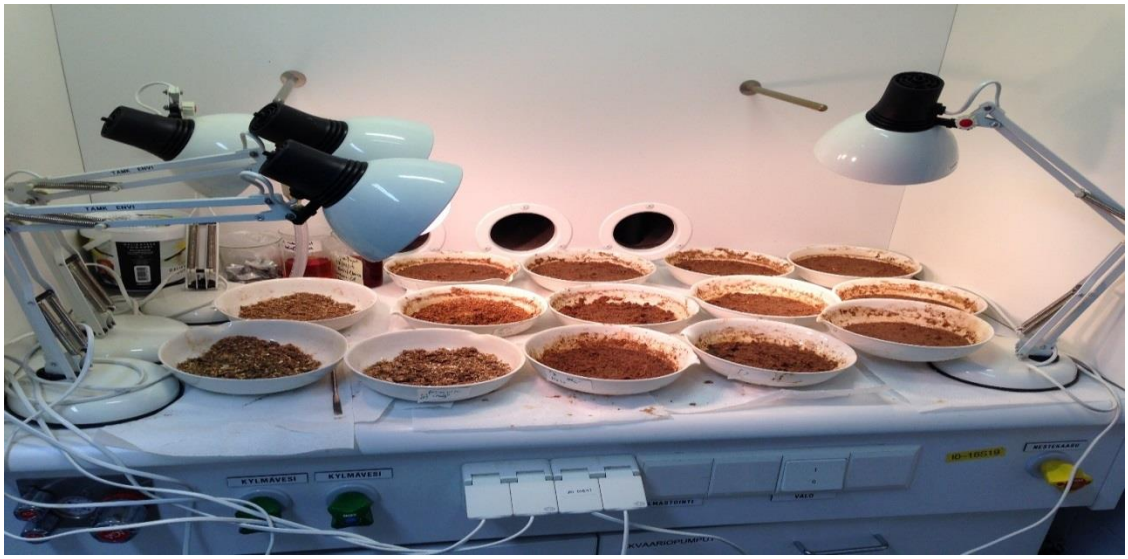
The moisture content measured the amount of total humidity of the soil mixed with the sawdust. The initial humidity of the treatment has to be the same until before the soil is put to be dry to find the final TPH.

2.7. Analytical balance

The balance was to measure the correct amount of the sawdust and contaminated soil, it is important that the volume of these materials be in the range of the experiment.

2.8. Lamps

For this experiment heat is important because with the heat the microorganisms will grow and reproduce. The optimum temperature for bioremediation of mineral oil hydrocarbons under temperature climates is in the range of 20-30 ^{o}C. (Milton and Rachakonda 2005, 179). The lumination in this experiment was 3000 lux measured with luminosity device. The Lamps used were Underwriters laboratory portable lamp of the model UV lamp which produced light of the wavelength between 254 and 365 nm and an illumination of 3000 lux; four combined.



Picture 2 Dried samples ready for temperature measurement (Rodolfo Casimiro 2014)

2.9. Experiment setup

This bioremediation experiment was done in the laboratory of TAMK. There were 4 treatments with different ratio of sawdust and water. Every treatment included three or four replicate treatments. The only common proportion of all was the contaminated soil of 100g, because the aim was to see how different ratio of water and sawdust would affect the experiment. The target was to reveal which treatment would remediate faster. The experiment lasted for 22 days.

Table 1 shows how the experiment was divided into four treatments; each treatment had 100grams of contaminated soil plus different proportion of sawdust and water.

Table 1 The experimental setup

Treatment number	contaminated soil (g)	Sawdust (g)	Water (ml)	Humidity (%)	Number of replicates	
0	100	15	0	0	3	
1	100	50	300	80	4	
2	100	30	250	60	4	
3	100	15	125	30	3	

The figure number 1 represents the experiment setups, the level of the contaminated soil, the sawdust level, the water level and the humidity level.
In the treatment number 1, the contaminated soil was mixed with 50 grams of sawdust + 300ml of tap water that made the humidity of the soil 80%. The optimal rates of biodegradation of oil sludge in soil have been reported at 30 % to 90% water saturation (Dibble and Bartha, 1979)

In the treatment number 2 the mixture was done with less humidity and less sawdust, the mixture was done with 250 ml of water + 30 grams of sawdust, the soil humidity was 60%.

In this treatment number 3 the mixture was done with even less humidity and sawdust, it is all part of the experiment, the reason is to find the setup with better results.
In this setup the water was 125ml + 15 grams of sawdust, the soil humidity was 30%.

In the treatment number 4 of the setup, the contaminated soil was a mixture with 15 grams of sawdust. This setup was done to find out how much sawdust alone can absorb the oil from the contaminated soil, just by mixing it under the temperature of 28^{o} C.

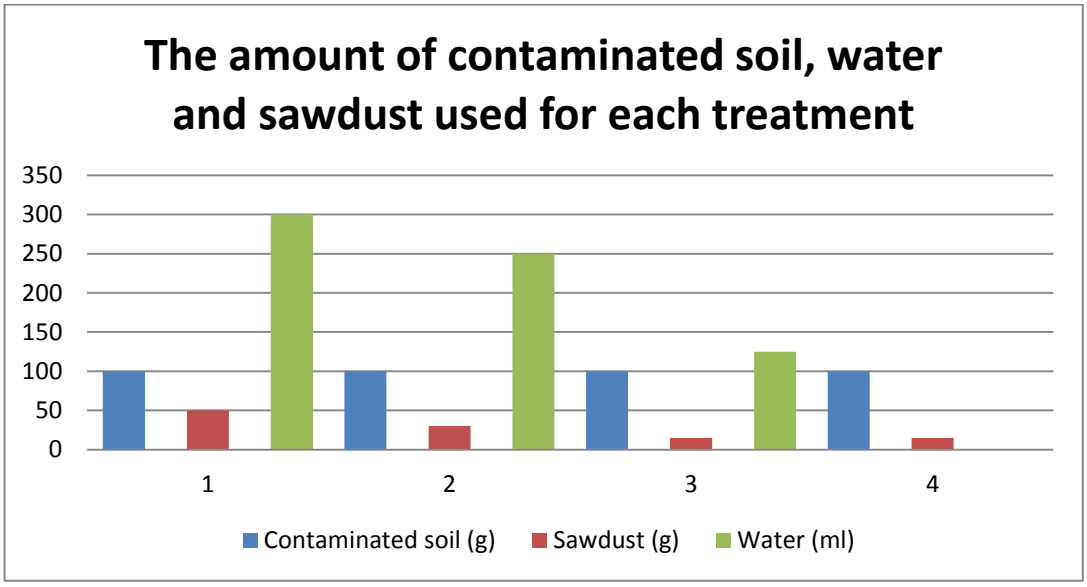


Figure 1. The amounts of contaminated soil, sawdust and water used for each treatment.

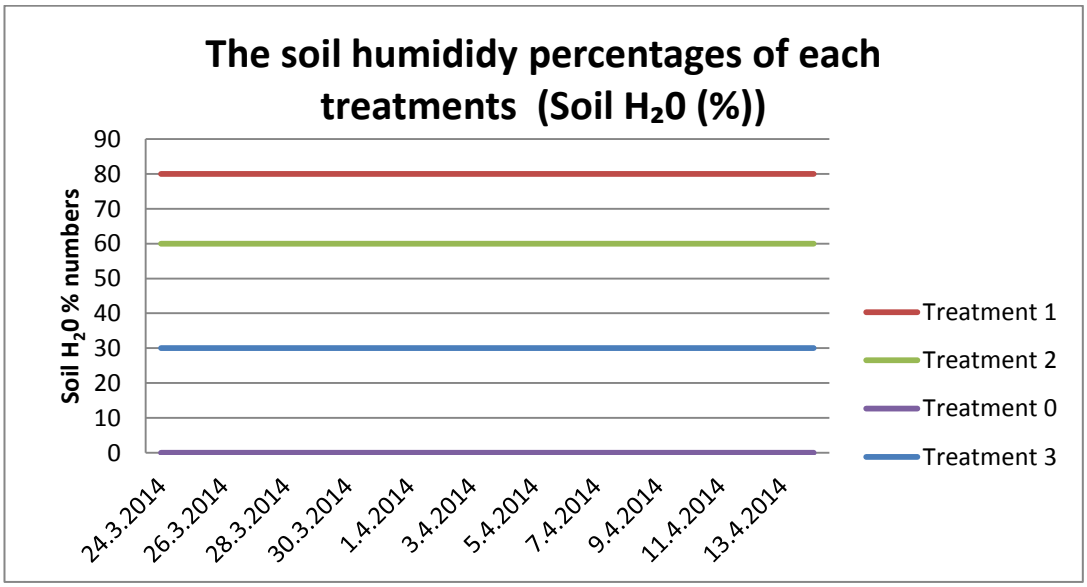


Figure 2. The figure shows the amount of soil H_2O for each treatment numbers

2.10. Measurements

The physical parameters that affect microbial growth had been measured daily, from Monday until Friday five days a week. The parameters measured during the experiment were soil temperature, pH, the humidity and illumination that had to be done in order for the treatment to be in the range of the experiment. The measurements were recorded on a daily basis since the setup had to be realistic. The temperature had to be in the range of 20 to 30ºC, pH being in between 7.0 -7.5 and the humidity according to the values mentioned in table 1.

2.11. pH

The pH in the picture number 1 was measured with 10 grams of contaminated soil and 150 ml of distilled water, it was measured in order to be in the range between 7.0-7.5, daily check-up had to be done (Milton and Rachakonda 2005, 181).

2.12. Temperature

The temperature of the test media was measured with the temperature thermometer, which was done by introducing the electrode into the soil for about a minute. The room temperature of the laboratory was varying from one day to another, which has affected the samples temperature as well.

2.13. Soil humidity

The moisture content was measured to have the values of the treatment's soil humidity. The measurement was done daily from Monday until Friday, so as to meet the values planned at the beginning of the experiment. In order to keep the moisture content constant during the whole experiment period, water was added on a regular basis.

2.14. Hydrocarbons

In the experiment PetroFLAG® was used in order to find the total initial hydrocarbons and the total final hydrocarbons of the contaminated soil.

Picture 3 PetroFLAG® kit (Rodolfo Casimiro 2014)

2.15. Procedure

The procedure was done in two phases. The first one was to measure the initial amounts used for the experiment e.g. the contaminated soil, sawdust and water. The initial hydrocarbons were measured at the beginning of the experiment, in the middle then at the end, in order to see how much hydrocarbons were evaporated. During the 22 days period of the experiment, the pH, Temperature and soil humidity were measured on a daily basis.

2.16. Measuring the initial hydrocarbon content in the soil

For sample preparation, the following items are required for each soil sample;

soil tube, extract ampule, developer vial, filter assembly. “Once the calibration is completed weigh 10 grams of soil sample into the plastic soil tube. Break off the top of the ampule and pour entire content into soil tube and shake vigorously for 4 minute and allow the sample to sit for 1 minute. Start the time for ten minutes and turn on the PetroFLAG® then insert the developer vial then read on.”(Dexsil corporation, 1997)

2.17. Measure of sawdust before applying it with the soil sample

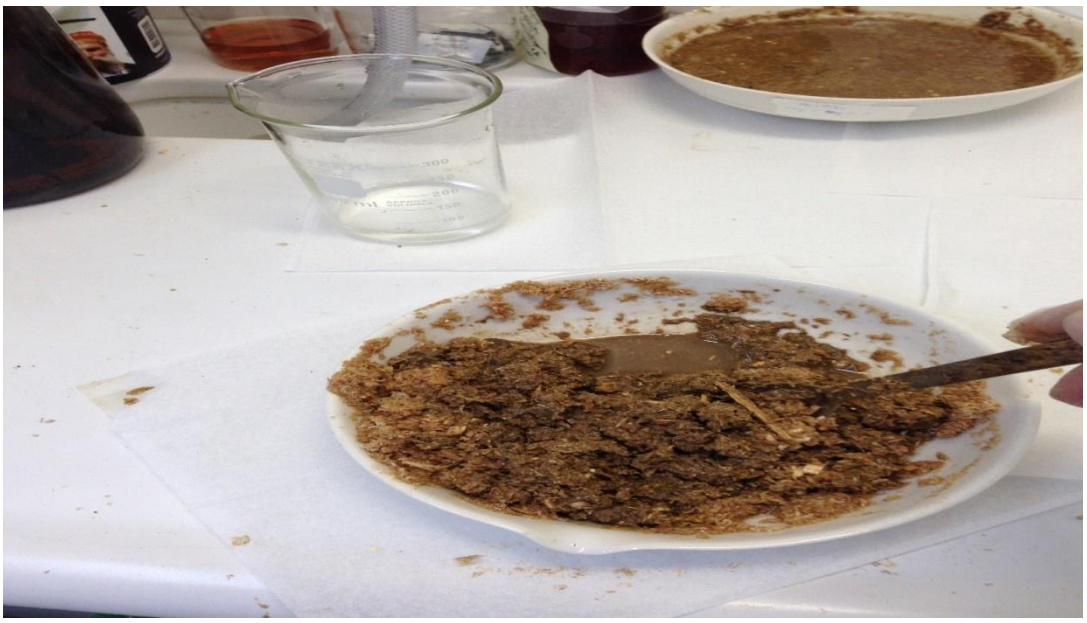
The sawdust is the most important ingredient in this experiment, since it is a dry material that will absorb the oil from the contaminated soil. It is necessary for the sawdust to be in the proper amount as planned for the experiment (table 1). The same amount will go for the replicates also; the moisture level of the sawdust was the same for all experiment.

2.18. Measure the moisture of the sample

According to the table 1, the moisture level had to be constant during the whole experiment. For this reason it was measured daily as the water was added to maintain it back to the humidity level required.

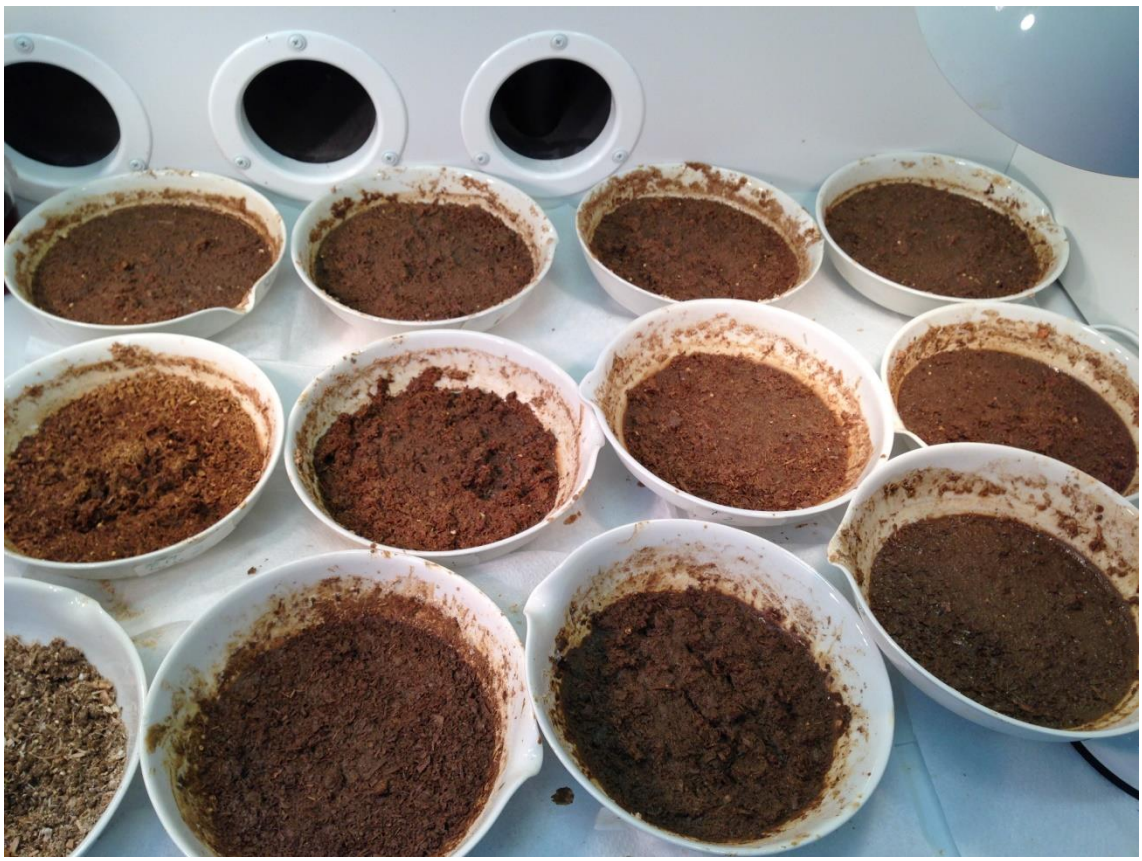
2.19. Blending the sawdust and contaminated soil

The materials had to be well mixed with a spoon, the reason is because sawdust is an organic material and at some point it will start decompose letting the microorganisms consuming or cutting down hydrocarbons. For that chemistry to happen, daily mixing was substantial. To allow air to penetrate the soil and enriching the bacteria with oxygen (Milton and Rachakonda 2005, 186).



Picture 4. How the mixture of the experiment was done with sawdust, contaminated soil and water (Rodolfo Casimiro 2014)

In the picture number 6 it shows that the treatments were already mixt (picture 5) and place according to the treatment numbers and the replicates. The mixture of the experiment was done the same for the all treatments and for their replicates. (picture 5).



Picture 5. The mixture of contaminated soil, sawdust and water (Rodolfo Casimiro 2014)

3 Results

The observation table shows the recorded date of the parameter obtained during the 22 days of the experiment. The table number 2 presents the temperature, the pH and the humidity of each treatment. The values presented in table 2 are averages of the replicates.

Table 2 Observation table during 22 days (Soil H_20 for Moisture)

Data	Treatment 0			Treatment 1			Treatment 2			Treatment 3		
	T°	pH	Soil H_20 %	T°	pH	Soil H_20 %	T°	pH	Soil H_20 %	T°	pH	Soil H_20 %
24/03/2014	19	7.4	0	22	7	80	23	7,3	60	23	6,4	30
25/03/2014	23	7	0	23	7	80	24	7,5	60	23	6,5	30
27/03/2014	23,4	7	0	22	7,5	80	23	7	60	22	6.7	30
28/03/2014	23,8	7	0	21	7	80	22	6.5	60	21	6.9	30
31/03/2014	22	6.5	0	24	6.8	80	22	6.5	60	22	7	30
01/04/2014	26	6,5	0	26	7	80	27	7	60	27	7	30
02/04/2014	27	6	0	27	7	80	28	6.5	60	26	7.5	30
03/04/201	26	7,5	0	27	7	80	28	7	60	27	7.3	30
04/04/2014	28	7.5	0	27	7	80	27	7	60	27	7	30
07/04/2014	28	7	0	27	7	80	26	7	60	28	7	30
08/04/2014	25	7	0	28	7,4	80	28	7,6	60	28	7,5	30
09/04/2014	26	7	0	28	7	80	28	7	60	27	7	30
10/04/2014	27	7	0	27	7.5	80	28	7	60	27	7	30
11/04/2014	27	7	0	28	7	80	28	7	60	27	7,4	30
14/04/2014	28	7	0	28	7,2	80	28	7,3	60	27	7	30

The figure number 3 represents the average of the treatment 0, represents the variation curve of the temperature, pH and moisture during the 22 days of the experiment.

The blue curve represents the temperature during 22 days of the experiment in the low temperature the ambient temperature inside the laboratory was cold and did affect the temperature of the treatment. The pH and moisture graphs were constant over the experiment period. Table 2 presents the treatment 0 in more details.

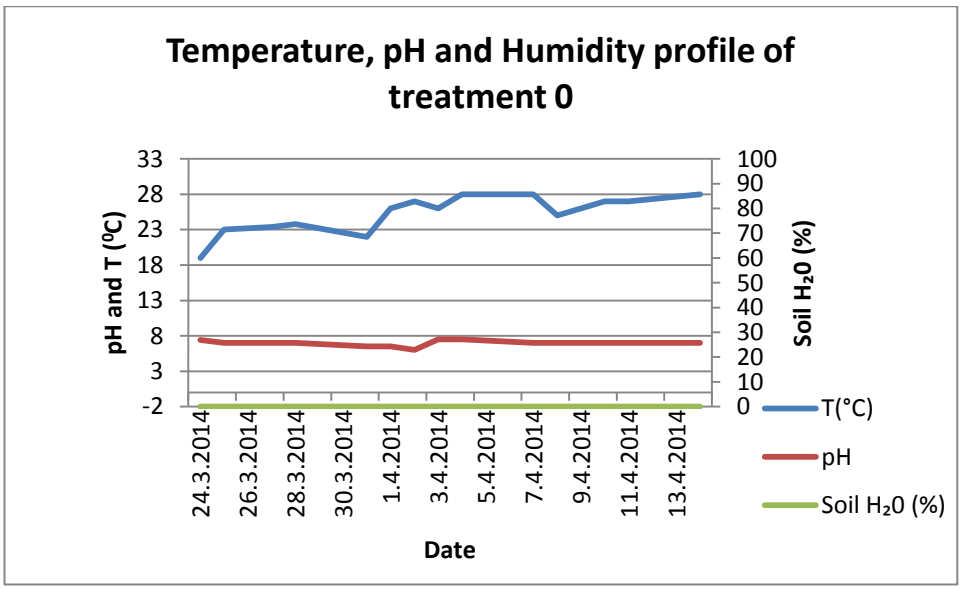


Figure 3. Representation of the variation curve of temperature, pH and Soil moisture of the 22 days of the treatment 0.

The figure number 4 represents the treatment 1. In this graph the green curve represent moisture level that is 80%, according to the table setup number 1. The blue curve represents the temperature. The temperature is varying because of the inside ambient sometimes colder than other days. The red represents the pH level which was constant during the 22 days.

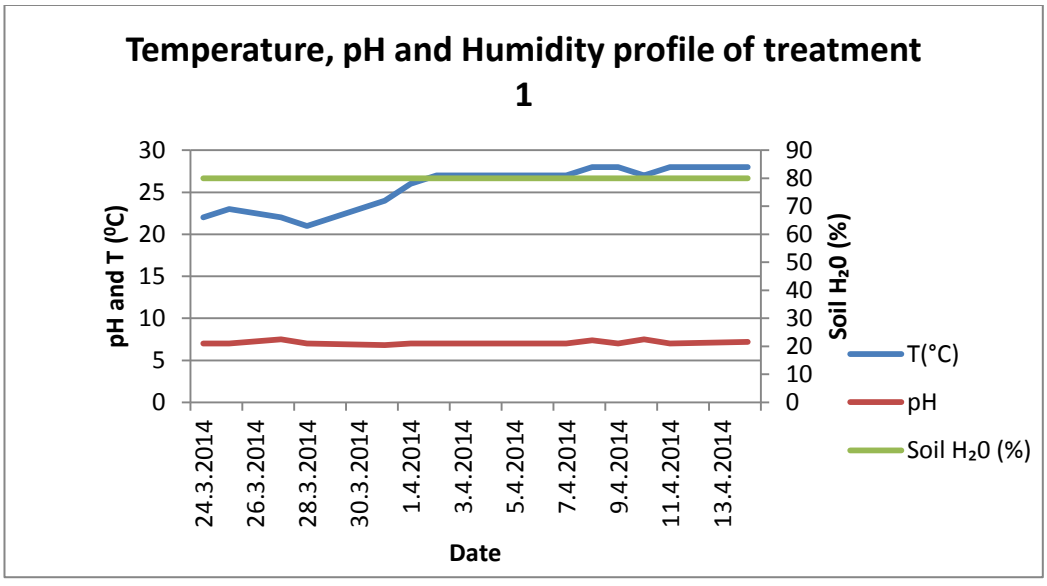


Figure 4. Representation of the variation curve of temperature, pH and Soil moisture of the 22 days of the treatment 1.

The figure number 5 represent treatment number 2, the green curve represent the moisture of the treatment is 60% the blue curve represent the temperature and the red represent pH level, that both were observed to be constant.

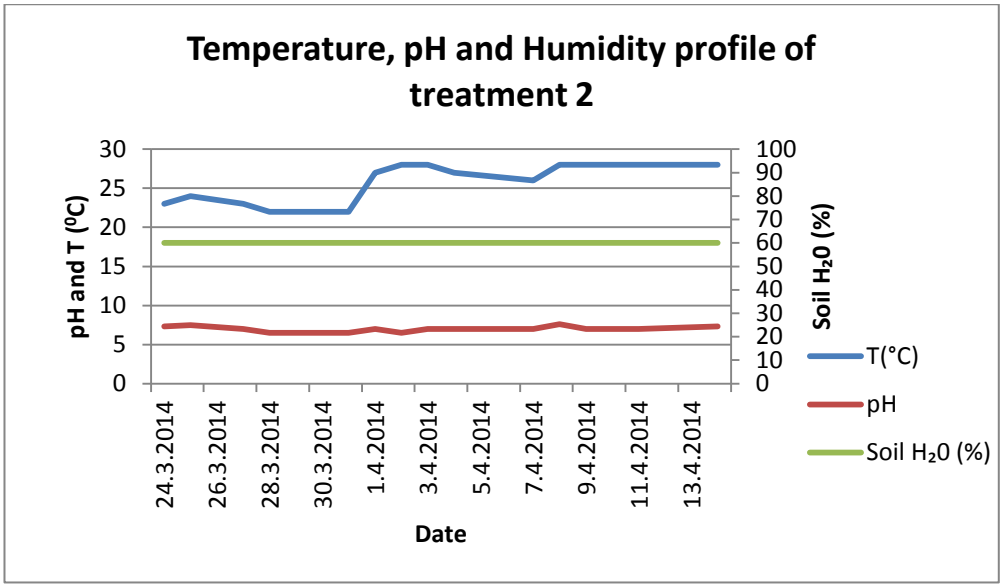


Figure 5. Representation of the variation curve of temperature, pH and Soil moisture of the 22 days of the treatment 2

Figure number 6 represents the result of the treatment 3, the green curve represent moisture level that is 30%. The blue curve represents temperature level that was getting higher during the days of the experiment. The red one represents pH level which didn't vary that much.

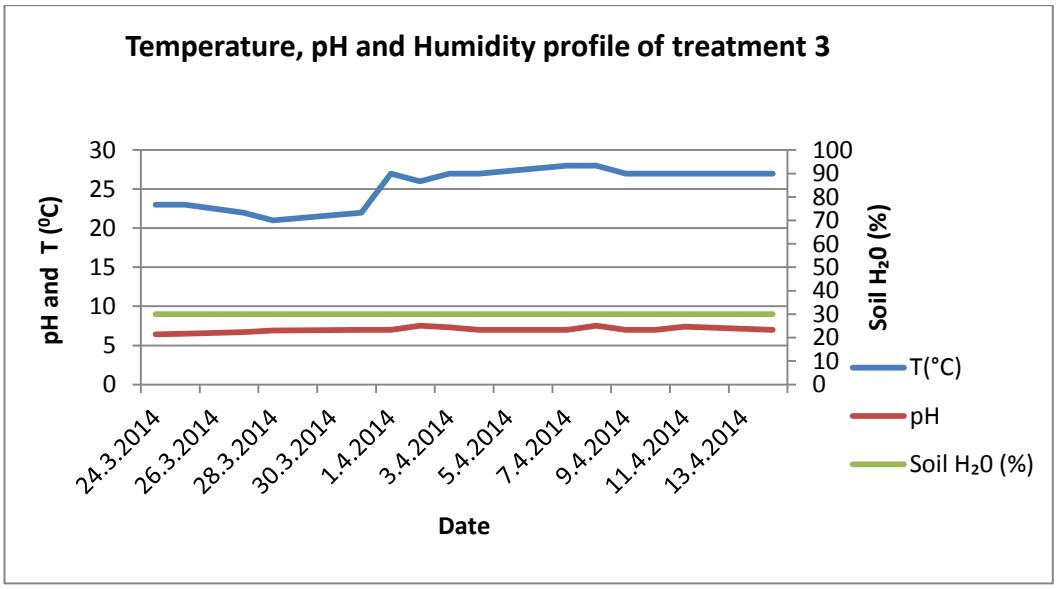


Figure 6. Representation of the variation curve of temperature, pH and Soil moisture of the 22 days of the treatment 3

The different temperature variations observed in the figure can be tracked down not only to the ambient air conditions inside the laboratory, but also it is or it can be due to microbial activity.

The calculation was to find the percentages of the TPH evaporated within 11 days and 22 of the experiment. Table 4 presents the reduction values of the TPH after 11 and 22 days.

Treatment 0 Percentages after 11 days

$$(\frac{22060 - 21020}{22060} X100\% = 4.71\%)$$

Percentage after 22 days

$$(\frac{22060 - 19600}{22060} X100\% = 11.15\%)$$

Treatment 1 Percentage after 11 days

$$(\frac{22060 - 16070}{22060} X100\% = 27.15\%)$$

Percentage after 22 days

$$(\frac{22060 - 7690}{22060} X100\% = 65.14\%)$$

Treatment 2 Percentage after 11 days

$$(\frac{22060 - 16820}{22060} X100\% = 23.75\%)$$

Percentage after 22 days

$$(\frac{22060 - 9780}{22060} X100\% = 55.66\%)$$

Treatment 3 Percentage after 11 days

$$(\frac{22060 - 14920}{22060} X100\% = 32.36\%)$$

Percentage after 22 days

$$(\frac{22060 - 9520}{22060} X100\% = 56.84\%)$$

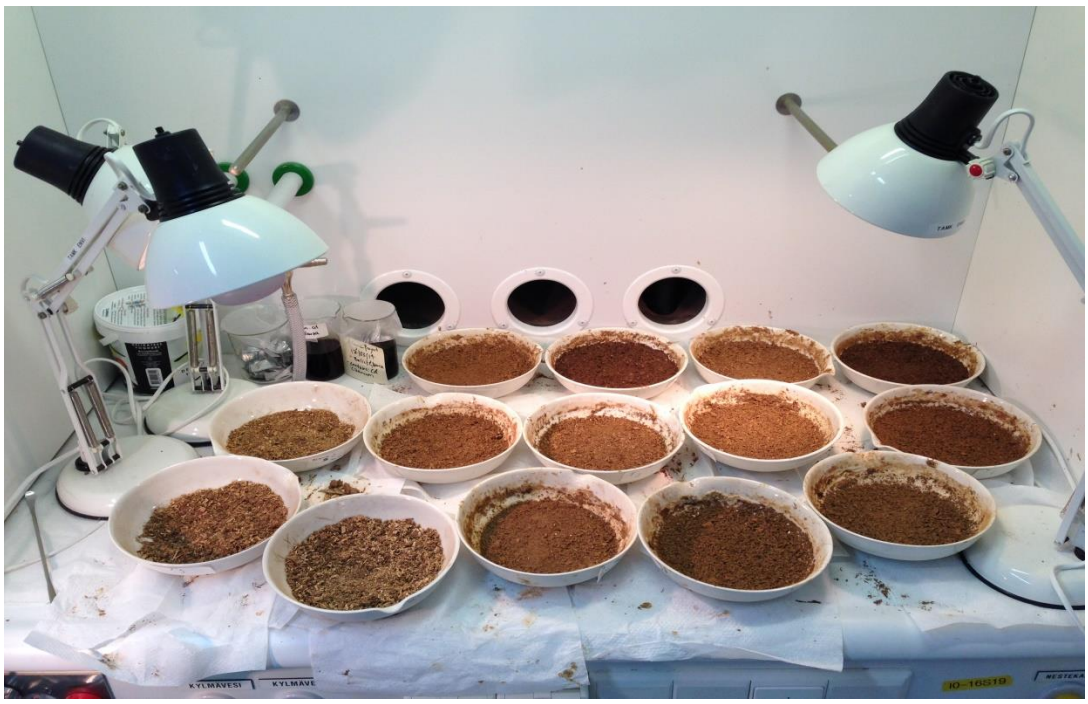
The table number 3 represent the initial, middle and the final TPH of the contaminated soil, treatment was done under those dates with PetroFLAG® kit.

The table below displays the results obtained during the experiment

Table 3 Initial, Middle and Final TPH values of different soil sample treatments in ppm

		Initial values of TPH (ppm)	TPH average values after 11 days (ppm)	TPH average values after 22 days (ppm)
Treatment 0	Rep 1	22060	21020	19600
	Rep 2	22060		
	Rep 3	22060		
Treatment 1	Rep 1	22060	16070	7690
	Rep 2	22060		
	Rep 3	22060		
	Rep 4	22060		
Treatment 2	Rep 1	22060	16820	9780
	Rep 2	22060		
	Rep 3	22060		
	Rep 4	22060		
Treatment 3	Rep 1	22060	14920	9520
	Rep 2	22060		
	Rep 3	22060		

At the beginning of the experiment all samples prepared were of the same contaminated soil source. For this reason the values for each initial replicate is 22060ppm. The blank treatment (treatment 0) has shown a little reduction in the amount of TPH, since no water was added however sawdust was amended to it. Treatment number 1 reduced in values. This treatment was of a moisture content of almost 80%. For the treatment 2 TPH values also shows significant data where the water content was of 60%. The final treatment displays a reduction of the TPH amount where the humidity level was 30%. One important factor affecting these treatments is the amount of light received for each replicates, which was not even as the picture below shows.



Picture 6 the distribution of light for the treatments (Rodolfo Casimiro 2014)

Table 4 TPH percentage values treated within 11 days and 22 days of the experiment, treatments amendment are presented in table1

Treatment numbers	THP percentages of contaminated soil after 11 days	TPH removal efficiency	Treatment numbers	TPH percentages of contaminated soil after 22 days	TPH removal efficiency
0	21020ppm	4.71%	0	19600ppm	11.15%
1	16070ppm	27.15%	1	7690ppm	65.14%
2	16820ppm	23.75%	2	9780ppm	55.66%
3	14920ppm	32.36%	3	9520ppm	56.84%

The figure number 7 represents the dates and the amount of the TPH during the 22 days of the treatment number 0, the loss of TPH observed amongst the treatments. The initial TPH of the contaminated soil was measured 22060ppm in the first day of the experiment. The 21020ppm was measured in the middle of the experiment. The 19600ppm was measured on the final day measured of the experiment. 11.15% of the TPH was treated within 22 days. The blue curve represents the amount of TPH evaporated, while the second curve shows the percentage of the evaporation during the experiment. This clearly shows a significant reduction of the TPH along the experiment.

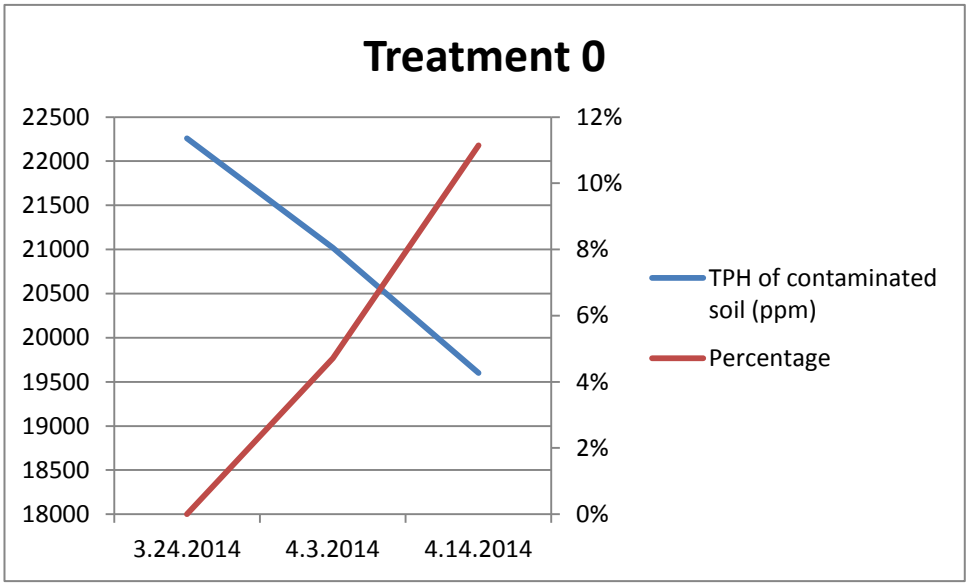


Figure 7. This figure represents the initial, middle and final TPH of the treatment number 0

The figure number 8 represents the dates and the amount of the TPH during the 22 days of the treatment number 1, which was the maximum loss of TPH observed amongst the treatments. The initial TPH of the contaminated soil was measured 22060ppm in the first day of the experiment. The 16070 ppm was measured in the middle of the experiment. The 7690ppm was measured on the final day measured of the experiment.65.14% of the TPH was treated within 22 days. The blue curve represents the amount of TPH evaporated, while the second curve shows the percentage of the evaporation during the experiment. This clearly shows a significant reduction of the TPH along the experiment.

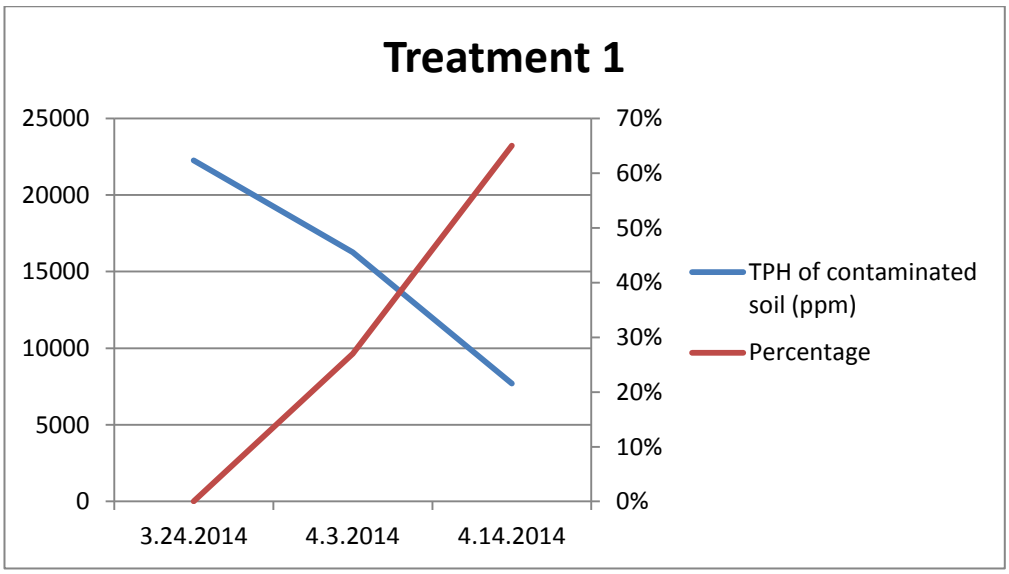


Figure 8. This figure represents the initial, middle and final TPH of the treatment number 1

The figure number 9 represents the dates and the amount of the TPH during the 22 days of the treatment number 2, the loss of TPH observed amongst the treatments. The initial TPH of the contaminated soil was measured 22060ppm in the first day of the experiment. The 16820ppm was measured in the middle of the experiment. The 9780ppm was measured on the final day measured of the experiment. 55.66% of the TPH was treated within 22 days. The blue curve represents the amount of TPH evaporated, while the second curve shows the percentage of the evaporation during the experiment. This clearly shows a significant reduction of the TPH along the experiment.

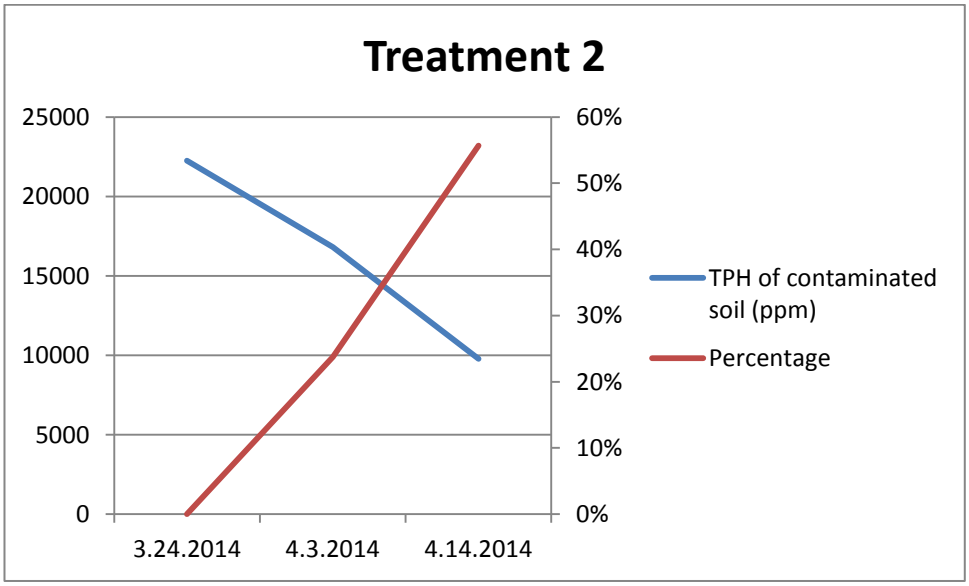


Figure 9. This figure represents the initial, middle and final TPH of the treatment number 2

The figure number 10 represents the dates and the amount of the TPH during the 22 days of the treatment number 3, the loss of TPH observed amongst the treatments. The initial TPH of the contaminated soil was measured 22060ppm in the first day of the experiment. The 14920ppm was measured in the middle of the experiment. The 9520ppm was measured on the final day measured of the experiment. 56.84% of the TPH was treated within 22 days. The blue curve represents the amount of TPH evaporated, while the second curve shows the percentage of the evaporation during the experiment. This clearly shows a significant reduction of the TPH along the experiment.

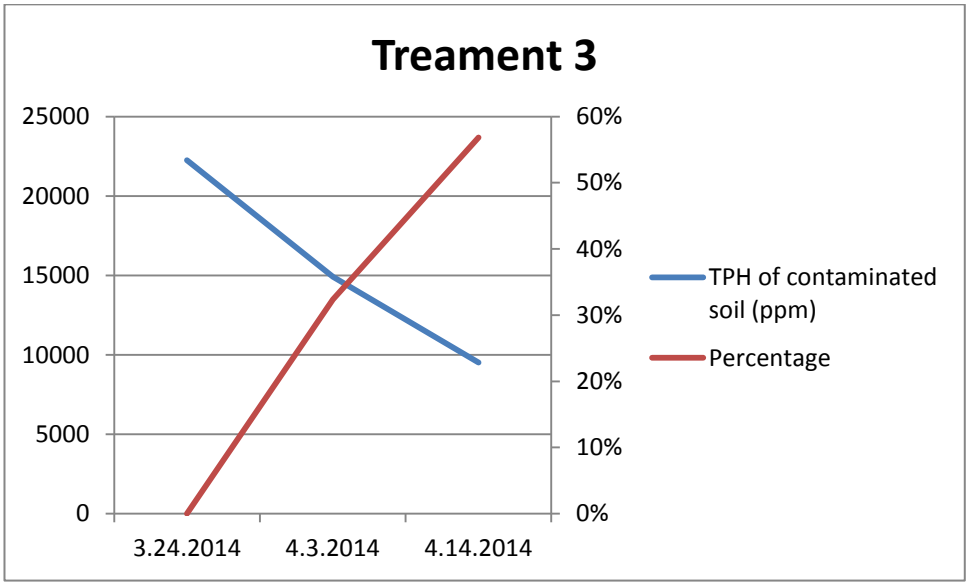


Figure 10. This figure represents the initial, middle and final TPH of the treatment number 3

4 Discussion and Conclusion

The experiment was to test the potential of remediating contaminated soil containing crude oil with sawdust and water, in the experiment four treatments with different proportion of sawdust and water was prepared. The experiment was done in 22 days.

The results obtained during the experiment from the treatment 0, 1, 2, and 3. The result with more reduction of hydrocarbons was the treatment number 1, in the figure 6 shows an reduction of the TPH during the first 11 days of the treatment number 1, which was recorded to be a 27.15% reduction of its amount. While for the rest of the experiment the amounts of the TPH kept on reducing but slower than the treatment number 1. The total reduction of the treatment number 1 in just 22 days was 65.14%. This clearly shows the potential of using sawdust combined with water to bio remediate contaminated soils with crude oil.

However during this bio process many other physicochemical, hydrological and microbiological factors interfere with the efficiency of such bio remedial work. These factors are mentioned and explained as follows.

One way to improve this experiment would be rather to use some reflective material, which will spread the light more evenly for the samples; for instance aluminium foil.

4.1. Temperature

Temperature has a considerable influence on petroleum biodegradation by its effect on the composition of microbial community and its rate of hydrocarbon metabolism, and its physical nature and chemical composition of the oil (Atlas 1981)

Some small alkane's component of petroleum oil are more soluble at $0^{o}C$ than at 25 ^{o}C (Polak and Lu 1973) and elevated temperature can influence non-biological losses, mainly by evaporation.

In some cases the decrease in evaporation of toxic components at lower temperature was associated with inhibited degradation (Foodgate 1984)

Atlas and bartha (1992) found that the optimum temperature for bioremediation of mineral oil hydrocarbons under temperature climates is in the range of 20-30 ^{o}C. (Milton and Rachakonda 2005, 179)

4.2. Moisture

The moisture effect in this experiment is a key role to find the setups that will have the significant loss of TPH. The moisture will also affect the growth of bacteria and with the temperature of the experiment in the range of 20 to 30ºC the humidity will evaporate quickly and will release the TPH.

Bacteria rely upon the surrounding water film when they exchange materials with the surrounding medium through the cell membrane. At soil saturation, however, all pore spaces are filled with water. At a 10% moisture level in soil the osmotic and matrix forces may reduce metabolic activity to marginal level. Soil moisture level in the range of 20-80% of saturation generally allows suitable biodegradation to take place. (Milton and Rachakonda 2005, 180)

Leahy and Colwell (1990) in their review of microbial degradation of hydrocarbons in the environment suggested that hydrocarbon biodegradation in terrestrial ecosystem may be limited by the water available (a_w ranges from 0.0 to 0.99) for microbial growth and metabolism. Optimal rates of biodegradation of oil sludge in soil have been reported at 30% to 90% water saturation. (Milton and Rachakonda 2005, 182) Evaporation is usually the most important weathering process as it has the greatest effect on the fate of oil. At 15^{o}C and over a two day period, gasoline evaporates completely, while about 60% of diesel fuel evaporates, about 40% of a light crude, about 20% of a heavy crude, and about 3% of Bunker C. The formation of water in oil emulsions is the second most important weathering process because it can drastically change the properties of the oil. Example, a liquid oil can become a viscous and heavy mass (M. Fingas 1978, 41).

4.3. The role of Sawdust

Sawdust was used in this experiment to prove a point, was to prove that the bioremediation can be done by just using bio-product. Sawdust in this case was the absorbent of the crude oil and water added in the setups of the experiment.

Sawdust had absorb the crude oil and water of the each setups, by absorbing it the sawdust degraded and created bacteria that had eaten the hydrocarbons and reduced the pollutant in the contaminated soil.

In this experiment sawdust and soil was used as carbon source, sawdust is wood bio product according to (MSDS 2), which represents the main source of Carbon.

4.4. Oxygen

In most petroleum contaminated soils, sediment, and water, oxygen usually is the limiting requirement for hydrocarbon biodegradation, because the bioremediation methods for reclamation of these contaminated sites are mainly based on aerobic process. Bacteria and fungi in their breaking down of aliphatic cycle and aromatic hydrocarbons involve oxygenize enzymes for which molecular oxygen is required. (Milton and Rachakonda 2005, 180)

4.5. pH

In soil and poorly buffered treatment situations, organic acids and mineral acids from various metabolic processes can significantly lower the pH. The overall biodegradation rate of hydrocarbons is generally higher under slightly alkaline condition.

An appropriated monitoring and adjustments should be made to keep such systems in pH range of 7.0 – 7.5. The pH of the soil is an important factor for anthracene and pyrene degradation activity of introduced bacteria. (Milton and Rachakonda 2005, 181)

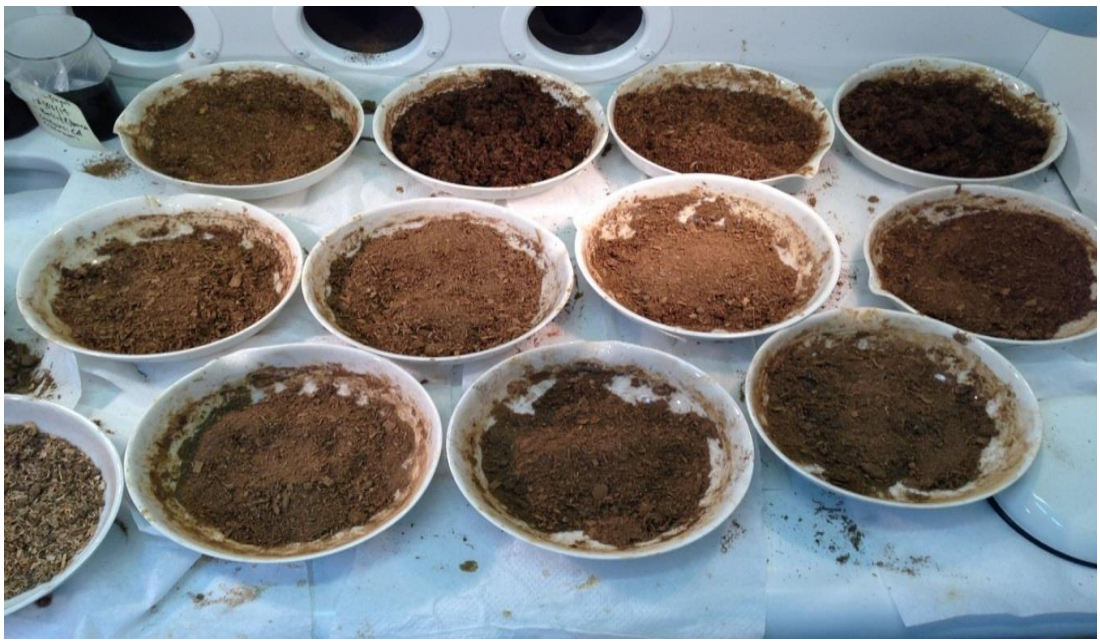
The beginning of the experiment the contaminated soil was like mud soil all wet and more or like clay soil. Picture number 8 was taken after three days of the experiment. The difference is that, the soil before the treatment was dark muddy and one could smell the HC from far away. After the tenth day of the experiment as it shows in the picture number 9, the contaminated soil was much lose and was changing to lighter brown color.

Picture 7. Soil outlook after the third day of the experiment (Rodolfo Casimiro 2014)

Picture 8. Pre-treated soil, picture taken after the tenth day of the experiment (Rodolfo Casimiro 2014)

At this stage of the experiment after 11 days the hydrocarbon in the soil are starting to become lose and mixing with sawdust and water. Now with the temperature higher than 25^0C the evaporation will start to take place which is beneficial to the experiment, meaning that the rapidly the water evaporate, more water have to be added in order to have the amount of humidity according to the table 1. By doing this the percentage of hydrocarbon contamination will decrease and the sawdust will decompose faster.

The picture number 10 was taken after 22 days one can observe that the soil is very lose and some treatments are dryer than others. If we have to compare to the initial contaminated soil, picture 10 looks much better and treated. The picture number 10 was taken in the last day of the experiment, although it look very loose but not totally remediate, one can still smell a little bit of hydrocarbon in the soil. In this picture number 10 not all the sample was dry, the reason is because of the light, some of the sample did not get enough light (temperature), the space was very small not enough room for the air to circulate and take the smell away.

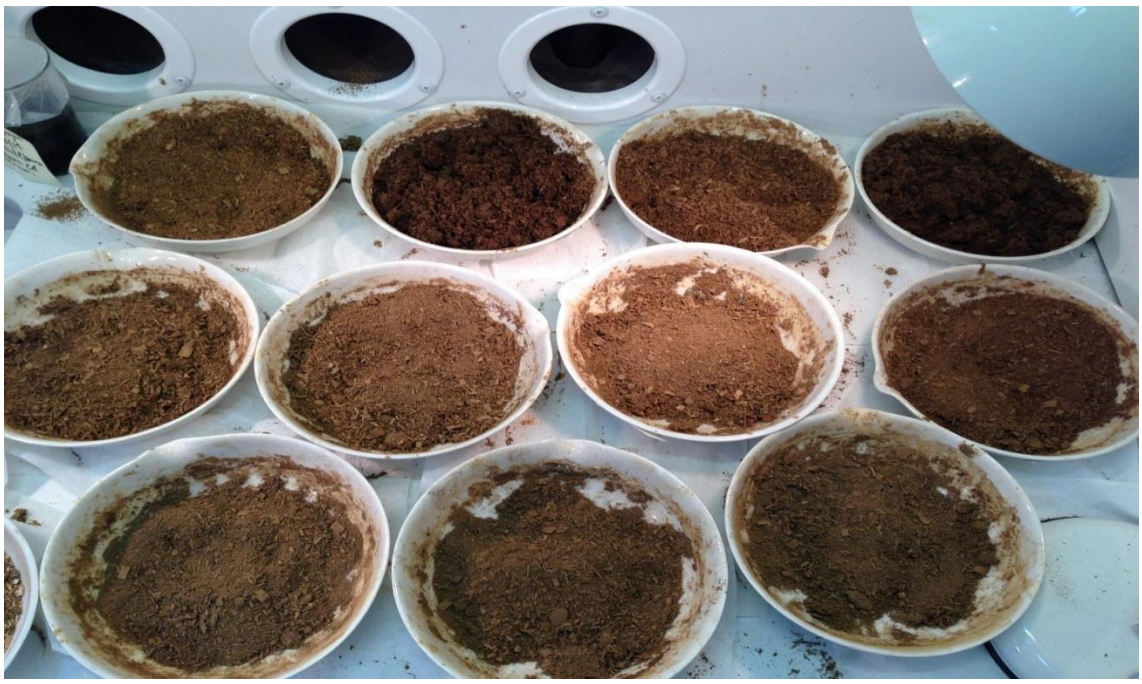


Picture 9 The treatment result after 22 days of the experiment (Rodolfo Casimiro 2014)

The treatment number 1 the contaminated soil was reduced 65.14% of the TPH was treated just in the period of 22 days (see picture 11), the final TPH of the treatment number 1 was 7690 ppm.

According to the setup and the results given in this report it could be possible to experiment this process in large scale. The all idea of the experiment was to find out if this experiment had the potential to be treated in large scale to and with available materials locally.

The procedure performed is simple and inexpensive. It allows the owners of the land polluted with crude oil to be able to clean up their land and soil for the better environment. This procedure allows giving the soil biological activity which it did not have before.



Picture 10 Final outlook of the soil samples after 22 days (Rodolfo Casimiro 2014)

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6 APPENDICES

6.1. Appendix 1

QUALITY STANDARDS AND RECOMMENDATIONS FOR WATE
INTENDED FOR HUMAN CONSUMPTION

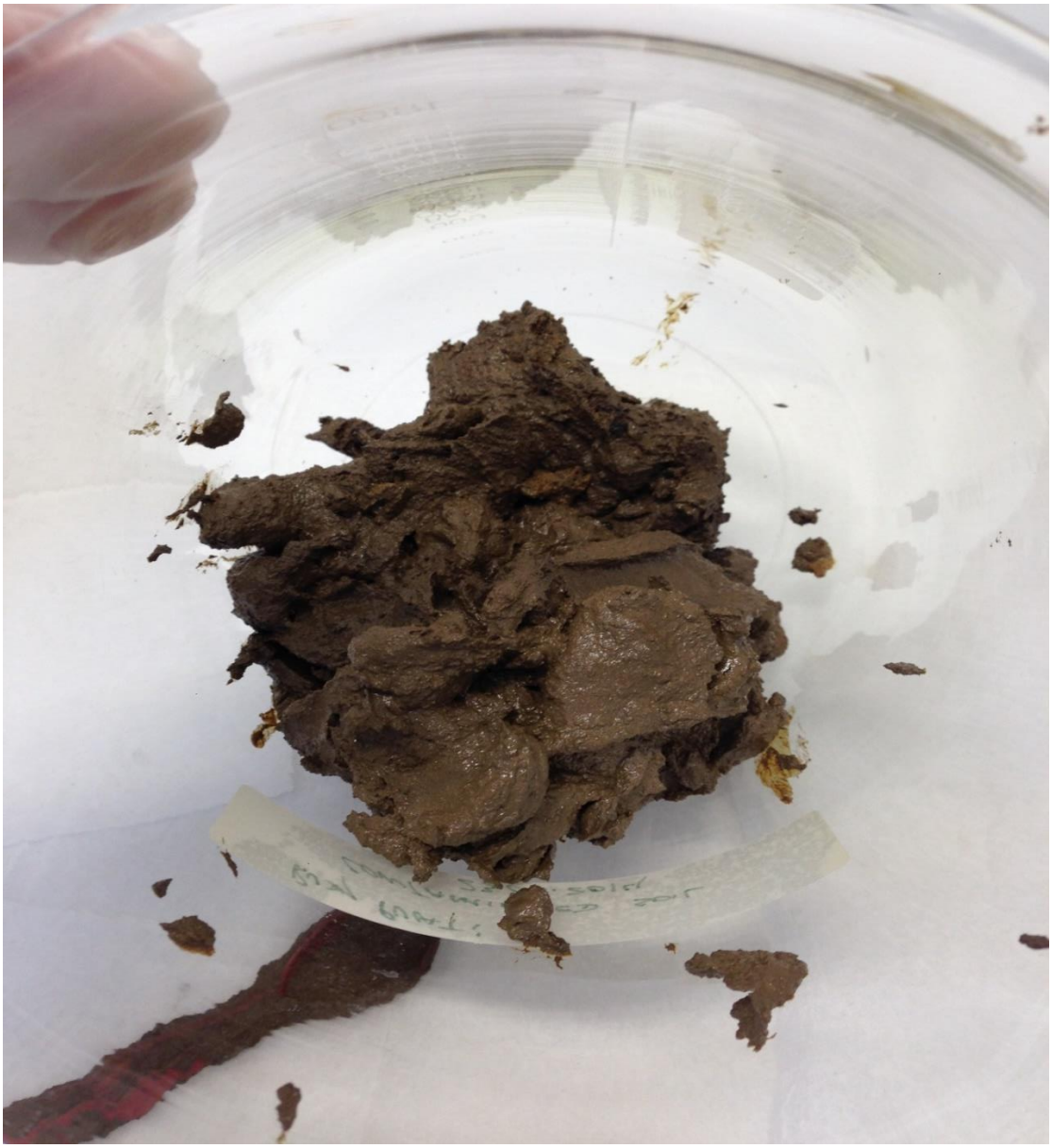
Table 1. Microbiological standards (maximum density)

Escherichia coli	0 cfu/100 ml
Enterococci	0 cfu/100 ml

Table 2. Chemical standards (maximum concentration)

			Note
Acrylamide		0,10 µg/l	(1)
Antimony		5,0 "	
Arsenic		10 "	
Benzene	1,0 "		
Bentso(a)pyrene		0,010 "	
Boron		1,0 mg/l	
Bromate	10 µg/l	(2)	
Cadmium		5,0 "	
Chromium			50 "
Copper		2,0 mg/l	(3)
Cyanide	50 µg/l		
1,2-dichloroethane		3,0 "	
Epichlorohydrin		0,10 "	(1)
Fluoride	1,5 mg/l		
Lead		10 µg/l	(3)
Mercury	1,0 "		
Nickel		20 "	(3)
Nitrate (NO_3^-)		50 mg/l	(4)
Nitrate nitrogen (NO^-_3-N)		11,0 "	
Nitrite (NO_2^-)		0,5 "	(4)
Nitrite nitrogen (NO_2^--N)		0,15 "	
Pesticides		0,10 µg/l	(5 and 6)
- " - total		0,50 "	(5)
Polycyclic aromatic hydrocarbons		0,10 "	(7)
Selen	10 "		
Tetrachloroethene and Trichloroethene, sum	10 "		
Trihalomethanes, total		100 "	(2 and 8)
Vinyl chloride		0,50 "	(1)

6.2. Appendix 2 Picture of contaminated soil with crude oil before treatment.

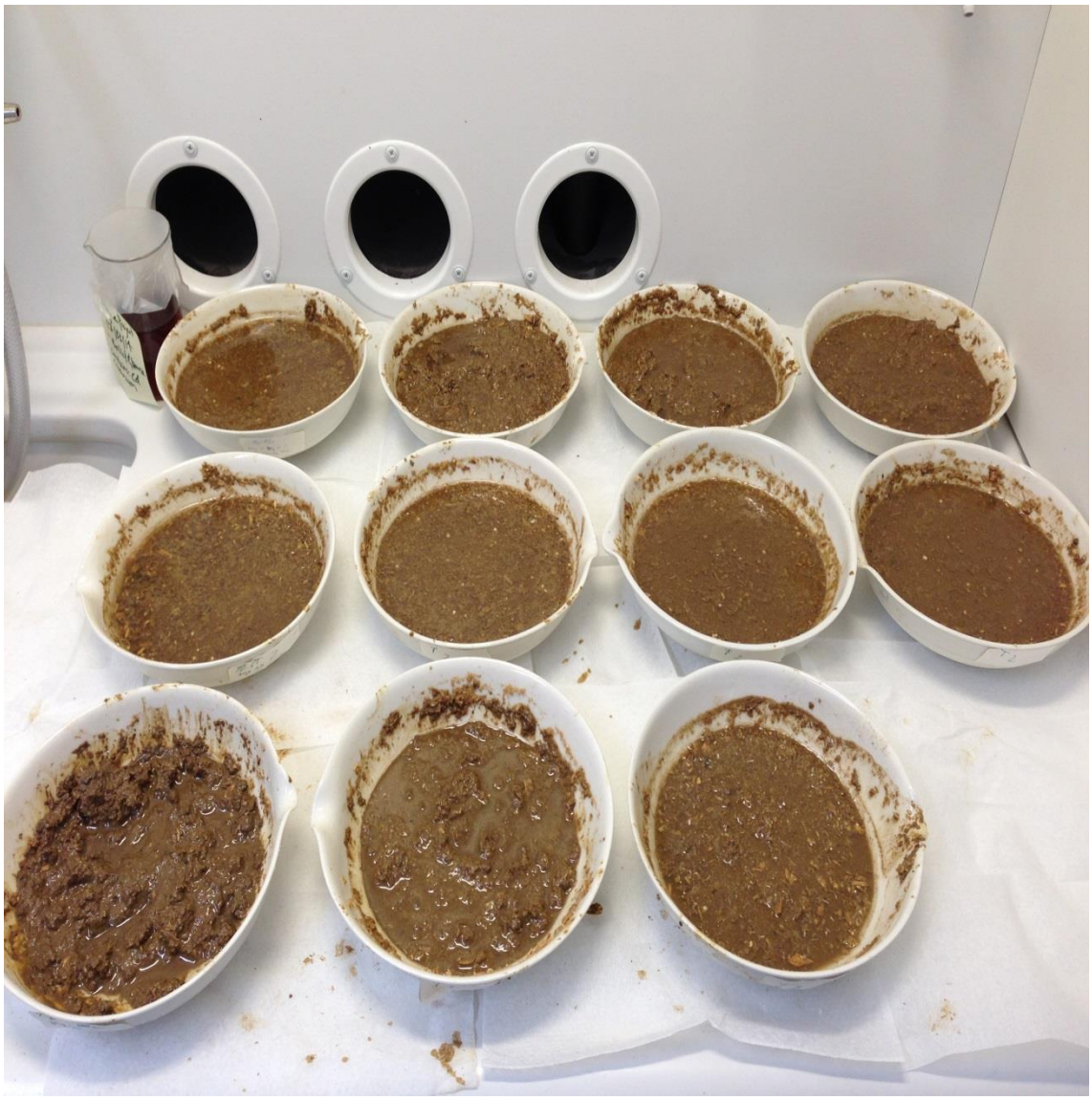


6.3. Appendix 3

Sawdust and contaminated soil before the mixing

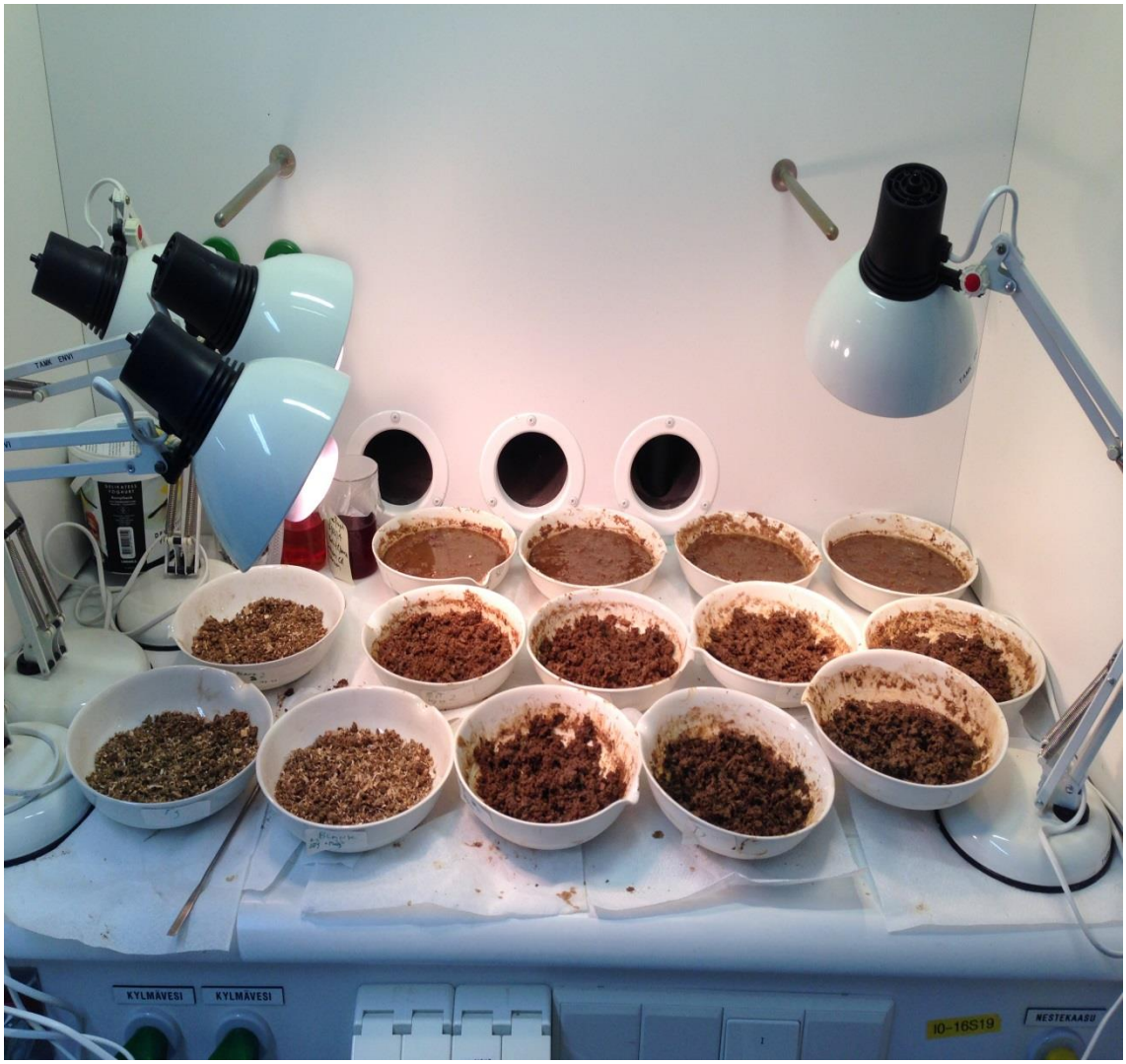
6.4. Appendix 4

Sawdust + contaminated soil + water, after the mixing



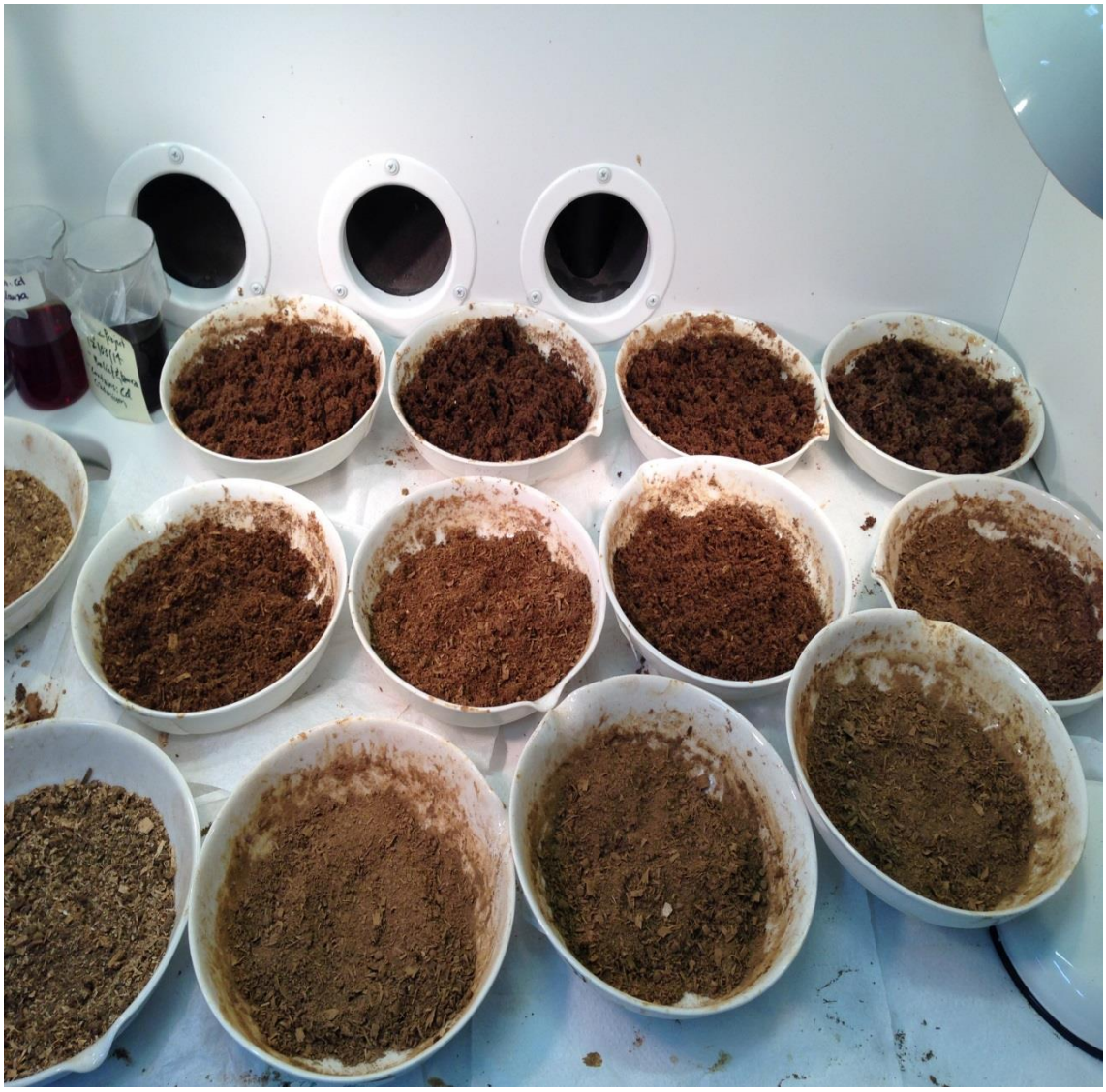
6.5. Appendix 5

The treatment under the hood been monitored.



6.6. Appendix 6

The treatment dried under the hood



6.7. Appendix 7

The soil mixture at the end of the treatment after 22 days of the experiment