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Research Article

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The Effects of the Application of Random Sampling on the Analysis of the Biodegradation of Oil Spills in Soil

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Abstract

Random sampling is a part of the sampling technique in which each sample has an equal probability of being chosen. A sample chosen randomly is meant to be an unbiased representation of the total population. If for some reasons, the sample does not represent the population, the variation is called a sampling error. Thus, an unbiased random sample is important for drawing conclusions in all issues, especially when the issue relates to environmental cleanup. Biodegradation focuses largely on the cleanup of petroleum hydro carbons and the past decades have seen challenges arise in order to make biodegradation technology appropriate and productive. This is because oil spills composition varies in locations as crude oil is a mixture of thousands of organic compounds that vary from one source to another.

The purpose of this work was to determine the reliability of the random sampling method on oil spills which clean up sometimes cause s more harm than the oil spill itself. Random sampling is the basis for all probability sampling techniques used in soil sampling and serves as a reference point from which modifications to increase the efficiency of sampling are evaluated. Therefore its effectiveness was measured using Oil and Grease and Total Petroleum Hydrocarbon analysis of the biodegraded soil.

Keywords: Random sampling, biodegradation, analysis sampling errors, microorganisms

Introduction

Random sampling is a part of the sampling technique in which each sample has an equal probability of being chosen. A sample chosen randomly is meant to be an unbiased representation of the total population. If for some reasons, the sample does not represent the population, the variation is called a sampling error.

Random sampling is one of the simplest forms of collecting data from the total population. Under random sampling, each member of the subset carries an equal opportunity of being chosen as a part of the sampling process. For example, if the total number of objects in a study group is 200 and to conduct a survey, a sample group of 20 objects is selected to do the survey, the population is the total number of objects and the sample group of 20 objects is the sample. Each object in the groups has an equal opportunity of being chosen because all of the objects which were chosen to be part of the survey were selected randomly. Nevertheless, there is always a distinct possibility that the group or the sample does not represent the population as a whole; in that case, any random variation is often dismissed as a sampling error.

However, an unbiased random sample is important for drawing conclusions in all issues, especially when the issue relates to environmental cleanup. Hence, variations can occur when drawing conclusions based on the analysis of the 20 sample and one of the disadvantages of random sampling is the fact that it requires a complete list of the total objects in the original 200. Thus, if a researcher is planning to carry out an environmental survey of a polluted area and intends to deploy random sampling, it must be recognized that there will be local (and regional) variations in the area to be samples especially, in the current context, when soil is the material on to which the spilled chemical is spilled (deposited). Variations in soil make up such as the relative amounts of organic matter, minerals, gases, liquids, and organisms that together support life in the soil. In addtion to such variations in soil character on one site, the potential for variations from site-to-site is potentially enormous. In such cases, the effects of any random sampling protocol on the analytical outcome must be given very serious consideration.

It is the purpose of this work to report the effects of random sampling on the outcomes of the analysis of spills, such as spills of crude oil, on soil and the meaning of the data in terms of any trends in the random sampling method and the precise variation of the trends when a sample of crude oil is added to soil.

Crude Oil Spills and Biodegradation

From a chemical standpoint petroleum is an extremely complex mixture of hydrocarbon compounds, usually with minor amounts of nitrogen-, oxygen-, and sulfur-containing compounds as well as trace amounts of metal-containing compounds. Thus crude oil is not a uniform material and each of the chemical types within the oil will behave differently when in contact with an adsorbent, such as soil [1,2]. This is particularly true when the material involved in the spillage in heavy crude oil.

Spillage of crude oil can occur from a number of sources, including tankers and oil extraction and storage facilities. In the case of spills on to the land, to prevent widespread damage when spills occur, responding personnel attempt to contain the spill using absorbent barriers. Another useful method is *in situ burning*, where oil is burned off the surface of the soil, which can lead to atmospheric pollution. New bioremediation technologies are constantly being developed using microorganisms to break the hydrocarbons down into less harmful compounds. But, because it is impossible to completely eliminate the risk of an oil spill during the extraction, processing, and transportation of oil, it is important to have a well-designed plan in place that can be used for cleanup in order to limit negative impacts. However, the negotiation of increasingly stringent rules and regulations, along with continued research, make it possible for oil spills to become even more sporadic and less catastrophic. Another method of cleanup is biodegradation but, whichever method is chosen, there is the need for careful analysis to determine the extent of the spill, the extent of any of the oil constituents adsorbed on to the soils, the effectiveness of the cleanup method. It is the biodegradation method and the reliability of the analytical data that is the subject of this work.

Biodegradation is one of the most primary mechanisms to remove petroleum hydrocarbons from polluted environments. It is a feasible and cheap method of dealing with widespread environmental contaminations because it utilizes indigenous materials when compared to customary (physical and chemical) remediation methods. Also, the microorganisms engaged are capable of performing almost any detoxification reaction. Biodegradation studies provide information on the fate of a chemical or mixture of chemicals (waste streams, oil spills, etc.) in the environment. It is nature's way of getting rid of wastes by breaking down organic matter into nutrients that can be used by other organisms.

The commercial practice of biodegradation society focuses primarily on the cleaning up of spilled petroleum hydrocarbons. Therefore it has been a challenge to make biodegradation technology appropriate and productive. This requires an analysis of the contaminated sites, find the best method suited for the environment, optimize these techniques and even assist in the emergence of new techniques.

However, bioremediation is only as successful as the ability of the microorganisms to convert the petroleum hydrocarbons to benign products and return the affected (contaminated) area to its original condition. This, in turn, is dependent upon the extent of the contamination of the soil and the ability of the microorganisms to convert the spilled oil. Thus, before and during the bioremediation process, analyses are necessary to determine (i) the horizontal extent of the contaminated area, (ii) the vertical extent of the contaminated area, (iii) the concentration of oil in the contaminated area, and (iv) ensuring that the amount of microorganisms to be used is adequate to the clean-up task.

Soil testing has become an important method tool for assessing soil contamination and for arriving at proper clean-up recommendations. Soil testing is also a valuable management aid for studying soil changes resulting from oil spills and for diagnosing specific bioremediation methods. However, soil variability and dispersion of the oil spill is a major concern when deciding how to collect a representative soil sample. Soil samples submitted for analysis should be representative of the affected area. Therefore, sampling from a part of the affected area may not always produce a typical (or average) representation of the magnitude of the oil spill.

The ability to collect and preserve a sample that is representative of the site is critically important step [3]. Obtaining environmental samples is always a challenge, due to heterogeneity of different sample matrixes. For soil sampling, four variables are generally considered. These are the spatial distribution of samples across the landscape, the depth of sampling, the time of year when samples are taken and how often an area is sampled. Presently, the sampling methodologies on oil spills vary tremendously. This is because of the challenges in sampling solids for environmental analysis to collect a relatively small portion of the sample that accurately represents the composition of the whole [1]. Therefore imprecise, inaccurate, and inconsistent soil sampling techniques are a major source of uncertainty in calculations.

Soil samples can be collected using a variety of methods depending on the depth of the desired sample, the type of sample required (disturbed vs. undisturbed), and the soil type. These soil sampling techniques range from sub sampling or two-stage sampling, double sampling, composite sampling, random sampling, and stratified sampling. But the basis for most sampling plans in environmental sampling is the concept of random sampling. With random sampling each sample point within the site has an equal probability of being selected [4].

Therefore, in the study and selection of a site, the main consideration in physical soil sampling is the method of randomization of the soil samples because the sample distribution usually depends on the degree of variability in a given area. For those situations where there is inadequate information for developing a conceptual model for a site or for stratifying the site, it may be necessary to use a random sampling design.

In terms of sampling environmental items such as soils, the sample must be chosen from a population for investigation. A random sample is one chosen by a method involving an unpredictable component. Random sampling can also refer to taking a number of independent observations from the same probability distribution, without involving any real population. A probability sample is one in which each item has a known probability of being in the sample.

As part of the sampling protocol, a simple random sample is selected so that all samples of the same size have an equal chance of being selected from the population. Alternatively, a self-weighting sample (also known as an equal probability of selection method, EPSEM) is one in which every individual, or object, in the population of interest has an equal opportunity of being selected for the sample. Simple random samples are self-weighting. On the other hand, stratified sampling involves selecting independent samples from a number of subpopulations, group or strata within the population. Great gains in efficiency are sometimes possible from judicious stratification. Finally,cluster sampling involves selecting the sample units in groups. The analysis of cluster samples must consider the intra-cluster correlation which reflects the fact that units in the same cluster are likely to be more similar than two units picked at random.

Whatever method is employed, the sample usually will not be completely representative of the population from which it was drawn– this random variation in the results is known as sampling error. In the case of random samples, mathematical theory is available to assess the sampling error. Thus, estimates obtained from random samples can be accompanied by measures of the uncertainty associated with the estimate. This can take the form of a standard error, or if the sample is large enough for the central limit theorem to take effect, confidence may be calculated.

Random sampling is the basis for all probability sampling techniques used in soil sampling and serves as a reference point from which modifications to increase the efficiency of sampling are evaluated. Where there is a lack of information, as with oil spills, the simple random sampling design is the only design other than the systematic grid that can be used. The simple random sampling is the basis for all probability sampling techniques used in soil sampling and serves as a reference point from which modifications to increase the efficiency of sampling are evaluated.

Soil samples used in various studies are usually collected from various locations and depths on the contaminated sites [5]. Random sampling has been employed in past investigations of oil spills where the sampled soil was used for Total Petroleum Hydrocarbon analyses, testing the effect of various concentrations of crude oil on fungal populations of soil [6]. Hydrocarbondegradation of refined petroleum hydrocarbon in soils treated with 5% gasoline, kerosene and diesel oil that was being investigated or even crude oil that was isolated from oil-contaminated soil in Bangkok, Thailand [7-9].

The purpose of this work was to determine the reliability of the random sampling *method* after an oil spill recognizing that when an oil spill occurs the oil is not distributed evenly on the oil either horizontally and vertically. Furthermore, the oil does not always penetrate deeply into the oil – adsorption occurs and the maximum depth of vertical penetration in many cases is less than four inches, assuming sampling is taken immediately after the spill. In addition, oxidation of the oil occurs as soon as the oil is exposed to the air and the oxidation processes produced chemical functionality within the formerly hydrocarbon oil that enhances adsorption of the oil on to the soil. Soil containing clay constituents is an even stronger adsorbent for non-oxidized oil and for oxidized oil.

Materials and Methods

Twelve wooden trays (1m x 1m) were filled with approximately 30 to 33kg of virgin soil collected from flat land in Central Trinidad. The soil was placed in the trays and was covered with varying volumes of oil randomly spilled; this was done through a simulated oil spill. The soil placed in Tray 1 and 2 were heated in an oven to serve as the controls for the experimental work completed over a six month period. This was to destroy any indigenous microbes that were in the soil so as to analyze the effects of natural conditions on the soil.

The remaining trays were set up according to a designed plan (Table 1). Oil/soil sample was collected from wooden trays using a random sampling method on a weekly basis based on the grids in the wooden trays. The microorganisms present in trays 3 to 12 were the indigenous microorganisms found in these soil samples, no additional microorganisms were added.

Table 1: Trays arrangement for experimental work

Tray Number	Microorganisms Present In The Soil	Soil Samples Were Tilled	Volume of Oil Added To The Tray/mL
1	No	No	1500
2	No	Yes	1500
3	Yes	Yes	500
4	Yes	No	500
5	Yes	Yes	1000
6	Yes	No	1000
7	Yes	Yes	1500
8	Yes	No	1500
9	Yes	Yes	2000
10	Yes	No	2000
11	Yes	Yes	3000
12	Yes	No	3000

Size of trays: 100 cm x 100 cm, depth:15 cm

The microbes were tested by using an adaptation of pour plate method [10]. The pour plate method was used with an incubation temperature of 35°C (95°F) for 48 hours, using plate count agar. The composition of the oil sample was analyzed by magnetic resonance spectrometry, UV/visible spectrometry and infrared spectrometry.

The oil and grease and total petroleum hydrocarbon analysis of the soil used in biodegradation were completed using sox let extraction for oil and grease) and n-hexane gravimetric analysis (total petroleum hydrocarbon (EPA Method 3540C). For the oil and grease analysis, the solid sample was mixed with anhydrous sodium sulfate, placed in an extraction thimble and extracted using dichloromethane in the sox let extractor. In the method, the solvent is heated and refluxed through the soil sample continuously for approximately sixteen hours. The extract was then dried, using the rotary evaporator with aspirator pump and sample weighed.

For total petroleum hydrocarbon, the samples were randomly taken from the trays to create a composite final sample for analysis. Hexane was used to extract total petroleum hydrocarbon from the soil before determination by gravimetric analysis. The precipitate was collected by filtration, washed, dried to remove traces of moisture from the solution, and weighed. The amount of analyze in the original sample was calculated from the mass of the precipitate and its chemical composition.

Results and Discussion

Due to the number of variables present in each tray, a vast scatter of data was collected over a six months period. Oil and grease analyses account for the oil and petroleum products plus animal fats, vegetable oils, soaps, and other biological oils in the soil, however the apparent increase in oil and grease content from 15 weeks to 24 weeks cannot be not logically explained.

Oil and grease in the first weeks of biodegradation ranged from 2.6% - 26.5%, while at the end, values were 1.75% - 13.3% (Figure 1). As the oil to soil ratio increased the percentage of oil and grease degraded decreased. The overall fluctuations in the biodegradation graph was as a result of the random sampling method used to collect the samples, although the representative samples were taken with the assumption that the oil was evenly placed on the soil samples.

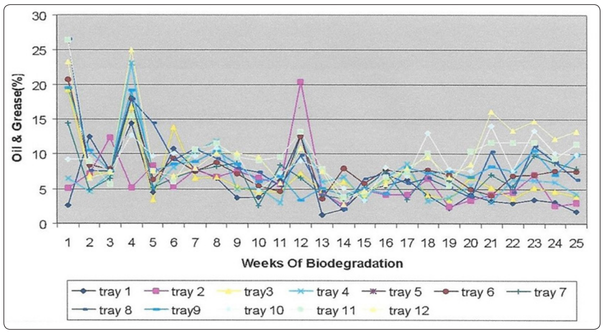

Figure 1: Oil and grease results on the twelve trays

Simple random sampling is the basis for all probability sampling techniques used in soil sampling and serves as a reference point from which modifications to increase the efficiency of sampling are evaluated. Given the nature of the study where there is a lack of information about the area to be studied and about the pollution distribution, the simple random sampling design is best suited. Random sampling gives a suitable indication as to what is happening with time. From the results obtained from this sampling methodology, specific test methods can be implemented for further analysis.

The total petroleum hydrocarbon (TPH) results (Figure 2) also showed fluctuations from the initial to final stages, with a steady decrease in the overall percentage. The 4th month (week 14) showed a drastic lowering of TPH in all the soil samples as compared to the other weeks. This occurrence also took place for oil and grease on the 15th week. These results showed that the 4thmonth was the optimum month for biodegradation of the oil samples based on random sampling on the soil.

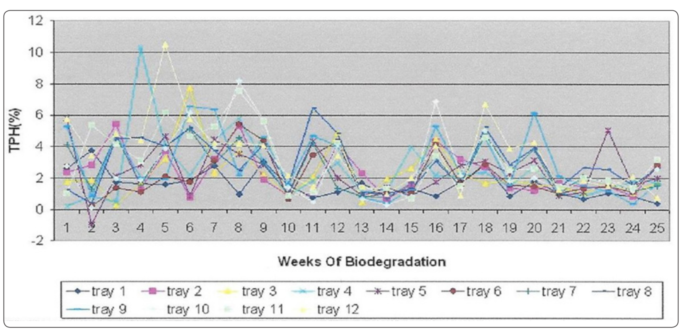

Figure 2: Total petroleum hydrocarbon results on the twelve trays

At the same time, the regression analysis (Figure 4) shows that the amount of total petroleum hydrocarbons (TPH) in the soil decreases steadily over the 24-week time period. It is anticipated that the material remaining in the soil is the residue from the oil that is not extractable by the method for total petroleum hydrocarbons. In summary, the bacteria preferentially consume the volatile hydrocarbon constituents of the sample before decreasing in numbers due to the toxicity of the non-volatile constituents start to overcome the bacteria and reduce their population count. Whilst the continuing decrease in the total petroleum hydrocarbons and the decrease in the oil and grease (up to 14 weeks) is in keeping with the known actions of bacteria on hydrocarbons in soil, the oil and grease data in the post-14 week period leaves questions about the interpretation of the data and, particularly, about the reliability of the regression analysis.

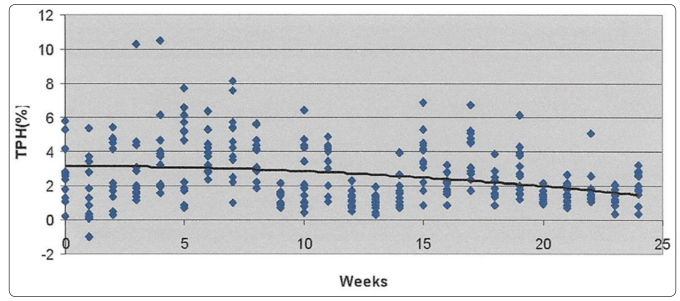

Figure 4: Total petroleum hydrocarbon regression curve for the sixmonths period

The scatter of points (Figure 1, Figure 2, Figure 3 and Figure 4) are all the data obtained for oil and grease and TPH analysis gathered for the twelve trays over the six months period. In particular, the data (Figure 3) show that using regression analysis gives minimum oil and grease content in approximately 14 weeks after beginning the experiment. The samples collected were not mixed collectively but were analyzed as individual samples. This was because each sample was exposed the varying volumes of oil in the soil and different methods of aeration (tilled vs. untilled). Therefore the integrity of the individual samples was not disturbed. The vast range in the scatter of data for biodegradation may be related to the four basic problems of sampling frames. These four problems are missing elements, foreign elements, duplicate entries and groups (clusters). Each element of the frame has an equal probability of selection: the frame is not subdivided or partitioned.

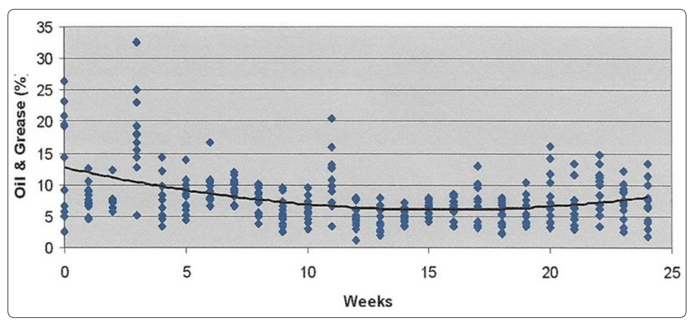

Figure 3: Oil and grease regression curve for the six months period

Furthermore, any given pair of elements has the same chance of selection as any other such pair (and similarly for triples, and so on). This minimizes bias and simplifies analysis of results. In particular, the variance between individual results within the sample is a good indicator of variance in the overall population, which makes it relatively easy to estimate the accuracy of results. However, simple random sampling can be vulnerable to sampling error because the randomness of the selection that may result in a sample that doesn't reflect the makeup of the population. Random sampling assumes that the units to be sampled are included in a list, also termed a sampling frame. This is usually not the case given the nature of the region to be sampled.

Conclusions

Random soil sampling has been, and remains, the traditional approach for areas where a spill is uniform. Random sampling provides an average of all cores taken throughout the affected area and is not always truly representative of the dispersion of the oil.

Using random sampling in this experimental work on biodegradation of heavy oils helped determine (but did not conclusively identify to what extent) that (i) there is a decrease in the amount of total petroleum hydrocarbons in the soil over the 24-week period of the work, (ii) there is a decrease in the amount of oil and grease over the initial 14-week period, and (iii) the regression analysis is dependent upon the method used and can present erroneous data.

Moreover and particularly important, is that observation that in spite of random sampling method showing trends, the precise variation of the trends could only be roughly estimated because of the variability of the data derived using the random sampling method.

The amounts of contamination varied significantly from one point to another and the level of soil contamination and impact of oil on the soil quality greatly depended on the means by which the oil was dispersed as a function of the spill. If follows that the oil has the potential to cause major changes in the chemical properties of the soil and to determine the success of the bioremediation process.

Random sampling is beneficial for unknown parameters, but the inconsistencies that are shown in the results from this experimental work proves that random sampling does not assist in making accurate decisions. On large scale field testing, random sampling would affect the accuracy of in situ and ex situ restoration of contaminated soil. Therefore it is more adaptable to situations where general conclusions are required as opposed to accuracy.

Oil spills are never evenly distributed in the soil; therefore the process of developing bioremediation techniques depends on the results obtained from the samples collected. As a result, if the collected sample is not representative of the overall contaminated site, the resulting remediation process would not be as effective as desired.

Furthermore, the random sampling method should only be used when there is limited data on the area being investigated. After which, deductions based on the results would assist in choosing a more appropriate sampling method for data collection.

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