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A comparative study on effectiveness of soapnut, rhamnolipid and EDTA in cleaning diesel oil contaminated soil from a commercial site in Edinburgh

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ABSTRACT— This study presents performances of soapnut, rhamnolipid and EDTA in washing soil contaminated with diesel oil. The soil was collected from a business park in Riccarton area of Edinburgh. The soil was contaminated with vehicle grade commercial diesel oil (BS EN590) available from a local gas station. While soapnut is derived from plant Sapindus mukorossi, rhamnolipid is a class of microbial surfactant produced by Pseudomonas aeruginosa and EDTA is an amino-polycarboxyilic acid, a synthetic chemical, widely used as a cleaning/chelating agent in industry. The optimum operating conditions for all three cleaning agents were established by Box-Behnken method. When such operating conditions were used for column washing, EDTA had the highest cumulative removal efficiency of 43.3%, followed by rhamnolipid and soapnut, which recorded 40.7% and 39.3% respectively. The cumulative diesel oil removed by distilled water was 3.7%. However, considering the low cost of soapnut, it will be more economical to use soapnut than Rhamnolipid or EDTA.

KEYWORDS: Soil cleaning, diesel oil, soapnut, EDTA, rhamnolipid

1. INTRODUCTION

Petroleum products often contaminate land surrounding petrol stations, ports and service terminals, where oil is handled or distributed [1]. Some of the health issues, which have been related to oil contamination include infections and parasitic diseases as well as diseases of the blood, skin and heart [2]. Diesel oil is a complex mixture of hydrocarbons, primarily containing saturated hydrocarbons as well as low and high molecular weight poly-aromatic hydrocarbons (PAHs) [3]. Diesel oil contamination of soil and groundwater can occur due to pipeline leakages, accidental spills or leakage from storage tanks and automobiles [4]. Remediation of contaminated land has therefore become an urgent need for many countries, encouraging the growth of various technologies, which could be used to ameliorate oil contamination. Some of the remediation technologies which have been recorded to minimise diesel oil contamination include electro-remediation technology [5], use of Fenton's reagent (hydrogen peroxide with ferrous iron) [6], solidification and stabilization [7] and ozonation [8]. Other remediation techniques, which have recorded success in the treatment of soil contaminated by diesel oil include bioventing and composting [9], treatment by jet-fluidized bed [10] and bio-stimulation [11]. The use of surfactants in remediation of contaminated soil is a technique that has been explored for various pollutants. This technology has recorded significant success in the removal of heavy metals, persistent organic chemicals, crude oil, and its products. The mechanism of removal of oil from soil with surfactants works in a manner that enhances removal of the non-aqueous phase liquid (NAPLs) by reducing their surface/interfacial tension at air-water as well as water-oil interfaces [12]. Surfactants are classified based on their hydrophilic group such as ionic, non-ionic, and cationic. They can also be classified based on their origin as synthetic surfactants and biosurfactants. Those produced from materials that are from chemical sources are classified as synthetic surfactants while those produced from materials that are from biological sources are classified as biosurfactants [13]. Biosurfactants are either plant based or microbial surfactants. Plant based surfactants are surface agents produced from plant seeds, leaves and other parts of plants while bacteria and yeast produce the microbial surfactants. Biosurfactants are anionic in nature and far less toxic than synthetic surfactants. They are biodegradable and possess good surface-active properties [14]. Soil washing by surfactants has received growing attention as many studies have investigated their ability in solubilisation and biodegradation of different NAPLs [15], [12], [16]. The technology has recorded great success in the washing of heavy metals, pesticides and oil from soil [17], [18], [19], [20], [21], [22], [23], [24].

1.1 Rationale for the study

Diesel oil is a complex hydrocarbon and any spill has chronic and acute effects on the environment. For example, diesel oil contaminated soil has serious adverse effects on plant germination and growth [3]. Once the soil is contaminated, it becomes not only difficult for the cultivation of crops but also unsafe for residential development as it presents health risks and high chance of groundwater contamination. For redevelopment of such contaminated lands, which have been termed as "brownfields", and for the restoration of the soil fertility, removal of contaminants is necessary to safeguard public health and comply with local legislation. This requires the design, development, and implementation of soil washing techniques.

1.2 Aim and objectives of the study

This study aims to assess the removal of diesel oil from contaminated soil via soil washing, using synthetic surfactants, plant-based surfactants and microbial surfactants in various concentrations and washing methods. To fulfil this aim, experiments were carried out with the following objectives.

(i) Conducting soil washing based on two methods, namely, batch and column;
(ii) evaluating the success of the two different soil washing methods in the removal of diesel oil from contaminated soil;
(iii) assessing the removal of diesel oil components (petroleum hydrocarbons) from the soil;
(iv) evaluating the efficacy of different types of surfactants considering the effects of surfactant concentration, washing time, flow rate and pore volumes on oil desorption from contaminated soil.

2. Methods

For this study, soil was collected from a business park in Riccarton area of Edinburgh. Characteristics of this soil are listed in Table 2. The soil was contaminated with vehicle grade commercial diesel oil (BS EN590) available from a local gas station. Three cleaning agents, namely soapnut, rhamnolipid and EDTA were used to wash diesel-oil contaminated soil in batch and column washing modes, which represent ex-situ and in-situ washing methods in principle.

2.1 Batch experiment

Batch washing was carried out using three different surfactants in three different concentrations for each type. 5 g of the contaminated soil sample was poured into a 250 cm^3 flask. Then 50 cm^3 of known concentration of surfactant solution was poured into the flask. All soil-surfactant slurry samples were shaken in a shaker (Orbital incubator S1600 manufactured by STUART) for 12 hrs, 200 rpm, $28^{O}C$. After washing, the mixtures were allowed to settle for 1 hour. The time of settling was selected based on the type of soil particle following the work published earlier [25]. The supernatant was decanted, and the settled soil was rinsed with 5 cm^3 distilled water by shaking for 5 mins (at 200 rpm, $28^{O}C$). Rinsing was carried out at 200 rpm and 28^{O}C.

2.2 Optimization method

The optimum condition for soil washing was investigated using Box-Behnken experimental design for 3 factors. The three factors Box-Behnken design is illustrated in Fig. 1.

Run	x_1	x_2	x_3
1	−1	−1	0
2	−1	1	0
3	1	−1	0
4	1	1	0
5	−1	0	−1
6	−1	0	1
7	1	0	−1
8	1	0	1
9	0	−1	−1
10	0	−1	1
11	0	1	−1
12	0	1	1
13	0	0	0
14	0	0	0
15	0	0	0

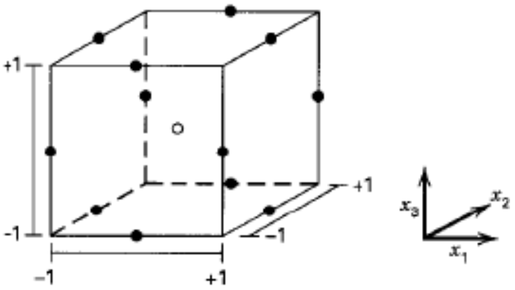

Figure 1: Box-Behnken three factor design

Three factors in the study were, surfactant type (soapnut, rhamnolipd and EDTA), surfactant concentration (0.25%, 0.5%, and 1%), and washing time (6, 12, and 24 hours). A total of 15 batch washing runs were carried out following the Box-Behnken experimental design to model a response surface. Table 1 shows the experimental runs with the set of input parameters which were chosen for this work.

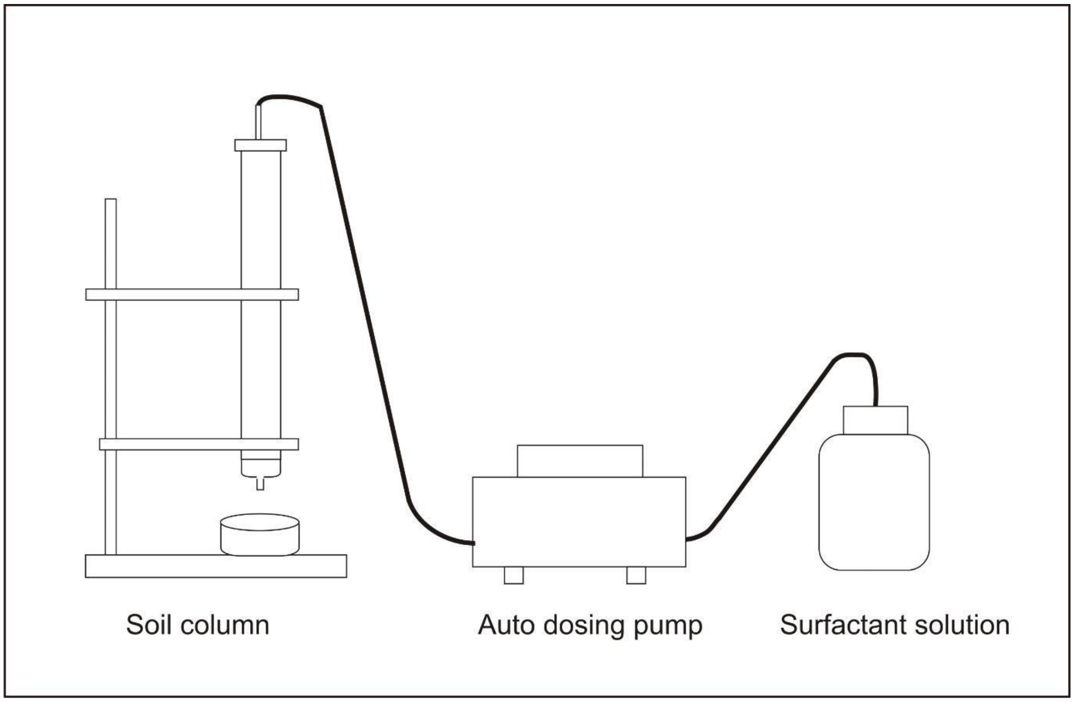

Figure 2: Diagram of column washing experimental set-up

Table 1: Box-Behnken design for the optimization experiment

Run order	Surfactant type	Surfactant concentration (%)	Washing time (Hours)
1	Soapnut	0.25	12
2	Soapnut	1	12
3	EDTA	0.25	12
4	EDTA	1	12
5	Soapnut	0.5	6
6	Soapnut	0.5	24
7	EDTA	0.5	6
8	EDTA	0.5	24
9	Rhamnolipid	0.25	6
10	Rhamnolipid	0.25	24
11	Rhamnolipid	1	6
12	Rhamnolipid	1	24
13	Rhamnolipid	0.5	12
14	Rhamnolipid	0.5	12
15	Rhamnolipid	0.5	12

2.2.1 Statistical Analysis

The data obtained from the results of Box-Behnken design were analysed using Minitab. Analysis of variance (ANOVA) for the quadratic polynomial models for diesel oil removed was used to test the variation among three factors and also to obtain their interactions. Contour and Surface plots were used to show the details of relationships that exist between the responses. In contour plots, two-dimensional views, which have the same response value, are connected to produce contour lines represented 3- dimensions. Similarly, surface plot shows the 3-dimensional view of the surface in x, y and z axis. Both contour and surface plot are used to indicate the optimum range of values of the experimental factors and for predicting response.

2.3 Column washing

The washing was carried out using distilled water as well as three different surfactants. 200 g of the contaminated soil was packed into a plastic column giving a soil bulk density of 0.8 g/cm^3. The height of the column was 17.5 cm, while the internal diameter was 5 cm. The column was secured vertically using a tripod stand. The soil column had a porosity of 68%, which resulted in a pore volume (PV) of 146.8 cm^3. 200-micron wire mesh was used in both the inlet and outlet of the column to prevent any loss of soil particles. The air trapped in the column was removed via the introduction of distilled water into the soil column at the rate of 2 cm^3/minute. Six pore volumes of the surfactant solution w used for the washing. The flushing solutions were pumped into the saturated soil from the top to achieve a down- flow mode of washing. For every pore volume, the effluent was collected, and the washed oil was extracted using n-hexane. Table 3 shows the experimental plan, which describes the control factors and their level or condition for the column washing.

Table 2: Control factors and their levels for the experimental deign

Control factor	Level					
	1	2	3	4	5	6
Washing solution (A)	Water	Soapnut	Rhamnolipid	EDTA		
Pore volume (B)	PV 1	PV2	PV3	PV4	PV5	PV6

2.4 Analysis of oil in soil

Any oil residue in soil can be extracted with solvents such chloroform and n-hexane. In this work, the amount of oil in the soil was quantified by using a UV-Visible spectrophotometer (Model 7305 spectrophotometer manufactured by JENWAY). N-hexane, which is a non-polar solvent with less toxicity than chlorinated solvents like chlorofluorocarbon, was used for oil extraction. Moreover, n- hexane is recommended as the extraction solvent for the determination of oil and grease and petroleum hydrocarbons [26, 27].

2.5 Determining oil removal

The initial oil in the soil before washing was estimated from the pre-determined amount of diesel oil that was used to contaminate a known amount of soil (the contamination was such that 1 g of soil contains 55 mg of diesel oil). The procedure for determining the extracted diesel oil from the contaminated soil is as follows: 5 g of the diesel oil contaminated soil was weighed and emptied into a flask. Thereafter, 10 cm^3 of n-hexane was measured and introduced into the flask containing the contaminated soil and shaken for 10 mins (at 200 rpm, $28^{O}C$). The oil/n-hexane mixture was decanted; following this, the absorbance was measured at 400 nm using pure hexane as a control. The extraction process was carried out at $28^{0}C$ and 200 rpm for 10 mins, and repeatedly for 3 times. The mixture was centrifuged for 20 minutes at 3000 rpm to remove any suspended particles. Absorbance was measured at 400 nm and concentration calculated using the equation from the calibration curve. For the batch washing, diesel oil removal efficiency was estimated from the diesel oil remaining in the washed soil as the initial oil content in the soil is known. To determine the percentage oil removal, Eqn. 1 was used as shown below:

$$\text{Crude oil removed}\,(\%) = \frac{O_i - O_r}{O_i} \times 100$$

……………………….. (1)

Where O_i is the amount of oil in the contaminated soil initially before washing (g) and O_r is the amount of remaining diesel oil in the washed soil (g).

For the column washing, diesel oil removal was determined based on the concentration of diesel oil in the effluent after washing.

To calculate error, the measurements (absorbance) were repeated for multiple times and the average of the values was taken:

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i \quad \text{……………………………..} (2)$$

Where n is the number of measurements taken, and x_i is the value for the i^{th} measurement.

The variance s^2 was defined thus:

$$s^2 = \frac{1}{n-1}\sum_{i=1}^{n} (x_i - \bar{x})^2 \quad \text{……………………} (3)$$

And standard deviation s was defined thus:

$$s = \sqrt{s^2} = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2} \quad \text{…………….} (4)$$

Following which, the absolute error was defined as:

$$\omega = \frac{x_i - \bar{x}}{\bar{x}} \times 100\% \quad \text{………………………...} (5)$$

3. Results and discussion

3.1 Soil characterization

Soil characterization was carried out to determine some of the important properties of the soil. Table 3 shows the results of the experiments.

Table 3: Soil characterization

Properties	Values	Units
Soil moisture content	23.1	%
Soil porosity	68	%
Soil pH in water	7.22	
Soil bulk density	0.8	g/cm
Soil organic matter content	8	%
Soil particle size	2	mm
Soil permeability	3.2	cm/hr

3.2 Surfactants characterization

3.2.1 Surface tension

The surface tension values of the surfactants measured at various concentrations are shown in Fig. 3. The results indicate that the CMC of soapnut solution occurred at a concentration of 0.1, while that of rhamnolipid solution occurred at a concentration of 0.02, and that of EDTA occurred at a concentration of 0.2. Since factors such as temperature, water hardness, and electrolyte affect the CMC of the surfactant solution, the results of this study were compared with those reported in literature [28], [29], and were found to be in agreement. The ability of surfactants in reducing the air-water surface tension corresponds to its ability to reduce interfacial force between the diesel oil and soil.

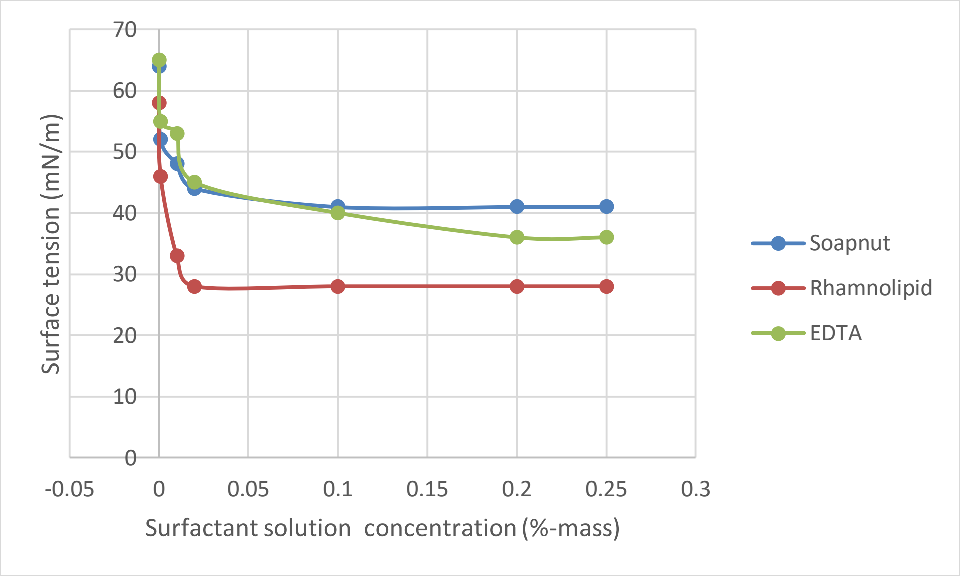

Figure 3: Surface tension of surfactant solutions

3.2.2 Interfacial tension

The interfacial tension data are shown in Fig. 4. The oil-water forces were found to decrease, which shows the surfactants' ability to remove oil from the soil by reducing the capillary force thereby increasing the contact angle between the oil and the soil to enhance mobilization.

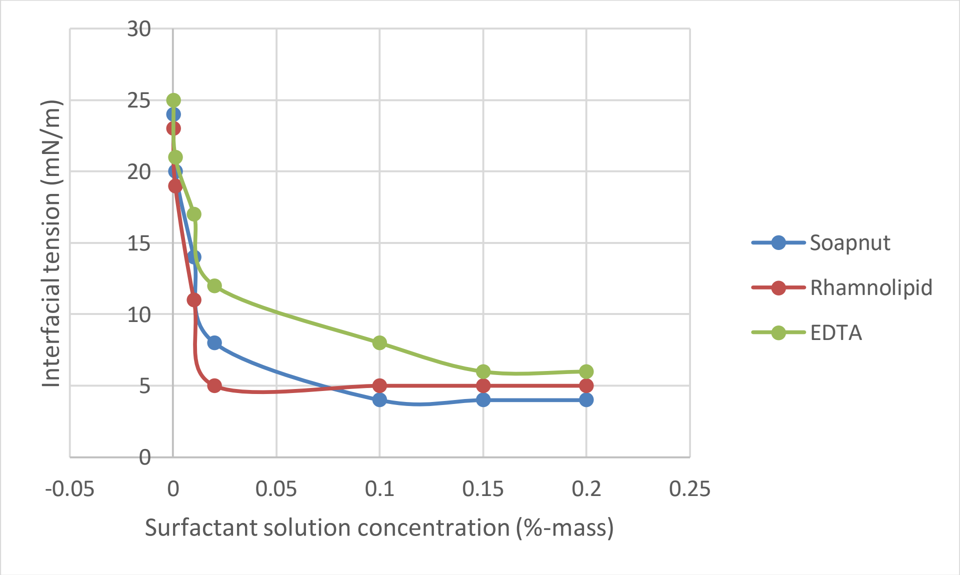

Figure 4: Interfacial tension of surfactant with crude oil.

3.2.3 Surfactant pH

Surfactant pH measured at various concentrations is shown in Table 4. This is the natural pH of the solution, which was not adjusted. There is slight difference in the pH as the concentration changes. This follows the common knowledge that the pH of an acid solution decreases, as the solution is more concentrated, while the pH of a base increases, as the solution is more concentrated.

Table 4: Different surfactants and their pH at different concentration

Surfactant	**pH for conc. of 0.25%-mass**	**pH for conc. of 0.5%-mass**	**pH for conc. of 1%-mass**
Soapnut solution	4.54	4.51	4.50
Rhamnolipid solution	9.01	9.04	9.10
	pH for conc. of 0.025M	**pH for conc. of 0.05M**	**pH for conc. of 0.1M**
EDTA solution	2.85	2.83	2.81

3.3 Batch washing

Batch washing was used to investigate the potential of ex-situ washing using different surfactants in different concentrations. 5 g of the contaminated soil sample was measured using AX224/E electronic balance manufactured by OHAUS CORA, and poured into a 250 cm^3 flasks used for shaking. Then 50 cm^3 of known concentrations of surfactant solutions were poured into the flask containing the contaminated soil. They were fixed in a shaker (Orbital incubator S1600 manufactured by STUART) and shacked for 12 hrs, 200 rpm, 28^{O}C. After washing, the mixture was allowed to settle for 1 hour. The time of settling was selected based on the type of soil particle according to [25]. The settled solution went through decantation as the supernatant was decanted and the settled soil was then rinsed using 5 cm^3 distilled water by shaking for 5 mins in order to remove the oil that must have stuck on the walls of the flask and also to remove the remaining surfactant solution in the soil. Soapnut (saponin), which is a plant-based surfactant was used in three different concentrations in carrying out batch washing of diesel oil contaminated soil and it resulted in 87.8% oil removal for 0.25% w/v soapnut solution, 88.0% oil removal for 0.5% w/v soapnut solution and 85.1% oil removal for 1% w/v soapnut solution. Rhamnolipid, which is a biosurfactant, is a class of glycolipid produced by Pseudomonas aeruginosa was also used in three different concentrations and 0.25% w/v rhamnolipid was able to remove 81.2% oil, 0.5% w/v rhamnolipid removed 85.9% diesel oil, while 1% w/v rhamnolipid removed 87.3% oil. In the case of EDTA, which is a chelating agent, three different concentrations were used to carry out batch washing. In this case, 0.025 M EDTA solution was able to remove 83.5% oil while 0.05 M and 0.1 M EDTA solution removed 86.3% and 88.1% diesel oil respectively. The diesel oil removed from the contaminated soil by three different surfactants of different origin was much more effective than the distilled water alone, which was only 32% of the total oil in soil. This shows that the removal of diesel oil from the contaminated soil can be attributed to the decrease in surface and interfacial tensions of surfactant solutions, which aided the increase in mobility of the diesel oil and thus enhanced the separation of oil from the soil.

3.3.1 Effect of surfactant concentration

The enhancement in removal of diesel oil by increasing surfactant concentration is shown in Fig. 5. The phenomenon can also be explained by the fact that reduction in the attraction between soil and diesel oil due to the increase in contact angle and change in wettability of the system because of the presence of surfactant" [30]. For soapnut (saponin) solution, there is no increase but rather decrease in the removal of oil between concentration of 0.5% w/v and 1% w/v, the observation is similar to the previous report by [31], which explains that surfactant solution may not enhance the removal of oil from soil at concentrations greater than their CMC value. Therefore, at a concentration below CMC, higher diesel oil removal was achieved; this observation suggests that mobilization due to reduction in interfacial tension may have enhanced diesel oil removal. The reduction of removal of diesel oil exhibited by greater concentration soapnut solution can also be attributed to the change in micelle shape and sizes, which may be observed for surfactants with bulky molecular structure that may cause micelle instability and reduction of detergency [32].

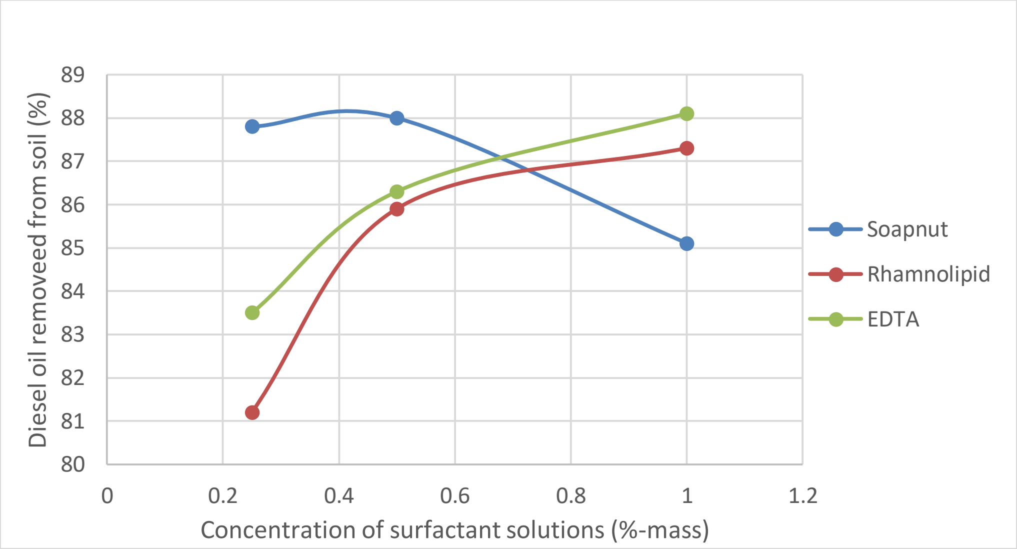

Figure 5: Diesel oil removal based on concentration of surfactant solutions

3.3.2 Scanning Electron Microscope

Scanning Electron Microscope (SEM) study helps to give a picture of the surface structure of the soil samples. SEM is a form of electron microscope that generates the images of the sample by a scanning mechanism. The surface of the sample is scanned with a focused beam of electrons which interact with the atoms in the samples thereby giving output signals about the sample's surface topography and composition. The SEM images of the surface structure of the contaminated and washed soils (Fig. 6 and 7) indicate that the surface roughness appearance becomes clearer as the concentration of the surfactant solution increases with respect to the contaminated soil.

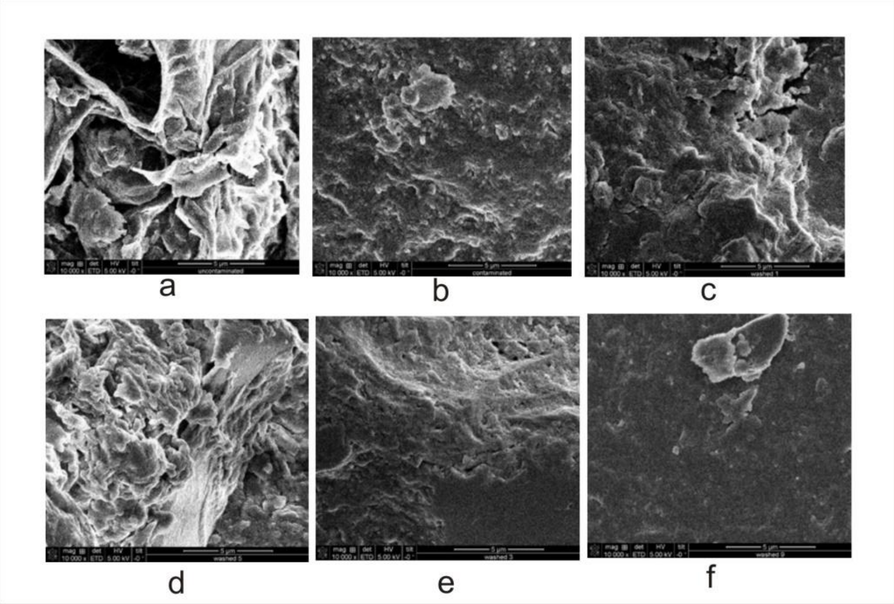

Figure 6: SEM image of (a) Uncontaminated soil (b) Soil contaminated with diesel oil (c) Soil washed with 0.25%-mass soapnut solution (d) Soil washed with 0.5%-mass soapnut solution (e) Soil washed with 1%-mass soapnut solution (f) Soil washed with 0.25%-mass rhamnolipid solution.

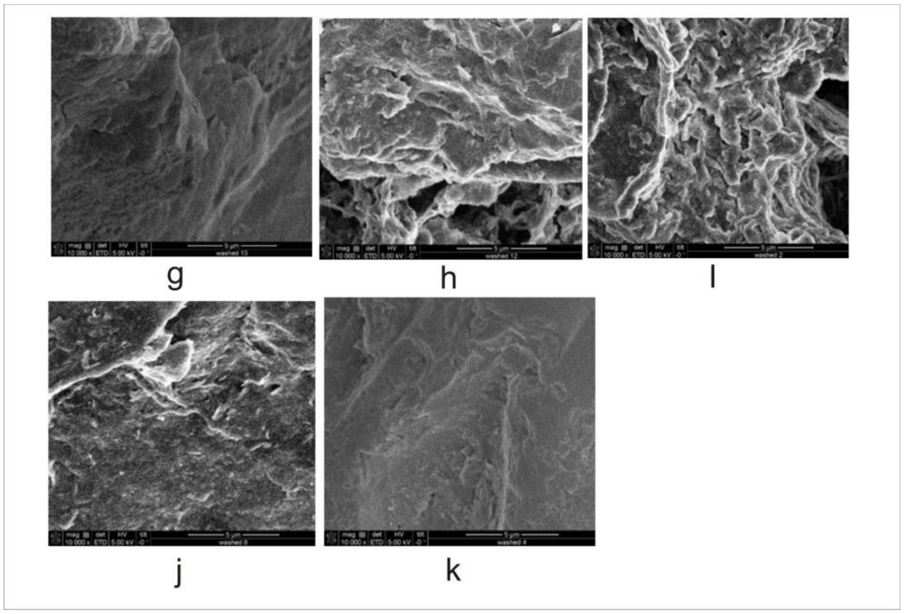

Figure 7: SEM image of (g) Soil washed with 0.5%-mass rhamnolipid solution (h) Soil washed with 1%-mass rhamnolipid solution (i) Soil washed with 0.025 M EDTA solution (j) Soil washed with 0.05 M EDTA solution (k) Soil washed with 0.1 M EDTA solution.

3.3.3 Gas Chromatograph/Mass Spectrometric analysis

Gas chromatography-mass spectrometry (GC/MS) analysis was carried for both soil and oil extract to determine if there is any preferential removal of specific diesel oil components from the soil. In this study, n-hexane was used for extraction purposes. The diesel oil/n-hexane extracts were analysed using GC/MS as shown in Figs. (8-11). The purpose of this GC study carried out for each of the diesel oil/n-hexane extracts was to observe the trend of the hydrocarbons remaining in the washed soil and the results were compared with that of the contaminated soil.

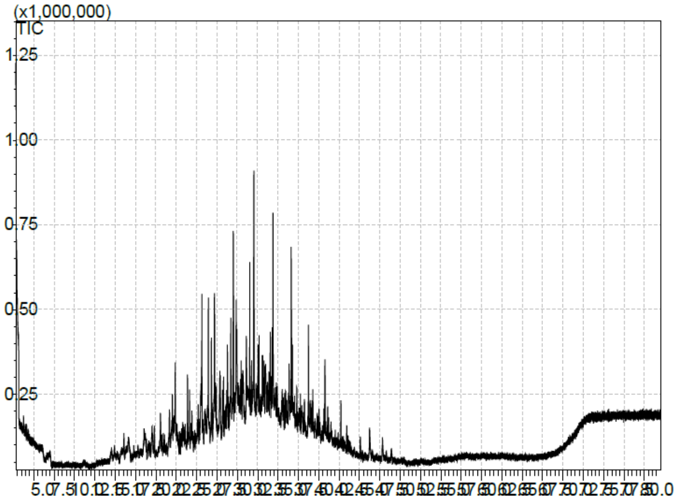

Figure 8: GC/MS chromatogram of the diesel oil contaminated soil.

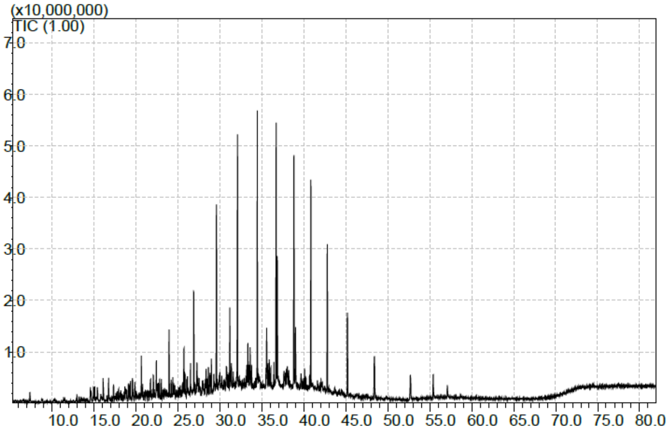

Figure 9: GC/MS chromatogram of the diesel oil contaminated soil washed with 0.5%-mass soapnut solution.

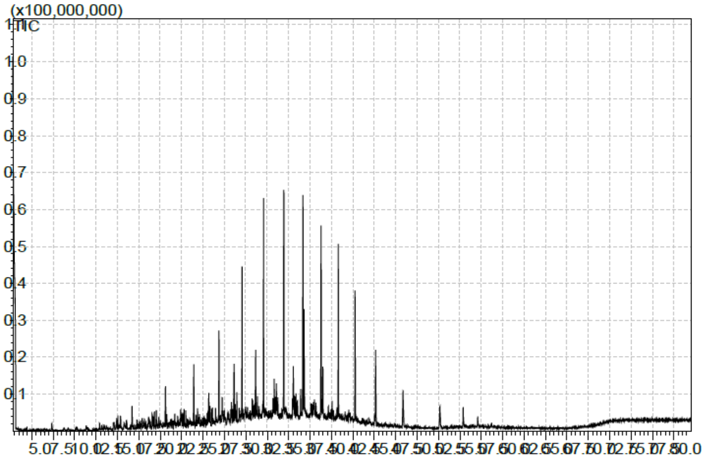

Figure 10: GC/MS chromatogram of the diesel oil contaminated soil washed with 1%-mass rhamnolipid solution.

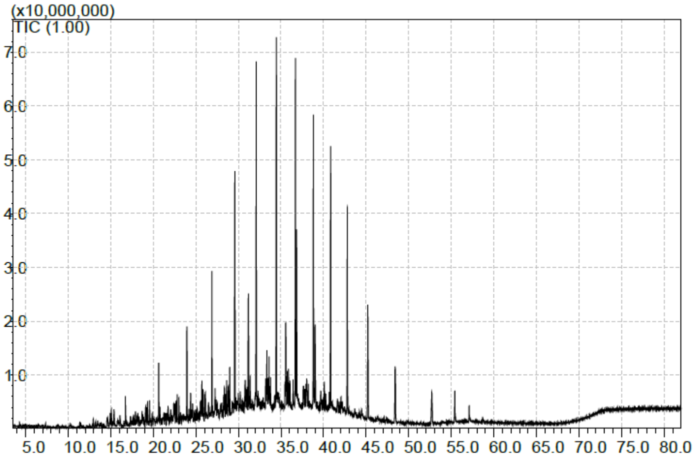

Figure 11: GC/MS chromatogram of the diesel oil contaminated soil washed with 0.1M EDTA solution.

3.4 Column Washing

Column washing in a down flow mode was carried out to simulate an in-situ washing method. Optimum concentrations of the three surfactant solutions obtained from the batch experiment were used; other wash conditions were, 6 pore volumes (PV) of each surfactant at a flow rate of 5 cm^3/mins for 40 mins residence time, reckoning pore volume measures 147 cm^3. Distilled water was also used for the column washing, following same washing conditions. The oil removed was extracted from the effluent for each pore volume and measured, after which the diesel oil removed was calculated in percentage (r, %). The cumulative diesel oil removal (R, %) was determined by adding each PV as shown in Eqn. 6.

$$R = r_{n-1} + r_n \text{ .... } (6)$$

The data for the cumulative diesel oil removal from the contaminated soil as shown in Fig. 12 indicate that EDTA solution has the highest cumulative diesel oil removed from the contaminated soil with the cumulative diesel oil removal of 43.3%, followed by rhamnolipid solution which has the cumulative diesel oil removal of 40.7% and soapnut solution with the cumulative diesel oil removal of 39.3%. The cumulative diesel oil removed by distilled water is 3.7%.

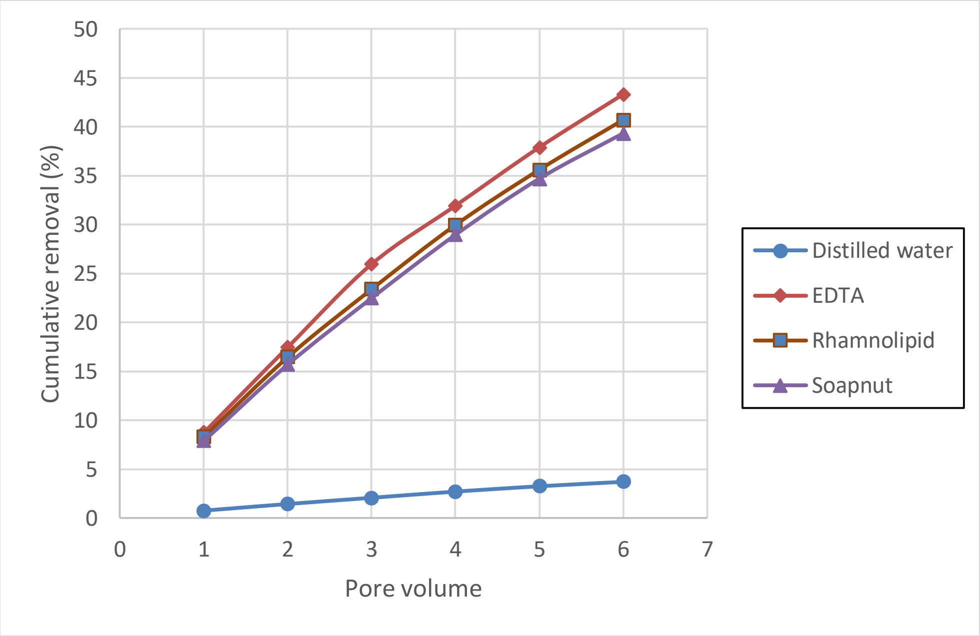

Figure 12: Cumulative diesel oil removal from a down flow column washing of contaminated soil using EDTA solution, rhamnolipid solution, Soapnut solution and distilled water.

3.4.1 Flow rate

The flow rate is an important factor as it is indirectly proportional to the retention time. The flow rate for the washing was 5 cm^3/minute for 40 minutes' residence time. The residence time was determined with one PV of the washing solution. The flow rate was not a limiting factor in the diesel oil removal since the soil had high permeability.

3.4.2 Pore volume

The down flow column washing was carried out using 6 PV of the surfactant solution and washed oil was measured for each PV in order to investigate the rate of diesel oil removal. The trend was quite similar for all the washing solutions including distilled water, as there is greater removal at the initial PVs and the removal decreases as PV increases as shown in Fig. 13. The low concentration of surfactant solution in the effluent of the initial PVs can be considered as an indication that the surfactant solution at the initial PV went through adsorption process on the soil particles, which enables rapid extraction of the contaminant by micellar solubilisation, while the higher concentration of surfactant solution in the effluent of later PVs can be best explained by the fact that the adsorption of the surfactant in the soil decreases as the washing progresses.

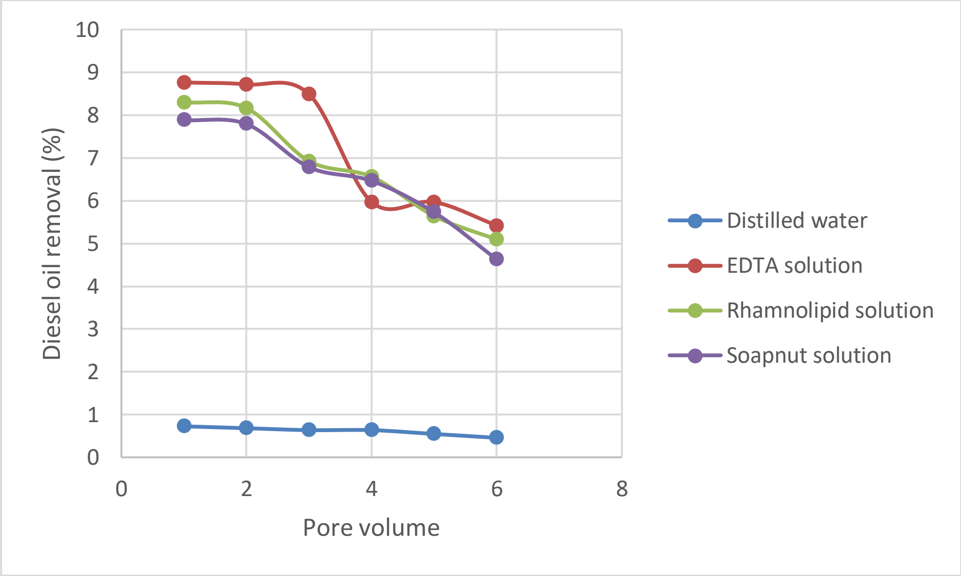

Figure 13: Diesel oil removal at various pore volumes.

3.4.3 Distilled water washing

The soil column was washed with distilled water to investigate its ability to remove oil that resulted in cumulative oil removal of only 3.7%, which is 10.7 times lower than that of soapnut solution, 11 times lower than that of rhamnolipid solution and 11.7 times lower than that of EDTA solution as can be seen in Fig. 14. Thus, it is evident that only a tiny portion of the diesel oil in the contaminated soil could be removed by water in column washing.

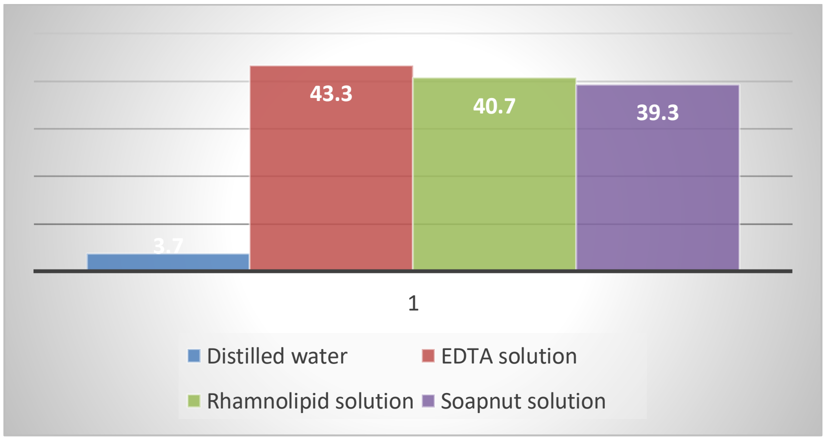

Figure 14: Diesel oil removal by distilled water, EDTA solution, rhamnolipid solution, and Soapnut solution.

3.5 Optimization of diesel oil removal

Optimization of the batch soil washing was carried out in order to determine which set of conditions is best for washing diesel oil contaminated soil. The batch washing was done according to the Box-Behnken experimental design as shown in Table 2. The percentage of diesel oil removal was calculated for all the 15 runs and was used for the optimization analysis. With the Box-Behnken experimental design, a polynomial equation was generated in order to evaluate the interaction of the 3 factors. To evaluate the experimental errors, five replicates were used. Table 5 shows the main and interaction effects of the factors affecting the diesel oil removal process with the model F value as 2.90. The regression model had the R^2 value of 83.93% (adj. 55.00%). The first plot in Fig. 15 shows the normal probability plot of residuals, which helps in the normality checks and is described by the fitting of the points on the straight line, while the second plot shows the plot of the actual versus predicted values, which explains the relationship between the fitted and the real data. The regression equation generated as an empirical model for the diesel oil removal is given in Eqn. 7 below.

$$\text{(\% oil removal)} = 86.182 - 0.430\,A + 1.314\,B + 0.295\,C + 1.347\,A*A - 1.355\,B*B + 0.045\,C*C + 1.793\,A*B - 0.393\,A*C - 0.589\,B*C \quad \ldots\ldots (7)$$

Table 5: Analysis of variance for quadratic polynomial models for diesel oil removal

Source	DF	Adj Sum of Square	Adj Mean Square	F-Value	P-Value
Model	9	45.4474	5.0497	2.90	0.127
Linear	3	15.9820	5.3273	3.06	0.130
A - Surfactant type	1	1.4776	1.4776	0.85	0.399
B - Surfactant conc.	1	13.8096	13.8096	7.93	0.037
C – Washing time	1	0.6948	0.6948	0.40	0.555
Square	3	14.6028	4.8676	2.80	0.148
A*A	1	6.6953	6.6953	3.85	0.107
B*B	1	6.7770	6.7770	3.89	0.105
C*C	1	0.0075	0.0075	0.00	0.950
2-Way Interaction	3	14.8626	4.9542	2.85	0.145
A*B	1	12.8555	12.8555	7.39	0.042
A*C	1	0.6176	0.6176	0.35	0.577
B*C	1	1.3895	1.3895	0.80	0.413
Error	5	8.7030	1.7406		
Lack-of-Fit	3	8.6194	2.8731	68.71	0.014
Pure Error	2	0.0836	0.0418		
Total	14	54.1504			

Model summary (Table 5)

Standard deviation	R- square	R- square (adj)	R- square (pred)
1.31932	83.93%	55.00%	0.00%

For evaluating the effects of the three different variables on diesel oil removal process, the Box-Behnken model was used and the relationship among these three factors was established by a surface plot as a function of two factors and by fixing the third factor at a certain level. The regression surface obtained was plotted as a function of the different variables of the process, which are surfactant type, surfactant concentration and washing time. Fig. 17 depicts the effect of surfactant concentration; it can be seen from the 3D surface plot, based on the fitted second order polynomial equation that the surfactant concentration (B) has higher interaction with the washing time (C) than with the surfactant type (A). As the surfactant concentration increases as well as the washing time, the diesel oil removal also increases slightly.

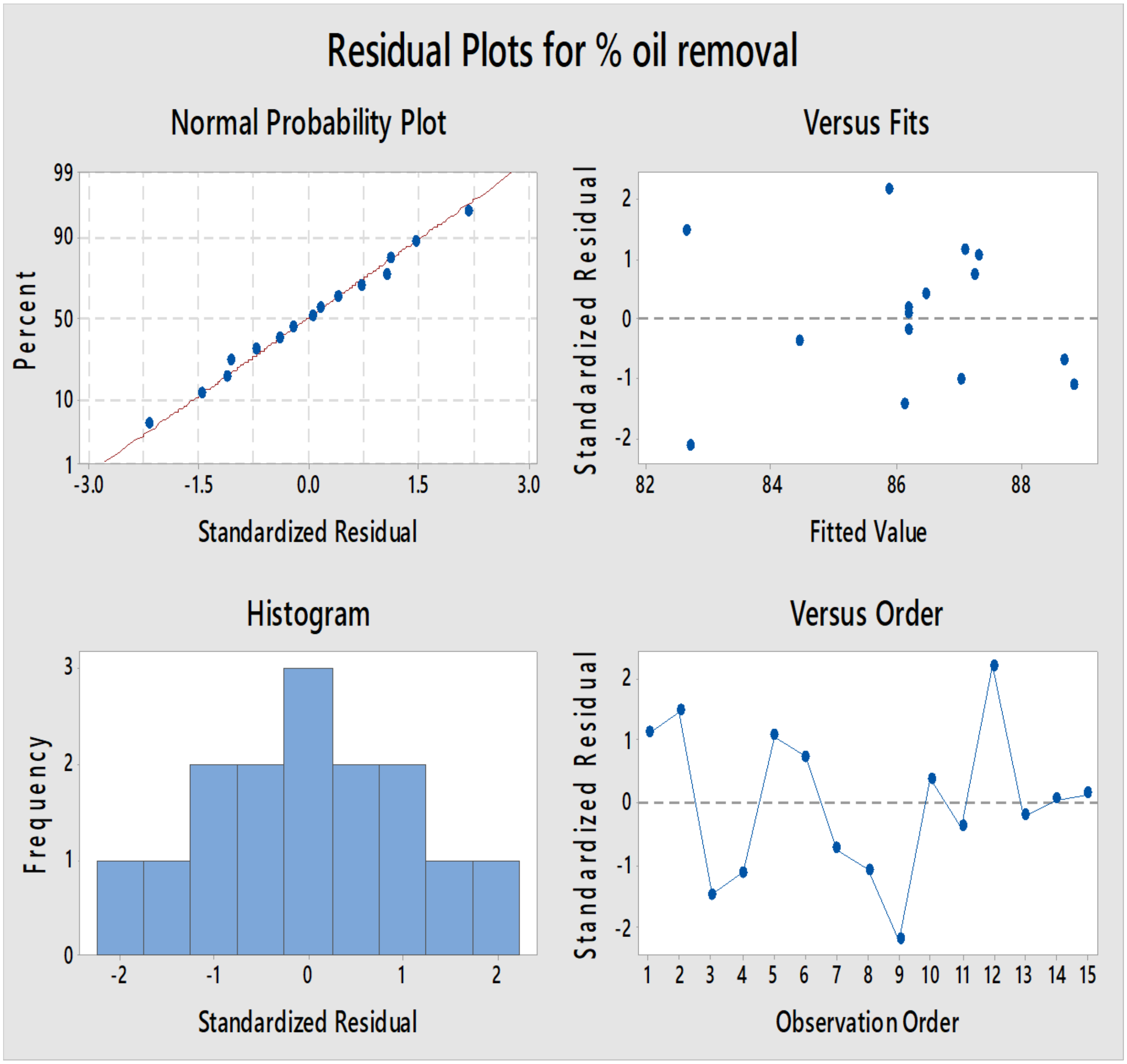

Figure 15: Residual plots for diesel oil removal

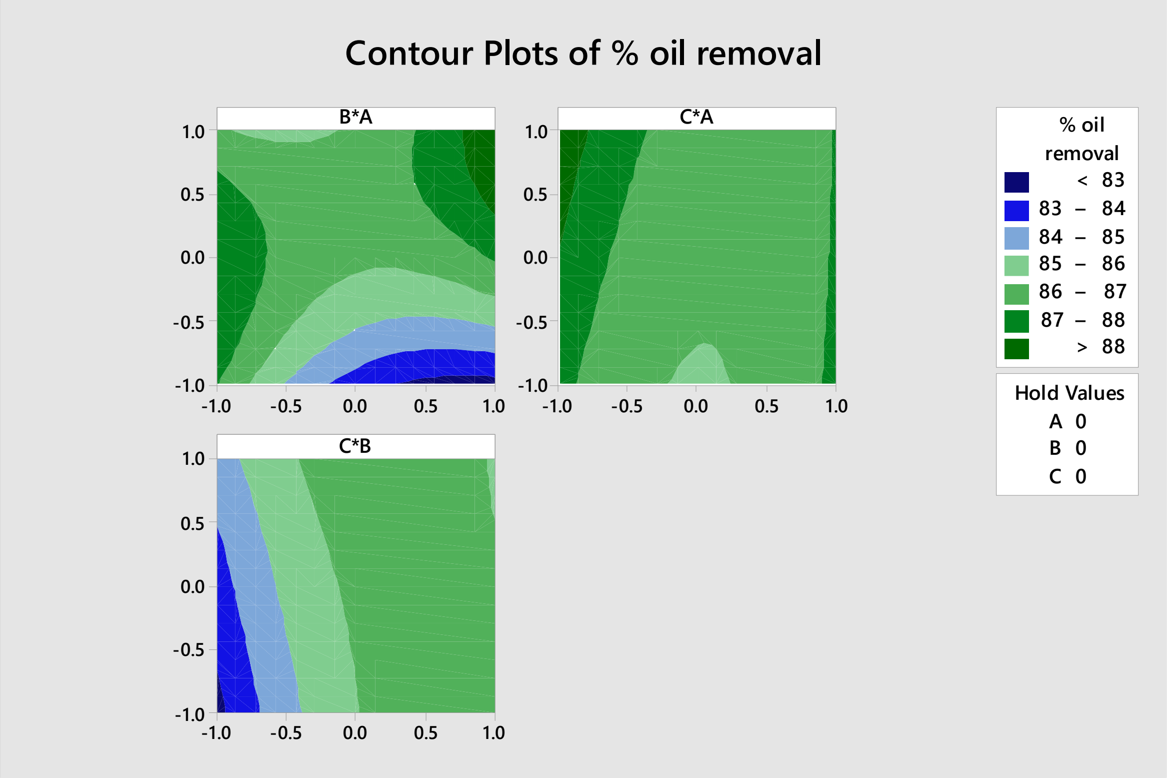

Fig 16: Contour plot for diesel oil removal

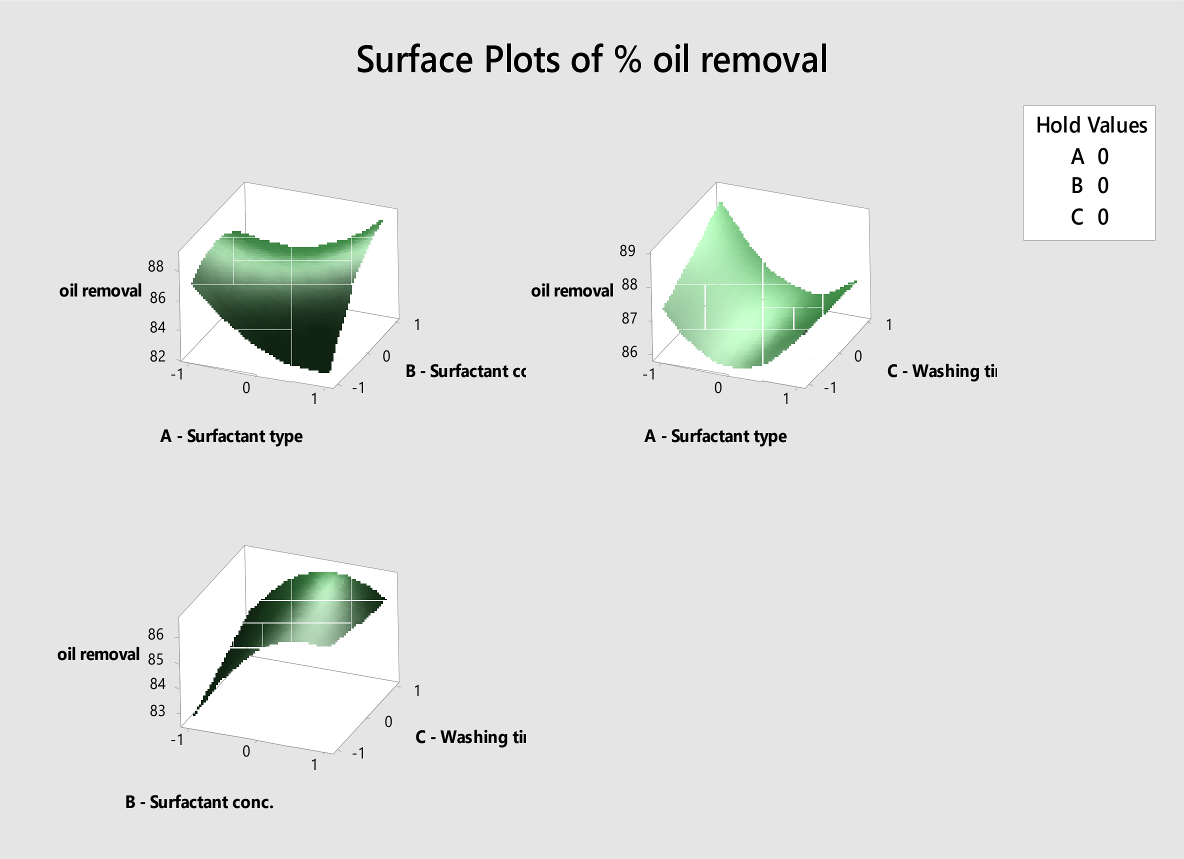

Figure 17: Surface plot of diesel oil removal

3.6 Biosafety

Plant saponins play an important role in defence against insects and pathogens. Figen [33] reported various biological roles of saponins that are present in plants, which defend the plants against phytopathogenic fungi. [34] have published a detailed report on the effect of saponin on ruminants. Plants containing saponin that are commonly foraged by ruminants have a beneficial effect such as defaunation; this improves nitrogen utilisation by ruminants, which in turn may increase growth and milk production. It is also reported that high saponin content in the feed may decrease protozoal count in the rumen but this is transient and the count normalises quickly within a span of 1-2 weeks. [35], in a review article presented strong evidence in support of saponin in protection of plants against root-infecting fungi. [36] reported the traditional uses of Sapindus mukorossi including medicinal uses in expectorant, lice removal from scalp as well as various medical formulations. On the other hand, [37] reported that common lettuce plant (L. sativa) shows high sensitivity to rhamnolipid. At a concentration level of 4-6 mg. g-1 of rhamnolipid, both germination index (GI) and acute toxicity (EC 50) of L. sativa decreased, while at lower concentration of 1 mg. g-1 the GI value increased, suggesting release of essential nutrients from soil that helped germination. [38] observed that field pea seeds soaked in rhamnolipid bio-complex prior to planting in fields contaminated with petroleum residues, 2-8% (w/w), reduced the oil content by 60-65%. Therefore, no conclusive evidence can be drawn on the toxicity of rhamnolipid on plants. While saponin from plant and microbial sources is unlikely to be a threat to plant, animal and human lives as well as soil health, EDTA on the other hand, has more adverse effects on soil properties despite its global popularity as a remediation as well as cleaning agent. [39] observed that while it improved phytoremediation efficiency of heavy metal contaminated soil, it had toxic effects on soil microbial community. It could also degrade soil by dissolving mineral components.

4. Conclusions

This study seeks to evaluate the removal of diesel oil from contaminated soil via two different soil-washing methods: batch and column washing, using synthetic surfactant (EDTA) as well as a plant based (soapnut) and a microbial (rhamnolipid) surfactant in various concentrations. Batch washing was carried out using three different concentrations of soapnut, rhamnolipid, and EDTA, while column washing was carried out using optimum concentration of these surfactants. Batch as well as column methods of soil washing were carried out in this study in order to remove diesel oil from the soil. The batch method depicts the ex-situ soil washing while the column method depicts the in-situ soil washing. The results of the experiment show that soil washing by batch method had higher removal of diesel oil from the soil than soil washing by of column method. This suggests that ex-situ soil washing can be more effective than in-situ soil washing with surfactants. This is in line with the general observation for in-situ “pump and treat” remediation methods, as they are considered to be less effective because of the low water solubility of NAPLs and mass-transfer constraints [30]. This observation, however, does not account for enormous removal and transportation cost of soil from the contaminated site as well as off-site treatment. In summary, the results suggest that diesel oil contaminated land can be remediated with all three surfactant types investigated in this study; however, plant-based surfactant appears to be the more efficient and economical than the other two surfactants as rhamnolipid is the most expensive, followed by EDTA. Moreover, considering biosafety aspect, plant based saponins may be chosen over rhamnolipid or EDTA for soil remediation. In addition, the batch washing method was found to be more effective in removing diesel oil from soil than the column washing method.

5. Acknowledgement

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7. Appendix

7.1 Calibration of oil

To obtain the appropriate wavelength to be used in the UV-spec analysis, n-hexane was used to make a stock solution of diesel oil. The stock solution contains 1 g of diesel oil which was emptied into a 100 cm3 volumetric flask and was completed with n-hexane. It was centrifuged at 3000 rpm for 20 mins. The absorbance of the stock solution was measured at different wavelength starting from 390 to 450 nm as shown in the graph in Fig. 18. The wavelength which has the highest absorption of light that goes through the solution was taken as the appropriate wavelength. 400 nm was the wavelength with the highest absorbance, and this was used as the wavelength throughout the analysis. In order to obtain a calibration curve with which the concentration of diesel oil (%-mass) in the extract will be determined, the stock solution was diluted into several concentrations ranging from 0.1(%-mass) to 1(%-mass) and their absorbance was measured at the appropriate wavelength of 400 nm. The absorbance was plotted against the stock solution concentration and then the linear relationship equation was obtained as shown in Fig. 19.

The equation obtained from the linear relationship of the curve is shown in equation 14 and this implies that any absorbance that is measured to be above the linear part, the sample will be diluted, and new absorbance measured.

$$y = 2.1403x \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (14)$$

Where y is the absorbance measured at 400 nm and x is the concentration of oil remaining in the soil after washing (%-mass).

7.2 Sample of oil removal calculation

Five grams of diesel oil contaminated soil was washed for 12 hrs using 10 cm^3 of 0.25% soapnut in a shaker at 200 rpm and 28^{o}C. The supernatant was removed and the diesel oil remaining in the soil extracted with n-hexane as described in section 3.7.3. The amount of diesel oil in the 5 g of contaminated soil before washing was 0.237817 g. After measuring absorbance, the absorbance of the extract was 0.124.

Using the equation from the calibration curve, the x (%-mass) becomes:

$y = 2.1403x$

$0.124 = 2.1404x$

$x = 0.057936$ %-mass

Therefore, the mass of diesel oil remaining in 5 g of the contaminated soil becomes

$= 0.057936 \times 50/100$

$= 0.028968$ g

Therefore, the mass of oil removed from the 5 g contaminated soil was calculated thus:

$= 0.237817 – 0.028968 = 0.208849$ g

The percentage oil removal performance of the 0.25% Soapnut was calculated thus:

= (mass of oil removed from soil/mass of initial oil in soil) × 100

$= (0.208849/0.237817) \times 100$

$= 87.82\%$

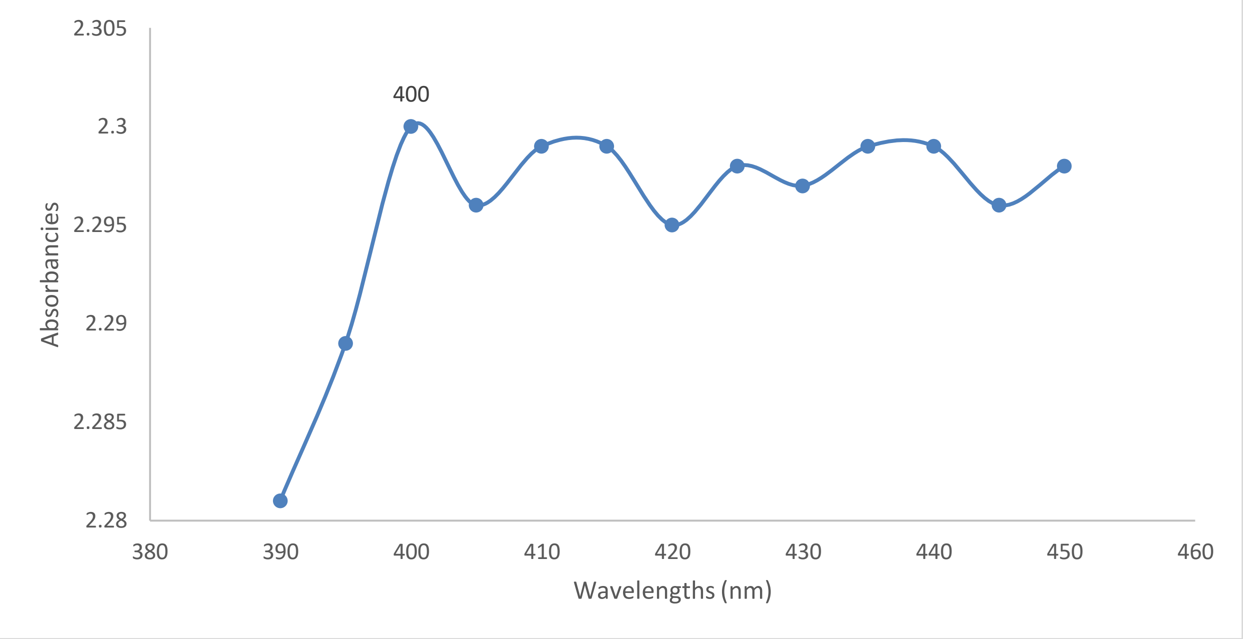

Figure 18: Wavelength of diesel oil stock solution

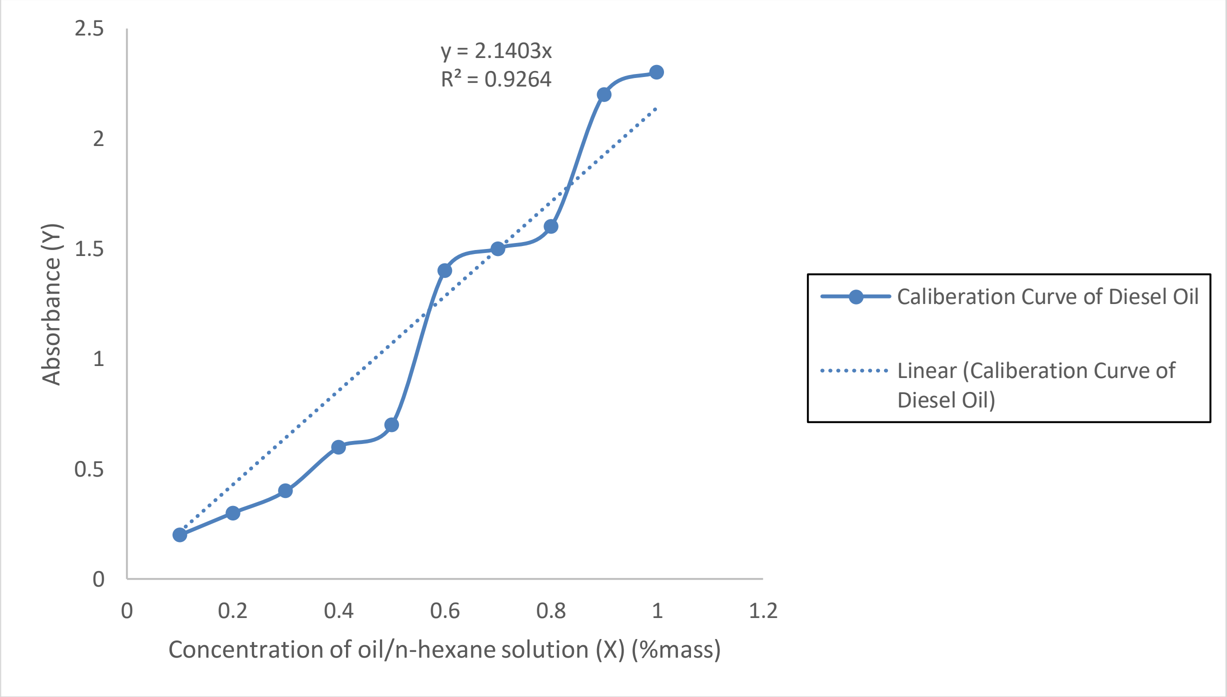

Figure 19: Calibration curve of diesel oil