EFFECTIVENESS OF SAPONIN ON PHYTOREMEDIATION OF PETROLEUM-CONTAMINATED SOIL

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Abstract

This study aims to evaluate efficiency of saponin, a natural surfactant, in remediation the soils which are smeared by crude oil and it was conducted based on the soil properties of oil ring no. (52), Nyaungdon Twonship, Ayeyarwaddy Region in 2019. For this purpose, soil properties were initially studied in this zone including soil texture, pH, moisture, nitrogen content, phosphorus content and potassium content. Crude saponin was extracted from the vegetable waste materials such as Onion (*Allium cepa* L.) skin, Ka Nyut (*Asparagus officinalis* L.) hard stem and Hin Nu Nwe (*Amaranthus paniculatus* L.) hard stem by the yield percent of 14.55 %, 2.53 % and 2.12 %, respectively. The studied phases included variables of saponin concentration within ranges of 250, 500 and 1000 ppm, contact time (10 week) and concentration of oil within ranges of 0.1-10 % in soil. The findings from assessment showed that the conditions under concentration of saponin (1000 ppm) on 0.1 % oil contaminated soil. The removal efficiency percentage of crude oil on 10 weeks contact time was observed 24.89 %. A laboratory study was extended to assess the potentials of local grass (Bermuda grass) under normal environmental conditions with or without surfactants in remediating soil contaminated with crude oil. The results of the phytoremediation of contaminated soil showed that significant reduction 76.3 % of crude oil was observed by natural saponin. It was also observed that the crude oil in the polluted soil was reduced by 72.4 % as a result of plant only, similar to commercial surfactant (SLES) treatment 72.1 %. This research indicates the soil remediation by Onion skin saponin, making better use of Bermuda grass and contributing to environmental protection.

Keywords: vegetable waste, saponin, Onion skin, petroleum contaminated soil, oil extraction, phytoremediation, Bermuda Grass

Introduction

As raw material for production of petroleum and other chemicals, crude oil has become one of the most important energy sources in the world. However, the soil, water and air has contaminated by crude oil as a result of exploration, production, maintenance, transportation, storage and accidental release, add hazardous chemicals to the ecosystem. So, pollution has become a major global problem. Management of pollution is still a challenge to the humans. Among them, soil can be contaminated by many different human activities. Soil can be subjected to remediation techniques for the purposes of site decontamination.

Fortunately, surfactants can promote the removal of organic compounds and heavy metals from contaminated soils. Saponins are steroid or triterpene glycoside compounds found in variety of plants and derive their name from the soapwort plant. Saponins are traditionally used as natural detergents. Saponins show the unique properties of foaming and emulsifying agents (Bajad and Pardeshi, 2015). It is also called natural surfactant.

Surfactants are substances whose molecules consist of a water soluble (hydrophilic) and an oil soluble (hydrophobic) part. They have the tendency of accumulating at oil-soil and oil-water interfaces. The addition of surfactant to the washing solution (crude oil contaminated soil) to reduce the surface and interfacial tension at the air-water and oil-water interface, thereby reducing the capillary force that holds the oil and soil, which may lead to the mobilizing and (or) the solubility of the oil. The mechanisms behind surfactant removal of petroleum oil from soil studied (Deshpande *et al*., 1999).

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Currently, chemical surfactants have been used in enhanced solubilization for organic compounds and removal of heavy metals, usually synthesized by chemical materials (Wei *et al.,* 2015). Compared with chemical surfactants, biosurfactants were isolated from plants produced by microorganisms. It shows the excellent performance for remediation process owing to their lower toxicity, better surface activity, readily biodegradable and huge environmental compatibility (Mnif *et al*., 2015).

Bioremediation is the use of biological processes to degrade, transform, or essentially remove contaminants from soil. This process relies on microorganisms (bacteria and/or fungi) and plants. For this reason, bioremediation is widely used to remediate organic contaminants and can be an effective means of mitigating.

Phytoremediation is applied to provide long-term rehabilitation of the residual oil-contamination. Phytoremediation is a bioremediation process that uses various types of plants to remove, transfer, stabilize, and (or) destroy contaminants in soil. Use of green plants and their microorganisms to reduce environmental problems without the need to excavate the contaminant material and dispose of it elsewhere.

Bermuda grass was selected for phytoremediation. Myanmar name is Myin-sa-myet, Mye-sa-myet. It was distributed in Myanmar, India, Philippines and South Africa. The stem of Bermuda grass has 12-24 cm high, slender, round, shoots and roots arise from nodes, most of the stems and growing points are covered with leaf sheath. The leaf of Bermuda grass has linear, 2-3 mm wide, blue green. Bermuda grass has a deep root system; in drought situations with penetrable soil, the root system can grow to over 2 m deep. Bermuda grass has been used as a phytoextraction (Adomako et al. 2010). It has also been used for arsenic and mercury (Weaver *et al*., 1984). Certain mechanisms such as phytoextraction, phytostabilisation, phytodegradation, rhizoremediation and rhizofilteration occur, allowing plants to remediate both organic and inorganic substances (Zoller and Reznik, 2009).

The aim of the present research work is to study the phytoremediation of petroleum contaminated soil by using natural surfactant (saponin) from vegetable wastes on soil washing process and by using the Bermuda grasses.

Materials and Methods

Collection and Preparation of Vegetable Waste Samples

Vegetable waste samples were collected in June, 2018 from Kamayut Township, Yangon Region of Myanmar. They were identified by the Department of Botany, University of Yangon.

The two collected vegetable waste samples, Hin Nu Nwe and Ka Nyut were washed with tap water and dried for a while to remove water. These two fresh samples were sliced very small. The dried sample of Onion skin was also sliced very small. All samples are used directly without further modification (Laufenberg *et al*., 2003).

Extraction and purification of saponin

The selected waste samples (5 g) were placed in beakers 250 mL, extracted with 100 mL of aqueous ethanol in a water bath for 2 h with continuous stirring until discolored. The mixture was filtered and combined this solution and was concentrated to about 40mL. The residue was transferred to separation funnel, and defatted with petroleum ether. The aqueous layer was recovered while the petroleum ether layer was discarded. The purification process was repeated. 60 mL of *n*-butanol was added. The *n*-butanol extracts were washed with 10 mL of 50 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to constant weight and the saponins content was calculated (De Geyter *et al*., 2007).

Identification of saponin

Saponin was identified by spectroscopy method and chemical method. Firstly, the crude saponin was identified by FT IR. And then. the crude saponin was determined by foaming test and emulsifying test.

In foaming test, the selected waste samples were extracted with water, and filtered, the filtrate in test tubes with various volume ratios. Then, the permanent foaming was observed and measured the foaming height. In emulsifying test, the selected waste samples were extracted with water, and filtered, the filtrate in test tubes, and it was warmed in water bath, the stable persistent froth, was mixed with liquid paraffin and shaken vigorously, then observed for the formation of emulsion, indicate the presence of saponins. Emulsifying capacity was measured at some important variables such as contact time and saponin concentration were selected in 1:1 volume ratio of saponin to paraffin. The operation variables used were saponin concentration (25-200 µg/mL) and contact time (15-60 min).

Collection of Soil Sample

The soil sample was collected from the upper layer (0-20 cm) of oil ring no. (52), Nyaungdon Township, Ayeyarwaddy Region. The soil was air-dried, crushed and passed 40mm sieve to remove the larger clods and trash.

Characterization of Soil Sample

Soil texture and pH were characterized by pipetting method based upon the Strobe's law and standard method by using pH meter. The moisture content in the soil was determined by oven dry method (Schneekloth *et al*., 2002). The organic matter was investigated by Walkley and black method based upon the oxidizable organic matter content. And then, total nitrogen and phosphorous content in the soil were determined by Kjeldahl's method, Olsen method for neutral and alkaline soil (measured by spectrophotometer). Finally, the total potassium and humus content were characterized by Ammonium Acetate extraction method (measured by Flame photometer) and ignition method.

Preparation of contaminated soil sample

The collected soil sample was dried at room temperature. The dried sample was sieved (40 mm) mesh. The soil was spread on plastic tray and air dried. A 40 mm mesh screen separated the larger clods and trash. The soil was mixed by hand with lubricant oil in various concentrations (0.1, 1, and 10 %).

Bench Study

The collected soil sample was treated with lubricant (0.1 %, 1 % and 10 %) and three different concentrations of saponin. This mixture was added n-hexane and acetone (5:1) ratio, shaken 10 min at the shaker, filtered and centrifuged this mixture. It was evaporated and calculated the constant weight of dried extract.

Phytoremediation

Phytoremediation is a bioremediation process that uses various types of plants to remove, transfer, stabilize, and (or) destroy contaminants in soil. Use of green plants and their microorganisms to reduce environmental problems without the need to excavate the contaminant material and dispose of it elsewhere.

The tropical grass sample (Bermuda grass) was used for this phytoremediation process. The collected petroleum contaminated soil sample was treated with 1% lubricant oil and saponin concentration (250 ppm). The composite soil mixture was filled into plastic pots (1kg soil per pot) and treated with four different designs (plant only, plants with natural surfactant, plants with commercial surfactant (SLES) and without plant).

The oil remaining in soil was determined by using (5:1) ratio of n-hexane and acetone. Twenty millilitres of this solvent mixture was added to the soil, shaken for 10 min and filtered. And then, the liquid decanted was added in the centrifuge tube and was centrifuged at 2500 rpm for 15 min. Weekly, crude oil residue was extracted from spiked soil samples from each vessel and studied on removal efficiency according to this procedure (Phyu Phyu Myint *et al*., 2013).

Results and Discussion

Extraction and Identification of Saponin

Crude saponin was extracted from the selected vegetable waste samples by lead-acetate method. The yield percent of crude saponin were calculated. The yield % of saponin were found 14.55 % in Onion skin, 2.53 % in Ka Nyut hard stem, and 2.12 % in Hin Nu Nwe hard stem. Onion skin has the highest saponin content. The results obtained are present in Table 1.

Table 1 Yield % of Saponin Content in Collected Samples

No.	Sample Name	Sample Condition	Yield (saponin content)
1	Onion skin (*A.cepa*)	dried	14.55
2	Ka Nyut hard stem (*A.officinalis*)	fresh	2.53
3	Hin Nu Nwe hard stem (*A. paniculatus*)	fresh	2.12

According to FT IR spectral data, saturated and unsaturated H/C group and cyclic alcohol group were observed in the extracted crude saponin. FT IR was identified by PerkinElmer Spectrum Two at University of Yangon. This spectral data is present in Figure 1.

FT IR analysis of the vegetable waste samples has absorption bands and wave numbers (cm^{-1}) of the prominent peaks obtained are described in Table 2. The peaks of frequency of above 3000 cm^{-1}, 1500 cm^{-1}, 1300 cm^{-1}, 1000 cm^{-1} and 700 cm^{-1} were strong while the others vary from medium to weak. The peak at 3500-3200 cm^{-1} was assigned to the OH stretching. The peak intensity at nearly 1600 cm^{-1} was assigned to C=C skeletal stretching of alkene. The absorption band at 1390-1380 cm^{-1} was assigned to CH bending. The peak at 700 ± 20 cm^{-1}, CH bending will present and at 1390-1310 cm^{-1} was assigned to the OH bending.

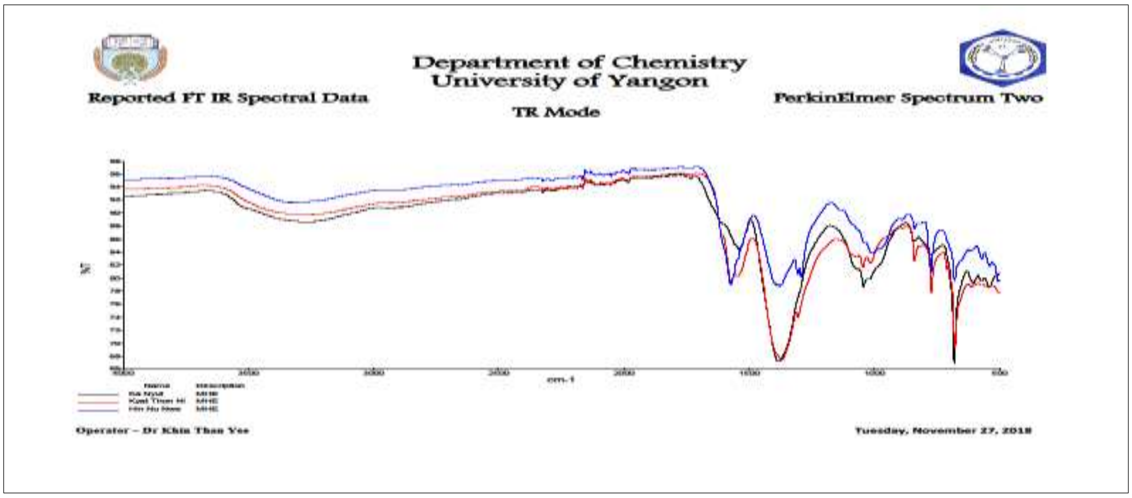


Figure 1 FT IR spectra of saponin from the selected samples

Table 2 FT IR Spectral Data of Saponin from the Selected Samples

Wave number (cm^{-1})			Band Assignment
Onion skin	**Ka Nyut hard stem**	**Hnin Nu Nwe hard stem**	
3242.51	3271.37	3326.32	ν_{OH} of hydroxyl group
1571.62	1541.27	1571.10	$\nu_{C=C}$ of alkene
1370.76	1380.36	1376.06	δ_{CH} of gem dimethyl group
1045.35	1043.20	1007.00	$\nu_{CH\text{-}OH}$ in cyclic alcohol
771.80	773.53	772.55	δ_{CH} out of plane wagging

Characterization of Saponin

Permanent foaming capacity of crude saponin extract was determined by Froth test using various volume ratio. By Froth test, higher concentration of saponin increases the permanent foaming high as shown in Figure 2. These results obtained are present in Table 3. The foam value was calculated by using the method developed by taking the foam height after 2 min dissipation and subtracting the foam interface (Dini *et al*., 2009).

Changes of emulsifying capacity as a function of saponin concentration

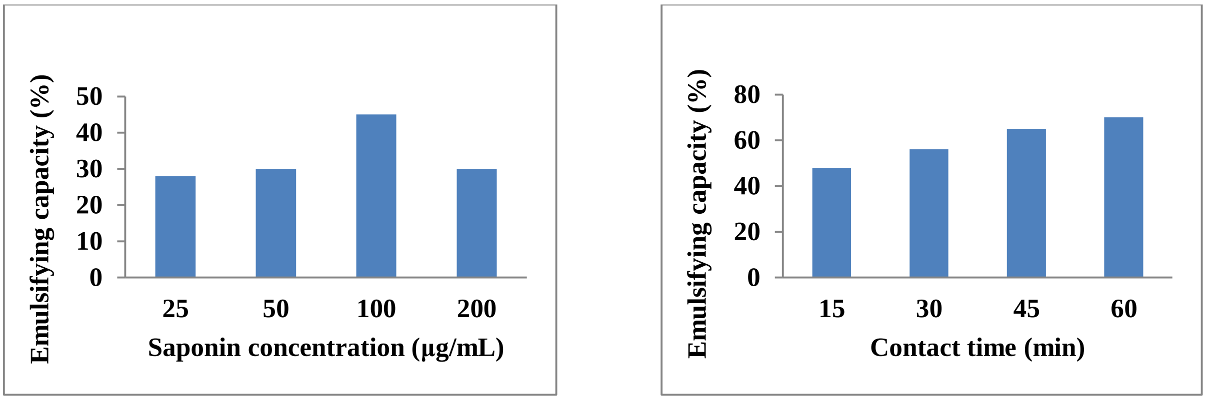
Although three samples showed higher forming capacity of their saponin extracts, Onion skin was chosen for further studies since it possesses the highest amount of saponin in compare with other two samples. Thus, emulsifying capacity of Onion skin saponin was determined by the variation of time and saponin concentration**.** According to these data, emulsifying capacity changes with contact time and saponin concentration (100 µg/mL) indicated the highest emulsifying capacity. Emulsifying capacity was changed with saponin concentration are present in Figure 3 (a) and varied with contact time are shown in Figure 3 (b).

Table 3 Permanent Foaming Capacity of Saponin Extracted from Different Vegetable Wastes

Saponin extract (v/v % in H_2O)	Permanent foaming capacity (%)		
	Onion skin	Ka Nyut hard stem	Hnin Nu Nwe hard stem
100	40	40	40
80	25	25	33.3
60	14.28	23.08	25
40	7.69	14.29	3.32
20	4	7.69	3.32

(a) (b) (c)

Figure 2 Foaming capacity of saponin extracted from (a) Onion Skin (b) Ka Nyut hard stem and (c) Hnin Nu Nwe hard stem



(a) **(b)**

Figure 3 Changes of emulsifying capacity as a function of (a) saponin concentration and (b) contact time

Physicochemical Properties of Soil Sample

The physicochemical properties including soil texture, pH, moisture, nitrogen content, phosphorus content and potassium content of soil sample were characterized and the result obtained were present in Table 4. The soil texture was determined by laboratory analysis. The soil samples were averagely sandy loam and (33.12 % sand, 52.00 % silt and 14.88 % clay) with very low contents of nitrogen, phosphorous and potassium (<1 %) were shown in Table 4. Oceanic waters contain only about 0.04 percent of potassium in contrast with about 2.45 percent in the earth's crust. A soil with 0.29 % of total potassium would be rated very low in this element. Potassium as nitrogen and phosphorous must be in an available form, or in other words soluble in the soil moisture, before plants can utilize it. Without sufficient available of potassium in the soil crop plants suffer in reduced vigor, greater susceptibility to disease, impairment of growth process. Potassium might be considered to stand between nitrogen and phosphorous in its effects on plant growth. A lack of available potassium in the soil is very likely to result in poor quality of the crop (Zhang and Kang, 2013).

Table 4 Characteristics and Physico-chemical Properties of Crude Oil Contaminated Soil

Test Parameter	Result
Texture	Sandy loam
Sand (%)	33.12
Silt (%)	52.00
Clay(%)	14.88
pH	9.16
Moisture content (%)	0.13
Humus	1.20
Organic matter content (%)	1.27
Total Nitrogen content (%)	0.11
Total Phosphorus content (ppm)	1.00
Potassium content (mg/100 g)	0.29

Efficiency of Saponin on Remediation of Petroleum Contaminated Soil (Bench Study)

A laboratory bench study was conducted to assess the removal efficiency of saponin on 1 % crude oil contaminated soil. Various concentration (250, 500 and 1000 ppm) of saponin treatments along with control (no saponin) were applied. All treatments showed the results of residual crude oil 96.97 %, 90.99 %, 75.11 % in soil treatment by 250, 500 and 1000 mg saponin/kg of soil whereas 95.94 % residue was found in control soil. The results were shown in Table 5. According to these data, the concentration of saponin (1000 ppm) decreases the highest residual percent of crude oil in soil during 10 weeks treatment.

Efficiency of Saponin on Remediation of Petroleum Contaminated Soil (Phytoremediation)

A laboratory study was extended to assess the potentials of local grass (Bermuda grass) under normal environmental conditions with or without surfactants in remediating soil contaminated with crude oil. Each 1 kg of the contaminated soil sample (1% oil in soil) was distributed into nine plastic pots. The Bermuda grasses were transplanted into six pots for two treatments (three pots for plant with commercial surfactant (sodium lauryl ether sulfate-SLES) and three pots for plants with natural surfactant). The other three pots served as Control with plant only. The results of the phytoremediation of contaminated soil showed that significant reduction (76.3 %) of crude oil was observed by natural saponin. It was also observed that the crude oil in the polluted soil were reduced by 72.4 % as a result of plant only, similar to surfactant (SLES) treatment (72.1 %). These data were present in Table 8. According to phytoremediation data, the contaminated soil with plant decreases the residual percent of crude oil in soil.

Table 5 Changes of Residual Percent of Crude Oil in Soil during 10 Weeks Treatment

Time (week)	Residual crude oil (%) in treated soil by			
	Saponin (0 ppm)	Saponin (250 ppm)	Saponin (500 ppm)	Saponin (1000 ppm)
0	100.00	100.00	100.00	100.00
2	104.80	103.95	102.21	91.27
4	97.42	97.69	96.98	89.01
6	96.48	97.66	96.40	85.63
8	97.77	98.45	92.32	79.40
10	95.84	96.97	90.99	75.11

Table 6 Residual Percent of Crude Oil in Saponin Enhanced Phytoremediation

Time (week)	Residual crude oil (%) in treated soil by		
	Soil+Oil+Plant	Soil+Oil+ Plant+Saponin	Soil + Oil+ Plant+SLES
0	100.0	100.0	100.0
4	23.7	27.0	27.3
6	24.3	25.8	27.3
8	26.0	24.7	27.2
10	27.6	23.7	27.9

SLES = Sodium Lauryl Ether Sulfate

Conclusion

The yield % of saponin was found to be highest of 14.55 % in Onion skin, in compare with that of Ka Nyut hard stem (2.53 %) and Hin Nu Nwe hard stem (2.12 %). By Froth test, higher concentration of saponin increases with the permanent foaming high. According to FT IR spectral data, saturated and unsaturated H/C group and cyclic alcohol group were observed in the extracted crude saponin. Emulsifying capacity of Onion skin saponin was changed with saponin concentrations and then saponin (100 µg/mL) possesses the highest emulsifying capacity. Emulsifying capacity was also changed with contact time between saponin solution and paraffin. Crude saponin extracted from Onion skin is potentially applied on the petroleum biodegradation in soil sample. A laboratory study was conducted to assess the potentials of natural saponin and local grasses under normal environmental conditions in remediating soil contaminated with engine oil. Results obtained showed a considerable reduction in the crude oil level of the soil samples compared to the unplanted control soil samples, moreover, the grasses and the saponin showed great promising potential as phytoremediating agents in order to clean-up of crude oil contaminated soil.

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