



4-2016

## Evaluation of an Alternative Natural Surfactant for Non Aqueous Phase Liquid Remediation

Brian A. Beach

Follow this and additional works at: [https://scholarworks.wmich.edu/masters\\_theses](https://scholarworks.wmich.edu/masters_theses)



Part of the Earth Sciences Commons

---

### Recommended Citation

Beach, Brian A., "Evaluation of an Alternative Natural Surfactant for Non Aqueous Phase Liquid Remediation" (2016). *Master's Theses*. 680.

[https://scholarworks.wmich.edu/masters\\_theses/680](https://scholarworks.wmich.edu/masters_theses/680)

This Masters Thesis-Open Access is brought to you for free and open access by the Graduate College at ScholarWorks at WMU. It has been accepted for inclusion in Master's Theses by an authorized administrator of ScholarWorks at WMU. For more information, please contact [wmu-scholarworks@wmich.edu](mailto:wmu-scholarworks@wmich.edu).



EVALUATION OF AN ALTERNATIVE NATURAL SURFACTANT FOR NON  
AQUEOUS PHASE LIQUID REMEDIATION

by

Brian A. Beach

A Thesis submitted to the Graduate College  
in partial fulfillment of the requirements  
for the degree of Master of Science  
Geosciences  
Western Michigan University  
April 2016

Thesis Committee:

Daniel P. Cassidy, Ph.D., Chair  
Duane R. Hampton, Ph.D.  
David A. Barnes, Ph.D.

## EVALUATION OF AN ALTERNATIVE NATURAL SURFACTANT FOR NON AQUEOUS PHASE LIQUID REMEDIATION

Brian A. Beach, M.S.

Western Michigan University, 2016

Applications of surfactant technology in the environmental remediation industry can greatly enhance the success of non-aqueous phase liquid (NAPL) remediation. Saponins derived from the bark of the Quillaja soapbark tree were evaluated as an alternative natural surfactant. Properties including the critical micelle concentration, emulsion kinetics and the solubilization enhancement of sixteen PAHs (polycyclic aromatic hydrocarbons) were measured. The critical micelle concentration (CMC) for Quillaja saponin was found to be 60 mg/L. Soil contaminated with NAPL from a former manufactured gas plant was used to evaluate saponin's ability to enhance the solubilization of PAHs commonly found in NAPL. Although solubilization enhancement was observed for all of the PAHs analyzed, the greatest enhancements occurred for PAHs having a larger number of aromatic rings. The solubilization enhancement data were, in turn, used to quantify the solubilization capacity of saponins and shown to have a strong correlation with the intrinsic properties of the PAHs. A novel determination was made with respect to emulsion kinetics. The optimal resting period determined by this research to maximize the effectiveness of saponins was found to be approximately 14 days. Overall, this research showed that saponins are an effective alternative for NAPL remediation.

Copyright by  
Brian A. Beach  
2016

## **ACKNOWLEDGEMENTS**

Thank you to Dan Cassidy for advising me through this knowledge-filled journey. Much of this research could not have been completed without your guidance and personal stash of contaminated soil. You provided access to not only material for research, but also to resources to assist in collecting data beyond what my part-time graduate career could handle. I sincerely hope that our paths will meet again on an academic or professional level. Thanks for bearing with me and getting me through. Thank you also to my family, I know my sacrifices are never my own.

Brian A. Beach

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	ii
LIST OF TABLES.....	iv
LIST OF FIGURES.....	v
1.0 INTRODUCTION .....	1
1.1 Literature Review.....	3
1.2 Research Objectives .....	14
2.0 MATERIALS AND METHODS .....	16
2.1 Materials.....	16
2.2 Methods.....	18
2.2.1 CMC Measurements.....	18
2.2.2 Solubilization Enhancement of Polycyclic Aromatic Hydrocarbons.....	19
2.2.3 Emulsion Kinetic Measurements .....	20
2.3 Analysis .....	21
3.0 RESULTS.....	23
3.1 CMC .....	23
3.2 Solubility Enhancement of PAHs Using Saponins.....	26
3.3 Saponin Emulsion Kinetics .....	34
3.4 Alternative Quantification .....	35
4.0 CONCLUSIONS.....	38
REFERENCES.....	40

## LIST OF TABLES

1. Effect of pH and Electrolyte Concentrations on the CMC Values of Quillaja Saponin .....	8
2. Comparison of Saponin Solubilization for Phenanthrene With Other Surfactants .....	9
3. Properties of the Homogenized Contaminated Soil Before Treatment .....	17
4. Measured Surface Tension Data for Quillaja Saponins .....	25
5. Measured Physical Properties of Contaminated Soil .....	27
6. Baseline PAH Concentrations of Contaminated Soil .....	28
7. PAH Concentrations (mg/L) at Respective Dose Above CMC (60 mg/L) .....	30
8. Saponin Solubilization Capacity .....	35

## LIST OF FIGURES

1. Diagram of a Micelle .....	1
2. Winsor Type Emulsions .....	2
3. Diagram Showing Ethoxylation .....	5
4. Molecular Structure of Quillaja Saponin .....	7
5. Changes in Phenanthrene Solubilization at Different pH .....	9
6. Sorption Isotherms for Saponin on Soil with Different Organic Content ....	10
7. Sorption Isotherms of Saponin and TX 100 on a Soil .....	11
8. Effects of Electrolytes on the Desorption of Cadmium with Saponin .....	13
9. Surface Tensiometer .....	18
10. 2.5 L Vessels Used to Determine the “Apparent” Solubility of the PAH in the Test Soil .....	20
11. 600 mL Beakers Used for Emulsion Kinetics Tests .....	21
12. Measured Surface Tension Values for Quillaja Saponin .....	26
13. Apparent Solubility of 2 and 3 Ring PAHs .....	31
14. Apparent Solubility of 4 Ring PAHs .....	31
15. Apparent Solubility of 5 and 6 Ring PAHs .....	32
16. Solubility Enhancement of 2 and 3 Ring PAHs .....	32
17. Solubility Enhancement of 4 Ring PAHs .....	33
18. Solubility Enhancement of 5 and 6 Ring PAHs .....	33
19. Saponin Emulsion Kinetics .....	34

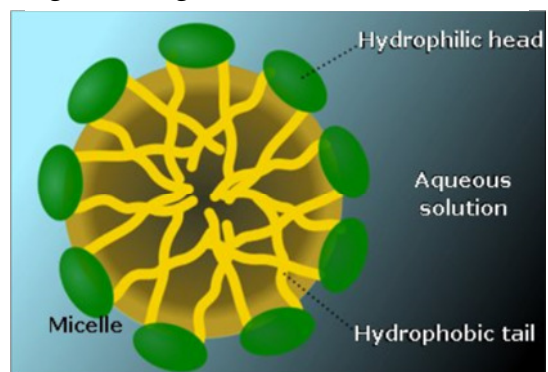


## 1.0 INTRODUCTION

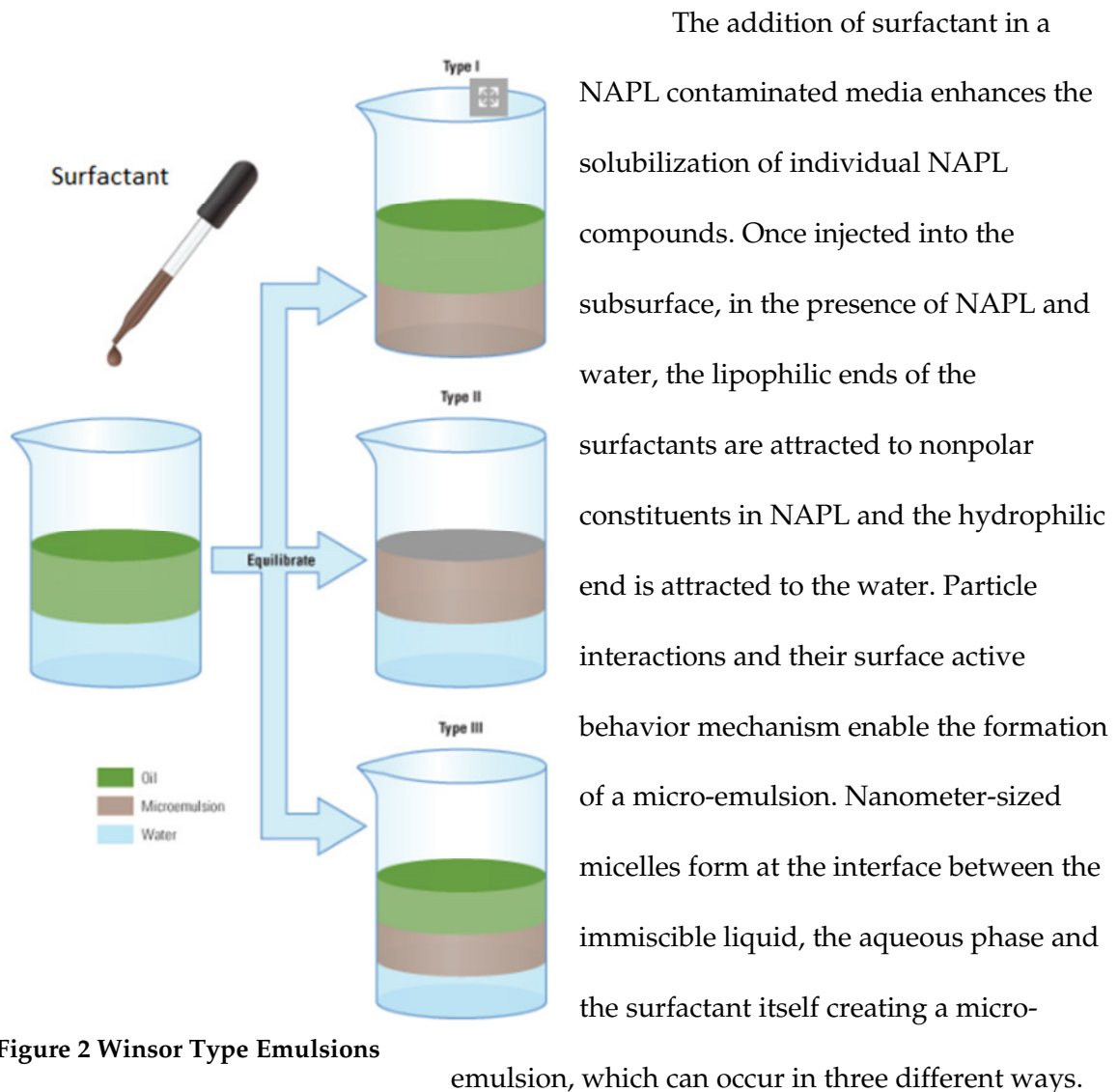
Surfactants have applications in many aspects of life. Because of their ability to lower the surface tension of water, thus enhancing the solubilization of various compounds, surfactants are paramount for their uses as detergents, emulsifiers and foaming agents. Surfactants are currently utilized in the cosmetics industry, the pharmaceutical industry, the medical industry, and specially engineered in the petroleum industry for a variety of applications including enhanced oil recovery and washing drilling cuttings.

The molecular structure of a surfactant molecule consists of two parts; a hydrophobic (non-polar) “tail” and a hydrophilic (polar) “head” which give the structure its amphipathic properties. The design to be both soluble and insoluble in water enables surfactants to become reactive at interfaces including the liquid-air interface; the liquid-solid interface and the oil-water (liquid-liquid) interface. Surfactants introduced in water in the presence of other non-aqueous phase liquid (NAPL) constituents have the tendency to aggregate and configure their molecular structure to form a micelle (Figure 1). The spherical geometric structure of a micelle thus acts as a vehicle to facilitate the solubilization of a normally insoluble compound. The many different nonpolar organic compounds that make up a NAPL greatly reduce the NAPL’s aqueous solubility based on each individual compound’s mole fraction in the NAPL and

**Figure 1 Diagram of a Micelle**



activity coefficient, as described by Raoult's Law (Mulligan, 2005). This results in decreased mobilization resulting in persistent residual impact that can be unaffected by common pump and treat remediation systems (Fountain et al., 1996) or other NAPL recovery methods.



**Figure 2 Winsor Type Emulsions**

Figure 2 depicts the different categories of micro emulsions which are found to be thermodynamically stable, homogeneous and optically isotropic solutions (Castro Dantas et al., 2003). According to Castro Dantas et al., an excess of oil-in-water is an "oil

continuous”, Winsor Type I micro emulsion, and an excess of water-in-oil is a “water continuous”, Winsor Type II micro emulsion system.

The effectiveness of a surfactant is evaluated based on its ability to lower the surface tension while using the minimum amount of surfactant to cause micelle formation (Mulligan, 2005). The point at which the concentration of a surfactant enables the formation of micelles is termed the critical micelle concentration (CMC) and this property is influenced by several factors including pH, temperature, ionic strength and salinity (Bera et al., 2013; Mulligan, 2005; and Zhou et al., 2011). Manipulating surfactants and exploiting their surface active behavioral properties has led to a multitude of possible applications that have relatively recently been shown to be promising alternatives in the environmental remediation industry.

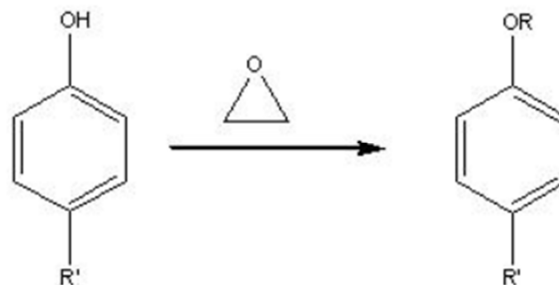
## **1.1 Literature Review**

Some of the earlier applications of surfactants in the environmental industry were at contaminated sites undergoing surfactant enhanced aquifer remediation (SEAR). The technical basis to support these applications was to increase the effectiveness of simple groundwater pump and treat systems and enhance the mobilization and recovery of residual NAPL. The addition of relatively inexpensive surfactants in SEAR would reduce the operating time of pump and treat systems and achieve regulatory cleanup goals. Among the challenges faced with SEAR, even today, are meeting regulatory requirements to recover the volume of material injected into the subsurface, and developing a complete conceptual site model that fully defines the hydrogeological system that controls the movement and chemical interactions of injected material in the subsurface.

Fountain et al. (1996) demonstrates the practice of creating micro-emulsions in a field scale study to enhance the effectiveness of a pump and treat system designed for the remediation of a dense non-aqueous phase liquid (DNAPL) plume at a Canadian military base. Their research modified a pump and treat system with the addition of ethoxylated surfactants to increase the solubility and mobilization of the DNAPL constituents, specifically tetrachloroethylene (PCE). They designed column experiments using soil from the site to evaluate the effectiveness of lowering the interfacial tension (IFT) of PCE using nonylphenol ethoxylate and a phosphate nonylphenol ethoxylate. One interesting discovery made from the column experiments was that the mixture of the two surfactants was more efficient at emulsifying the DNAPL than using nonylphenol ethoxylate alone. This was attributed to the formation of more viscous emulsions by nonylphenol ethoxylate.

Surfactants are both naturally occurring in several varieties of plants, including alfalfa, soy beans and soapwort, and they can be synthesized from petroleum based products. Both perform comparably and natural surfactants are arguably more biodegradable and less toxic than synthesized surfactants. However, synthetic surfactants can be engineered to mimic the properties of natural surfactants, including biodegradability, but there are higher costs associated with some of these specialized engineering practices. This, in turn, may make some of the natural surfactants more desirable to practitioners. Common synthetic surfactants used in contaminated site remediation include sodium-dodecyl-sulfate (SDS), Triton X-100, Tween-80, Brij-30, VeruSol-3 and E600 (Wyrwas et al., 2011; Bandala et al., 2010; Singh et al. 2013; Wang et al., 2013; Rodriguez-Cruz et al., 2005; and Iturbe et al., 2008). The synthesis of these

ethoxylated surfactants utilizes a common industrial process where ethylene oxide is added to alcohols and phenols in the presence of a catalyst (Figure 3)



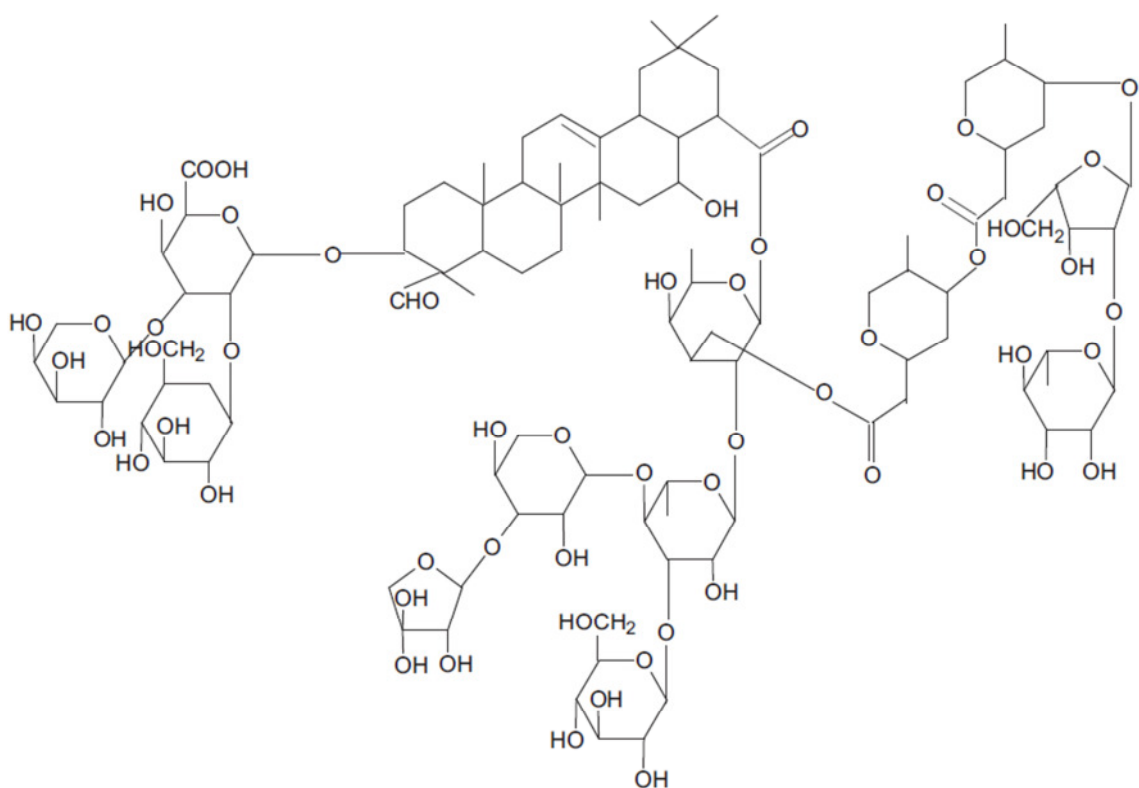
**Figure 3 Diagram Showing Ethoxylation**

(Wikipedia, 2015). This process has been attributed to the potential formation of 1-4 dioxane which has been identified as a known carcinogen (Wikipedia, 2015). Additionally, ethoxylated alcohols have been found to exhibit acute toxicity to *daphnia magna* (water flea) and certain ethoxylated alkylphenols have been determined to degrade relatively slowly and produce toxic biodegradation products (Roberts et al., 2007; Kibbey and Hayes, 2000). Alternatively, researchers have turned to evaluating natural surfactants for their applications for contaminated site remediation in the environmental industry.

Ahmadi et al. (2014) investigated the use of a natural surfactant derived from micro particles of the mulberry leaf on lowering the IFT in a mixture of distilled water and kerosene. Their research was able to determine that a mixture of just 1 wt% of the mulberry leaf derived surfactant could effectively lower the IFT of kerosene by 60%. Furthermore, they applied their findings to design an experiment using a core displacement apparatus to show that the naturally derived surfactant could increase the sweep efficiency of brine flooding in enhanced oil recovery (EOR) from 49% to 66.8% of the original oil in place. This research concluded that there may be more economical, naturally occurring surfactants available that could achieve the objectives of EOR while eliminating the use of industrial surfactants that may be less biodegradable and more toxic to the environment.

A comparison of the effectiveness of natural surfactants versus synthetic surfactants on the removal of crude oil contamination from soils was conducted by Urum et al. (2005). In their research, they used rhamnolipid and saponins as their source for natural surfactants and SDS as the synthesized surfactant. Their research was accomplished by using GC/MS to measure the concentrations of hydrocarbons on soil washed with each surfactant compared to a control. The results of their research indicated that, although SDS showed a greater overall effectiveness at removing crude oil contamination from soil, comparatively, each surfactant performed differently at removing specific constituents found within the composition of crude oil. Urum et al. (2005) showed that SDS was best at removing the aliphatic constituents and that rhamnolipids and saponins were better at removing the aromatic hydrocarbons.

Saponins are a family of surfactants that, along with natural occurrence as plant-derived surfactants, have a unique molecular structure with the potential for remediating mixed contaminated sites. In general, co-contaminated sites pose more difficult challenges to environmental remediation practitioners. The base structure of saponins is categorized as a triterpene sapogenin, which acts as the hydrophobe. Attached to this are various hydrophilic functional groups including acids, carbohydrates and other glycosides (Zhou et al., 2011). The molecular weight of saponin was determined by Mitra and Dungen (1997) to be approximately 1,650 g/mol. The configuration of the saponin molecule is unique because it doesn't take on the elongated chain-like configuration that is common in other surfactant compounds which has attributed to saponin's ability to act as an effective chelator for various heavy metals



**Figure 4 Molecular Structure of Quillaja Saponin**

including cadmium, zinc, copper, lead and nickel (Hong et al. 2002, Song et al., 2008, Chen et al., 2008, Castro Dantas et al., 2003 and Lu et al., 2014).

Utilizing saponins from the Quillaja soapbark tree to wash soil contaminated with polycyclic aromatic hydrocarbons (PAHs) was evaluated by Zhou et al. (2011). Their findings showed that the effective properties of saponins (10% purity) are not immune to changes in environmental conditions, and that the CMC of saponins varied with changing pH as well as with increasing concentrations of electrolytes (Table 1). Overall, there was a decrease in the CMC of saponins as pH decreased and as the concentration of the NaCl electrolyte solution increased (Zhou et al., 2011). As with the CMC, changes in pH were also shown to affect the saponin's ability to enhance the solubilization of PAHs, specifically phenanthrene (Figure 5). Saponins were shown to be

most effective at enhancing the solubilization of phenanthrene when its CMC was

**Table 1 Effect of pH and Electrolyte Concentrations on the CMC Values of Quillaja Saponin (from Zhou et al., 2011 p. 1202)**

Conditions	CMC ( $\mu\text{mol/L}$ )	
pH	4.0	16.5
	5.0	28.1
	6.0	58.6
	7.0	85.3
	8.0	92.5
NaCl concentrations (M)	0.01	30.9
	0.05	24.2
	0.10	22.0
	0.50	10.1
	1.00	5.2

lowest, which occurs in conditions of low pH or in the presence of elevated concentrations of electrolytes (Zhou et al., 2011).

Ultimately, Zhou et al. were able to show that saponins have a greater capacity at enhancing PAH solubility than other tested

synthetic surfactants and biosurfactants. Their evaluations included calculating the molar solubility ratio (MSR), weight solubility ratio (WSR) and the micelle-water partition coefficient ( $K_m$ ) of phenanthrene. Table 2 below presents the quantitative results of their evaluations in comparison with other synthetic surfactants and biosurfactants.



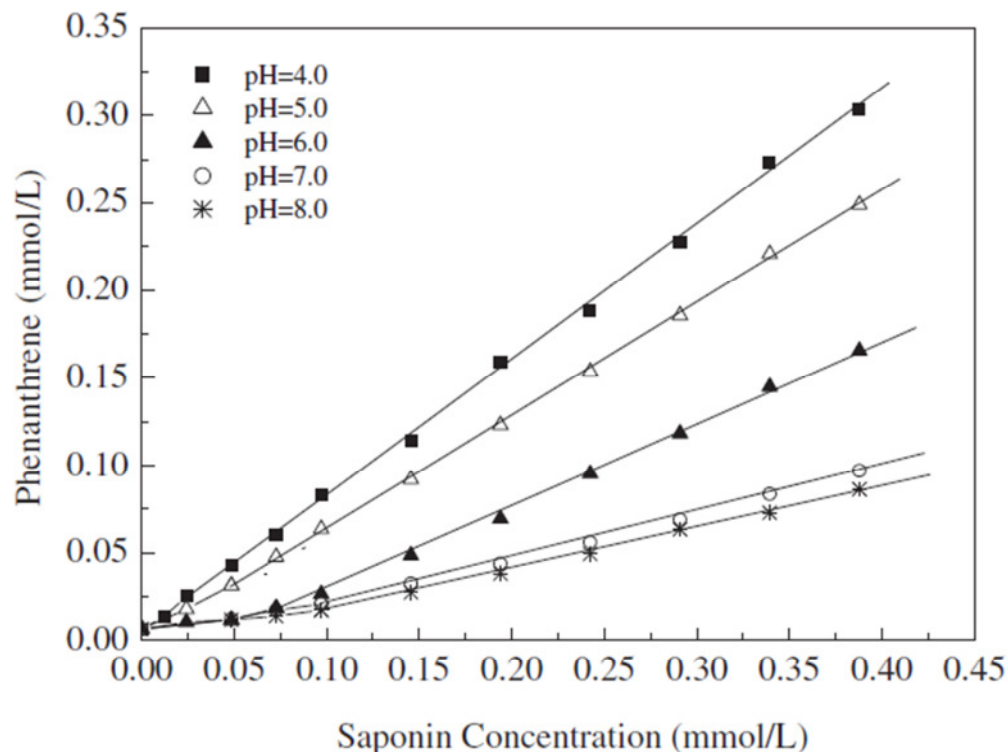


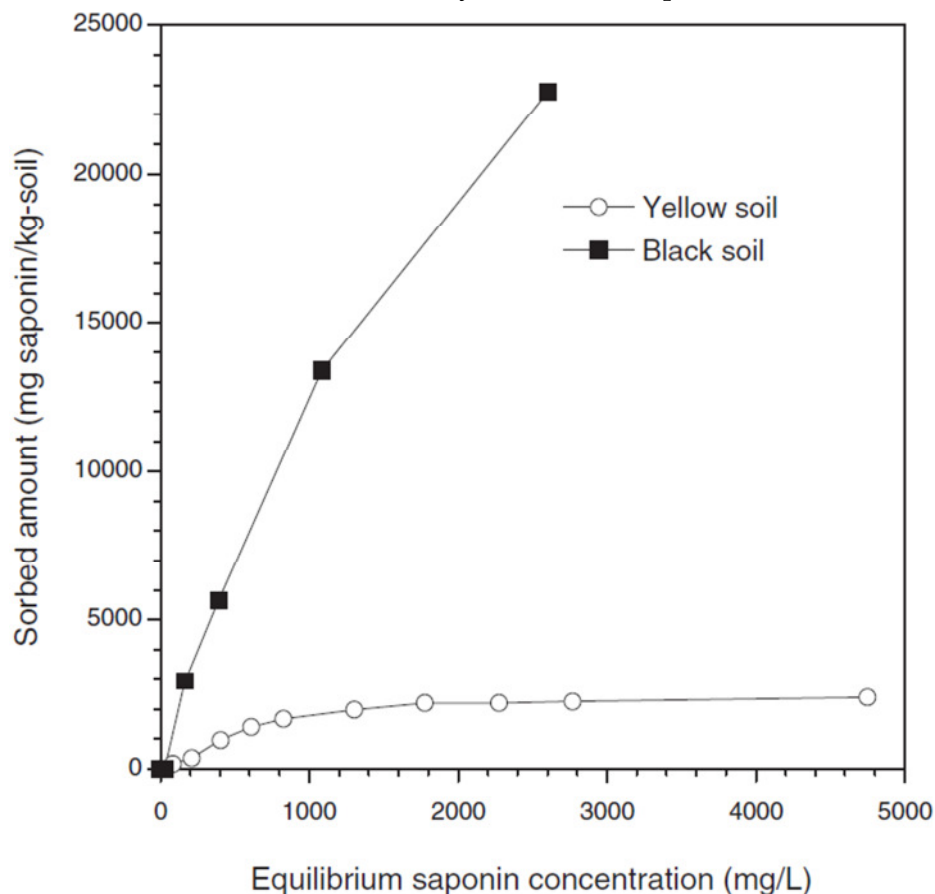
Figure 5 Changes in Phenanthrene Solubilization at Different pH (from Zhou et al., 2011 p. 1202)

Table 2 Comparison of Saponin Solubilization for Phenanthrene With Other Surfactants (from Zhou et al., 2011 p. 1201)

Surfactants	MW	MSR	WSR	LgK <sub>m</sub>	Ref.
Saponin	1650	0.606	0.0655	6.50	This study
Rhamnolipid	—	0.0425	—	—	Shin et al., 2004
Monorhamnolipid	504	0.057	0.02	—	Zhang et al., 1997
Dirhamnolipid	650	0.021	0.006	—	Zhang et al., 1997
Brij-58	1124	0.199	0.0316	6.12	This study
		0.18	0.0285	6.2	Prak and Pritchard, 2002
Brij-35	1198	0.13	0.0193	6.1	Prak and Pritchard, 2002
Tween-80	1310	0.265	0.0361	6.22	This study
		0.19	0.0258	6.2	Prak and Pritchard, 2002
Tween-20	1228	0.14	0.0203	6.1	Prak and Pritchard, 2002
TX-100	625	0.141	0.0402	5.99	This study
		0.111	0.0316	5.70	Edwards et al., 1991
Igepal CA-720	735	0.104	0.0252	5.68	Edwards et al., 1991
Tergitol NP-10	683	0.160	0.0417	5.72	Edwards et al., 1991

Similarly, PAH impacted soil washed with Quillaja saponins was also evaluated by Kobayashi et al., 2012. While they were able to obtain similar results as Zhou et al. (2011) with respect to solubilization enhancement of certain PAHs, their research evaluated the tendency for saponins to adsorb onto soil particles of artificially spiked soils having different organic content profiles. Two different soils were tested and the influence of organic content was represented on a sorption isotherm plot showing the sorbed amount of saponins at increasing concentrations in each respective soil (Figure 6). Although their findings determined that saponins were effective at enhancing the solubilization of PAHs, they demonstrated that its effectiveness can be limited by the organic content of the soil itself.

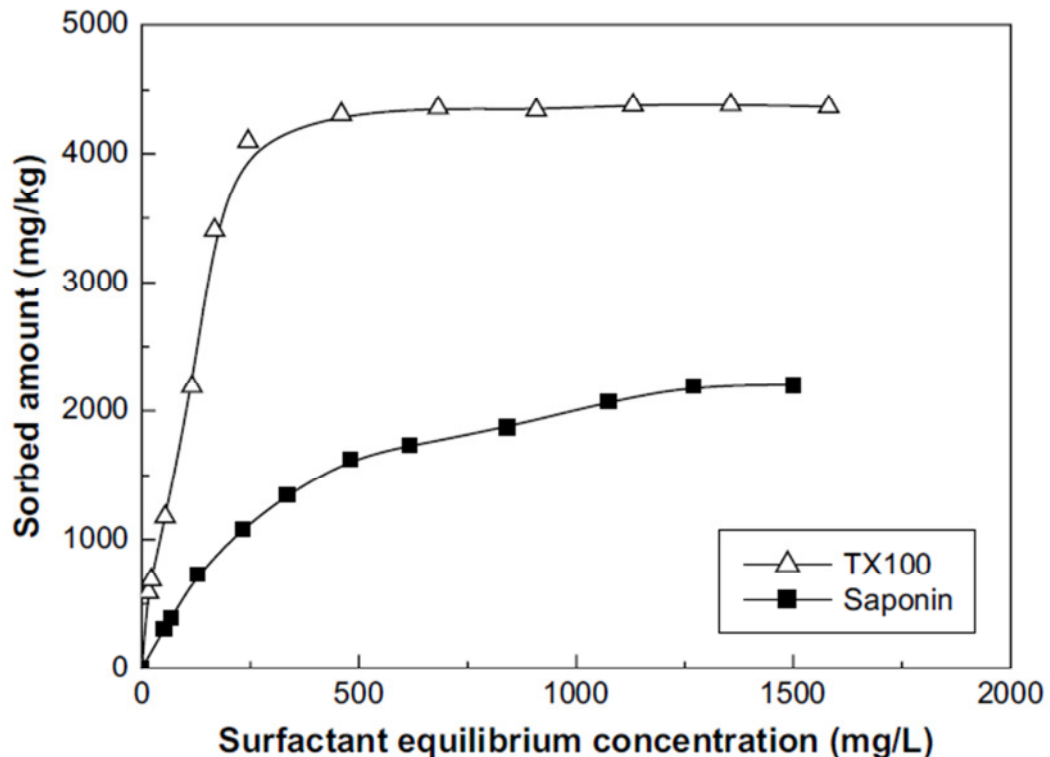
**Figure 6 Sorption Isotherms for Saponin on Soil with Different Organic Content (from Kobayashi et al., 2012 p. 1143)**



Heavy metal chelation abilities of saponins were demonstrated in research conducted by Hong et al. (2002), which utilized Quillaja saponins. They determined that given the optimum pH range (5.0 – 5.5 s.u.), saponins successfully removed 90% - 100% cadmium and 85% - 98% zinc from different soil types. Reduction of heavy metal concentrations were found to follow first order kinetics with equilibrium being reached in approximately 6 hours according to the following reaction rate hierarchy; zinc > cadmium > copper > lead.

Song et al. (2008) further demonstrated the soil washing capabilities of saponins in a mixed contaminant scenario. Their research showed that saponins effectively increased the desorption of phenanthrene from soil, which was also shown to perform better than a comparable synthetic surfactant, Triton X100 (Figure 7). Although Triton X100 showed success at partitioning phenanthrene into its micelle, there was an increase

Figure 7 Sorption Isotherms of Saponin and TX 100 on a Soil (from Song et al., 2008 p. 1369)



in the potential for the surfactant to adsorb onto solid soil particles as the concentration of the surfactant increased. This, in turn, caused the phenanthrene to persist in the soil as well. In contrast, saponins were shown to have an increase in phenanthrene partitioning with increasing surfactant concentration without the increase in adsorption onto the soil particles. The structure of the saponin enriched micelle further allowed cadmium present in the soil to chelate with the polar carboxyl group located on the exterior of the micelle. Essentially, Song et al. were able to show that saponins may be effective for the remediation of mixed contaminant sites. Mixed contaminant sites pose their own challenges because while one remedial technique might work to clean up one contaminant at a site, the technique may not have an impact on other contaminants present at the site which may exacerbate the impacts of other contaminants present on the site.

Lu et al. (2014) demonstrated the effectiveness of soil washing methods using natural saponins derived from tea seed (tea saponin). Their research showed that tea saponin was effective at removing cadmium from artificially contaminated soil samples spiked with cadmium nitrate, at an efficiency ranging from 64.6% to 74.5%. Higher desorption rates were achieved nearly two-fold by adding  $\text{NaNO}_3$  as a background electrolyte (Figure 8). This was attributed to the cation exchange taking place between the  $\text{Na}^+$  and  $\text{Cd}^{2+}$  ions whereby the  $\text{Na}^+$  showed greater affinity to replace the  $\text{Cd}^{2+}$  ions on the particle surfaces. Desorption of cadmium was shown to occur relatively quickly, reaching equilibrium within 20 minutes of reaction time.

This reaction time was quicker than desorption studies performed by Chen et al. (2008). In their research, they used saponin derived from the bark of the quillaja tree to

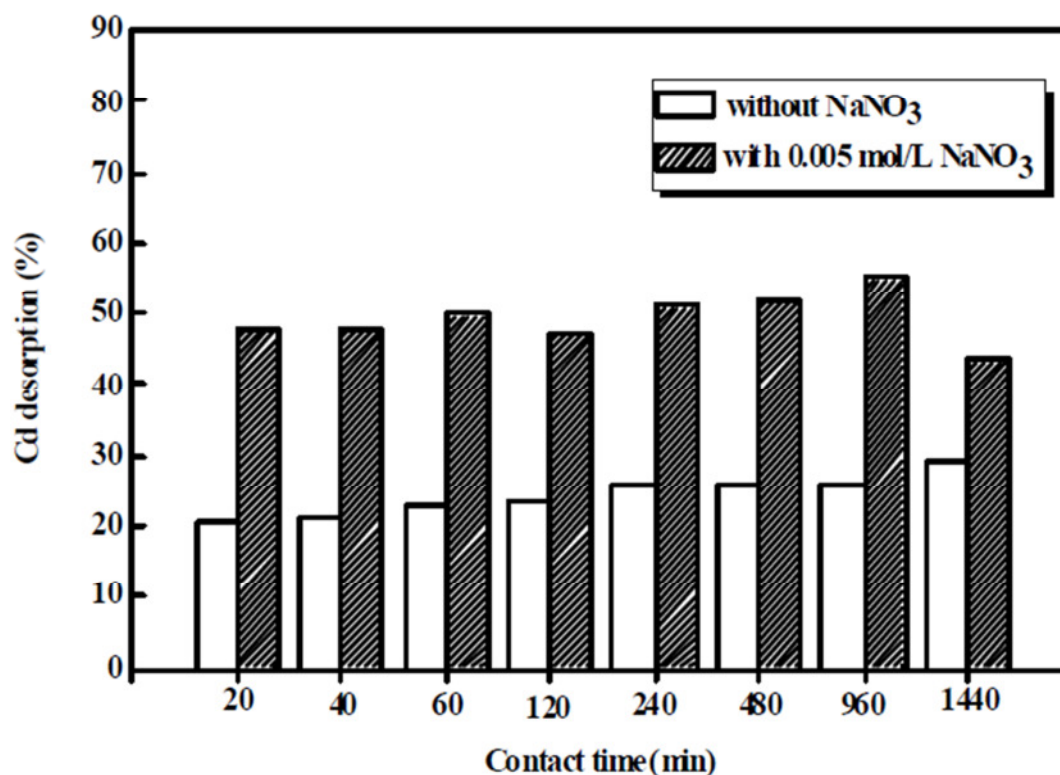


Figure 8 Effects of Electrolytes on the Desorption of Cadmium with Saponin  
(from Lu et al., 2014 p. 162)

treat kaolin clay soils artificially contaminated with cadmium, copper and lead. Chen et al. (2008) presented a comparison of metal desorption efficiencies using quillaja bark saponin and SDS with an ethylenediaminetetraacetic acid (EDTA) chelate. Their research showed that saponin effectively competes with the kaolin clay for metal complexation based on Lewis Acid-Base interaction induced by pH, structure size or charge of surfactant solution. Several important characteristics of saponins were determined by Chen et al. (2008) including the effects of pH on the CMC of aqueous saponin alone and with saponin complexed with copper and nickel. They found that the CMC of saponin at a near-neutral pH of 6.5 s.u. remained unchanged when chelated with heavy metals and that micelles were more difficult to generate at a higher alkaline pH of 10 s.u. The effectiveness of removing heavy metals from a kaolin soil system using saponins was shown to be a viable alternative when compared to EDTA or SDS.

Although greater removal was observed using EDTA, the results using saponins were comparable and performed much better than SDS.

Through the development of surfactant technology, researchers have found that the applications for surfactants in site remediation can vary depending on what type of surfactant is used as well as in the methods of implementation. The typical application of surfactants in environmental remediation has been to inject the aqueous surfactant into the subsurface. Promising alternative applications of surfactants that have been successful in remediating contaminated sites include soil washing, enhanced bioremediation, enhanced *in situ* chemical oxidation, enhanced pump and treat and activation of *in situ* chemical oxidants. Moreover, surfactants such as saponins have been shown to be successful at remediating sites with mixed contaminants.

## **1.2 Research Objectives**

In addition to presenting a review of some of the current applications of surfactants for environmental remediation, the main objectives of this research is to evaluate Quillaja saponins for use as an alternative surfactant. Properties of the Quillaja saponins that will be explored include determining the CMC. Based on research conducted by Mitra and Dungen (1997) it is important to determine the CMC of the tested saponins because the intrinsic properties were shown to vary between slight differences in molecular structure and impurities present in industrial sources of the product. Secondly, this research evaluated Quillaja saponins with respect to their ability to enhance the solubilization of a total of sixteen PAHs. And lastly, this research was

used to address a common misconception in the environmental remediation industry regarding surfactant emulsion kinetics.

## **2.0 MATERIALS AND METHODS**

### **2.1 Materials**

All chemicals used were reagent grade. Saponins, extracted from Quillaja Saponaria Molina was purchased from Acros Organics (Thermo Fisher Scientific, New Jersey, USA). The 16 individual PAHs quantified were purchased from Aldrich (Milwaukee, Wisconsin, USA). For comparison of CMC data, Biosolve™, a commonly used surfactant in environmental remediation, was obtained for testing and used as received.

#### **2.1.1 Contaminated Soil**

The soil used in these studies was collected from a former manufactured gas plant (MGP) site in Ashland, WI. The soil was first sieved to remove particles larger than 2 mm. The sieved soil was homogenized in a portable cement mixer for 2 hours. Characterization of the homogenized soil was done according to Methods of Soil Analysis (ASA, 1994), and the results of the analyses are listed in Table 3.



**Table 3****Properties of the Homogenized Contaminated Soil Before Treatment.**

Analyte	Result	Units
sand-sized particles	80	% (w w <sup>-1</sup> )
silt-sized particles	18	% (w w <sup>-1</sup> )
clay-sized particles	2	% (w w <sup>-1</sup> )
native organic material (NOM)	0.2	% (w w <sup>-1</sup> )
total carbonates	3.0	% (w w <sup>-1</sup> )
pH	7.9 ± 0.1 <sup>a</sup>	
total PAH concentration	52,057 ± 3,210	mg kg <sup>-1</sup>

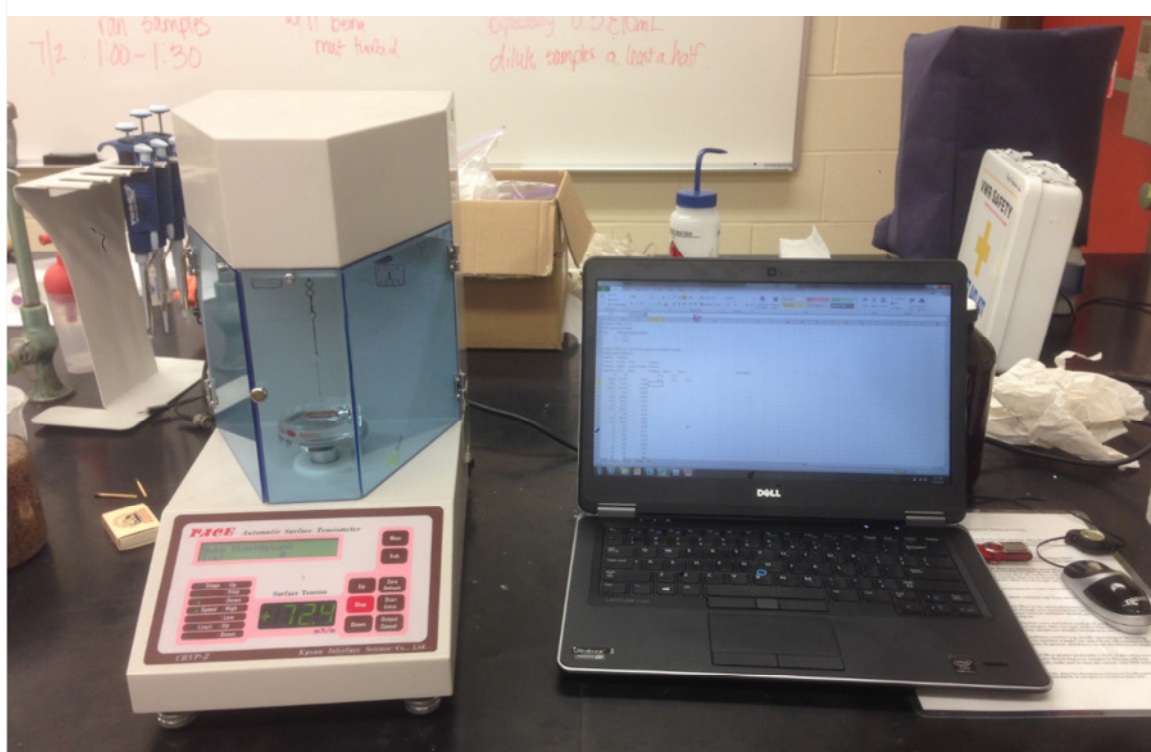
<sup>a</sup> mean ± standard deviation (number of measurements).

## 2.2 Methods

### 2.2.1 CMC Measurements

The CMCs of the Quillaja saponins and Biosolve were measured using the Wilhemy Plate method to measure the change in surface tension with increasing concentration of surfactant. Surface tension measurements were collected using a DyneMaster DY-300 from Kyowa Interface Science Co, Ltd. (Figure 9) which offers full automatic measurements of surface tension including IFT. The procedures for analyzing the surface tension of a sample were followed based on the instrument's user manual. For the Quillaja saponins, an initial 250 mL stock solution of 1 g/L aqueous saponins was prepared to evaluate a range of serial dilutions. Similarly, a 0.5% v/v stock solution of Biosolve was prepared to allow for a series of serial dilutions. Serial dilutions were

**Figure 9 Surface Tensiometer**



prepared by pipetting select volumes of the stock solution into a clean, plastic graduated sample vessel and diluting the sample to a total volume of 25 mL with distilled and de-ionized (DDI) water. The sample vessel was subsequently capped and inverted several times to ensure mixing. Lastly, the 25 mL sample was transferred into a clean glass dish and placed onto the instrument stage to be measured.

Prior to measuring the surface tension of the sample, the Wilhelmy plate was rinsed several times with DDI water and dried using an alcohol lamp which burned denatured alcohol. The plate was allowed to cool for a minimum of 30 seconds prior to collecting surface tension measurements. Data was collected by the surface tensiometer in standard, fully automatic mode in triplicate and recorded in Microsoft® Excel. The arithmetic mean was calculated and plotted on a graph of surfactant concentration vs. surface tension. The CMC was determined from the graphs to be the point at which a nominal increase in surfactant concentrations resulted in a minimal decrease in surface tension. The CMC was also determined quantitatively by plotting the linear regression of the data points both above and below the suspected CMC and observing the intersection of the two regressions (Zhou et al., 2011, Mitra and Dungen, 1997 and Edwards et al., 1991).

### **2.2.2 Solubilization Enhancement of Polycyclic Aromatic Hydrocarbons**

The apparent solubility of the 16 PAHs in the test soil was measured in a 2.5 L reaction vessel (Figure 10). The vessel received 1.5 L of site soil and 0.5 L of groundwater from the site. A separate reaction vessel was used for each reaction scenario (i.e., control with no added saponin, 120 mg/L saponin, 240 saponin, 360 mg/L saponin, and 600 mg/L saponin). The soil and water were mixed to maximize the rate and extent of

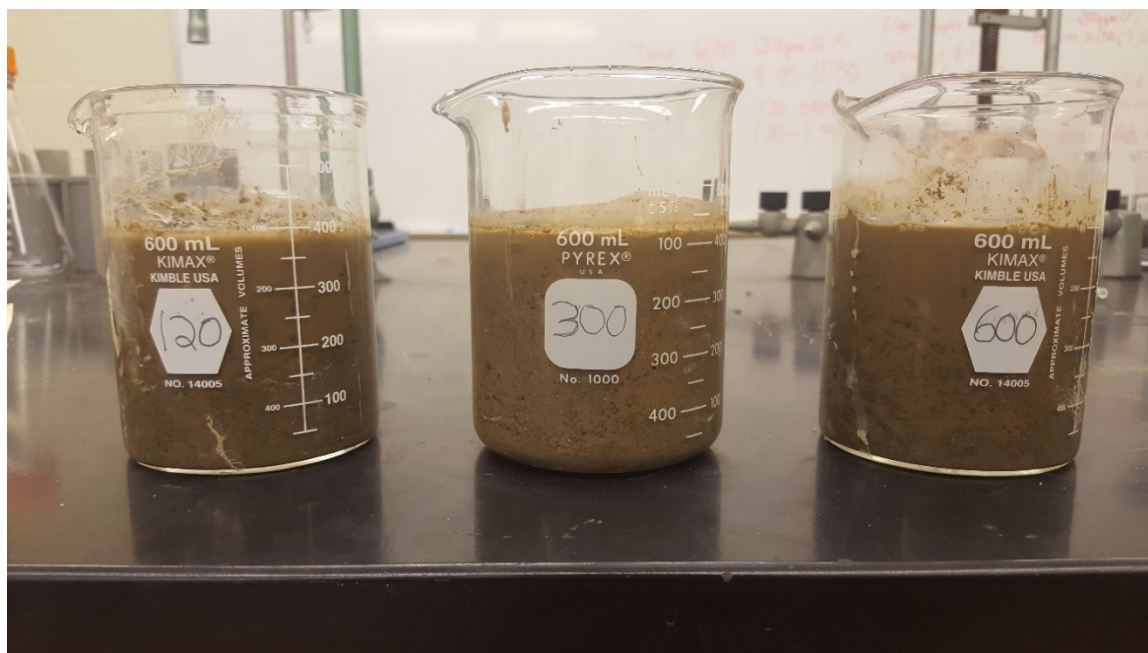


**Figure 10 2.5 L Vessels Used to Determine the “Apparent” Solubility of the PAH in the Test Soil**

dissolution of the PAHs. The reaction vessels were designed to be air tight, and were equipped with a dedicated mechanical mixer. The reactors were mixed for 2 weeks to allow dissolution to take place. After this time, the water from the reactor was removed and filtered (0.2  $\mu\text{m}$ ), and PAH concentrations were measured in the filtrate. PAH concentrations were also measured in the residual soil to demonstrate that each of the 16 PAHs quantified were present in excess.

### **2.2.3 Emulsion Kinetic Measurements**

The kinetics experiments to measure PAH emulsion for each of the 4 saponin doses (i.e., 120 mg/L, 240 mg/L, 360 mg/L, and 600 mg/L) was prepared using 600 mL beakers. Each beaker received 400 mL of homogenized soil and 120 mL of groundwater, which was sufficient to saturate the soil pores and leave a thin layer of standing water on top of the soil (Figure 11). At various time intervals (time-zero, and after 1, 2, 4, 7, 10, 14, 21, 28 days) an entire beaker was sacrificed for sampling. Time-zero samples were taken immediately after the reactors were set up. The reactors were sampled by filtering the pore water (0.2  $\mu\text{m}$ ) and then measuring the 16 PAHs in the filtrate at each sampling



**Figure 11 600 mL Beakers Used for Emulsion Kinetics Tests**

time. No pH buffers were added to any of the reactors. The reactors were maintained in a temperature controlled room at 20°C.

### **2.3 Analysis**

The pH of the filtrate from the emulsion kinetics studies and the solubility enhancement studies was measured with an Orion pH probe. The surface tension of the filtrate was also measured to confirm that saponins were present above the CMC in the filtrate. Surface tension was measured using a Tantec CBVP-Z Tensiometer. PAH concentrations were quantified with EPA Method 8270, using a Hewlett-Packard 5890 GC with a Supelco SPB-5 fused silica capillary column (30 m x 0.53 mm i.d.). The carrier gas was helium and the makeup gas was nitrogen. The injector and detector temperatures were 260°C and 320°C, respectively. The oven temperature started at 100°C for 2 min., increased to 280°C at a rate of 4°C/min, and then to 300°C at 24°C/min, where it was held for 10 minutes. Recovery of 2-fluorobiphenyl was in excess

of 95%, which indicates that the extraction method was quite effective. PAH concentrations in the whole slurry are reported as mg/kg, and in filtrate as mg/L.

### 3.0 RESULTS

#### 3.1 CMC

The CMC of saponins derived from Quillaja bark (10% purity) has been presented by Zhou et al., (2011) and was found to range from as low as 16.5  $\mu\text{mol/L}$  (30 mg/L) in a solution buffered to a pH of 4.0 s.u., to as high as 92.5  $\mu\text{mol/L}$  (150 mg/L) in a solution buffered to a pH of 8.0 s.u. Chen et al., (2008) determined the CMC of their saponins (13.9% purity) to range from 100 to 200 mg/L at a near neutral pH of 6.5. Other research conducted by Stanimirova et al., (2011), presented their CMC data for saponins from Quillaja bark to be approximately 0.15 mM (approximately 247.5 mg/L) or 0.025 wt. %. Relatively higher CMCs were also identified in earlier research conducted by Mitra and Dungen (1997) which measured the CMC of Quillaja saponins from three different manufacturers. Their CMC data ranged from 0.51 to 0.72 g/L at 298 K, which they attributed to the various manufacturers and Quillaja bark sources, which they suggest may have included non-surface active impurities, or higher proportions of glucose attached to the hydrophilic head group than in other sources of saponins (Mitra and Dungen, 1997). Notably, it was determined that the CMC of the Quillaja saponins varied between manufacturers and source and, thus, implies that it is necessary to determine the specific CMC for the product used in this research. As such, the Quillaja saponins evaluated as a part of this research was determine to have a CMC of approximately 60 mg/L (0.006 wt%) at a pH of approximately 4.5 s.u. (1 g/L stock solution). This value is within the range of CMC values determined by others presented herein. Table 4 provides a summary of the surface tension measurements taken at various saponin concentrations and these results are depicted in graphical form in

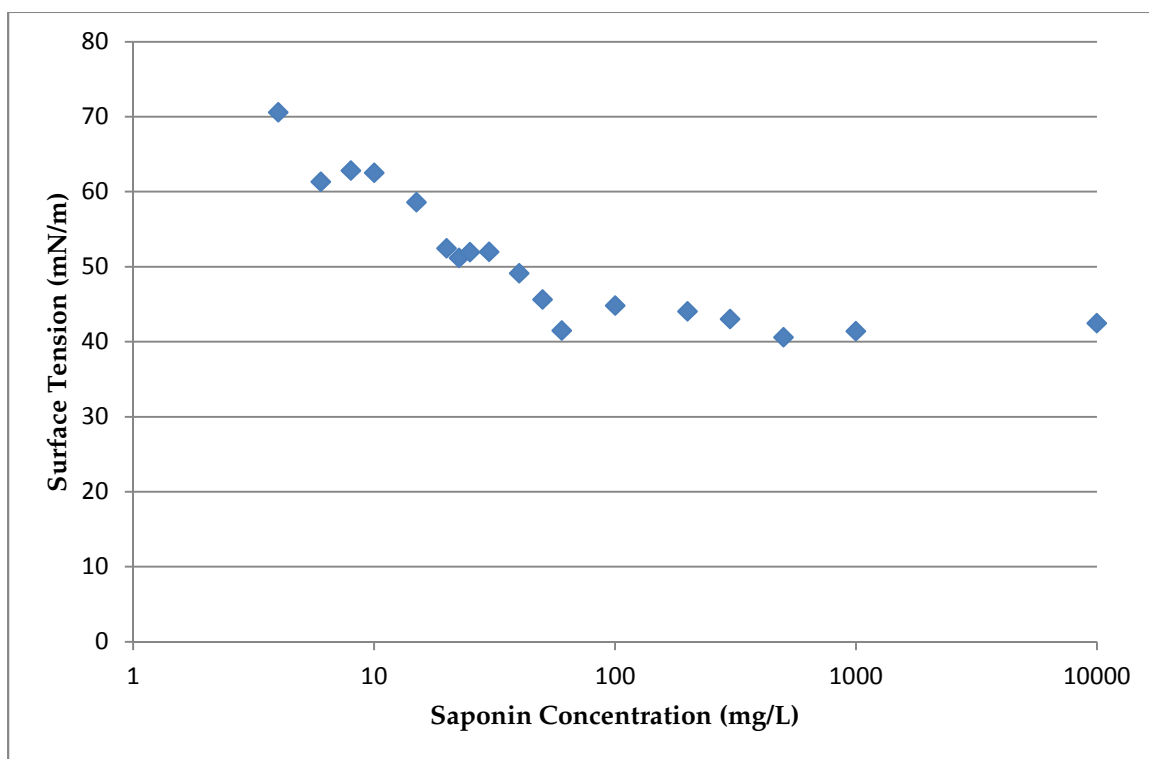
Figure 12. As proposed by Oakenfull (1986), the relative break in surface tension observed around the estimated CMC is an indication that saponins form micelles as opposed to other aggregate types. For comparison purposes, the CMC for Biosolve was evaluated and determined to be approximately 0.1% v/v, which is lower than the CMC determined for Quillaja saponin.



**Table 4****Measured Surface Tension Data for Quillaja Saponins**

Saponin Concentration (mg/L)	Average Surface Tension (mN/m)
0	72.20
4	70.57
6	61.30
8	62.82
10	62.50
15	58.60
20	52.43
22.5	51.17
25	51.93
30	51.97
40	49.13
50	45.63
60	41.47
100	44.80
200	44.03
300	43.00
500	40.60
1,000	41.40
10,000	42.47

**Figure 12 Measured Surface Tension Values for Quillaja Saponin**



### 3.2 Solubility Enhancement of PAHs Using Saponins

Data regarding the condition of the contaminated soil prior to the solubility enhancement studies is presented in Table 5. The average concentrations for each of the sixteen PAHs are presented in the table along with the standard deviation calculated from the results. The initial data shows that concentrations for each PAH evaluated were present in excess to adequately service as a testing media for this evaluation. Table 6 presents data regarding the physical properties of the PAHs analyzed including the water solubility ( $S_w$ ) and the octanol-water partitioning coefficient ( $K_{ow}$ ) for each compound. A notable increase in  $K_{ow}$  is observed as the incremental number of aromatic rings increases for each compound.

**Table 5**  
**Baseline PAH Concentrations of Contaminated Soil**

PAH	PAH Concentration (µg/Kg)					
	Sample 1	Sample 2	Sample 3	AVG	STDEV	Conc. (mg/kg)
Acenaphthene	1,200,000	1,400,000	1,100,000	1,233,333	124,722	1,233
Acenaphthylene	690,000	730,000	840,000	753,333	63,421	753
Anthracene	1,100,000	860,000	990,000	983,333	98,093	983
Benzo(a)anthracene	840,000	990,000	940,000	923,333	62,361	923
Benzo(a)pyrene	2,800,000	2,300,000	2,500,000	2,533,333	205,480	2,533
Benzo(b)fluoranthene	1,800,000	2,100,000	2,200,000	2,033,333	169,967	2,033
Benzo(ghi)perylene	2,800,000	2,800,000	2,800,000	2,800,000	0	2,800
Benzo(k)fluoranthene	890,000	810,000	950,000	883,333	57,349	883
Chrysene	1,400,000	1,600,000	1,300,000	1,433,333	124,722	1,433
Dibenzo(ah)anthracene	970,000	1,100,000	1,200,000	1,090,000	94,163	1,090
Fluoranthene	5,100,000	5,200,000	5,600,000	5,300,000	216,025	5,300
Fluorene	1,200,000	1,400,000	1,500,000	1,366,667	124,722	1,367
Indeno(123cd)pyrene	2,600,000	2,200,000	2,200,000	2,333,333	188,562	2,333
1-Methylnaphthalene	960,000	960,000	850,000	923,333	51,854	923
2-Methylnaphthalene	3,000,000	3,200,000	3,500,000	3,233,333	205,480	3,233
Naphthalene	8,700,000	9,900,000	9,600,000	9,400,000	509,902	9,400
Phenanthrene	8,300,000	8,100,000	8,700,000	8,366,667	249,444	8,367
Pyrene	6,500,000	6,000,000	6,900,000	6,466,667	368,179	6,467
<b>Total</b>	<b>50,850,000</b>	<b>51,650,000</b>	<b>53,670,000</b>	<b>52,056,667</b>	<b>3,210,000</b>	<b>52,057</b>

Table 6

## Measured Physical Properties of Contaminated Soil

PAHs (no. of rings)	Chemical Formula	MW	S <sub>w</sub> (mg/L) <sup>a</sup>	logK <sub>ow</sub> <sup>a,b,c,d</sup>
naphthalene (2)	C10H8	128.2	31.7	3.29
acenaphthene (3)	C12H10	154.2	0.82	4.03
acenaphthylene (3)	C12H8	152.2	0.85	3.93
anthracene (3)	C14H10	178.2	0.69	4.53
dibenzofuran (3)	C12H8O	168.2	0.78	4.12
fluorene (3)	C13H10	166.2	0.75	4.18
phenanthrene (3)	C14H10	178.2	0.69	4.56
benzo[a]anthracene (4)	C18H12	228.3	0.012	5.61
chrysene (4)	C18H12	228.3	0.012	5.90
fluoranthene (4)	C16H10	202.3	0.018	4.90
pyrene (4)	C16H10	202.3	0.018	5.18
benzo[a]pyrene (5)	C20H12	252.3	0.0011	6.04
benzo[e]pyrene (5)	C20H12	252.3	0.0011	6.21
benzo[b]fluoranthene (5)	C20H12	252.3	0.0011	6.12
benzo[k]fluoranthene (5)	C20H12	252.3	0.0011	6.14
dibenzo[a,h]anthracene (5)	C22H14	278.3	0.0006	6.50
benzo[g,h,i]perylene (6)	C22H12	276.3	0.0006	6.58
indeno[1,2,3-cd]pyrene (6)	C22H12	276.3	0.0006	6.58

Note: MW = Molecular weight (g/mol)

<sup>a</sup> from U.S. EPA (1984) and LaGrega et al.. (2001).

<sup>b</sup> from CDC-NIOSH (2016)

<sup>c</sup> from Hansch et al.. (1995)

<sup>d</sup> from USPHS (1990)

The data showing the apparent solubility ( $S_w^*$ ) of PAHs at saponin concentrations 2X, 4X, 6X and 10X the CMC are presented in Table 7. Apparent solubility is measured as the concentration of PAHs detected in the emulsified filtrate extracted from the experimental reactors. Graphical representations of the apparent solubilities of the sixteen PAHs tested are provided in Figures 13 through 15. Based on the apparent solubility results, higher concentrations of PAHs having fewer aromatic rings (two and three ring aromatics) were identified in the emulsion filtrate as compared to PAHs with a larger number of aromatic rings (five and six ring aromatics). However, when comparing the apparent solubility to the true solubility ( $S_w$ ) values for each respective PAH compound, it was noted that solubility enhancement was greater for PAHs having a larger number of aromatic rings than those with fewer aromatic rings. A depiction of the solubility enhancement of PAHs is presented as a function of the saponin concentration and the proportion of apparent solubility and true solubility in Figures 16 through 18. It should be noted that, based on the results of apparent solubility and solubility enhancement, these properties would increase linearly with the further increase in saponin concentrations above the CMC. Concentrations of saponins above 600 mg/L (10 X the CMC) were not evaluated because the relative cost related to the feasibility of this application was taken into consideration.

**Table 7****PAH Concentrations (mg/L) at Respective Dose above CMC (60 mg/L)**

<b>PAHs</b>	<b>Control</b>	<b>2 x CMC</b>	<b>4 x CMC</b>	<b>6 x CMC</b>	<b>10 x CMC</b>
naphthalene (2)	31.2	67.4	134.6	175.5	317.4
acenaphthene (3)	0.73	16.7	41.2	57.7	91.3
acenaphthylene (3)	0.77	20.2	39.1	64.4	95.4
anthracene (3)	0.65	18.9	40.7	66.2	105.3
dibenzofuran (3)	0.72	17.9	39.9	57.9	97.3
fluorene (3)	0.68	25.2	47.1	72.2	116.8
phenanthrene (3)	0.64	21.34	42.68	64.66	106.7
benzo[a]anthracene (4)	0.008	1.191	2.389	3.852	6.142
chrysene (4)	0.011	1.398	2.996	4.505	7.001
fluoranthene (4)	0.018	1.977	3.984	5.878	9.242
pyrene (4)	0.014	1.686	3.424	5.068	8.191
benzo[a]pyrene (5)	0.0008	0.2183	0.5188	0.7414	1.3138
benzo[e]pyrene (5)	0.0009	0.2438	0.4976	0.7325	1.1794
benzo[b]fluoranthene (5)	0.0009	0.2585	0.5573	0.8161	1.3423
benzo[k]fluoranthene (5)	0.0008	0.2945	0.5909	0.8958	1.4851
dibenzo[a,h]anthracene (5)	0.0005	0.2234	0.4552	0.6733	1.1183
benzo[g,h,i]perylene (6)	0.0004	0.2455	0.4748	0.7453	1.2121
indeno[1,2,3-cd]pyrene (6)	0.0004	0.2494	0.4782	0.7587	1.2671

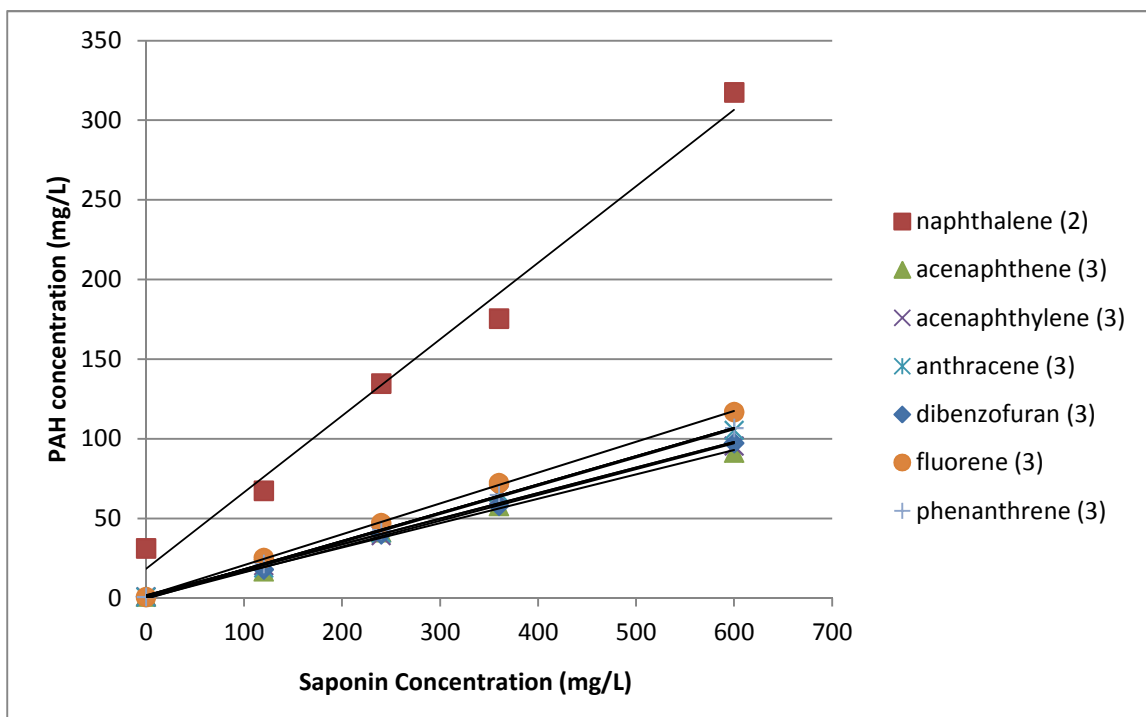


Figure 13 Apparent Solubility of 2 and 3 Ring PAHs

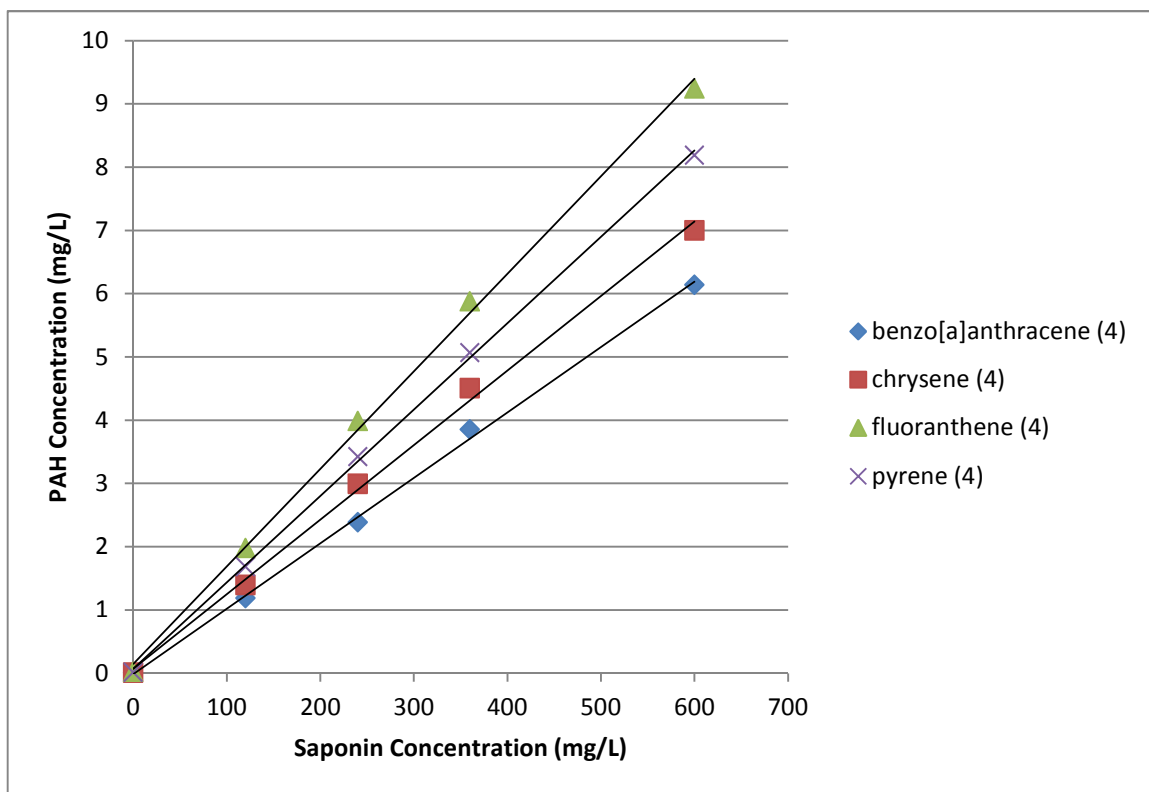


Figure 14 Apparent Solubility of 4 Ring PAHs

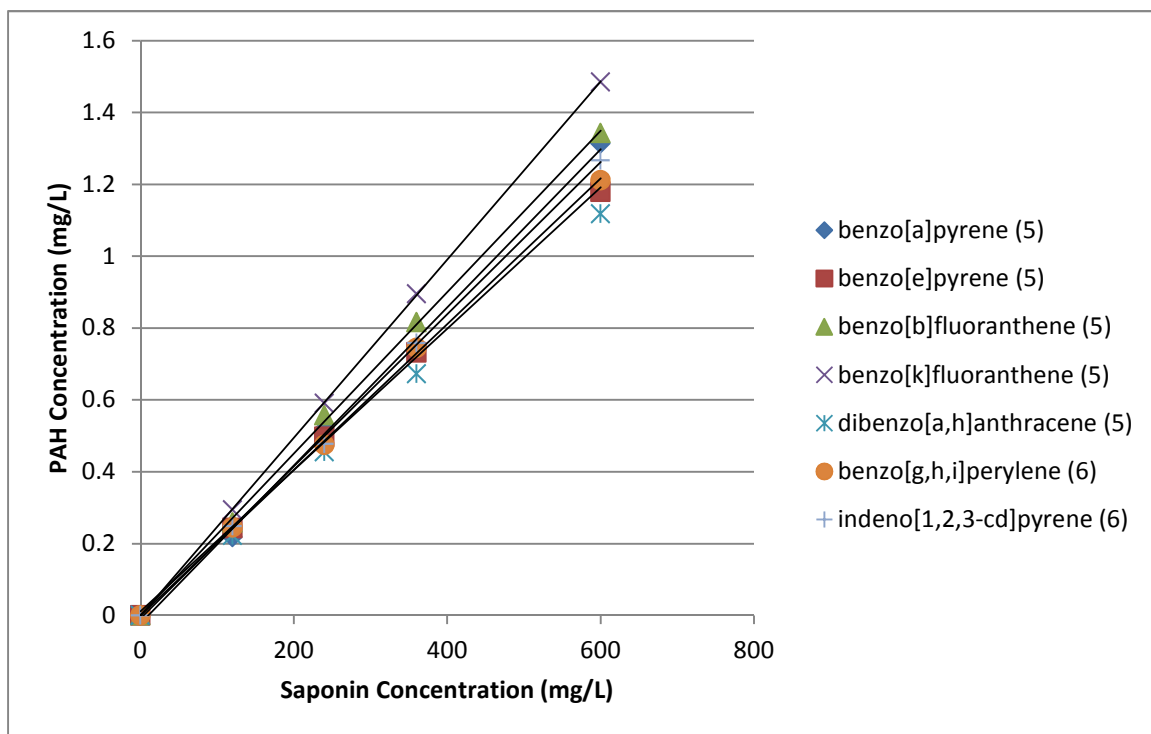


Figure 15 Apparent Solubility of 5 and 6 Ring PAHs

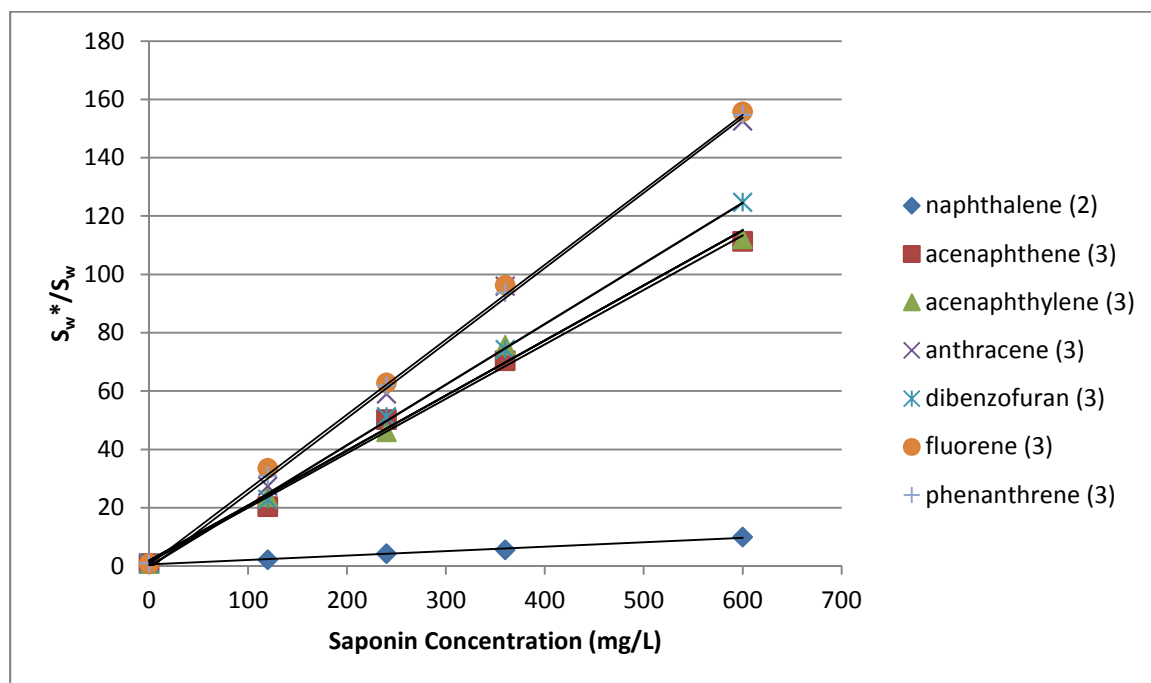


Figure 16 Solubility Enhancement of 2 and 3 Ring PAHs



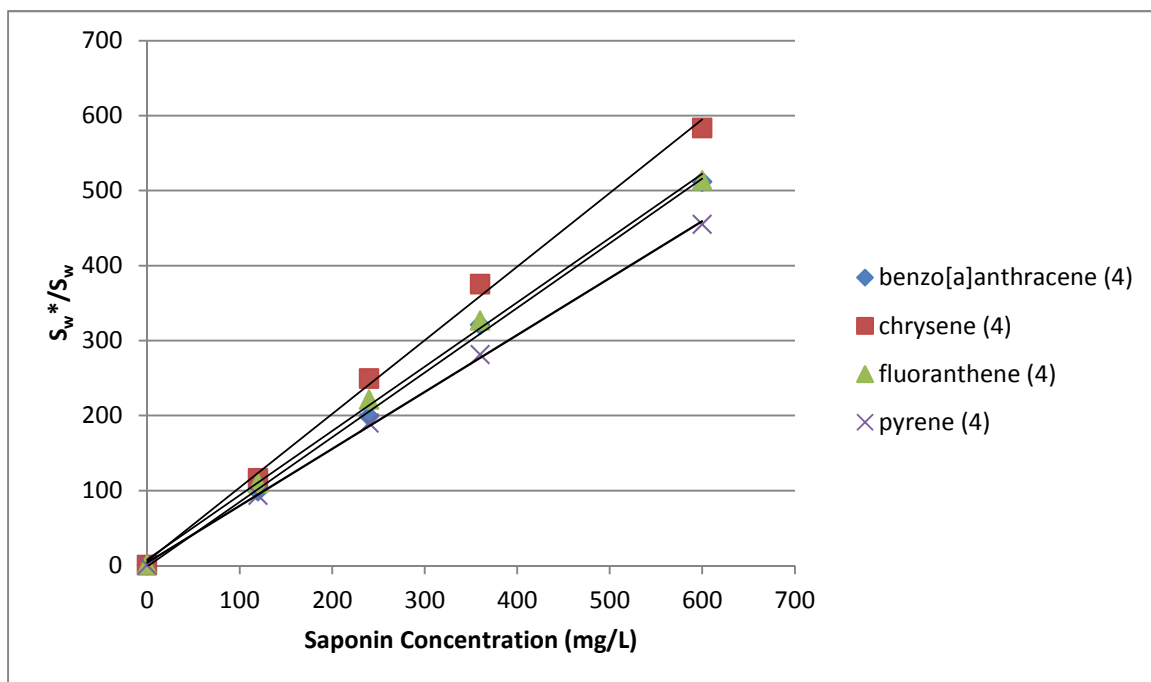


Figure 17 Solubility Enhancement of 4 Ring PAHs

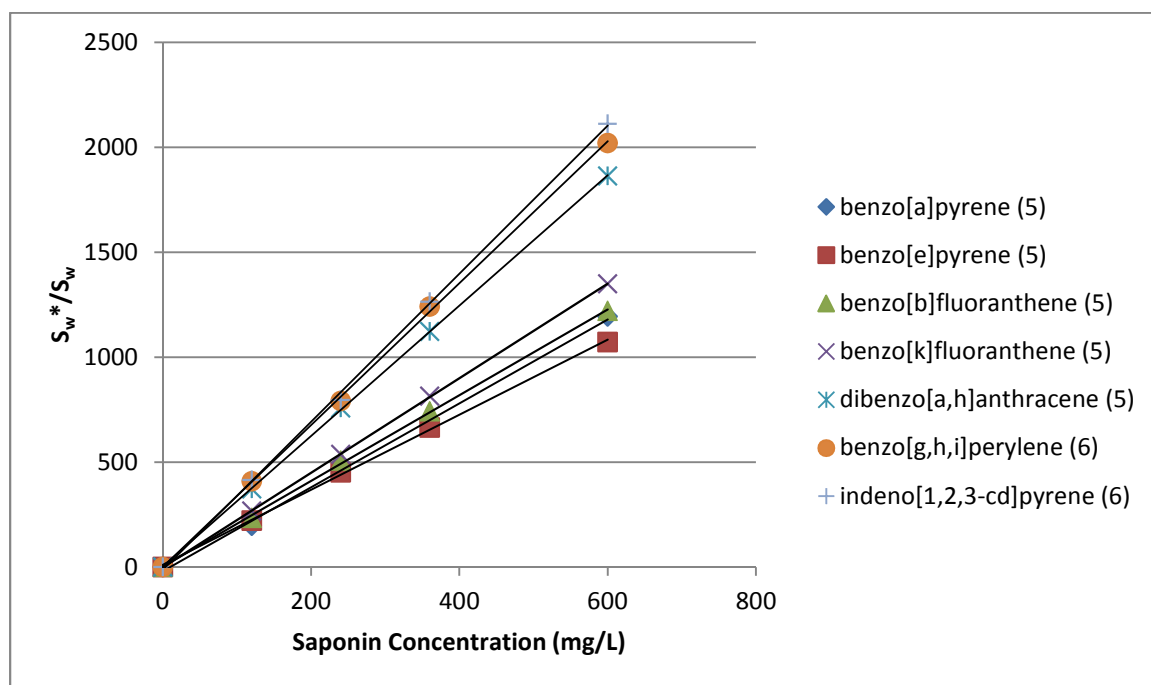


Figure 18 Solubility Enhancement of 5 and 6 Ring PAHs

### 3.3 Saponin Emulsion Kinetics

The rate at which saponins emulsified NAPL in a contaminated soil was measured over a period of 28 days. Measurements of total PAH concentration were collected from the pore water extracted from sacrificial reactors at pre-determined intervals throughout the testing period. Saponin reactors at four different concentrations were evaluated, 2X, 4X, 6X and 10X the CMC. Figure 19 depicts the total PAH concentration with time for the different saponin concentrations. Based on the results, a relatively significant increase in the total PAH concentrations measured in the pore water was observed until an elapsed interaction time of approximately 14 days was reached. After 14 days, only a minimal increase in total PAH concentration is observed.

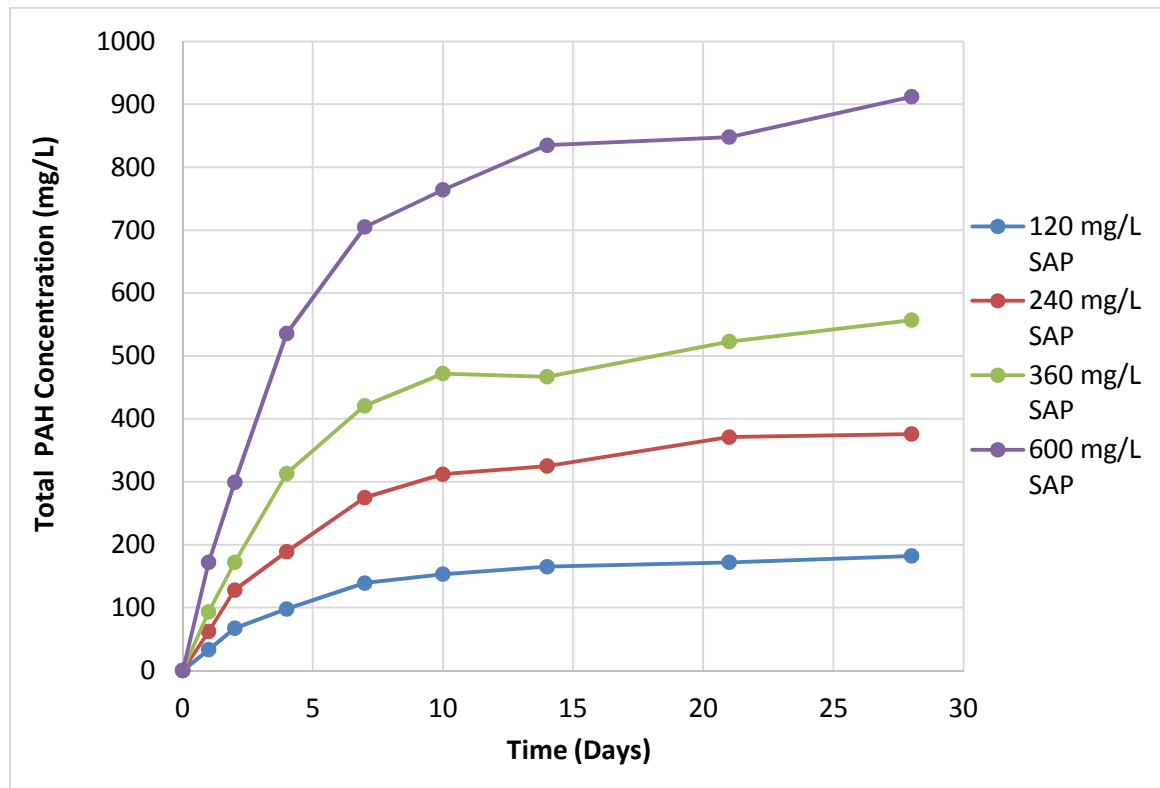


Figure 19 Saponin Emulsion Kinetics

### 3.4 Alternative Quantification

An alternative method for quantifying the solubilization capacity of saponins has been presented by Edwards et al. (1991). The molar solubility ratio (MSR) can be determined and expressed as the number of moles of solute solubilized per moles of surfactant added using the following equation by Edwards et al. (1991):

$$MSR = (S - S_{cmc}) / (C_S - CMC) \quad (1)$$

Where:

S is the apparent solubility of solute in surfactant solution at a particular surfactant concentration greater than CMC;

$S_{cmc}$  = the apparent solubility of solute with the surfactant concentration at CMC; and

$C_S$  = is the surfactant concentration at which S is evaluated.

Additionally, the relationship between the  $K_{ow}$  and the micelle-water partition coefficient ( $K_m$ ) can be represented by first calculating the respective  $K_m$  values for each PAH using the equation provided by Edwards et al. (1991):

$$K_m = MSR / [(1 + MSR)S_{cmc}V_w] \quad (2)$$

Where:

$V_w$  is the molar volume of water (0.01805 L/mol).

Next, because there is a correlation observed between higher  $\log K_{ow}$  values and the enhanced solubilization results ( $S_w^*/S_w$ ), the calculated  $\log K_m$  can be plotted on an axis versus  $\log K_{ow}$  values from Table 6 to obtain a linear relationship between these

properties. Doing this, the relationship between  $\log K_{ow}$  and  $\log K_m$  can be represented by the following expression:

$$\log K_m = 0.254 \log K_{ow} + 4.65 \quad (R^2 = 0.84) \quad (3)$$

A summary of the quantified solubilization capacity data is presented in Table 8.

**Table 8**  
**Saponin Solubilization Capacity**

PAHs (no. of rings)	Molar Solubility Ratio	Km	logKm
naphthalene (2)	6.23	1.45E+05	5.16
acenaphthene (3)	1.65	5.29E+05	5.72
acenaphthylene (3)	1.78	4.94E+05	5.69
anthracene (3)	1.66	6.26E+05	5.80
dibenzofuran (3)	1.6	6.24E+05	5.80
fluorene (3)	1.94	5.05E+05	5.70
phenanthrene (3)	1.66	5.74E+05	5.76
benzo[a]anthracene (4)	0.07	1.56E+06	6.19
chrysene (4)	0.09	1.37E+06	6.14
fluoranthene (4)	0.13	1.23E+06	6.09
pyrene (4)	0.11	1.35E+06	6.13
benzo[a]pyrene (5)	0.015	1.86E+06	6.27
benzo[e]pyrene (5)	0.013	1.46E+06	6.16
benzo[b]fluoranthene (5)	0.015	1.57E+06	6.20
benzo[k]fluoranthene (5)	0.016	1.63E+06	6.21
dibenzo[a,h]anthracene (5)	0.011	1.60E+06	6.20
benzo[g,h,i]perylene (6)	0.012	1.59E+06	6.20
indeno[1,2,3-cd]pyrene (6)	0.013	1.70E+06	6.23

## 4.0 CONCLUSIONS

The findings from this research indicate that saponins derived from the bark of the Quillaja soapbark tree effectively enhanced the solubilization of all sixteen PAHs tested. The highest degree of solubility enhancement was observed for PAHs having more aromatic rings in their molecular structure, and a lesser degree was observed for PAHs with fewer rings. The CMC for Quillaja saponins measured in this research was lower than the CMC measured for Biosolve, a common proprietary surfactant. Lower CMC values typically indicate that a lesser volume of the surfactant is needed in order to become useful for its intended purpose, which also typically leads to cost savings. One common misconception associated with the use of surfactants in environmental remediation is that emulsification of NAPLs occur instantaneously. The experimental data presented herein indicate that regardless of the concentration of saponins used to emulsify NAPL in contaminated soil, an elapsed time of approximately 14 days are recommended in order for the saponins to become fully effective. Saponin solubilization capacity data was quantified and presented herein. This information can be quantified for other surfactants and used to evaluate how each surfactant compares and which surfactant would be best for a specified purpose.

Based on the results of this research and the literature reviewed on this topic, Quillaja saponins are an effective alternative for NAPL remediation. Additionally, this naturally occurring surfactant has the potential for remediating mixed contaminant sites based on its unique molecular structure. Furthermore, relatively inexpensive extraction methods described by Oleszeck et al. (1992) could support the possibility to make saponins a cost effective alternative. Industry sectors that could benefit from using

saponins for remediation purposes include the petroleum and mining sectors. As a naturally occurring, plant-derived alternative, saponins would offer a less toxic and more biodegradable option to industry practitioners while maintaining the effective properties of a desirable surfactant.

## REFERENCES

- Ahmadi**, M.A., Y. Arabsahebi, S.R. Shadizadeh, and S.S. Behbahani. "Preliminary Evaluation of Mullberry Leaf-Derived Surfactant on Interfacial Tension in an Oil-Aqueous System: EOR Application." *Fuel* 117 (2014): 749 – 755.
- Bandala**, E.R., F. Aguilar, and L.G. Torres. "Surfactant-Enhanced Soil Washing for the Remediation of Sites Contaminated with Pesticides." *Land Contamination and Reclamation* 18.2 (2010): 151 – 159.
- Bera**, A., T. Kumar, K. Ojha, and A. Mandal. "Adsorption of Surfactants on Sand Surface in Enhanced Oil Recovery: Isotherms, Kinetics, and Thermodynamic Studies." *Applied Surface Science* 284 (2013): 87 – 99.
- Castro Dantas**, T.N., Dantas Neto, A.A., Moura, M.C.P.A., Barros Neto, E.L., Forte, K.R. and Leite R.H.L. "Heavy Metals Extraction by Microemulsion". *Water Research* 37 (2003): 2709 – 2717.
- Centers** for Disease Control and Prevention. National Institute for Occupational Safety and Health – International Chemical Safety Cards.  
<http://www.cdc.gov/niosh/ipcs/default.html>. March 2016
- Chen**, W.J., L.C. Hsiao, and K.K.Y. Chen. "Metal Desorption from Copper (II)/Nickel (II)- Spiked Kaolin as a Soil Component Using Plant-Derived Saponin Surfactant." *Process Biochemistry* 43 (2008): 488 – 498.
- Edwards**, D.A., Luthy, R.G. and Liu, Z. "Solubilization of Polycyclic Aromatic Hydrocarbons in Micellar Nonionic Surfactant Solutions." *Environmental Science and Technology* 25 (1991): 127 – 133.
- Fountain**, J.C., R.C. Starr, T. Middleton, M. Beikirch, C. Taylor, and D. Hodge. "A Controlled Field Test of Surfactant Enhanced Aquifer Remediation." *Ground Water* 34.5 (1996): 910 – 916.
- Hansch**, C., Leo, A., D. Hoekman. *Exploring QSAR - Hydrophobic, Electronic, and Steric Constants*. Washington, DC: American Chemical Society., 1995., p. 96
- Hong**, K.J., S. Tokunaga, and T. Kajiuchi. "Evaluation of Remediation Process with Plant-Derived Biosurfactant for Recovery of Heavy Metals from Contaminated Soils." *Chemosphere* 49 (2002): 379 – 387.
- Iturbe**, R., J. Lopez, and L.G. Torres. "Microbiological and Physicochemical Changes Occurring in a Contaminated Soil After Surfactant-Enhanced Soil Washing." *Environmental Geosciences* 15.4. (2008): 173 – 181,
- Kibbey**, T.C.G., and K.F. Hayes. "Effects of Surfactant Concentration and Sorbent Properties on the Sorption and Transport of Ethoxylated Nonionic Surfactant Mixtures." *Journal of Contaminant Hydrology* 41 (2000): 1 – 22.



**Kobayashi, T., Kaminaga, H., Navarro, R.R., & Iimura, Y.** "Application of Aqueous Saponin on the Remediation of Polycyclic Aromatic Hydrocarbons-Contaminated Soil." *Journal of Environmental Science and Health, Part A.* 47:8 (2012): 1138-1145.

**Lu, X., Z. Yan, X. Ma, H. Liu, and H. Xia.** "An Environmental Benign Approach for Cadmium Removal from Soils Via Tea Saponin Desorption." *Nature Environment and Pollution Technology* 13.1 (2014): 159 – 163.

**Mitra, S. and Dungan, S.R.** "Micellar Properties of Quillaja Saponins. 1. Effects of Temperature, Salt and pH on Solution Properties." *Journal of Agricultural and Food Chemistry* 45 (1997): 1587 – 1595.

**Mulligan C.N.** "Environmental Applications for Biosurfactants." *Environmental Pollution* 133 (2005): 183 – 198.

**Oakenfull, D.** "Aggregation of Saponins and Bile Acids in Aqueous Solution." *Australian Journal of Chemistry* 39 (1986): 1671 – 1683.

**Oleszeck, W., Jurzysta, M., Ploszynski, M., Colquhoun, I. J., Price, K. R. and Fenwick, G. R.** "Zahnic Acid Tridesmoside and Other Dominant Saponins from Alfalfa (*Medicago sativa* L.) Aerial Parts." *Journal of Agricultural and Food Chemistry* 40 (1992): 191 – 196.

**Roberts, J.F., S.J. Marshall, and D.W. Roberts.** "Aquatic Toxicity of Ethoxylated and Propoxylated Alcohols to *Daphnia Magna*." *Environmental Toxicology and Chemistry* 26.1 (2007): 68 – 72.

**Rodriguez-Cruz, M.S., M.J. Sanchez-Martin, and M. Sanchez-Camazano.** "A Comparative Study of Adsorption of an Anionic and a Non-ionic Surfactant by Soils Based on Physicochemical and Mineralogical Properties of Soils." *Chemosphere* 61 (2005): 56 – 64.

**Ruyssen, R.** "The Measurement of Surface Tension and Interfacial Tension of Saponin Solutions." *Recueil des Travaux Chimiques des Pays-Bas* 65.8 (1946): 580 – 584.

**Salager, J.L.** "Surfactant Types and Uses." *Univeridad de los Andes – Laboratory of Formulation, Interfaces, Rheology, and Processes v.2* (2002): 1 – 48.

**Singh, S.K., and J. Siby.** "Surfactant-Enhanced Remediation of Soils Contaminated with Petroleum Hydrocarbons." *International Journal of Environment and Waste Management* 11.2 (2013): 178 – 192.

**Song, S., L. Zhu, and W. Zhou.** "Simultaneous Removal of Phenanthrene and Cadmium from Contaminated Soils by Saponin, a Plant-Derived Biosurfactant." *Environmental Pollution* 156 (2008): 1368 – 1370.

**Stanimirova, R., Mirinova, K., Tcholakova, S., Denkov, N.D., Stoyanov, S. and Pelan, E.** "Surface Rheology of Saponin Adsorption Layers." *Langmuir* 27 (2011): 12486 – 12498.

**US Public Health Service** (USPHS). 1990. Toxicological Profile for Polycyclic Aromatic Hydrocarbons. U.S. Department of Health and Human Services, PHS, Agency for Toxic Substances and Disease Registry. 231 p.

**Urum**, K., S. Grigson, T. Pekdemir, and S. McMenamy. "A Comparison of the Efficiency of Different Surfactants for Removal of Crude Oil from Contaminated Soils." *Chemosphere* 62 (2006): 1403 – 1410.

**Wang**, W.H., G.E. Hoag, J.B. Collins, and R. Naidu. "Evaluation of Surfactant-Enhanced *in situ* Chemical Oxidation (S-ISCO) in Contaminated Soil." *Water Air Soil Pollution* 224 (2013): 1713 – 1722.

**Wikipedia** contributors. "Ethoxylation." *Wikipedia, The Free Encyclopedia*. Wikipedia, The Free Encyclopedia, 18 Mar. 2016. Web. 30 Mar. 2016.  
<https://en.wikipedia.org/w/index.php?title=Ethoxylation&oldid=710675392>

**Wyrwas**, B., L. Chrzanowski, L. Lawniczak, A. Szulc, P. Cyplik, W. Bialas, A. Szymanski, and A. Holderna-Odachowska. "Utilization of Triton X-100 and Polyethylene Glycols during Surfactant-Mediated Biodegradation of Diesel Fuel." *Journal of Hazardous Materials* 197 (2011): 97 – 103.

**Zhou**, W., Yang, J., Lou, L. and Zhu, L. "Solubilization Properties of Polycyclic Aromatic Hydrocarbons by Saponin, a Plant-Derived Biosurfactant". *Environmental Pollution* 159 (2011): 1198 – 1204.