ECOTOXICITY OF ARABIAN LIGHT CRUDE OIL AND OIL BASED DRILLING MUD ON THE LOCAL ARABIAN KILLIFISH (*Aphanius dispar*) AND COMMERCIAL BRINE SHRIMP (*Artemia sp.*)

BY

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This thesis is dedicated to my parents and family, may Allah forgive their sins, have Mercy on them and grant them al-Jannat ul Firdaus, Aamiin

"My Lord! Bestow on them Your Mercy as they did bring me up when I was small."

[Quran; Surah Al-Isra, Verse 24]

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LIST OF ABBREVIATIONS

ALCO - Arabian Light Crude Oil

OBM - Oil-Based Mud

PSU - Practical Salinity Unit

SDS - Sodium Dodecyl Sulfate

SP - Solid Phase

SPP - Suspended Particulate Phase

WSF - Water Soluble Fraction

PAH - Polycyclic Aromatic Hydrocarbon

TPH - Total Petroleum Hydrocarbon

ABSTRACT

Full Name : Arum Albuntana

Thesis Title : [Ecotoxicity of Arabian Light Crude Oil and Oil-Based Drilling Mud on the Local Arabian Killifish (*Aphanius dispar*) and Commercial Brine Shrimp (*Artemia sp*.)

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Oil spills are one of the common occurrences in the Arabian Gulf either due to accidental spills from oil-related activities or due to marine transportation of oil. Studies on the toxicity of Arabian Light crude oil on marine aquatic organisms are limited. This work aims to study the toxicity of the Arabian Light crude oil, Water Soluble Fraction (WSF) of Arabian Light crude oil, Arabian Light crude oil plus a dispersant, Suspended Particulate Phase (SPP) of oil-based mud, and Solid Phase (SP) of oil-based mud on two animal models viz., the local Arabian Killifish (*Aphanius dispar*) and commercial Brine Shrimp (*Artemia sp.)* from Ocean nutrition, USA. Varying concentrations of Arabian Light crude oil and oil-based mud were prepared to determine the LC_{50} of Arabian Killifish (*Aphanius dispar*) for 96h, and the LC_{50} and EC_{50} of Brine Shrimp (*Artemia sp*.) for 48h of exposure. The results show that the LC_{50} of Arabian killifish (*Aphanius dispar*) exposed to Arabian Light crude oil plus oil dispersant for 96h is 164.19 mgL^{-1}, 262.90 mgL^{-1} for Dispersant, and 137565.36 mgL^{-1} for Solid Phase (SP) of Oil-Based Mud. Whilst, LC_{50} of *Artemia sp.* exposed to the SPP of oil-based mud for 48h is 38.82%. Furthermore, the short-term (48h) toxicity tests show that EC_{50} of the cysts of the Brine Shrimp (*Artemia sp.)* exposed to the SPP of Oil-Based Mud is 5.01%, and 5.95% for WSF of Arabian Light Crude Oil. The study indicates that Arabian Killifish (*Aphanius dispar*) can be used for short-term (96 h) toxicity tests using the Arabian Light Crude Oil plus the dispersant

Surfatron and the SP of oil-based mud. However, the WSF of Arabian Light crude oil and the SPP of oil-based mud do not show any mortality in Arabian Killifish (*Aphanius dispar*) for short-term (96 hours) toxicity testing. This may be due to the concentration of toxic compounds (total PAHs) in WSF of Arabian light crude oil (39.55 ng/ml), and SPP of oil-based mud (345.58 ng/ml) was not high enough to kill the Arabian killifish for an exposure of 96 hours. On the contrary, experiments on the Brine Shrimp (*Artemia sp.)* can be concluded that the Brine Shrimp (*Artemia sp*.) can be used and is more feasible for short-term toxicity tests on Arabian Light crude oil and oil-based mud (OBM).

Keywords: Ecotoxicity, LC_{50}, EC_{50}, Arabian Light Crude Oil (ALCO)**,** Oil-Based Mud (OBM)

ملخص الرسالة

الاسم الكامل : أروم ألبنتانا

عنوان الرسالة : [السمية البيئية للزيت العربي الخفيف للنفط الخام والحفر المستخرج من الزيت على الكليفش العربي (أفانيوس ديسبار) والجنبري التجاري للمحلول الملحي (أرتميا سب)].

المجال الرئيسي : [العلوم البيئية]

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انسكابات النفط هي واحدة من الأحداث الشائعة في الخليج العربي إما بسبب الانسكابات العرضية من الأنشطة المرتبطة بالنفط أو بسبب النقل البحري للنفط، إن الدراسات حول سمية النفط الخام العربي الخفيف على الكائنات المائية البحرية محدودة. وهذه الدراسة، جرت محاولة مدى سمية النفط الخام العربي الخفيف، الجزء الذائب للذوبان في الماء من الزيت الخام الخفيف العربي، الزيت الخام الخفيف العربي بالإضافة إلى مشتتة، مرحلة الجسيمات (العالقة سب) الطين، والصلبة (الطينية سب) من الطين القائم على النفط على اثنين من النماذج الحيوانية، والكليفيش العربي المحلي (أفانيوس ديسبار) والجنبري البراري التجاري (أرتيميا سب) من المحيط التغذي، الولايات المتحدة الأمريكية، وقد تم تحضير تراكيز مختلفة من الزيت العربي الخفيف والزيت المستخرج من الزيت لتحديد كمية LC_{50} من الكليفيش العربي (أفانيوس ديسبار) لمدة 96 ساعة، و LC_{50} و EC50 لجنبري البرمة (أرتيميا سب) لمدة 48 ساعة من التعرض، أظهرت النتائج أن LC_{50} من الأنواع المحلية (أفانيوس ديسبار) المعرضة للزيت الخام العربي بالإضافة إلى النفط المشتت لـ 96 h هو 164.19 ملغل-1، 262.90 ملغل 1- لمشتت فقط، و 137565.36 ملغل 1-لمرحلة (الصلبة سب) من الطين القائم على النفط. في حين، LC_{50} من أرتيميا سب يتعرض لـ سب من الطين القائم على النفط لـ 48 h هو38.82 ٪. وعلاوة على ذلك، فإن اختبارات السمية قصيرة الأجل (48 ساعة) تبين أن EC_{50} من الخراجات من الجنبري المالح التجاري (أرتيميا سب) التي تتعرض لـ سب من الطين القائم على النفط هو5.01 ٪، و5.95 ٪ لـ وسف من النفط الخام الضوء العربي . وأظهرت الدراسة أن الأنواع المحلية كيليفيش العربية (أفانيوس ديسبار) يمكن استخدامها على المدى القصير (96ساعة) اختبارات السمية باستخدام النفط الخام العربي الخفيف بالإضافة إلى تشتت سورفاترون و سب من الطين القائم على النفط. ومع ذلك، لا يظهر الصندوق العالمي للنفط الخام الخام العربي و سب من الطين القائم على النفط أي وفيات في الأنواع المحلية كيليفيش العربية (أفانيوس ديسبار) على المدى القصير (96ساعة) اختبار السمية، وقد يعزى ذلك إلى تركيز المركبات السامة مجموع المركبات الهيدروكربونية المشبعة بالهيدروجين في الصندوق السعودي الفرنسي للنفط الخام العربي الخفيف (39.55 نانوجرام / مل) ولم يكن سب من الطين القائم على النفط (345.58 نانوجرام / مل) مرتفعا بما فيه الكفاية لقتل لمدة 96 ساعة. وعكس ذلك، يمكن أن تستنتج التجارب على الجنبري البراري التجاري (أرتيميا سب) أن الجنبري البراري التجاري (أرتيميا سب) يمكن استخدامها وأكثر جدوى للاختبارات السمية على المدى القصير على النفط الخام العربي الخفيف والنفط القائم الطين (أوب).

الكلمات الرئيسية :السمية البيئية،LC_{50}، EC_{50} ، الزيت العربي الخفيف، الطين القائم على النفط (أوب).

CHAPTER 1
INTRODUCTION

1.1 Background

Ecotoxicology is the study of the detrimental effects of chemicals or toxins on biological organisms. The effects could range from physiological disturbances in tissues and/or organs, which could eventually lead to mortality, resulting in a decline of the organism at the population level (Manahan, 2010). The initial stages of a marine organism such as the embryos and nauplii, juveniles of shrimps and juveniles of fish have been widely used in studies in the last decade to understand the quality of water in the marine environment. Organisms at this stage have a higher sensitivity than the adults and are highly recommended to evaluate the effects of toxicity. (Fathallah et al., 2011; Morroni et al., 2016).

Aquatic toxicity or whole effluent toxicity (WET) tests are normally employed to evaluate the impacts associated with discharges into the marine environment from industries, oil and gas exploration, or production activities (Holdway, 2002; Martínez et al., 2007). An ideal candidate species for toxicity bioassay should have the following requirements: i) sensitivity to the contaminant, ii) abundance and availability throughout the year, and iii) amenable to culture in the laboratory (Stringer et al., 2012; Haschek et al., 2013). Acute aquatic toxicity would normally be determined using a fish for 96 hours LC_{50}, or a crustacean species for 48 hours EC_{50}, and/or an algal species for 72 or 96 hours EC_{50} (USEPA, 2002).

Crude oils pumped from reservoirs contain a mixture of chemicals that vary greatly depending on the source. They contain both hydrocarbons and non-hydrocarbons as constituents. Alkanes (straight, branched, or cyclic), aromatics (benzene, alkyl-benzenes, or naphthalene), and polycyclic aromatic hydrocarbons (PAHs) are the major hydrocarbon fractions in crude oil (Wang et al., 2003; Worton et al., 2015). On the other hand, non-hydrocarbons including S, N, O, and metals (Gogoi et al., 2003) are also present. Total petroleum hydrocarbons (TPH) is a term used to identify several hundred organic chemical compounds originating from crude oil. When TPH is discharged directly to the marine ecosystem through oil spills or leaks, certain TPH fractions will float in water surface and make a thin layer film (Gros et al., 2014). Other heavier crude oil fractions will deposit on the sediments in the seabed, which may have a detrimental effect on fish and other marine organisms (Rodrigues et al., 2010).

Water soluble fraction (WSF) is the solution containing low molecular mass hydrocarbons naturally formed by petroleum hydrocarbon mixtures in contact with seawater (Ziolli and Jardim, 2003). WSF of crude oil can have significant developmental effects on marine embryos even at ppm concentrations, leading to a decline in the survival of marine animals to reach adulthood (Incardona et al., 2012, 2014, 2015; Nahrgang et al., 2017). Suspended particulate phase (SPP) testing can be used for the evaluation of the potential impacts of dissolved and suspended contaminants resulting from dredged materials (drilling mud) on marine organisms

(Apitz et al., 2005). The embryos and nauplii are very sensitive to oil spills because they are considered to be the most vulnerable life stages of marine organisms, which have a direct link to population consequences and resilience (Albers, 2002).

The Arabian Gulf is one of the most productive areas in the world in terms of oil and gas exploration and production. There are more than 800 offshore oil and gas platforms and 25 major terminals in the Arabian Gulf. Some of them are the largest oilfields with the largest infrastructure for oil production in the world (Albano et al., 2016; Sheppard et al., 2010). An offshore oil spill is the release of liquid petroleum hydrocarbons into the marine environment due to human activities, such as oil tanker accidents, blow outs, and carry over from drilling exploration, or due to natural disasters (Law and Kelly, 2004). In the 1991 Gulf oil spill, not less than 10.8 million barrels of crude oil was deliberately released into the Arabian Gulf. Much of the intertidal habitat remains severely contaminated with very large volumes of oiled sediments, even 12 years after the oil spill (Bejarano and Michel, 2010; Hussain and Gondal, 2008). Marine pollution can acutely affect the marine ecosystem because of the physical deterioration and toxic effects, which will have a devastating impact on humans and wildlife (Kaushik, 2006).

The Arabian Gulf marine ecosystems are under significant stress both due to natural stresses such as high temperature and salinities, and anthropogenic stresses such as developmental activities in the coastal areas, including oil and gas exploration, loading and unloading of oil tankers, etc. (Danish, 2010). Even though bioassays allow the preliminary testing for chemical effects on ecosystems, they are inadequate for predicting the effects on the natural populations and attributes of the ecosystem. (Reid and MacFarlane, 2003). The use of a wide range of sensitive marine species from several trophic levels is a more efficient and important approach for forecasting the detrimental hazards faced by the marine ecosystem (Farré and Barceló, 2003).

Therefore, a wide range of sensitive aquatic species (fish, shrimp, and worms) were selected for marine ecotoxicity testing to understand the detrimental effects if any (USEPA, 2002). The Arabian Killifish (*Aphanius dispar*) is a fish commonly found in the coastal areas in the Arabian Gulf throughout the year. The use of this fish in Arab countries for disinfectant testing in the water cooling systems and toxicity of pesticides (Saeed et al., 2015; Shoaib et al., 2012) is still limited. Also, studies on toxicity testing of crude oil and drilling mud have not been widely published. Another species that can be used for bioassays is shrimp. Brine Shrimp (*Artemia sp.*) is a commonly used saltwater organism for acute aquatic toxicity test. The aim of this study is to evaluate the feasibility of using these marine organisms as potential candidates for testing the ecotoxicity of Arabian Light crude oil (ALCO) and oil-based mud.

1.2 Significance of the Study

Studies focusing on using the local species Arabian Killifish (*Aphanius dispar*) and Brine Shrimp (*Artemia sp.*) are scarce. In this study, the detrimental effects of Arabian Light crude oil and oil-based mud were tested on two candidate species, viz., Arabian Killifish (*Aphanius dispar*) and Brine Shrimp (*Artemia sp.*). Researchers have reported that the Arabian Killifish (*Aphanius dispar*) can be commonly used to determine the toxicity of pesticides and insecticides in Oman (Ba-Omar et al., 2011; Shoaib et al., 2012), and the effect of disinfectant in a cooling system plant in Qatar (Saeed et al., 2015).

1.3 Research Objectives

The main objective of the study is to evaluate the feasibility of using the local and commercial marine species as potential candidates for testing the toxicity of Arabian light crude oil and oil-based drilling mud.

The specific objectives of the study are to study the feasibility of using:

1. the local Arabian Killifish (*Aphanius dispar*) in acute toxicity (LC_{50}) studies using Arabian light crude oil and oil-based drilling mud.

2. the commercial Brine Shrimp (*Artemia sp.)* in acute toxicity (LC_{50}) studies using Arabian light crude oil and oil-based drilling mud.

3. the commercial Brine Shrimp (*Artemia sp.*) in sublethal toxicity (EC_{50}) studies using Arabian light crude oil and oil-based drilling mud.

CHAPTER 2
LITERATURE REVIEW

2.1 Selection of Marine Organism for Ecotoxicity Test

Selection of a suitable marine organism as a potential candidate for ecotoxicity testing is important because each organism responds differently to any known toxicant, which largely depends on the type of species and the ecosystems in they are found (Maltby et al., 2005). Some of the marine species extensively used in research and applied toxicology are microalgae for testing the effect of insecticide in the aquatic environment (Maltby et al., 2005), shrimp for testing the ecological impacts of oil refineries (Wake, 2005), fish for determining the toxic effects of an oil spill on fish early life stages (Edwards et al., 2003), and sea worms for toxicity assessment of heavy metals (Banni et al., 2009).

Local species may provide more representative results for the assay of the sensitivity of marine animals to the toxic substances in the ecosystem (Embry et al., 2010). Arabian Gulf is a marginal, semi-enclosed sea unique for its environmental setting. Depth in the Gulf decreases from east to west with a maximum depth of 90 m in the Strait of Hormuz (Agah et al., 2009). The water sea surface temperature in the offshore waters range from 16ºC in the winter to 32ºC in the summer, while coastal bays and lagoons have an even wider temperature fluctuation ranging from 10ºC to as high as 40ºC. The lack of significant river runoff, high evaporation rates (2m/yr.), and limited water exchange with the Indian Ocean raise the overall salinity in the Arabian gulf up to 42 PSU offshore and over 60 PSU in some restricted shallow coastal areas of the Western Arabian Gulf (KFUPM/RI, 2008, 2010).

This uniqueness of environmental conditions has led the marine organisms in the Arabian Gulf to demonstrate a specific pattern of adaptation to environmental stresses and anthropogenic stresses (Sheppard et al., 2010). Among the numerous toxicants in the Arabian Gulf are residual chlorine, ammonia, heavy metals, hydrocarbons, and other organic compounds such as pesticides (Neff et al., 2000). This study will consider the effects of oil related activities such as drilling exploration that produce drilling mud and crude oil.

2.2 Potential Toxicants from Oil and Gas Exploration

Three types of drilling mud are commonly used in oil and gas exploration; oil-based mud (OBM), synthetic-based mud, and water-based mud (WBM) (Edge et al., 2016). Oil-based mud (OBM) is prepared with diesel, kerosene, fuel oil, selected crude oil or mineral oil, or low-toxic linear olefins and paraffin. Typically, OBM contains lime to maintain an elevated pH, resist adverse effects of H_2S and CO_2, and enhance emulsion stability (Neff et al., 2000).

Worldwide, regulations prohibit the discharge of waste containing OBM into the marine waters. Such waste is normally shipped onshore for treatment and disposal. The biological effects of oil-based muds appear to be higher than those of water-based muds on marine organisms as the oil-based muds have higher concentrations of PAHs (Rodrigues et al., 2010). Many studies have reported that the oil-based muds affect the gills of fishes, bivalves, and coral polyps (Erftemeijer et al., 2012).

Furthermore, offshore oil exploration, shipping, and transportation have increased the number of oil spills in the marine environment (Lari et al., 2016). Alkanes (straight, branched, and cyclic), aromatics (benzene, alkylbenzenes, and naphthalene), and PAHs are the major hydrocarbons in crude oil (Xue et al., 2015). Aromatics among the petroleum hydrocarbons, particularly PAHs, are generally thought to be the principal determinant of the toxicity of oil to marine organisms (Fernández et al., 2006). Polycyclic aromatic hydrocarbons are considered to be responsible for the effects of reducing the size at hatch, spinal malformations, pericardium, and yolk sac edemas of fish (Carls et al., 2008; Turcotte et al., 2011).

Oil spills cause significant detrimental effects to marine organisms. High concentrations of toxicants can cause serious damage to the sensitive ecosystem of the marine environment (Lari et al., 2016).

2.3 Local Species Arabian Killifish (*Aphanius dispar*)

The Arabian Killifish (see Figure 1) is commonly found in the coastal areas of the Mediterranean Sea, the Red Sea, the Arabian Gulf, and the Arabian Sea (Reichenbacher et al., 2009). The Arabian Killifish has a wide tolerance limit and can survive in both freshwater and marine environments with high salinity. The fish is found in some freshwater ponds and landlocked seas in Egypt and Saudi Arabia, and typically has a minimum size of 5 cm and can reach a maximum size of 7-8 cm (Teimori et al., 2011). The fish breeds year-round with a slight peak in the breeding season from April to June (Carpenter et al., 1997).

The fish is a potential candidate organism for evaluating toxicity as it is found in large numbers in the coastal waters, throughout the year (Teimori et al., 2011). The fish does not have any commercial importance in terms of the fisheries industry. However, many recent studies have used the Arabian Killifish as an experimental organism in the control of mosquito nauplii in streams (Haq and Yadav, 2011) and as a model organism for bioassay studies to test the toxicity of discharges from water cooling systems at power plants (Saeed et al., 2015). In the United Arab Emirates, it has been introduced into a number of mountain streams, as well as various water ponds to control mosquito nauplii (Al-Kahem-Al-Balawi et al., 2008). Many studies have reported that Arabian Killifish can be commonly used to determine the toxicity of pesticides and insecticides both in Oman (Ba-Omar et al., 2011) and in Pakistan (Shoaib et al., 2012). Saeed et al., (2015) have reported the advantages of using the embryos of the Arabian Killifish as a model for bioassay, due to its sensitivity to the toxicants, and ease of culturing as it breeds year-round.

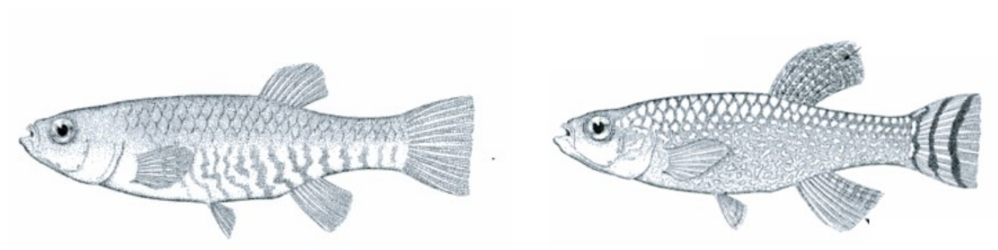


Figure 1. *Aphanius dispar* with the common names (English: Arabian killifish or Arabian pupfish, Arabic: Harsun) (Carpenter et al., 1997)

2.4 Commercial Brine Shrimp (*Artemia sp.*)

The Brine Shrimp (*Artemia sp*.) (see Figure 2) is a commonly used salt water organism for acute aquatic toxicity tests (Libralato et al., 2016). The brine shrimp lethality tests are extensively used in research and applied toxicology. Many studies have used the brine shrimp as an experimental organism as a model species to test the toxicity of produced formation water (PFWs) after separation oil from Bass Strait Platforms, (Holdway, 2002), for testing the toxicity of surfactant (Sodium dodecyl sulfate) has been widely applied in cleaning products (Zheng et al., 2006), and as a model species for determining the toxicity of drilling fluid components (Yunqian et al., 2009).

Brine Shrimp can be found in saline and hypersaline aquatic environments and has been recorded to be distributed in over 600 coastal and inland sites in the world (Castro et al., 2006; Nunes et al., 2006). The cysts are not active and are available in dry condition. Upon immersion in seawater for about 24-30 hours the membrane of the cyst will break and rupture releasing the free-swimming nauplii (Muñoz et al., 2008; Asem et al., 2010). Some advantages of using Brine Shrimp *Artemia sp.* in acute aquatic toxicity testing are the short duration, cost-effectiveness, and convenience of hatching from commercially available cysts (eggs) (Krishnakumar et al., 2007; Libralato et al., 2016). Other benefits include the well-known biology, morphology, and ecology, ease of manipulation in the laboratory conditions, and the small body size allowing culture in small containers or micro-plates (Nunes et al., 2006; Kokkali et al., 2011).

Figure 2. The Brine Shrimp (*Artemia sp.*) (USEPA, 2002).

CHAPTER 3
METHODOLOGY

3.1 The Experimental Design

The local species Arabian Killifish (*Aphanius dispar*) and the commercial Brine Shrimp (*Artemia sp.*) from Ocean Nutrition, USA, were used to determine the detrimental effects of Arabian Light crude oil, WSF of Arabian Light crude oil, dispersant of Arabian Light crude, and SPP and Solid Phase (SP) of OBM). Varying concentrations of WSF and SPP (1.5%, 3%, 6%, 12%, 24%, 48%, 96%, and 100%) were prepared to determine the LC_{50} of Arabian Killifish after 96 hours of exposure, and the LC_{50} and EC_{50} of Brine Shrimp after 48 hours of exposure. Artificial seawater without toxicants served as the negative control, and $CuSO_4$ served as the positive control. All the experiments were conducted in a controlled environment (growth chamber - Nuve TK 252). Experimental parameters during the experiment were as follows: salinity (25-30 PSU for *Artemia sp.*), (40-45 PSU for *Aphanius dispar*), temperature (21ºC±1), and photoperiod (12L:12D). Samples of Arabian Light crude oil and oil-based mud were obtained from the Drilling & Workover Department of Saudi Aramco.

3.2 Apparatus and Equipment

Details of equipment that were used while taking the Arabian killifish from Tarut Bay, Saudi Arabia are Bongo net with the net size 1 mm and fish net 15x10 cm, plastic bucket 10L for taking natural seawater, the fish sample transferred into plastic sample 30x60 and then aerated by oxygen (Oxygen Tank DOT-3 AL-3000). Whilst, the equipment that were used on the lab are analytical balance for weighing the sample (Precisa XT220A - calibrated on March 2017), count register for counting the number of nauplii of *Artemia sp.*, microtiter 300µl (Costar 3516), for conducting the toxicity test of *Artemia sp.*, thermometer glass, beaker glass (Schott-Duran 25 ml, 100 ml, 500 ml, and 1000 ml), separatory funnel (Normax 500 ml, 1000 ml), micropipette (Eppendorf 0.1-20 µl, 2-200 µl, and 50-1000 µl), aquarium cylinder glass (2L), aquarium glass (45x25 cm), GC-MS (Agilent Technologies 6890 N - calibrated on November 2017), microscope (Olympus DP-72, 12.8 megapixel-calibrated on September 2017) for taking the picture, digital orbital shaker (Thomas scientific) and mud shaker (IKA-Eurostar 200) for shaking the toxicants, magnetic stirrer (VELP Scientifica - AREC.T), pH and salinity meter (HI 3512 HANNA Instruments), DO meter (HI 2400 HANNA Instruments), aerator pump (Resun LP 60), hand gloves (Microflex), parafilm (American National Can.tm).

3.3 Reagents and Consumables

Details of reagents and consumables that were used during the study are Hexane (Sigma Aldrich, USA), Dichloromethane (Sigma Aldrich, USA), Cupric sulfate (Fisher scientific, USA), commercial brine shrimp (Ocean Nutrition, USA), commercial reef salt powder (Aquaforest, Poland), distilled water, Arabian Light Crude Oil and Oil Based Mud.

3.4 Collection of Arabian Killifish (*Aphanius dispar*) from Tarut Bay, Saudi Arabia

Samples of Arabian Killifish, *Aphanius dispar* were collected from the sea using a bongo net with a size of 1 mm and were transferred into a bucket with natural seawater (see Figure 3). They were identified according to the method described by (Carpenter et al., 1997) in "Living Marine Resources of Kuwait, Eastern Saudi Arabia, Bahrain, Qatar, and the United Arab Emirates". In situ parameters such as temperature, pH, and salinity of seawater were measured. The collected fish samples were immediately brought in an aerated bag to the laboratory at the Center for Environment & Water at the Research Institute of KFUPM.

In the lab, fishes were acclimatized for 1 hour before being transferred into the aquarium containing artificial seawater. The procedure to make artificial seawater is to dissolve the salt in the previously prepared demineralized water. Water temperature should be about 24°C. For salinity of 40-45 ppt dissolve about 5.12 kg of salt in 100 liters of water. Stir the solution vigorously for about 15 minutes. Once the salt is fully dissolved and the solution is clear, the saline water is ready to use. Fish were fed with commercial fish feed 2 times a day.

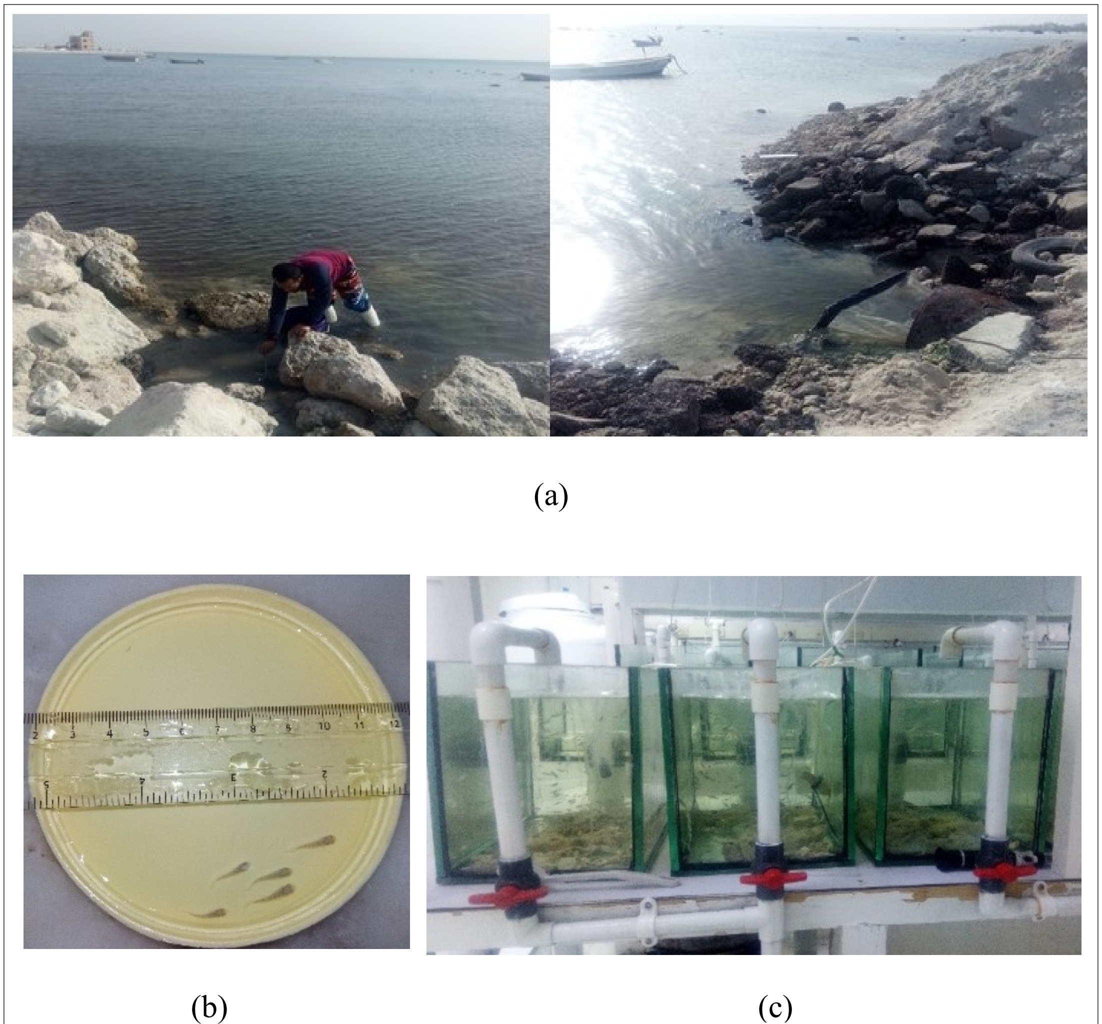


Figure 3. (a) Sampling the local Arabian Killifish (*Aphanius dispar*) in the coastal area in Tarut Bay, KSA using a Bongo net, (b) Juveniles of Arabian Killifish (*Aphanius dispar*) with a length of 0.5 - 1 cm, and (c) Acclimatized Arabian Killifish (*Aphanius dispar*) in the aquarium with artificial seawater of 45 PSU salinity.

3.5 Preparation of the Solution Test

Samples of Arabian Light crude oil and oil-based mud were obtained from the Drilling Department of Saudi Aramco and was immediately shipped to the Ecotoxicology Laboratory at KFUPM on blue ice. Once in the lab, the samples were stored in Refrigerator (4^{o}C) until the time of testing. Composition of Arabian light Crude Oil (see Appendix 1), Oil Based Mud (see Appendix 2), and Oil Dispersant (Surfatron – Champion Chemical, 2016) (see Appendix 3) are given in the appendices.

3.6 Water Soluble Fraction (WSF) of Arabian Light Crude Oil

The WSF of Arabian Light crude oil was prepared according to the method described by (Barron et al., 1999). One part of crude oil (200 ml) was gently mixed with nine parts filtered (1800 ml) artificial seawater (vol:vol) in a separating funnel. The separating funnel was sealed and mixed using an orbital shaker (Thom Sci Shaker 3500) at room temperature for 24 hours. After 24 h of shaking, the crude oil was in the upper layer above the WSF layer (see Figure 4). The aqueous phase (WSF) has allowed to flow into an appropriate storage bottle.

The separated solution was identified as 100% WSF. Solutions of WSF at other concentrations were prepared by the appropriate dilution of 100% WSF solution with water. The concentrations of the test solutions used were as follows:1.5%, 3%, 6%, 12%, 24%, 48%, 96%, and 100%. Artificial seawater without toxicants served as the negative control, and $CuSO_4$ served as the positive control.

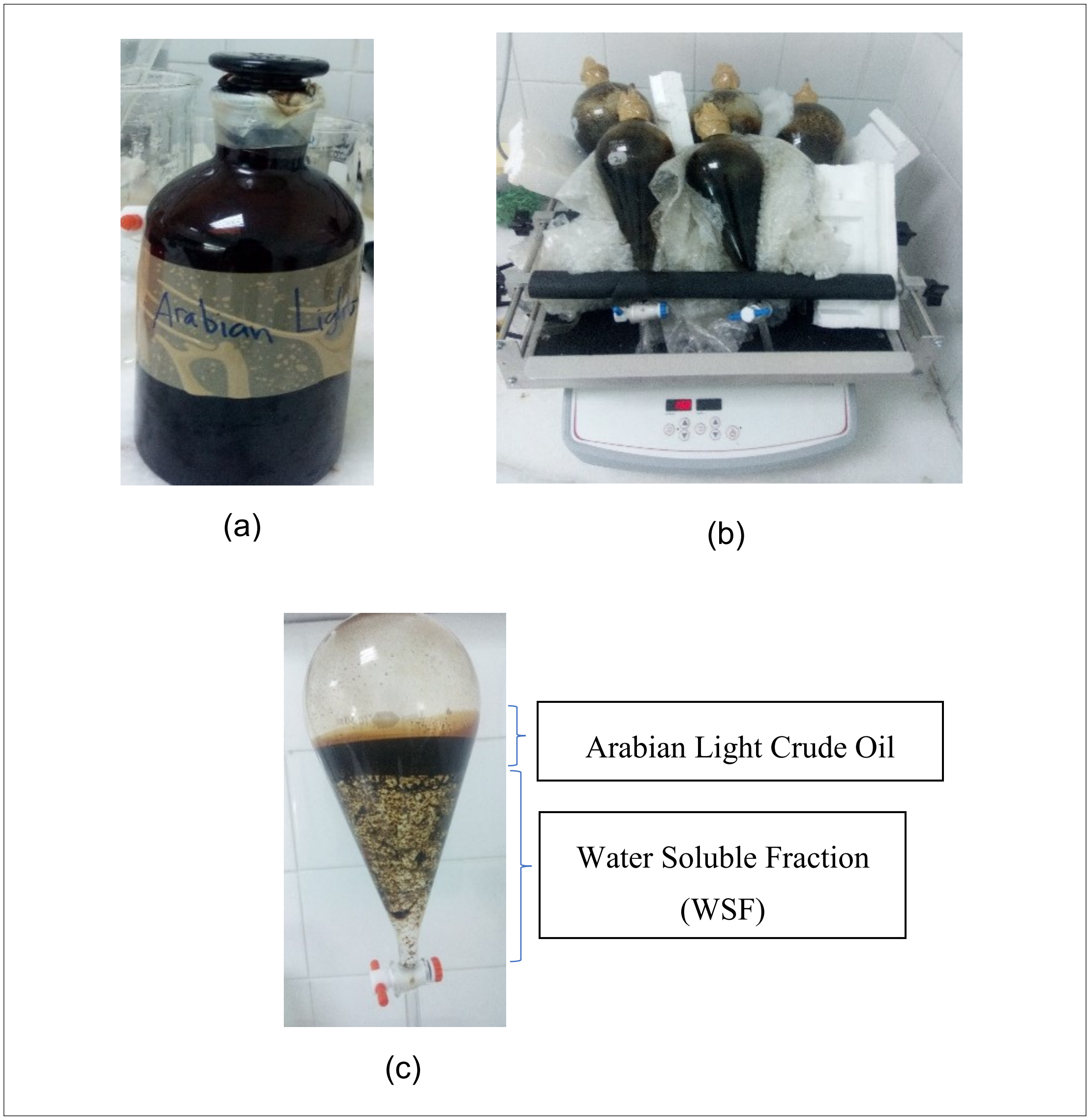


Figure 4. (a) Arabian light crude oil, (b) Mixing of Arabian light crude oil with artificial seawater in a horizontal shaker at 500 rpm, and (c) WSF allowed to separate from crude oil in a separatory funnel.

3.7 Test Solutions of Toxicants Prepared with Arabian Light Crude Oil

The toxicants containing Arabian Light crude oil was prepared following the method reported by (Ndimele et al., 2010). Arabian light crude oil was directly introduced to the artificial seawater (see Figure 5). The concentrations of the test solutions used were: 0.1, 0.2, 0.4, 0.6, 0.8, and 1 mL of Arabian Light crude oil (specific gravity of Arabian Light crude oil: 0.90053 mL^{-1}) in 2 L of the artificial seawater corresponding to 45, 90, 180, 270, 360, and 450 mgL^{-1} of Arabian light crude oil, respectively, per liter of seawater. Artificial seawater without toxicants served as the negative control, and 0.2, 0.39, 0.78, 1.18, 1.57, 3.14, 6.29, 12.57, 25.15, 50.3, 100.6, and 201.2 mgL^{-1} of $CuSO_4$ served as the positive control.

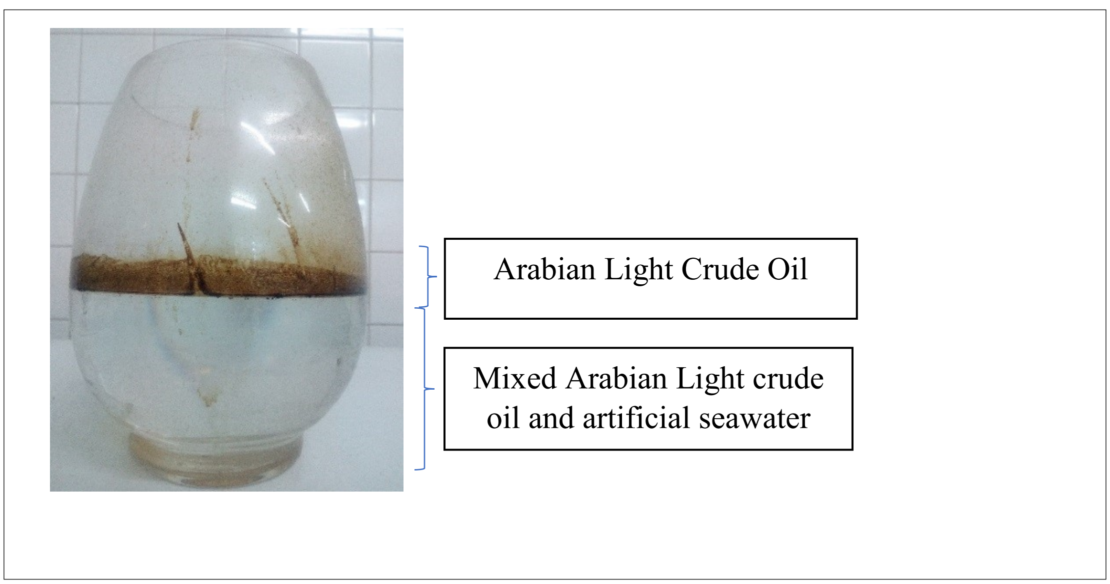


Figure 5. Arabian Light crude oil in artificial seawater of 45 PSU salinity.

3.8 Toxicant Test Solutions of Arabian Light Crude Oil Plus Dispersant

Dispersions of Arabian Light crude oil was prepared according to the method reported by (Ndimele et al., 2010). The Arabian light crude oil and dispersant Surfatron were directly introduced into artificial seawater (see Figure 6). Dispersions with varying concentrations were prepared as follows: 0.1, 0.2, 0.4, 0.6, 0.8, and 1 mL of Arabian light crude oil plus the dispersant Surfatron (specific gravity of Arabian light crude oil: 0.90053 mL^{-1}, and Surfatron: 0.99576 mL^{-1}) were added to 2 L of the artificial seawater corresponding to 95, 190, 379, 569, 759, 858, 948, and 1356 mgL^{-1} of Arabian Light crude oil plus dispersant, respectively per liter of artificial seawater.

At the same time, Dispersant only with varying concentrations were prepared as follows: 0.1, 0.2, 0.4, 0.6, 0.8, and 1 mL were directly introduced into artificial seawater corresponding to 50, 100, 199, 299, 398, and 498 mgL^{-1}, respectively per liter of artificial seawater. Whilst, Artificial seawater without toxicants served as the negative control, and $CuSO_4$ served as the positive control.

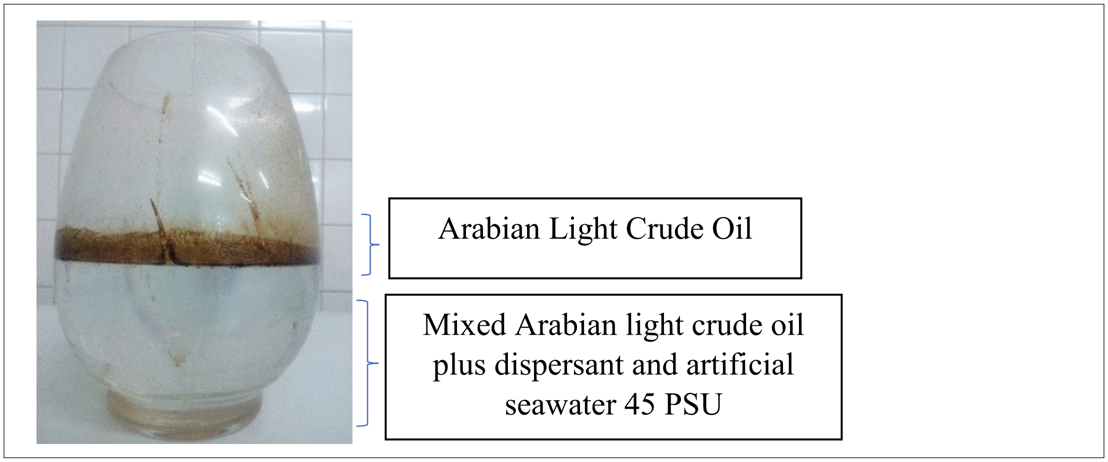


Figure 6. Arabian Light crude oil plus dispersant in artificial seawater of 45 PSU.

3.9 Suspended Particulate Phase of Oil-based Mud

The procedure to make the SPP of the oil-based mud is similar to the procedures described by (Barron et al., 1999; Nunes et al., 2006). One part of oil-based mud (200 ml) was gently mixed with nine parts filtered (1800 ml) artificial seawater (vol:vol) in a separating funnel. The separating funnel was sealed, and the contents were mixed using an orbital shaker (Thom Sci Shaker 3500) at room temperature for 24 hours (see Figure 7).

After 24 h of shaking, the SPP was in the upper layer. The separated phase containing SPP was identified as 100% SPP. Media with other concentrations of SPP was obtained by appropriately diluting 100% SPP with water. The concentrations of used were as follows: 1.5%, 3%, 6%, 12%, 24%, 48%, 96%, and 100%. Artificial seawater without toxicants served as the negative control and $CuSO_4$ served as the positive control.

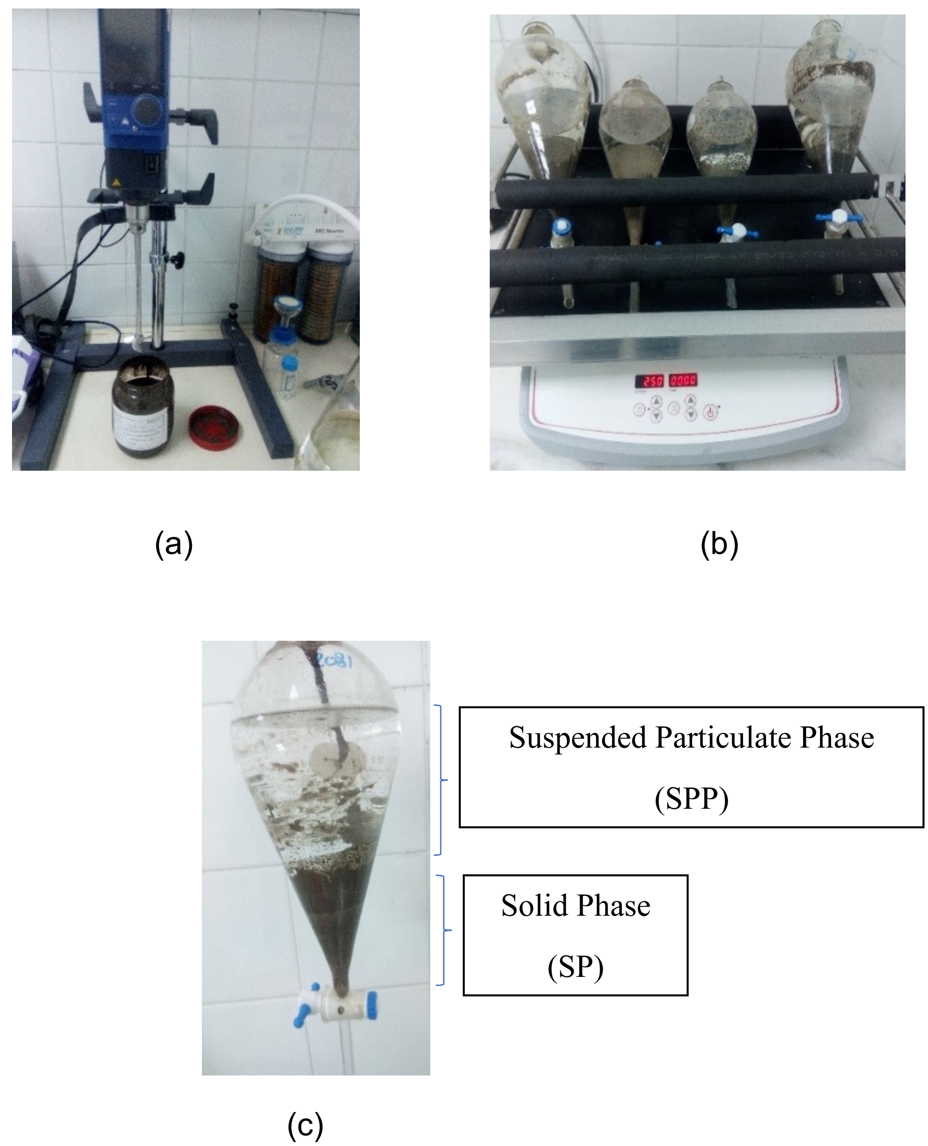


Figure 7. (a) OBM (Oil-based Mud), (b) Mixing of OBM with the artificial seawater using a horizontal shaker at 500 rpm, and (c) SPP and SP in a separatory funnel.

3.10 Solid Phase of the Oil-based Mud

The SP of oil-based mud was prepared according to the method reported by (Nunes et al. 2006). The lower layer formed in the preparation of SPP described in Section 3.9 SP was separated and collected in a glass beaker. The concentrations of the test media were: 10000 mg, 100000 mg, 250000 mg, and 750000 mg of SP of oil-based mud in 1 L of the artificial seawater (see Figure 8).

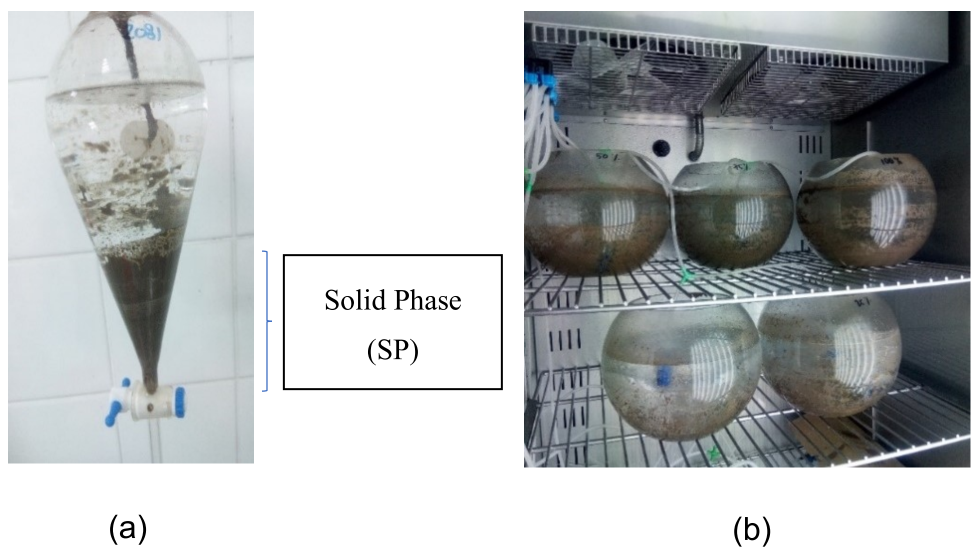


Figure 8. (a) Solid Phase of oil-based mud, and (b) Toxicity set up tests with different concentration 10000 mgL^{-1}, 100000 mgL^{-1}, 250000 mgL^{-1}, 500000 mgL^{-1}, and 750000 mgL^{-1} of SP of oil-based mud.

3.11 Lethality (LC_{50}) Test with Arabian Killifish (*Aphanius dispar*)

The lethality test (LC_{50}) with Arabian Killifish (*Aphanius dispar*) was conducted in a 2000 ml glass aquarium under static conditions using juvenile fish of *Aphanius dispar* with a size of 0.5 cm (see Figure 9). In each of the glass aquariums ten (10) fishes of length 0.5 cm were introduced into toxicant solutions of the following concentrations: WSF and SPP :1.5%, 3%, 6%, 12%, 24%, 48%, 96% and 100%, SP: 0.01%, 0.1%, 1%, 10%, 25%, 50%, and 75%, Arabian light crude oil: 45, 90, 180, 270, 360, and 450 mgL^{-1}, Arabian light crude oil plus dispersant Surfatron: 95, 190, 379, 569, 759, 858, 948, and 1356 mgL^{-1}, and the concentration of oil dispersant (Surfatron) tested were: 50, 100, 199, 299, 398, and 498 mgL^{-1}

Mortality of fish was observed after 96 hours. Animals were considered dead if they did not show any movement during 10 seconds of observation. Lethality rates in each of the glass aquariums with different concentrations were recorded, and then the mean percentage of lethality rates was estimated for 96 hours of exposure.

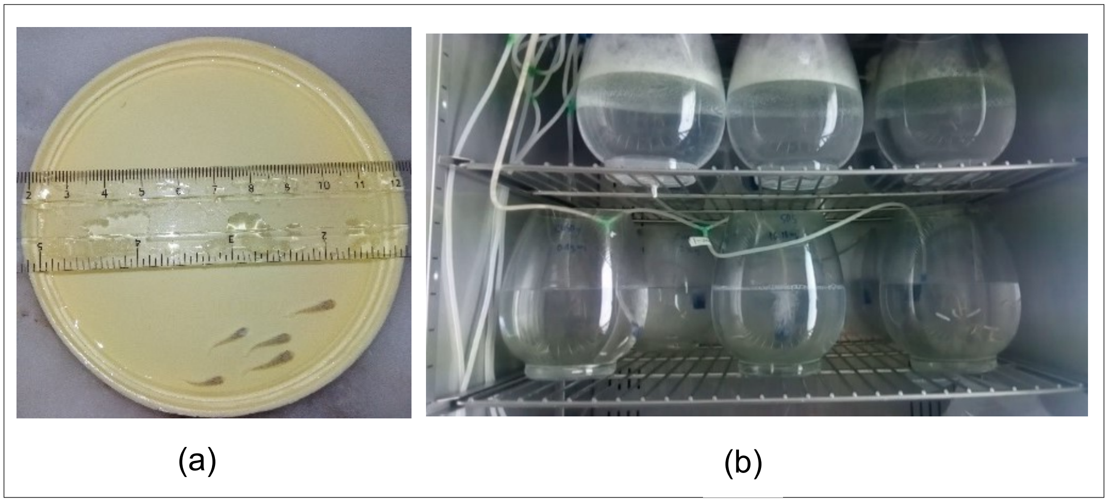


Figure 9. (a) Local species Arabian Killifish, *Aphanius dispar* with a length of 0.5 -1 cm, and (b) Lethality (LC_{50}) test being conducted in the growth chamber at 21ºC ±1.

3.12 Lethality Test (LC_{50}) with Brine Shrimp (*Artemia sp.*)

The lethality test (LC_{50}) with Brine Shrimp *Artemia sp.* was conducted in 300-µl multi-well plates under static conditions using 30-48-hour old nauplii. Twenty (20) nauplii of *Artemia sp.* were introduced into each well with toxicant solutions of concentrations: 0.19%, 0.38%, 0.75%,1.50%, 3%, 6%, 12%, and 24% and the mortality of nauplii was examined under a microscope (see Figure 10). The nauplii of *Artemia sp*. was considered dead if they did not show any movement during ten (10) seconds of observation. Lethality rates in each multi-well plate containing toxicant solutions with different concentrations was recorded, and the mean percentage of lethality rates was estimated after 48 hours of exposure.

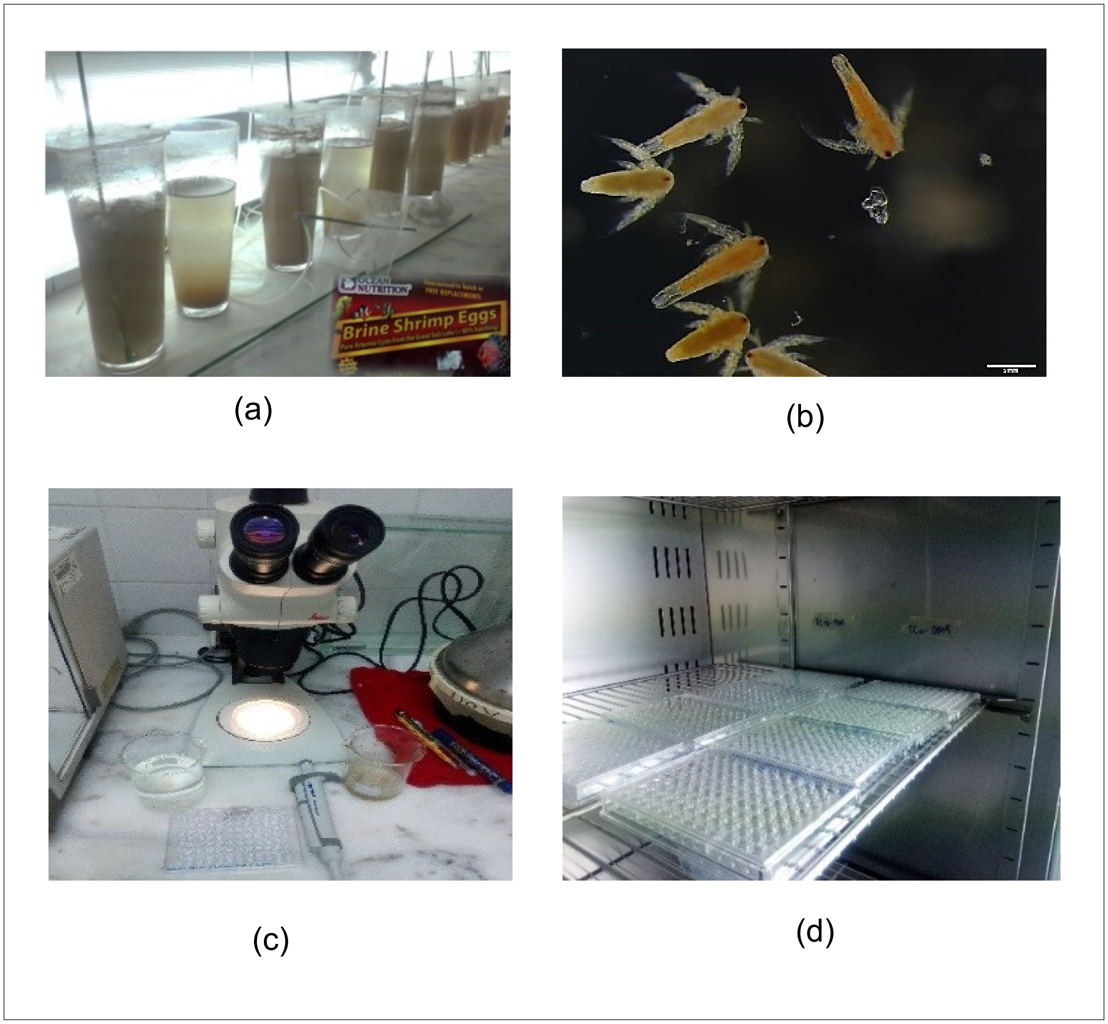


Figure 10. (a) Hatching of commercial Brine Shrimp (*Artemia sp.*) cysts, (b) Nauplii of *Artemia sp.* with a size of 470-550 µm, (c) Toxicity test of *Artemia sp*. conducted in a multi-well plate, and (d) Lethality (LC_{50}) test being conducted in the growth chamber at 21ºC ± 1.

3.13 Hatching Efficiency (EC_{50}) Test

Experiments on the efficiency of the success of cyst hatching (EC_{50}) was conducted in 300-µl multi-well plates under static conditions using commercial *Artemia salina* (Ocean Nutrition, USA) (see Figure 11). In each well, twenty (20) cyst *Artemia sp.* were introduced into each well containing toxicant solutions of concentrations: 0.19%, 0.38%, 0.75%,1.50%, 3%, 6%, 12%, and 24%.

The hatching efficiency (EC_{50}) test was considered complete if the nauplii of *Artemia salina* did not emerge from the egg membranes and has considered to be affected due to toxicants. The unhatched cysts in each well with different toxicant concentrations was recorded, and the mean percentage was estimated after 48 hours of exposure.

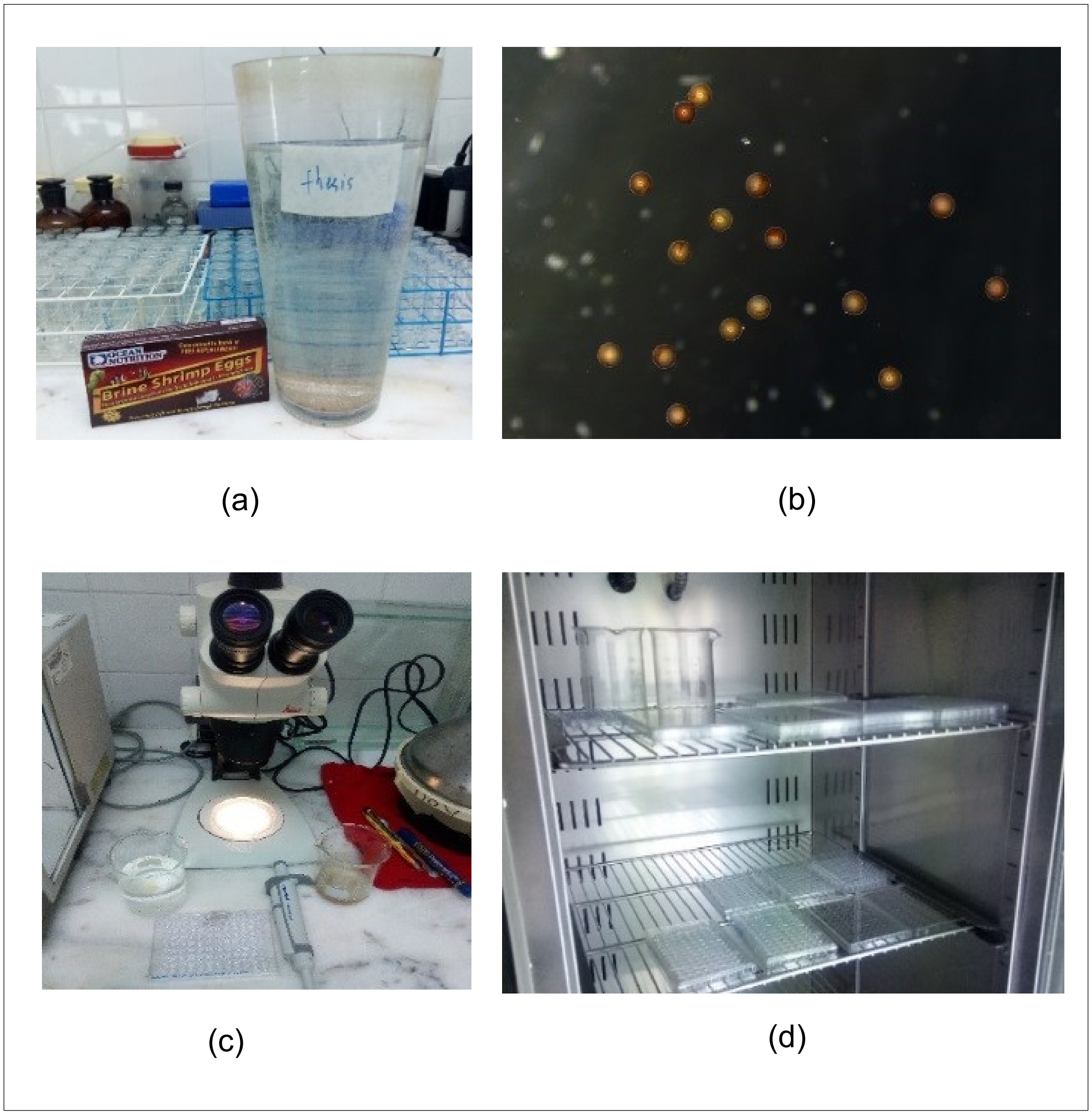


Figure 11. (a) Commercial Brine Shrimp (*Artemia sp.*) cysts, (b) Cysts of *Artemia sp.* with a size of 200-250 µm, (c) The Hatching efficiency (EC_{50}) test conducted in a multi-well plate, and (d) Hatching efficiency (EC_{50}) test being conducted in the growth chamber at 21ºC ± 1.

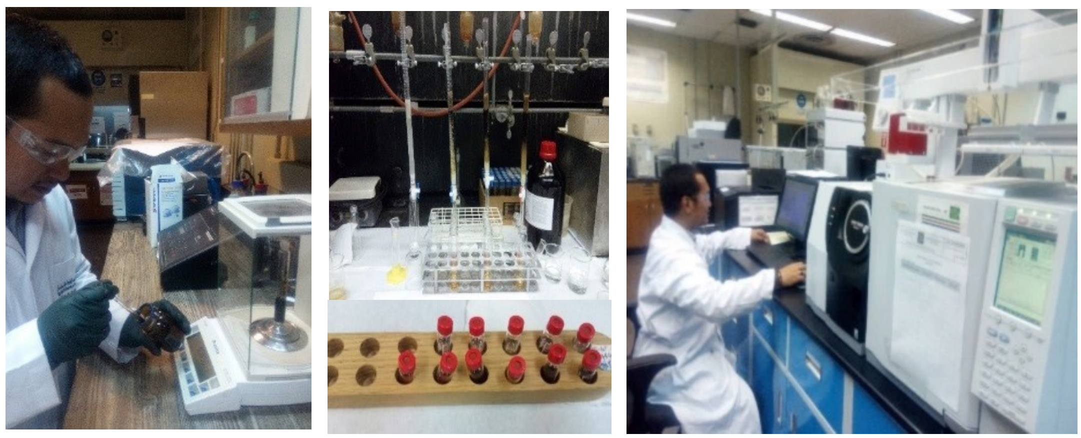
3.14 Chemical Analysis

Samples of Arabian Light crude oil and oil-based mud were analyzed using an Agilent Technologies 6890N gas chromatograph (GC-MS) according to the US EPA method 8015 in a chemistry laboratory of Center for Environment & Water at KFUPM-RI (see Figure 12). The purpose of the chemical analysis was to determine the concentration of PAHs in the samples.

PAHs analysis in Arabian Light crude oil was prepared as follow: about 0.4 grams of Arabian Light crude oil was dissolved in Hexane (about 1.5 ml). The solution was passed thru column for separation of PAHs. Eluate for PAHs was concentrated to about 3 ml and solvent exchange by adding 5 ml hexane and was further concentrated to a volume of about 1.5 ml. Whilst, WSF and SPP were prepared as follow: an amount of 100 ml of samples were extracted by using a separatory funnel with 1:1 (dichloromethane: hexane, 15 ml for three times). And then, the samples were concentrated to about 3 ml and solvent exchange by adding 5 ml hexane and were further concentrated to a volume of about 1.0 ml.

PAHs analysis in Oil Based-Mud and Oil Dispersant were prepared as follow: about 1 to 5 grams of samples was extracted by Ultrasonic extraction with 1:1 (dichloromethane: hexane, 10 ml for three times). And then, the samples were concentrated to about 3 ml and solvent exchange by adding 5 ml hexane and were further concentrated to a volume of about 1.0 ml.

Then 2 μl of all samples were injected into the GC-MS for PAHs analysis. Analysis by GC-MS, ion selected are: masses 128, 142, 152, 154, 166, 178, 202, 228, 252, 276, and 278. PAHs quantitation was made using four or five points PAHs Standard Curve.



(a) (b) (c)

Figure 12. (a) Weighing the Arabian Light crude oil, oil-based mud, and dispersant (b) Column chromatography performed in the chemistry lab to separate individual chemical compounds from mixtures of compounds, and (c) Chemical analysis using gas chromatography.

3.15 Observation during the Test

In the LC_{50} fish testing, the test solution in each aquarium was renewed every 24 hours. Set the temperature in the growth chamber in accordance with the 21±1° C during the experiment. The glass thermometer is placed inside the growth chamber as a quality control to ensure that the set temperature is equal to the desired conditions during the experiment.

3.16 Data Analysis

Lethal and effective concentrations (LC_{50}/EC_{50}) were estimated by fitting two parameter log-logistic functions with binomial type using the SPSS and are expressed as mgL^{-1}.

CHAPTER 4
RESULTS

4.1. Lethality (LC_{50}) Test of Arabian Killifish *Aphanius dispar*

Lethality test of Arabian Killifish (*Aphanius dispar*) in acute toxicity (LC_{50}) studies used Arabian light crude oil (ALCO), Water Soluble Fraction of ALCO, Oil-Based Mud (OBM), Suspended Particulate Phase (SPP) of OBM, and $CuSO_4$ as the positive control.

4.1.1 Determination of LC_{50} in $CuSO_4$ as the Positive Control

$CuSO_4$ served as the positive control for the lethality (LC_{50}) test with Arabian Killifish *Aphanius dispar*. In each glass aquarium, 10 individuals of Arabian Killifish were added, and the test was conducted in three replicates. The concentration of $CuSO_4$ used to determine the LC_{50} of the Arabian Killifish at 96 hours were 0.2, 0.39, 0.78, 1.18, 1.57, 3.14, 6.29, 12.57, 25.15, 50.3, 100.6, and 201.2 mgL^{-1} (see Table 3). The LC_{50} of Arabian Killifish (*Aphanius dispar*) exposed to $CuSO_4$ for 96 hours as the positive control is 29.65 mgL^{-1} (95% Lower Conf. Limit: 1.067; 95% Upper Conf. Limit: 3.703). The calculation of LC_{50} by using SPSS with 95% confidence limit can be seen in Appendix 4.

Table 1. The Results of the Positive Control Test ($CuSO_4$)

Concentration (mgL^{-1})	Number of death			Average
	a	b	c	
0.2	0	0	0	0
0.39	0	0	0	0
0.78	0	0	0	0
1.18	0	0	0	0
1.57	0	0	0	0
3.14	0	0	0	0
6.29	0	0	0	0
12.57	3	3	2	2.67
25.15	5	4	5	4.67
50.3	7	8	6	7
100.6	8	8	9	8.33
201.2	10	10	10	10

4.1.2 Determination of LC_{50} in Arabian Light Crude Oil

Ten individuals of Arabian Killifish were placed in each glass aquarium, and the test was conducted in three replicates. The concentration of Arabian Light crude oil (see Table 2) used to determine the LC_{50} of Arabian Killifish after 96 hours were: 45, 90, 180, 270, 360, and 450 mgL^{-1} (see Table 3). All Arabian Killifish exposed to Arabian Light crude remained alive after 96 hours.

Table 2. The Concentration of The Arabian Light Crude Oil Test Solution

Specific gravity of Crude Oil is 0.90053 g			
Concentration (ml)	in 2L	gL^{-1}	mgL^{-1}
0.1	2	0.045	45
0.2	2	0.090	90
0.4	2	0.180	180
0.6	2	0.270	270
0.8	2	0.360	360
1	2	0.450	450

Table 3. The Results of the LC_{50} Test in Arabian Light Crude Oil

Concentration (ml)	[mgL^{-1}]	Number of death			Average
		a	b	c	
0.1	45	0	0	0	0
0.2	90	0	0	0	0
0.4	180	0	0	0	0
0.6	270	0	0	0	0
0.8	360	0	0	0	0
1	450	0	0	0	0

4.1.3 Determination of LC_{50} in Arabian Light Crude Oil Plus Dispersant

Ten individuals of Arabian Killifish were placed in each glass aquarium containing the test solutions, and the tests were conducted in three replicates. The concentration of Arabian light crude oil (see Table 2) plus oil dispersant (Surfatron) (see Table 4) tested were: 95, 190, 379, 569, 759, 858, 948, and 1356 mgL^{-1} (see Table 5). The LC_{50} of local Arabian Killifish (*Aphanius dispar*) exposed to Arabian light crude oil plus oil dispersant (Surfatron) for 96 hours is 164.2 mgL^{-1} (95% Lower Conf. Limit: -1.227; 95% Upper Conf. Limit: 6.925). The calculation of LC_{50} using SPSS with 95% confidence limit can be seen in Appendix 5.

Table 4. The Concentrations of the Oil Dispersant (Surfatron) Test Solutions

Specific gravity of Oil Dispersant is 0.99576			
Concentration (ml)	in 2L	gL^{-1}	mgL^{-1}
0.1	2	0.050	50
0.2	2	0.100	100
0.4	2	0.199	199
0.6	2	0.299	299
0.8	2	0.398	398
1	2	0.498	498
2	2	0.996	996

Table 5. Results of the LC_{50} Test in Arabian Light Crude Oil Plus Dispersant

Concentration (ml)	[mgL^{-1}]	Number of death			Average
		a	b	c	
0.1+0.1	95	2	3	2	2.3
0.2+0.2	190	7	6	6	6.3
0.4+0.4	379	8	7	8	7.7
0.6+0.6	569	9	10	9	9.3
0.8+0.8	759	10	10	10	10.0
0.8+1	858	10	10	10	10.0
1+1	948	10	10	10	10.0
0.8+2	1356	10	10	10	10.0

4.1.4 Determination of LC_{50} in Oil Dispersant

Ten individuals of Arabian Killifish were placed in each glass aquarium containing the test solutions, and the tests were conducted in three replicates. The concentration of oil dispersant (Surfatron) (see Table 4) tested were: 50, 100, 199, 299, 398, and 498 mgL^{-1} (see Table 6). The LC_{50} of local Arabian Killifish (*Aphanius dispar*) exposed to oil dispersant for 96 hours is 262.9 mgL^{-1} (95% Lower Conf. Limit: 1.426; 95% Upper Conf. Limit: 7.953). The calculation of LC_{50} using SPSS with 95% confidence limit can be seen in Appendix 6.

Table 6. Results of the LC_{50} Test in Oil Dispersant

Concentration (ml)	[mgL^{-1}]	Number of death			Average
		a	b	c	
0.1	50	1	0	0	0.33
0.2	100	1	1	0	0.67
0.4	199	3	4	4	3.67
0.6	299	5	6	6	5.67
0.8	398	8	7	6	7.00
1	498	10	10	10	10.00

4.1.5 Determination of the LC_{50} in the Solid Phase of Oil-based Mud

Ten individuals of Arabian Killifish were placed in each glass aquarium containing test solutions of the following concentrations of Solid Phase of oil-based mud:10000 mg, 100000 mg, 250000 mg, 500000 mg, and 750000 mg into 1L of artificial seawater (see

Table 7). The LC_{50} of local species Arabian Killifish (*Aphanius dispar*) exposed to SP of the oil-based mud for 96 hours is 137565.4 mgL^{-1} (95% Lower Conf. Limit: 1.116; 95% Upper Conf. Limit: 5.247). The calculation of LC_{50} using SPSS with 95% confidence limit can be seen in Appendix 7.

Table 7. Results of the LC_{50} Test in the Solid Phase of Oil-based Mud

Concentration (mgL^{-1})	Number of death a	b	c	Average
10000	1	1	1	1
100000	4	3	4	3.7
250000	9	8	9	8. 7
500000	10	9	9	9.3
750000	10	10	10	10

4.1.6 Determination of the LC_{50} in the Water-Soluble Fraction of Arabian Light Crude Oil

Ten individuals of Arabian Killifish were placed in each glass aquarium containing the water-soluble fraction of Arabian Light crude oil and the test was conducted in 3 replicates. The concentration of Arabian Light crude oil used were: 1.5%, 3%, 6%, 12%, 24%, 48%, 96%, and 100% (see Table 8). After 96 hours, all local species Arabian Killifish (*Aphanius dispar*) were alive in all test solutions.

Table 8. Results of the LC_{50} Test in the Water-Soluble Fraction of Arabian Light Crude Oil

Concentration (%)	Number of death			Average
	a	b	c	
1.50%	0	0	0	0
3%	0	0	0	0
6%	0	0	0	0
12%	0	0	0	0
24%	0	0	0	0
48%	0	0	0	0
96%	0	0	0	0
100%	0	0	0	0

4.1.7 Determination of the LC_{50} in the Suspended Particulate Phase of Oil-based Mud

Ten individuals of Arabian Killifish were placed in each glass aquarium containing SPP of oil-based mud, and the test was conducted in three replicates. The concentration of the SPP of oil-based mud tested were: 1.5%, 3%, 6%, 12%, 24%, 48%, 96%, and 100% (see Table 9). After 96 hours of exposure, all Arabian Killifish (*Aphanius dispar*) were alive in all test solutions.

Table 9. Results of the LC_{50} Test in the Suspended Particulate Phase of Oil-Based Mud

Concentration	Replicates			Average
(%)	a	b	c	
1.50%	0	0	0	0
3%	0	0	0	0
6%	0	0	0	0
12%	0	0	0	0
24%	0	0	0	0
48%	0	0	0	0
96%	0	0	0	0
100%	0	0	0	0

4.2. Lethality (LC_{50}) Test of Brine Shrimp *Artemia sp.*

4.2.1 Determination of LC_{50} in $CuSO_4$ as the Positive Control

Twenty individuals of the Brine Shrimp *Artemia sp.* were placed in each micro-well plate containing the test solution. The concentration of $CuSO_4$ tested were: 0.98, 1.96, 3.93, 7.86, 15.72, 31.43, 62.87, and 125.74 mgL^{-1} (see Table 10). The LC_{50} of commercial Brine Shrimp (*Artemia sp.*) exposed to $CuSO_4$ for 48 hours as the positive control is 10.23 mgL^{-1} (95% Lower Conf. Limit: 1.034; 95% Upper Conf. Limit: 2.075). The calculation of LC_{50} using SPSS with 95% confidence limit can be seen in Appendix 8.

Table 10. Results of the Positive Control Test using $CuSO_4$

Concentration (mgL^{-1})	Number of death					Average
	a	b	c	d	e	
0.98	1	0	0	1	1	0.6
1.96	3	2	1	3	2	2.2
3.93	5	4	3	4	3	3.8
7.86	11	11	10	10	11	10.6
15.72	15	16	15	15	15	15.2
31.43	16	16	15	16	15	15.6
62.87	17	17	16	16	16	16.4
125.74	18	18	18	19	20	18.6

4.2.2 Determination of the LC_{50} in the Water-Soluble Fraction of Arabian Light Crude Oil

Twenty individuals of the Brine Shrimp *Artemia sp.* were placed in each micro-well plate containing WSF test solutions of Arabian Light crude oil. The concentrations tested were: 1.5%, 3%, 6%, 12%, 24%, 48%, 96% and 100% (see Table 11). The LC_{50} of commercial Brine Shrimp (*Artemia sp.)* exposed to WSF of Arabian Light crude oil for 48 hours was not calculated as some of the animals stayed alive even in the maximum concentration of the test (100%).

Table 11. Results of LC_{50} Test in the Water-Soluble Fraction of Arabian Light Crude Oil

Concentration (%)	Number of death					Average
	a	b	c	d	e	
1.50%	1	1	0	0	1	0.6
3%	1	1	1	0	1	0.8
6%	1	1	1	0	2	1
12%	2	2	1	1	2	1.6
24%	3	2	1	2	2	2
48%	3	2	2	3	2	2.4
96%	5	5	4	4	5	4.6
100%	5	6	4	4	5	4.8

4.2.3 Determination of the LC_{50} in the Suspended Particulate Phase of Oil-based Mud

Lethality (LC_{50}) test of Brine Shrimp *Artemia sp.* in the suspended particulate phase of oil-based mud was conducted. Twenty individuals of the Brine Shrimp *Artemia sp.* were placed in each micro-well plate containing the toxicants and the test was conducted in five replicates. Media concentration of SPP of oil-based mud in the range of 1.5%, 3%, 6%, 12%, 24%, 48%, 96%, and 100% (see Table 12) was prepared to determine the LC_{50} of the Brine Shrimp *Artemia sp.* The LC_{50} of commercial Brine Shrimp (*Artemia sp.*) exposed to the SPP of oil-based mud for 48 hours is 36.8% (95% Lower Conf. Limit: -1.91; 95% Upper Conf. Limit: 2.383). The calculation of LC_{50} using SPSS with 95% confidence limit can be seen in Appendix 9.

Table 12. Results of the LC_{50} Test in the Suspended Particulate Phase of Oil-Based Mud

Concentration	Number of death					Average
(%)	a	b	c	d	e	
1.50%	4	4	4	3	4	3.8
3%	6	6	5	5	6	5.6
6%	7	6	6	8	8	7
12%	8	9	8	8	9	8.4
24%	9	10	9	11	10	9.8
48%	10	12	11	12	11	11.2
96%	15	15	15	14	14	14.6
100%	15	16	16	15	16	15.6

4.3. Hatching Efficiency (EC_{50}) Test of Brine Shrimp Cysts

4.3.1 Determination of the EC_{50} in the Suspended Particulate Phase (SPP) of Oil-Based Mud

The un-hatched efficiency (EC_{50}) test of cysts of Brine Shrimp *Artemia sp.* was conducted in the SPP of oil-based mud. Twenty cysts of the Brine Shrimp *Artemia sp.* were placed in each micro-well plate containing the toxicants and the test was conducted in five replicates. The variation of Media with a concentration of the SPP of oil-based mud in the range of 0.19%, 0.38%, 0.75%,1.50%, 3%, 6%, 12%, and 24% (see Table 13) were prepared to determine the un-hatched efficiency (EC_{50}) of cysts of Brine Shrimp *Artemia sp.* The EC_{50} of the cysts of the commercial Brine Shrimp

(*Artemia sp.*) exposed to the SPP of oil-based mud for 48 hours is 5% (95% Lower Conf. Limit: 0.446; 95% Upper Conf. Limit: 8.735). The calculation of EC_{50} using SPSS with 95% confidence limit can be seen in Appendix 10.

Table 13. Results of the EC_{50} test in the SPP of Oil-based Mud

Concentration (%)	Number of un-hatched					Average
	a	b	c	d	e	
0.19%	9	9	9	9	9	9
0.38%	10	9	10	10	10	9.8
0.75%	10	10	10	11	11	10.4
1.50%	11	11	11	11	11	11
3%	11	11	12	12	11	11.4
6%	15	15	16	16	15	15.4
12%	20	20	20	20	20	20
24%	20	20	20	20	20	20

4.3.2 Determination of the EC_{50} in the Water-Soluble Fraction (WSF) of Arabian Light Crude Oil

The un-hatched efficiency (EC_{50}) test of the cysts of Brine Shrimp *Artemia sp.*in the WSF of Arabian Light crude oil was conducted. Twenty cysts of the Brine Shrimp *Artemia sp.* were placed in each micro-well plate containing the toxicants and the test was conducted in five replicates. Solutions of the WSF of Arabian Light crude oil with concentration in the range of 0.19%, 0.38%, 0.75%,1.50%, 3%, 6%, 12%, and 24% (see Table 14) were prepared to determine the EC_{50} of cysts of the Brine Shrimp *Artemia sp.* The EC_{50} of the cysts of the commercial Brine Shrimp (*Artemia sp.*)

exposed to the WSF of Arabian Light crude oil for 48 hours is 5.92% (95% Lower Conf. Limit: 0.403; 95% Upper Conf. Limit: 8.440). The calculation of EC_{50} using SPSS with 95% confidence limit can be seen in Appendix 11.

Table 14. Results of the EC_{50} test in the Water-Soluble Fraction of Arabian Light Crude Oil

Concentration (%)	Number of un-hatched					Average
	a	b	c	d	e	
0.19%	9	9	9	9	9	9
0.38%	10	9	10	10	10	9.8
0.75%	10	10	10	11	11	10.4
1.50%	11	11	11	11	11	11
3%	11	11	12	12	11	11.4
6%	15	15	16	16	15	15.4
12%	20	20	20	20	20	20
24%	20	20	20	20	20	20

4.4. Results of Chemical Analyses

Table 15. Constituents of Arabian Light Crude Oil (ALCO), WSF of Arabian Light Crude Oil, Oil-Based Mud (OBM) and SPP of Oil-Based Mud (OBM)

No	PAHs Identity	in ppb (ng/ml)			
		ALCO	WSF of ALCO	OBM	SPP of OBM
1	Naphthalene	5148.5	21.5	68730	98.4
2	Methyl-Naphthalene	13778.6	16.9	136665	184
3	Acenaphthylene	2780.4	-	1477	-
4	Acenaphthene	242.3	-	5304	13
5	Fluorene	881.7	-	6743	5.21
6	Phenanthrene	2473	1.15	15988	3.32
7	Anthracene	264	-	7599	21.8
8	Fluoranthene	525.2	-	873	-
9	Pyrene	703	-	6718	-
10	Benzo(a)-anthracene	259	-	95	-
11	Chrysene	322	-	442	5.11
12	Benzo(b)-fluoranthene	-	-	563	3.08
13	Benzo(k)-fluoranthene	-	-	66	3.5
14	Benzo(a)-pyrene	-	-	192	3.59
15	Indeno(1,2,3cd)-pyrene	-	-	150	1.6
16	Dibenzo(a,h)-anthracene	-	-	109	1.4
17	Benzo(g,h,i)-perylene	-	-	183	1.57
18	Total PAHs	27377.7	39.55	251897	345.58

Table 16. PAHs Concentration in Arabian Light Crude Oil plus Dispersant

No	PAHs Identity	in ppb (ng/ml)							
		A	B	C	D	E	F	G	H
1	Naphthalene	-	-	-	-	1.38	3.57	8.65	2.76
2	Methyl-Naphthalene	-	-	1.69	2.79	6.16	13.66	31.13	10.48
3	Acenaphthylene	-	-	-	-	-	-	-	-
4	Acenaphthene	-	-	-	-	-	-	-	-
5	Fluorene	-	-	-	-	-	1.66	3.07	1.25
6	Phenanthrene	-	-	-	-	1.37	2.58	5.17	2.16
7	Anthracene	-	-	-	-	-	-	-	-
8	Fluoranthene	-	-	-	-	-	-	-	-
9	Pyrene	-	-	-	-	-	-	-	-
10	Benzo(a)-anthracene	-	-	-	-	-	-	-	-
11	Chrysene	-	-	-	-	-	1.47	-	1.94
12	Benzo(b)-fluoranthene	-	-	-	-	-	-	-	-
13	Benzo(k)-fluoranthene	-	-	-	-	-	-	-	-
14	Benzo(a)-pyrene	-	-	-	-	-	-	-	-
15	Indeno(1,2,3cd)-pyrene	-	-	-	-	-	-	-	-
16	Dibenzo(a,h)-anthracene	-	-	-	-	-	-	-	-
17	Benzo(g,h,i)-perylene	-	-	-	-	-	-	-	-
18	Total PAHs	0.00	0.00	1.69	2.79	8.91	22.93	48.01	18.59

Note: Concentration of Arabian Light Crude Oil: Dispersant (v: v);

A = 0.1 ml + 0.1 ml, B= 0.2 ml + 0.2 ml, C= 0.4 ml + 0.4 ml, D= 0.6 ml + 0.6 ml, E= 0.8 ml + 0.8 ml, F= 0.8 ml + 1ml, G= 0.8 ml + 2 ml, H=1 ml + 1 ml

Table 17. PAHs Concentration of Solid Phase of Oil-Based Mud in Artificial Seawater

No	PAHs Identity	in ppb (ng/ml)				
		A	B	C	D	E
1	Naphthalene	-	5.54	13.85	27.69	41.54
2	Methyl-Naphthalene	-	9.41	23.53	47.05	70.58
3	Acenaphthylene	-	-	-	-	-
4	Acenaphthene	-	-	1.13	2.26	3.39
5	Fluorene	-	-	1.57	3.14	4.70
6	Phenanthrene	-	-	1.53	3.05	4.58
7	Anthracene	-	-	-	-	-
8	Fluoranthene	-	-	-	-	-
9	Pyrene	-	-	-	-	1.05
10	Benzo(a)-anthracene	-	-	-	-	-
11	Chrysene	-	-	-	-	1.13
12	Benzo(b)-fluoranthene	-	-	-	-	-
13	Benzo(k)-fluoranthene	-	-	-	-	-
14	Benzo(a)-pyrene	-	-	-	-	-
15	Indeno(1,2,3cd)-pyrene	-	-	-	-	-
16	Dibenzo(a,h)-anthracene	-	-	-	-	-
17	Benzo(g,h,i)-perylene	-	-	-	-	-
18	Total PAHs	0.00	14.95	41.59	83.19	126.97

Note: Concentration of Oil-Based Mud in 1L of Artificial seawater

A = 10000 mgL^{-1}, B = 100000 mgL^{-1}, C = 250000 mgL^{-1}, D = 500000 mgL^{-1},

E = 750000 mgL^{-1}

Table 18. Water Solubilities of PAHs

No	PAHs Identity	Solubilities of PAHs	
		T(ºC)	Solubility (μmolL^{-1})
1	Naphthalene	25	249
2	Methyl-Naphthalene	25	-
3	Acenaphthylene	25	-
4	Acenaphthene	25	29
5	Fluorene	25	11
6	Phenanthrene	25	7.2
7	Anthracene	25	0.37
8	Fluoranthene	25	1.2
9	Pyrene	25	0.72
10	Benzo(a)-anthracene	25	0.048
11	Chrysene	25	0.013
12	Benzo(b)-fluoranthene	25	0.006
13	Benzo(k)-fluoranthene	25	0.003
14	Benzo(a)-pyrene	25	0.016
15	Indeno(1,2,3cd)-pyrene	25	0.00069
16	Dibenzo(a,h)-anthracene	25	0.0020
17	Benzo(g,h,i)-perylene	25	0.0020

(Pearlman et al., 1984)

CHAPTER 5
DISCUSSION

The local species Arabian Killifish (*Aphanius dispar*) was used to determine the detrimental effects of the Arabian light crude oil, WSF (water-soluble fraction) of Arabian Light crude oil, Arabian Light crude oil plus dispersant, the SPP of oil-based mud and the SP of oil-based mud (OBM) for 96 hours of exposure. In addition, the commercial Brine Shrimp *Artemia sp* were used to determine the detrimental effects of the WSF of Arabian Light crude oil, and the SPP of oil-based mud.

According to the (USEPA, 2002) guidelines for acute toxicity testing of fish, fish caught in the wild can be used for toxicity testing if size, age, and source requirements are satisfied. These requirements are as follows; The longest should not be more than twice the length of the shortest. The fish must be of the same size, originate from the same source and population, and fish may not be fed during the treatment period (acute testing must be performed for a minimum of 96 h).

In the first of experiment, this study focused on WSF and SPP as the toxicants. Media with concentrations of WSF and SPP in the range of 1.5%, 3%, 6%, 12%, 24%, 48%, 96%, and 100% were prepared to determine the LC_{50} of Arabian Killifish. During the experiments, an aerator pump was used to create conditions similar with the natural conditions of the original ecosystem under controlled conditions inside the laboratory.

The results do not show a significant mortality rate among the juveniles of Arabian killifish (*Aphanius dispar*). This may be due to the concentration of toxic compounds (total PAHs) in WSF of Arabian light crude oil (39.55 ng/ml), and SPP of oil-based mud (345.58 ng/ml) was not high enough to kill the Arabian killifish for an exposure of 96 hours. Another reason that makes the low rate of mortality of *Aphanius dispar* in this experiment is the low solubility of PAHs in the artificial seawater (see Table 18).

Similar results have been obtained by (Agamy, 2013), who showed that the mortality rate is not significant among juveniles of Rabbit fish *Siganus canaliculatus* exposed to 3--100% of the WSF of Arabian Light crude oil from Sharjah, UAE (United Arab Emirate). Further research shows that the 96-hour LC_{50} could not be calculated as deaths did not occur during the test of the WSF of a Nigerian light crude oil on *Clarias gariepinus* (Makinde, 2015).

Thus, the thesis experiment method was changed following other references and the experiments for estimating the toxicity of Arabian light crude oil and oil-based mud was modified. The Arabian light crude oil and Arabian light plus dispersant were prepared according to the method reported by (Ndimele et al., 2010). And the SP of oil-based mud was prepared according to the method reported by (Nunes et al., 2006)

In the second experiment, the Arabian Light crude oil was directly introduced to artificial seawater at different concentration. The test solutions were prepared with 0.1, 0.2, 0.4, 0.6, 0.8, and 1 mL of Arabian Light crude oil (specific gravity of Arabian light crude oil: 0.90053 mL^{-1}) in 2 L of the artificial seawater corresponding to 45, 90, 180, 270, 360, and 450 mgL^{-1} of Arabian light crude oil, respectively, per liter of seawater. Ten individuals of Arabian Killifish were placed in each glass aquarium, and the tests were conducted in three replicates. As none of the local species (*Aphanius dispar*) died in 96 hours of exposure to Arabian Light crude oil an LC_{50} could not be calculated.

Due to the immiscibility of Arabian Light crude oil and seawater, they separate into two phases in the aquarium even after vigorous stirring. Hence, the toxicants in the Arabian Light crude do not dissolve in natural seawater (Lessard and DeMarco, 2000; Manahan, 2010). This conclusion is supported by the results of the chemical analysis, which indicates that the total concentration of PAHs in Arabian light crude oil is 27377.7 ng/ml, while it is only 39.55 ng/ml in artificial seawater. The toxic compounds (total PAHs) in Arabian light crude oil cannot properly dissolve in the artificial seawater. Thus, the total concentration of PAHs in artificial seawater (39.55 ng/ml) is not high enough to kill the Arabian killifish in the 96-hour experiment.

Furthermore, the Arabian killifish can survive in solutions of toxic compounds due to its specific adaptation to the harsh conditions in the Arabian Gulf. Other species may respond quite differently to solutions of toxicants depending on the type of species and the ecosystem where they live (Maltby et al., 2005).

In the third experiment, Arabian light crude oil and a dispersant (Surfatron) were directly introduced to the artificial seawater at different concentrations. Volumes of 0.1, 0.2, 0.4, 0.6, 0.8, and 1 mL of Arabian Light crude oil and the dispersant Surfatron (specific gravity of Arabian light crude oil: 0.90053 mL^{-1}, and dispersant Surfatron: 0.99576 mL^{-1}) were added separately to 2 L of artificial seawater corresponding to 95, 190, 379, 569, 759, 858, 948, and 1356 mgL^{-1} of Arabian light crude oil plus dispersant per liter of artificial seawater.

Ten individuals of Arabian Killifish (*Aphanius dispar*) were placed in each glass aquarium containing the toxicants, and the test was conducted in three replicates. The LC_{50} of local species (*Aphanius dispar*) exposed to Arabian light crude oil plus oil dispersant Surfatron for 96 hours is 164.19 mgL^{-1} and 262.90 mgL^{-1} for Dispersant only (see Table 19). Based on the calculations performed using Software SPSS, the values of LC_{50} obtained are indicated in Table 19.

Table 19. Result for LC_{50} of Local Species Arabian killifish *Aphanius dispar* exposed to toxicants calculated using SPSS

No	*Aphanius Dispar*			
	Toxicants	LC_{50}	y	R^2 Linear
1	LC_{50} of $CuSO_4$	29.65 mgL-1	y= -2.57+1.78*x	0.996
2	LC_{50} of ALCO + Dispersant	164.19 mgL^{-1}	y=-5.89+2.65*x	0.962
3	LC_{50} of Dispersant	262.90 mgL^{-1}	y=-6.75+2.78*x	0.968
4	LC_{50} of Solid Phase of OBM	137565.36 mgL^{-1}	y=-8.12+1.66*x	0.92

Dispersant Surfatron is produced by Nalco-Champ, USA with the following composition; petroleum naphtha (30-60%), light aromatic solvent naphtha (30-60%), ethanolamine-organic acid salt (30-60%), 1,2,4 trimethyl-benzene (10-30%), and other ingredients (see Appendix 3). The dispersant can be attracted to both Arabian light crude oil and artificial seawater because one end which is hydrophilic (or 'waterloving') can attach to water molecules, and the other end which is hydrophobic can attach to organic molecules in Arabian light crude oil (Lessard and DeMarco, 2000; Technologies, 2009). Oil dispersant Surfracton induces changes in the concentration of polycyclic aromatic hydrocarbons (PAHs) in the aqueous phase, which affects the mortality of Arabian Killifish in this experiment. Similar results were obtained by (Couillard et al., 2005) who showed that dispersed-Mesa light crude oil affected larval survival, body length, or ethoxyresorufin-O-deethylase (EROD) activity of *Fundulus heteroclitus*.

Dispersant Corexit EC9500 increases the solubility of crude oil in water so that the toxic compounds in the crude oil can dissolve completely. It increased the uptake of PAHs by rainbow trout fish (*Oncorhynchus mykiss*) exposed to crude oil (Ramachandran et al., 2004). Other studies have shown that Corexit 9500 increases the solubility of Iraqi crude oil in water. Dispersed oil is more toxic to larvae of common carp (*C. Carpio*), Carassin (*C. auratus*) and grass carp (*C. idella*) than floating oil (Farid et al., 2016).

In the fourth experiment, the SP of oil-based mud was tested to the local Arabian killifish *Apahanius dispar* with several concentrations; 10000 mg, 100000 mg, 250000 mg, 500000 mg, and 750000 mg of Oil-Based Mud per liter of seawater. The LC_{50} of local species (*Aphanius dispar*) exposed to the SP of oil-based mud for 96 hours is 137565.36 mgL^{-1} (see Table 19).

Artificial seawater 45 PSU salinity without toxicants was used as the negative control, while $CuSO_4$ was used as the positive control. In the negative control (artificial seawater without toxicants) response is not expected. On the other hand, in the positive control ($CuSO_4$) a known response is expected (Mitchell et al., 2008).

$CuSO_4$ was used as the positive control for the Arabian Killifish *Aphanius dispar* lethality (LC_{50}) test. Ten individuals of the Arabian Killifish were placed in each glass aquarium containing $CuSO_4$ solutions, and the test was conducted in three replicates. Solutions of $CuSO_4$ with a of concentration of 0.2, 0.39, 0.78, 1.18, 1.57, 3.14, 6.29, 12.57, 25.15, 50.3, 100.6, and 201.2 mgL^{-1} were prepared to determine the LC_{50} of the Arabian Killifish. The LC_{50} of local species Arabian Killifish (*Aphanius dispar*) exposed to the positive control $CuSO_4$ for 96 hours is 29.65 mgL^{-1} (see Table 19).

In the fifth experiment, the lethality test (LC_{50}) of Brine Shrimp *Artemia sp.* was conducted in 300-µl multi-well plates under static conditions using 30-48-hour old nauplii. Twenty nauplii of *Artemia sp.* were placed in each well containing varying concentrations of the WSF of Arabian Light crude oil and the SPP of oil-based mud (1.5%, 3%, 6%, 12%, 24%, 48%, 96% and 100%). The lethality of nauplii of *Artemis sp.* was examined under a microscope. The nauplii of *Artemia sp*. was considered dead if they did not show any movement during ten (10) seconds of observation.

The LC_{50} of commercial Brine Shrimp (*Artemia sp*.) exposed to the WSF of Arabian Light crude oil for 48 hours cannot be calculated due to the mortality is more than 100%. Whilst, the LC_{50} of commercial Brine Shrimp (*Artemia sp.*) exposed to the SPP of oil-based mud for 48 hours is 36.82% (see Table 20).

Table 20. Result for LC_{50} of commercial Brine shrimp (*Artemia sp.)* exposed to toxicants calculated using SPSS

No	*Artemia sp.*			
	Toxicants	LC_{50}	y	R^2 Linear
1	LC_{50} of $CuSO_4$	10.23 mgL^{-1}	y=-1.65+157*x	0.949
2	LC_{50} of SPP of OBM	36.82%	y=-1.04+0.81*x	0.962

Other similar studies have shown that LC_{50} of Brine Shrimp exposed to the WSF of US-National oil is 72.1--43.0% (Cavender et al., 1995). A short-term toxicity test has shown that the LC_{50} of Pink Shrimp *Farfantepenaeus duorarum*, larvae exposed to the WSF of Macondo Canyon (MC) 252 crude oil is > 100 mg/L (Laramore et al., 2016). The LC_{50} of *Litopenaeus setiferus* exposed to drilling fluids commonly used in petroleum perforation, and extraction in the Campeche Sound of the Gulf of Mexico is about 475000 to 700000 ppm SPP (Nunes et al., 2006).

Crude oils or drilling mud pumped from underground reservoirs contain a mixture of chemicals that vary greatly depending on the source. Each source of oil produces a specific oil with the toxicity levels different from oil from another source. Thus, in the toxicity test, different types of toxicants give different results for the value of the mortality rate of each species. Each species responds quite differently to toxicant solutions, depending on the type of species and the ecosystem where species live (Maltby et al., 2005).

Same is true for the experiments on the local species Arabian killifish (*Aphanius dispar*). $CuSO_4$ served as the positive control for the lethality (LC_{50}) test of Brine

Shrimp (*Artemia sp.*). The LC_{50} of commercial Brine Shrimp (*Artemia sp.*) exposed to the positive control $CuSO_4$ for 48 hours is 10.23 mgL^{-1} (see Table 2). Artificial seawater of 30 PSU salinity without toxicants served as the negative control.

In the sixth experiment, the lowest concentration (12-24%) in the fifth experiment capable of killing the least number of *Artemia sp*. (1-2 individuals only) was used. The experiments on the efficiency of cyst hatching success (EC_{50}) were conducted in 300-µl multi-well plates under control conditions using commercial *Artemia salina* (Ocean Nutrition, USA). Twenty cysts of *Artemia sp.* was placed in each well containing varying concentrations of the WSF of Arabian Light crude oil and the SPP of oil-based mud (0.19%, 0.38%, 0.75%,1.50%, 3%, 6%, 12%, and 24%). The cyst un-hatched efficiency (EC_{50}) test was considered complete if the nauplii of *Artemia sp.* did not emerge from the egg membranes affected by the toxicants.

The EC_{50} of the cysts of the commercial Brine Shrimp (*Artemia sp.*) exposed to the WSF of Arabian Light crude oil for 48 hours is 5.95% (see Table 21). PAHs is one of chemical that caused detrimental effect in hatching process in Artemia sp. PAHs concentration in 5.95% is equal with 2.35 ng/ml. Whilst, the EC_{50} of the cysts of the commercial Brine Shrimp (*Artemia sp*.) exposed to the SPP of oil-based mud for 48 hours is 5.01%. PAHs concentration in 5.01% is equal with 17.31 ng/ml.

Table 21. Result for EC_{50} of the cysts of the commercial Brine shrimp (*Artemia sp.)* exposed to toxicants calculated using SPSS

No	*Artemia sp.*			
	Toxicants	EC_{50}	y	R^2 Linear
1	EC_{50} of SPP of OBM	5.01%	y=0.14+0.48*x	0.771
2	EC_{50} of WSF of ALCO	5.95%	y=-0.1+0.33*x	0.942

Other similar studies have shown that EC_{50} of species exposed to the WSF of effluent from an oil refinery at Mangalore, India were 40.6% (in the dry season) and 73.4% (in the wet season) (Krishnakumar et al., 2007). The EC_{20s} of *Mysidopsis bahia* exposed to the WSF of spilled oil at a coastal California oil field was 0.32 to 5.7 μgL^{-1}. PAHs are generally assumed to be the toxic fraction of spilled petroleum.

The total concentration of PAHs in the WSF of Arabian Light crude oil is 39.55 ng/ml and in the SPP of oil-based mud is 345.58 ng/ml, where Methyl-Naphthalene has the highest concentration among other PAHs (see Table 15). Naphthalene in the WSF of spilled oil at a coastal California oil field oil had the greatest toxicity to *Mysidopsis bahia* in the toxicity tests (Barron et al., 1999). Furthermore, the EC_{50} of microbial toxicity test (bacteria *Vibrio fisheri*) exposed to the weathered Arabian medium crude oil was 1.10 mg/l (Fuller et al., 2004).

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the experiments conducted, LC_{50} of local species (*Aphanius dispar*) exposed to Arabian Light crude oil plus the oil dispersant Surfatron for 96 hours is 164.19 mgL^{-1}, and LC_{50} of local species Arabian killifish (*Aphanius dispar*) exposed to the SP of oil-based mud for 96 hours is 137565.36 mgL^{-1}. This study shows that the local species Arabian Killifish (*Aphanius dispar*) can be used for short-term (96 h) toxicity tests using the Arabian Light crude oil plus the dispersant Surfatron and the SP of oil-based mud. However, the WSF of Arabian Light crude oil and the SPP of oil-based mud do not show any mortality in local species Arabian Killifish (*Aphanius dispar*) for short-term (96 hours) toxicity testing.

On the contrary, experiments on the commercial Brine Shrimp (*Artemia sp.)* show LC_{50} of commercial Brine Shrimp (*Artemia sp.*) exposed to the WSF of Arabian Light crude oil for 48 hours is 20,441,370 mgL^{-1} and the LC_{50} of commercial Brine Shrimp (*Artemia sp.*) exposed to the SPP of oil-based mud for 48 hours is 170,769.4 mgL^{-1}. Furthermore, the short-term (48 hours) toxicity tests show that the EC_{50} of the cysts of the commercial Brine Shrimp (*Artemia sp.*) exposed to the SPP of Arabian Light crude oil for 48 hours is 5.01% and the EC_{50} of the cysts of the commercial Brine Shrimp

(*Artemia sp.*) exposed to the WSF of Arabian Light crude oil for 48 hours is 5.95%. Thus, it can be concluded that the commercial Brine Shrimp (*Artemia sp.*) can be used and is more feasible for short-term toxicity tests on Arabian Light crude oil and oil-based mud (OBM).

6.2 Future Recommendations

Exposure to crude oil can cause several biological effects including increased mortality, early-life stage developmental defects, reduced reproductive capacity, genetic damage, impaired immune function and disease resistance, and changes in behavior. Many publications have reported that early-life stages (embryos and larvae) of fish are more sensitive to oil exposure than adults (Dupuis and Ucan-Marin, 2015; Esenowo and Ugwumba, 2010).

Therefore, I highly recommend that the embryos of the local species Arabian killifish (*Aphanius dispar*) could be evaluated to assess the detrimental and pathological effects of Arabian light crude oil and oil-based mud.

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Appendix 1 Composition of Arabian Light Crude Oil

No	Crude Oil Properties	
1	API GRAVITY	33-34
2	SEDIMENT CONTEN	0.1
3	ASTM STABILISED GRAVITY	34.5
4	Wax-WT PERCENT	2.9
5	Vanadium ppm V200	11
6	GROSS HEATING VALUE	19.23
7	REID VAPOUR PRESSURE	2
8	SALT CONTENT, PPM NaCl	3.8
9	SULPHUR, WT PERCENT	1.5 Max
10	ASH, PPM	100
11	COMP. CARBON RESIDUE. WT PERCENT	3.1
12	VISCOCITY, CP	55
13	POUR POINT	35.0

(Source: Drilling & Workover Department of Saudi Aramco)

Appendix 2 Composition of Oil Based Mud

Recommended mud receipt in order of addition is as follow:

No	Product	Unit	82 pcf	84 pcf	Properties	Units	Value
1	Safra Oil	bbl	0.58	0.57	Density	pcf	82-84
2	Invermul HT or equivalent	gal	1.0-1.5	1.0-1.5	PV	cP	ALAP*
3	Lime	ppb	6-8	6-8	Yield Point	$lb/100ft^2$	22-25
4	Duratone or equivalent	ppb	10-12	10-12	10 sec.gel	$lb/100ft^2$	8-12
5	Fresh water	bbl	0.1	0.1	10 min.gel	$lb/100ft^2$	12-16
6	Geltone/VG 69 or equivalent	ppb	6-8	6-8	6 rpm		7-10
7	EZ Mul or equivalent	gal	0.5-1.0	0.5-1.0	Fitrate HTHP 220ºF	ml/30 min	< 2 All oil
8	$CaCl_2$ (77)	ppb	20	20	Chlorides (Water Phase Salinity)	mg/l	+/- 250,000
9	$CaCO_3$ Fine	ppb	165	180	OWR		85/15
10	$CaCO_3$ Med	ppb	50	50	ES	volts	>400
11	*Omniplex or equivalent	ppb	As reqd.	As reqd.			

(Source: Drilling & Workover Department of Saudi Aramco)

Appendix 3 Composition of Oil Dispersant

No	Name	Weight (%)
1	Petroleum Naphtha	30-60
2	Light aromatic solvent Naphtha	30-60
3	1,2,4-Trimethylbenzene	10-30
4	Isopropyl Alcohol	5-10
5	1,3,5-Trimethylbenzene	5-10
6	Cumene	1-5
7	Diethyl benzene	1-5
8	Benzene, tetra propylene	1-5
9	Xylene	1-5
10	Ethanolamine, Organic Acid Salt	30-60

(Technologies, 2009)

Appendix 4 Analysis Result for LC_{50} of $CuSO_4$ in *Aphanius dispar*

Probit Analysis

[DataSet1]

Data Information

		N of Cases
Valid		6
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	14	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
PROBIT[a]	Concentration	2.385	.672	3.547	.000	1.067	3.703
	Intercept	-3.511	1.305	-2.691	.007	-4.815	-2.206

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Covariances and Correlations of Parameter Estimates

		Concentration	Natural Response
PROBIT	Concentration	.452	.648
	Natural Response	.107	.060

Covariances (below) and Correlations (above).

Natural Response Rate Estimate[a]

	Estimate	Std. Error
PROBIT	.000	.246

a. Control group is not provided.

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	1.733	3	.630[a]

a. Since the significance level is greater than .500, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

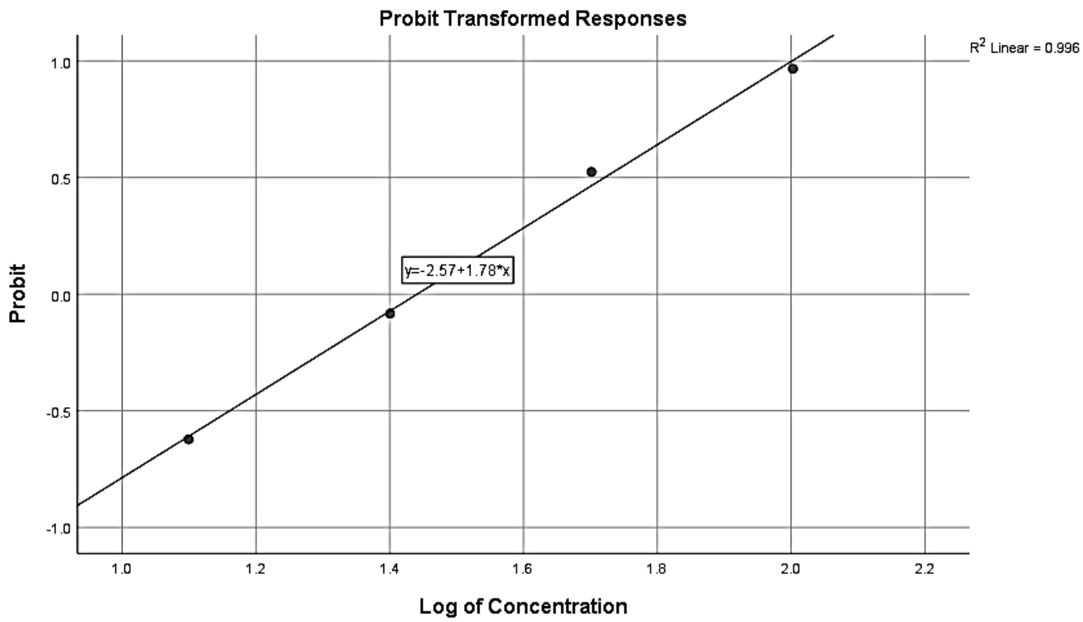
Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	.799	10	0	.541	-.541	.054
	2	1.099	10	3	1.871	.799	.187
	3	1.401	10	5	4.323	.347	.432
	4	1.702	10	7	7.080	-.080	.708
	5	2.003	10	8	8.972	-.642	.897
	6	2.304	10	10	9.763	.237	.976

Confidence Limits

		95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	3.138	.056	10.949	.497	-1.251	1.039
	.020	4.082	.100	13.115	.611	-1.000	1.118
	.030	4.824	.144	14.720	.683	-.841	1.168
	.040	5.470	.190	16.065	.738	-.722	1.206
	.050	6.058	.237	17.258	.782	-.626	1.237
	.060	6.608	.286	18.348	.820	-.544	1.264
	.070	7.132	.338	19.367	.853	-.472	1.287
	.080	7.636	.391	20.331	.883	-.407	1.308
	.090	8.125	.448	21.255	.910	-.349	1.327
	.100	8.603	.506	22.146	.935	-.295	1.345
	.150	10.900	.842	26.313	1.037	-.074	1.420
	.200	13.156	1.258	30.274	1.119	.100	1.481
	.250	15.459	1.770	34.242	1.189	.248	1.535
	.300	17.870	2.399	38.355	1.252	.380	1.584
	.350	20.438	3.169	42.729	1.310	.501	1.631
	.400	23.215	4.115	47.489	1.366	.614	1.677
	.450	26.261	5.280	52.783	1.419	.723	1.722
	.500	29.648	6.721	58.805	1.472	.827	1.769
	.550	33.472	8.514	65.827	1.525	.930	1.818
	.600	37.863	10.763	74.254	1.578	1.032	1.871
	.650	43.009	13.614	84.720	1.634	1.134	1.928
	.700	49.189	17.274	98.283	1.692	1.237	1.992
	.750	56.859	22.051	116.844	1.755	1.343	2.068
	.800	66.815	28.436	144.184	1.825	1.454	2.159
	.850	80.641	37.293	188.940	1.907	1.572	2.276
	.900	102.172	50.485	275.857	2.009	1.703	2.441
	.910	108.182	53.996	304.055	2.034	1.732	2.483
	.920	115.113	57.950	338.766	2.061	1.763	2.530
	.930	123.247	62.466	382.541	2.091	1.796	2.583
	.940	133.012	67.720	439.489	2.124	1.831	2.643
	.950	145.098	73.989	516.697	2.162	1.869	2.713
	.960	160.707	81.745	627.616	2.206	1.912	2.798
	.970	182.217	91.894	801.541	2.261	1.963	2.904
	.980	215.331	106.527	1118.244	2.333	2.027	3.049
	.990	280.158	132.612	1916.356	2.447	2.123	3.282

a. Logarithm base = 10.


Probit Transformed Responses
R2 Linear = 0.996
y=-2.57+1.78*x
Probit
1.0
0.5
0.0
-0.5
-1.0
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Log of Concentration

Appendix 5 Analysis Result for LC_{50} of ALCO plus Dispersant in *Aphanius dispar*

Probit Analysis

Data Information

		N of Cases
Valid		5
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	13	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
PROBIT[a]	Concentration	2.849	2.080	1.370	.171	-1.227	6.925
	Intercept	-6.311	5.910	-1.068	.286	-12.221	-.401

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Covariances and Correlations of Parameter Estimates

		Concentration	Natural Response
PROBIT	Concentration	4.326	.934
	Natural Response	1.665	.734

Covariances (below) and Correlations (above).

Natural Response Rate Estimate[a]

	Estimate	Std. Error
PROBIT	.000	.857

a. Control group is not provided.

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	1.009	2	.604[a]

a. Since the significance level is greater than .500, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

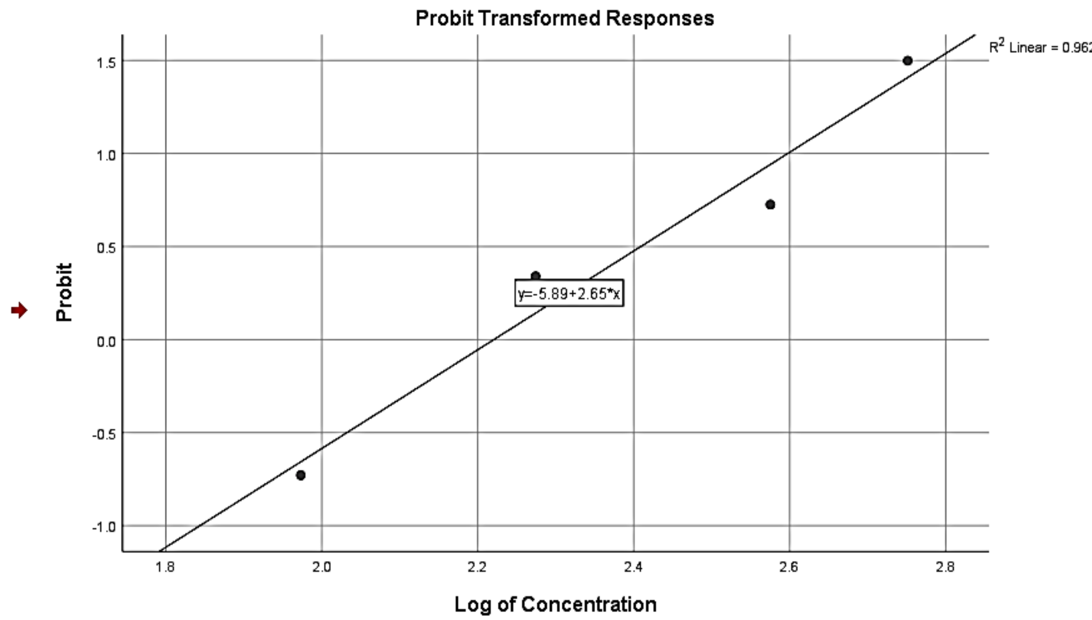
Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	1.973	10	2	2.451	-.121	.245
	2	2.274	10	6	5.665	.665	.567
	3	2.575	10	8	8.473	-.813	.847
	4	2.751	10	9	9.366	-.036	.937
	5	2.876	10	10	9.701	.299	.970

Confidence Limits

		95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	25.048	.	.	1.399	.	.
	.020	31.222	.	.	1.494	.	.
	.030	35.907	.	.	1.555	.	.
	.040	39.888	.	.	1.601	.	.
	.050	43.450	.	.	1.638	.	.
	.060	46.732	.	.	1.670	.	.
	.070	49.812	.	.	1.697	.	.
	.080	52.743	.	.	1.722	.	.
	.090	55.557	.	.	1.745	.	.
	.100	58.280	.	.	1.766	.	.
	.150	71.049	.	.	1.852	.	.
	.200	83.165	.	.	1.920	.	.
	.250	95.193	.	.	1.979	.	.
	.300	107.470	.	.	2.031	.	.
	.350	120.256	.	.	2.080	.	.
	.400	133.792	.	.	2.126	.	.
	.450	148.337	.	.	2.171	.	.
	.500	164.195	.	.	2.215	.	.
	.550	181.747	.	.	2.259	.	.
	.600	201.506	.	.	2.304	.	.
	.650	224.187	.	.	2.351	.	.
	.700	250.859	.	.	2.399	.	.
	.750	283.213	.	.	2.452	.	.
	.800	324.174	.	.	2.511	.	.
	.850	379.455	.	.	2.579	.	.
	.900	462.594	.	.	2.665	.	.
	.910	485.267	.	.	2.686	.	.
	.920	511.160	.	.	2.709	.	.
	.930	541.228	.	.	2.733	.	.
	.940	576.904	.	.	2.761	.	.
	.950	620.473	.	.	2.793	.	.
	.960	675.883	.	.	2.830	.	.
	.970	750.829	.	.	2.876	.	.
	.980	863.476	.	.	2.936	.	.
	.990	1076.307	.	.	3.032	.	.

a. Logarithm base = 10.



Probit Transformed Responses
R2 Linear = 0.962
1.5
1.0
0.5
0.0
-0.5
-1.0
Probit
y=-5.89+2.65*x
1.8
2.0
2.2
2.4
2.6
2.8
Log of Concentration

Appendix 6 Analysis Result for LC_{50} of Oil Dispersant in *Aphanius dispar*

Probit Analysis

[DataSet0]

Data Information

		N of Cases
Valid		6
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	12	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	4.689	1.665	2.816	.005	1.426	7.953
	Intercept	-11.347	4.191	-2.708	.007	-15.538	-7.157

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Covariances and Correlations of Parameter Estimates

		Concentration	Natural Response
PROBIT	Concentration	2.772	.492
	Natural Response	.048	.003

Covariances (below) and Correlations (above).

Natural Response Rate Estimate[a]

	Estimate	Std. Error
PROBIT	.038	.058

a. Control group is not provided.

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	2.047	3	.563[a]

a. Since the significance level is greater than .500, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

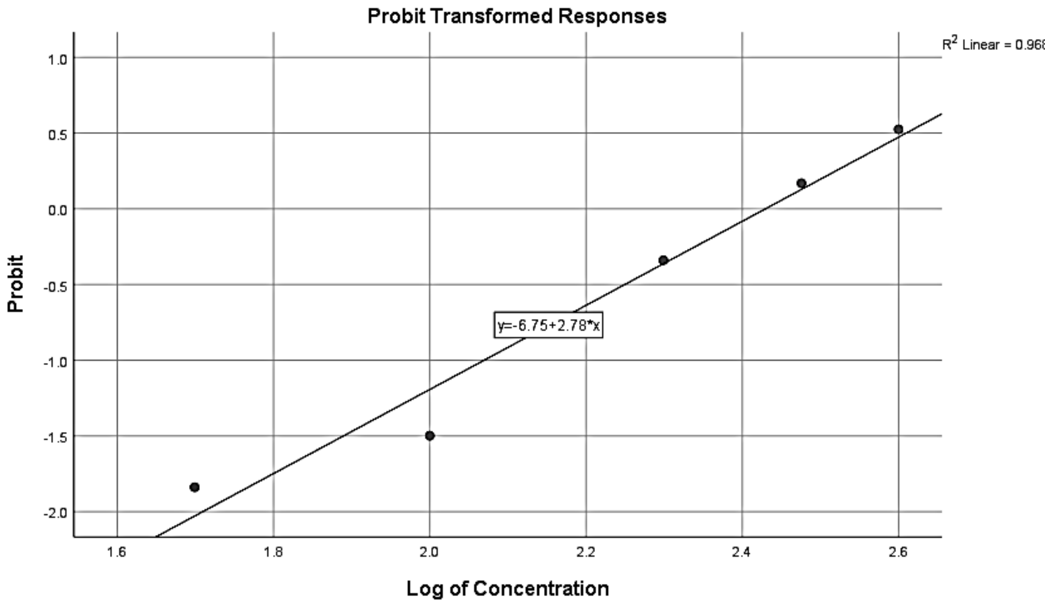
Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	1.699	10	0	.385	-.055	.039
	2	2.000	10	1	.618	.052	.062
	3	2.299	10	4	3.126	.544	.313
	4	2.476	10	6	6.185	-.515	.619
	5	2.600	10	7	8.084	-1.084	.808
	6	2.697	10	10	9.071	.929	.907

Confidence Limits

		95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	83.889	3.659	150.693	1.924	.563	2.178
	.020	95.904	5.665	163.585	1.982	.753	2.214
	.030	104.404	7.472	172.395	2.019	.873	2.237
	.040	111.292	9.200	179.377	2.046	.964	2.254
	.050	117.228	10.895	185.297	2.069	1.037	2.268
	.060	122.530	12.579	190.518	2.088	1.100	2.280
	.070	127.375	14.267	195.242	2.105	1.154	2.291
	.080	131.876	15.968	199.593	2.120	1.203	2.300
	.090	136.107	17.689	203.655	2.134	1.248	2.309
	.100	140.122	19.434	207.487	2.147	1.289	2.317
	.150	158.043	28.659	224.402	2.199	1.457	2.351
	.200	173.908	38.951	239.266	2.240	1.591	2.379
	.250	188.781	50.583	253.285	2.276	1.704	2.404
	.300	203.219	63.824	267.154	2.308	1.805	2.427
	.350	217.582	78.962	281.418	2.338	1.897	2.449
	.400	232.149	96.311	296.648	2.366	1.984	2.472
	.450	247.170	116.194	313.568	2.393	2.065	2.496
	.500	262.901	138.893	333.243	2.420	2.143	2.523
	.550	279.633	164.527	357.380	2.447	2.216	2.553
	.600	297.726	192.846	388.827	2.474	2.285	2.590
	.650	317.658	223.001	432.320	2.502	2.348	2.636
	.700	340.109	253.640	495.356	2.532	2.404	2.695
	.750	366.121	283.677	589.441	2.564	2.453	2.770
	.800	397.434	313.253	733.824	2.599	2.496	2.866
	.850	437.329	344.123	968.028	2.641	2.537	2.986
	.900	493.263	380.276	1397.086	2.693	2.580	3.145
	.910	507.813	388.832	1529.405	2.706	2.590	3.185
	.920	524.106	398.112	1688.385	2.719	2.600	3.227
	.930	542.625	408.325	1883.465	2.734	2.611	3.275
	.940	564.082	419.778	2129.437	2.751	2.623	3.328
	.950	589.592	432.940	2451.067	2.771	2.636	3.389
	.960	621.040	448.595	2893.713	2.793	2.652	3.461
	.970	662.011	468.199	3552.072	2.821	2.670	3.550
	.980	720.688	495.009	4670.235	2.858	2.695	3.669
	.990	823.906	539.353	7203.198	2.916	2.732	3.858

a. Logarithm base = 10.


Probit Transformed Responses
R^2 Linear = 0.968
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
Probit
y=-6.75+2.78*x
1.6
1.8
2.0
2.2
2.4
2.6
Log of Concentration

Appendix 7 Analysis Result for LC_{50} of Solid Phase (SP) of Oil-Based Mud in *Aphanius dispar*

Probit Analysis

Data Information

		N of Cases
Valid		5
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	17	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
PROBIT[a]	Concentration	3.182	1.054	3.019	.003	1.116	5.247
	Intercept	-16.348	5.627	-2.905	.004	-21.976	-10.721

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Covariances and Correlations of Parameter Estimates

		Concentration	Natural Response
PROBIT	Concentration	1.110	.186
	Natural Response	.018	.009

Covariances (below) and Correlations (above).

Natural Response Rate Estimate[a]

	Estimate	Std. Error
PROBIT	.097	.092

a. Control group is not provided.

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	.719	2	.698[a]

a. Since the significance level is greater than .500, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

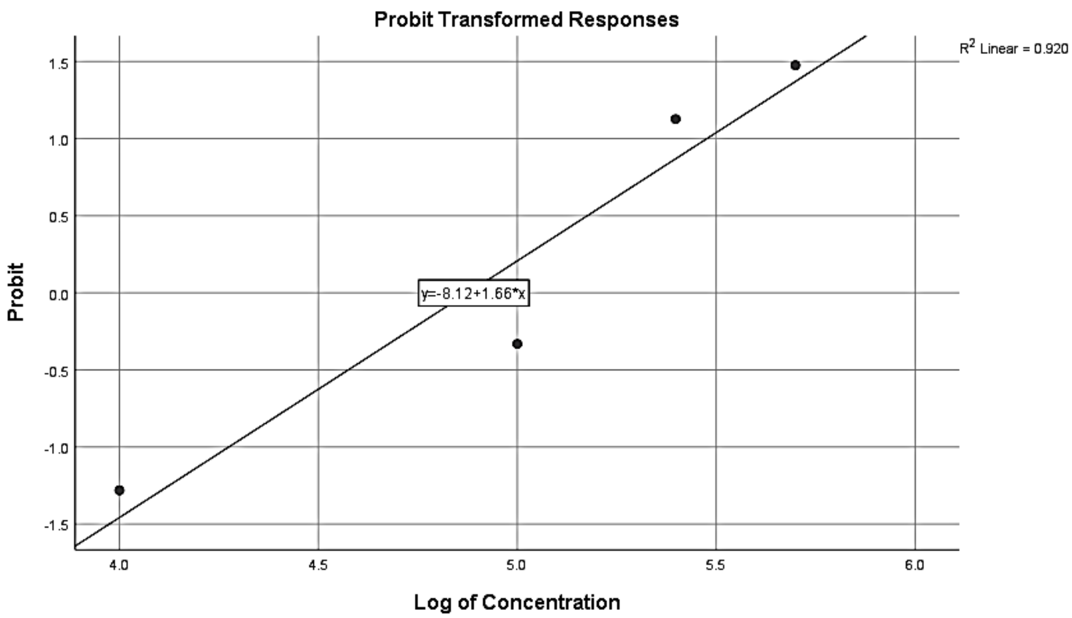
Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	4.000	10	1	.975	.025	.098
	2	5.000	10	4	3.950	-.250	.395
	3	5.398	10	9	8.154	.546	.815
	4	5.699	10	9	9.663	-.363	.966
	5	5.875	10	10	9.914	.086	.991

Confidence Limits

		95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	25545.076	462.562	63521.438	4.407	2.665	4.803
	.020	31116.351	805.849	72111.872	4.493	2.906	4.858
	.030	35265.561	1145.193	78215.397	4.547	3.059	4.893
	.040	38747.645	1491.018	83185.161	4.588	3.173	4.920
	.050	41832.118	1847.352	87490.835	4.622	3.267	4.942
	.060	44650.178	2216.359	91356.887	4.650	3.346	4.961
	.070	47276.842	2599.452	94909.917	4.675	3.415	4.977
	.080	49759.559	2997.695	98229.150	4.697	3.477	4.992
	.090	52130.520	3411.971	101367.875	4.717	3.533	5.006
	.100	54412.727	3843.068	104363.908	4.736	3.585	5.019
	.150	64974.714	6276.165	117990.524	4.813	3.798	5.072
	.200	74812.886	9239.193	130486.211	4.874	3.966	5.116
	.250	84432.188	12835.017	142687.273	4.927	4.108	5.154
	.300	94120.251	17187.826	155105.742	4.974	4.235	5.191
	.350	104087.307	22449.609	168169.053	5.017	4.351	5.226
	.400	114519.316	28806.323	182327.379	5.059	4.459	5.261
	.450	125606.486	36483.760	198137.472	5.099	4.562	5.297
	.500	137565.360	45751.539	216365.263	5.139	4.660	5.335
	.550	150662.826	56921.774	238145.215	5.178	4.755	5.377
	.600	165249.225	70336.762	265255.510	5.218	4.847	5.424
	.650	181811.104	86340.096	300620.854	5.260	4.936	5.478
	.700	201064.362	105237.061	349276.166	5.303	5.022	5.543
	.750	224135.233	127290.496	420347.866	5.351	5.105	5.624
	.800	252954.125	152877.472	531672.692	5.403	5.184	5.726
	.850	291255.276	183041.118	722930.213	5.464	5.263	5.859
	.900	347790.475	221043.439	1105300.784	5.541	5.344	6.043
	.910	363016.292	230283.626	1230318.984	5.560	5.362	6.090
	.920	380313.421	240381.276	1384388.554	5.580	5.381	6.141
	.930	400285.368	251578.341	1578773.511	5.602	5.401	6.198
	.940	423833.206	264231.227	1831559.261	5.627	5.422	6.263
	.950	452385.133	278895.483	2173865.725	5.656	5.445	6.337
	.960	488396.857	296507.180	2664541.901	5.689	5.472	6.426
	.970	536620.647	318833.316	3431245.223	5.730	5.504	6.535
	.980	608176.334	349878.529	4819573.134	5.784	5.544	6.683
	.990	740817.058	402620.760	8283230.996	5.870	5.605	6.918

a. Logarithm base = 10.



Probit Transformed Responses
R² Linear = 0.920
y=-8.12+1.66*x
Probit
Log of Concentration
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
4.0
4.5
5.0
5.5
6.0

Appendix 8 Analysis Result for LC_{50} of $CuSO_4$ in the Commercial Brine Shrimp *Artemia sp.*

Probit Analysis

Data Information

		N of Cases
Valid		8
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	12	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	1.554	.266	5.853	.000	1.034	2.075
	Intercept	-1.570	.412	-3.808	.000	-1.982	-1.157

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Covariances and Correlations of Parameter Estimates

		Concentration	Natural Response
PROBIT	Concentration	.071	.635
	Natural Response	.016	.009

Covariances (below) and Correlations (above).

Natural Response Rate Estimate[a]

	Estimate	Std. Error
PROBIT	.000	.092

a. Control group is not provided.

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	4.758	5	.446[a]

a. Since the significance level is less than .500, a heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Cell Counts and Residuals

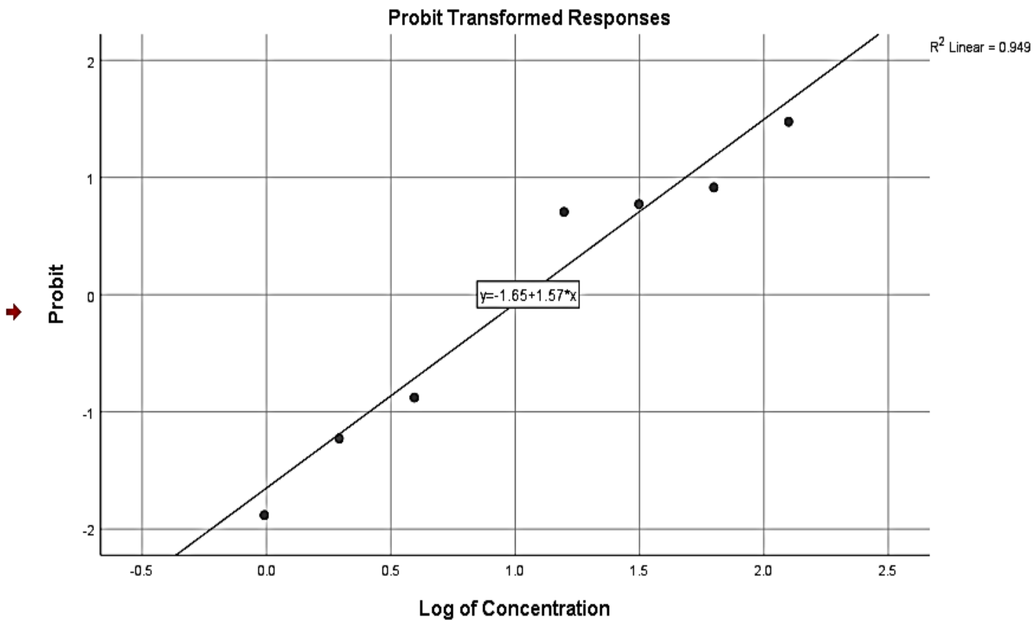
	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	-.009	20	1	1.134	-.534	.057
	2	.292	20	2	2.647	-.447	.132
	3	.594	20	4	5.184	-1.384	.259
	4	.895	20	11	8.588	2.012	.429
	5	1.196	20	15	12.282	2.918	.614
	6	1.497	20	16	15.514	.086	.776
	7	1.798	20	16	17.797	-1.397	.890
	8	2.099	20	19	19.097	-.497	.955

Confidence Limits

		95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[b]		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	.010	.326	.011	1.344	-.487	-1.957	.128
	.020	.488	.022	1.801	-.311	-1.655	.255
	.030	.631	.034	2.170	-.200	-1.463	.337
	.040	.765	.048	2.499	-.116	-1.320	.398
	.050	.895	.063	2.804	-.048	-1.203	.448
	.060	1.022	.079	3.094	.010	-1.104	.491
	.070	1.149	.096	3.375	.060	-1.017	.528
	.080	1.276	.115	3.648	.106	-.939	.562
	.090	1.404	.135	3.916	.147	-.869	.593
	.100	1.532	.157	4.182	.185	-.804	.621
	.150	2.203	.291	5.502	.343	-.537	.741
	.200	2.940	.472	6.869	.468	-.326	.837
	.250	3.766	.713	8.340	.576	-.147	.921
	.300	4.704	1.029	9.965	.672	.012	.998
	.350	5.780	1.439	11.798	.762	.158	1.072
	.400	7.029	1.971	13.912	.847	.295	1.143
	.450	8.492	2.657	16.402	.929	.424	1.215
	.500	10.230	3.543	19.406	1.010	.549	1.288
	.550	12.323	4.689	23.133	1.091	.671	1.364
	.600	14.889	6.177	27.913	1.173	.791	1.446
	.650	18.103	8.117	34.291	1.258	.909	1.535
	.700	22.245	10.667	43.226	1.347	1.028	1.636
	.750	27.784	14.062	56.528	1.444	1.148	1.752
	.800	35.590	18.699	77.966	1.551	1.272	1.892
	.850	47.497	25.350	116.622	1.677	1.404	2.067
	.900	68.291	35.911	200.376	1.834	1.555	2.302
	.910	74.551	38.884	229.407	1.872	1.590	2.361
	.920	82.003	42.324	266.171	1.914	1.627	2.425
	.930	91.061	46.375	313.992	1.959	1.666	2.497
	.940	102.364	51.259	378.369	2.010	1.710	2.578
	.950	116.977	57.332	469.087	2.068	1.758	2.671
	.960	136.832	65.224	605.394	2.136	1.814	2.782
	.970	165.919	76.186	831.049	2.220	1.882	2.920
	.980	214.372	93.249	1271.865	2.331	1.970	3.104
	.990	321.033	127.251	2506.404	2.507	2.105	3.399

a. A heterogeneity factor is used.

b. Logarithm base = 10.


Probit Transformed Responses
R^2 Linear = 0.949
y=-1.65+1.57*x
Probit
Log of Concentration
2
1
0
-1
-2
-0.5
0.0
0.5
1.0
1.5
2.0
2.5

Appendix 9 Analysis Result for LC_{50} of Suspended Particulate Phase (SPP) in the Commercial Brine Shrimp *Artemia sp.*

Probit Analysis

Data Information

		N of Cases
Valid		8
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	15	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
PROBIT[a]	Concentration	1.096	.657	1.669	.095	-.191	2.383
	Intercept	-1.716	1.406	-1.221	.222	-3.122	-.310

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Covariances and Correlations of Parameter Estimates

		Concentration	Natural Response
PROBIT	Concentration	.431	.913
	Natural Response	.132	.049

Covariances (below) and Correlations (above).

Natural Response Rate Estimate[a]

	Estimate	Std. Error
PROBIT	.161	.221

a. Control group is not provided.

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	.784	5	.978[a]

a. Since the significance level is greater than .500, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

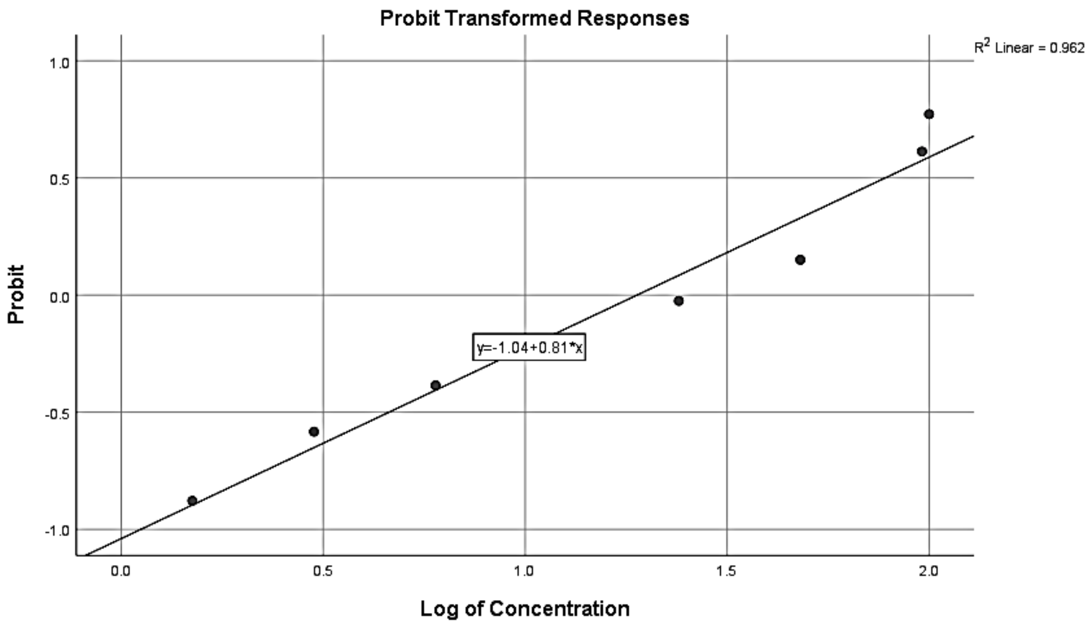
Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	.176	20	4	4.284	-.484	.214
	2	.477	20	6	5.166	.434	.258
	3	.778	20	7	6.468	.532	.323
	4	1.079	20	8	8.195	.205	.410
	5	1.380	20	10	10.251	-.451	.513
	6	1.681	20	11	12.449	-1.249	.622
	7	1.982	20	15	14.558	.042	.728
	8	2.000	20	16	14.675	.925	.734

Confidence Limits

		95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	.277	.	.	-.557	.	.
	.020	.492	.	.	-.308	.	.
	.030	.707	.	.	-.150	.	.
	.040	.930	.	.	-.032	.	.
	.050	1.161	.	.	.065	.	.
	.060	1.403	.	.	.147	.	.
	.070	1.657	.	.	.219	.	.
	.080	1.922	.	.	.284	.	.
	.090	2.200	.	.	.343	.	.
	.100	2.492	.	.	.397	.	.
	.150	4.171	.	.	.620	.	.
	.200	6.281	.	.	.798	.	.
	.250	8.923	.	.	.951	.	.
	.300	12.232	.	.	1.087	.	.
	.350	16.383	.	.	1.214	.	.
	.400	21.619	.	.	1.335	.	.
	.450	28.272	.	.	1.451	.	.
	.500	36.816	.	.	1.566	.	.
	.550	47.941	.	.	1.681	.	.
	.600	62.695	.	.	1.797	.	.
	.650	82.731	.	.	1.918	.	.
	.700	110.812	.	.	2.045	.	.
	.750	151.899	.	.	2.182	.	.
	.800	215.812	.	.	2.334	.	.
	.850	324.979	.	.	2.512	.	.
	.900	543.930	.	.	2.736	.	.
	.910	615.985	.	.	2.790	.	.
	.920	705.121	.	.	2.848	.	.
	.930	818.088	.	.	2.913	.	.
	.940	965.778	.	.	2.985	.	.
	.950	1167.030	.	.	3.067	.	.
	.960	1457.678	.	.	3.164	.	.
	.970	1915.993	.	.	3.282	.	.
	.980	2755.675	.	.	3.440	.	.
	.990	4886.456	.	.	3.689	.	.

a. Logarithm base = 10.


Probit Transformed Responses
R2 Linear = 0.962
y=-1.04+0.81*x
Probit
1.0
0.5
0.0
-0.5
-1.0
0.0
0.5
1.0
1.5
2.0
Log of Concentration

Appendix 10 Analysis Result for EC_{50} of Suspended Particulate Phase (SPP) in the Commercial Brine Shrimp *Artemia sp.*

Probit Analysis

Data Information

		N of Cases
Valid		8
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	20	No[a]

a. Parameter estimates did not converge.

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	4.591	2.114	2.171	.030	.446	8.735
	Intercept	-3.213	1.788	-1.797	.072	-5.001	-1.425

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Covariances and Correlations of Parameter Estimates

		Concentration	Natural Response
PROBIT	Concentration	4.471	.321
	Natural Response	.038	.003

Covariances (below) and Correlations (above).

Natural Response Rate Estimate[a]

	Estimate	Std. Error
PROBIT	.499	.056

a. Control group is not provided.

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	1.175	5	.947[a]

a. Since the significance level is greater than .500, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

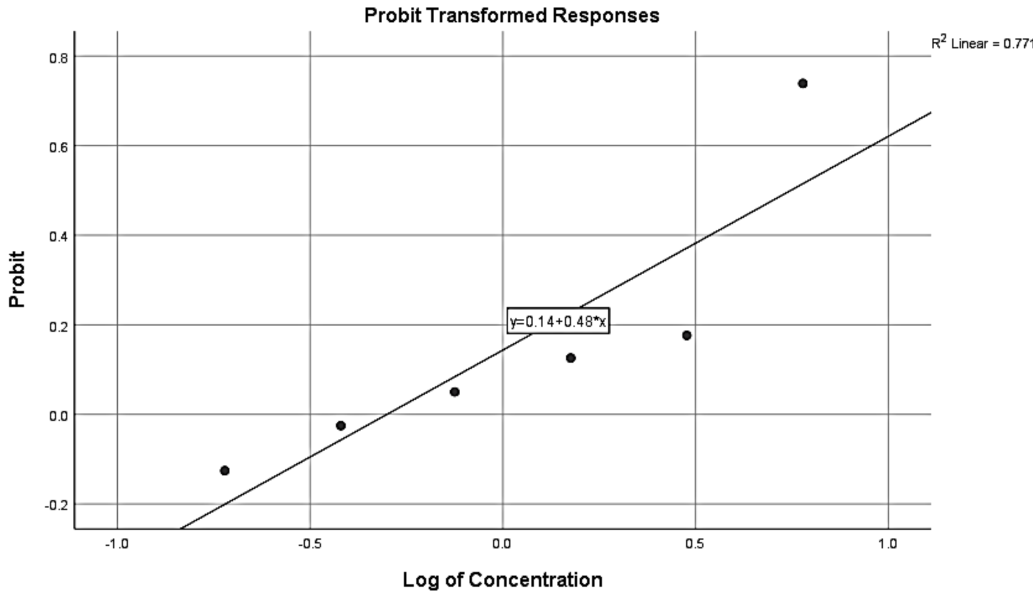
Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	-.721	20	9	9.982	-.982	.499
	2	-.420	20	10	9.982	-.182	.499
	3	-.125	20	10	9.982	.418	.499
	4	.176	20	11	10.063	.937	.503
	5	.477	20	11	11.516	-.116	.576
	6	.778	20	15	16.396	-.996	.820
	7	1.079	20	20	19.591	.409	.980
	8	1.380	20	20	19.991	.009	1.000

Confidence Limits

		95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	1.560	.000	3.281	.193	-5.714	.516
	.020	1.789	.000	3.544	.253	-5.106	.549
	.030	1.951	.000	3.723	.290	-4.720	.571
	.040	2.082	.000	3.866	.319	-4.430	.587
	.050	2.196	.000	3.987	.342	-4.194	.601
	.060	2.297	.000	4.095	.361	-3.993	.612
	.070	2.390	.000	4.192	.378	-3.817	.622
	.080	2.477	.000	4.281	.394	-3.660	.632
	.090	2.558	.000	4.365	.408	-3.517	.640
	.100	2.635	.000	4.444	.421	-3.385	.648
	.150	2.980	.001	4.797	.474	-2.841	.681
	.200	3.285	.004	5.111	.517	-2.410	.709
	.250	3.573	.009	5.412	.553	-2.041	.733
	.300	3.852	.019	5.716	.586	-1.711	.757
	.350	4.130	.039	6.036	.616	-1.407	.781
	.400	4.413	.076	6.387	.645	-1.121	.805
	.450	4.705	.142	6.792	.673	-.847	.832
	.500	5.011	.262	7.286	.700	-.582	.862
	.550	5.337	.475	7.937	.727	-.323	.900
	.600	5.690	.849	8.886	.755	-.071	.949
	.650	6.080	1.473	10.474	.784	.168	1.020
	.700	6.519	2.411	13.602	.814	.382	1.134
	.750	7.029	3.561	20.782	.847	.552	1.318
	.800	7.643	4.678	39.156	.883	.670	1.593
	.850	8.428	5.661	93.068	.926	.753	1.969
	.900	9.530	6.601	301.564	.979	.820	2.479
	.910	9.818	6.801	403.515	.992	.833	2.606
	.920	10.140	7.011	554.810	1.006	.846	2.744
	.930	10.506	7.234	788.971	1.021	.859	2.897
	.940	10.930	7.478	1171.413	1.039	.874	3.069
	.950	11.435	7.749	1842.323	1.058	.889	3.265
	.960	12.059	8.063	3143.142	1.081	.907	3.497
	.970	12.872	8.446	6076.589	1.110	.927	3.784
	.980	14.039	8.954	14642.277	1.147	.952	4.166
	.990	16.096	9.769	58854.629	1.207	.990	4.770

a. Logarithm base = 10.



Probit Transformed Responses
R2 Linear = 0.771
0.8
0.6
0.4
0.2
0.0
-0.2
Probit
y=0.14+0.48*x
-1.0
-0.5
0.0
0.5
1.0
Log of Concentration

Appendix 11 Analysis Result for EC_{50} of Water Soluble Fraction (WSF) in the Commercial Brine Shrimp *Artemia sp.*

Probit Analysis

Data Information

		N of Cases
Valid		8
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	20	No[a]

a. Parameter estimates did not converge.

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
PROBIT[a]	Concentration	4.421	2.050	2.157	.031	.403	8.440
	Intercept	-3.425	1.880	-1.822	.068	-5.305	-1.546

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Covariances and Correlations of Parameter Estimates

		Concentration	Natural Response
PROBIT	Concentration	4.203	.364
	Natural Response	.041	.003

Covariances (below) and Correlations (above).

Natural Response Rate Estimate[a]

	Estimate	Std. Error
PROBIT	.425	.055

a. Control group is not provided.

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	3.828	5	.574[a]

a. Since the significance level is greater than .500, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

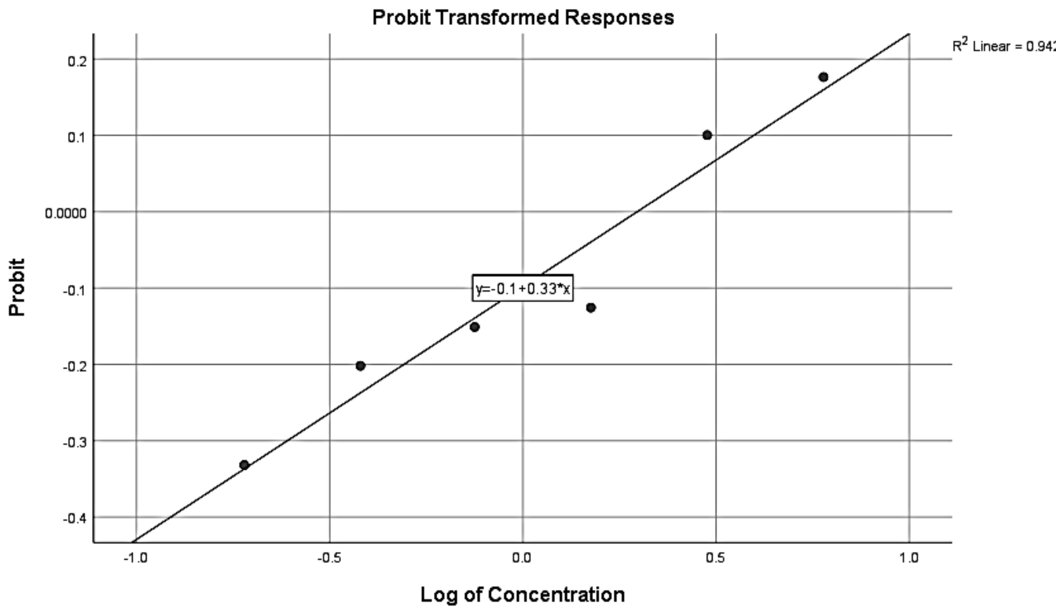
Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	-.721	20	7	8.503	-1.103	.425
	2	-.420	20	8	8.503	-.103	.425
	3	-.125	20	9	8.503	.297	.425
	4	.176	20	9	8.550	.450	.427
	5	.477	20	11	9.585	1.215	.479
	6	.778	20	11	14.322	-2.922	.716
	7	1.079	20	20	18.976	1.024	.949
	8	1.380	20	20	19.957	.043	.998

Confidence Limits

		95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	1.772	.000	3.786	.249	-6.289	.578
	.020	2.043	.000	4.097	.310	-5.614	.612
	.030	2.235	.000	4.309	.349	-5.186	.634
	.040	2.392	.000	4.477	.379	-4.865	.651
	.050	2.527	.000	4.620	.403	-4.603	.665
	.060	2.649	.000	4.746	.423	-4.381	.676
	.070	2.760	.000	4.860	.441	-4.186	.687
	.080	2.864	.000	4.965	.457	-4.011	.696
	.090	2.961	.000	5.063	.471	-3.852	.704
	.100	3.054	.000	5.156	.485	-3.706	.712
	.150	3.470	.001	5.568	.540	-3.102	.746
	.200	3.840	.002	5.933	.584	-2.623	.773
	.250	4.189	.006	6.281	.622	-2.213	.798
	.300	4.530	.014	6.630	.656	-1.847	.822
	.350	4.870	.031	6.994	.688	-1.508	.845
	.400	5.217	.065	7.392	.717	-1.189	.869
	.450	5.575	.131	7.847	.746	-.883	.895
	.500	5.952	.260	8.401	.775	-.586	.924
	.550	6.355	.507	9.133	.803	-.295	.961
	.600	6.792	.972	10.219	.832	-.012	1.009
	.650	7.275	1.807	12.120	.862	.257	1.084
	.700	7.822	3.108	16.200	.893	.492	1.210
	.750	8.458	4.649	26.603	.927	.667	1.425
	.800	9.227	6.019	55.888	.965	.780	1.747
	.850	10.212	7.156	150.915	1.009	.855	2.179
	.900	11.602	8.237	568.849	1.065	.916	2.755
	.910	11.966	8.469	788.600	1.078	.928	2.897
	.920	12.373	8.714	1126.425	1.092	.940	3.052
	.930	12.837	8.977	1669.851	1.108	.953	3.223
	.940	13.376	9.265	2596.225	1.126	.967	3.414
	.950	14.019	9.589	4301.965	1.147	.982	3.634
	.960	14.813	9.965	7800.533	1.171	.998	3.892
	.970	15.852	10.427	16246.115	1.200	1.018	4.211
	.980	17.346	11.046	43193.811	1.239	1.043	4.635
	.990	19.992	12.048	202547.554	1.301	1.081	5.307

a. Logarithm base = 10.



Probit Transformed Responses
R² Linear = 0.942
y=-0.1+0.33*x
Probit
0.2
0.1
0.0000
-0.1
-0.2
-0.3
-0.4
-1.0
-0.5
0.0
0.5
1.0
Log of Concentration

Curriculum Vitae

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2006 – 2010: B.Sc. in Biology, University of Indonesia, Jakarta, Indonesia.

Conferences Attended:

4th Water Arabia Conference & Exhibition on October 17, 18 & 19 February 2015, at Al-Khobar, Saudi Arabia.

8th Petro Environment Conference & Exhibition on 22-24th February 2016, Al Khobar Saudi Arabia,

20th Middle East Oil & Gas Show and Conference on 6 - 9 March 2017, Bahrain.

5th Water Arabia Conference & Exhibition on October 17, 18 & 19, 2017, at Al-Khobar, Saudi Arabia.

Exploration and Producing Technical Exchange (EPTEK) on October 15-17, 2017, at KFUPM, Dhahran, Saudi Arabia.

Publications:

Albuntana, A & Tawabini, B.S. 2016. Study of Potential Gelling Extract Cactus *Opuntia ficus-indica* for Water Purification: An Alternative Natural Adsorbent for Phenol Removal. 8th PetroEnvironment Symposium, Dhahran, Saudi Arabia.

Albuntana, A., Manikandan, K.P., Tawabini, B.S., Qurban, M.A., Hall, J.A., Abdulghani, W. 2018. Ecotoxicity of Arabian Light Crude Oil and Oil-based Drilling Mud on The Local Arabian Killifish (*Aphanius dispar*) and Commercial Brine Shrimp (*Artemia sp*.). *Environmental Toxicology. (Under Review)*