İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

OIL POLLUTION TOXICITY ASSESSMENT

M.Sc. Thesis by
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Our modern daily lives are dependent to petroleum and petroleum products. There is a big concern started to grow up according to the vast usage of petroleum products lead to oil spills to environment. The damage directed to the environment by oil spills can only be quantified by toxicity analysis. There are difficulties in determining the toxicity of oils because petroleum and petroleum products do not dissolve in water.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

OIL POLLUTION TOXICITY ASSESSMENT

SUMMARY

Recently, petroleum and petroleum products has become the major sustainer of our lifestyles and also the industrial life. Because of petroleum and petroleum products used in vast amounts, increased the chance of oil spills by accidents, malfunction and during transportation. This lead to an increase in the damage introduced to the environment and raised a global awareness.

The main purpose of this study is to predict the toxicity of the oils, but because of petroleum having a low solubility in water, this causes difficulties in the toxicity evaluation. For this purpose apart from the water soluble fraction of oils, they mixed with different solvents and then introduced to the water medium in the experiments conducted in this study prior to toxicity testing. The solvents used in the experiments tested for toxicity for determining the toxicities of the solvents and then compared with the toxicities of the oil-solvent mixtures. This helps us to evaluate the toxicity of the solvent extractable part of the oils. In addition the oil samples were poured onto sand samples and extracted by solvents and these extracts tested for toxicity.

In the studies three oil samples are used. These are motor oil, used motor oil and bunker c fuel oil. The extractions of these oil samples planned to done with 3 different solvents which are tetrahydrofuran (THF), dimethylsulfoxide (DMSO) and n-hexane but because of n-hexane is insoluble with water, only 2 of these 3 solvents used.

As toxicity testing in this study BioTox^{TM} toxicity bioassay is used. This test determines the toxicity by the light reduction in the test reagent bioluminescent marine bacterium *Vibrio Fischeri*. The test procedure determines the toxicity according to the reduction of the light output of the test reagent before and after mixing with the sample oil mixtures. The light reduction measured by a luminometer and transferred the data to a computer program. The computer program calculates the effective concentration (EC_{50}) by the 50% decrease in the light output of the test reagent.

In the experiments first solvents tested for toxicity. In the next step 2 grams of motor oil, used motor oil and fuel oil mixed with 100 ml of DMSO followed by 1 grams of motor oil and used motor oil mixed with 50 ml of THF and 1 gr of fuel oil mixed with 100 ml of THF. These samples are tested for toxicity. As the third step of the experiments 0,5 grams of motor oil, used motor oil and fuel oil poured onto 50 grams of sand. These mixtures extracted by both 50 ml of THF and 50 ml of DMSO from a Zero Headspace Extractor (ZHE) according to the Toxicity Characteristic Leaching Procedure (TCLP) then all the sample extracts tested for toxicity. The

final experiments done in order to determine the toxicity of the water soluble fraction of oils in both distilled and sea water. 5 grams of three sample oils poured into 100 ml of distilled and sea water respectively. It is known that wave action increase the solubility of oils therefore in order to simulate these conditions the samples were sonicated for 10 min followed by orbital shaking for 72 hours at 200 rpm at room temperature. Samples than filtered from a ZHE and the water soluble fraction of extracts tested for toxicity.

PETROL KİRLİLİĞİNİN ZEHİRLİLİK DEĞERLENDİRMESİ

ÖZET

Günümüzde petrol ve petrol ürünleri hayatımızın ve endüstriyel hayatın en önemli hammaddesi haline gelmiştir. Petrol ve petrol ürünlerinin büyük miktarlarda kullanılması, bu ürünlerin kazalar, makine arızaları ve taşınma sırasında dökülmeleri neticesinde doğal çevreye verilen zararın önemli boyutlara ulaşmasını sağlamış ve küresel bir farkındalık yaratmıştır.

Bu çalışmadaki ana amaç petrolün toksisitesinin (zehirliliğinin) saptanmasıdır; fakat petrol suda çok düşük miktarlarda çözündüğü için toksisite değerlendirmesinde zorluklar çıkarmaktadır. Bu sorunun aşılması için yapılan deneylerde petrolün suda çözünmüş bölümünün yanında değişik solventlerle (çözücülerle) karıştırılarak bu solvent-petrol karışımının toksisitesine bakılmıştır. Petrolün toksisitesinin belirlenmesi için tek başına solventlerin zehirlilikleri ile solvent-petrol karışımının zehirliliklerine bakılarak petrol ve türevlerinin çözünmüş olması durumunda ne kadar bir zehirlilik oluşturacakları karşılaştırılmıştır. Çalışmanın son kısmında ise kum numuneleri üzerine dökülen petrol örnekleri solventlerle ekstrakte edilerek toksisitelerine bakılmıştır.

Çalışmalarda üç adet petrol örneği kullanılmıştır. Bunlar, motor yağı, kullanılmış motor yağı, ve No.6 fuel oildir. Deneylerde 3 solvent (tetrahidrofuran (THF), dimetilsülfoksit (DMSO) ve n-Hekzan) kullanılması planlanmasına karşı n-Hekzan ın sudaki düşük çözünürlüğü sonucu kullanmaktan vazgeçilmesiyle 2 adet solventle çalışılmıştır.

Toksisite ölçümünde BioTox^{TM} toksisite testi kullanılmıştır. Bu test *Vibrio Fischeri* ışık saçan bakterilerinin ışınımındaki azalmayı ölçerek toksisite değerlendirmesi yapmaktadır. Değişik seyreltiler halinde hazırlanan örneklerin, aklime edilmiş bakteri kültürüne eklenerek karışımdan önceki ve sonraki ışınım değerlerindeki azalmayı bir luminometre yardımıyla ölçerek, bilgisayar programı aracılığı ile organizmaların ışınımındaki %50 düşüşe denk gelen efektif konsantrasyon (EC_{50}) değerinin bulunmasını sağlamıştır.

Deneylerde ilk olarak solventlerin tek başlarına toksisitelerine bakılmıştır. Daha sonraki adımda 2gr motor yağı, kullanılmış motor yağı, ve No.6 fuel oil 100'er ml DMSO ile ardından ise 1gr motor yağı, kullanılmış motor yağı 50'şer ml THF, ve 1 gr No.6 fuel oil 100 ml THF ile karıştırılarak karışımlardan alınan örnekler üzerinde toksisite testi yapılmıştır. Test düzeneğinde üçüncü adım olarak 0,5gr motor yağı, kullanılmış motor yağı, ve No.6 fuel oil 50'şer gr kum ile karışırılmıştır. Bu karışımlar 50 ml DMSO ve 50 ml THF ile ZHE (Zero Headspace Extractor) ile 0,6 µm filtreden basınçlı hava yardımıyla ekstrakte edilmiş ve örnekler tek tek toksisite testine sokulmuştur. Son olarak deneylerde örneklerin sudaki çözünmüş kısımlarının

oluşturduğu toksisitenin ölçülmesi amaçlanmıştır. Bu amaç doğrultusunda 5gr motor yağı, kullanılmış motor yağı, ve No.6 fuel oil 100'er ml deniz suyu daha sonra 100'er ml distile su ile karıştırılmıştır. Su ortamındaki dalga hareketlerinin çözünme etkisini arttırmaktadır. Bu durumun canlandırılması için örnekler önce 10 dakika sonike (ses dalgalarıyla karıştırılmış) edildikten sonra ise 72 saat boyunca orbital karıştırıcıda bırakılmıştır. Daha sonra bu örnekler ZHE ile süzülerek toksisite testine tabi tutulmuştur.

1. INTRODUCTION

Oil is a necessity in our industrial society, and a major sustainer of our lifestyle. In recent years, major oil spills attract the attention of the public and the media, which created a global awareness of the risks of oil spills and their damage reflected to the environment. According to trends in energy usage, it is not likely to decrease much in the future. Industries use oil and petroleum derivatives to manufacture such vital products as plastics, fertilizers, and chemical feedstocks, which will still be required in the future. In fact, the production and consumption of oil and petroleum products are increasing worldwide and the threat of oil pollution is increasing accordingly. The movement of petroleum from the oil fields to the consumer involves as many as 10 to 15 transfers between many different modes of transportation including tankers, pipelines, railcars, and tank trucks. Oil is stored at transfer points and at terminals and refineries along the route. Accidents can happen during any of these transportation steps or storage. Oil spills are a frequent occurrence, particularly because of the heavy use of oil and petroleum products in our daily lives. Oil is a common soil contaminant from a variety of sources, including leaking fuel storage tanks, crude oil spills and production waste products.

30 to 50 percent of oil spills are directly or indirectly caused by human error; whereas, 20 to 40 percent of these spills caused by equipment failure or malfunction. About 10 million tons of oil and petroleum products are used worldwide every day. In both Canada and the United States, most oil spills reported are more than 4000 L (about 1000 gallons). In Canada, there are about 12 such oil spills every day, of which only about one is spilled into navigable waters. These 12 spills amount to about 40 tons of oil or petroleum product. In the United States, there are about 25 spills per day into navigable waters and an estimated 75 spills on land. It is mostly believed that oil spills from tankers are the primary source of oil pollution in the marine environment. While it is true that most of the large spills are from tankers, it must be recognized that these spills still make up less than about 5% of all oil pollution entering the sea. Half of the oil spilled in the seas is the runoff of

oil and fuel from land-based sources, usually from wastewater. Much lubricating oil finds its way into wastewater, which is often discharged directly into the sea. About 24% of oil spilled into the sea comes from the transportation sector, which includes tankers, freighters, barges, and other vessels. Natural sources of oil constitute about 11% of the input. Atmospheric sources constitute 13% of oil pollution in the sea. This pollution is the result of hydrocarbons in the air from a variety of sources and causes such as inefficient combustion. Much of the material is re-precipitated onto land and subsequently ends up in the sea (Fingas, 2000)..

Although the chronic or acute effects of the oil pollution after an accident on the aquatic plants and animals can not be quantified since an inventory is not available before accident, the lethal and adverse effects of the pollution on biota is certain and quantity and properties of the hazardous material are also known (Talınlı et al., 2003). The toxicity of a hazardous material is one of the most important hazard criteria. Therefore, determination of the toxicity is an important stage on assessing the hazard level of the environmental components and their protection. The assessment of environmental damage can be made by monitoring the toxicity, which may change by natural attenuation and cleanup operations (Toxicity measurements by BioTox^{TM} bioassay give EC_{50} values).

1.1 Aim and Scope

The main problem in this study is, because oils do not dissolve in water, predicting the hazards of petroleum products and oils which penetrated to the aquatic environment are not possible according to the low solubility. Therefore according to the toxicity analysis of oils in the literature, oils had to be transferred into liquid phase by solvents and tested for toxicity. This study focuses on calculating the toxicity of petroleum products and oils by extracting them with solvents, than introducing into BioTox^{TM} bioassay.

In this study three oil samples (Motor Oil (MO), Used Motor Oil (UMO), and Bunker C Fuel Oil (FO)) were used and the extractions were made by solvents (Tetrahydrofuran (THF) and Dimethylsulfoxide (DMSO)) and the experiments were conducted by BioTox^{TM} toxicity test using *Vibrio Fischeri* on the samples in order to determine the toxicity of oils. The toxicities of individual solvents tested for blank toxicity and integrated with the toxicity data of solvent-oil mixtures. Moreover, in order to determine the extraction power of the solvents and also to simulate the behavior of sand on oil spills, oil samples were mixed with sand and extracted by solvents with Zero Headspace Extractor (ZHE) according to the Toxicity Characteristic Leaching Procedure (TCLP). As the final approach the water soluble fraction of oils and their toxicities were also determined.

The aims and scope of this study may be summarized as:

- Determination of the toxicity of oils and water soluble fraction of oils.
- Investigation of the suitability of BioTox^{TM} bioluminescence test for the determination of toxic effects of petroleum and petroleum products.
- Comparison of the most suitable solvent for oil extraction.
- Determination of the effect of sand on oil extractability.

2. OIL POLLUTION

2.1 Petroleum and Petroleum Products

The word *petroleum*, derived from the Latin *petra* and *oleum*, meaning literally "rock oil" issued to describe a myriad of hydrocarbon-rich fluids that have accumulated in subterranean reservoirs. Petroleum (also called *crude oil*) varies dramatically in color, odor, and flow properties that reflect the diversity of its origin (Speight, 2002).

Petroleum is a naturally occurring liquid found in formations in the Earth consisting of a complex mixture of hydrocarbons (mostly alkanes) of various lengths and also includes compounds of sulfur, nitrogen, oxygen, metals, and other elements. Petroleum is used mostly, by volume, for producing fuel oil and gasoline, both important "primary energy" sources. 84% (37 of 42 gallons in a typical barrel) of the hydrocarbons present in petroleum is converted into energy-rich fuels (petroleum-based fuels), including gasoline, diesel, jet, heating, and other fuel oils and liquefied petroleum gas. Petroleum is also the raw material for many chemical products, including solvents, fertilizers, pesticides, and plastics; the 16% not used for energy production is converted into these other materials (Wong, 2004).

Petroleum can be an environmental hazard for all organisms. Petroleum contains PAHs which are individual Polycyclic Aromatic Hydrocarbons can be toxic to organisms, but they are most commonly associated with illnesses in humans. Petroleum spills raised some environmental concern during the early twentieth century when ocean transport of large volumes of crude oil began. World War I caused a large number of oil spills that had a noticeably adverse effect on marine birds. The subsequent conversion of the economy of the world from coal to oil, followed by World War II, greatly increased the petroleum threat to marine life. Efforts to deal with a growing number of oil spills and intentional oil discharges at sea continued during the 1950s and 1960s. The wreck of the *Torrey Canyon* off the coast of England in 1967 produced worldwide concern about the consequences of massive oil spills in the marine environment. Research on the environmental fate and biological effects of spilled petroleum increased dramatically during the 1970s (Albers, 2003). The *Exxon Valdez* oil spill in Prince William Sound, Alaska, in 1989, and the massive releases of crude oil into the Arabian Gulf during the 1991 Gulf War again captured international attention and resulted in an increase in environmental research. Despite considerable progress in developing methods to clean up spills, the adoption of numerous national and international controls on shipping practices, and high public concern (e.g., passage of the Oil Pollution Act of 1990 [33 USCA Sec. 2701-2761] in the United States), petroleum continues to be a widespread environmental hazard.

Known reserves of petroleum are typically estimated at around 1.2 trillion barrels with at least one estimate as high as 3.74 trillion barrels. Consumption is currently around 84 million barrels per day, or 31 billion barrels per year. At current consumption levels, current known reserves would be gone in about 32 years, around 2039 (Albers, 2003).

There are multiple sources of petroleum that can contribute to the Unresolved Complex Mixture (UCM) in the extracts of environmental samples. These include acute and chronic oil spills, urban runoff, atmospheric decomposition and waste treatment plants. The National Academy of Sciences estimated that U.S. coastal regions annually receive 2,7 tonnes of fresh or weathered petroleum hydrocarbons including crude oil and refined products such as kerosene, home heating oil, diesel fuel, industrial heating fuel, bunker oil and motor lubricating oils. UCM may contain a full array of alkanes, branched alkanes, cycloalkanes, mono aromatics, multi ring aromatics, heteroatomic aromatics, steranes and cyclic triterperoids that are part of crude or refined petroleum. Second evaporation, dissolution and chemical and biological oxidation weather the petroleum. Evaporation removes the most volatile compounds, dissolution removes the more polar and water soluble compounds and biodegradation generally attacks the linear alkanes, branched alkanes and then the cycloalkanes and aromatics (Frysinger et al., 2003).

Historically, physical properties such as boiling point, density, odor, and viscosity have been used to describe oils. Petroleum may be called *light* or *heavy* in reference to the amount of low-boiling constituents and the relative density (specific gravity).

Likewise, odor is used to distinguish between *sweet* (low sulfur) and *sour* (high sulfur) crude oil (Speight, 2002). Density is the property used by the petroleum industry to define light or heavy crude oils (Fingas, 2000).

In general, motor oil, is an oil suitable for use in an engine crankcase. This term also can sometimes apply to oils used to lubricate electric motors. Motor oil is one of the hundreds of different lubricant products that are made by many refineries and compounders. Motor oil is used to lubricate the parts of an automobile engine, in order to keep everything moving smoothly. Crankcase oil consists of virgin lubricating base oil and additives; it is used in the crankcase as a hydrodynamic lubricant to reduce friction, as a coolant, and to form a compression seal. Before they are used, crankcase oils consist of a base lubricating oil (a complex mixture of hydrocarbons, 80 to 90% by volume) and performance enhancing additives (10 to 20% by volume). Most engine oils are petroleum based materials comprised primarily of saturated hydrocarbons. The most important characteristic of a lubricating oil for automotive use is its viscosity. For example, the common SAE viscosity number "10W30" used in most automobiles corresponds to specific ranges of viscosity at 0 degrees F and 210 degrees F. According to the US Coast Guard Emergency Response Notification System (ERNS), unused motor oil is one of the most commonly spilled petroleum products the U.S.. Private vehicles use approximately 75% of the crankcase oils sold for gasoline engines, and light commercial vehicles that run on gasoline or propane use the remaining 25%. Heavy, commercial on-road vehicles use 70% of the crankcase oils sold for diesel engines, and industrial off-road vehicles use the remaining 30%. About half of the volume of crankcase oil used in the automotive sector is burned and released from exhaust during engine use and lost from crankcase leaks into the environment (Irwin, 1997).
Waste crankcase oil (WCO) is defined as used lubricating oils removed from the crankcase of internal combustion engines. Before they are used, crankcase oils consist of a base lubricating oil (a complex mixture of hydrocarbons, 80 to 90% by volume) and performance enhancing additives (10 to 20% by volume). Crankcase oils are altered during use because of the breakdown of additives, contamination with the products of combustion, and the addition of metals from the wear and tear of the engine. Therefore, the composition of waste oil is difficult to generalize in exact chemical terms. It is recognized that the major components consist of aliphatic

and aromatic hydrocarbons (such as phenol, naphthalene, benz(a)anthracene, benzo(a)pyrene, and fluoranthene). Used motor oil is generally similar to new motor oil except for the addition of additional:

- Metals including zinc, magnesium, barium, lead, aluminum, chromium, copper, iron, manganese, nickel, silicon, and tin.
- Water, gasoline, antifreeze, and thermal or breakdown products of various hydrocarbons.

According the US Coast Guard Emergency Response Notification System (ERNS), used motor oil is one of the most commonly spilled petroleum products the U.S. (Irwin, 1997).

No. 6 fuel oil is a dense, viscous oil produced by blending heavy residual oils with a lighter oil (often No. 2 fuel oil) to meet specifications for viscosity and pour point. When spilled on water, No. 6 fuel usually spreads into thick, dark colored slicks, which can contain large amounts of oil (Irwin, 1997).

2.1.1 The composition of oil

Petroleum consists of crude oils and a wide variety of refined oil products. Crude oils vary in chemical composition, color, viscosity, specific gravity, and other physical properties. Color ranges from light yellow-brown to black. Viscosity varies from free flowing to a substance that will barely pour. Specific gravity of most crude oils varies between 0.73 and 0.95 (Albers, 2003). Crude oils are mixtures of hydrocarbon compounds ranging from smaller, volatile compounds to very large, non-volatile compounds. This mixture of compounds varies according to the geological formation of the area in which the oil is found and strongly influences the properties of the oil. Likewise, crude oils that consist primarily of large compounds are viscous and dense. On the other hand petroleum products such as gasoline or diesel fuel are mixtures of fewer compounds and thus their properties are more specific and less variable (Fingas, 2000).

Hydrocarbon compounds are composed of hydrogen and carbon, which are therefore the main elements in oils. Oils also contain varying amounts of sulfur, nitrogen, oxygen, and sometimes mineral salts, as well as trace metals such as nickel, vanadium, and chromium. In general, the hydrocarbons found in oils are characterized by their structure. The hydrocarbon structures found in oil are the saturates, olefins, aromatics, and polar compounds. The saturate group of components in oils consists primarily of alkanes, which are compounds of hydrogen and carbon with the maximum number of hydrogen atoms around each carbon. Thus, the term “saturate” is used because the carbons are saturated with hydrogen. The saturate group also includes cyclo-alkanes, which are compounds made up of the same carbon and hydrogen constituents, but with the carbon atoms bonded to each other in rings or circles. Larger saturate compounds are often referred to as waxes (Fingas, 2000).

The olefins, or unsaturated compounds, are another group of compounds that contain fewer hydrogen atoms than the maximum possible. Olefins have at least one double carbon-to-carbon bond that displaces two hydrogen atoms. Significant amounts of olefins are found only in refined products. The aromatic compounds include at least one benzene ring of six carbons. Three double carbon-to-carbon bonds float around the ring and add stability. Because of this stability, benzene rings are very persistent and can have toxic effects on the environment. The most common smaller and more volatile compounds found in oil are often referred to as BTEX, or benzene, toluene, ethyl-benzene, and xylenes. Polyaromatic hydrocarbons, or PAHs, are compounds consisting of at least two benzene rings. PAHs make up between 0 and 60% of the composition of oil. Polar compounds are those that have a significant molecular charge as a result of bonding with compounds such as sulfur, nitrogen, or oxygen. The “polarity” or charge that the molecule carries results in behavior that, under some circumstances, is different from that of unpolarized compounds. In the petroleum industry, the smallest polar compounds are called “resins,” which are largely responsible for oil adhesion. The larger polar compounds are called “asphaltenes” because they often make up the largest percentage of the asphalt commonly used for road construction. The following are the oils to illustrate the fate, behavior, and cleanup of oil spills (Fingas, 2000) :

- Gasoline — as used in automobiles
- Diesel fuel — as used in trucks, trains, and buses
- Light crude oil — as produced in great abundance in western Canada or Louisiana

- Heavy crude oil — as imported to North America from Arabic countries or similar to that produced off the coasts of Newfoundland and California
- Intermediate fuel oil (IFO) — a mixture of a heavy residual oil and diesel fuel used primarily as a propulsion fuel for ships (the intermediate refers to the fact that the fuel is between a diesel and a heavy residual fuel)
- Bunker fuel — such as Bunker C which is a heavy residual fuel remaining after the production of gasoline and diesel fuel in refineries and often used in heating plants
- Crude oil emulsion — such as an emulsion of water in a medium crude oil

In a recent (Upshall et al, 1993) study, used motor oil had a density of 0.828 g/ml and contained 14% aromatics and 65.4% aliphatics (by weight). In this study, the sum of 26 individual PAHs represented 0.17% of the oil, or 1.2% of the aromatic fraction. Used motor oil typically has much higher concentrations of PAHs than unused motor oil. Combustion-derived PAHs have been linked to mutagenesis and carcinogenesis, and some heavy metals have a toxic effect. The 4- to 7-ring PAHs have been especially implicated in the carcinogenic effect of used oil.

Hydrocarbons from used motor oil can move to atmosphere or settle through water to bottom sediments, where they may persist for years. Metals from oil may build up in various media. The concentration of various PAHs is much higher in used oil than in (fresh) lubricating oil.

As an oil used in a crankcase, motor oil breaks down to give a wide variety of oxygenated and aromatic hydrocarbons. Other organic compounds found in waste oil include toluene, benzene, xylenes, and ethylbenzene. Also present are organic and inorganic compounds of chlorine, sulfur, phosphorus, bromine, nitrogen, and metals such as zinc, magnesium, barium, and lead resulting from oil additives and contamination during use or disposal.

Used engine oil is a contaminant of concern, with large volumes entering aquatic ecosystems through water runoff. The major source of petroleum contamination in urbanized estuaries comes from waste crankcase oil (Irwin, 1997).

- In rural areas a considerable portion of PAHs in streams comes from highways.
- Another source of UMOs entering the aquatic environment is through improper disposal of used motor oil.

2.1.2 Physical properties of oil

Crude oils vary widely in their physical and chemical properties because of their varying composition and constituents. As their composition varies, each type of oil or petroleum product has certain unique characteristics or properties. These properties influence how the oil behaves when it is spilled at sea and determine the effects of the oil on the environment. They also influence the efficiency of cleanup operations (Fingas, 2000). The main physical properties, which affect the behavior and the persistence of oil, are briefly explained below.

2.1.2.1. Specific gravity

Specific gravity is the density of a substance compared to the density of water. Since most oils have a specific gravity below 1 and are lighter than sea water which has a specific gravity of about 1.025, they float on top of it. Heavier oils, vegetable oils, and animal fats may sink and form tar balls or may interact with rocks or sediments on the bottom of the water body (USEPA, 1999b; ITOPF, 2002).

Oil spill specific gravities range from 0.75 to 1.03. The lower values represent highly refined products such as gasoline, kerosene, and diesel fuels. The upper values represent residual oils. Crude oils have specific gravities between 0.8 and 1.0; however, this increases rapidly when the light ends (fractions) evaporate (Liu, 1999).

The American Petroleum Institute gravity scale (°API) is commonly used to describe the specific gravity of crude oils and petroleum products, and is calculated as follows:

$$^{0}API = \frac{141.5}{specific\ gravity} - 131.5 \tag{2.1}$$

In addition to determining whether the oil will float, the specific gravity can also give a general indication of other properties of the oil. For example, oils with a low specific gravity (high °API) tend to contain a high proportion of volatile components and to be of low viscosity (ITOPF, 2002).

2.1.2.2. Solubility

Solubility in water is the measure of how much of an oil will dissolve in the water column on a molecular basis. Solubility is important in that the soluble fractions of the oil are sometimes toxic to aquatic life, especially at higher concentrations. As the amount of oil lost to solubility is always small, this is not as great a loss mechanism as evaporation (Fingas, 2000). However, this small fraction of the fuel oil soluble in water could cause long-term effects. This water-soluble fraction (WSF) has been observed to consist mainly of a variety of polyaromatic hydrocarbons (PAHs) and some quantities of aliphatic hydrocarbons (Navas et al., 2006).

2.1.2.3. Viscosity

The viscosity of a fluid is a measure of its resistance to flow and is expressed as Saybolt Universal Seconds (SUS), Saybolt Furol Seconds (SFS), or centistokes (cSt, kinematic viscosity) (Speight, 2002). The higher the viscosity of the oil, the greater the tendency for it to stay in one place. The viscosity of the oil is largely determined by the amount of lighter and heavier fractions that it contains. The greater the percentage of light components such as saturates and the lesser the amount of asphaltenes, the lower the viscosity (Fingas, 2000; USEPA, 1999b).

As with other physical properties, viscosity is affected by temperature, with a lower temperature giving a higher viscosity. For most oils, the viscosity varies as the logarithm of the temperature, which is a very significant variation (Fingas, 2000). Since sea temperatures are often lower than cargo or bunker temperatures on board a vessel, viscosity-dependent cleanup operations such as skimming and pumping generally become more difficult as the spilled oil cools (ITOPF, 2002).

2.1.2.4. Surface tension

The surface tension, sometimes called oil/water interfacial tension, is the force of attraction or repulsion between the surface molecules of oil and water. Together with viscosity, surface tension is an indication of how rapidly and to what extent an oil will spread on water. The lower the interfacial tension with water, the greater the extent of spreading (Fingas, 2000).

Because increased temperatures can reduce a liquid's surface tension, oil is more likely to spread in warmer waters than in very cold waters (USEPA, 1999b).

2.1.2.5. Vapor pressure

The vapor pressure of an oil is a measure of how the oil partitions between the liquid and gas phases, or how much vapor is in the space above a given amount of liquid oil at a fixed temperature. Because oils are a mixture of many compounds, the vapor pressure changes as the oil weathers. Vapor pressure is difficult to measure and is not frequently used to assess oil spills (Fingas, 2000). Vapor pressure controls evaporation rate and air concentrations of hydrocarbons and, therefore, the fire hazard in the vicinity of spills (NRC, 1985).

2.1.2.6. Flash point

The flash point of an oil is the temperature at which the liquid gives off sufficient vapors to ignite upon exposure to an open flame. The flash point is used primarily as an index of fire hazards (Speight, 2002). A liquid is considered flammable if its flash point is less than 60°C. There is a broad range of flash points for oils and petroleum products, many of which are considered flammable, especially when fresh. Gasoline, which is flammable under all ambient conditions, poses a serious hazard when spilled. Many fresh crude oils have an abundance of volatile components and may be flammable for as long as 1 day until the more volatile components have evaporated. On the other hand, Bunker C and heavy crude oils generally are not flammable when spilled (Fingas, 2000).

2.1.2.7. Pour point

Pour point is the temperature below which an oil will not flow. The pour point is a function of the wax and asphaltene content of the oil. As an oil cools, it will reach a temperature, the so-called 'cloud point', at which the wax components begin to form crystalline structures. This increasingly hinders flow of the oil until it eventually changes from liquid to semi-solid at the pour point (ITOPF, 2002).

The pour point is the lowest temperature at which the crude oil will flow under specified conditions. The maximum and minimum pour point temperatures provide a temperature window where a petroleum product, depending on its thermal history, might appear in the liquid as well as the solid state. (Speight, 2002).

The pour point represents a consistent temperature at which an oil will pour very slowly and therefore has limited use as an indicator of the state of the oil (Fingas, 2000).

2.1.2.8. Distillation fractions

Distillation characteristics of oil describe its volatility. As the temperature of an oil is raised, different components reach their boiling point one after another and evaporate. The distillation characteristics are expressed as the proportions of the parent oil, which distil within given temperature ranges. Some oils contain bituminous, waxy or asphaltenic residues which do not readily distil, even at high temperatures. These are likely to persist for extended periods in the environment (ITOPF, 2002). This data also provides useful insights into the chemical composition of oils. For example, while 70% of gasoline will boil off at 100°C, only about 5% of a crude oil will boil off at that temperature and an even smaller amount of a typical Bunker C. The distillation fractions correlate strongly to the composition as well as to other physical properties of the oil (Fingas, 2000).

2.1.3. Natural attenuation

Natural attenuation processes include a variety of physical, chemical, or biological processes that, under favorable conditions, act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in the receiving media. These processes include weathering processes like biodegradation; dispersion; dilution; sorption; volatilization; and chemical or biological stabilization, transformation, or destruction of contaminants (USEPA, 1999a). Weathering processes contributing to natural attenuation are described in the following section.

Natural attenuation (no treatment – recovery without intervention) is a suitable spill response option where active cleanup techniques would cause more damage than leaving the environment to recover naturally, when response techniques would not accelerate natural recovery, or when safety considerations place response personnel at risk. Figure 2.1 shows the relevant processes contributing towards natural attenuation of petroleum hydrocarbons in receiving media (USEPA, 1999a).

Natural attenuation has become a preferred response option for use on shoreline sites of limited public use that are contaminated with low concentrations of nonpersistent oil (Lee et al., 2003). Natural weathering processes of evaporation, dissolution and biodegradation can account for up to 30% removal of spilled petroleum in the first 72 h after release (Page et al., 2002).

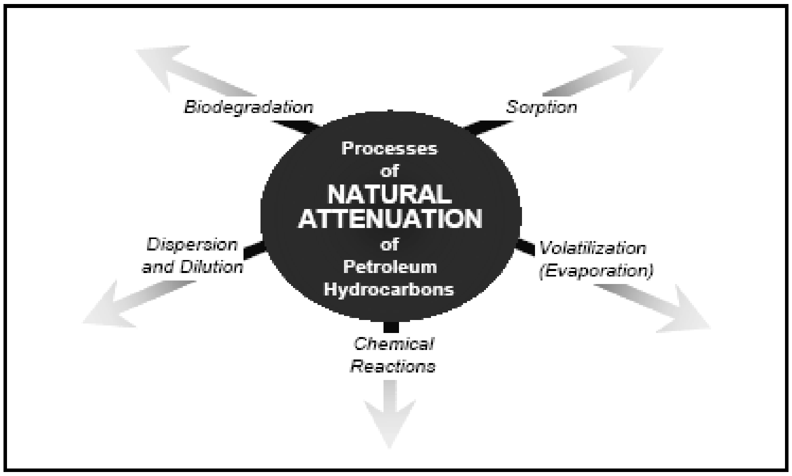


Figure 2.1: Processes of natural attenuation of petroleum hydrocarbons (USEPA, 1999a)

2.1.4. Weathering processes contributing to natural attenuation

Petroleum introduced to the marine environment goes through a variety of physical, chemical, and biological transformations during its transport by the advective and spreading processes. These physical, chemical, and biological processes leading to the dispersion and final removal of oil in the environment are collectively termed as weathering processes (French-McCay, 2004).

A detailed understanding of weathering processes is required to assess exposure, to estimate environmental damage, and to develop effective restoration strategies. Figure 2.2 shows the weathering processes affecting fuel oil spills also indicating the time scale. Figure 2.3 shows the chemical and biological fate of oil spills both on land and marine environment. Spreading, evaporation, dispersion, emulsification and dissolution are most important during the early stages of a spill whilst oxidation, sedimentation and biodegradation are longer-term processes, which determine the ultimate fate of oil. An understanding of the way in which weathering processes interact is important when attempting to forecast the changing characteristics of an oil during the lifetime of a slick at sea.

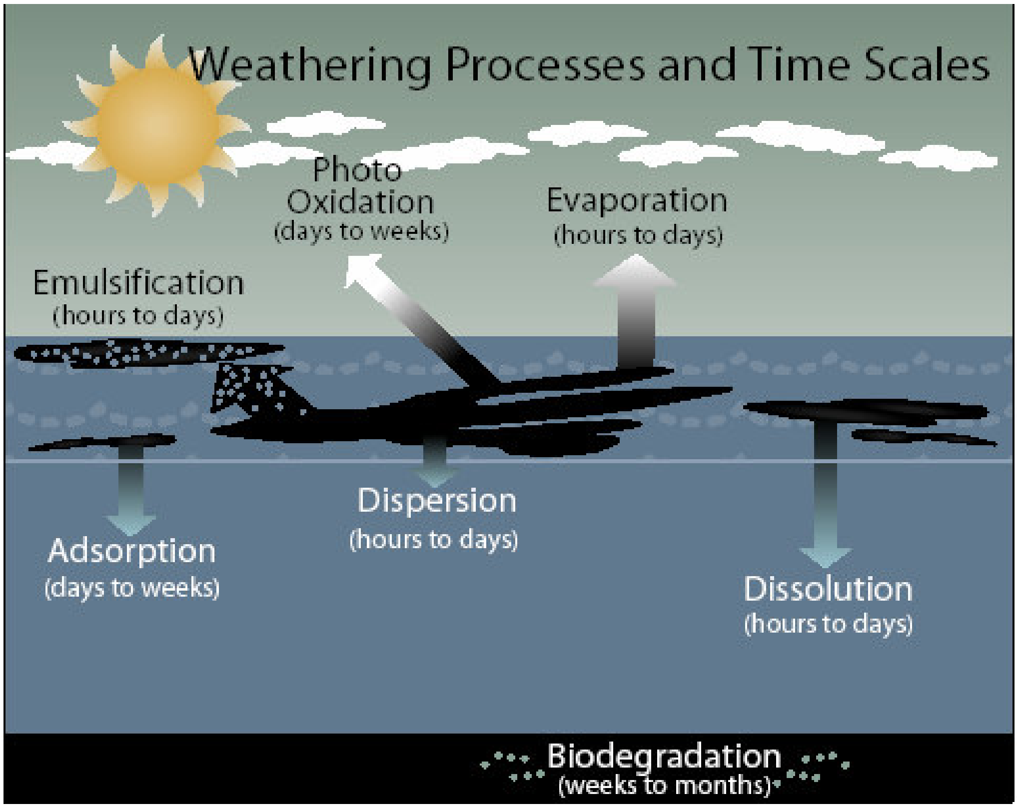


Figure 2.2: Processes affecting fuel oil spills (NOAA, 2002).

Spreading and dissolution occurs immediately after the spill, and the rate of dissolution decreases rapidly after the spill as soluble substances are quickly depleted. Some of the soluble compounds also evaporate rapidly (Fingas, 2000). Additionally, photochemical oxidation of some of the components of petroleum can be induced by sunlight. Dark or auto oxidation may also occur. The products of these processes include hydrocarbon fractions and reaction products introduced to the atmosphere, slicks and tar lumps on the surface of the ocean, dissolved and particulate hydrocarbon materials in the water column, and similar components in the sediments.

While physical and chemical processes are occurring, biological processes also act on the different fractions of the original petroleum in various ways. The biological processes considered include degradation of petroleum by microorganisms to carbon dioxide or organic components in intermediate oxidation stages, uptake by larger organisms and subsequent metabolism, storage, or discharge (NRC, 1985).

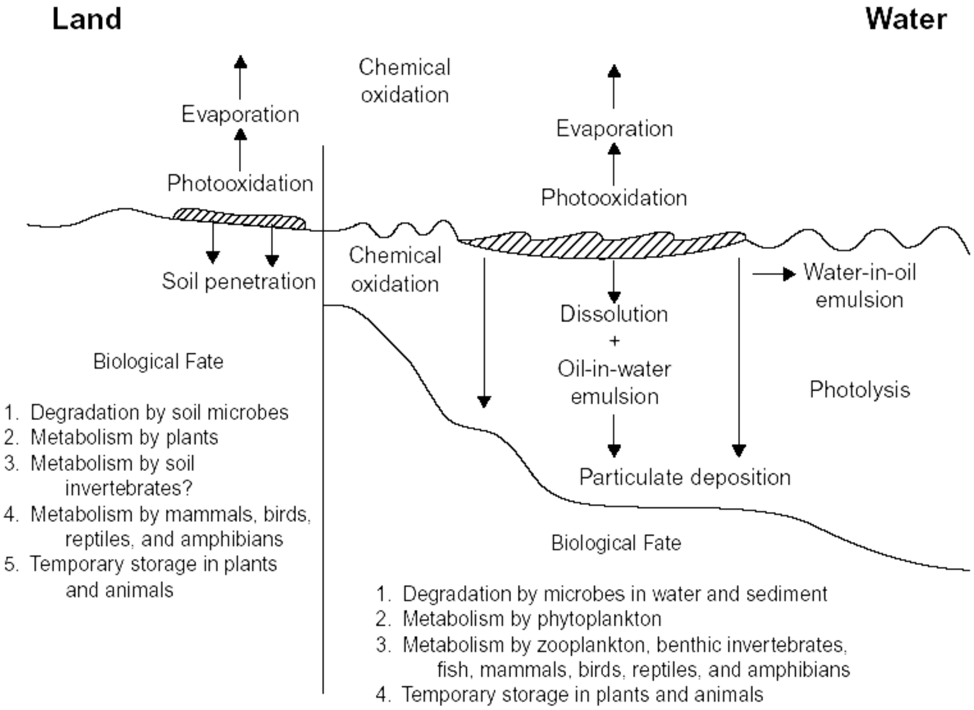


Figure 2.3: Chemical and biological fate of oil spills both on land and marine environment (Albers, 2003).

2.1.4.1. Dispersion

Natural dispersion occurs when fine droplets of oil are transferred into the water column by wave action or turbulence. Small oil droplets (less than 20 µm or 0.020 mm) are relatively stable in water and will remain so for long periods of time. Large droplets tend to rise and larger droplets (more than 100 µm) will not stay in the water column for more than a few seconds. Natural dispersion is dependent on both the oil properties and the amount of sea energy. Heavy oils such as Bunker C or a heavy crude will not disperse naturally to any significant extent, whereas light crudes and diesel fuel can disperse significantly if the saturate content is high and the asphaltene and resin contents are low (Fingas, 2000).

2.1.4.2. Dissolution

Through the process of dissolution, some of the most soluble components of the oil are lost to the water under the slick. These include some of the lower molecular weight aromatics and some of the polar compounds, broadly categorized as resins.

The significance of dissolution is that the soluble aromatic compounds are particularly toxic to fish and other aquatic life. If a spill of oil containing a large amount of soluble aromatic components occurs in shallow water and creates a high localized concentration of compounds, then significant numbers of aquatic organisms can be killed (Fingas, 2000).

2.1.4.3. Evaporation

Evaporation has the greatest effect on the amount of oil remaining on water or land after a spill. Over a period of several days, a light fuel such as gasoline evaporates completely at temperatures above freezing, whereas only a small percentage of a heavier Bunker C oil evaporates. The rate at which an oil evaporates depends primarily on the oil's volatility. About 80% of evaporation occurs in the first few days after a spill (Fingas, 2000).

2.1.4.4. Spreading

After an oil spill on water, the oil tends to spread into a slick over the water surface. This is especially true of the lighter products such as gasoline, diesel fuel, and light crude oils, which form very thin slicks. Heavier crudes and Bunker C spread to slicks several millimeters thick. Heavy oils may also form tar balls and tar mats and thus may not go through progressive stages of thinning. Oil spreads horizontally over the water surface even in the complete absence of wind and water currents. This spreading is caused by the force of gravity and the interfacial tension between oil and water. The viscosity of the oil opposes these forces. As time passes, the effect of gravity on the oil diminishes, but the force of the interfacial tension continues to spread the oil. The transition between these forces takes place in the first few hours after the spill occurs (Fingas, 2000).

2.1.4.5. Emulsification

Emulsification is the process by which one liquid is dispersed into another one in the form of small droplets. The mechanism of emulsion formation is not yet fully understood, but it probably starts with sea energy forcing the entry of small water droplets, about 10 to 25 µm in size, into the oil. If the oil is only slightly viscous, these small droplets will not leave the oil quickly. On the other hand, if the oil is too viscous, droplets will not enter the oil to any significant extent. The conditions required for emulsions of any stability to form may only be reached after a period of evaporation. Evaporation lowers the amount of low-molecular weight aromatics and increases the viscosity to the critical value. The formation of emulsions is an important event in an oil spill. First, and most importantly, it substantially increases the actual volume of the spill. Emulsions of all types contain about 70% water and thus, when emulsions are formed, the volume of the oil spill more than triples (Fingas, 2000).

A crude spill can become a 40% water emulsion in a single day due to open sea action. In 5 days, this can increase to 80%. Increased shearing rates and action decreases water droplet size and increases emulsion stability. Pumping emulsions with free water may result in up to 98% water in the oil emulsions, which are so formed (Liu, 1999).

2.1.4.6. Oxidation

Oxidation occurs when oil contacts the water and oxygen combines with the oil hydrocarbons to produce water-soluble compounds. This process affects oil slicks mostly around their edges. Thick slicks may only partially oxidize, forming tar balls (USEPA, 1999a).

Photooxidation can change the composition of an oil. It occurs when the sun's action on an oil slick causes oxygen and carbons to combine and form new products that may be resins. The resins may be somewhat soluble and dissolve into the water or they may cause water-in-oil emulsions to form. It is not well understood how photooxidation specifically affects oils, although certain oils are susceptible to the process while others are not. For most oils, photooxidation is not an important process in terms of changing their fate or mass balance after a spill (Fingas, 2000).

2.1.4.7. Biodegradation

A large number of microorganisms are capable of degrading petroleum hydrocarbons. Many species of bacteria, fungi, and yeasts metabolize petroleum hydrocarbons as a food energy source. Hydrocarbons metabolized by microorganisms are generally converted to an oxidized compound. The aquatic toxicity of the biodegradation products is sometimes greater than that of the parent compounds. The rate of biodegradation is greatest on saturates, particularly those containing approximately 12 to 20 carbons. Aromatics and asphaltenes, which have a high molecular weight, biodegrade very slowly, if at all. The rate of biodegradation is also highly dependent on the availability of oxygen. It is estimated that it would take all the dissolved oxygen in approximately 400,000 L of sea water to completely degrade 1 L of oil. The rate of degradation also depends on the availability of nutrients such as nitrogen and phosphorus (Fingas, 2000).

Biodegradation of an oil spill in an aquatic environment, which is essentially a massive heterotrophic event with a rapid increase of oil-degrading bacteria and a concomitant demand for oxygen in the water column, cascades into a potentially lethal anoxic environment for fish and invertebrates (Johnson and Romanenko, 1998).

2.1.4.8. Sedimentation

Sedimentation is the process by which oil is deposited on the bottom of the sea or other water body. Sedimentation occurred when oil droplets reached a higher density than water after interacting with mineral matter in the water column. When oil is on the bottom, it is usually covered by other sediment and degrades very slowly. Oil slicks and oil on shorelines sometimes interact with mineral fines suspended in the water column and the oil is thereby transferred to the water column. Particles of mineral with oil attached may be heavier than water and sink to the bottom as sediment or the oil may detach and re-float (Fingas, 2000).

2.2. Oil Spills

Petroleum products and oils that enter the marine environment have distinct effects, according to their composition and concentration. Oil pollution modifies the environmental conditions and can be translated into: transformations of the chemical composition of the environment and alterations in its physical properties; destruction of the marine biomass nutritional capital; danger to human health; and changes in the environmental biological equilibrium (Pinho et al, 2004). The most viscous no. 6 oils will often breakup into discrete patches and tarballs when spilled instead of forming slicks. Oil recovery by skimmers and vacuum pumps can be very effective, early in the spill. Very little of this viscous oil is likely to disperse into the water column.

In aquatic environment an oil spill immediately introduces three types of stress factors – physical, physiological, and toxicological – that may impact the health, vigor and general well being of the ecosystem (Alexander, 1999).

Bunker C Fuel Oil is a persistent oil; only 5-10% is expected to evaporate within the first hours of a spill. Consequently, the oil can be carried hundreds of miles in the form of scattered tarballs by winds and currents. The tarballs will vary in diameter from several meters to a few centimeters and may be very difficult to detect visually or with remote sensing techniques. Thus, spilled oil can float, suspend in the water column, or sink. Small changes in water density may dictate whether the oil will sink or float. Floating oil in a high sediment environment (rivers, beaches) could potentially sink once it picks up sediment, resulting in subsurface tarballs or tarmats. These oils can occasionally form an emulsion, but usually only slowly and after a period of days. Because of its high viscosity, beached oil tends to remain on the surface rather than penetrate sediments. Light accumulations usually form a "bathtub ring" at the high-tide line; heavy accumulations can pool on the beach. Shoreline cleanup can be very effective, before the oil weathers, becoming stickier and even more viscous. Natural degradation rates for these heavy oils are very slow (Irwin, 1997). Recovery of the environment from an oil spill can take many years, so there is considerable incentive to quickly clean up these areas after a spill, but the efforts can be expensive and themselves destructive (Page et al., 2002).

2.2.1. Oil spill statistics

The movement of vast quantities of petroleum products over great distances is usually over water. Mishandling and accidents in the transport of such large quantities are inevitable (API, 2000). The sources of oil spills worldwide are shown in Figure 2.4. Half of the oil spilled in the seas is the runoff of oil and fuel from land-based sources, usually from wastewater. Much lubricating oil finds its way into wastewater, which is often discharged directly into the sea. About 24% of oil spilled into the sea comes from the transportation sector, which includes tankers, freighters, barges, and other vessels. Natural sources of oil constitute about 11% of the input. Natural sources include the many natural "seeps" or discharges from oil-bearing strata on the ocean floor that reach the surface. Atmospheric sources constitute 13%

of oil pollution in the sea. This pollution is the result of hydrocarbons in the air from a variety of sources and causes such as inefficient combustion. Much of the material is re-precipitated onto land and subsequently ends up in the sea.

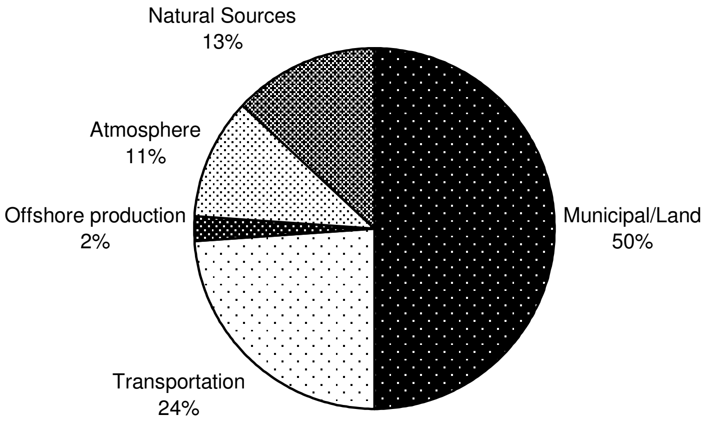


Figure 2.4: Sources of spilled oils into seas (Fingas, 2000).

As another fact, most oils spilled into the sea worldwide are fuels (48%) and then crude oils (29%). Fuels consist primarily of Bunker oils and intermediate fuel oils (IFO) which consist of Bunker oils mixed with fuels such as diesel. Grounding is the leading cause of oil spills from vessels (26%), followed by collision at 22%. Some other accidental causes of oil spills are explosion / fire (9%), ramming (9%), and sinking (7%), with human error (5%) and mechanical failure (2%) causing the least number of spills (Fingas, 2000).

Ships today are better equipped and built than ever before, with all new-build large tankers having double hulls. Ship-source pollution, which averaged over 400,000 tons a year at its peak in the late 1970s, averaged 27,000 tonnes per year from 2000-2005, a fall of 93% (ITOPF, 2006).

A brief list of the largest 20 oil spills caused by tankers in the past 40 years given in the Table 2.1. The most catastrophic oil spill was in 1979 which caused 287.000 tonnes of oil spill to marine environment from the ship Atlantic Empress. On the other hand according to the statistics the Gulf War Oil Spill which was in 1991, caused 780.000 – 1.500.000 tonnes of crude oil to spill the Persian Gulf. In addition Ixtoc I Oil Well caused 454.000 – 480.000 tonnes of crude oil spill to the Gulf of Mexico from 1979 to 1980.

Table 2.1: Major Oil Spills since 1967 (ITOPF, 2005)

Position	Shipname	Spill Size (tonnes)	Location	Year
1	Atlantic Empress	287,000	Off Tobago, West Indies	1979
2	ABT Summer	260,000	700 nautical miles off Angola	1991
3	Castillo de Bellver	252,000	Off Saldanha Bay, South Africa	1983
4	Amoco Cadiz	223,000	Off Brittany, France	1978
5	Haven	144,000	Genoa, Italy	1991
6	Odyssey	132,000	700 nautical miles off Nova Scotia, Canada	1988
7	Torrey Canyon	119,000	Scilly Isles, UK	1967
8	Sea Star	115,000	Gulf of Oman	1972
9	Irenes Serenade	100,000	Navarino Bay, Greece	1980
10	Urquiola	100,000	La Coruna, Spain	1976
11	Hawaiian Patriot	95,000	300 nautical miles off Honolulu	1977
12	Independenta	95,000	Bosphorus, Turkey	1979
13	Jakob Maersk	88,000	Oporto, Portugal	1975
14	Braer	85,000	Shetland Islands, UK	1993
15	Khark 5	80,000	120 nautical miles off Atlantic coast of Morocco	1989
16	Aegean Sea	74,000	La Coruna, Spain	1992
17	Sea Empress	72,000	Milford Haven, UK	1996
18	Katina P	72,000	Off Maputo, Mozambique	1992
19	Nova	70,000	Off Kharg Island, Gulf of Iran	1985
20	Prestige	63,000	Off the Spanish coast	2002

2.2.2. Oil spill cleanup

Oil spilled on water is seldom completely contained and recovered and some of it eventually reaches the shoreline. It is more difficult and time-consuming to clean up shoreline areas than it is to carry out containment and recovery operations at sea.

Physically removing oil from some types of shoreline can also result in more ecological and physical damage than if oil removal is left to natural processes.

Factors that affect the type of cleanup method used include the type of oil spilled, the geology of the shoreline and rate of water flow, and the type and sensitivity of biological communities in the area. Natural processes, such as evaporation, oxidation, and biodegradation, help to clean the shoreline.

Because natural removal processes are often too slow to prevent an oil slick from reaching the shoreline, active measures to remove the slick from the water may be required. These processes include mechanically removing the oil from the open water to prevent oil from reaching shorelines and adding materials to the slick to enhance natural removal processes (Reis, 1996).

Damage to spill-contaminated shorelines and dangers to other threatened areas can be reduced by timely and proper use of containment and recovery equipment. Different individual methods or a combination of them may be applied for cleanup purposes depending upon the factors discussed above. Methods applied may include (USEPA, 1999b):

- Mechanical Containment
    - Booms
    - Skimmers
    - Sorbents
- Chemical and Biological Treatment
    - Dispersing agents
    - Biological agents
- In-situ Burning

Containment of an oil spill refers to the process of confining the oil, either to prevent it from spreading to a particular area, to divert it to another area where it can be recovered or treated, or to concentrate the oil so it can be recovered, burned, or otherwise treated. Booms are the basic and most frequently used piece of equipment for containing an oil spill on water. A boom is a floating mechanical barrier designed to stop or divert the movement of oil on water. Booms resemble a vertical curtain with portions extending above and below the water line and are used to enclose oil and prevent it from spreading, to protect harbors, bays, and biologically sensitive areas, to divert oil to areas where it can be recovered or treated, and to concentrate oil and maintain an even thickness so that skimmers can be used or other cleanup techniques, such as in situ burning, can be applied. A boom's performance and its ability to contain oil are affected by water currents, waves, and winds. Either alone or in combination, these forces often lead to boom failure and loss of oil.

Skimmers are mechanical devices designed to remove oil from the water surface. The effectiveness of a skimmer is rated according to the amount of oil that it recovers, as well as the amount of water picked up with the oil. Removing water from the recovered oil can be as difficult as the initial recovery. Effectiveness depends on a variety of factors including the type of oil spilled, the properties of the oil such as viscosity, the thickness of the slick, sea conditions, wind speed, ambient temperature, and the presence of ice or debris.

Sorbents are used for final cleanup of a spill or in very small spills. They can be synthetic materials such as plastic, organic materials such as peat moss, or inorganic materials such as clay. Sorbents are materials that recover oil through either absorption or adsorption. They play an important role in oil spill cleanup and are used to clean up the final traces of oil spills on water or land. Treating the oil with specially prepared chemicals is another option for dealing with oil spills.

Dispersant is a common term used to label chemical spill-treating agents that promote the formation of small droplets of oil that "disperse" throughout the top layer of the water column. Dispersants contain surfactants, chemicals like those in soaps and detergents, which have molecules with both a water-soluble and oil-soluble component. The effectiveness of a dispersant is determined by measuring the amount of oil that it puts into the water column and comparing it to the amount of oil that remains on the water surface. Dispersants are designed to chemically disperse an oil slick so that the oil enters the water column, minimizing opportunities to strand on shorelines. As wave energy is added to chemically dispersed oil (CDO) at sea, the slick breaks up into small oil droplets that disperse into the water column. Rapid dispersal of an oil slick in open waters is almost always less costly from an economic standpoint than mechanical shoreline cleanup once the oil reaches land. Wave action can keep these small droplets suspended in the water column, as opposed to resurfacing or being deposited on sediments. Moreover dispersants may enhance oil bioavailability by creating more surface area in terms of multiple small oil droplets, allowing for increased biodegradation of the oil (Page et al., 2002).

Biodegradation agents are used primarily to accelerate the biodegradation of oil in the environment. They are used primarily on shorelines or land. They are not effective when used at sea because of the high degree of dilution and the rapid movement of oil.

In-situ burning is an oil spill cleanup technique that involves controlled burning of the oil at or near the spill site. The major advantage of this technique is its potential for removing large amounts of oil over an extensive area in less time than other techniques. The major disadvantage is concerns about toxic emissions from the large black smoke plume produced (Fingas, 2000).

2.2.3. Effects of oil spills

When an oil spill occurs, many factors determine whether that spill will cause heavy, long lasting biological damage; comparatively little or no damage; or some intermediate degree of damage. Factors affecting impact of oil (NRC, 1985):

- Oil type
- Oil dosage and impact area
- Geographic location
- Oceanographic conditions
- Meteorological conditions
- Season

Toxic effects are classified as chronic or acute, which refers to the rate of effect of toxin on an organism. Acute means toxic effects occur within a short period of exposure in relation to the life span of the organism. Chronic toxicity refers to long-term effects that are usually related to changes in such things as metabolism, growth, reproduction, or ability to survive (Fingas, 2000).

New motor oil contains a higher percentage of fresh and lighter (often more volatile and water soluble as well as lighter) hydrocarbons that would be more of a concern for short term (acute) toxicity to aquatic organisms, whereas used motor oil contains more metals and heavy polycyclic aromatic hydrocarbons (PAHs) (some partly the result of combustion processes) that would contribute to chronic (long-term) hazards including carcinogenicity. Although the concentration of several PAHs is much higher in used oil than in (fresh) lubricating oil, new lubricating oils do nevertheless contain many hazardous PAHs. Long-term potential hazards of the some of the lighter, more volatile and water soluble compounds (such as benzenes, toluene, xylenes) in new oils would include contamination of groundwater. Long-term water uses threatened by spills include potable (ground) water supply. Long-term effects are also a potential problem associated with PAHs, alkyl PAHs, and alkyl benzene (such as xylene) constituents of new oils. Although PAHs do not make up as large percentage of new oils by weight, there are some PAHs in new lubricating oils. Due to their relative persistence and potential for various chronic effects, PAHs, (and particularly the alkyl PAHs), can contribute to long term (chronic) hazards of new oils in contaminated soils, sediments, and groundwaters. New motor oil contains fresh and lighter hydrocarbons that would be more of a concern for short-term (acute) toxicity to aquatic organisms, whereas used motor oil contains more metals and heavy polycyclic aromatic hydrocarbons (PAHs) that would contribute to chronic (long-term) hazards including carcinogenicity. Metals of concern include lead; and often to a lesser degree, zinc, chromium, barium, and arsenic. Aromatics are considered to be the most acutely toxic component of petroleum products, and are also associated with chronic and carcinogenic effects. Aromatics are often distinguished by the number of rings they possess, which may range from one to five. Lighter, mono-aromatics (one ring) compounds include benzene, toluene, ethylbenzene, and xylenes. Waste crankcase oil contains several toxic components including up to 30% aromatic The Bunker C Fuel Oil may persist on beaches for months to years. Adverse effects of floating No. 6 fuel oil are related primarily to coating of wildlife dwelling on the water surface, smothering of intertidal organisms, and long-term sediment contamination. No. 6 fuel oil is not expected to be as acutely toxic to water column organisms as lighter oils, such as No. 2 fuel oil. Direct mortality rates can be high for seabirds, waterfowl, and fur-bearing marine mammals, especially where populations are concentrated in small areas, such as during bird migrations or marine mammal haulouts. On the other hand direct mortality rates are generally less for shorebirds because they rarely enter the water. Shorebirds, which feed in intertidal habitats where oil strands and persists, are at higher risk of sublethal effects from either contaminated or reduced population of prey (Irwin, 1997).

2.2.3.1. Effects on physical environment

A film of oil spread on the water surface, prevents air/sea exchanges necessary for marine biological cycles. Thus (IMO/UNEP, 1995);

- it diminishes the renewal of oxygen,
- it gets in the way of sunbeams, thus curbing the photosynthesis,
- it entails a rise in temperature and favors the proliferation of oxygen consuming microorganisms.

2.2.3.2. Effects on biological environment

Many freshwater biota respond to oil in a manner similar to their salt water counterparts.

While oil is less soluble in freshwater, this is largely offset by the fact that many freshwater bodies are much shallower than oceans. The effects of oil on the environment are varied and complex. Some appear immediately, others over a longer period. For the biological environment, toxicity caused by oil spills may be termed as the most important factor in the cause and effect relationship. In the case of crude oils, the most volatile fractions and the aromatic compounds are the most toxic (IMO/UNEP, 1995).

Fish: Oils may come into direct contact and contaminate their gills; the water column may contain toxic and volatile components of oil that may be absorbed by their eggs, larvae, and juvenile stages; and they may eat contaminated food. If chemicals such as dispersants are used to respond to a spill, there may be an increased potential for tainting of fish and shellfish by increasing the concentration of oil in the water column (EPA, 1999).

Mammals: The sensitivity of mammals to spilled oil is highly variable. The amount of damage appears to be most directly related to how important the fur and blubber are to staying warm, which is called thermoregulation (EPA, 1999).

Birds: Birds are the most visible biota affected by oil spills, especially in the aquatic environment. Oil contaminates feathers when the birds come into contact with slicks on water or shorelines. For sea birds, this is particularly dangerous because when their feathers are oily, their insulation and buoyancy properties are decreased. Once oiled, a bird rapidly loses its body heat, especially at sea and this may cause death.

Invertebrates: The invertebrates refers to the environment on the bottom of bodies of water and includes plankton, fish, and other species. Benthic species can be killed when large amounts of oil accumulate on the bottom sediments. This can occur as a result of sedimentation (EPA, 1999).

2.2.3.3. Effects on human health

Besides direct intoxication through inhaling or massive ingestion of petroleum products, the consumption of certain marine animals (fish, crustaceans, and shellfish) which have been in contact with oil can be dangerous for human health through cumulative effects.

However, most of the time, the harmful effects of pollution are felt indirectly through the economic and ecological impact (IMO/UNEP, 1995):

- Damage to biological resources: marine flora and fauna, consequently hindering certain maritime activities,
- Defacement of amenities and a blow to tourism,
- Lowering of the quality of sea water affecting its multiple beneficial usages.

3. TOXICOLOGY

3.1. General Information

The word toxicology comes from the Greek word for poison (toxicon) and scientific study (logos) and was coined in the 17th century. The early history of toxicology can be written as the history of pharmaceutics, but also the history of forensic medicine (Milles, 1999). Toxicology is the study of the adverse effects of chemicals on living systems, whether they are human, animal, plant or microbe. 'Adverse effect' can range from a life threatening injury to something that might be considered a minor annoyance. In the practice of toxicology it is often used to determine the route, concentration and migration of chemicals in water, soil and air, respectively (Schrager, 2007). Many chemical substances or mixtures exert a whole spectrum of activities, ranging from beneficial to neutral to lethal. Their effect depends not only on the quantity of the substance to which an organism is exposed, but also on the species and size of the organism, its nutritional status, the method of exposure, and a number of other related factors (Zakrzewski, 1991).

Toxicity is a relative property reflecting a chemical's potential to have a harmful effect on a living organism. It is a function of the concentration and composition/properties of the chemical to which the organism is exposed and the duration of exposure. Traditionally, toxicity data have been used in comparing chemical substances or the sensitivities of different species to the same substance. Information about the biological mechanism affected and the conditions under which the toxicant is harmful are also important for this comparison. Toxicity tests are therefore used to evaluate the adverse effects of a chemical on living organisms under standardized, reproducible conditions that permit comparison with other chemicals or species tested and comparison of similar data from different laboratories (Rand, 1995).

Toxicity of a substance can be affected by the pathway of exposure (is the toxin applied to the skin, ingested, inhaled, injected), the time of exposure (a brief encounter or long term), the number of exposures (a single dose or multiple doses over time), the physical form of the toxin (solid, liquid, gas), the genetic makeup of an individual, an individual's overall health, and many others.

3.1.1. Subdisciplines of toxicology

Toxicology has a broad scope. It deals with toxicity studies of chemicals used (1) in medicine for diagnostic, preventive, and therapeutic purposes, (2) in the food industry as direct and indirect additives, (3) in agriculture as pesticides, growth regulators, artificial pollinators, and animal feed additives, and (4) in the chemical industry as solvents, components, and intermediates of plastics and many other types of chemicals. It is also concerned with the health effects of metals, petroleum products, paper and pulp, toxic plants, and animal toxins. The public is exposed to a variety of toxicants. The sources of these substances, their transport, degradation, and bioconcentration in the environment, and their effects on humans are dealt with in environmental toxicology. (Lu and Kacew, 2002).

Environmental toxicology is defined as the study of the fate and effects of chemicals in the environment. Environmental toxicology is typically associated with the study of environmental chemicals of anthropogenic origin. Environmental toxicology can be divided into two subcategories: environmental health toxicology and ecotoxicology. Environmental health toxicology is the study of the adverse effects of environmental chemicals on human health, while ecotoxicology focuses upon the effects of environmental contaminants upon ecosystems and constituents thereof (fish, wildlife, etc.). Ecotoxicology involves the study of the adverse effects of toxicants on numerous of organisms that compose ecosystems. Further, comprehensive insight into the effects of chemicals in the environment requires assessments auxiliary to toxicology such as the fate of the chemical in the environment, and toxicant interactions with abiotic (nonliving) components of ecosystems (Hodgson, 2004).

Regulatory toxicology relies on risk assessment and experimental data to determine the risk and benefits, or the costs and benefits of exposure to certain chemicals, to determine whether such chemical will be allowed in the public sphere and to what extent its use and exposure will be regulated. Clinical toxicology involves the application of toxicological principles within a diagnostic setting, usually to determine whether a presenting adverse effect or disease or injury is due to some type of chemical exposure. Forensic toxicology focuses on medical-legal aspects of chemical exposure and toxic injury (Schrager, 2007).

Ecotoxicology and aquatic toxicology

The term ecotoxicology was first used by Truhaut in 1969 as a natural extension from toxicology, the science of the effects of poisons on individual organisms, to the ecological effects of pollutants. In the broadest sense, ecotoxicology has been described as toxicity testing on one or more components of any ecosystem. This definition can be further expanded as the science of predicting effects of potentially toxic agents on natural ecosystems and non-target species (Hoffman et al., 2003).

Ecotoxicology is the science of assessing the effects of toxic substances on ecosystems with the goal of protecting entire ecosystems and not merely isolated components (Hoffman et al., 2003).

Aquatic toxicology is the study of the effects of toxic agents on aquatic organisms. This broad definition includes the study of the toxic effects at the cellular, individual, population, and community levels. The field of aquatic toxicology has grown out of the disciplines of water pollution biology and limnology. Aquatic toxicology studies evolved from simple tests conducted over intervals as short as a few hours to standard acute lethality tests lasting 48 or 96 hours, depending on the species. Most early toxicity tests consisted of short term exposure of chemicals or effluents to a limited number of species. Acute toxicity tests were followed by the development of various short sublethal tests and tests with prolonged exposures such as partial life-cycle studies and full life cycle studies (Hoffman et al., 2003).

The vulnerability of the aquatic environment to chemical insult depends on several factors, including (1) physical and chemical properties of the chemical and its transformation products; (2) concentrations and total loading of the chemical entering the ecosystem; (3) duration and type of inputs (acute or chronic, intermittent spill or continuous discharge); (4) properties of the ecosystem that enable it to resist changes that could result from the presence of the chemical (e.g., pH buffering capacity of seawater dissolved organic matter concentrations) or return to its original state after the chemical is removed from the system (e.g., flushing of water from estuaries by tidal action); and (5) location of the ecosystem in relation to the release site of the chemical. Because aquatic ecosystems involve complex interactions of physical, chemical, and biological factors, it is difficult to understand the response of a system to a chemical unless the relationships among components of the system are well defined (Rand, 1995).

3.2. Toxic Effects

Toxic effects are greatly variable in nature, potency, target organ, and mechanism of action. A better understanding of their characteristics can improve assessment of the associated health hazards. It can also facilitate the development of rational preventive and therapeutic measures. All toxic effects result from biochemical interactions between the toxicants (and/or their metabolites) and certain structures of the organism. The structure may be non-specific, such as any tissue in direct contact with corrosive chemicals. More often, it is specific, involving a particular sub-cellular structure. A variety of structures may be affected (Lu and Kacew, 2002).

Some toxic effects are reversible and the others are irreversible. Effects may be reversible by normal repair mechanisms, such as by regeneration of damaged or lost tissue and recovery from narcosis. In many cases, effects are reversible only if the organism can escape the toxic medium and find a toxicant-free environment. Serious damage or injury to an organism may be irreversible and may eventually result in death (Rand, 1995).

Functional effects are in general reversible, whereas morphologic effects are not, and functional changes are generally detected earlier or in animals exposed to lower doses than those with morphologic changes. In addition, functional tests are valuable in following the progress of effects on target organs in long-term studies in animals and humans (Lu and Kacew, 2002).

Adverse or toxic effects can be produced in the laboratory or in the natural environment by acute or chronic exposure to chemicals or other potentially toxic agents. In acute exposure, organisms come in contact with the chemical delivered either in a single event or in multiple events that occur within a short period of time, generally hours to days. Acute exposures to chemicals that are rapidly absorbed generally produce immediate effects, but they may also produce delayed effects similar to those caused by chronic exposure. During chronic exposure, organisms are exposed to low concentrations of a chemical delivered either continuously or at some other periodic frequency over a long period of time (weeks, months, or years), measured in relation to the organism's life cycle. Chronic exposure to chemicals may induce rapid, immediate effects similar to acute effects, in addition to effects that develop slowly (Rand, 1995).

3.3. Dose – Response Relationship

The science of toxicology is based on the principle that there is a relationship between a toxic reaction (the response) and the amount of poison received (the dose). An important assumption in this relationship is that there is almost always a dose below which no response occurs or can be measured. A second assumption is that once a maximum response is reached any further increases in the dose will not result in any increased effect (EXTOXNET, 1993).

The relationship of dose to response can be illustrated as a graph called a dose-response curve. There are two types of dose-response curves: one that describes the graded responses of an *individual* to varying doses of the chemical and one that describes the distribution of responses to different doses in a *population* of individuals. The dose is represented on the x-axis. The response is represented on the y-axis. Enzyme inhibition, DNA damage, death, behavioral changes, and other responses can be described using this relationship (Landis and Yu, 1998).

Two parameters of this curve are used to describe it: (1) the concentration or dose that results in 50% of the measured effect and (2) the slope of the linear part of the curve that passes through the midpoint. Both parameters are necessary to describe accurately the relationship between chemical concentration and effect. The midpoint is commonly referred to as a LD_{50}, LC_{50}, EC_{50}, and IC_{50}. The definitions are relatively straightforward (Landis and Yu, 1998):

- **LD_{50}:** The dose that causes mortality in 50% of the organisms tested estimated by graphical or computational means.
- **LC_{50}:** The concentration that causes mortality in 50% of the organisms tested estimated by graphical or computational means.

- **EC_{50}:** The concentration that has an effect on 50% of the organisms tested estimated by graphical or computational means. Often this parameter is used for effects that are not death.
- **IC_{50}:** Inhibitory concentration that reduces the normal response of an organism by 50% estimated by graphical or computational means. Growth rates of algae, bacteria, and other organisms are often measured as an IC_{50}.

A typical dose-response curve is shown in Figure 3.1, in which the percentage of organisms or systems responding to a chemical is plotted against the dose. For most types of toxic responses, there is a dose, called a threshold, below which there are no adverse effects from exposure to the chemical. The NOEL (no observable effect level) is the highest dose or exposure level of a chemical that produces no noticeable toxic effect. Although this is generally accepted for most types of chemicals and toxic effects, for chemical carcinogens acting by a genotoxic mechanism the shape of the curve is controversial and for regulatory purposes, their effect is assumed to be a no-threshold phenomenon (Hodgson, 2004).

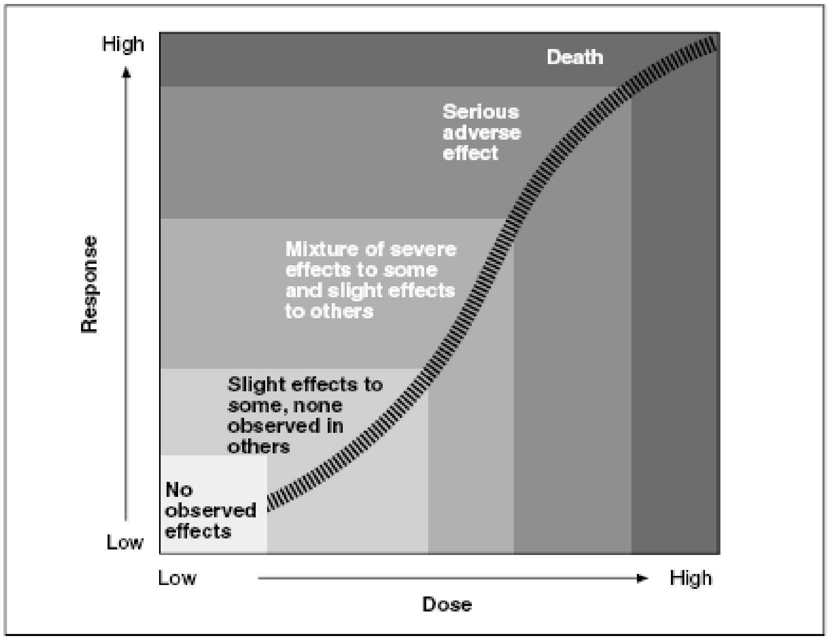


Figure 3.1: The dose response curve (Moore, 1999)

The various segments (Figure 3.1) of the curve are represented as follows (Hodgson, 2004):

Segment I. This portion of the line has no slope and is represented by those doses of the toxicant that elicited no mortality to the treated population of organisms.

Segment II. This segment represents those dosages of the toxicant that affected only the most susceptible members of the exposed population. Accordingly, these effects are elicited at low doses and only a small percentage of the dosed organisms are affected.

Segment III. This portion of the line encompasses those dosages at which most of the groups of organisms elicit some response to the toxicant. Because most of the groups of exposed organisms respond to the toxicant within this range of dosages, segment III exhibits the steepest slope among the segments.

Segment IV. This portion of the line encompasses those dosages of the toxicant that are toxic to even the most tolerant organisms in the populations. Accordingly, high dosages of the toxicant are required to affect these organisms.

Segment V. Segment V has no slope and represents those dosages at which 100% of the organisms exposed to the toxicant have been affected. A well-defined dose-response curve can then be used to calculate the LD_{50} for the toxicant.

3.4. Toxicity Tests

Microbial tests have been widely used in environmental toxicity screening procedures due to the similarity of complex biochemical functions in bacteria and higher organisms, ease of handling, short testing time and reproducibility among laboratories. In addition, the use of bioassays to evaluate toxic effects of complex mixtures of chemicals has the advantage that the influence of multiple factors such as pH, solubility, synergism/antagonism, and bioavailability are taken into account (Mowat, 2000).

An aquatic toxicity test is frequently called as a bioassay. A bioassay is performed to measure the degree of response produced by a specific level of chemical concentration. A biological assay (bioassay) is an experiment for estimating the nature, constitution, or potency of a material (or of a process), by means of the reaction that follows its application to living matter (Rand, 1995). Bioassays used in aquatic toxicology have taken a prominent position among analytical test for identifying and measuring environmental hazards. In particular, chronic toxicity tests have been developed for testing effluents, surface water, and sediment samples to estimate the safe or no effect sample concentration (Ostrander, 1996).

The fundamental principle upon which all toxicity tests are based is the recognition that the response of living organisms to the presence (exposure) of toxic agents is dependent upon the dose (exposure level) of the toxic agent. Aquatic toxicity tests are designed to describe a concentration-response relationship, referred to as the concentration-response curve when the measured effect plotted graphically with the concentration. Acute toxicity tests are usually designed to evaluate the concentration-response relationship for survival, whereas chronic studies evaluate sub-lethal effects such as growth, reproduction, behavior, tissue residues or chemical effects and are usually designed to provide an estimate of the concentration that produces no adverse effects (Hoffman et al., 2003).

During the last decade, significant effort has been expended in developing rapid toxicity assays. There has been an increasing need to assess toxicity of various sample types in minutes to hours instead of days. The use of assays (such as BioTox™ assay) can speed up the toxicity identification evaluation (TIE) process considerably (Hoffman et al., 2003).

Bioassays using luminescent bacteria are routinely used to assess the acute toxicity of environmental samples. Luminescent bacteria posses several attributes that support their practical use for toxicity testing. Their small cell size provides a high surface-to-volume ratio, which maximizes exposure potential. This structural characteristic plus (1) lack of membrane-aided compartmentalization; (2) location of most respiratory pathways (including enzymes required for bioluminescence) on or near the cell membrane; and (3) a metabolic rate 10 to 100 times mammalian cells, provide a dynamic metabolic system which can be easily quantities by measuring the rate of light output. The close association of the light production pathway with the bacteria's respiratory system provides a convenient and sensitive biological system for quantization a metabolic inhibition due to the presence of toxic chemicals (Ostrander,1996; Ren and Frymier, 2003).

Acute Toxicity Tests: These are tests designed to evaluate the relative toxicity of a chemical to selected aquatic organisms upon short-term exposure to various concentrations of test chemical. Common effect criteria for fish are mortality; for invertebrates, immobility and loss of equilibrium; and for algae, growth. These tests may be conducted for a predetermined length of (time-dependent test) to estimate the 24- or 96-h LC_{50} or the 48- or 96-h EC_{50}. An acute toxicity test may also have a duration that is not predetermined, in which case it is referred to as a *time-independent* (TI) test. In a TI test, exposure of the test organisms continues until the toxic response manifested has ceased or economic or other practical considerations dictate that the test be terminated. For example the acute TI test should be allowed to continue until acute toxicity (mortality or a defined sub-lethal effect) has ceased or nearly ceased and the toxicity curve indicates that a threshold or incipient effect concentration can be estimated (Rand, 1995). Bioassays using luminescent bacteria are routinely used to asses the acute toxicity of environmental samples. The fact that a chemical does not have adverse effects on aquatic organisms in acute toxicity tests does not necessarily indicate that it is not toxic to these species (Ostrander, 1996).

In the early development of acute toxicity tests, data were expressed as the *median tolerance limit* (TL_m or TL_{50}) the test material concentration at which 50% of the test organisms survive for a specified exposure time (usually 24-96 h). This term has been replaced by median lethal concentration (LC_{50}) and median effective concentration (EC_{50}) (Rand, 1995).

Chronic Toxicity Tests: Chronic tests are intended to predict adverse biological effects that result when organisms are exposed over their entire life cycle. Such tests are difficult and expensive to perform. Chronic toxicity tests permit evaluation of the possible adverse effects of the chemical under conditions of long-term exposure at sub-lethal concentrations. In a full chronic toxicity test, the test organism is exposed for an entire reproductive life cycle (e.g., egg to egg) to at least five concentrations of the test material. Partial life cycle (or partial chronic) toxicity tests involve only several sensitive life stages; these include reproduction and growth during the first year but do not include exposure of very early juvenile stages. In full chronic toxicity tests, exposure is generally initiated with an egg or zygote and continues through development and hatching of the embryo, growth and development of the young organism, attainment of sexual maturity, and reproduction to produce a second-generation organism. Tests may also begin with the exposed adult and continue through egg, fry, juvenile, and adult to fertilized eggs and criteria for effect include growth, reproduction, development of gametes, maturation, spawning, success, hatching success, survival of larvae or fry, growth and survival of different life stages, and behavior. The duration of a chronic toxicity test varies with the species tested; for instance, it is approximately 21 d for the water flea *Daphina magna* and can be 275-300 d for the fathead minnow, *Pimephales promels* (Rand, 1995).

From the data obtained in partial life cycle and complete life cycles test the *maximum acceptable toxicant concentration* (MATC) can be estimated. This is the estimated threshold concentration of a chemical within a range defined by highest concentration tested at which no significant deleterious effect was observed (NOEC) and the lowest concentration tested at which some significant deleterious effect was observed (LOEC). Because it is not possible to test an unlimited number of intermediate concentrations, an MATC is generally reported as being greater than the NOEC and less then the LOEC (NOEC < MATC < LOEC) (Rand, 1995).

Acute toxicity test results (LC_{50}) were used to predict chronic effect levels by means of an application factor (AF). The approach has generally been calculated as the $LC_{50} \div GMCV$, where GMCV is the geometric mean of the no observed effect concentration (NOEC) and the lowest observed effect level (LOEC), termed the chronic value (CV). The chronic value has also been alternatively referred to as the geometric mean maximum acceptable toxicant threshold (GM-MATC) (Hoffman et al., 2003).

Most studies on the effects of oil spills have been focused on short-term effects based on the monitoring of highly visible indicators, such as evidence of surface recolonization by macrophytes and/or bethnic invertebrate species. Some studies have suggested that residual oil within the sediments of intertidal reaches of salmon streams induced long-term chronic effects. These impacts were attributed to longer 3 and 4 ring PAHs that cause cellular and genetic effects rather than narcotic monoaromatic hydrocarbons that are typically responsible for acute mortalities. Total PAH concentrations in the water as low as 1 µg/L could impair the productivity of populations. In contrast, an 11 year monitoring program reported that spill residues on the oiled shorelines rapidly lost their toxicity through weathering. The heaviest of all fuel oils, Bunker C contains high concentrations of $n^{12}C$ through $n^{34}C$ alkanos and appreciable quantities of PAHs, with concentrations of 4 to 6 ring condensed aromatic hydrocarbons representing 5% or more of the total weight. Short and Heintz (1997) have also suggested that masses of persistent PAHs buried within sediments may be redistributed by physical disturbances into a film or droplets which can rapidly release dissolved, highly bioavailable PAHs. Based on a series of toxicity studies on weathered crude oils and a diesel fuel, Neff reported that PAHs contribute 58-94% of the hazardous potential (Lee et al., 2003).

Toxicity assessment is the determination of the potential of any substance to act as a poison, and characterization of its actions under the conditions which this potential will be realized.

4. MATERIALS AND METHODS

4.1. Experimental Approach

In this study three different oil samples used to project the oil pollution and tested for toxicity. These samples are Motor Oil (MO), Used Motor Oil (UMO) and Bunker C Fuel Oil (FO). In order to predict the toxicity of oils, because of the low solubility, oils have to transferred into liquid phase. For this purpose 3 different types of solvents are used. These are tetrahydrafuran (THF), n-Hexane and dimethylsulfoxide (DMSO). Because of the low solubility of n-hexane the tests conducted with 2 solvents which are tetrahydrafuran (THF) and dimethylsulfoxide (DMSO).

In this study in order to determine the toxicity of oil samples which are they first mixed with solvents which are tetrahydrafuran (THF) and dimethylsulfoxide (DMSO) and shaked prior to toxicity bioassay. UMO sample is taken from the Ataturk Autoindustrial Field which is located at Maslak. MO is a Castrol HD30 is taken from a gas station. At the second set of experiments, in order to simulate an oil spill reaching the shore and also an oil spill ended up in the bottom of the sea, oil samples are poured on sand and extracted by the help of solvents in a Zero Headspace Extractor (ZHE) than toxicities of these extracts tested. In this part of the experiments the behavior of oil on sand is projected and also solvents extraction potential is compared. The final set of experiments oil samples are poured onto Sea Water (SW) and also Distilled Water (DW) these mixtures are sonicated and shaked in orbital shaker and extracted according to the Toxicity Characteristic Leaching Procedure (TCLP) by Zero Headspace Extractor (ZHE) in order to determine the water soluble fraction of oils and their toxicities. The distilled water used in the experiments supplied from the laboratory and the sea water is taken from Ayvalık. Effective Concentrations (EC_{50}) of samples are determined by toxicity bioassay and converted to Toxic Unit (TU).

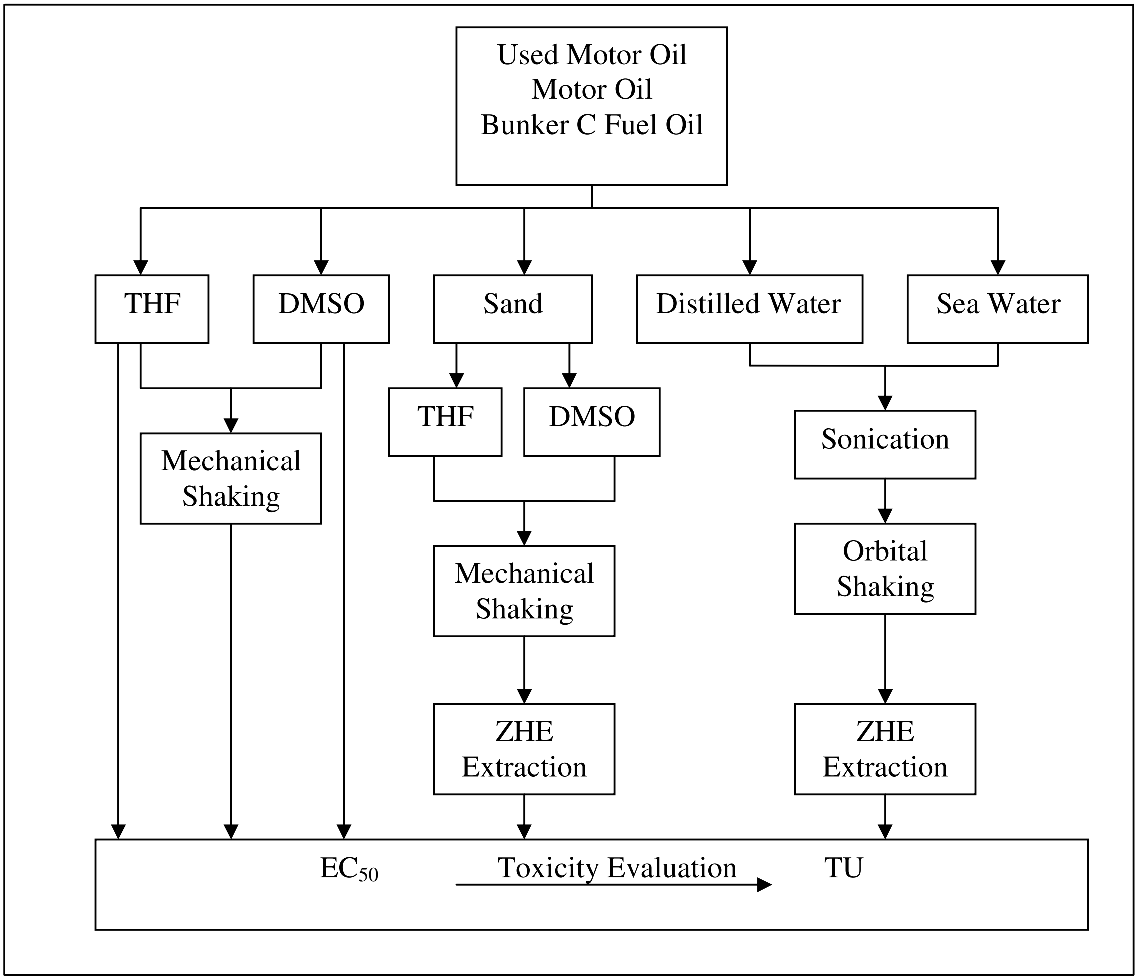


Figure 4.1: Experimental approach design

4.1.1. Sample preparation

As stated before, oils can not be tested individually in bioassays since the low solubility. Therefore oils have to be transferred into liquid phase by the help of solvents. For this purpose first 2 gr of sample oils mixed with 100 ml of DMSO and 1 gr of sample oils mixed with 50 ml of THF with the exception of FO which is mixed with 100 ml of THF. Also pure solvents were also prepared to determine the blank toxicity. The experimental framework is given in the Table 4.1.

Table 4.1 : Sample Preparation of oil-solvent mixtures for Experimental Framework

Sample Code	Amount of oil	Amount of solvent	Experimental Procedure
THF	-	10 ml	BioTox
THF MO	1 gr	50 ml	BioTox
THF UMO	1 gr	50 ml	BioTox
THF FO	1 gr	100 ml	BioTox
DMSO	-	10 ml	BioTox
DMSO MO	2 gr	100 ml	BioTox
DMSO UMO	2 gr	100 ml	BioTox
DMSO FO	2 gr	100 ml	BioTox

After preparing the solvent oil mixture samples, the next step is to prepare the oil sand mixture samples. The 50 gr of sand samples, which are collected from Florya, Istanbul are weighed and mixed with 0,5 gr of oil samples. Afterwards these mixtures are mixed with 50 ml of solvents and extracted according to the Toxicity Characteristic Leaching Procedure (TCLP) with a Zero Headspace Extractor (ZHE). Samples taken from this extracts are tested for toxicity. The experimental framework for oil-sand mixtures is summarized in the Table 4.2.

Table 4.2 : Sample Preparation of oil-sand mixtures for Experimental Framework

Sample Code	Amount of oil	Amount of sand	Amount of solvent	Experimental Procedure
THF MO SAND	0,5 gr	50 gr	50 ml	TCLP with ZHE, BioTox
THF UMO SAND	0,5 gr	50 gr	50 ml	TCLP with ZHE, BioTox
THF FO SAND	0,5 gr	50 gr	50 ml	TCLP with ZHE, BioTox
DMSO MO SAND	0,5 gr	50 gr	50 ml	TCLP with ZHE, BioTox
DMSO UMO SAND	0,5 gr	50 gr	50 ml	TCLP with ZHE, BioTox
DMSO FO SAND	0,5 gr	50 gr	50 ml	TCLP with ZHE, BioTox

The final samples which are used in this study are oil and water mixtures. 5 gr of oil samples are weighed and mixed with 100 ml of sea water and distilled water. The sea water is collected from Ayvalık and the distilled water is obtained from the laboratory. In order to increase the soluble fraction of oils these mixtures are sonicated for 10 minutes to disperse the oil samples, followed by 72 hours of orbital shaking in a closed bottle at 200 rpm. Before testing for toxicity these water-oil mixtures are extracted from the non-soluble solid part by the TCLP procedure with a zero headspace extractor. The extracts contain only the soluble part of the dissolved oil. The experimental framework for oil-water mixture samples are given in the Table 4.3.

Table 4.3 : Sample Preparation of oil-water mixtures for Experimental Framework

Sample Code	Amount of oil	Amount of water	Experimental Procedure
MO DW	5 gr	100 ml	Sonication, Orbital Shaking, TCLP with ZHE, BioTox
UMO DW	5 gr	100 ml	Sonication, Orbital Shaking, TCLP with ZHE, BioTox
FO DW	5 gr	100 ml	Sonication, Orbital Shaking, TCLP with ZHE, BioTox
MO SW	5 gr	100 ml	Sonication, Orbital Shaking, TCLP with ZHE, BioTox
UMO SW	5 gr	100 ml	Sonication, Orbital Shaking, TCLP with ZHE, BioTox
FO SW	5 gr	100 ml	Sonication, Orbital Shaking, TCLP with ZHE, BioTox

4.2. Methods

The standardized methods used in the experimental part of the study can be classified as extraction procedure method and toxicity procedure method. Biologically directed fractionation methods, often called toxicity identification evaluation (TIE) methods, are an effective means to identify the causes of chemical toxicity in environmental samples. Many common sediment contaminants are hydrophobic and present in interstitial water at very low concentrations, which limits the chemical mass that can be recovered via interstitial water. An alternative approach to the bioavailability issue is to use an extraction method that is based on solvent extraction rather than chemical activity. Dilution series can be prepared using with different volumes of extract, allowing the quantization of toxicity and an assessment of recovery. However the potential for artifactual toxicity should always be kept in mind, and procedural blanks should be included and tested for toxicity as part of any fractionation scheme. An alternative is to solvent extract sediments and than conduct toxicity tests on dilutions of the extract and/or fractions thereof. The relative toxicological potencies of extracted chemicals should be more or less conserved if the extract is diluted with water and tested for toxicity (Heinis et al., 2004). The aim of the extraction is to transfer products to be analyzed into a mobile gas, liquid or even supercritical phase. The goal of solvent extraction is to create conditions for a new equilibrium so that the bulk of the consistent under investigation transfers into the solvent phase. These methods are explained in this chapter.

4.2.1. Extraction Procedure Method

There are 2 set of samples which are oil-sand mixtures and oil-water mixtures were extracted from the solid part. Oil-sand samples and oil-water samples are extracted by both Millipore Zero Headspace Extractor (ZHE) according to Toxicity Characteristic Leaching Procedure (TCLP) given by USEPA and "Protocol for the Basic Test Using Organic Solvent Sample Solubilization" (USEPA, 1992; Azur Environmental, 1998; Johnson and Long, 1998; Lee et al., 2003).

The TCLP, or Toxicity Characteristic Leaching (not Leachate) Procedure is designed to determine the mobility of both organic and inorganic analytes present in liquid, solid, and multiphasic wastes. For liquid wastes (i.e., those containing less than 0.5% dry solid material), the waste, after filtration through a 0.6 to 0.8 µm glass fiber filter, is defined as the TCLP extract.

Zero-Headspace Extraction Vessel (ZHE) is for use only when the waste is being tested for the mobility of volatile analytes. The ZHE allows for liquid/solid separation within the device, and effectively precludes headspace. This type of vessel allows for initial liquid/solid separation, extraction, and final extract filtration without opening the vessel The vessels shall have an internal volume of 500-600 mL, and be equipped to accommodate a 90-110 mm filter. For the ZHE to be acceptable for use, the piston within the ZHE should be able to be moved with approximately 15 psi or less. The device shall be capable of supporting and keeping in place the glass fiber filter and be able to withstand the pressure needed to accomplish separation (50 psi). When the waste is evaluated for other than volatile analytes, any filter holder capable of supporting a glass fiber filter and able to withstand the pressure needed to accomplish separation may be used. Suitable filter holders range from simple vacuum units to relatively complex systems capable of exerting pressures of up to 50 psi or more. Filters shall be made of borosilicate glass fiber, shall contain no binder materials, and shall have an effective pore size of 0.6 to 0.8 µm, or equivalent. Glass fiber filters are fragile and should be handled with care (Millipore, 2002).

The Millipore ZHE allows for liquid/solid separation within the device, and effectively precludes headspace. This vessel allows for initial liquid/solid separation, extraction, and final extract filtration without opening the vessel. The vessel has an internal volume of 500 ml, and be equipped to accommodate a 90 mm diameter 0.6 μm pore sized filter. There are 2 metal filter protective parts are present in order to protect filter from tearing by the pressurized solid particles.

In the most related oil spill studies, solvents such as DCM, Acetone, DMSO and n-hexane and their mixtures used. In this study only the most soluble solvents are selected and used such as THF and DMSO.

4.2.2. Toxicity Procedure Method

BioTox^{TM} is a standardized toxicity test system which is rapid, sensitive, reproducible, ecologically relevant and cost effective. It is recognized and used throughout the world as a standard test for aquatic toxicity testing. The BioTox^{TM} system is a bioassay to test the acute toxicity on environmental samples and pure compounds. The bioassay's strongest attribute is its usefulness as a primary screening test for a broad spectrum of toxicants and its monitoring capability over time. The BioTox^{TM} procedure can be used for testing either water (marine or fresh) or associated sediments. The BioTox^{TM} toxicity bioassay uses freeze dried luminescent bacteria *Vibrio fischeri* (*Photobacterium phosphoreum*) as the test organisms and based on the measurement of light output of the bioluminescent marine bacterium. In presence of pollutant agents, the natural bioluminescence of *Vibrio fischeri* is reduced and the toxicity is expressed as the agent concentration which produces a 50% reduction of the initial luminescence (EC_{50}). Light production is the result of a chemical reaction involving the oxidation of a substrate, generally called luciferin, mediated by a protein called luciferase in the presence of an ionic cofactor; the intensity of produced light is proportional to the amount of reagents involved in the chemical reaction. A decrease in the intensity of the light produced therefore indicates alteration of one of the events leading to light production: either the chemical reaction (e.g., configurational inactivity of reagents), the expression of genes coding for the reagents, and/or any physiological control associated with the process (Deheyn et al., 2004). Bacteria bioluminescence is intimately associated with cell respiration and any inhibition of cellular activity results in a changed rate of respiration and a corresponding change in the rate of bioluminescence. The more toxic the sample, the greater the percent light loss from the test suspension of luminescent bacteria. The inhibition of natural luminescence of bioluminescent bacteria is regarded as the toxicity endpoint. Bacterial bioluminescence has proved to be a convenient measure of cellular metabolism and consequently, a reliable sensor for measuring the presence of toxic chemicals in aquatic samples (AZUR Environmental, 1998).

There are two specific diluents for BioTox ^{TM}, the extra purity 20% NaCl solution for liquid samples and the 35% NaCl solution for all the types of solid samples such as soils and sediments. The main reason for using these diluents is to warrant an opportune osmotic microenvironment for the bacteria so that the unique reasonable cause of the reduction of luminescence depends on the effect of pollutants. The producer's recommendations report that the bioassay or toxicity test must be performed within a pH range 6-8 and at an equivalent of salinity higher than 20% w/w expressed as NaCl (Onorati et al., 2002).

A suspension of luminescent bacteria, *Vibrio fischeri*, formerly *Photobacterium phosphoreum* was thawed and hydrated. An aliquot of 10 µL of the bacterial suspension was transferred to a test vial containing the standard diluent (2% NaCl) and equilibrated to first 4˚C and acclimated for 30-45 minutes than the temperature rise to 15˚C using a temperature controlled incubating device and acclimated for 30-45 minutes prior to toxicity testing. The amount of light loss per sample was proportional to the toxicity of that test sample. Light loss was expressed as a gamma value and defined as the ratio of light lost to light remaining. DMSO was compatible with the BioTox^{TM} system because of its low test toxicity and its ability to solubilize a broad spectrum of non-polar organic compounds. The concentration of the extract that inhibited luminescence by 50% after a 15 minute exposure period (EC_{50}) was determined. All EC_{50} values reported were 15 minute readings with 95% confidence intervals. All tests were performed in duplicate.

The BioTox^{TM} system is ecologically relevant in a more fundamental way. The more common application is for testing complex chemical mixtures such as effluents, leachates or sediment pore waters. It is well recognized that there is no single test which can detect a range of compounds and the toxic effects, but if the ultimate aim of toxicity testing is to protect the environment from toxic stress then the test must have good sensitivity to a broad range of toxic substances as has been demonstrated with BioTox^{TM} (AZUR Environmental, 1998).

EC_{50} values, defined as the concentration, which provokes a 50% light reduction on *V. fischeri* measured in the analyzer of BioTox^{TM} basic test protocol, are calculated by regression analysis between toxic material concentration and light intensity ratio (ISO, 1999; Fulladosa et al., 2005). Although EC_{50} value represent a concentration of toxicity for an individual material, the obtained values based on a concentration of percent from mixtures or wastes such as oil, hazardous waste, may indicate the type of toxic interaction such as antagonistic (implying that the observed toxicity of the mixture is lower than the sum of toxicities), synergistic (implying that the observed toxicity of the mixture is higher than the sum of toxicities) or additive.

The extent of deviation from a simple additive effect generally depends on (Fulladosa et al., 2005):

1. The measured parameter,
2. The chemical nature of toxicants, and
3. The relative contribution of each toxicant to the toxicity of the mixture.

In this case, it is assumed that each material act independently to provoke the toxic effect by a specific way. For this reason and for a clearer presentation, the computed mixture toxicities must be expressed as toxicity units (TU), defined as $TU=100/EC_{50}$ (Fulladosa et al., 2005). Greater toxicity is reflected by higher TU values.

The inhibition of the luminescence was determined by combining different dilutions of the test sample with luminescent bacteria. The decrease of light intensity was measured with Aboatox 1253 luminometer after a contact time of 15 minutes. Filtered seawater was used as emulsification water for only synthetic samples and the salinity of the samples was adjusted within 2% sodium chloride by adding standard diluent solutions of the Aboatox. The pH was adjusted to 7±0.2. All samples were tested in duplicates. The inhibitory effect of dilutions was compared to a toxin free control to give the percentage inhibition. The value was plotted against the dilution factor and the resultant curve was used to calculate the EC_{50} of the sample. The standard dose-response curve method was used to determine a 50 percent loss of light in the test bacteria. The luminometer and supporting computer software with a standard log-linear model were used to calculate EC_{50} values (AZUR Environmental, 1998).

The BioTox^{TM} Software performs automatically all needed calculations according to the equations below.

$$KF = \frac{IC_t}{IC_0} \tag{4.1}$$

$$INH\,\% = 100 - \frac{IT_t}{KF \times IT_0} \times 100 \tag{4.2}$$

Where

INH % = Inhibition percentage
KF = Correction factor
IC_t = Luminescence intensity of control after control time
IC_0 = Initial luminescence intensity of control sample
IT_t = Luminescence intensity of test sample after control time
IT_0 = Initial luminescence intensity of the test sample

5. RESULTS AND DISCUSSION

Toxicity data obtained from the set of experiments as EC_{50} values are converted to Toxic Units (TU) and summarized in the Table 5.1.

Table 5.1. BioTox^{TM} Test Results of Experiments as TU

Sample	EC_{50} (%)	Toxic Units (TU)
THF	0,19	526
THF + MO	0,28	357
THF + UMO	0,22	455
THF + FO	0,17	588
THF + MO + Sand	0,19	526
THF + UMO + Sand	0,19	526
THF + FO + Sand	0,20	500
DMSO	11,9	8
DMSO + MO	10,6	9
DMSO + UMO	10,95	9
DMSO + FO	0,22	455
DMSO + MO + Sand	11,9	8
DMSO + UMO + Sand	9,84	10
DMSO + FO + Sand	11,48	9
MO + SW	18	5
UMO + SW	15,92	6
FO + SW	-	-
MO + DW	-	-
UMO + DW	26,66	4
FO + DW	-	-

5.1. Individual solvent samples

In order to help determination of the toxicities of oil samples, as first step the solvents are tested for toxicity alone. According to the BioTox ^{TM} bioassay testing procedure first 10 ml of DMSO is mixed with 1,1 ml of 20% NaCl solution to reach the desired salinity level of the sample to be tested. After the reagent is acclimated at both 4 and 15 ºC mixed with the dilutions of the sample solvent, DMSO. Tests run in duplicate and the computerized results given as a graph with the 95% confidence limit in the Figure 5.1. EC$_{50}$ value of DMSO is found to be 11,9 %. Therefore having a density of 1,1 gr/cm^3 resulted an EC$_{50}$ value of 131 g/L for DMSO.

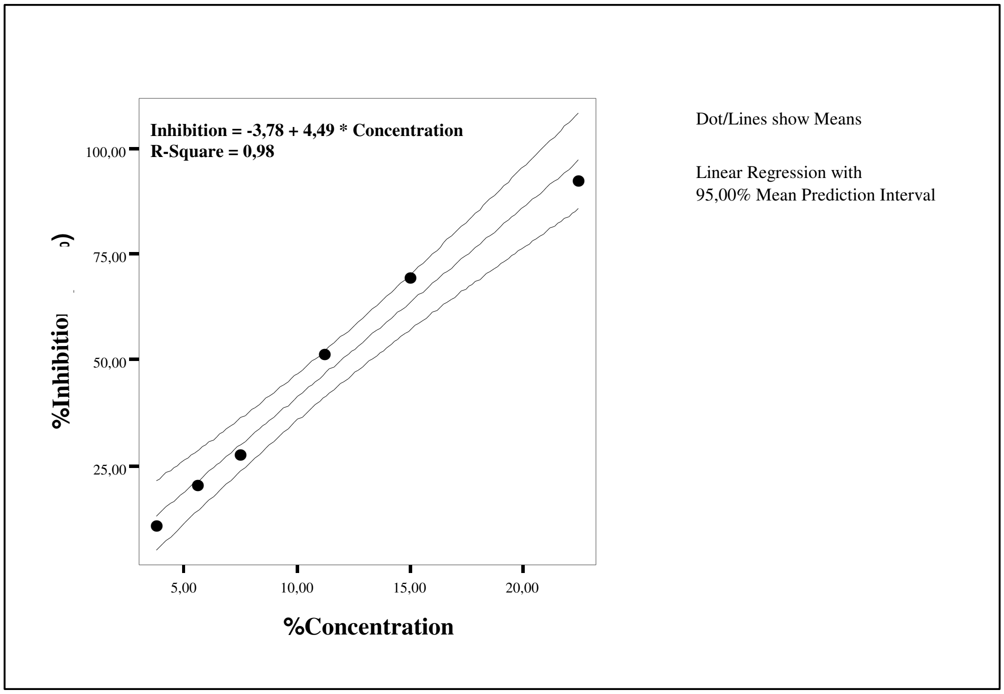


Figure 5.1. Toxicity of DMSO

The same testing procedure was repeated for THF. In order to obtain the salinity level of the sample 10 ml of THF is mixed with 1,1 ml of 20% NaCl stock solution and different dilutions of the sample tested for toxicity in duplicate. EC$_{50}$ value of the THF calculated as 0,19%. From the density of THF which is 0,89 g/cm^3 EC$_{50}$ value calculated as 1,7 g/L. The bioassay results are plotted on the graph by the computer program and given in the Figure 5.2. The THF found to be more toxic than the DMSO and has to be diluted several times in order to reach the compatible limits to predict EC$_{50}$ values in the BioTox $^{\text{TM}}$ bioassay test.

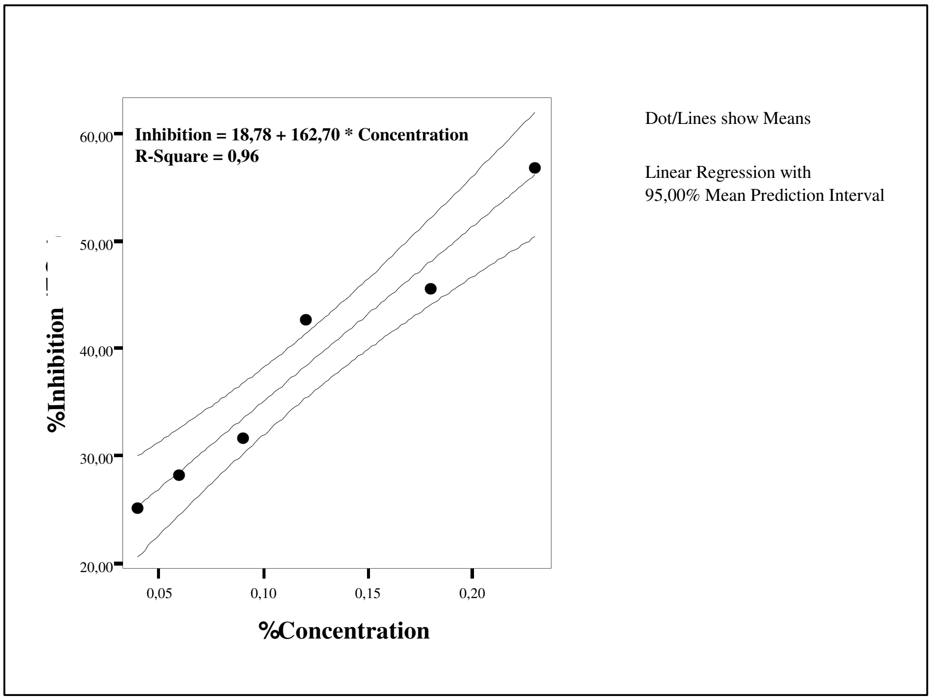


Figure 5.2. Toxicity of THF

5.2. Oil-solvent mixture samples

After determining the toxicities of solvents, in other words blank toxicity, the next step is to integrate these results with the toxicities of oil-solvent mixtures. For this purpose 2 gr of Motor Oil is dissolved with 100 ml of DMSO. 10 ml of this mixture is taken and mixed with 1,1 ml of 20% NaCl stock solution prior to toxicity testing. Computerized test results is given in Figure 5.3 and the EC_{50} calculated as 10,6%. As the second step of oil-solvent toxicity tests 2 gr of Used Motor Oil is dissolved in 100 ml of DMSO and 10 ml of this sample tested for toxicity after salinity level is brought to the desired level. The results of the toxicity bioassay, given as a graph in the Figure 5.4. The EC_{50} of the DMSO and Used Motor Oil mixture determined as 10,95% which is a bit less toxic than DMSO and Motor Oil mixture.

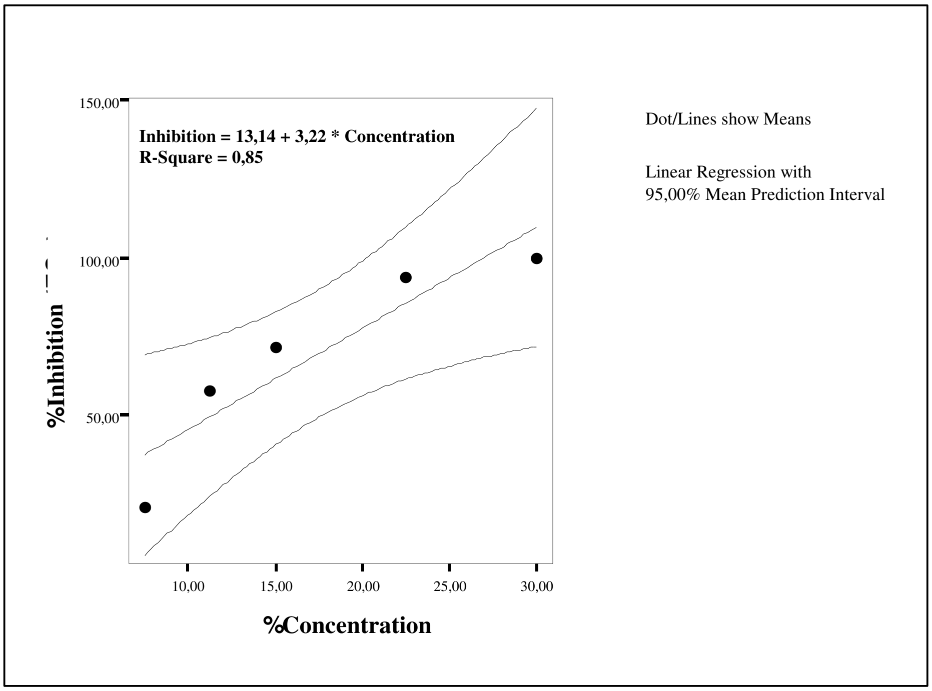


Figure 5.3. Toxicity of Motor Oil and DMSO

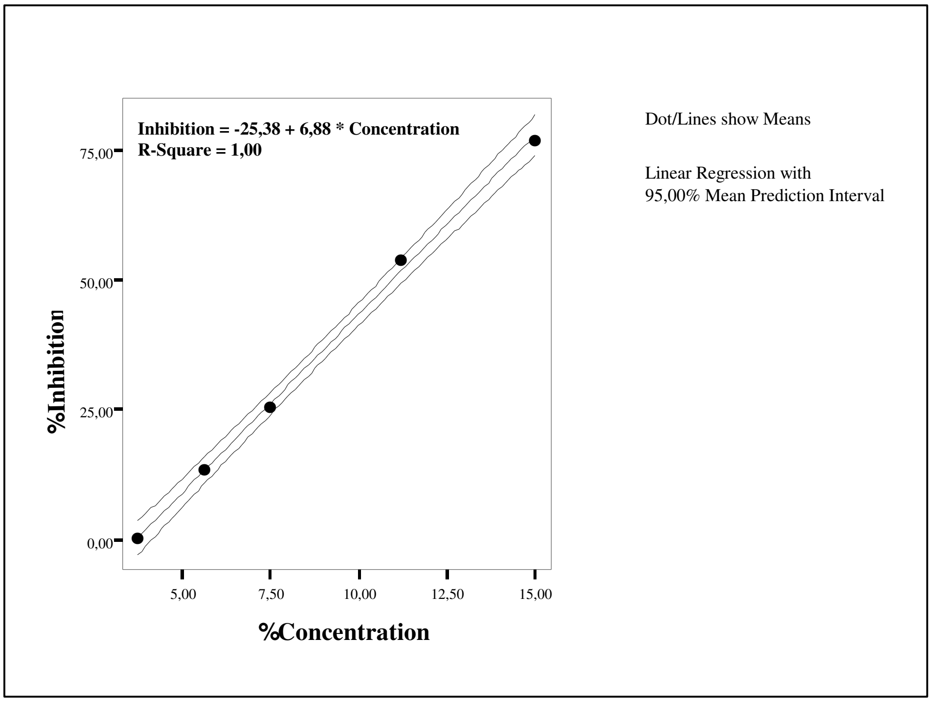


Figure 5.4. Toxicity of Used Motor Oil and DMSO

The last oil sample mixed with 100 ml of DMSO is 2 gr of Fuel Oil. 10 ml of this sample mixture tested for toxicity after mixing with 1,1 ml of 20% NaCl stock solution. 3 different set of experiments are conducted in order to obtain the EC_{50} values of the mixture. This mixture found to be the most toxic among the DMSO-Oil mixtures with an EC_{50} value of 0,22%. The graph of the toxicity results of this sample is given in the Figure 5.5.

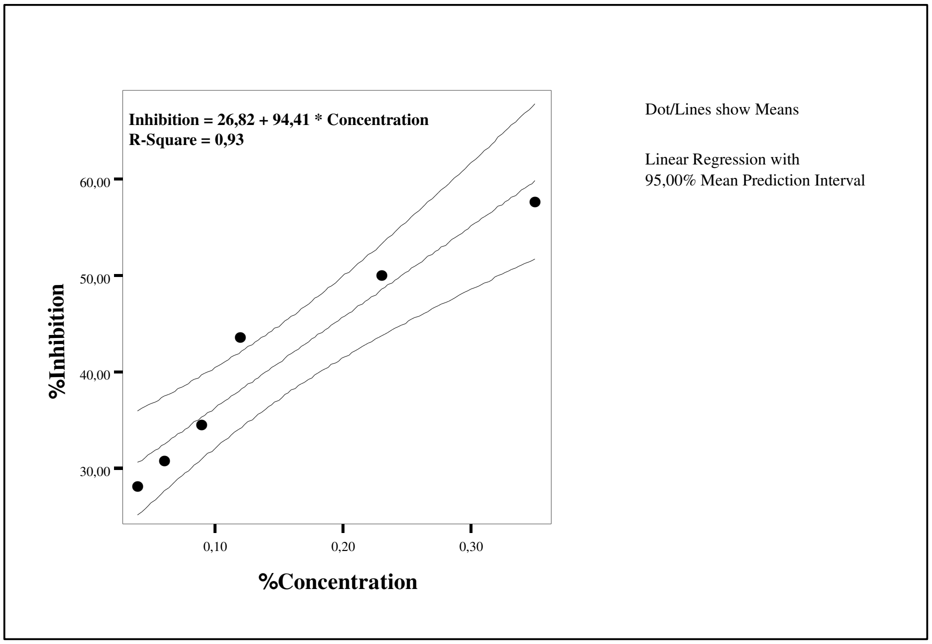


Figure 5.5. Toxicity of Fuel Oil and DMSO

After determining the toxicities of Oil-DMSO mixture samples next step is to conduct the toxicity tests for Oil-THF samples. Therefore 1 gr of Motor Oil is dissolved in 50 ml of THF afterwards 10 ml of this sample is taken and mixed with 1,1 ml of 20% NaCl solution to adjust the desired salinity level prior to toxicity bioassay. The results of the toxicity test represented as a graph in the Figure 5.6. the EC_{50} value is determined as 0,28% which is lower than the toxicity of the solvent by itself. Same procedure is repeated for the mixture of 1 gr of Used Motor Oil dissolved in 50 ml of THF and the results given in the Figure 5.7. The EC_{50} value of the sample found 0,22% which is more toxic than Motor Oil and THF mixture but still lower than the toxicity of THF itself alone.

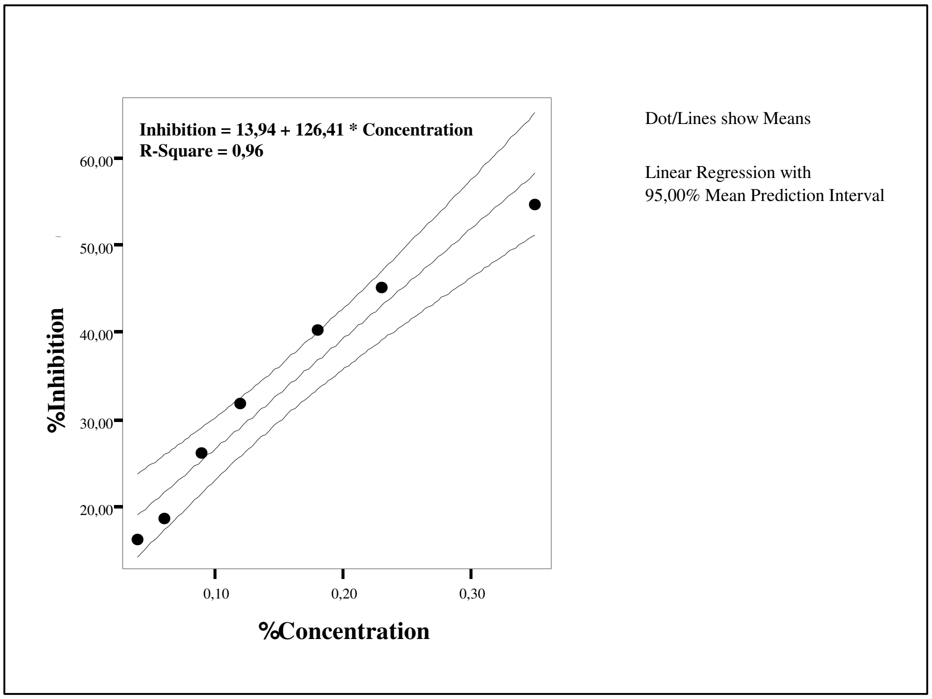


Figure 5.6. Toxicity of Motor Oil and THF

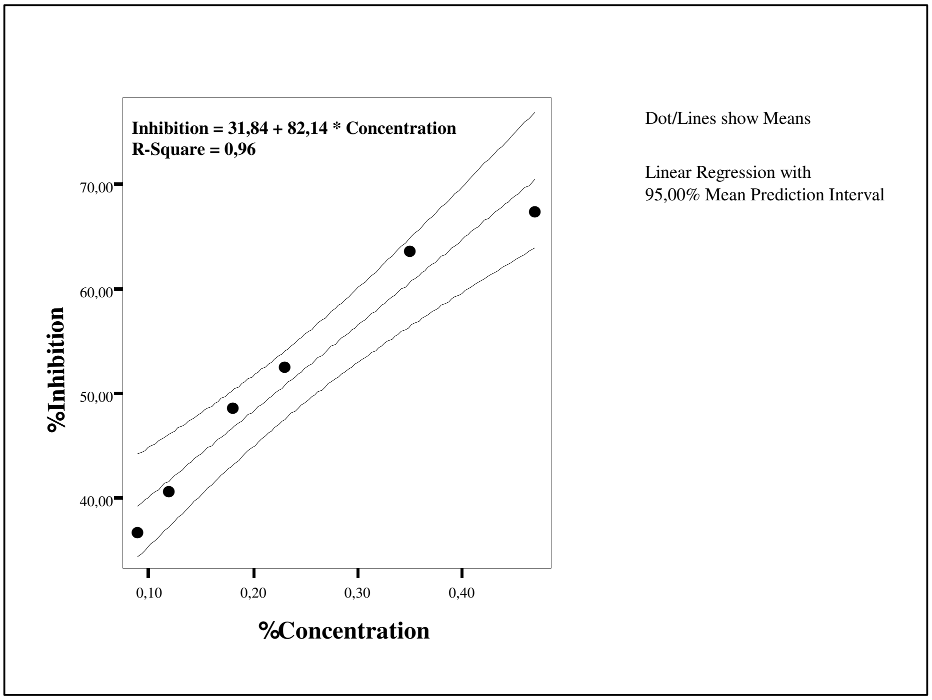


Figure 5.7. Toxicity of Used Motor Oil and THF

The final sample of Oil-Solvent mixtures is the mixture of THF and Fuel Oil. For this experiment 1 gr of Fuel Oil dissolved in100 ml of THF and 10 ml of this sample tested for toxicity after the salinity levels arranged by 20% NaCl solution. The EC_{50} value of the sample found as 0,17% which is the most toxic mixture of all oil-solvent mixtures and also the mixture found to be more toxic than the solvent itself. This shows the most toxic oil is Fuel Oil among others. The experimental data shown as a graph in Figure 5.8.

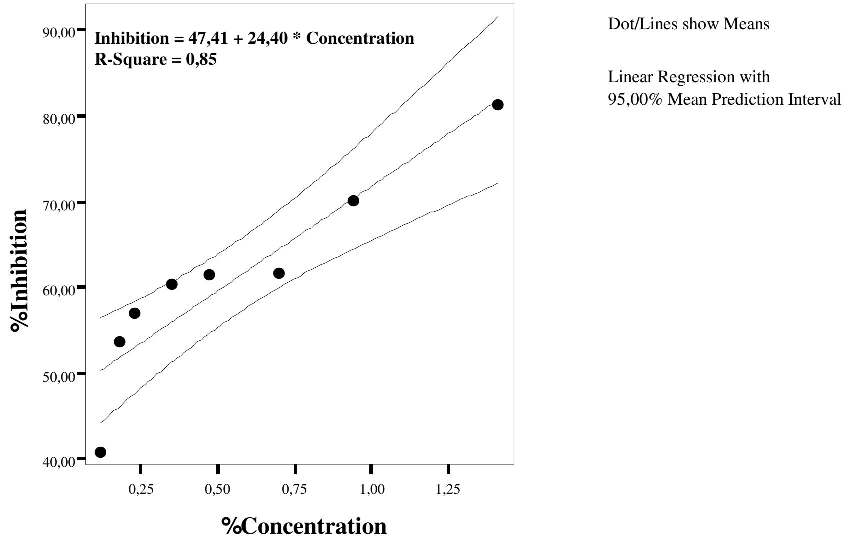
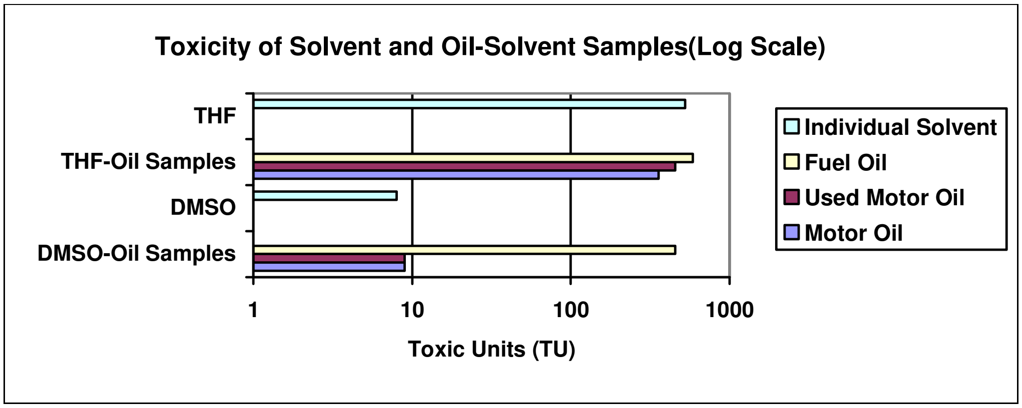


Figure 5.8. Toxicity of Fuel Oil and THF

According to the first set of experiments Fuel Oil found to be the most toxic oil among all oil samples. On the other hand the toxicities of Motor Oil and Used Motor Oil show significant changes according to the type of the solvent. When THF used as solvent Used Motor Oil found to be more toxic than the Motor Oil; whereas Motor Oil is a bit more toxic than Used Motor Oil when DMSO used as solvent. Therefore different solvents dissolve different parts of the oil samples causing a change in the toxicities. The toxicities of Oil-Solvent mixtures and individual solvents shown in the Table 5.2 in Toxic Units.

Table 5.2. Toxicity of Oil-Solvent Mixtures



5.3. Oil-sand mixture samples

At the second set of experiments oil samples are mixed with sand samples taken from Florya, Istanbul. These mixtures mixed with solvents and extracted according to the Toxicity Characteristic Leaching Procedure (TCLP) with a Zero Headspace Extractor (ZHE) prior to toxicity testing. First 0,5 gr of Fuel Oil mixed with 50 gr of sand then the mixture mixed with 50 ml of DMSO and shaked mechanically. This mixture poured into the ZHE and extracted from 0,6 μm glass fiber filter according to the TCLP. 10 ml of this extract mixed with 1,1 ml of 20% NaCl stock solution to reach the desired salinity level prior to toxicity testing. The EC_{50} value of the extract determined as 11,48% from the toxicity bioassay. The computerized results of the toxicity data given as a graph in Figure 5.9. Afterwards 0,5 gr of Motor Oil is mixed with 50 gr of sand and 50 ml of DMSO added to this mixture prior to extraction. 10 ml of the extract from the ZHE mixed with 1,1 ml of 20% NaCl solution to modify the salinity of the sample. The EC_{50} results of this mixture found to be 11,9% and given as a graph in the Figure 5.10. As a result there is no significant difference between the toxicities of the DMSO solvent itself and extract from the Motor Oil and Sand mixture by DMSO.

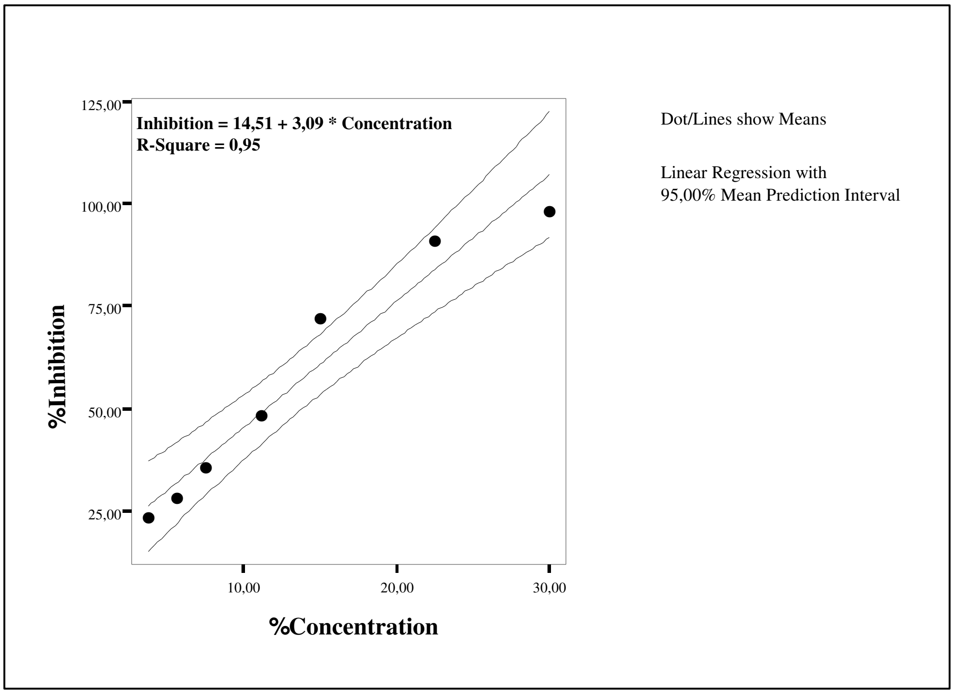


Figure 5.9. Toxicity of DMSO Extract of Sand and Fuel Oil

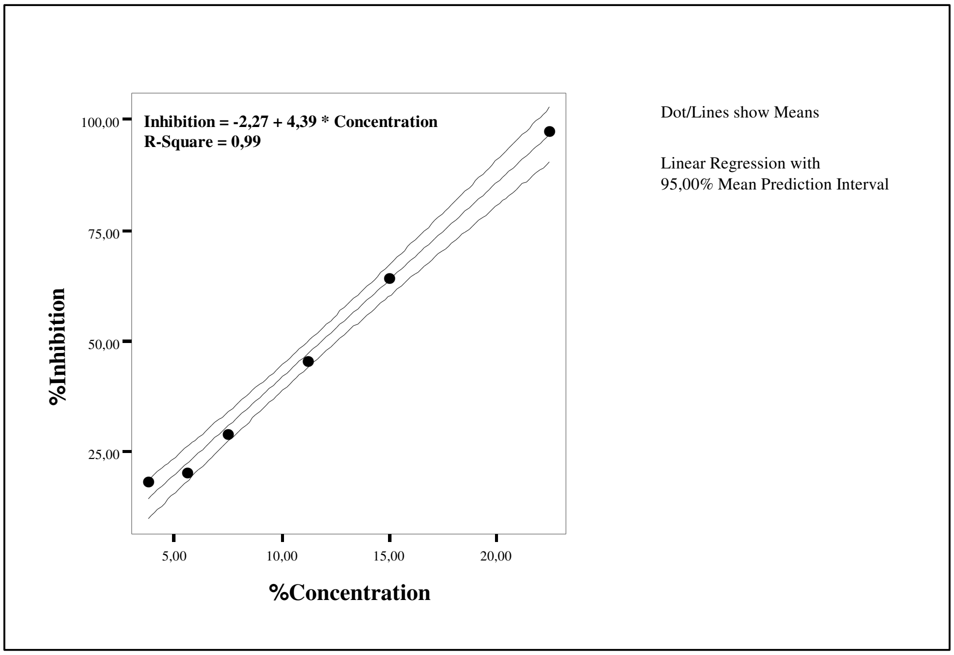


Figure 5.10. Toxicity of DMSO Extract of Sand and Motor Oil

The final DMSO extraction made with the mixture of Used Motor Oil and Sand. For this experiment set 0,5 gr of Used Motor Oil mixed with 50 gr of sand. After adding 50 ml of DMSO the mixture shaked mechanically and extracted from the ZHE. 10 ml of this mixture sample tested for toxicity after the desired salinity level is reached by adding 1,1 ml of 20% NaCl stock solution. The EC_{50} value of this mixture found as 9,84 and the computerized results given in the Figure 5.11.

According to the bioassay test results the toxicity of the DMSO extract of the Used Motor Oil and Sand samples found to be the most toxic compound followed by Fuel Oil and Sand mixture and there is no significant difference between the toxicity of DMSO and the DMSO extract of the Motor Oil and Sand mixture.

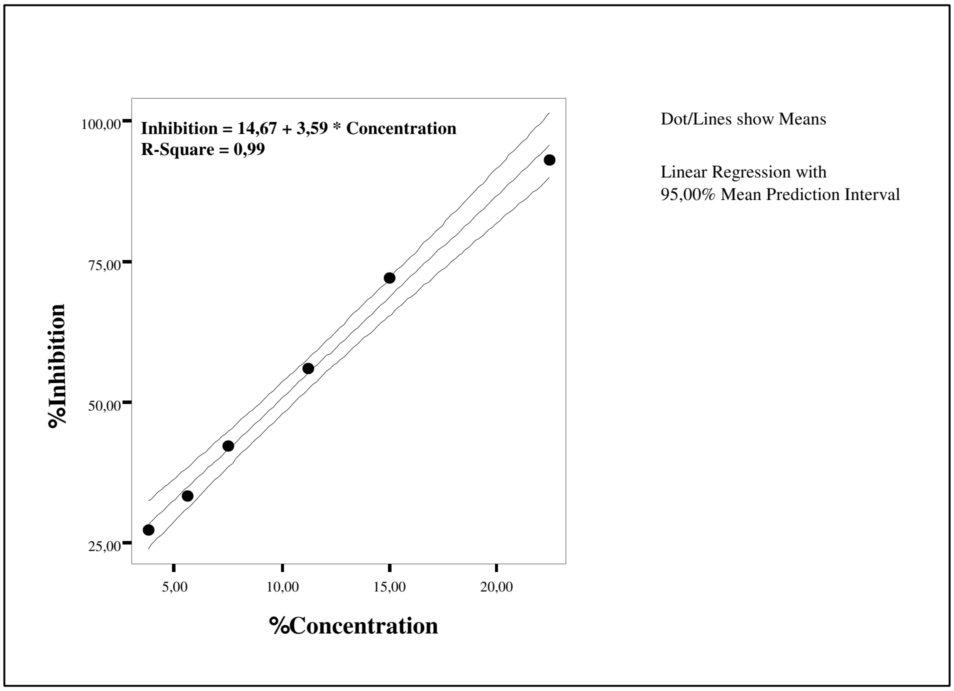


Figure 5.11. Toxicity of DMSO Extract of Sand and Used Motor Oil

Second part of this experiment set is the extracts of the Oil-Sand samples extracted by THF to determine the effect of sand on oil extractability and also the changes in the toxicities of the oil when sand added to samples. For this purpose first 0,5 gr of Fuel Oil is poured onto 50 gr of sand. This mixture is mixed with 50 ml of THF and extracted by a ZHE. 10 ml of the extract mixed with 1,1ml 20% NaCl stock solution to reach the desired salinity level. The EC_{50} value determined from the dilutions found to be 0.20% and shown as a graph in the Figure 5.12. The following toxicity test is done with the THF extract of the mixture of 0,5 gr of Motor Oil and 50 gr of sand. The THF used in this experiment is 50 ml. and the EC_{50} value is found as 0,19%. The data of the toxicity bioassay result is summarized as a graph in the Figure 5.13.

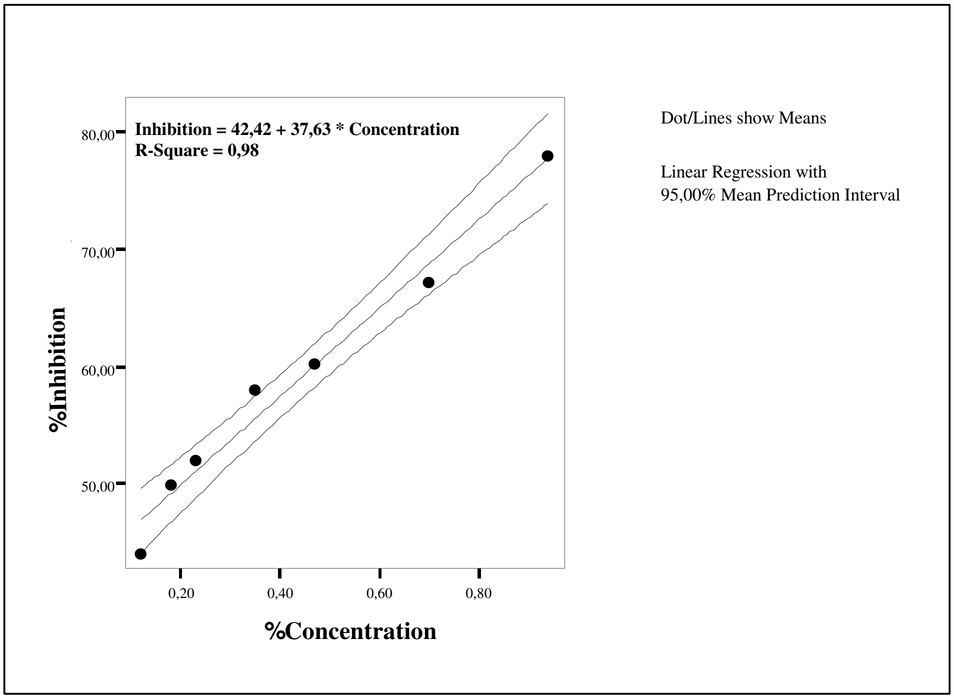


Figure 5.12. Toxicity of THF Extract of Sand and Fuel Oil

The final extraction is made by 50 ml of THF. The sample is 0,5 gr of Used Motor Oil and 50 gr of sand mixture. 10 ml taken from this extract is mixed with 1,1 ml of 20% NaCl stock solution and tested for toxicity. The toxicity test results is given as a graph in the Figure 5.14. The EC_{50} value of this sample found as 0,19%.

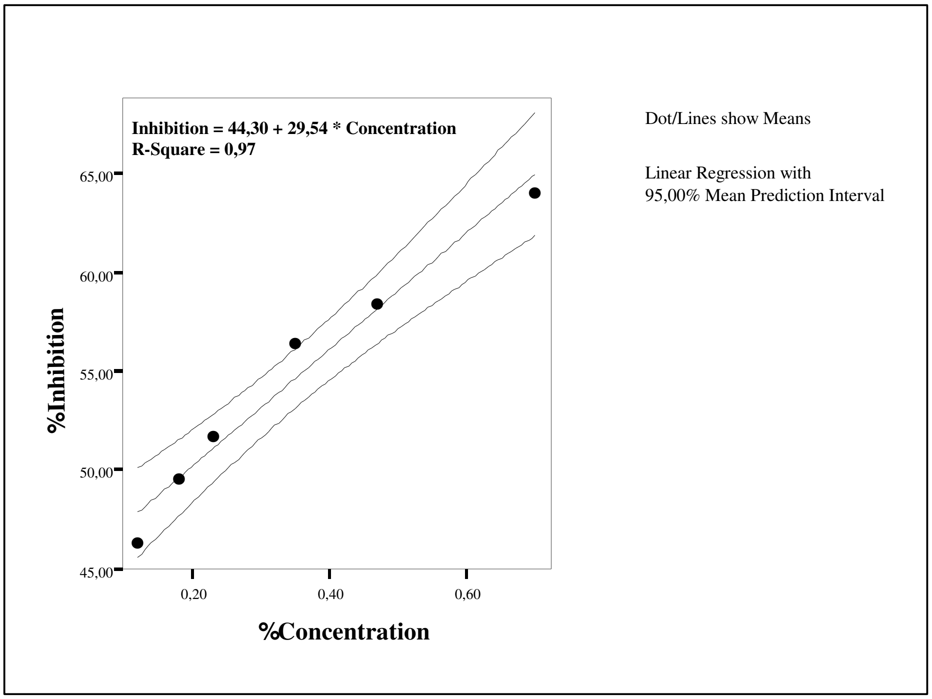


Figure 5.13. Toxicity of THF Extract of Sand and Motor Oil

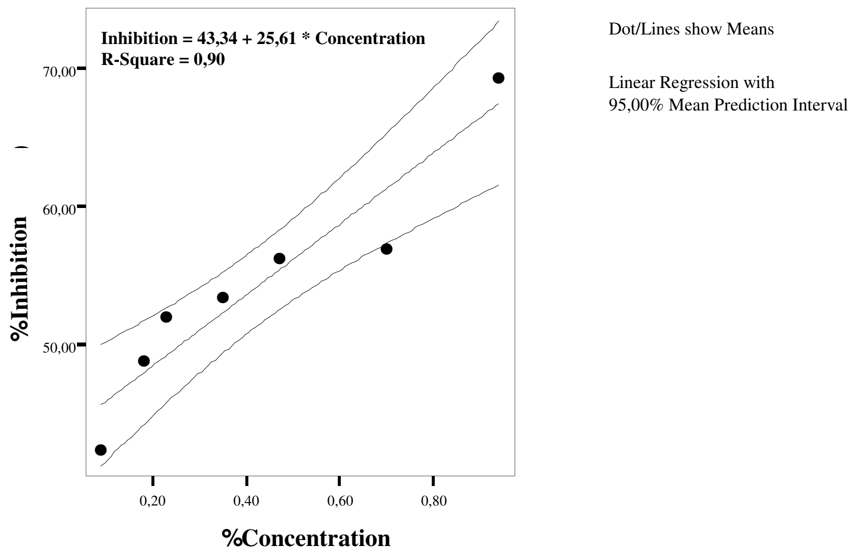
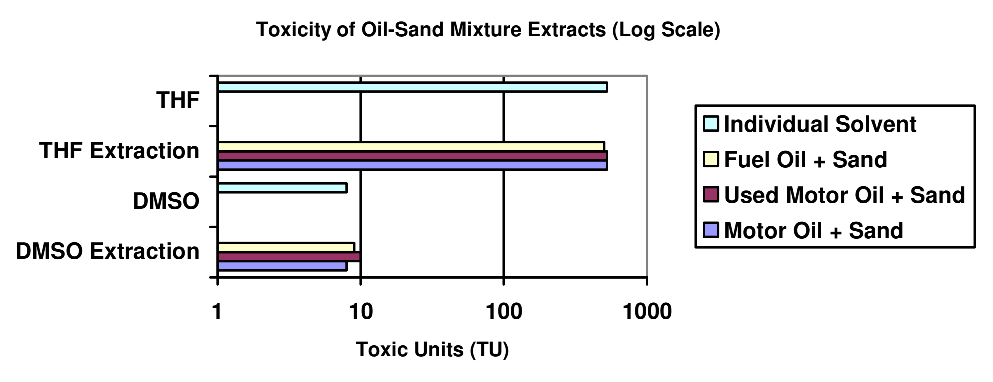


Figure 5.14. Toxicity of THF Extract of Sand and Used Motor Oil

The results of the second part of the experiments which is the solvent extract of sand and oil mixtures are summarized in the Table 5.3. Also the toxicities of the individual solvents are expressed in the table in order to compare them among each other.

Table 5.3. Toxicity of Oil-Sand Mixture Extracts



5.4. Oil-water mixture samples

The final set of experiments which is the determination of toxicities of the water-oil mixture samples done in order to identify the toxicities of the water soluble fraction of oils both in sea water and distilled water. For this purpose first 5 gr of Used Motor Oil mixed with 100 ml of Sea Water. This mixture is than sonicated for 10 min to increase the water soluble fraction and also to simulate the behavior of oil in sea in the presence of waves and tides. Afterwards this sample is shaked in an orbital shaker for 72 hours at 200 rpm at room temperature. Finally this sample extracted from a ZHE to remove the non-soluble part of the oil and the extract is directly tested for toxicity because of sea water having the desired salinity. The EC_{50} value found to be 15,92% and the computerized data of the bioassay test given in the Figure 5.15 as a graph.

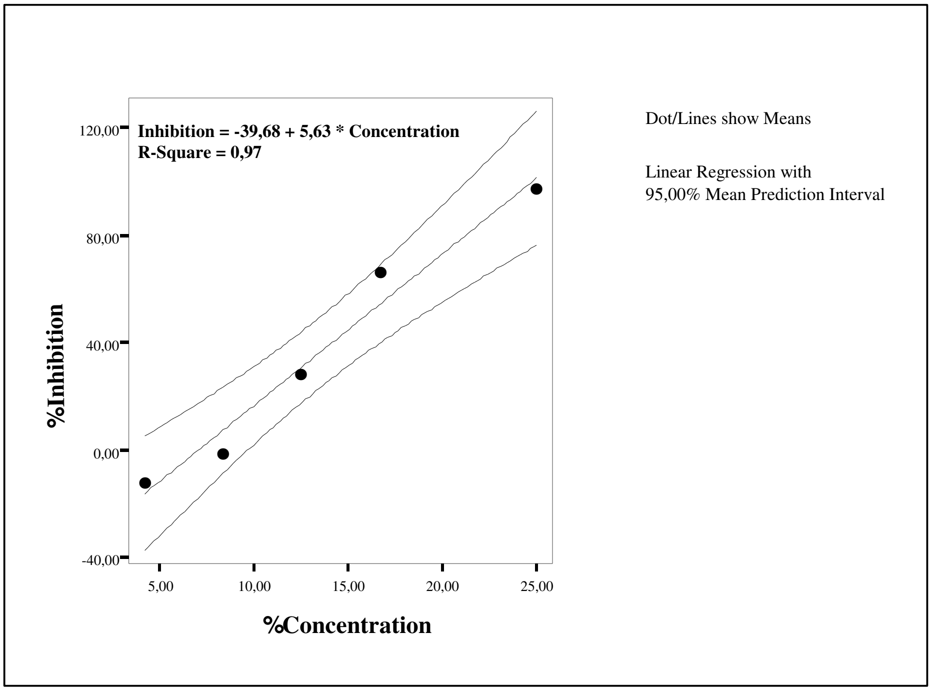


Figure 5.15. Toxicity of WSF of Used Motor Oil in Sea Water

The next experiment done with the mixture of 5 gr of Motor Oil and 100 ml of sea water. The same sonication and shaking processes done for this sample as in the Used Motor Oil and sea water sample. 10 ml of this sample is tested for toxicity in different dilutions and the EC_{50} value found as 18 %.

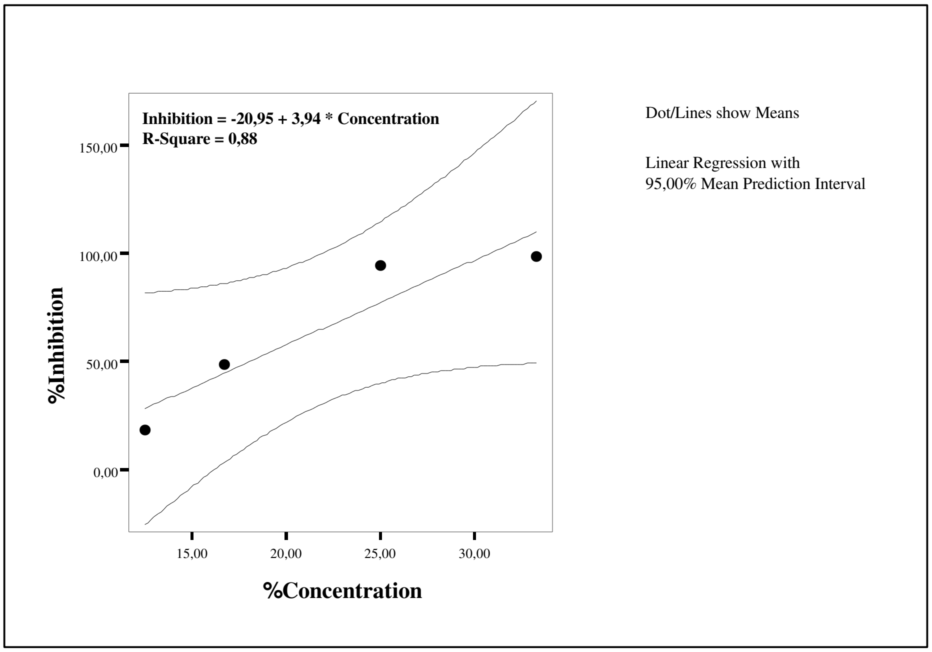


Figure 5.16. Toxicity of WSF of Motor Oil in Sea Water

5 gr of Fuel Oil is poured onto 100 ml of sea water and the sample sonicated for 10 minutes. Than shaked in an orbital shaker for 72 hours at 200 rpm at room temperature. Finally this sample extracted through 0,6 µm glass fiber filter by ZHE and the non-soluble part is completely separated. 10 ml of this sample tested for toxicity in the BioTox TM toxicity bioassay. There was no significant inhibition moreover there was an increase in the light output of the reagent vibrio fisheri. This result showed that Fuel Oil is very slightly soluble with water and

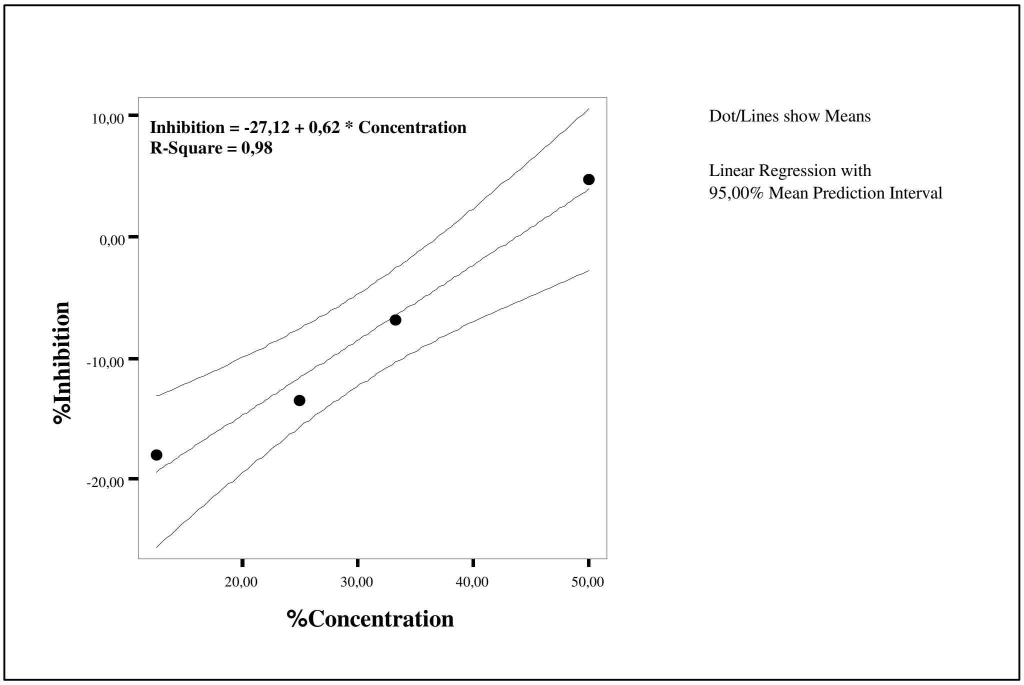


Figure 5.17. Toxicity of WSF of Fuel Oil in Sea Water

The second part of the final set of experiments is the mixtures of distilled water and oil samples. Therefore 5 gr of Fuel Oil mixed with 100 ml of Distilled Water and sonicated for 10 minutes in a sonicator. Afterwards shaked in a orbital shaker for 72 hours at 200 rpm at room temperature. The sample then extracted from a ZHE and the non-soluble part is removed completely. 10 ml of this sample mixed with 1,1 ml of 20% NaCl stock solution prior to toxicity testing to reach the desired salinity level. There was no toxicity detected from the toxicity bioassay. The same sonication and shaking procedure applied to the motor oil and distilled water sample. 5 gr of motor oil is mixed with 100 ml of distilled water and extracted by a ZHE and tested for toxicity. However there was a slight toxicity the most concentrate dilution even cannot give a meaningful EC_{50} value.

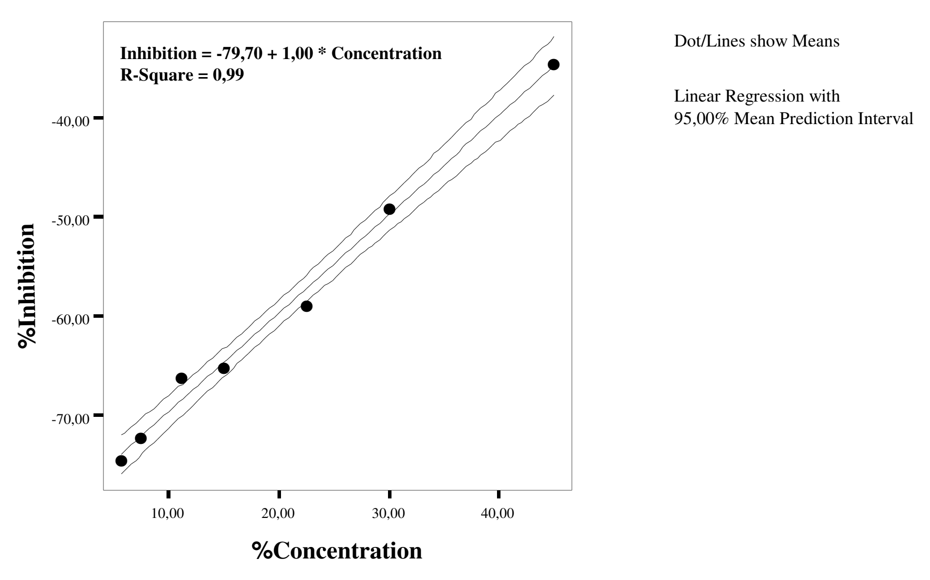


Figure 5.18. Toxicity of WSF of Fuel Oil in Distilled Water

The final experiment of the study conducted with the mixture of distilled water and Used Motor Oil. 5 gr of Used Motor Oil poured onto 100 ml of distilled water and sonicated for 10 minutes. The sample then shaked in an orbital shaker for 72 hours at 200 rpm at room temperature. After extracting the non-soluble part from the ZHE 10 ml of the sample mixed with 1,1 ml of 20% NaCl. According to the BioTox bioassay the EC_{50} value of the sample found 26,66% the computerized results is given in the Figure 5.19.

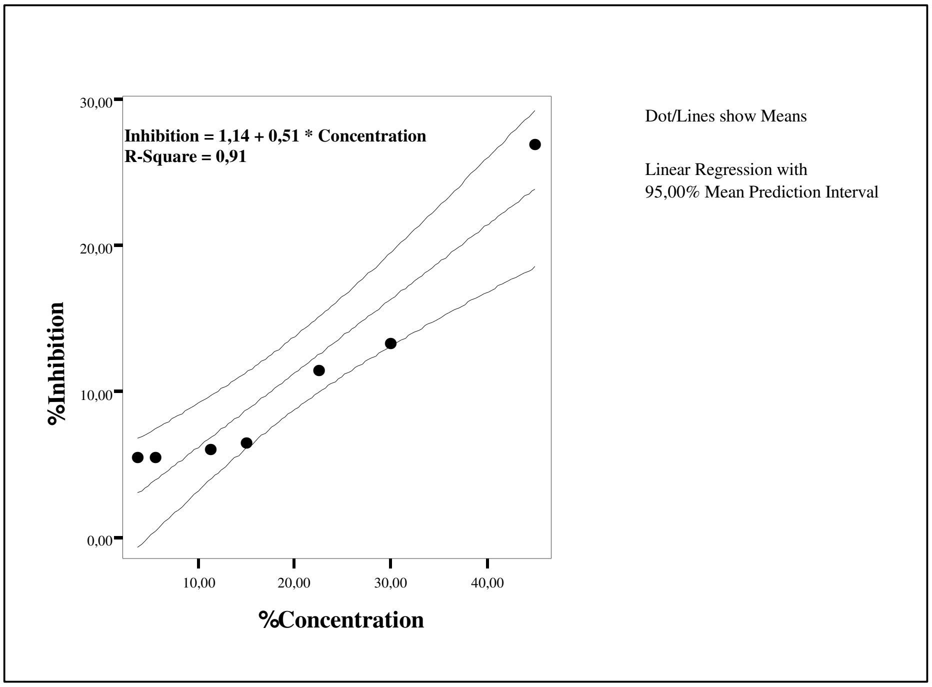


Figure 5.19. Toxicity of WSF of Motor Oil in Distilled Water

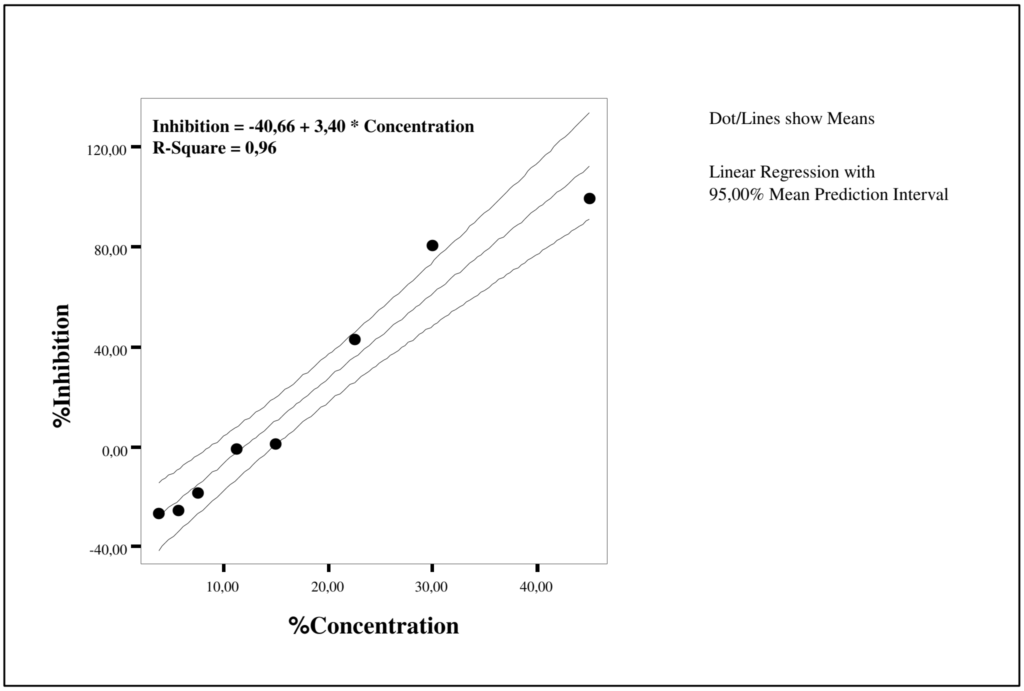
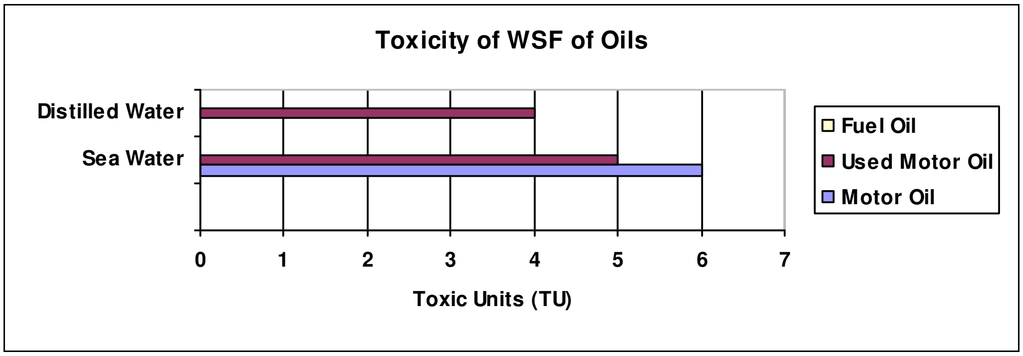


Figure 5.20. Toxicity of WSF of Used Motor Oil in Distilled Water

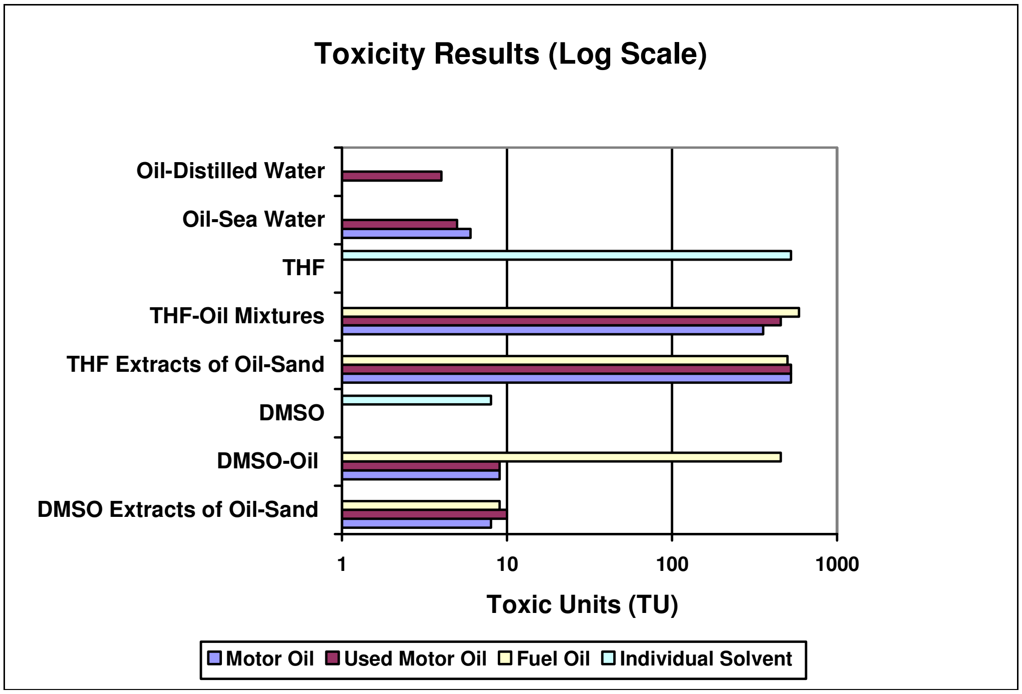
The toxicities of the water soluble fraction of oils in both sea water and distilled water are summarized in the Table 5.4. in toxic units. According to the toxicity bioassay results the only oil which provoked significant toxicity is Used Motor Oil. For sea water both used motor oil and motor oil gave significant toxicity whereas the toxicity of used motor oil is greater than the toxicity of motor oil. On the other hand fuel oil showed no toxicity because of low solubility.

Table 5.4.: Toxicity of Water Soluble Fraction of Oils



All the toxicity results of the experiments conducted in this study are summarized in toxic units in the Table 5.5. according to the table the most toxic oil found to be fuel oil when extracted with solvents, whereas; because of the low solubility of fuel oil, it gave no significant toxicity as WSF. Another results that can be obtained from the table is extraction efficency in solid phase is lower than the liquid phase.

Table 5.5. : Overall Toxicity Test Results



6. CONCLUSION

Heavy fuel oil cannot conjugate to toxicity in the BioTox^{TM} bioassay because of its low soluble fraction in the sea and water. Therefore, it can be measured by using tests based on organic extraction of sediment and beach sand. Dimethylsulfoxide (DMSO) is a solvent compatible with the BioTox^{TM} system because of its low test toxicity and its ability to solubilize a broad spectrum of non-polar organic compounds. Also tetrahydrofuran (THF) is evaluated for the BioTox^{TM} toxicity bioassay in this study and found more toxic than DMSO. If the toxicity tests use THF for testing the toxicities of non-polar organic compounds such as petroleum and petroleum products.

- The samples have to be diluted several times because of THF's toxicity is being greater than DMSO. In addition this will result as a increase in the experimental error due to the dilution which also cause a decrease in the solvent's extraction efficiency. In this study a suitable solvent searched for organic compound extraction, hence; it is concluded that BioTox^{TM} system using DMSO is a suitable method for toxicity because of having both low toxicity and high solubility.

- The oil-solvent mixture samples showed that DMSO is not a strong solvent compared to THF. Besides, not only the extraction efficiency of THF is greater than DMSO but also the toxicity is greater. In the oil-solvent mixtures for both solvents, the higher toxicity is caused by Fuel Oil whereas; the Motor Oil and Used Motor Oil mixture samples' toxicities are very similar with the toxicities of the solvents. Because of the main part of the toxicity of these samples result from the toxicites of the solvents the toxicity of the. solvent extractable part of the Motor Oil and Used Motor Oil do not provoke significant toxicity.

- The toxicity test results of the organic extraction of beach sand pointed out that the toxicity of Fuel Oil lower than the oil-solvent mixtures resulting that Fuel Oil and sand mixtures extraction effciency is lower than the Fuel Oil itself alone. It is concluded that obviously the extraction efficiency for both solvents is lower in the solid phase.

- According to the test results of the water soluble fraction of oils, Fuel Oil seems to be least toxic oil of all the sample oils. Fuel Oil is very slightly soluble with sea water and completely insoluble with distilled water both samples do not caused any significant toxicity.

- Soluble fractrions of motor oils in sea water are more toxic than in distilled water. The reason of the Motor Oil having the highest toxicity could be caused by the additives added to Motor Oil in order to resist the high temperatures in the engines.

- Used Motor Oil give significant toxicity in both sea and distilled water, whereas the toxicity in sea water is higher than in distilled water because of having inorganic compounds from the crankcase of the engine such as heavy metals and also impurities from the combustion.

- As a summary of the water soluble fraction of oils it is seemed to be that all the toxicities of the sample oils in sea water is greater than in distilled water.

- This can be concluded as, because of sea water contains elements dissolved in itself, it contributes to a better dissolution of the samples due to sonication and shaking. These two processes' efficency can be rised according to the dissolved matter in the water medium, in other words higher ionic strength causes beter dissolution.. Collusion of the paticles cause oil samples to dissolve more effectively, therefore provide a rise in the toxicities of the samples. This should be kept in mind and the environmental damage must be assessed according to the dissolved matter content of the water medium and the wave action where the spill is occured.

- These factors will increase the soluble fraction of oils resulting a rise in the toxicity.

- As a result because of heavy fuels have very low soluble fractions in water it is important to quantify the toxicity of these samples. As the recent and most compatible tests for determining the toxicity are toxicity biassays, these tests cannot quantify the real toxicity directed to the environment, it can only determine the toxicity of water soluble fraction of heavy fuels. Therefore the toxicity bioassay results show no significant toxicity for heavy fuels. This is not because of heavy fuels are not toxic, it is because heavy fuels cannot dissolve in water which retards the toxicity testing in toxicity bioassays. When the heavy fuels extracted by a solvent it can be seen that these fuels cause significant toxicites, therefore; great concern should be subjected to heavy fuels and heavy fuel spills. The toxicity bioassays conducted where a heavy fuel spill is occured do not project the real consequences of the toxic materials entered the environment.

- In conclusion the toxicities caused by the individual substances can be quantified by introducing them into toxicity bioassays.

But the toxicities subjected to the environment by complex substances such as oil and grease as collective parameters can only be quantified by the toxicity conjugates of these complex substances. Even in this situation the environmental conditions and environmental effects connot be included. The chemical content of the environment will also have a strong effect on changing the toxicity by synergistic and antagonistic effects .

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