İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

COD COUNTERPART OF OIL AND GREASE AND ASSESSMENT OF ITS TOXICITY

M.Sc. Thesis by

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Department : Environmental Engineering

Programme : Environmental Sciences and Engineering

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YAĞ VE GRESİN KOİ EŞLENİĞİNİN VE TOKSİSİTESİNİN DEĞERLENDİRİLMESİ

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SUMMARY

The oil and grease content of domestic and industrial wastewaters, and of treatment sludges, is an important consideration in the handling and treatment of these materials for ultimate disposal. Oil and grease have poor solubility in water and its separation from the aqueous phase is helpful for treatment. Oil and grease which cannot be removed from wastewaters complicate the transportation of wastes through pipelines, disposal into receiving waters, and inhibit the biological treatment units. Though just a minor amount of oil is soluble in water, this minor fraction of oil might cause severe negative effects. In this study, oil and grease and its soluble fraction in aqueous medium is investigated. Determining COD counterpart of oil and grease and its possible toxic effects will give advantages in evaluating its environmental damage. Total organic carbon analyses were applied to extraction solvets and water soluble fractions of oils. COD and TOC correlation is also investigated. Another important parameter affecting the treatment is toxicity. It is very important to determine the toxicity which is one of the criteria used for determining hazardous waste. Toxic pollutants threaten the ecosystem with their characteristics. They can inhibit the biological treatment units. In this research, oil and grease amount of wastewater will be determined and COD equivalent of oil-grease were analyzed. Toxicity tests were applied on the wastewater samples that are highly polluted by oil and grease. The method of toxicity tests were BioTox^{TM} test using *Vibrio fischeri*, a luminescent bacteria. Extraction of the water soluble fractions of oils from aqueous medium were obtained by Zero Head Space Extractor (ZHE) based on Toxicity Characterization of Leaching Procedure (TCLP) and the EC_{50} values of the extracts were evaluated. During experiments three organic solvents were used for extractions specimen namely; hexane, dimethylsulfoxide (DMSO), tetrahydrafuran (THF). In this study, oil and grease contents of motor oil, used motor oil and fuel oil (no:6) that can be spilled somehow during their usage or transportion, were determined, and COD counterpart of these oil and grease contents, and their toxicity were also measured.

YAĞ VE GRESİN KOİ EŞLENİĞİNİN VE TOKSİSİTESİNİN DEĞERLENDİRİLMESİ

ÖZET

Evsel ve bazı endüstriyel atıksuların ve çamurların arıtma ve nihai uzaklaştırmasında yağ ve gres ele alınması gereken önemli bir parametredir. Yağ ve gresin atıksuda çözünmeyerek ayrı bir faz oluşturması, yağ ve gresin fiziksel arıtımını kolaylaştırmaktadır. Arıtılamamış, su ortamında kalmış yağ-gres ise boru hatlarında sorun yarattığı gibi biyolojik arıtma ünitelerinde tahribatlara ve alıcı ortam deşarjlarında sorunlara neden olmaktır. Bununla beraber, su ortamında küçük miktarlarda da olsa yağın çözülebilir bir kısmı mevcuttur ve küçük miktarlarına karşın bu çözünmüş kısmın kirletici etkileri olabilir. Bu çalışmada yağ gresle beraber yağ gresin sudaki fraksiyonunun etkileri de incelenmiştir. Atıksulardaki yağ-gresin ne kadar kimyasal oksijen ihtiyacı (KOİ) miktarına denk geldiğinin ve ne kadar toksik etkiye sahip olduğunun bilinmesi yağ-gresin yaratacağı çevresel etkinin tahmininde ve kontrolünde avantaj sağlayacaktır. Toplam organik karbon analizleri ekstraksiyon solventlerine ve çözünmüş yağ gres örneklerine uygulanmıştır. Ayrıca, KOİ ve TOK arasındaki korelasyon araştırılmıştır. Zararlı atık tespitinde kullanılan tehlike kriterlerinden birisi olan toksisitenin belirlenmesi oldukça önemlidir. Toksik kirleticiler özellikleri itibari ile ekosistemi tehdit etmektedirler. Ayrıca, biyolojik arıtmayı inhibe edebilmektedirler. Bununla beraber yağ-gres örneklerine zehirlilik testleri uygulanmış, yöntem olarak ta *Vibrio fischeri* bakterilerini kullanan BioTox^{TM} testi kullanılmıştır. Yağların sudaki çözünebilir kısımlarının tespiti amacıyla su-yağ karışımlarının ekstraksiyonu Toxicity Characterization of Leaching Procedure (TCLP) metoduna bağlı kalınarak Zero Head Space Extractor (ZHE) ile elde edilen ve ekstraktların EC_{50} değerleri değerlendirilmiştir. Deneylerde ekstraksiyonlar için 3 solventle (tetrahidrofuran (THF), dimetilsülfoksit (DMSO) ve n-Hexane) çalışılmıştır. Bu çalışmada, kullanımı ve taşınması sırasında herhangi bir şekilde dökülüp çevreye karışabilecek bir adet motor yağı, kullanılmış motor yağı ve 6 numara fuel oilin yağ-gres içerikleri belirlenmiş ve bu yağ gres içeriklerinin KOİ ve toksisite eşdeğerleri saptanmıştır.

1. INTRODUCTION

The definiton of oil and grease includes compound classes with otherwise unrelated chemical structures, properties, and uses, including vegetable oils, petrochemical oils and volatile essential oils. The oil and grease content of domestic and certain industrial wastewaters which vary from petroleum refineries to otomotiv, metal, food, tannery, meat-packing, textile industries, and of sludges, is an important consideration in the handling and treatment of these materials for ultimate disposal. Besides, frequent oil spills result from accidents at sea and from the discharge of oil-laden bilge water as ships approach port to pick up new cargo, and oil and grease leaking from automobiles are considerable environmental problems.

In enviromental engineering, oil and grease in the water is equally undesirable because of gross pollution of land and water with their high biochemical oxygen demand (BOD) and chemical oxygen demand (COD). They can also be harmful to aquatic biota, fish food in water, and consequently fish themselves. Therefore, oil and grease may threat ecosystem with toxic characteristics. It is usually required to reduce the grease content of the industrial wastes below 100 mg per liter before it is discharged to a municipal system (Cammarota and Freire, 2006)

Chemical oxygen demand (COD) is a significant parameter commonly used for estimating the organic content of waters and wastewaters. It is known that high COD values of oily wastewaters are mainly originated from oil and grease content of the wastewater. As per date, alternative purification methods have been offered according to oil and grease concentration in wastewaters and their effects on high concentrations have been searched. Predominantly, COD and oil and grease (O&G) parameters are investigated individually. Even though COD and O&G interaction is obvious in oily wastewaters, there are few researches in literature except Wang et.al. (2003) about the correlation between these two parameters.

The organic carbon determination is free of the many variables such as organic solvents which plague the COD and BOD analyses with more reliable and

reproducible data being the net result. Total organic carbon analyses were applied to extraction solvents and water soluble fractions of oils. COD and TOC correlation is also investigated.

Another important parameter is toxicity which is one of the criteria used for determining hazardous waste. Toxic pollutants threaten the ecosystem with their characteristics. The toxicity of water soluble fractions of oil and grease is analyzed.

In this study, COD counterpart of oil and grease and its toxicity were determined with the help of series of experiments. Petroleum and mineral oil based samples were chosen. The BioTox^{TM} test using *Vibrio fischeri* a luminescent bacteria was used for the determination of toxicity. Extraction of the water soluble fractions of oils from aqueous medium were obtained by procedure proposed by Navas et.al. and the EC_{50} values of the extracts were evaluated. Correlations and interactions of O&G, COD and toxicity were assessed according to all analysis.

1.1. Aim and Scope

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

- Determining the collective parameters such as COD counterpart of Oil and Grease (O&G) which is a group parameter, and its toxicity
- Assesing oil and grease content of sample oils
- Determining of COD, TOC counterparts of water soluble fraction of oils and its toxicity
- Assesing correlations between COD and TOC parameters
- Applying COD and TOC analysis for solvents
- Evaluating of solvent extraction performance for oil and grease samples in toxicity analysis

2. OIL AND GREASE

2.1 General Consideration

Oil and grease is not a specific substance. Rather, it is a group of substances determined on the basis of their common solubility in an organic extraction agent. The terms grease and oil as used in wastewater treatment denote a variety of materials, including fats, waxes, free fatty acids, calcium and magnesium soaps, mineral oils and other nonvolatile materials that are soluble in and can be extracted by hexane from an acidified sample (Patnaik, 1997; Liu and Lipták, 1999).

Although a variety of extraction agents have been used for the estimation of oil and grease concentrations in wastewaters, including trichlorotrifluoroethane, n-hexane or a mixture of n-hexane and methyl-tert-butyl ether commonly is used, and oil and grease may be alternatively described as hexane extractable materials Among these solvents, freon has been extensively used in the oil and grease extraction. However, because of its ozone depletion action, the manufacture and use of Freon is currently being curtailed, and other solvents are now being used.

These substances that are classified under the above definition of oil and grease belong to both the biological lipids and the petroleum hydrocarbons and include straight chain and branched hydrocarbons, fatty acids, and the esters of fatty acids. Certain organic dyes, sulfur compounds, and chlorophyll are also extracted, and contribute to the measurement (Patnaik, 1997; USEPA, 2002).

They are also usually related to spills or other releases of petroleum products. Minor oil and grease problems can result from wet weather runoff from highways or the improper disposal in storm drains of motor oil. They are insoluble in water, but dissolve in organic solvents such as petroleum, chloroform, and ether. Fats and oils are contributed in domestic wastewater in butter, lard, margarine, and vegetable fats and oils. When these glycerides of fatty acids are liquid at ordinary temperature they are called oils, and those that are solids are called fats (Spellman, 2003).

2.2 Classification of Oil and Grease

USEPA differentiates between and establish separate classes for various types of oils, specifically: animal fats and oils and greases, and fish and marine mammal oils; oils of vegetable origin; petroleum oils, and other non-petroleum oils and greases. In differentiating between these classes of oils, Federal agencies are directed to consider differences in the physical, chemical, biological, and other properties, and in the environmental effects, of the classes (USEPA, 2002).

2.2.1 Oils

Oil is a general term that describes a wide variety of natural substances of plant, animal, or mineral origin, as well as a range of synthetic compounds. The many different types of oil are made up of hundreds of major compounds and thousands of minor ones. 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 and determine the effects of the oil on living organisms in the environment (Fingas, 2000).

Oily materials may be classified according to their properties. Physically, light hydrocarbons or solvents, such as kerosene or gasoline, are less viscous and more volatile than heavy hydrocarbons like tars and residual fuel oils. Chemically, some materials are classed as aliphatic, i.e. straight or branched chain, saturated or unsaturated. Still, others are classed as aromatic (unsaturated and with ring structure). They may have chemical functionality. Their structure may contain acid, carbonyl or other functional groups and have elements other than carbon, hydrogen and oxygen, such as nitrogen or sulfur (Liu and Lipták, 1999).

Oils are, of course, liquids. Fats and oils are actually esters of glycerol and fatty acids Examples of fats are butter, lard, and margarine; and examples of oils are the vegetable oils cottonseed oil, linseed oil, and palm oil. Fats and oils are abundant in meat and meat products (Sincero, 2003).

The machine tool industry uses considerable volumes of oil for lubrication and cutting. All mechanized industries require oil for lubrication. In the steel industry, fabricated metals are frequently dipped in oil to prevent rust during storage (Liu and Lipták, 1999).

2.2.2 Grease

In environmental engineering, waxes and complex lipids (fats, oils, and phospholipids) and mineral oils such kerosene, crude oil, and lubricating oil and similar products are collectively called *grease*. All these materials have a "greasy feel" and are associated with the problems in waste treatment related to grease. Grease is among the most stable of the organic compounds that, as such, is not easily consumed by microorganisms. Mineral acids can attack it liberating the fatty acids and glycerol. In the presence of alkali, glycerol is liberated, and the fatty acids, also are equally resistant to degradation by microorganisms (Sawyer, 2003; Sincero, 2003).

The term "grease" refers to the softer, lower melting inedible fats used for fatty acid production or for soapmaking. Inedible tallows and greases produced by meat packinghouses may contain either hog or beef fat. They are defined in terms of their hardness rather than their origin; a fat with a titer below 40ºC is a grease, and a fat with a titer over 40 ºC is an inedible tallow (Swern et al.,1979).

Meanwhile, grease is a solid to semisolid product that consists of a lubricating fluid that has been gelled with a thickening agent so that the lubricant can be retained more readily into the required area. Grease is used to prolong the life and increase the efficiency of mechanical devices by reducing friction and wear and there are specific performance requirements. Generally, a fluid lubricant (such as lubricating oil) is difficult to retain at the point of application and must be replenished frequently. On the other hand, a thickened fluid lubricant (grease) is easier to retain at the point of application, and lubrication intervals can be extended.

Grease varies in texture from soft to hard and in color from light amber to dark brown and, in contrast to liquid lubricants, will stay in place in a bearing assembly with comparatively elementary mechanical seals. Grease also assists in sealing against extraneous material and will lubricate without constant replenishment. Grease is, essentially, a two-phase system comprised of a liquid-phase lubricant (the liquid phase) containing a uniformly dispersed, finely divided thickener (the solid phase).

The largest volume of grease in use is made from petroleum products produced from naphthenic, paraffinic, blended, hydrocracked, hydrogenated, and solvent-refined

stocks. In addition to petroleum oils, other lubricating fluids, such as esters, diesters, silicones, polyethers, and synthetic hydrocarbons, are also used.

Grease is lubricating oil to which a thickening agent has been added for the purpose of holding the oil to surfaces that must be lubricated. The most widely used thickening agents are soaps of various kinds, and grease manufacture is essentially the mixing of soaps with lubricating oils.

The soaps used in grease making are usually made in the grease plant, usually in a grease making kettle. Soap is made by chemically combining a metal hydroxide with a fat or fatty acid Frequently a fat is separated into its fatty acid and glycerin components, and only the fatty acid portion is used to make soap. Commonly used fats for grease making soaps are cottonseed oil, tallow, lard, and degras. Among the fatty acids used are stearic acid (from tallow), oleic acid (from cottonseed oil), and animal fatty acids (from lard) (Speight, 2002).

2.3 Properties of Oil and Grease

Oil and grease can be characterized in three ways: by polarity, biodegradability, and physical characteristics. Polar greases and oils are normally derived from animal and vegetable materials, and are the characteristics form found in food-processing wastewaters. Nonpolar oil and greases are derived from petroleum or mineral sources. Generally, polar oils and greases are biodegradable while nonpolar forms are considered bioresistant (Patterson, 1985).

Most oils are insoluble in water, aiding in their separation. Furthermore, most insoluble oils are lighter than water, and therefore they float on its surface. A few oils are heavier than water, and these settle to the bottom of water. Wastewater treatment facilities must also make separate provisions to separate and collect these heavy oils. Oils that create the greatest problem are those whose density is close to that of water. These oils separate from water slowly—in some cases, too slowly for normal gravitational separation to be effective. These soluble oils can be naturally soluble but are rendered soluble or miscible due to man’s activities. Oils can be rendered miscible by adding detergents and emulsifiers, or using mechanical processes that result in homogenization. In all cases, soluble and miscible oils are in the same category for treatment(Liu and Lipták, 1999).

There are five categories to describe the physical forms of oil in wastewater (Patterson, 1985) :

I. *Free oil.* Rises rapidly to the surface under quiescent conditions.

II. *Mechanical dispersions.* Fine droplets ranging in size from microns to a few milimeters in diameter, which are stabilized by electrical charges or other forces but not through the influence or surface active agents.

III. *Chemically stabilized emulsions.* Oil droplets similar to mechanical dispersions but with enhanced stability resultingfrom surface active agents at the oil/water interface.

IV. *"Dissolved" oil.* Truly soluble species in the chemical sense plus very finely divided oil droplets (typically less than 5 µ diameter). This form generally defies removal by normal physical means.

V. *Oil-wet solids.* Oil adhered to the surface of particulate material in the wastewater.

2.4 Sources

2.4.1 Industrial Sources

One of the principal industrial sources of oily wastes is the petroleum industry. Oily wastes result from producing, refining, storing, or transporting operations, or in the use of this industry's products. Another major source is the metals industry. In the metals industry two major sources of oily wastes are steel manufacture and metal working. Oily wastes include both emulsified and free oils. In steel manufacture, stel ingots are rolled into desired shapes in either hot or cold rolling mills. Rinse and coolant waters from the cold rolling mills may contain several thousands mg/l oil, of which %25 or more may be emulsified and thus difficult to separate from the wastewater. Oily wastewaters from metal-working processes contain grinding oils, cutting oils, and lubrication fluids. Coolant oil-water emulsions are also emplyed in many metal-working processes (Patterson, 1985 ; Liu and Lipták, 1999).

In processing meat, fish and poultry, oily wastes are produced from cleaning, slaughtering, and processing by-products. A major source of oily wastes is the rendering process. Cooking plant tissues, seeds, grains, and nuts aids in extracting

their oils for commercial purposes; cooking and extraction processes result in oily wastewaters.

Most oily wastes in the textile industry result from scouring fibers in an early process step, especially scouring wool. The waste liquor yields valuable lanolin, but the wastewater is also high in extractables and difficult to process.

Oily wastes in the transportation industry result from leaks, spills, or cleaning operations. Tankers, barges, and tank trucks transporting oily materials must be cleaned to prevent possible product contamination. The cleaning solutions contain oily materials and create pollution if discharged without treatment.

Some oily materials are introduced into water systems during heating or cooling steps. Oily materials may be derived from leaks in seals, condensers, or heat exchangers from the process side of the equipment. When steam used for direct heating of fatty or oily products, the recovered condensate will likely be contaminated. Run-off from industrial areas following storms may be contaminated with oily materials. The rain washes processing units, walkways, buildings, and surrounding grounds, carrying away oily materials deposited there (Liu and Lipták, 1999).

2.4.2 Municipal Sources

The major sources of oily wastes are from food preparation, garbage disposal, and cleaning. Cleaning includes laundry, car washing, and general cleaning jobs where water is the main solvent and carrier. Grease and oily materials are removed at sewage treatment plants. Road oil and degraded asphalt are washed from roads into storm sewers and streams. Rainwater also contains soot and various hydrocarbons washed from the faces of buildings and other structures in communities (Liu and Lipták, 1999).

2.4.3 Natural Sources

Coniferous trees and shrubs contribute oily materials to run-off water, particularly in areas wooded with pine trees (Liu and Lipták, 1999).

2.5 Treatment Problems

The oil and grease content of domestic and certain industrial wastes, and of sludges, is an important consideration in the handling and treament of these materials for ultimate disposal. Oil and grease are singled out for special attention because of their poor solubility in water and their tendency to separate from the aqueous phase. Although this characteristic is advantageous in facilitating the separation of oil and grease by use of flotation devices, it does complicate the transportation of wastes through pipelines, their destruction in biological treatment units, and their disposal into receiving waters.

Wastes from the meat-packing industry, particularly where hard fats from the slaughtering of sheep and cattle are involved, and from restaurants have resulted in serious decreases in the carrying capacty of sewers. Such experiences, and other factors releated to treatment or ultimate disposal, have served as the basis for ordinances and regulations governing the discharge is permitted.

A number of problems are caused by oil and grease in waste treatment practice. Very few plants have provisions for the separate disposal of these materials to scavengers or by incineration; consequently, that which seperates as scum in primary settling tanks is normally transferred with the settled solids to disposal units (Sawyer, 2003).

The grease content of wastewater can cause many problems in wastewater treatment unit processes. For example, high grease content can cause clogging of filters, nozzles, and sand beds. Moreover, grease can coat the walls of sedimentation tanks and decompose and increase the amount of scum. Additionally, if grease is not removed before discharge of the effluent, it can interfere with the biological processes in the surface waters and create unsightly floating matter and films. In the treatment process, grease can coat trickling filters and interfere with the activated sludge process; this can interfere with the transfer of oxygen from the liquid to the interior of living cells (Spellman, 2003).

Separation of floating grease is final settling tanks has been a problem in some treatment plants employing high-rate processes. This has been attributed to short-term contact of the waste with limited amounts of biological growths that destroy the emulsifiyng agents present but do not have sufficient adsorptive powers to hold the

grease that is released, nor time to oxidize it. As a result, the grease is free to separate under quiescent conditions such as occur in final settling tanks or receiving waters.

All too frequent spills of crude and refined petroleum from ships used for their transport have resulted in loss of fish, mammals, and waterfowl, and the fouling of beaches. Such spills may result from accidents at sea or from the discharge of oil-laden bilge water as ships approach port to pick up new cargo. Oil and grease leaking from automobiles result in high concentrations in storm runoff from streets, contaminating waterways into which storm water drains. Thus, it is not only the oil and grease in wastewaters that are of environmental concern. In oceanic spills, detailed analyses of oil and grease components are commonly used to determine the source of spills, which many times are not readily apparent (Sawyer, 2003).

2.5.1 Treatment technologies

Oil in the water is equally undesirable. Optical detection methods for both types of contamination require regular, conscientious maintenance for continuous, reliable performance. Oil and grease cause gross pollution of land and water with their high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) and can also be toxic to aquatic biota, fish food in water, and fish themselves. It is usually required to reduce the grease content of the industrial wastes below 100 mg per liter before it is discharged to a municipal system. A large number of pretreatment systems (grease-trap, tilted plate separators, dissolved air flotation systems and physical–chemical treatment) are employed to remove oil and grease (O&G) from these wastewaters prior to the main treatment process itself, which is generally of a biological nature (Liu and Lipták, 1999; Cammarota and Freire, 2006).

One of the major purposes of waste treatment facilities is to remove unsightly and obnoxious floating matter, of which oil and grease are major constituents. Oil and grease determinations on raw and settled wastewasters give a measure of the effectiveness of primary settling tanks, and determinations on final effluents provide a record of the efficiency of secondary treatment units, as well as the amounts actually discharged to receiving waters. The latter is particularly important where disposal is into recreational areas (Sawyer, 2003).

Treatment of oily waste is similar in concept to treatment of domestic sewage. In domestic sewage treatment a primary level of treatment is employed to separate the

easily settleable solids fro the liquid-and in treatment of oily waste, primary treatment separates the floatable (free and nonemulsified) oils from the water and emulsified oil. A secondary treatment phase is then required to break the oil-water emulsion and separate the remaining oil and water. The performance of any oil-water separation process will depend on the distribution of the oil among its physical categories. Furthermore, since many oily wastes contain other constituents whose presence may have an impact on the treatment process performance, the successful treatment approach may be unique to the particular waste involved.

Where a significant fraction of the oil is adhered to the surface of settleable solids (oil-wet solids), simple gravity sedimentation as in a primary clarifier can result in appreciable reductions in the wastewater oil concentration(Patterson, 1985).

Density separation is a process whereby the water and contaminant are separated based on their individual densities. The most common oil–water separators are the American Petroleum Institute (API) gravity separators and the parallel-plate separators. The design of an oil–water separator is based on the amount of oil present in the water, the oil droplet size distribution, the presence of surfactants, the specific gravity of the oil, and the water temperature. Insoluble oils lighter than water can be easily separated in a settling tank with an adjustable skimming weir. These oils readily float to the surface, and the depth of the weir is adjusted according to the amount of oil in the water. Insoluble oils heavier than water can be recovered in a settling basin with a bottom sludge separator. The withdrawal rate of the sludge from the bottom must be proportional to the amount of heavy oil separated from these liquids. All coagulation techniques use the settling principle; the oil is rendered insoluble and heavier than the water so that it can be removed by settling with sludge removal. For coagulated solids, wastewater treatment facilities use filtration through various media (Liu and Lipták,1999).

Secondary treatment technologies include filtration and coalescence, ultrafiltration and reverse osmosis, chemical coagulation followed by air flotation or sedimentation, and electrical technologies. Biological and activated carbon treatment is also utilized for secondary oil and grease removal.

Dissolved air flotation (DAF) is normally considered a secondary level treatment technology, since in order to assure maximum effectiveness it is treatment aspects of

DAF are extremely important with respect to the removal of colloidal or emulsified oil (Patterson, 1985).

Oil and grease removals by DAF improve from 60 to 80 percent without chemical addition to 85 to 99 percent with chemical addition. There are many advantages to a DAF system, including its low installation costs, compact design, ability to accept variable loading rates, and low level of maintenance. The mechanical equipment involved in the DAF system is fairly simple, requiring limited maintenance attention for such things as pumps and mechanical drives (USEPA, 2002).

2.5.2 Environmental problems

Oily materials can be classified as to use, such as fuels, lubricants, coatings, cleaners, solvents, cutting and rolling fluids, hydraulic fluids, carriers, and cooking fats and oils. Many uses are both domestic and industrial. Light hydrocarbons and solvents are found in industrial waste streams, as a result of degreasing or extraction, cleaning, painting, and coating operations. Their vapors represent potential fire and explosion hazards, and their presence makes the removal of heavier oily materials more difficult. The pollution potential of all hydrocarbons is moderately high; however light hydrocarbons are more readily oxidized biologically than heavier fuels, tars, and residues. They are also a potential source of air pollution (Liu and Lipták, 1999).

The transportation of the oil and oil products by seaway involves significant environmental risks such as tanker accidents near the shoreline. 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).

Oil floating on water forms a mechanical barrier between the air and water, preventing oxygenation and killing oxygen- producing vegetation on the banks of streams. By coating the gills of fish, these materials prevent breathing and cause fish to suffocate. Therefore, ships and municipal and industrial waste treatment plants must monitor outfalls and control oil removal to prevent oil-bearing wastes from entering natural waves. Continuous monitors are available to detect any hydrocarbon floating on the surface of water (Liu and Lipták, 1999).

2.6 Oil And Grease Analysis

The term “oil and grease” refers to a broad class of organic substances recovered from the sample matrices by extraction with an appropriate solvent. Such recovery, therefore, is characteristic of certain physical properties of the compounds, primarily the volatility of the compounds and their solubility in the extraction solvent. The solvent must be immiscible in water and volatile, as well as readily distilled on a water bath. Many solvents or mixed-solvent systems should be suitable for the extraction of oil and grease in aqueous and nonaqueous samples. These include petroleum ether, *n*-hexane, methylene chloride, methyl *tert*-butyl ether, and trichlorotrifluoroethan (freon). These solvents are listed in Table 2.1(Patnaik, 1997).

Table 2.1: Extraction Solvents for Oil and Grease

Extraction Solvent	Not applicable to substances votalizing below	Solvent layer in separatory funnel
Trichlorotrifluoroethan (Freon).	70ºC	Lower
n-hexane	85ºC	Upper
n-hexane/methyl tertbutyl ether	85ºC	Upper
methylene chloride	70ºC	Lower
Petroleum ether	70ºC	Upper

Hexane is currently “Standard Methods” solvent of choice for oil and grease determinations because it is a good solvent for all the materials normally associated with the term grease and has a minimum solvent power for other organic compounds. While hexane has been the standard for this purpose for many years, it was replaced in recent times by 1,1,2-trichloro-1,2,2-trifluoroethane, commonly known as CFC-113, because it exhibited less of an explosion hazard. However, all chlorofluorocarbons (CFCs) including CFC-113, which are excellent solvents and refrigerants and were in the past widely used industrially and commercially, are responsible for the depletion of the beneficial ozone in the stratosphere. For this reason they are being phased out of commercial use. As a result, “Standard Methods” has returned to hexane, except in the partition-infrared procedure. Extraction

procedures that recover the solvent upon evaporation are of importance for environmental and safety reasons (Patnaik, 1997, Sawyer, 2003).

Samples are acidified with hydrochloric acid to a pH of about 1.0 to release the free acids for analysis. The reaction involved may be represented by the following equation:

$$Ca(C_{17}H_{35}COO) + 2H^{+} \rightarrow 2C_{17}H_{35}COOH + Ca^{2+} \quad \textbf{(2.1)}$$

Four different procedures are available for measuring oil and grease in water and wastewater samples. They all involve an initial extraction into hexane or CFC-113 (infrared procedure only). In the partition – gravimetric method, the hexane is then separeted from water and evaporated, the residue remaining being used as a measure of the oil and grease content. In the partition – infrared method, the CFC-113 extracted materials are measured with infrared scanning. The accuracy of this procedure depends upon the use of oil and grease standards for calibration that are similar in composition to the oil and grease in the samples being analyzed. The advantage over the gravimetric procedure is speed of analysis. The third, or Soxhlet extraction, procedure involves an initial step of acidification and filtration to remove oil and grease from the aqueous phase, and then hexane extraction. This procedure tends to retain more of the volatile hydrocarbons than the gravimetric procedure, but is more time intensive.

Filtration is considered acceptable procedure, since it effectively separates those materials normally referred to as oil and grease and allows low-molecular-weight and soluble materials, which are of no consequence, to escape in the filtrate. Drying of he filtered material removes water so that the solvent can penetrate the sample readiliy and accomplish separation of the grease in the 4-h extraction period normally provided. It also eliminates the possibility of appreciable amounts of water being carried into the extract and thereby simplifies the drying procedure. A soxhlet type of extractor that provides intermittent batchwise extraction is used (equation 2.1).

A fouth standard method for water and wastewater is designed to more selectively determine hyrdrocarbons associated with petroleum products and to exclude fatty acids and other fatty materials that are more associated with animal and vegetable materials. Thus this is a *hydrocarbon* analysis, rather than an oil and grease analysis. Here, silica gel is added to the hexane extract and selectively removes the fatty

materials, the remaining materials in the hexane solvent are then analyzed by any of the first three procedures (Sawyer, 2003).

2.6.1. Gravimetric method

The aqueous sample is acidified to pH 2 or lower with 1:1 HCl. A measured volume of sample (1 L) is transferred to a separatory funnel. A 30-mL portion of extracting solvent is added to the sample. The content are shaken vigorously for 1 to 2 min. The organic layer separated is collected in a distillation flask. The aqueous layer is reextracted two more times using 30 mL portion of solvent each time. The extracts are combined together in tared (or weighted) distillation flask. The solvent is distilled from the flask on a water bath. After all the solvent distills out, the residue in the flask is dried under air flow and vacuum. The distillation flask containing the oil and grease residue is cooled in a desiccator and weighed to a constant weight (Patnaik, 1997).

2.6.2. Infrared method

The extraction of oil and grease by infrared method is same as above for the gravimetric method. The only difference, however, is that the solvent extract is not evaporated nor is the solvent distilled out. The absorbance of the solvent extract is measured at 2930 cm using a 1 cm path-length cell and compared against the calibration standards solutions. Thus, oil and grease measured by both the methods are susceptible to show variation. While gravimetric method measures “all” substances that are solvent extractables and “nonvolatile” under the conditions of distillation and drying, infrared method measures the absorbance of carbon-hydrogen bond of substances extracted. Also, compounds boiling below the distillation temperature of the extraction solvent may occur in the extract and contribute to oil and grease measured by the infrared method (Patnaik, 1997).

2.6.2. USEPA method 1664

A 1-L sample is acidified to pH <2 and serially extracted three times with n-hexane in a separatory funnel. The extract is dried over sodium sulfate. The solvent is distilled from the extract and the n-hexane extractable material (HEM) is desiccated and weighed. If the HEM is to be used for determination of silica gel treated SGT-HEM, the HEM is redissolved in n-hexane. For SGT-HEM determination, an amount

of silica gel proportionate to the amount of HEM is added to the solution containing the redissolved HEM to remove polar materials. The solution is filtered to remove the silica gel, the solvent is distilled, and the SGT-HEM is desiccated and weighed. Quality is assured through calibration and testing of the extraction, distillation, and gravimetric systems.

HEM and SGT-HEM are method-defined analytes; i.e., the definitions of both HEM and SGT-HEM are dependent on the procedure used. The nature of the oils and/or greases, and the presence of extractable non-oily matter in the sample will influence the material measured and interpretation of results.

Glassware is cleaned by washing in hot water containing detergent, rinsing with tap and distilled water, and rinsing with solvent or baking. Boiling flasks that will contain the extracted residue are dried in an oven at 105–115ºC and stored in a desiccator. Sodium sulfate and silica gel fines have the potential to inflate results for HEM and SGTHEM by passing through the filter paper. If the filter paper specified in this method is inadequate for removal of these fines, use of a 0.45-micron filter is recommended.

Interferences extracted from samples will vary considerably from source to source, depending upon the diversity of the site being sampled. For those instances in which samples are thought to consist of complex matrices containing substances (such as particulates or detergents) that may interfere with the extraction procedure, a smaller sample may need to be collected for analysis (USEPA, 1999).

3. SIGNIFICANT ORGANIC PARAMETERS

3.1. General Information

It is essential that the nature and characteristics of the wastewater be evaluated with respect to the unit processes considered. When one speaks of pollution,, organic and oxygen demanding substances are of immediate concern. However, the single or conjunctive use of many parameters, both organic and inorganic, may be necessary to provide the proper analysis of a wastewater (Adams et.al, 1981).

Oxygen demand is an important parameter for determining the amount of organic pollution in water. The test has its widest application in measuring waste loadings of treatment plants and in evaluating the efficiency of treatment processes. Other applications include testing lake and stream water samples for organic pollution. Oxygen demand testing does not determine the concentration of a specific substance; rather, it measures the effect of a combination of substances and conditions. Because oxygen demand is not a pollutant, it poses no direct threat to fish or other life. It can, however, pose an indirect threat to living organisms by reducing the level of dissolved oxygen. There are three widely-used methods of measuring oxygen demand. Two measure oxygen demand directly: Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). A third method— Total Organic Carbon (TOC)—measures oxygen demand indirectly (Boyles, 1997).

The oxygen demand of a water sample is the amount of elemental oxygen required to react with oxidizable or biodegradable material, dissolved or suspended in the sample. This amount is expressed as milligrams of oxygen per liter of sample. When the agent required to effect the oxidation reaction is a population of bacteria, the oxygen required is called BOD. When the oxidation is carried out with a chemical oxidizing reagent such as potassium dichromate, the oxygen equivalent is called the COD. Other means also effect the oxidation of material in a water sample, including heating the sample in a furnace in the presence of oxygen, TOD, or in the presence of carbon dioxide, resulting in a total carbon dioxide demand (TCO_2D) measurement.

The BOD test is the most important oxygen demand measurement for analyzing effluents and receiving waters (streams, lakes, and rivers). The BOD test measures the amount of oxygen used by microorganisms feeding on organic water pollutants under aerobic conditions. In this test, a bacterial culture is added to the sample under well-defined conditions, and oxygen utilization is measured. Although test procedures are carefully defined, obtaining reproducible results is difficult and the procedure is subject to the influence of many variables, particularly when the wastewater contains a variety of complex materials. Factors contributing to variations in BOD results are:

• Biological seed used

• pH if not near neutrality

• Temperature if other than 20°C

• Sample toxicity

• Incubation time

When the incubation time and temperature are not stated, the general assumption is that the test was run at 20°C for a period of 5 days (Liu and Liptak, 1999).

The presence of toxic materials in the wastewater sample may have a bio-toxic or bio-static effect on the seed microorganisms. This effect is usually evidenced by "sliding" BOD values, where the BOD magnitudes increase with increasing sample dilutions. This is indicative of the presence of toxic materials, and it is therefore necessary to predetermine the dilution value above which the BOD yields are consistent (Adams et.al, 1981).

3.2. Chemical Oxygen Demand

The chemical oxygen demand (COD) is a measure of the oxygen equivalent of the organic fraction in the sample which is susceptible to permanganate or dichromate oxidation in an acid solution. This parameter has been used for over a quarter of a century in estimating the organic content of waters and wastewaters. However, correct interpretation, of COD values is still a problem and one must understand those variables which affect the COD value of the sample in question(Adams et.al, 1981).

Interpretations of COD values are difficult since this method of oxidation is markedly different from the BOD method. Although ultimate BOD values can agree with COD values, a number of factors can prevent this concordance including:

1. Many organic materials are oxidizable by dichromate but not biochemically oxidizable and vice versa. For example, pyridine, benzene, and ammonia are not attacked by the dichromate procedure.
2. A number of inorganic substances such as sulfide, sulfites, thiosulfates, nitrites, and ferrous iron are oxidized by dichromate creating an inorganic COD that is misleading when the organic content of wastewater is estimated. Although the seed acclimation factor gives erroneously low results on BOD tests, COD results do not depend on acclimation.

3. The BOD results may be affected by lack of seed acclimation, giving erroneously low readings. The COD results are independent of this variable.

4. Certain organic compounds (e.g. straight chain, saturated aliphatic acids and alcohols) are not efficiently oxidized by $Cr_20_7^{2-}$. A silver sulfate catalyst is added to ensure efficient oxidation of these compounds.

5. Chlorides interfere with the COD analysis and their effect must be minimized for consistent results. The standard procedure provides for a limited amount of chlorides in the sample. Despite these limitations, the dichromate COD is useful in the control of wastewater effluents containing caustic and chlorine, dyeing and textile effluents, organic and inorganic chemicals, paper, paints, plating, plastics, steel, aluminum, and ammonia. Erroneously high readings will occur by the oxidation of chlorides by dichromate:

$$6Cl^- + Cr_2O_7^{2-} + 14H^+ \rightarrow 3C1_2 + 2Cr^{3+} + 7H_2O \qquad \textbf{(3.1)}$$

This interference can be eliminated by the addition of $HgSO_4$ to the mixture. Hg^{2+} combines with Cl^- to form the poorly dissociated complex, $HgC1_2$, which is not oxidized by $Cr_2O_7^{2-}$.

$$Hg^{2+} + 2C1^- \rightarrow H_gCl_2\ (_{aq}) \qquad \textbf{(3.2)}$$

For Cl^- concentrations of greater than 1,000 mg/l, it is recommended that the standard and blank determinations be performed with identical Cl^- concentrations to the

sample. A stock solution of NaCI can be used to spike the standard and blank sample. If insufficient amounts of $HgSO_4$ are added (a 10:1 weight ratio of $HgSO_4$:Cl^- is recommended in *Standard Methods*), the excess Cl^- will precipitate with the silver catalyst as follows:

$$Ag + Cl^- \rightarrow AgCl_{(S)} \quad \textbf{(3.3)}$$

and incomplete and unpredictable oxidation of $AgCl_{(S)}$ by $Cr_20_7^{2}$, takes place (Adams et.al, 1981, Liu and Liptak, 1999).:

There are two methods commonly used for COD analysis. These are:

1. A two-hour reflux time Dichromate Oxidation; and
2. A rapid COD test.

3.2.1. Two-hour reflux time dichromate oxidation

This method uses potassium dichromate as the oxidant. An Ag_2SO_4 catalyst is used and $HgSO_4$ is added to complex chloride. The sample and oxidant are digested under reflux in a 50 percent v/v sulfuric acid solution for two hours. The excess dichromate is titrated with ferrous ammonium sulfate. The extent of oxidation of organics in this test is affected by the reflux time. The exact effect of reflux time on dichromate oxidation depends on the nature of the wastewater. The final precision of the COD value depends on reflux time for particular wastewaters. Consequently, with unfamiliar or complex wastes, it is recommended that the reflux time be checked to determine its influence on final results (Adams et.al, 1981).

3.2.2. Rapid COD test

A COD test using a shortened digestion time has already been proposed. An aliquot of wastewater sample is added to a dichromateacid-silver solution and mixed. The contents are heated to 165°C and digested for 15 minutes. The sample is then diluted with distilled water and titrated with ferrous ammonium sulfate. Using this method the organics in domestic sewage are oxidized to about 66 percent of the value obtained using the twohour reflux test. This yield may be higher or lower for other wastes, depending on the nature of the organic constituents (Adams et.al, 1981).

3.3. Total Organic Carbon

TOC is the acronym for total organic carbon. The first TOC methods were developed to correlate information obtained from chemical oxygen demand (COD) and biochemical oxygen demand (BOD) tests in drinking and wastewater. The TOC methods were designed to be more efficient than the COD and BOD tests, which required the use of hazardous chemicals and multiple days to complete. Today new government regulations are making TOC analysis a standard test for all industry water types. The water's characteristics such as hardness, particulates, bacterial levels, conductivity, and TOC levels significantly influence downstream processing. Measurement of TOC is a direct reflection on the quality of the water being produced (Cooper, 2002).

The recently developed carbon analyzer has provided a rapid and simple means of determining organic carbon levels in aqueous samples, enhancing the popularity of TOC as a fundamental method of analysis. The organic carbon determination is free of the many variables which plague the COD and BOD analyses, with more reliable and reproducible data being the net result (Adams et.al, 1981).

It is measured by oxidizing the dissolved organic carbon (DOC) and quantitating the evolved carbon dioxide. One way to do this is to sweep the released CO_2 into a nondispersive IR (NDIR) photometer that has been tuned to measure CO_2. This is accomplished by measuring the absorbance of the characteristic C–O stretching vibration. Two analytical approaches to the quantitative determination of TOC for aqueous samples that contain DOC have emerged over the years: oxidation of carbon to CO_2 via high-temperature catalyzed combustion and persulfate–ultraviolet irradiation.

A TOC measurement involves oxidizing organic carbon in an aqueous sample, detecting and quantifying the oxidized carbon as CO_2, and presenting the results in terms of the mass of carbon per unit volume of the aqueous sample (Loconto, 2006).

UV-promoted persulfate oxidation involves exposing an aqueous sample to persulfate ions and UV radiation. This produces highly reactive sulfate and hydroxyl free radicals. The CO2 produced from the persulfate oxidation reactions is swept by a stream of inert carrier gas, such as nitrogen, to the NDIR detector. IC and POCs are removed by acidification and sparging in the IC sparger. An aliquot of the sparged

sample is then transferred to the UV reactor and persulfate reagent is added to oxidize the organic carbon. The *high-temperature combustion* (HTC) technique uses heat (680°C or higher), in the presence of a titanium dioxide-based platinum catalyst, with a stream of hydrocarbon free compressed air or oxygen to oxidize organic carbon. DOC and particulates that contain carbon fully oxidize to CO_2 under these conditions. Following IC removal, an aliquot of the sparged aqueous sample is transferred to the combustion furnace to oxidize the organic carbon to form CO_2. The catalytic combustion oxidation products are continuously swept through the NDIR detector, which is selective to CO_2 and whose analog output signal is proportional to the concentration of CO_2 in the carrier gas, and thus in the original sample (Loconto, 2006).

The total organic carbon concentration in a wastewater is a measure of organic content. While TOC measurements give no indication of the oxidation state of the carbon, correlations can often be made between TOC and occasionally BOD values for individual wastes. Because the analysis time using the carbon analyzer is only several minutes, the efficacy of using this parameter is apparent, particularly when a TOC-BOD or TOC-COD correlation can be established (Adams et.al, 1981).

3.4. Total Oxygen Demand

Another recently developed analyzer has provided a means for rapidly determining the oxygen demand of a sample rather than its carbon content.-The measurement is obtained by continuously monitoring the oxygen content of a mixed N_2/O_2 gas stream. This gas mixture flows through a platinum catalyzed combustion chamber, where the oxidizable constituents of the sample are con- verted to stable oxides by the O_2. The depletion of O_2 is measured in an electro- lytic detector cell and is directly related to the oxygen demand of the sample.

The total oxygen demand (TOD) of a substance as measured by this analyzer includes organic and inorganic substances, but at varying reaction efficiencies. The chemical reactions that apparently take place in the apparatus are as follows (Adams et.al, 1981):

1. Carbon is converted to carbon dioxide;
2. Hydrogen is converted to water;

3. Nitrogen in a -3 valence state is converted to nitric oxide;

4. The sulfite ion is partially converted to sulfate; and,

5. The sulfide ion is partially converted to sulfate.

The reaction efficiencies for each of the oxidations are reported in Table 3.1. It should be recognized that nitrates cause significant interference in the TOD analysis by providing oxygen to the carrier gas (Adams et.al, 1981).

Table 3.1 : Total Oxygen Demand Reaction

Reaction	Highest Stable Oxidation State	Reaction Efficiency (%)
$C + O_2$	CO_2	95-100
$2H_2 + O_2$	$2H_2O$	95-100
$2N^{3-} + O_2$	$2NO$	≈ 95
$S^{2-} + O_2$	SO_4^{2-}	≈ 78
$SO_3^{2-} = + ½ O_2$	SO_4^{2-}	≈ 72

3.5. Comparative Analysis of the Organic Parameters

Many regulatory agencies recognize only the BOD or COD measurements of the pollution load as the basis for pollution control. They are concerned with the pollution load on the receiving water, which is related to lowering the dissolved oxygen (DO) due to bacterial activity. When considering the analysis of industrial wastes, it is imperative to evaluate the BOD and COD values for various classes of compounds. The BOD values for many pure organic compounds common to industrial discharges have been reported. The COD and BOD values and the fractions of the theoretical oxygen, demand (ThOD) represented by these values for alphatics, aromatics, nitrogenous organics and refractories are presented in Table 3.2. The degree to which the COD and BOD tests reflect the theoretical oxygen demand be evaluated from this table. In attempting to correlate BOD and COD of an industrial waste with TOC, one should recognize those factors which might constrain or negate the correlation. These factors include:

Table 3.2 : COD, BOD and Theoretical Oxygen Demand For Test Organic Chemicals

Chemical Group	Th. OD (mg/mg)	Measured COD (mg/mg)	COD Th.OD (%)	Measured BOD_5 (mg/mg)	BOD_5 Th.OD (%)
ALIPHATICS					
Methanol	1.50	1.05	70	1.12	75
Ethanol	2.08	2.11	100	1.58	76
Ethylene glycol	1.26	1.21	96	0.36	29
Isopropanol	2.39	2.12	89	0.16	7
Maleic acid	0.83	0.80	96	0.64	77
Acetone	2.20	2.07	94	0.81	37
Maleic ethylketone	2.44	2.20	90	1.81	74
Ethyl acetate	1.82	1.54	85	1.24	68
Oxalic acid	0.18	0.18	100	0.16	89
Group Average			91		56
ALIPHATICS					
Toluene	3.13	1.41	45	0.86	28
Benzaldehyde	2.42	1.98	80	1.61	67
Benzoic acid	1.96	1.95	100	1.45	74
Hydroquinone	1.89	1.83	100	1.00	53
o – Cresol	2.52	2.38	95	1.75	70
Group Average			84		58
NITROGENOUS ORGANICS					
Monoethanolamine	2.49	1.27	51	0.83	34
Acrylonitrile	3.17	1.39	44	nil	0
Aniline	3.18	2.34	74	1.42	44
Group Average			58		26
REFRACTORY					
Tetriary – butanol	2.59	2.18	84	0	0
Diethylene glycol	1.51	1.06	70	0.15	10
Pyridine	3.13	0.05	2	0.06	2
Group Average			52		4

1. A portion of the COD of many industrial wastes is attributed to the dichromate oxidation of ferrous iron, nitrogen, sulfites, sulfides, and other of these compounds;

2. The BOD and COD tests do not include many organic compounds which are partially or totally resistant to biochemical or dichromate oxidation. However, all of the organic carbon in these compounds is recovered in the TOC analysis; and,

3. The BOD test is susceptible to variables which include seed acclimation, dilution, temperature, pH, and toxic substances (Adams et.al, 1981).

Researchers in an interlaboratory comparative study employing a synthetic waste found standard deviations around the mean of 620% for BOD and 610% for COD. Another extensive study (Ford, Eller, and Gloyna 1971) made the following conclusions:

1. A reliable statistical correlation between wastewater BOD and COD and the corresponding TOC or TOD can frequently be achieved, particularly when organic strength is high and diversity in dissolved organic constituents is low.

2. The relationship is best described by a least squares regression with the degree of fit expressed by the correlation coefficient—this relationship applies to the characterization of individual chemical-processing and oil-refining wastewaters, not to all types of samples across the board.

3. The observed correspondence COD–TOD was better than COD–BOD for the wastewater mentioned (generally, correlating BOD with TOD was difficult, particularly when the wastewater contained low concentrations of complex organic materials)

4. The BOD–COD or BOD–TOC ratios of untreated wastewater indicate the biological treatment possible with wastewater. As these ratios increase, higher organic removal treatment efficiencies occur by biological methods.

Several papers indicate high correlation between BOD and other methods. This correlation is achieved when the nature of the pollutant is constant and only its amount changes. For complex and varying mixtures, obtaining good correlations is difficult (Liu and Liptak, 1999).

One would expect the stoichiometric COD/TOC ratio of a wastewater to approximate the molecular ratio of oxygen to carbon (32/12 = 2.66). Theoretically, the ratio limits would range from zero, when the organic material is resistant to dichromate oxidation, to 5.33 for methane or slightly higher when inorganic reducing agents are present. The BOD/TOC ratio of an industrial waste would be subject to many of the aforementioned variables and could not be expected to follow any particular pattern. This is underscored by the variability between the calculated and measured COD/TOC values for various compounds in Table 3.3.

Table 3.3: COD-TOC Relationships

Substance	COD/TOC (Calculated)	COD/TOC (Measured)
Acetone	3.56	2.44
Ethanol	4.00	3.35
Phenol	3.12	2.96
Benzene	3.34	0.84
Pyridine	3.33	nil
Salicylic Acid	2.86	2.83
Methanol	4.00	3.89
Benzoic Acid	2.86	2.90
Sucrose	2.67	2.44

This variability is attributed to the COD yield of the compounds, and wastestreams containing a portion of these substances would be subjected to a fluctuating COD/TOC ratio in the event of component concentration changes. The greater the variability in the character of an industrial wastestream, the more pronounced will be the change in its COD/TOC ratio. This in itself is a good indicator of the degree of consistency of wastewater constituents and can be a valuable aid in predicting the design organic load applied to a biological treatment facility.Reported COD, and TOC values for several industrial wastewaters are listed in Table 3.4, the COD/TOC ratio varying from 1.75 to 6.65. It has been difficult to correlate BOD with TOC for industrial wastes. (Adams et.al, 1981).

Table 3.4: Oxygen Demand and Organic Carbon Of Selected Industrial Wastewater

Type of Waste	COD (mg/l)	TOC (mg/l)	COD/TOC
Chemical*	4,260	640	6.65
Chemical*	2,440	370	6.60
Chemical*	2,690	420	6.40
Chemical	576	122	4.72
Chemical	41,300	9,500	4.35
Chemical-Refinery	580	160	3.62
Petrochemical	3,310	900	3.32
Chemical	1,900	580	3.28
Chemical	1,400	450	3.12
Chemical	17,500	5,800	3.02
Chemical	78,000	26,020	3.00
Chemical	143,000	48,140	2.96
Chemical	165,000	58,000	2.84
Chemical	15,000	5,500	2.72
Nylon Polymer	23,400	8,800	2.70
Petrochemical	--	--	2.70
Nylon Polymer	1 12,600	44,000	2.50
Olefin Processing	321	133	2.40
Butadiene Processing	359	156	2.30
Chemical	350,000	160,000	2.19
Synthetic Rubber	192	110	1.75

*= high concentration of sulfites

In summary, it can be stated that TOC and TOD are both valid measures of the organic character and both can be correlated to COD values in many applications. These are extremely good control parameters for most waste- waters because of the abbreviated analysis time associated with the respective analyzers. It is less probable that TOD, TOC, or COD can be correlated to BOD unless the constituents in the wastewater remain relatively constant. The conjunctive use of these parameters in terms of COD, TOC, and TOC ratios can be helpful in properly evaluating the organic nature of an unknown waste. The relationship between the aforementioned parameters in terms of accuracy (percent of theoretical oxygen demand or carbon concentration) is illustrated in Figure 2.1 (Adams et.al, 1981).

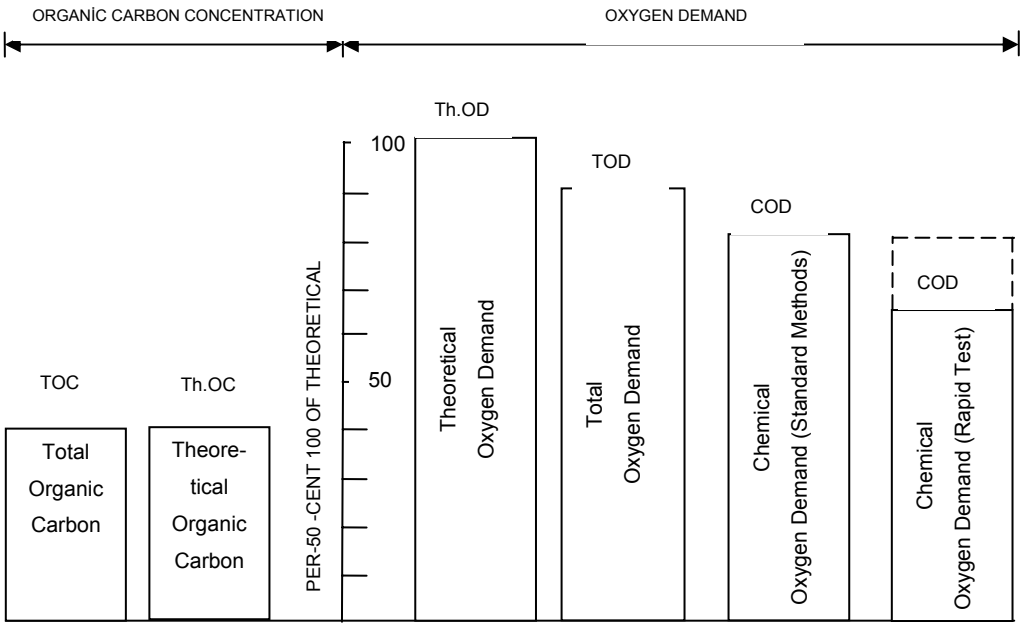


Figure 3.1: Relationship Between Oxygen And Carbon Parameters

3.6. Miscellaneous Organic Parameters

As attention has been focused on the TOD, TOC, COD and BOD parameters, it is necessary to recognize the importance of specific organic analyses such as oil and grease content, phenols, and organics containing toxic functional groups. Oil and phenol analyses are particularly significant when evaluating unit processes for the treatment of chemical, petrochemical, and refinery wastewaters (Adams et.al, 1981).

4. TOXICOLOGY

4.1. General Information

Toxicology is a branch of science that deals with poisons, and a poison can be defined as any substance that causes a harmful effect when administered, either by accident or design, to a living organism. Many complications exist beyond this simple definition, both in bringing more precise meaning to what constitutes a poison and to the measurement of toxic effects. Broader definitions of toxicology, such as "the study of the detection, occurrence, properties, effects, and regulation of toxic substances," are more descriptive. The study of toxicology serves society in many ways, not only to protect humans and the environment from the deleterious effects of toxicants but also to facilitate the development of more selective toxicants such as anticancer and other clinical drugs and pesticides

Toxicity itself can rarely, if ever, be defined as a single molecular event but is, rather, a cascade of events starting with exposure, proceeding through distribution and metabolism, and ending with interaction with cellular macromolecules (usually DNA or protein) and the expression of a toxic end point (Hodgson, 2004).

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).

There are numerous variables related to the ways in which organisms are exposed to toxic substances. One of the most crucial of these, dose. Another important factor is the toxicant concentration, which may range from the pure substance (100%) down to a very dilute solution of a highly potent poison. Both the duration of exposure per exposure incident and the frequency of exposure are important. The rate of exposure and the total time period over which the organism is exposed are both important situational variables. The exposure site and route also affect toxicity (Manahan, 2003)

4.1.1 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 (Lu and Kacew, 2002).

Because of its broad scope as well as the need to accomplish different goals, toxicology has a number of subdisciplines. If the identity of the exposed toxicant is not known, *analytical toxicology* will be called upon to identify the toxicant through analysis of body fluids, stomach contents, etc. Those engaged in *clinical toxicology* will administer antidotes, if available, to counter the specific toxicity, and take other measures to ameliorate the symptoms and hasten the elimination of the toxicant from the body. There may also be legal implications, and that will be the task of *forensic toxicology*. Intoxication may occur as a result of occupational exposure to toxicants, which is in the domain of *occupational toxicology*. 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. Regulatory toxicology* attempts to protect the public by setting laws, regulations, and standards to limit or suspend the use of very toxic chemicals (Lu and Kacew, 2002).

Economic toxicology is the study of chemicals that are developed expressly for the purpose of improving economic gain by selectively eliminating a species

(insecticides and herbicides), by improving health and productivity (drugs), by preserving foodstuffs (food additives), or for the manufacture of a marketable product (industrial solvents, cleaning agents, etc.) (Philip, 2000).

Environmental toxicology deals with the effects of environmental toxicants on health and the environment. Environmental toxicants are agents released into the general environment that can cause adverse effects on the health of living organisms, including humans, animals, and plants. The study of environmental toxicology stems from the recognition that (a) human survival depends on the well-being of other species and on the availability of clean air, water, and food; and (b) anthropogenic chemicals as well as naturally occurring chemicals can cause detrimental effects on living organisms and ecological processes (Yu, 2005).

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.). Assessing the toxic effects of chemicals on humans involves the use of standard animal models (i.e., mouse and rat) as well as epidemiological evaluations of exposed human populations (i.e., farmers and factory workers) (Hodgson, 2004).

Ecotoxicology has not generally included the fields of industrial and human health toxicology or domestic animal and agricultural crop toxicology, which are not part of natural ecosystems, but are rather imposed upon them. Yet there is a growing belief by some that humanity and its artifacts should be regarded as components of natural systems, not apart from them.

Ecotoxicology employs ecological parameters to assess toxicity. In a more restrictive but useful sense, it can be defined as 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 a branch of the science of ecotoxicology that is multidisiplinary in scope and interdisiplinery in practice. that is multidisiplinary in scope and interdisiplinery in practice. *Aquatic toxicology* is the study of the effects of manufacturated chemicals and other anthropogenic and natural materials and

activities (collectively termed toxic agents or substances) on aquatic organisms at various levels of organization, from subcellular through individual organisms to communities and ecosystems.

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 (Rand, 1995).

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).

4.1.2 Kinds Of Toxic Substances

Toxic substances come in a variety of forms from a number of different sources. Those that come from natural sources are commonly called **toxins** , whereas those produced by human activities are called **toxicants** . They may be classified according to several criteria, including the following:

• Chemically, such as heavy metals or polycyclic aromatic hydrocarbons, some of which may cause cancer

• Physical form, such as dusts, vapors, or lipid-soluble liquids

• Source, such as plant toxins, combustion by-products, or hazardous wastes produced by the petrochemical industry

• Use, such as pesticides, pharmaceuticals, or solvents

• Target organs or tissue, such as neurotoxins that harm nerve tissue

• Biochemical effects, such as binding to and inhibiting enzymes or converting oxygen-carrying hemoglobin in blood to useless methemoglobin

• Effects on organisms, such as carcinogenicity or inhibition of the immune system Usually several categories of classification are appropriate. For example, parathion is an insecticide that is produced industrially, to which exposure may occur as a mist from spray, and that binds to the acetylcholinesterase enzyme, affecting function of the nervous system. Since toxicological chemistry emphasizes the chemical nature of toxic substances, classification is predominantly on the basis of chemical class. Therefore, there are separate chapters on elemental toxic substances, hydrocarbons, organonitrogen compounds, and other chemical classifications of substances(Manahan, 2003).

4.2 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, involving a particular subcellular structure. A variety of structures may be affected (Lu and Kaew, 2002).

Toxicity can be divided into the broad categories; direct and indirect. Direct toxicity results from the toxic agent acting more or less directly at sites of action and/or on organisms; indirect toxicity occurs because of the influence of changes in the chemical, physical and/or biological environment (e.g. changes in the quality and/or biological environment organisms or habitat changes and/or losses). Although most indirect toxicity on a population or community may be tracked back to direct toxicity in a particular group and species, this is not always the case. Most experimental toxicology studies have been concerned with direct toxicity to individual species. The direct toxicity information gained is then used to estimate indirect effects or interpret site-specific situations (Rand, 1995).

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. These include carcinomas, mutations, damage to neurons, and liver cirrhosis. Certain effects are considered irreversible even though they dissappear some time after cessation of exposure. The effect produced by a toxicant may be reversible if the organism is exposed at a low concentration and/or for a short duration, whereas irreversible effects may be produced at higher concentration and/or for longer durations of exposure.

Local effect occur at the primary site of contact. An example of a local effect is a skin or gill reaction (e.g., discoloration, inflammation, or erosion) in fish exposed to various organic and inorganic compounds. *Systemic effects* result only after the toxicant has been absorbed and distributed to other parts of the body. Most toxicants exert their main effects on one or a few organs. These organs are referred to as the "target organs" of the toxicants (Rand, 1995, Lu and Kaew, 2002).

Many toxicants produce *immediate toxic effects*, which develop shortly after a single exposure, a notable example being cyanide poisoning. *Delayed effects* occur after a lapse of some time. *Carcinogenic effecs* generally become manifest 10-20 years after the initial exposure in humans; even in rodents, a lapse of many months is required. To detemine these and other delayed effects of toxicants, long term studies are essential.

Morphologic effects refer to gross and microscopic changes in the morphology of the tissues. Many of these effects, such as necrosis and neoplansia, are irreversible and serious. *Functional effects* usually represent reversible changes in the functions of target organs. Functions of the liver and kidney (e.g. rate of excretion of dyes) are commonly tested in toxicology studies. 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. However, the results are often more variable. Although all toxic effects are associated with biochemical alterations, in routine toxicity testing, " *biochemical effects*" usually refer to those without apparent morphologic changes. (Lu and Kacew, 2002)

Modifying factors of toxic effects

While toxicity is an inherent property of a substance, the nature and extent of the toxic manifestations in an organism that is exposed to the substance depend on a variety of factors. The obvious ones are the dose and duration of exposure.

They also include such less obvious factors as the species and strain of the animal its sex and age, and its nutritional and hormonal status. Various environmental (physical and social) factors also play apart. In addition, the toxic effect of a chemical may be influenced by simultaneous and consecutive exposure to other chemicals. (Lu and Kacew, 2002)

Species or the size of the organism reacting to the toxicant could be a biotic modifying factor. Physicochemical entitles such as the pH or the temperature of the water could be abiotic modifying factors. The environmental or abiotic entitles should properly be called masking factors defined by Fry as an identity which modifies the operation of a second identity on organism (Rand, 1995).

4.3 Factor That Influence Toxicity

4.3.1 Factors related to exposure

Characteristics such as whether a pollutant is solid, liquid, or gas; whether the pollutant is soluble in water or in lipid; organic or inorganic material; ionized or nonionized, etc., can affect the ultimate toxicity of the pollutant. One of the most important factors affecting pollutant toxicity is the concentration of the pollutant (Landis and Yu, 1998).

Toxicant concentration, may range from the pure substance (100%) down to a very dilute solution of a highly potent poison. The concentrationand time required to produce an adverse effect vary with the chemical, species of organism, and severity of the effect. The contact-reaction between the organism and the chemical is called *exposure.* In the assessment of toxicity the most significant factors related to exposure are the kind, duration, and frequency of exposure and the concentration of the chemical. The rate of exposure and the total time period over which the organism is exposed are both important situational variables.(Rand, 1995, Manahan, 2003).

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).

4.3.2 Factors related to the organism

Species differ in susceptibility to chemicals. This may be due to differences in accessibility, with certain species effectively excluding a toxic medium for short periods of time. In addition, rates and patterns of metabolism and excretion can substantially affect susceptibility. Differences in susceptibility to chemical agents among fish of different strains also result from genetic factors. Dietary factors also influence toxicity, by producing changes in body composition, physiological and biochemical functions, and nutritional status of the organism.(Rand, 1995)

The most obvious of these is the taxonomic classification of the subject, that is, the species and strain. With test animals it is important to consider the genetic status of the subjects, including whether they are littermates, half-siblings (different fathers), or the products of inbreeding. Body mass, sex, age, and degree of maturity are all factors in toxicity. Immunological status is important. Another area involves the general well-being of the subject. It includes disease and injury, diet, state of hydration, and the subject's psychological state as affected by the presence of other species and/or members of the opposite sex, crowding, handling, rest, and activity. (Manahan, 2003)

4.3.3 Factors related to the chemical

The toxicity of a chemical agent can be influenced by its compositon. Impurities or contaminants that are considerably more toxic than the chemical itself may be

present. Impruties may vary from one batch of the chemical to another, so that the results obtained with a particular batch may not be reproducible. Therefore, the identity and purity of chemicals are important in toxicity testing. Other factors that are directly related to the chemical are its physical and chemical properties such as solubility,vapor pressure, pH, and lipophilicity. These factors affect the persistence, transformation, bioavailability, and ultimate fate of the chemical in water (Rand,1995).

The other major class consists of environmental factors. Among these are ambient atmosphere, conditions of temperature, pressure, and humidity, as well as composition of the atmosphere. Light and noise and the patterns in which they occur are important. Social and housing (caging) conditions may also influence response of subjects to a toxicant.(Manahan, 2003)

4.4 Chemical Interaction

The biological effects of two or more toxic substances can be different in kind and degree from those of one of the substances alone. One of the ways in which this can occur is when one substance affects the way in which another undergoes any of the steps in either the kinetic phase or the dynamic phase. Chemical interaction between substances may affect their toxicities (Manahan, 2003).

The toxicity of a chemical in an organism may be increased or decreased by a simultaneous or consecutive exposure to another chemical. If the combined effect is equal to the sum of the effect of each substance given alone, the interaction is considered to be *additive*; for example , combinations of most organophosphorous pesticides on cholinesterase activity. If the combined effect is greater than the sum, the interaction is considered to be *synergistic;* for example, carbon tetrachloride and ethanol on the liver and asbestos exposure and cigarette smoking on the lung. The term *potentiation* is used to describe the situation in which the toxicity of a substance that alone has no toxic effect on that organ. The exposure of an organism to a chemical may reduce the toxicity of another. *Chemical antagonism* denotes the situation wherein a reaction between the two chemicals produces a less toxic product. *Functional antagonism* exists when two chemicals produce opposite effects on the same physicologic parameters. *Competitive antagonism* exists when the agonist and antagonist act on the same receptor. *Noncompetitive antagonism* exists when the

toxic effect of a chemical is blocked by another not acting on the same receptor (Lu and Kacew, 2002).

4.5 Dose–Response Relationships

Toxicants have widely varying effects on organisms. Quantitatively, these variations include minimum levels at which the onset of an effect is observed, the sensitivity of the organism to small increments of toxicant, and levels at which the ultimate effect (particularly death) occurs in most exposed organisms. Some essential substances, such as nutrient minerals, have optimum ranges above and below which detrimental effects are observed.

Dose–response relationship is one of the key concepts of toxicology. **Dose** is the amount, usually per unit body mass, of a toxicant to which an organism is exposed. **Response** is the effect on an organism resulting from exposure to a toxicant.

In between, there is a range of doses over which some of the organisms respond in the specified manner and others do not, thereby defining a dose–response curve. Dose–response relationships differ among different kinds and strains of organisms, types of tissues, and populations of cells (Manahan, 2003).

A typical dose-response curve is shown in Figure 1.2**,** in which the percentage of organisms or systems responding to a chemical is plotted against the dose. For many chemicals and effects there will be a dose below which no effect or response is observed. This is known as the *threshold dose*. This concept is of significance because it implies that a *no observed effect level* (NOEL) can be determined and that this value can be used to determine the safe intake for food additives and contaminants such as pesticides (Hodgson, 2004).

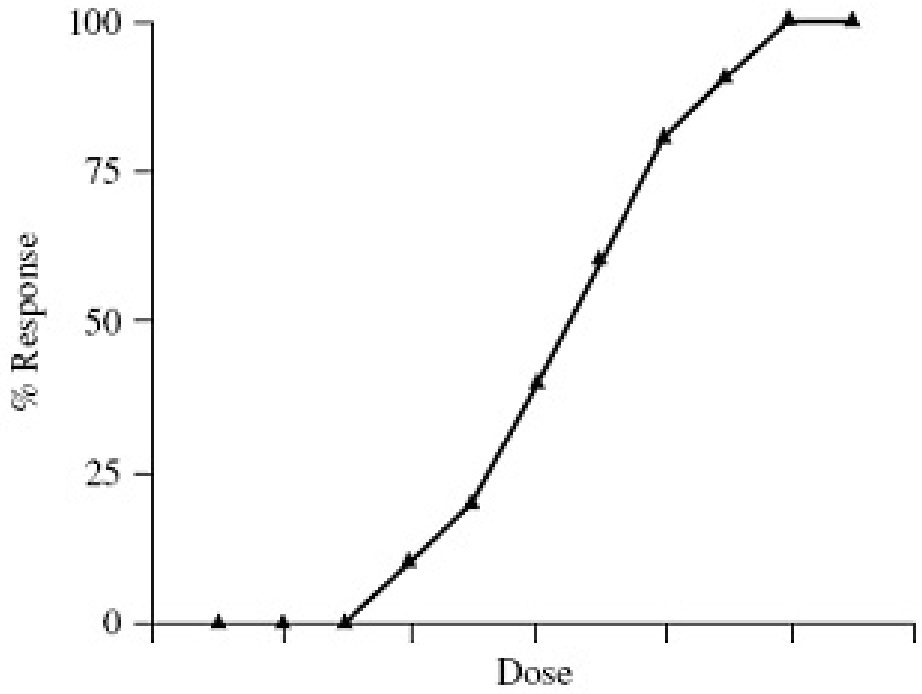


Figure 4.1: A typical dose-response curve

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}.

4.6 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).

Almost all ecosystems are contaminated by a more or less complex mixture of chemicals from anthropogenic source; many of these are synthetic chemicals. This does not necessarily mean that all of them will trigger a biological response or possibly harmful effects. The risk of ecotoxicity increases for compounds used in large amounts that are persistent, concentrate in the abiotic and biotic matter of ecosystems, are lipophilic, and are highly active (Rand, 1995).

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).

To determine the toxicity of a compound for a biological system, an observable and well-defined end effect must be identified. Turbidity or acid production, reflecting the growth or growth inhibition of a culture, may be used as an end point in bacterial systems. In some cases, such as in the study of mutagenesis, colony count may be

used. Similarly, measures of viable cells, cell protein, or colony count are useful end points in cell cultures. The most readily observable end point with in vivo experiments is the death of an animal, and this is frequently used as a first step in evaluating the toxicity of a chemical. Inhibition of a cell growth or death of animals are not the only concerns of toxicology. Many other end points may be chosen, depending on the goal of the experiment. Examples of such choices are inhibition of a specific enzyme, sleeping time, occurrence of tumors, and time to the onset of an effect (Zakrzewski, 1991).

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 T1 test should be allowed to continue until acute toxicity (mortality or a defined sublethal effect) has ceased or nearly ceased and the toxicity curve indicates that a threshold or incipient effect concentration can be estimated (Rand, 1995).

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: 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. Chronic toxicity tests permit evaluation of the possible adverse effects of the chemical under conditions of long-term exposure at sublethal 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; e.g., 0.5 ppm < MATC < 1.0 ppm). For regulatory purposes, the MATC is sometimes calculated as the

geometric mean of the LOEC and NOEC, so it can be used as a point estimate (Rand, 1995).

Toxicity assessment is the determination of the potential of any substance to act as a poison, the conditions under which this potential will be realized, and the characterization of its action. Risk assessment, however, is a quantitative assessment of the probability of deleterious effects under given exposure conditions. Both are involved in the regulation of toxic chemicals. Regulation is the control, by statute, of the manufacture, transportation, sale, or disposal of chemicals deemed to be toxic after testing procedures or according to criteria laid down in applicable laws.

Although for a variety of reasons extrapolation from experimental animals to humans presents problems, including differences in metabolic pathways, dermal penetration, mode of action, and others, experimental animals present numerous advantages in testing procedures. These advantages include the possibility of clearly defined genetic constitution and their amenity to controlled exposure, controlled duration of exposure, and the possibility of detailed examination of all tissues following necropsy (Hodgson, 2004).

5. MATERIALS AND METHODS

5.1. Experimental Approach

In this study determination of COD counterpart of oil and grease and its toxicity were aimed. According to the aim of the study, synthetic samples preferred for laboratory studies. These synthetic samples are motor oil, used motor oil and Bunker C fuel oil. Because of oils hydrophobic property, three different solvents (n-hexane, dimethylsulfoxide, and tetrahyrofuran) were used for extraction to make samples available for the analysis. Besides, to determine oil partitioning in water and its toxic effects, water soluble fractions were prepared. Codes for oil samples and solvents are shown in Table 5.1.

Table 5.1: Sample Coding

Sample Code	Sample definition
S_{MO}	Motor Oil
S_{UMO}	Used Motor Oil
S_{FO}	Bunker C Fuel Oil
S_{WSF} ($S_{WSF,MO}$, $S_{WSF,UMO}$, $S_{WSF,FO}$)	Water Soluble Fractions
A_1	n-Hexane
A_2	DMSO
A_3	THF

Oil and grease content of oil samples was firstly detected. COD experiments were done to obtain COD counterpart of oil samples, WSFs, and solvents. Afterwards, TOC anaysis applied on samples to determine a correlation between COD, TOC results, and their theoretical oxygen demands. Additionally, toxicity assessments were done for all samples. The experimental approach of this study is shown in Figure 5.1.

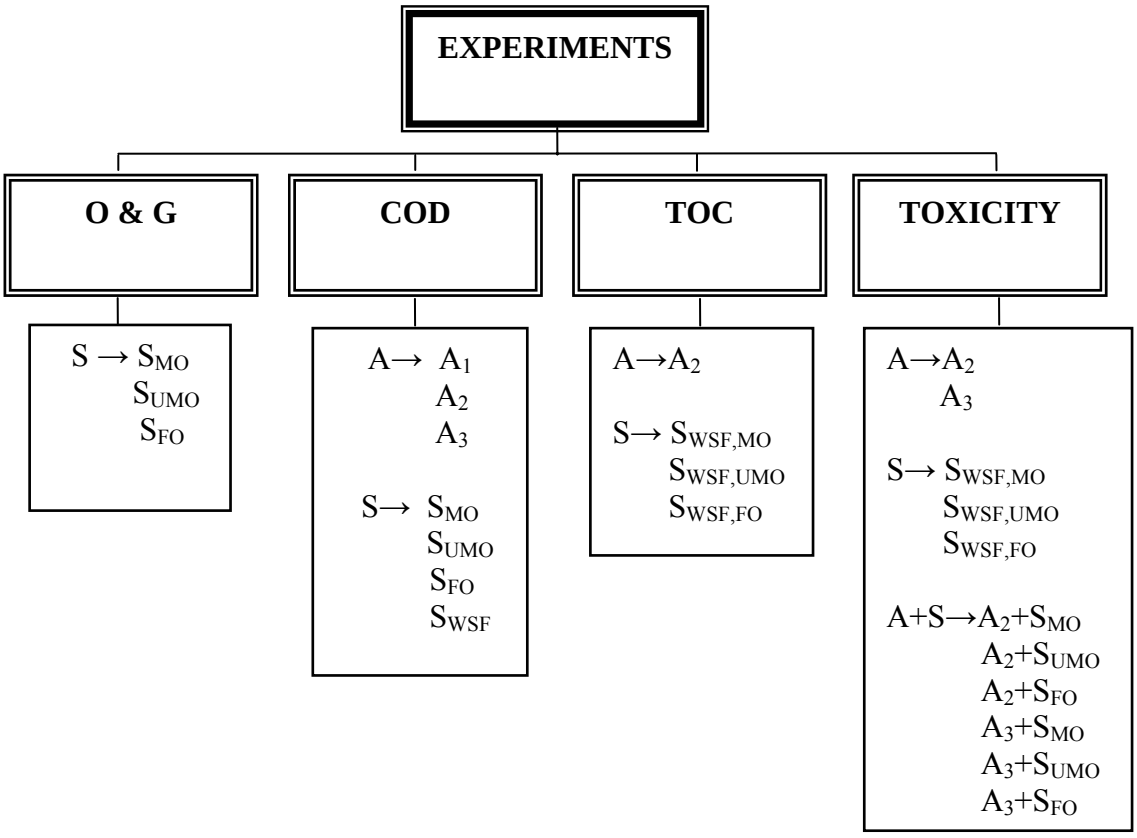


Figure 5.1: Experimental approach design

5.2. Sample Preparation

Experiments were applied on 3 different types of oil samples (motor oil, used motor oil, and fuel oil), solvents, water soluble fractions (WSFs), and oil-solvent combinations.

Motor oil and waste oil are hydrocarbon mixtures and are dominated by carbon chains with 20 or more carbon atoms. These typically have very high molecular weights and are less dense than water, relatively insoluble in water, and minimally mobile in the environment. Motor oil typically contains little or no mono-aromatic hydrocarbons. However, waste oil typically has a highly variable composition. It contains aromatic hydrocarbons as a result of engine blow-by and polynuclear aromatic hydrocarbons (PAH) as a result of exposure to high temperatures in the internal combustion engine. Waste oil can also contain a variety of other constituents including gasoline, solvents, and antifreeze, as a result of activities associated with collection Bunker, C fuel oil have progressively longer chains, higher molecular weights, lower vapor pressures, and are less soluble in water (Suthersan, 1999).

Castrol HD30 API SC/CC is a motor oil that has widely usage was choosen for motor oil (S_{MO}) sample from a gas station. This type of motor oils are mineral oil-based heavy duty premium monograde oils that are engineered for greater protection against thermal breakdown and normally aspirated diesel engines. It is API gravity of 0.882 at 60ºC, 7.32 pounds per galloon, viscosity of 11.5 CST at 100ºC, a pour point of – 3ºC, flash point of 230ºC, fire point of 243ºC, calcium content of 0.185%, zinc content of 0.09%, and phosphorus content of 0.08% by weight.

Used motor oil (S_{UMO}) contains metal impurities from engine and expectingly is more toxic than motor oils was taken from autoindustrial field at Maslak. Bunker C fuel oil (S_{FO}) is a heavy residual fuel.

Solvents are necessary for oil extraction. The performances of three extracting solvents, i.e. n-hexane (A_1), DMSO (A_2), and THF (A_3) were investigated.

One of the objective of this work was to characterize oxygen demand and the toxicity of the water soluble fraction (WSF) under laboratory conditions. In order to facilitate the use of the oil samples and to guarantee the total dispersion of the samples, 5 g of oils in 100 ml of distilled water were used. The mixtures were first sonicated for 10 min to disperse the oil samples, and then they were gently agitated for 72 h in a closed bottle and in obscurity. Finally, distilled water was filtrated with Millipore Zero Headspace Extractor (ZHE) in order to obtain only the WSF, avoiding any material in suspension.

Approximately 1 gr of motor oil (S_{MO}), used motor oil (S_{UMO}), and bunker C fuel oil (S_{FO}) were used for the oil and grease analysis. Meanwhile, for COD determination of oil and grease, 200 mg of oils were dissolved in a liter of n-hexane (A_1), from this main mixture different amounts were taken to COD flasks. Hexane is evaporated with the help of a rotary evaporator. Afterwards, COD experiments for pure oil samples were done. DMSO needed dilution preparations for COD experiment.

BioTox™ was chosen for the toxicity testing in the current study as it met a number of important criteria. It is a widely used test with a standardized, published, protocol. The test is rapid and relatively low cost, enabling large number of tests to be conducted, and results have been shown to be highly reproducible (Azur Environmental Ltd, 1995). The Effective Concentration (EC_{50}) of the chemical was that concentration which resulted in a 50% inhibition in light emitted by the

BioTox™ bacteria which was *Vibrio fischeri* in this study. The computed mixture toxicities was expressed as toxicity units (TU), defined as $TU=100/EC_{50}$. Toxicity assessments (analysis) were conducted on the 2 pure solvents, and 6 samples of solvent-oil combinations, and also WSFs, as appropriate. To estimate EC_{50} value for oils, a solvent extraction is needed to transfer oils into liquid phase for lack of oils' solubility.

DMSO and THF were used as an organic solvent for determination of toxicity of samples which are insoluble in water. DMSO is reported as one of the most appropriate solvent for toxicity analysis due to its non-toxic property and miscibility in water (Rand, 1995). 2 gr of sample oils (S_{MO}, S_{UMO}, S_{FO}) were dissolved in 100 ml of DMSO. The toxicity of pure DMSO was also determined as a blank toxicity. In the second step 1 gr of sample oils mixed with 50 ml of THF with the exception of fuel oil which is mixed with 100 ml of THF. Meanwhile, pure solvents were also prepared to determine the blank toxicity.

5.3. Analysis Methods

5.3.2. Oil and grease analysis

The sample is placed in a porous extraction thimble and immersed in the solvent. The extraction comprises a series of batch processes involving distillation and condensation of the solvent along with periodic fill-in and siphoning of the solvent in and out of the extraction chamber. This causes an intimate mixing of the sample with the solvent. The extraction also requires a relatively large quantity of solvent and usually a preconcentration step is necessary.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles, the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed, typically by means of rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded (Patnaik, 1997). The oil and grease result is expressed in milligrams per litre, is given by the formula

$$O\&G(mg/L) = \frac{(A-B).1000}{V_{numune}} \quad \textbf{(5.1)}$$

where,

A : weight of the container atfen distillation (g)

B : weight of the clean container (g)

Soxhlet extraction method is used for oil and grease analysis.

5.3.2. COD analysis

The chemical oxygen demand (COD) of water as determined by this dichromate method can be considered as an approximate measure of the theoretical oxygen demand, i.e. the amount of oxygen consumed in total chemical oxidation of the organic constituents to inorganic end products. The degree to which the test results approach the theoretical value depends. A test portion is refluxed in the presence of mercury (II) sulphate with a known amount of potassium dichromate and silver catalyst in strong sulphuric acid for a fixed period of time, during which part of the dichromate is reduced by the oxidizable material present. The remainder of the dichromate is titrated with ammonium iron (II) sulphate. The COD value is calculated from the amount of dichromate reduced. The chemical oxygen demand, COD, expressed in milligrams of oxygen per litre, is given by the formula

$$COD(mg/L) = \frac{(V_1 - V_2)\cdot c \cdot 8000}{V_0} \quad \textbf{(5.2)}$$

where

c : the concentration, in moles per litre, of the iron (II) ammonium sulphate solution;

V_0 : the volume, in millilitres, of the test portion before dilution (if any);

V_1 : the volume, in millilitres, of iron (II) ammonium sulphate solution used in the titration against the blank;

V_2 : the volume, in millilitres, of iron (II) ammonium sulphate solution used in the titration against the test portion;

8000 is the molar mass, in milligrams per litre, of ½ O_2 .

COD ISO 6060 method is used for COD analsis of samples (ISO,1999).

As is known, oil is lack of solubility and to obtain COD counterpart and still stay safe in range, preparing oil dilutions would be futile. For that reason oil is dissolved in n-hexane. Afterwards dilutions with n-hexane were taken to a Rotary Evaporator. The main components of a rotary evaporator are a vacuum system, consisting of a vacuum pump and a controller, a rotating evaporation flask which can be heated in a heated fluid bath, and a condenser with a condensate collecting flask. Water bath temperature was 75ºC which is above n-hexane's boiling point. With a speed of 30-90 rpm and 10 min. of evaporation time, n-hexane was completely evaporated.

5.3.3. TOC analysis

The TOC content of a sample of water is an important and non-compound-specific parameter. It is measured by oxidizing the dissolved organic carbon (DOC) and quantitating the evolved carbon dioxide. One way to do this is to sweep the released CO_2 into a nondispersive IR (NDIR) photometer that has been tuned to measure CO_2. This is accomplished by measuring the absorbance of the characteristic C–O stretching vibration.

Two analytical approaches to the quantitative determination of TOC for aqueous samples that contain DOC have emerged over the years: oxidation of carbon to CO_2 via high-temperature catalyzed combustion and persulfate–ultraviolet irradiation. A TOC measurement involves oxidizing organic carbon in an aqueous sample, detecting and quantifying the oxidized carbon as CO_2, and presenting the results in terms of the mass of carbon per unit volume of the aqueous sample. Some of the terms used in this important and nonselective determinative technique:

Total carbon (TC) is the measure of all the carbon in the sample, both inorganic and organic, as a single parameter. Generally, the measurement is made by placing the sample directly into the analyzer without pretreatment.

Total organic carbon (TOC) is the sum of all the organic carbon in the sample. TOC can be measured in one of two ways:

TOC measurement directly requires that inorganic carbon be removed by acidification and sparging. The DOC that remains is measured as TOC. Inorganic

carbon and purgeable organic compounds (POCs) are lost. POCs are generally present at 1% or less of total carbon.

TOC measurement by difference requires two quantitative determinations: one to measure TC and one to measure inorganic carbon. The difference between these two measurements is rigorously TOC.

Inorganic carbon (IC) includes carbonate, bicarbonate, and dissolved carbon dioxide. IC is determined in aqueous samples by acidifying with an inorganic acid to pH 3 or lower, and then sparging with a stream of inert gas. The acidification converts carbonates and bicarbonates to CO_2, which is then removed along with dissolved CO_2 by the gas stream and measured to provide an IC value.

Purgeable organic carbon (POC) is defined as the sum of volatile and semivolatile organic compounds sparged from an aqueous sample. However, these compounds are generally less than 1% of TC in an environmental aqueous sample.

The *high-temperature combustion* (HTC) technique uses heat (680°C or higher), in the presence of a titanium dioxide-based platinum catalyst, with a stream of hydrocarbon free compressed air or oxygen to oxidize organic carbon. DOC and particulates that contain carbon fully oxidize to CO_2 under these conditions (Loconto, 2006).

Shimadzu TOC-V_{CPN} analyzer is used for TOC analysis. TOC-V_{CPN} is a combustion catalytic oxidation/NDIR method, PC-controlled, standard model

5.3.4. Toxicity analysis

BioTox™ method was choosen for toxicity analysis that is the traditional and standardized way to measure the toxicity of chemicals or effluents by utilising photobacteria (ISO, 1999). The test is based on the fact that the light output of the bacteria is reduced, when it is introduced to toxic chemicals. The method is very rapid, it takes from 5 to 30 minutes to perform the test. The results with this system are comparable to published results with other photobacteria tests like Microtox® test. The principle of the standard photobacteria measurement is that the bacteria and the sample are mixed together and after an incubation period the light output is measured with Aboatox 1253 Luminometer.

BioTox™ toxicity bioassay is based on the measurement of light output of the bioluminescent marine bacterium *Vibrio fischeri*. 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).

EC_{50} values, defined as the concentration, which provokes a 50% light reduction on *V. fischeri* measured in the analyzer of BioTox™ 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.

Toxicity test were carried out in relevant with ISO 11348-3 standard test procedure. Pure cultures of bacteria in freeze dried form should be reconstituted with reconstitution solution in 15ºC in a chiller. Solutions were freshly prepared in order to minimize any time-related change in oil-solvent mixtures and WFSs. 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. 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. 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. The more toxic the substances tested, the lower the light produced by the bacteria relative to the control (ISO, 1999, AZUR Environmental, 1998).

A blank sample with no toxicant (control) was used for all sets of experiments to correct for the time-dependent change in the light production of the bacteria themselves in order to isolate the toxic effects of the sample alone, as well as to account for small effects due to dillution arising from sample transfer, pipette error, and introduction of reagents (Mowat and Bundy, 2002)

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

$$KF = \frac{IC_t}{IC_0} \quad \textbf{(5.3)}$$

$$INH\% = 100 - \frac{IT_t}{KF \times IT_0} \times 100 \quad \textbf{(5.4)}$$

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

5.3.5. Extraction procedure method

The Millipore 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 should have 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.

However, in this study ZHE was used for liquid/liquid separation to get a better performance for filtration of oil-water mixtures to obtain WSF.

6. RESULTS AND DISCUSSION

Oil samples were prepared with different amounts of different types of solvents for experiments. Also, water soluble fractions of oil and grease and (their) toxicity is detected. Initially oil and grease content of oil samples were assessed. The results are shown in Table 6.1.

6.1. Oil and Grease

Oil samples; Motor Oil (S_{MO}), Used Motor Oil (S_{UMO}), Bunker C Fuel (S_{FO}) were analyzed for their O&G content by soxhlet extraction method.

Table 6.1: O&G Extraction performance of Oil samples

Sample	Sample amount (g/L)	O&G (g/L)	O&G content of samples (%)
S_{MO}	0,951	0,763	**79**
	0,988	0,752	
	0,998	0,814	
	0,979*	**0,776 ***	
S_{UMO}	0,939	0,814	**83**
	1,000	0,795	
	0,976	NM	
	0,970 *	**0,804 ***	
S_{FO}	0,999	0,866	**85**
	0,997	0,804	
	0,990	0,892	
	0,995 *	**0,854 ***	

***** **:** Mean Values

NM **:** Not Meaningful

Because of an experimental error a significant amount of n-hexane was evaporated from soxhlet extractor during the soxhlet extraction for used motor oil (S_{UMO}). Therefore Oil and grease content for used motor oil (S_{UMO}) could not be measured. Triplicate O&G analysis were done to see experiment's reproducibility.

As can be seen from table 6.1 the oil and grease content of the three specimen namely Motor oil (S_{MO}), Used Motor Oil (S_{UMO}), and Bunker C fuel oil (S_{FO}) have been determined. Bunker C fuel oil (S_{FO}) has higher oil and Grease content than others. This shows that motor oil and used motor oil contain more complex material that can not be extracted by n-hexane as compared to Bunker C fuel oil.

6.2. COD

COD tests were applied to all oil samples, solvents, and WSFs according to ISO 6060. COD results for the three specimens namely Motor oil (S_{MO}), Used Motor Oil (S_{UMO}), and Bunker C fuel oil (S_{FO}) are given below in table 6.2.

Table 6.2: COD results for Motor oil, Used motor oil, and Bunker C fuel oil

Sample Volume (ml)	COD (mg/L)		
	S_{MO}	S_{UMO}	S_{FO}
7	478	466	425
5	372	370	334
3	227	258	217

The COD counterpart of O&G, Water Soluble fractions, and COD results for solvents were determined and are presented in the following sections.

6.2.1. COD counterpart of O&G

The oxygen demand of a water sample is the amount of elemental oxygen required to react with oxidizable or biodegradable material, dissolved or suspended in the sample. This amount is expressed as milligrams of oxygen per liter of sample. When the agent required to effect the oxidation reaction is a population of bacteria, the oxygen required is called BOD. When the oxidation is carried out with a chemical oxidizing reagent such as potassium dichromate, the oxygen equivalent is called the COD. The terms grease and oil as used in wastewater treatment denote a variety of materials, including fats, waxes, free fatty acids, calcium and magnesium soaps,

mineral oils and other nonvolatile materials that are soluble in and can be extracted by hexane from an acidified sample (Liu and Liptak, 1999).

COD is collective parameter, and oil and grease is group parameter. Oil is not soluble in water which makes it impossible to prepare a dilution solution suitable for COD determination. Therefore oil samples were dissolved in n-hexane and then hexane was evaporated with rotary evaporator as mentioned in materials and methods.

200 mg of oil samples were dissolved in a liter of n-hexane. From prepared sample 7, 5, 3 ml of mixtures were taken into COD flasks and placed in rotary evaporator for 10 minutes. Water bath temperature was approximately 75ºC which is above n-hexane boiling point (69ºC) and rpm was between 30 – 90. Weights of flasks were also checked to make sure all n-hexane was evaporated.

Amount of the sample oils that were taken from oil-hexane mixture into COD flasks is calculated according to the following equation:

$$(S_1, S_2, S_3)(mg) = \frac{200mg \times a}{1000ml} \qquad \textbf{(6.1)}$$

where a is the sample volume.

S_1, S_2, S_3 were calculated as 1.4, 1.0, and 0.6 ml respectively. For COD experiments 10 ml of distilled water was added into each COD flask.

Table 6.3: COD counterpart of Motor Oil

Sample Volume (ml)	**S_{MO} (mg/L)**	**O&G (mg/L)**	**COD (mg/L)**
7	140	111	478
5	100	79	372
3	60	48	227
Mean Values	100	79	359
COD / O&G	**4.5**		

The S values in table 6.3 show the concentrations of Motor oil used for the determination of COD presented as above. The percentage of O&G content for

Motor Oil was determined to be 79. Hence the O&G content of the samples are calculated using the following equation:

$$\text{O\&G (mg/L) calculated} = S_X(mg/L) \times \text{O\&G (\%) content} \quad \textbf{(6.2)}$$

It is determined in the results that the ratio of COD to Oil and Grease content of the motor oil is 4.5.

Similarly the percentage of O&G content for Used Motor Oil was determined to be 83%. Using equation 6.2 the corresponding O&G contents for the samples are calculated. The ratio of COD to Oil and Grease content of the used motor oil is determined to be 4.4 as shown in table 6.4.

Table 6.4: COD counterpart of Used Motor Oil

Sample Volume (ml)	**S_{UMO} (mg/L)**	**O&G (mg/L)**	**COD (mg/L)**
7	140	116	466
5	100	83	370
3	60	50	258
Mean Values	100	83	365
COD / O&G	**4.4**		

The percentage of O&G content for Bunker C fuel Oil was determined to be 85%. Using equation 6.2 the corresponding O&G contents for the samples are calculated. The ratio of COD to Oil and Grease content of the Bunker C fuel Oil is determined to be 3.9 as shown in table 6.5.

Table 6.5: COD counterpart of Bunker C Fuel Oil

Sample Volume (ml)	**S_{FO} (mg/L)**	**O&G (mg/L)**	**COD (mg/L)**
7	140	119	425
5	100	85	334
3	60	50	217
Mean Values	100	85	325
COD / O&G	**3.9**		

The results for mean COD to O&G content ratios for different samples are summarized in table 6.6 given below:

Table 6.6: COD/O&G ratios

Sample	**S_{MO}**		**S_{UMO}**		**S_{FO}**	
Analysis	O&G	COD	O&G	COD	O&G	COD
Result (mg&L)	79	359	83	365	85	325
COD / O&G ratios	**4.5**		**4.4**		**3.9**	

Approximately 1:4.5 ratio is determined as COD counterpart of oil and grease. This ratio indicates the contribution of O&G in COD concentration. COD/O&G correlations for three different concentrations are given in Figure 6.1.

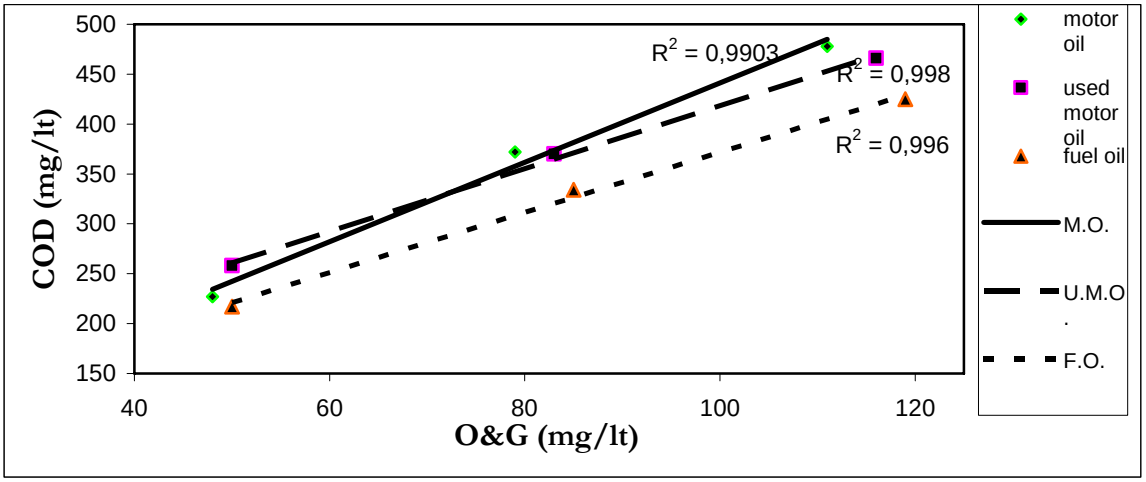


Figure 6.1: COD, and Oil and Grease correlations

Although the selected samples which are mineral based oils have different chemical properties, the COD/O&G ratios of them are similar.

6.2.2. COD counterpart of water soluble fractions of O&G

Oil has different physical forms in water. Dissolved oil is one of them. As it is known that just a minor amount is soluble in water, in this study this little amount of partioning was determined.

Water soluble fractions of oils were prepared as mentioned in materials and methods chapter. Water soluble fraction samples of 5 gr per liter of Motor oil (S_{MO}), used motor oil (S_{UMO}), and Bunker C fuel oil (S_{FO}) were prepared. Using samples' O&G contents given in table 6.1 before filtration, total oil and grease amount of samples prepared for WSFs were determined given below in table 6.7:

Table 6.7: O&G content of WFSs before filtration

Sample	Sample amount (gr/L)	O&G content of samples (%)	Total O&G content (gr/L)
S_{MO}	5	79	3.95
S_{UMO}	5	83	4.15
S_{FO}	5	85	2.25

After COD experiments were done for water soluble fractions of motor oil, used motor oil and Bunker C fuel oil, soluble oil and grease amount were estimated using the COD/O&G ratios determined in the previous section.

Table 6.8: COD and estimated soluble O&G results

Sample	COD / O&G	COD (mg/L)	Soluble O&G (mg/L) calculated
$S_{WSF,MO}$	4.5	409	91
$S_{WSF,UMO}$	4.4	1133	258
$S_{WSF,FO}$	4.9	39	10

In the table 6.7 and 6.8 total and soluble O&G contents were calculated. With the help of these results, water soluble fractions of oil samples were estimated.

Table 6.9: Water Soluble Fractions of Oil Samples

Sample	Total O&G content (mg/L)	Soluble O&G (mg/L) calculated	WFS (%)
$S_{WSF,MO}$	3950	91	0.2
$S_{WSF,UMO}$	4150	258	0.5
$S_{WSF,FO}$	2250	10	0.02

As it can be seen in the table, fuel oil has lowest solubility in water. In toxicity analysis, toxicity of these small partioning percentages on luminescent bacteria namely *vibrio fischeri* were analyzed.

6.2.3. COD results of solvents

Because of oils' poor solubility, they needed to be extracted. Main purpose 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. For this reason, Dimethlysulfoxide (DMSO), tetrahydrafuran (THF) and n-hexane were used as an extraction solvents. Firstly, it was aimed to determine their individual COD values.

6.2.3.1. n-Hexane

To determine COD counterpart of oil and grease, n-hexane was used as a solvent at the interlude step. In the table 6.10 individual n-hexane results are given.

Table 6.10: COD results for n-hexane

Sample volume (ml)	n-hexane (mg)	COD (mg/L)	Measured COD (mg/mg)
0.7	462	335	0.75
0.5	330	288	0.87
0.3	198	171	0.86
Mean Values	330	261	**0.82**

COD values for hexane were measured to be barely noticeable. Density of n-hexane is 0.66 kg/ L and molarity is 86.18 g/mol.

$$C_6H_{14} + {}^{19}\!/_{2}\, O_2 \rightarrow 6CO_2 + 7H_2O \qquad \textbf{(6.3)}$$

Theoretical oxygen demand of hexane can be estimated from equation (6.3) as 3.53 (mg/mg).

Table 6.11: COD and Th.OD comparision for n-hexane

Solvents	Th.OD (mg/mg)	Measured COD (mg/mg)	COD / Th.OD (%)
n-hexane	3,53	0.82	23

As it seems in table 6.11, ratio of COD and theoretical oxygen demand of n-hexane is low meaning potassium dichromate was unable to oxidize all hexane. Poor solubility of hexane might caused this result.

6.2.3.2. Dimethylsulfoxide

To determine COD of DMSO, many dilutions were prepared. Most of the dilutions failed. However dilutions that did not fail, show consistency as hexane results did. As can be seen in tablo 6.8, COD results for DMSO are independent from each other.

Table 6.12: COD results for DMSO

Dilution Ratio	Sample volume (ml)	DMSO (mg)	COD (mg/L)	Measured COD (mg/mg)
1/1000	10	11	232	21
1/1000	5	5.5	228	42
2/1000	1	2.2	1820	827
5/1000	1	5.5	2152	**391**

Density of DMSO is 1.10kg/L and molarity is 78,13g/ mol. Theorotical oxygen demand can be estimated from the equation below:

$$(CH_3)_2SO + 4O_2 \rightarrow 2CO_2 + 3H_2O + SO_2 \qquad \textbf{(6.4)}$$

From the equation, Th.OD is 1,64 (mg/mg). Measured COD (mg/mg) can not be bigger than the Th.OD. Hence, COD results for DMSO are unreliable.

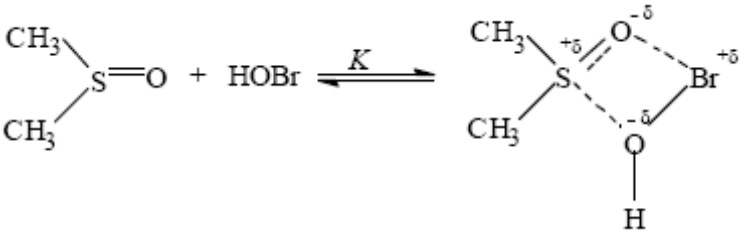
During the oxidation of DMSO by potasium dichromat – sulfuric acid in aqueous medium some problems might have been occured. It might be because of oxidation of DMSO by $NaBrO_3$–$NaHSO_3$ reagent that unreliable results have been achieved.

Aqueous solutions of $NaBrO_3$ and $NaHSO_3$ react together and generate HOBr completely first, and then kinetic run started with substrate DMSO. The course of the reaction was followed by estimating the unreacted HOBr iodometrically at regular intervals of time. The reaction product was identified as dimethyl sulphone. To see the effect of dissolved oxygen on the rates, nitrogen gas was bubbled through the

reaction mixture to expel dissolved oxygen and the reaction studied. It was found that there was no effect of oxygen on the rate. The stoichiometry was determined to be 1:1 (DMSO:HOBr).

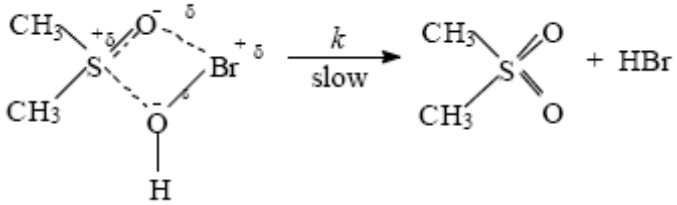
A fractional order in DMSO points to the formation of an intermediate adduct between DMSO and HOBr (6.5). This adduct decomposes in a rate-determining step to yield the product $DMSO_2$. HOBr has been well established to be the only effective oxidizing species 8–10. This is further supported by the fact that HOBr in aqueous solution is a weak acid (*K*a = $2*10^{-9}$ at 25°C) [10a]. Water is relatively a much weaker acid than HOBr (self ionization constant of water = $1* 10^{-14}$ at 25°C) [10b]. Hence protonation of HOB (i.e. formation of H_2OBr^+) is ruled out. The rates remain unaffected by added Na_2SO_4 salt indicating that reaction may involve two dipoles, i.e. DMSO and HOBr. Based on the product analysis, experimental observations, stoichiometry and foregoing discussions, the mechanism shown in (6.6) and (6.7) is proposed for the oxidation of DMSO by sodium bromate-sodium bisulphite reagent in neutral medium.(Viroopakshappa and Jagannadham, 2002)

Adduct formation:



(6.5)

Adduct decomposition:



(6.6)

As mentioned above an adduct formation and decomposition of DMSO might be the reason of high COD values despite little amounts of DMSO.

$$DMSO + HOBr \rightarrow DMSO_2 + HBr \quad \textbf{(6.7)}$$

$$DMSO + Cr_2O_7 + 2H_2O \rightarrow DMSO_2 + Cr^{3+} + SO_4^{2-} + H^+ \quad \textbf{(6.8)}$$

In addition, during the oxidation of DMSO by potassium dichromat – sulfuric acid, adduct formation and complex structure of Cr^{6+} might be ended up with masking of color.

6.2.3.3. Tetrahydrofuran

Lastly, COD determination of THF was planned. Unfortunately, after addition of potassium dichromat – sulfiric acid into COD flasks, THF reacted to that and color of the sample turned to black.

In COD tests, after oxidation, the excess potassium dichromate is titrated with ferrous ammonium sulfate (FAS). Typically, the oxidation-reduction indicator ferroin is added during this titration step as well. Once all the excess dichromate has been reduced, the Ferroin indicator changes from blue-green to reddish-brown.

Because of this color masking, COD determination of THF value is immeasurable.

Density of THF is 0.89kg/L and theorotical oxygen demand can be estimated as 2.44 from the equation below:

$$C_4H_8O + {}^{11}\!/_{2}\, O_2 \rightarrow 4CO_2 + 4H_2O \qquad \textbf{(6.9)}$$

Except n-hexane, solvents did not give meaningful COD results.

6.3. TOC

A TOC measurement involves oxidizing organic carbon in an aqueous sample, detecting and quantifying the oxidized carbon as CO_2, and presenting the results in terms of the mass of carbon per unit volume of the aqueous sample.

Oil samples (S_{MO}, S_{UMO}, S_{FO}) were unadaptable for TOC analysis not because they are hydrofobic and their viscose property is not acceptable for the analyzer.

n-Hexane could not be measured, also. Hexane is non-soluble solvent in aqueous medium, for that reason, it was unable to prepare a proper dilution for the analyzer.

However, DMSO has soluble property in water which helped to prepare proper dilutions for this test.

Table 6.13: TOC results, and TOC, Th.TOC comparison of DMSO

Solvents	Amounts (mg)	TOC mg/L	Th.TOC calculated(mg/mg)	Th.TOC measured(mg/mg)	Th.TOC/ Th.TOC measured
DMSO	1100	357	0.31	0.32	0.95

Theorotical total organic carbon is estimated from the formula of DMSO as 0.31. To determine measured theorotical total organic carbon, amount of DMSO was compared to TOC result of DMSO. Calculated and measured Th.TOC ratio shows that TOC analysis of DMSO is more reliable.

Water soluble fractions of Motor oil (S_{MO}), used motor oil (S_{UMO}), and Bunker C fuel oil (S_{FO}) were prepared as mentioned in materials and methods chapter. 20 ml of each samples were taken for TOC anaylsis. TOC results for WSFs are given below in table 6.14.

Table 6.14: TOC results for WSFs

Samples	$S_{4,1}$	$S_{4,2}$	$S_{4,3}$
TOC (mg/L)	84	50	7

In the previous sections, COD results and O&G content of WSFs were given. TOC, COD, and O&G relationship were determined.

Table 6.15: Comparing results of W.S.Fs

Samples	TOC (mg/L)	COD (mg/L) Measured	TOC/ COD	O&G (mg/L) Calculated	TOC/ O&G
$S_{WSF,MO}$	84	409	0,2	91	0,9
$S_{WSF,UMO}$	50	1133	0,04	258	0,2
$S_{WSF,FO}$	7	39	0,2	10	0,8

According to the table 6.15, it can be seen that water soluble fractions of oil samples were ended up with meaninngful results.

6.4. Toxicity

In this study, toxicity of three specimens namely Motor oil (S_{MO}), used motor oil (S_{UMO}), and Bunker C fuel oil (S_{FO}) with the help of two solvents (DMSO, THF) and water soluble fractions of oils ($S_{WSF,MO}$, $S_{WSF,UMO}$, $S_{WSF,FO}$) were detected. Toxicity data obtained from the set of experiments as EC_{50} values are converted to Toxic Units (TU) and summarized in the Table 6.16.

Table 6.16 : Results for Samples

Sample	EC_{50} (%)	Toxic Units (TU)
DMSO	12	8
DMSO + S_{MO}	11.47	9
DMSO + S_{UMO}	11.86	8
DMSO + S_{FO}	0.25	400
THF	0.19	526
THF + S_{MO}	0.32	357
THF + S_{UMO}	0.22	455
THF + S_{FO}	0.17	588
$S_{WSF,MO}$	-	-
$S_{WSF,UMO}$	27	4
$S_{WSF,FO}$	-	-

Oils are not soluble in water which makes it impossible to determine their toxicity on their own. Solvent extraction helped to determine toxicities of oil samples. Firstly, solvents individual toxicity were tested which would be counted as a blank toxicity. Afterwards, toxicity test applied on oil – solvent mixtures. Difference between solvents and oil-solvent mixtures would give individual oil toxicity.

Hexane was not appropriate solvent because of its lack of solubility, so DMSO, and THF were used in turn.

All toxicity tests were done according to the BioTox ^{TM} bioassay testing procedure. From each sample 10 ml was taken and mixed with 1,1 ml of 2% NaCl solution to adjust the salinity level of the sample to be tested. At -20 ºC freeze dried *vibrio fischeri* reagent was acclimated at both 4 and 15 ºC mixed with the dilutions of the sample solvent. For contact time 15 min. was choosen.

6.4.1. DMSO and DMSO-Oil Mixtures

Firstly, DMSO was tested indivudually. Tests run in duplicate and the computerized results given as a graph with the 95% confidence limit in the Figure 6.1. EC_{50} value of DMSO is found to be 12 %. Hence, having a density of 1,1 gr/cm^3 EC_{50} also corresponds for 131 g/L DMSO. All EC_{50} interpolation curves were convenient to common S-shaped (sigmoid) toxicity curves. R^2 value of linear regression of the test results for DMSO is 0.98. All points of the test results are in the 95% confidence limit for DMSO.

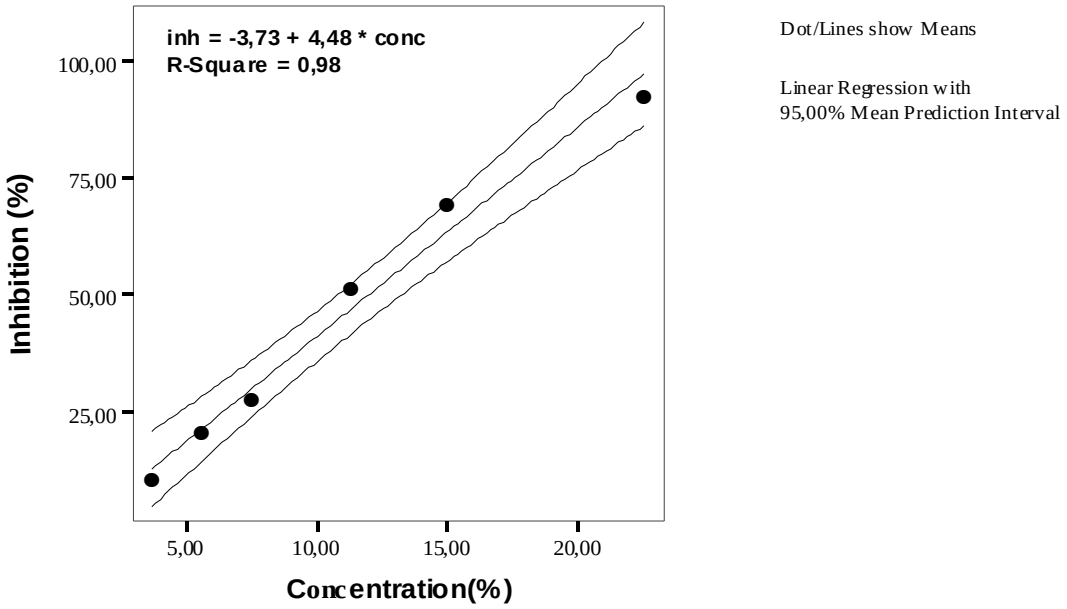


Figure 6.2: EC_{50} Interpolation curve for DMSO

After determining DMSO toxicity, DMSO-oil mixtures were prepared for the three specimens namely Motor oil (S_{MO}), used motor oil (S_{UMO}), and Bunker C fuel oil (S_{FO}), respectively. For this purpose 2 gr of Motor Oil was dissolved with 100 ml of DMSO. 10 ml of this mixture was taken and mixed with 1,1 ml of 2% NaCl stock solution prior to toxicity testing. Computerized test results is given in Figure 6.2 and the EC_{50} calculated as 11,47 %. Secondly, 2 gr of Used Motor Oil was 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 6.3. The EC_{50} of the DMSO and Used Motor Oil mixture determined as 11,86%. R^2 value of linear regression of the test results for DMSO+S_{MO} is 0.85 and

A_2+S_2 is 0.96 and for both test results, all of the points are in the 95% confidence limit.

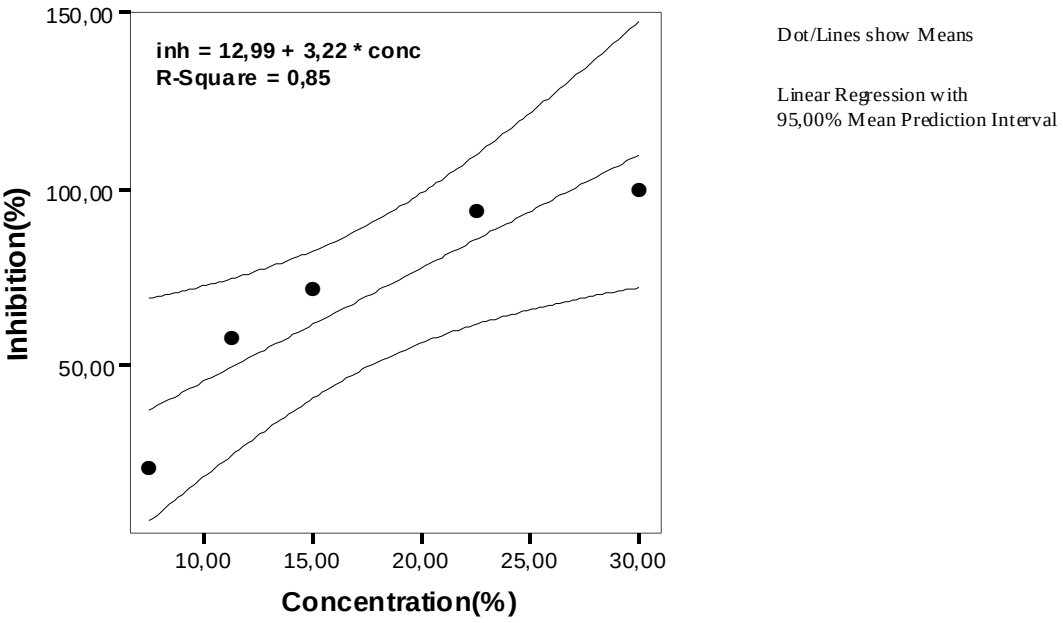


Figure 6.3: EC_{50} Interpolation curve for DMSO+S_{MO}

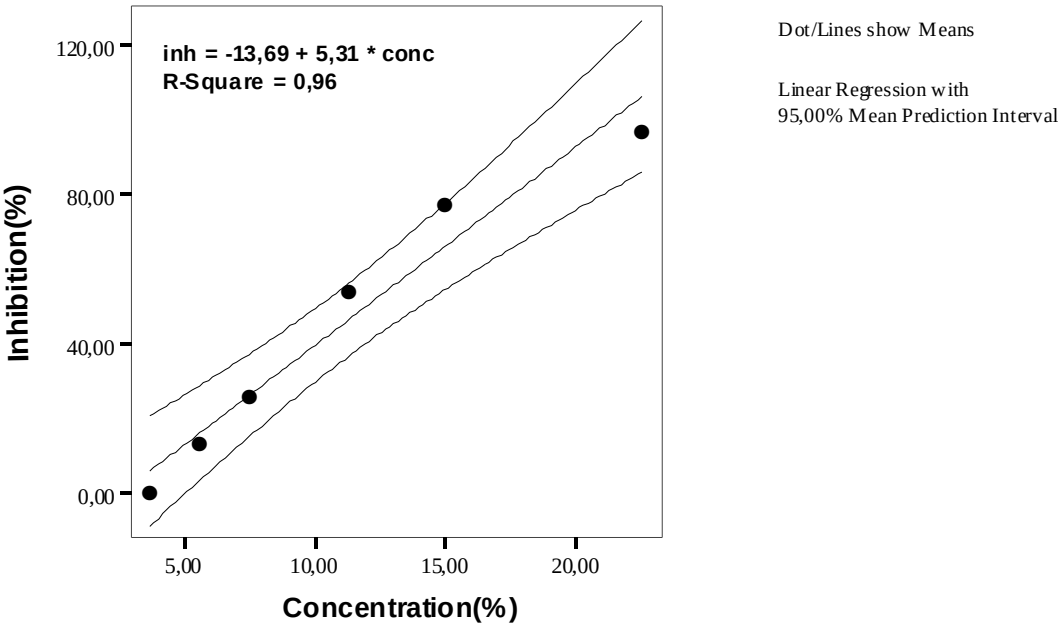


Figure 6.4: EC_{50} Interpolation curve for DMSO+S_{UMO}

Lastly, 100 ml of DMSO mixed with 2 gr of Bunker C Fuel Oil (S_{FO}). 10 ml of this sample mixture tested for toxicity after mixing with 1,1 ml of 2% NaCl stock solution. EC_{50} calculated for DMSO +S_{FO} as 0.25 % and R^2 value of linear regression of the test result is 0.93.

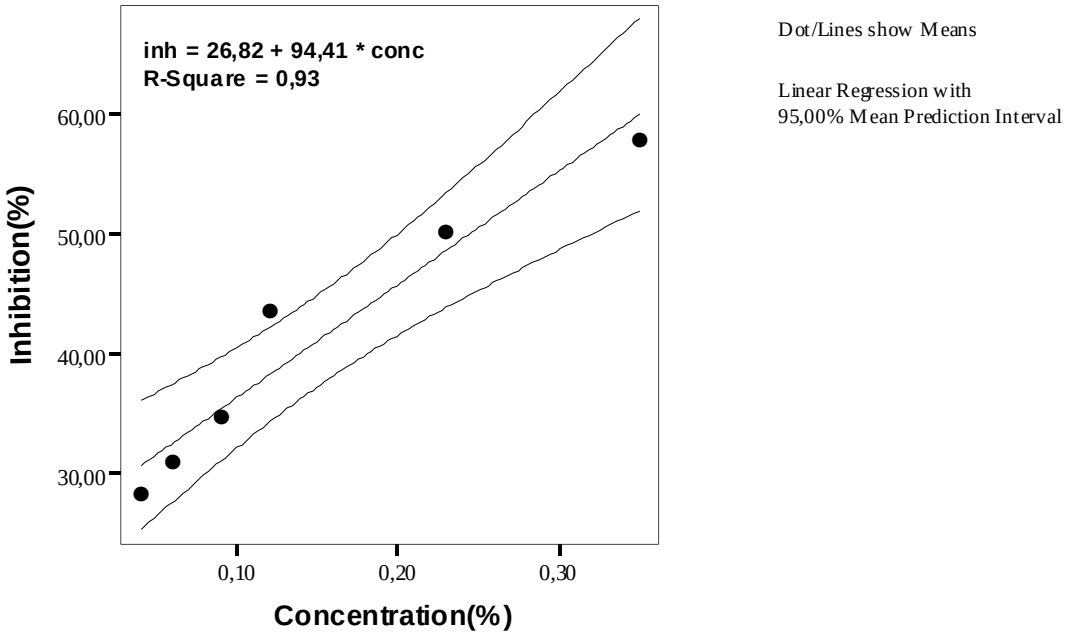


Figure 6.5: EC_{50} Interpolation curve for DMSO+S_{FO}

One point of test result is beyond 95% confidence limit for DMSO+S_{FO}. If this one point was removed, EC_{50} value would not be change much. Because the difference between EC_{50} values would be only %0.9. Apparently, EC_{50} value of DMSO – Bunker C fuel oil mixture DMSO+S_{FO} is lower than EC_{50} values of the other DMSO-oil mixtures. Comparing DMSO –oil mixture results to DMSO result, obviously motor oil and used motor oil are not toxic to luminescent bacteria. DMSO might be a better extraction solvent for bunker C fuel oil. It seems DMSO is not an adequate solvent for Motor Oil (S_{MO}), and Used Motor Oil (S_{UMO}).

6.4.2. THF and THF-Oil Mixtures

To evaluate THF toxicity, comparing to DMSO, more dilutions were prepared. The same testing procedure was applied to THF as DMSO. In order to obtain the salinity level of the sample 10 ml of THF is mixed with 1,1 ml of 2% NaCl stock solution and different dilutions of the sample tested for toxicity in duplicate. EC_{50} value of the THF calculated as 0,19%. EC_{50} value is calculated as 1.7 g/L. from the density of THF which is 0,89 g/cm^3. The bioassay results are plotted on the graph by the computer program and given in the Figure 6.5.

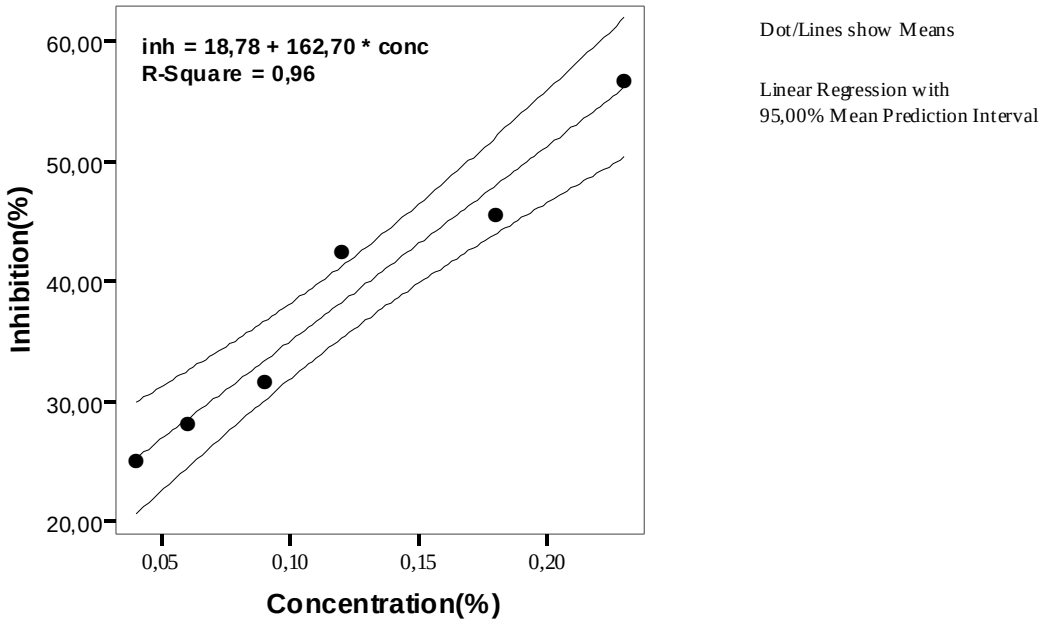


Figure 6.6: EC_{50} Interpolation curve for THF

R^2 value of linear regression of the test result for THF is 0.96. One point of the test result is beyond 95% confidence limit for THF. EC_{50} value would not be change much even if this one point was removed. Because the difference between EC_{50} values would be only %0.5. According to THF individual result, it is obvious that THF is toxic solvent, especially comparing to DMSO.

After determining the individual THF toxicity, THFwas mixtured with the three specimens namely Motor oil (S_{MO}), used motor oil (S_{UMO}), and Bunker C fuel oil (S_{FO}), respectively to obtain THF-oil mixtures toxicity. Hence, 1 gr of Motor Oil is dissolved in 50 ml of THF, and 10 ml of this sample was taken to mix with 1,1 ml of

2% NaCl solution to adjust the desired salinity level prior to toxicity bioassay. EC_{50} Interpolation curve for THF+S_{MO} shown in the Figure 6.6. The EC_{50} value is determined as 0.32% which is lower than the toxicity of the solvent by itself. R^2 value of linear regression of the test result for THF+S_{MO} is 0.92. Two points of the test result are beyond 95% confidence limit for THF+S_{MO}. EC_{50} value would not be change much even if this one point was removed. Because the difference between EC_{50} values would be only 0.9%.

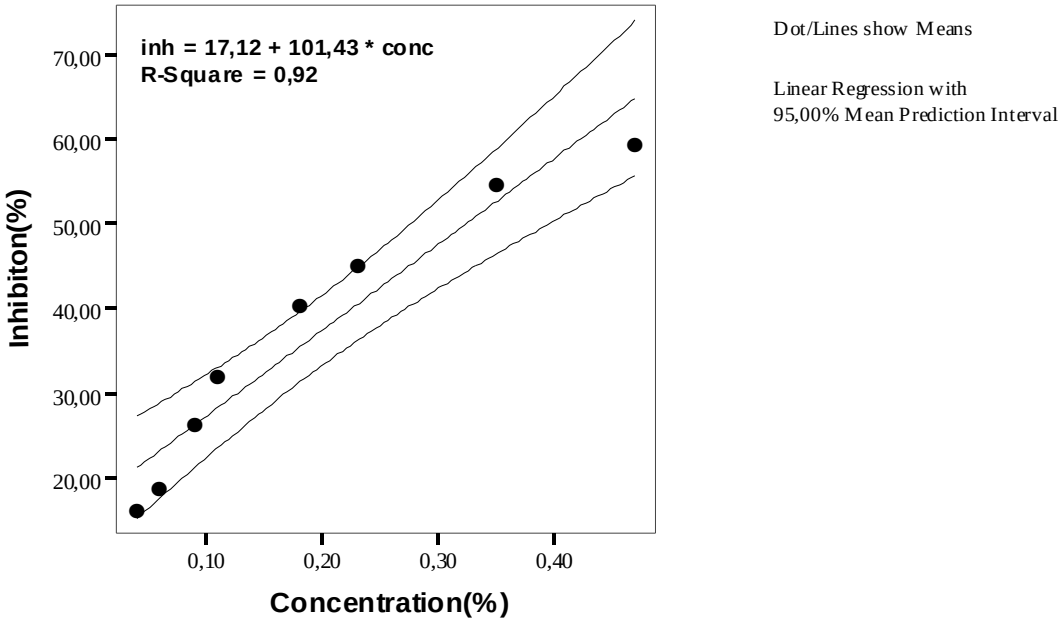


Figure 6.7: EC_{50} Interpolation curve for THF+S_{MO}

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 6.7. R^2 value of linear regression of the test result for THF+S_{UMO} is 0.96 and R^2 value of linear regression of the test results for THF+S_{FO} is 0.87 and all of the points are in the 95% confidence limit. The EC_{50} value of THF+S_{UMO} is found 0.22% which is higher than the EC_{50} value of THF+S_{MO}. Both THF mixtures with Motor Oil and Used Motor Oil have higher EC_{50} values than individual THF sample meaning that these samples are less toxic than THF by itself.

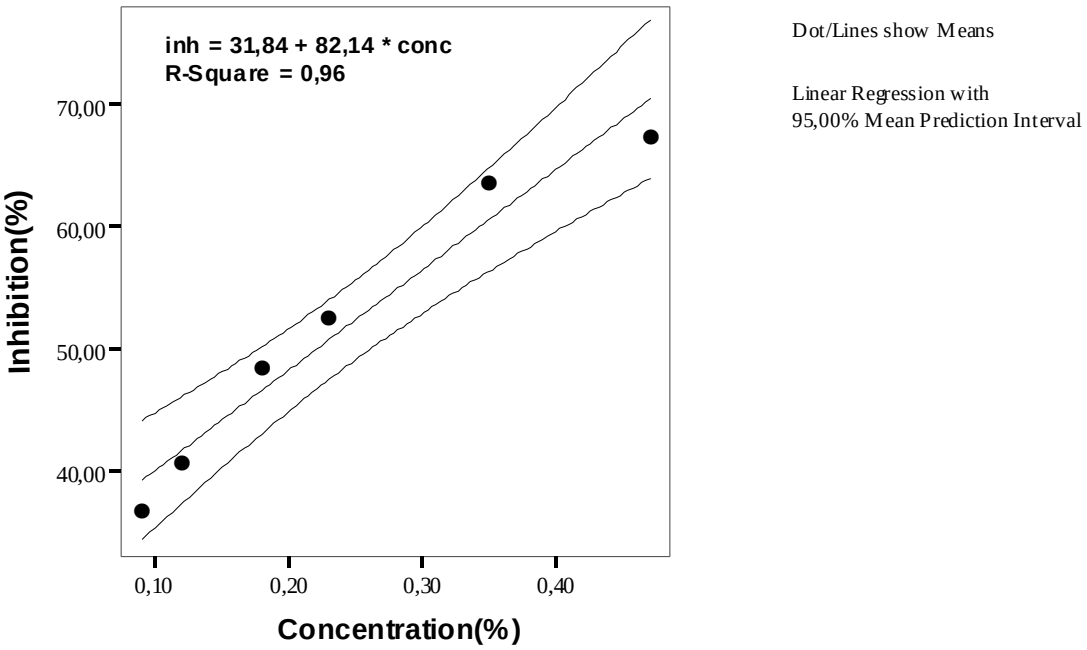


Figure 6.8 : EC_{50} Interpolation curve for THF+S_{UMO}

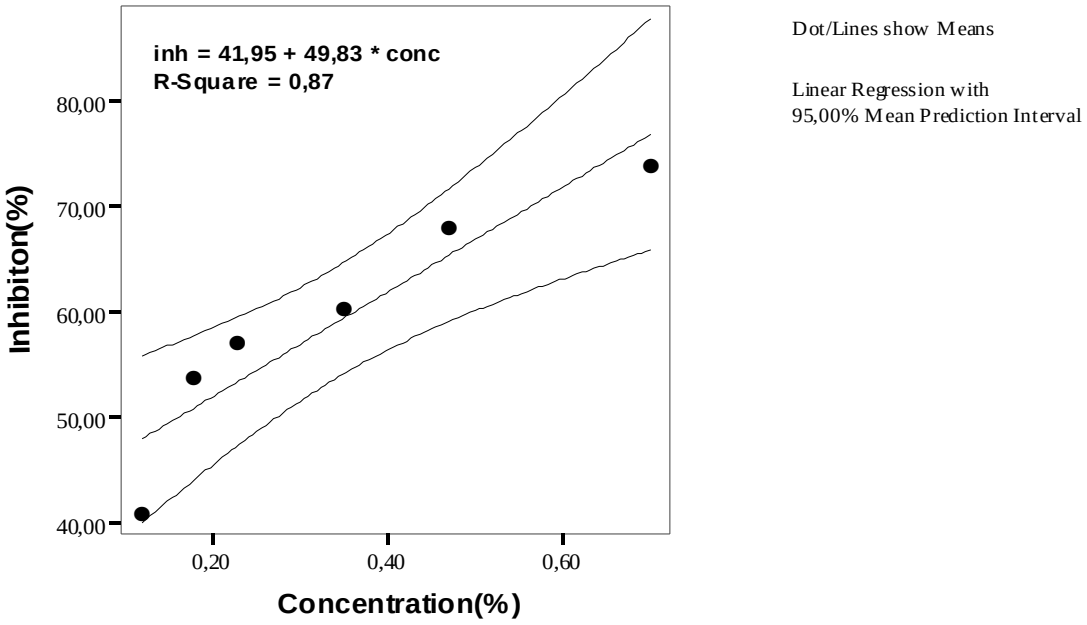


Figure 6.9 : EC_{50} Interpolation curve for THF+S_{FO}

Finally, THF and Bunker C Fuel Oil mixture was prepared for the BioTox ™ bioassay test. For this experiment 1 gr of Fuel Oil dissolved in 100 ml of THF and 10 ml of this sample tested for toxicity after the salinity levels arranged by 2% NaCl solution. Interpolation curve of THF+S_{FO} is given in Figure 6.8. R^2 value of linear regression of the test results for THF+S_{FO} is 0.87 and all of the points are in the 95% confidence limit. The EC_{50} value of the sample is found as 0.17% which is the most toxic mixture of all oil-solvent mixtures, and individual solvents. Meaning that most Bunker C Fuel Oil is the most toxic oil among others.

Meanwhile, Motor Oil and Used Motor Oil show significant changes according to the type of the solvent.Due to the oil-solvent mixtures, the EC_{50} values ought to be lower than individual solvents, expectively. When DMSO used as a solvent, mixtures of Motor Oil (S_{MO}), and Used Motor Oil (S_{UMO}) ended up with almost the same EC_{50} value as individual DMSO did. After all, mixtures of THF and both Motor Oil (S_{MO}), and Used Motor Oil (S_{UMO}) showed higher EC_{50} values than individual THF did. Hence, solvents (DMSO, THF) 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 6.17 in Toxic Units. As it can be seen from the table 6.17, THF is more toxic than the DMSO and Bunker C Fuel Oil can be both dissolve by DMSO, and THF, and gives the highest toxicity comparing to other oil samples (S_{MO},S_{UMO}).

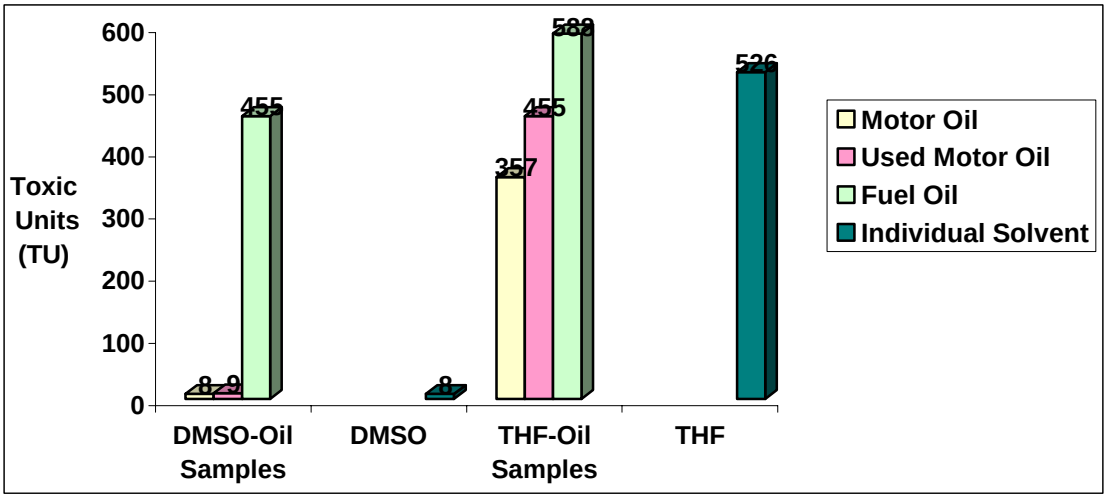


Figure 6.10: Toxicity of Solvents and Oil-Solvent Samples

6.4.3. Toxicity analysis for WSFs

Water soluble fractions of oils were prepared as mentioned in materials and methods chapter. 10 ml of $S_{WSF,MO}$, $S_{WSF,UMO}$, $S_{WSF,FO}$ were taken and mixed with 1,1 ml of 2% NaCl stock solution prior to toxicity testing, respectively. Computerized test results is given for $S_{WSF,MO}$ in Figure 6.9 . EC_{50} could not be determined accourding to all points are below EC_{50}. R^2 value of linear regression of the test results for $S_{WSF,MO}$ is 0.91 and all of the points are in the 95% confidence limit.

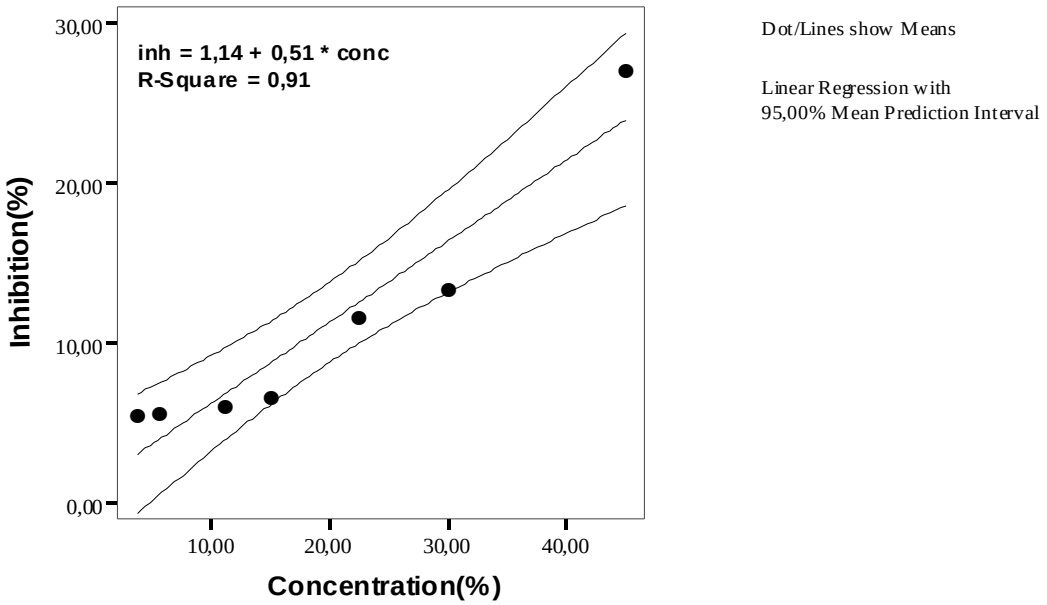


Figure 6.11 : Toxicity of WSF of Motor Oil

Only, EC_{50} value was determined for water soluble fraction of used motor oil ($S_{WSF,UMO}$). As it can be seen in Figure 6.10, 27% corresponds for EC_{50} value**.** R^2 value of linear regression of the test result for $S_{WSF,UMO}$ is 0.96 and except one point, all points are in the 95% confidence limit. Even if this one point was removed, EC_{50} value would not be changed because of the insignificant difference between EC_{50} values which is about 0.1 %. But still, this low value of toxic unit which is 4 shows that even WFS of used motor oil is observed lack of toxicity.

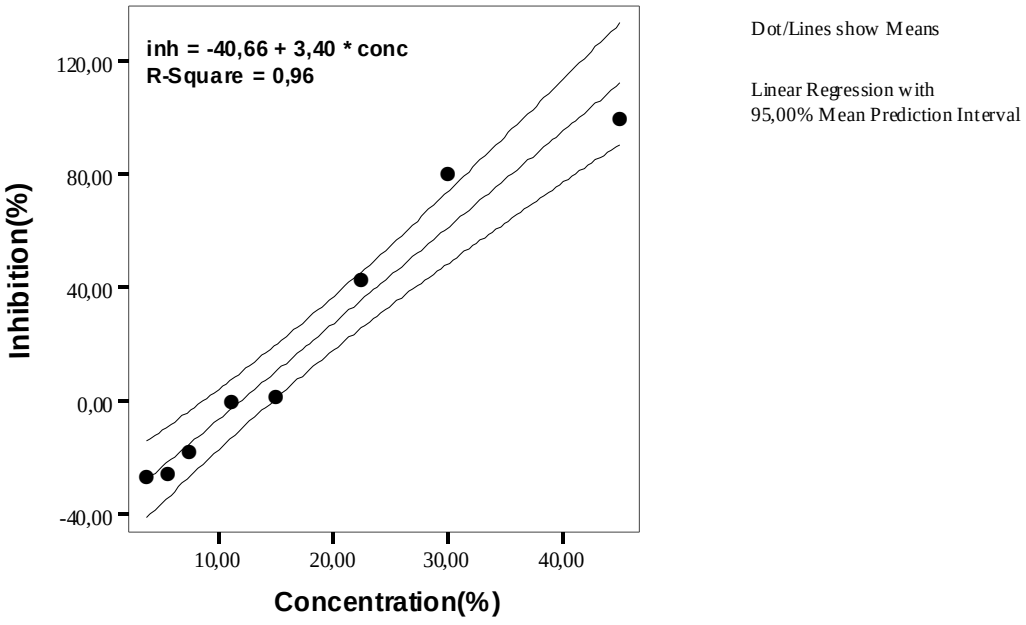


Figure 6.12 : Toxicity of WSF of Used Motor Oil

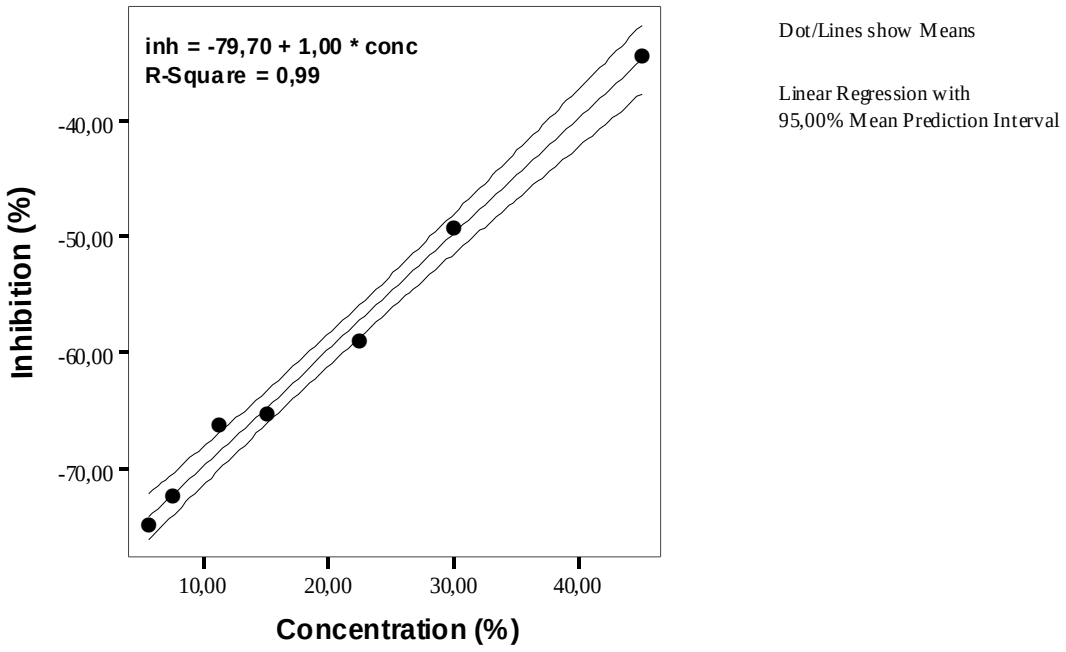


Figure 6.13 : Toxicity of WSF of Fuel Oil

An accurate determination of EC_{50} toxicity index for a particular toxicant is possible if responses of a test organism to several toxicant concentrations include values from 0% to 50% and above. When such responses are not observed, the EC_{50} is reported as a value, which is greater than the maximal tested concentration. Such expression of EC_{50} is very uncertain and limits its suitability for comparisons with toxicity values for other toxicants. This problem can be overcome by using more sensitive test organisms. The response of the given test organism can also be increased by facilitating transfer of a toxicant into water-soluble fraction (WSF). (Tsvetnenko and Evans, 2002).

There was no detectable toxicity to the luminescent marine bacteria, *Vibrio fischeri,* on exposure to water soluble fraction of bunker C fuel oil ($S_{WSF,FO}$), due to EC_{50} values being all bigger than 100% in the 100% test.

7. CONCLUSIONS

In enviromental engineering, oil and grease is an important collective parameter of the organic pollutants in water. In addition, chemical oxygen demand (COD) is another significant parameter, commonly used for estimating the organic content of waters and wastewaters. So far, it is known that high COD values of oily wastewaters mainly originate from oil and grease content of the wastewater. Besides, there are difficulties to understand different toxic characteristics between an individual material and collective parameters. The results which were obtained from experimenal study of COD, TOC counterparts of oil and grease, and its toxicity have been concluded as;

- For three specimen namely Motor oil, Used motor oil, and Bunker C fuel oil, oil and grease measurements by soxhlet extraction method have approximately 80 % efficiency. According to results, Bunker C fuel oil has higher oil and grease content than others. Even though, there is not a big difference between the oil and grease content (%) of samples, that little difference can be explained in terms of n-hexane extraction efficiency in the method. Motor oil and used motor oil might contain more metals and impurity content that cannot be extracted by n-hexane as compared to Bunker C fuel oil.
- Estimation of COD counterpart of oil and grease is not possible by using standard open reflux method. Oil is insoluble in water, which makes it impossible to prepare a proper dilution for COD determination. Therefore, to adjust oil samples to the COD experiment, transfering a specific amount of oil and grease into a COD flask by hexane extraction can be considered as a new method.
- Approximately 1:4.5 ratio is determined as COD counterpart of oil and grease. This ratio indicates the contribution of O&G in COD concentration. COD/O&G is an essential parameter during design of wastewater treatment plants because if O&G consequently COD is removed before main treatment units, the volumes of

the main treatment units would be smaller. This brings an important economic advantage during construction and operation of treatment plant.

- Although the selected samples which are mineral based oils have different chemical properties, the COD/O&G ratios of them are similar. It can be concluded that mineral based oils have similar COD/O&G ratios.
- Bunker C fuel oil has the lowest soluble fraction in water where used motor oil has the highest solubility. The reason used motor oil has a higher soluble fraction is because of its impurity content.
- Because of insolubility of Oils in water, solvents are used for their determination. In this study, dimethlysulfoxide (DMSO), tetrahydrafuran (THF) and n-hexane were used as extraction solvents to determine both COD counterpart of oil and grease, and its toxicity. For this reason, COD values of solvents were measured individually.
- At first COD and theoretical oxygen demand of n-hexane were determined, and results were compared. Ratio of COD and Th.OD of n-hexane was low meaning potassium dichromate was unable to oxidize all hexane. As is the case in oils, hexane has a poor solubility in water that might be reason of low COD/Th.OD ratio.
- Measured COD for DMSO cannot be bigger than its Th.OD. Hence, after the experiment it is seen that COD results for DMSO and its Th.OD are vice versa, meaning that results are unreliable. An adduct formation or decomposition of DMSO might be the reason of high COD values despite little amounts of DMSO. In addition, during the oxidation of DMSO by potasium dichromat – sulfuric acid, adduct formation and complex structure of Cr^{6+} might be ended up with color masking of titration in standart method.
- Lastly, COD test applied to THF, which was reacted with potassium dichromat – sulfiric acid, and formed black colored liquid. Because of this color masking, COD determination of THF value was immeasurable.
- For TOC analysis, samples have to be soluble in water. Hexane is insoluble solvent in aqueous medium, for that reason, it was not possible to prepare a proper dilution for the TOC analyzer and hence TOC result was not available.

- On the other hand, DMSO has soluble property in water thereby TOC was measured for DMSO. Calculated and measured Th.TOC ratio of DMSO shows that TOC analysis can be applied to DMSO successfully.. Obviously, TOC analysis can be also applied to all water soluble fractions of oil samples and can be compared with COD and soluble O&G content.

- Toxicity counterpart of O&G is unknown since O&G are insoluble in water as well as diluting the samples with water during determination of toxicity diminishes the solubility strength of the solvent. For that reason, to estimate EC_{50} values, solvent extraction is necessary using solvents such as DMSO, THF and hexane. Dimethylsulfoxide (DMSO) has low test toxicity and ability to solubilize a broad spectrum of non-polar organic compounds which makes it an adequate solvent for the BioTox^{TM} system.

- Toxicity of THF is considerably high comparing to DMSO. Solvents having high toxicity such as THF make preparation of dilutions difficult. Samples have to be diluted several times to be compatible with the limits to estimate EC_{50} values in the BioTox ^{TM} bioassay test. Hence, depending on the increase of dilution, solvent's extraction efficiency would decrease. So that, toxicity test might be ended up with an experimental error.

- It is seen that DMSO which has a low toxicity and high solubility showed a better efficiency than THF. Meaning that, DMSO is a more proper solvent for the BioTox^{TM} bioassay.

- According to the results of oil-solvent mixture samples, it can be said that toxicities of samples which were dissolved with THF are relatively higher than samples which were dissolved with DMSO because THF is a stronger solvent than DMSO.

- Motor Oil and Used Motor Oil mixture samples with DMSO, have very similar toxicities between each other and with the individual DMSO toxicity. The results show that DMSO extractable part of the Motor Oil and Used Motor Oil does not contain any substance that would cause a significant toxicity. Meanwhile, Motor Oil and Used Motor Oil mixture samples with THF have a lower toxicity than their individual THF toxicity. Decreasing extraction efficiency of THF during

dilution of the samples or an antagonistic effect might be considered as reasons for the results.

- By comparing differences between toxicities of solvents and oil-solvent mixtures, it can be concluded that the highest toxicity belongs to Bunker C fuel oil. However, water soluble fraction of Bunker C fuel oil showed no toxicity according its lack of solubility.
- According to the test results of the water soluble fractions of oils, motor oil, and fuel oil there is no detectable toxicity to the luminescent marine bacteria, *Vibrio fischeri.* Fuel Oil is completely insoluble with distilled water. Motor Oil is sligtly soluble in distilled water. Used Motor Oil give significant toxicity in distilled water because of having inorganic compounds from the crankcase of the engine such as heavy metals and also impurities from the combustion. These factors will increase the soluble fraction of oils resulting a rise in the toxicity. In this case, used motor oils have to be handled more specifically.
- Although oil and grease is insoluble in water, COD counterpart of oil and grease can be evaluated as weight by weight (w/w) and toxicity values can be determined with solvents mentioned above as Toxic Units (TU). So, oil pollution characteristics can be assessed based on organic collective parameters in literature.

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