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Bioremediation of Crude Oil Polluted Soils

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ABSTRACT
Turkey has recently been exhibiting an increasing development in terms of spreading oil pipe-line constructions. In a few months, Baku-Tiflis-Ceyhan pipe-line would be carrying 50 million ton crude oil per year from Ceyhan through all over the world. In the future, no doubt that new pipe lines will be connected to our oil transporting network so that Turkey would be much more effective to convert his geographical advantage to the income. However, we are lacking of knowledge on the impacts of oil pollution and its elimination from our lands. As well as its economic and geopolitical contributions, pipe-line transportation has a great risk in terms of oil contamination by spillage, leakage and accidents etc. on the natural sources. Improving our knowledge on the effects and remediation of oil-related pollution has therefore a critical significance in Turkey's future in the respect of sustainable use of the environment. For that reason, in the present review, we briefly discussed the methods used in the remediation of oil polluted soils. Of the technologies commonly used for the cleanup of polluted soils, bioremediation is a newly accepted idea that gaining a worldwide interest.

Key words: Soil, crude oil, pollution, remediation methods, bioremediation, soil microorganisms

INTRODUCTION
Crude oil is highly complex mixture, containing hundreds of thousands of hydrocarbons (Cooney, 1980). Compounds in crude oil can be divided into three general classes consisting of saturated hydrocarbons, aromatic hydrocarbons and polar organic compounds (Huesemann and Moore, 1993). Soils which are contaminated by hydrocarbons have extensive damage of local ecosystems since accumulation of pollutants in animals and plants tissues, may cause progency's death or mutation (Alvarez *et al*., 1991). Crude oil is physically, chemically and biologically harmful to soil because it contains many toxic compounds in relatively high concentrations (e.g., polycyclic aromatic hydrocarbons, benzene and its substituted, cycloalkane rings) (Franco *et al*., 2004). The presence of high molecular weight compounds with very low solubility in water prevents natural biodegradation process from working efficiently in hydrocarbon contaminated soils. These compounds also penetrate macro-and micropores in soil and thus limit water and air transport that would be necessary for organic matter conversion (Caravaca and Roldan, 2003). Generally, petroleum hydrocarbon compounds bind to soil components and are difficult to remove or degrade. Biosurfactans (BS) can emulsify hydrocarbons, thus enhancing their water solubility, decreasing surface tension and increasing the displacement of oily substances from soil particles (Banat, 1995a, b; Banat *et al*., 2000). Sites contaminated by petroleum compounds range from leaking household oil tanks to areas polluted by oil tanker spills, e.g., old and new petrol stations as well as areas surrounding oil storage facilities, pipelines, terminals and refineries.

In the respect of oil pollution, soil remediation methods aim preventing the further spread of the pollutant and also its removal from the soil. In the present study, we aimed to summarize the knowledge used in the remediaiton of oil polluted soils and to underline importance of bioremediation as a fresh study area in environmental and soil sciences.

Methods used in the cleanup of oil polluted soils: The methods used in the remediation of oil polluted soils can be grouped as physical-chemical processes (shallow soil mixing, oxidation-reduction, hydrolysis-neutralization, stabilization-solidification, mobilization-immobilization, soil flushing-washing), thermal processes use (heat to increase the volatility, to burn, decompose, destroy or melt the contaminants) and biological processes (bioventing, biopurging, hydraulic-pneumatic fracturing, soil bioinjection, air and water flushing, biopolymer shields and phytoremediation). The question of which method should be used in oil polluted lands depends on the chemical, physical and biological properties of both contaminant and soil source. Physical methods, such as stripping or sorption, are not as effective as biological methods for treating hazardous organic compounds (Knox *et al*., 1986). Chemical methods may have to be used to remove heavy metals. The chemical structure (and then the behavior) of pollutants is changed by means of chemical reactions to produce less toxic or better separable compounds from the matrix. Thermal processes use heat to increase the volatility, to burn, decompose, destroy or melt the contaminants. Chemical constituents are burned and chemically oxidized by applying a high heat input. The most important problem in incinerating hazardous wastes and soil is the generation of by-products such as polychlorinated dibenzofurans (PCDF), chlorinated benzenes, chlorinated phenols and nitrogen oxides (Song *et al*., 1992; Nito *et al*., 1997). In the remediation of oil polluted soils, physical and chemical methods i.e., aeration, excavation, transportation and incineration) have been used for the years. These methods however have been often expensive and laborious (Eckenfelder and Norris, 1993). The most important limitation to traditional cleanup methods is that they do not always remove the contaminants from soil completely.

What is bioremediation (What it is and How to do it): Of the technologies and methods that have been investigated for the cleaning up oil contaminated soils, bioremediation has appeared as the most desirable approach due to its low cost and ability to hinder the accumulation of contaminant (Bonnier *et al*., 1980; El-Nawawy *et al*., 1987). Shortly, soil bioremediation is the process in which most of the organic pollutants are decomposed by soil microorganisms and converted to harmless end products such as carbon dioxide, methane and water (Walter *et al*., 1997). As no single microbial species is capable of degradation all components of crude oil, complete oil degradation requires simultaneous action of different microbial populations. One of the factors limiting biodegradation of soil pollutants is their limited availability to microorganisms (Providenti *et al*., 1995). Soil microorganisms are a fundamentally important component of terrestrial habitats. Their primary roles are governing the nutrient cycles and involving in genesis and maintenance of soil structure. A characteristic feature of soil microorganisms is their complexity, both in terms of numbers of organisms and their genetic and functional diversity. This feature is usually termed as soil microbial diversity and describes the variability of biological organization at different levels in soil. In soil microbial universe, certain microbes have a distinctive ability to degrade or to convert organic pollutants to harmless biological products. The fact of bioremediation mainly relies on the use of these talented microorganisms surviving in soil. There are many biological techniques used in the cleanup of land and water sources (i.e., bioventing, bioslurping, hydraulic-pneumatic fracturing, soil bioinjection, air and water flushing, biopolymer shields, electrobioreclamation and phytoremediation). However, most of these techniques are highly expensive, preffered for cleanup of deep soil layers and may be limited in terms of soil properties and environmental conditions. These several technologies that biostimulations involves the addition of oxygen, water and mineral nutrients (Orzech *et al.*, 1991; Turgay *et al.*, 2010); bioventing technique combines conventional adjectives soil venting with biodegradation (Van Eyk, 1994; Reisinger *et al.*, 1994). Comparing to expensive biological treatments mentioned above, phytoremediation and bioremediation are more convenient approaches in that they do not need high input and may remove the pollutant in a shorter time. Phytoremediation is the use of plants to partially or substantially remediate selected contaminants in contaminated soil, sediment, sludge, waste water, surface water and ground water. It utilizes a variety of plant biological processes and the physical characteristics of plants to aid in site remediation. Phytoremediation has also been called green remediation, botano-remediation, agroremediation and vegetative remediation. The root system of plants is an important area for these reactions to take place. This process is especially useful in tight soils which take an advantage over other biological methods (i.e., bioventing and biopurging) that do not work well in tight soils. Natural plants can help to remediate contaminated sites through accumulating contaminants or enhancing biodegradation (Johns and Nyer, 1996). Bioremediation has been defined by Madsen (1991) as a managed or spontaneous process in which biological, especially microbial, catalysis acts on pollutant compounds, thereby remedying or eliminating environmental contamination. Actually bioremediation aims to optimize conditions for microbial hydrocarbon degradation and is currently receiving increasing attention as a treatment technology (Wang and Bartha, 1990). In order to improve the natural tendency of soil microorganisms to decompose hydrocarbons from crude oil, many techniques of land farming i.e., mineral fertilization, organic amendments, (Turgay *et al.*, 2010) cropping systems have been proposed and tested (Sims and Sims, 1999). The intensity of hydrocarbon biodegradation in soil is influenced by several environmental factors such as involved indigenous microbial populations, oxygen supply, pH, soil moisture, temperature, nutrient availability, organic matter, quantity, quality and bioavailability of contaminants and soil properties (Atlas, 1995). Bioremedation techniques accelerate the naturally occurring biodegradation by optimizing conditions for biodegradation through acration, addition of nutrients and control of pH and temperature (Norris *et al.*, 1994; Atlas and Bartha, 1992; Morgan and Watkinson, 1989). Microorganisms, just like humans, eat and digest organic substances for nutrients and energy. In chemical terms, organic compounds are those that contain carbon and hydrogen atoms. Certain microorganisms can digest organic substances such as fuels or solvents that are hazardous to humans. The microorganisms break down the organic contaminants into harmless products mainly carbon dioxide and water. This idea can be simply outlined as in Fig. 1 (USEPA, 1996).

Bioremediation is a treatment process that uses microorganisms (yeast, fungi, or bacteria) to break down, or degrade, hazardous substances into less toxic or nontoxic substances. The ability to utilize hydrocarbons is widely distributed among diverse microbial populations. In general, population levels of hydrocarbon utilizers and their proportions within the microbial community appear to be a sensitive index of environmental exposure to hydrocarbons (Atlas, 1981). Petroleum hydrocarbon contamination usually exists as a complex mixture of hydrocarbons. Despite the huge potential of microorganisms to degrade organic compounds under favorable conditions, no single species of microorganism can degrade all the components of given oil (Office Technology Assessment, 1991). In unpolluted ecosystems, hydrocarbon utilizers generaly constitute less than

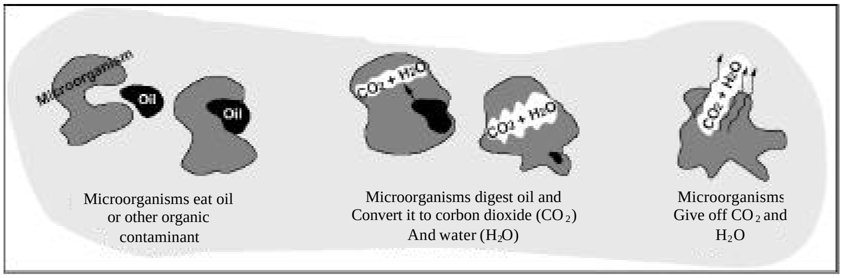

Fig. 1: Schematic diagram of aerobic biodegradation in soil

0.1% of the microbial community; in oil- polluted ecosystems, they can constitute up to 100% of the viable microorganisms. Individual organisms are not restricted to a certain range of oil types (Bausum and Taylor, 1986). Each organism attacks many different oils with comparable facility (Stone *et al.*, 1942). However, this is not the case when individual hydrocarbons or classes of hydrocarbons are considered (Bausum and Taylor, 1986). The overall effect of an organism on a complex substrate is limited by its capacity to attack only certain substances or to accumulate intermediates that it cannot further degrade. Many species of bacteria, cyanobacteria, fungi and yeast coexist in natural ecosystems and may act independently or in combination to metabolize aromatic hydrocarbons (Gibson, 1982; Cerniglia, 1984; Fedorak *et al.*, 1984).

Bacteria capable of biodegrading petroleum hydrocarbons may commonly be found in subssurface soils; however, natural breakdown of the compounds will occur too slowly without intervention to prevent accumulation of the pollutants to unacceptable levels (Lyman *et al.*, 1990). Combinations of bacteria, yeasts and fungi provide about twice as much as degradation of mixed hydrocarbon substrates as do bacterial or fungal strains individually (Walker and Colwell, 1947). Mixed microbial populations are almost always encountered in natural systems. Some of hydrocarbon degrading genera were shown in Table 1 (Shailubhai, 1986).

Mixed microbial populations are almost always encountered in natural systems. Extensive degradation of petroleum pollutants generally is accomplished by mixed microbial populations, rather than single microbial species (Atlas, 1978). For example Mishra *et al.* (2001) have studied plots A and B were treated with a bacterial consortium and nutrients, which resulted in 92 and 89% removal of TPH, compared to 14% removal of TPH in the control plot C.

A diverse group of bacteria and fungi has been identified that partially degrade, co-metabolically oxidize or mineralize some high-molecular-weight PAHs to less toxic products. According to the literature, the pyrene (PAH) mineralization by fungi is limited to certain species of white-rot fungi, such as Phanerocheate chyrsosporium (Hammel *et al.*, 1986), Crinepellis stipitaria (Lambert *et al.*, 1994; Lange *et al.*, 1994), some micromycetes (Ravelet *et al.*, 2000) and ectomycorrhizal fungi (Braun-Lulleman *et al.*, 1999).

Many authors have extensively studied bacterial metabolism of pyrene (PAH). The major species involved in degradation is Mycobacterium sp., (Kazunga and Aitken, 2000). Successful bioremediation strategies are those that are tailored to satisfy specific pollutant, site, public, regulatory, cost-and environmental effectiveness considerations, or can be well integrated into other waste cleanup schemes. For example, landfarming (which is described immediately below) will be sufficient for the cleanup of certain pollutants; however, the practice must be combined with very zaggressive interventions such as nutrient additions and alterations in environmental conditions

Table 1: Microbial genera degrading hydrocarbons in soil

Bacteria	Actinomycetes	Fungi	Yeast
Pseudomonas	Actinomyces	Aspergillus	Candida
Bacillus	Endomyces	Cephalosporium	Rhodotorula
Bacterium	Nocardia	Cunninghamella	Torula
Clostridium		Torulopsis	
Thiobacillus		Trichoderma	
Methanobacterium		Saccharomyces	
Enterobacter			

Table 2: Advantages and disadvantages of biodegradation

Advantages	Disadvantages
Can be done on site	Some chemical cannot be biodegraded
Permanently eliminate waste	Extensive monitoring is needed
Is cheaper	Site-specific requirements can affect applicability
Eliminates long-term liability	Toxicity of contaminates can be a problem
Minimizes site destruction	Unknown by-products can be produced
Eliminates transportation cost and liability	Perception of unproven technology may affect the use
Can be coupled with other treatment techniques	

(biostimulation) for expeditious and more effective cleanup of persistent wastes. Several organisms are known, each capable of degrading usually one or, at best, a few petroleum component at a time. Therefore, effective bioremediation of petroleum contamination requires a mixture of populations consisting of different general each capable of metabolizing the respective compounds. Bioremediation or bioreclamation refers to the enhancement of this native capability of the microorganisms. The bioremediation capacity of bacteria has been investigated more extensively because they are (1) easier to culture, (2) more amenable to molecular biology techniques, (3) capable of metabolizing chlorinated organics and (4) capable of mineralizing these chemicals and using them as carbon energy sources (Bouwer and Zehnder, 1993). Although, bioremediation is a convenient approach with low cost, its limitations and disadvantages should also be kept in mind. Table 2 shows major advantages and disadvantages for the bioremediation treatments.

CONCLUSION

Recent development in oil pipeline network in Turkey indicates that there is an urgent need to accumulate scientific knowledge and experience against to possible oil pollution risks in Turkey. In literature, there has been a considerable effort for soil bioremediation and many strategies have been explored and successfully implemented. Furthermore, combinations of various bioremediation approaches alone, or their integration with other cleanup strategies continue to grow. As a reflection of scientific information to practice, today, there are many bioremediation products in international market. Most of these products are commercial microbial inoculums with special formulation including oil degrading bacteria. On the other hand, some of these bacteria have been isolated from sewage sludge or hazardous mixtures which may bring health hazards for human. In addition, a number of the workers have stated their disappointments that ability of these products may be satisfactory only under strict scientific conditions and the natural populations may be often better competitors when supplied to polluted conditions with appropriate nutrients (OECD, 1994). Therefore, a bioremediation scenario should be carefully designed and evaluated before applying to a pollution problem.

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