

SOME INVESTIGATION ON BIOREMEDIATION OF PAHs CONTAMINATED SOIL IN IRAN TAR REFINERY

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Abstract The main purpose of this research is studying the possibility of bioremediation of polycyclic aromatic hydrocarbons (PAHs) contaminated soils in Iran's tar refinery area located in Isfahan. For many years a great quantity of tar produced by neighboring metallurgical plant has been stored in lagoons as natural reservoirs, causing the contamination of soil in this area. Microorganisms capable of transforming PAHs, such as anthracene and phenanthrene, are commonly found in soil, especially soil previously contaminated with PAHs and microorganisms capable of transforming PAH compounds have been used to decontaminate soil. In the first phase of our study, we focused on isolation and purification of PAHs utilizing microorganisms from soil. The results of site characterization and biofeasibility testing have shown the presence of a viable native population of community of specific contaminant-degrading microbes already in the site. Therefore, biostimulation becomes an available option. It is the essence of this option that the native (indigenous) microbial community is found to be capable of degrading the target site contaminants (PAHs) if managed properly. In this research for the first time in Iran we have studied, the possibility of bioremediation of PAHs contaminated sites by indigenous microorganisms. Microbiological analysis of soil chronically exposed to coal tar of an industrialized area near the city of Isfahan, resulted in the isolation of some bacteria which degraded, each of, naphthalene, phenanthrene, and, anthracene in an aqueous solution, when grown for two weeks in pure culture with organic nutrients. The initial PAH concentration was at the water solubility level and degradation proceeded to no detectable level, in some cases. They grew well at 25°C to 30°C. The use of these microorganisms may be an attractive alternative to existing physiochemical method for the remediation of PAH in the environment.

Key Words Bioremediation, Coal Tar, PAHs, Soil

چکیده درمان بیولوژیکی محیطهای آلوده به آلاینده‌های آلی و غیر آلی یکی از موثرترین روشهای جدید و در حال رشد قرن اخیر است. این روش در حقیقت یک کاربرد بسیار نو از شیوه قدیمی مورد استفاده در تصفیه فاضلاب بوده که امروزه در زمینه پاکسازی خاکها، لجن‌ها، آبهای زیر زمینی، آبهای سطحی آلوده به نفت خام و هیدروکربنهای نفتی، سوختها و حلالهای صنعتی بکار می‌رود. هدف اصلی این تحقیق مطالعه درباره امکان بکارگیری این روش در پاکسازی خاکهای آلوده به هیدروکربنهای آروماتیک چند حلقه ای در پالایشگاه قطران ایران واقع در اصفهان می‌باشد. در درمان بیولوژیکی از میکرو ارگانیزمهای تبدیل کننده هیدروکربنها برای پاکسازی استفاده می‌شود. این دسته از میکروبها در خاکها، بخصوص در خاکهای آلوده به هیدروکربنهای آروماتیک چند حلقه‌ای، وجود دارند. نتایج حاصل از بررسیهای منطقه‌ای و مطالعات بیولوژیکی خاک نشان دهنده وجود میکرو ارگانیزمهای بومی فعال تبدیل کننده این دسته از هیدروکربنها می‌باشد. بنابراین تحریک بیولوژیکی بعنوان یکی از روشهای درمان بیولوژیکی می‌تواند انتخاب شود. این روش که بخصوص در سالهای اخیر در سطح وسیعی مورد توجه قرار گرفته است، مرحله بعدی تحقیق خواهد بود.

INTRODUCTION

Bioremediation, or enhanced microbiological treatment of environments contaminated with a variety of organic and inorganic compounds is one

of the most effective and innovative technologies that have emerged in this century. Actually, it is simply a new application of a very old technology once primarily used in wastewater treatment. Today, bioremediation is routinely applied to soils,

sludge, groundwater, process water and surface waters contaminated with such chemicals as crude oil, petroleum hydrocarbons, fuels, industrial solvents and wood - treating agents.

Polycyclic aromatic hydrocarbons (PAHs) are chemicals containing two or more fused benzene rings, which are ubiquitous products of the incomplete combustion of fossil fuels and organic compounds [1]. Environmental levels of PAHs in industrialized countries have risen during the last century because of increases in anthropogenic sources as well as atmospheric deposition from natural sources [2]. The potential carcinogenic effects of PAHs in the environment and the possible health risks to humans are of concern [3].

Because of their genotoxicity, the U.S. Environmental Protection Agency has listed 16 PAHs as priority pollutants to be monitored in industrial effluents [4]. This interest has led to the development of technologies to contain and detoxify PAH - contaminated wastes.

Since these organic pollutants are hydrophobic and sparingly soluble, soil and groundwater contamination has become a common problem in many locations [5]. For about 100 years, chemicals as byproducts in the primary processing of coal tar. Metallurgical coke has been the main industrial source of aromatic compounds used as intermediates in organic synthesis [6]. In this process a great quantity of tar that is composed of

TABLE 1. Process Residuals from the Manufacture of Gas from Coal, Coke and Oil.

Process residuals	Physical form and principal chemical content	Coal carbonization	Carbureted water gas	Oil gas
Ammonia liquors-	Aqueous liquid: Inorganic, phenolics	X	-	-
Ash and clinker	Solid: metals (and unburned coke or coal)	X	X	-
Carbureted water Gas tar	Organic liquid: PAHs BTEX	-	X	-
Coal tar	Organic liquid: PAHs, BTEX and phenolics	X	-	-
Coke and coke breeze	Solid: pyrolyzed coal	X	-	-
Lampblack	Sludge: elemental carbon and oil tar	-	-	X
Light oils	Organic liquid: BTEX	X	X	X
Oil tar	Organic liquid, PAHs, BTEX	-	-	X
Spent oxide or lime, wood chips	Solid: metals, cyanide sulfur, tar	X	X	-
Tar sludge	Solid -liquid: PAHs, BTEX	X	X	X
Tar - oil – water Emulsions	Aqueous and organic liquids, PAHs, BTEX	X	X	X
Wastewater treatment sludge	Solids, aqueous, and organic liquids: inorganic			

X indicates that residual was produced, “-” indicates that residual was not produced in substantial amounts
PAH = Polycyclic aromatic hydrocarbons: BTEX = benzene, toluene, ethyl benzene, and xylene.

approximately 85% PAHs is produced [6]. Coal tar was usually stored in open lagoons causing contamination of soil.

On the other hand, prior to the widespread use of natural gas, combustible gas manufactured from coke, coal, and oil served as the major gaseous fuel for urban heating, cooking, and lighting in various countries [7].

For example, in the United States manufactured gas, or town gas, was produced at some 1000 to 2000 plants. Pipeline distribution of natural gas following World War II replaced manufactured gas as the major gaseous fuel, and as a result manufactured gas production came to an end in the 1950 s [8].

Today, soil and groundwater contamination problems exist at many former manufactured gases plant (MGP) sites because of prior process operations and residuals management practices [9].

Residuals that were produced in MGP processes are summarized in Table 1 for the three primary gas production methods: coal carbonization, carbureted water gas production, and oil gas production [10]. These process residuals are dominated by six primary classes of chemicals; namely, polycyclic aromatic hydrocarbons (PAHs), volatile aromatic compounds and phenolics inorganic compounds of sulfur, nitrogen and metals.

Tar residuals were produced from the volatile component of bituminous coals in coal carbonization, from the residue of gasifying oils in oil gas processes, and from the cracking of enriching oils used to increase gas Btu content in carbureted water gas production.

Tars are organic liquids that typically are denser than water, with a range of physical and chemical properties dependent on the feedstock and operating conditions of the production process [9-11]. Although some tar was used on site or sold, during certain periods, there was insufficient demand for all the tar that was produced.

Further, because of changes in tar composition due to changes in feedstock, problems with tar-water emulsions, and other factors, the intrinsic value of tars was of ten considered marginal. Consequently, tars were sometimes managed off site or were deposited on site in tar wells, sewers, nearby pits or streams.

SOIL CONTAMINATION AT COAL TAR DISPOSAL SITE

Each coal tar disposal site has unique aspects, but common operating and waste management practices of the past have led to similar patterns of soil contamination.

A subsurface cross section of a typical coal tar disposal site is, shown in Figure 1 [11]. These sites usually consist of a layer of fill (a mixture of soil, ash, and demolition debris) underlain by a layer of sand and gravel and a layer of silty clay or other fine-grained material. Shallow unconfined aquifers usually exist at these sites and are hydraulically connected to a water body.

The groundwater table is often shallow 0.15 to 4.5 meters beneath the surface. Contamination of soil with tar also occurred at many sites because of leaks and spills from on - site vessels and piping networks, incomplete separation of tar from aqueous liquids, storage of tar in unlined pits or shallow wells, and dismantling and decommissioning activities when the plant was taken out of service. In addition, tar would sometimes be mixed with other site wastes and used as fill in the low - lying areas of a plant site.

Tar released into the subsurface by the above processes migrates downward as a result of gravity until it encounters a low permeability layer that it cannot penetrate because of large capillary forces (Figure 1). If present in sufficient quantity the tar may pool on the low permeability material or move laterally, following the geologic gradient (dip) of these materials [12].

Contact of groundwater with the tar results in dissolution of tar constituents and generation of contaminated ground -water plumes.

Tar contamination along riverbanks and in the shallow sediments of rivers and lakes has also been found near these sites. These observations primarily reflect the discharge of tars directly into the adjacent water bodies through site sewers or ditches.

Much of the tar that escaped the plants in this manner did so as incidental carryover of hydrocarbon - water emulsions from the tar separators. Migration from tar wells and subgrade gasholder tanks has also contaminated some streams.

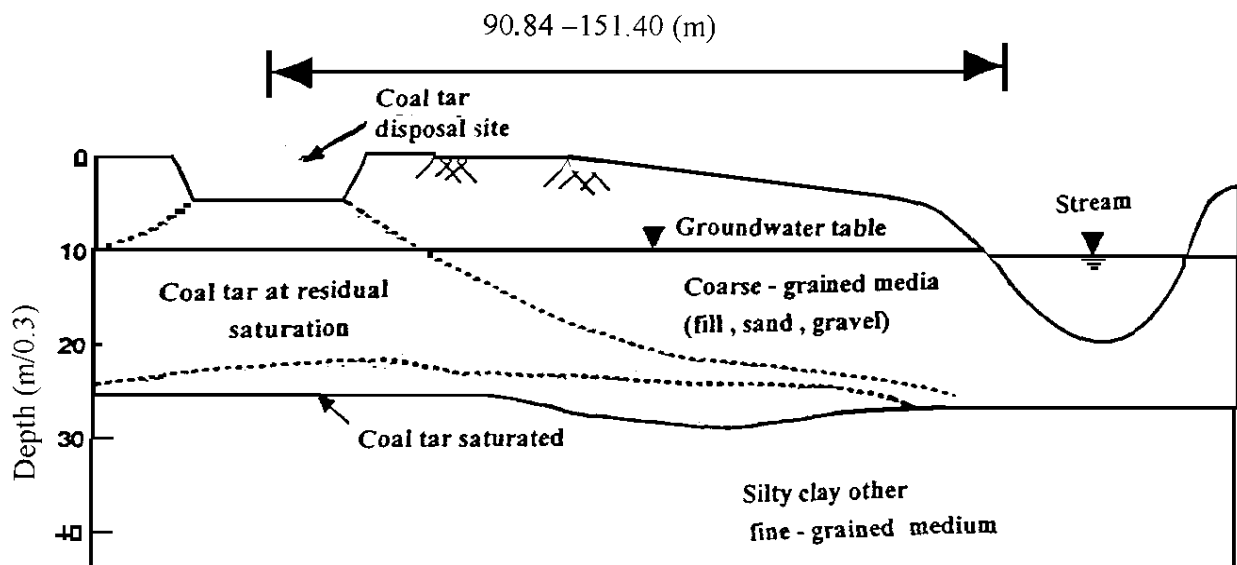


Figure 1. Generalized cross section of a typical coal tar disposal site.

REMEDIATING TAR CONTAMINATED SOILS

While characterization of tar contamination problems at industrial sites has progressed, there have only been limited remediation attempts. At the same time, there has been a significant amount of investigation of remediation approaches. Comprehensive reviews of potential remediation technologies for use at MGP sites have been prepared [10]. Most of these technologies have

been proposed based on their performance on comparable.

Non-aqueous-Phase Liquid (NAPL) wastes from industries such as petroleum refining, wood treating, byproduct coke manufacture, and synthetic fuel production.

Research efforts have focused on the development of low cost methods for site remediation, especially alternatives to excavation and incineration, and to land disposal for treatment of tar-contaminated soils. Various in situ

TABLE 2. Treatment of Excavated Soil. The Techniques Most Frequently Applied in Practice [11, 12].

Type of technique	Field of application	
	Type of soil	Pollutants
• Extraction/wet classification	sandy	all types
• Thermal treatment	all types	organics/Hg
• Biological treatment		
* Land farming	micro biodegradable sandy	sandy
* Heap pile systems		micro
* Slurry reactors		biodegradable
	all types	micro biodegradable

TABLE 3. In Situ Treatment of Soil. The Techniques Most Frequently Used in Practice.

Type of technique	Field of application	
	Pollutants	Type of soil
• In-situ extraction	sandy	water-soluble
• Soil vapor extraction	sandy	volatile
• Air sparring	sandy	volatile
• Bioremediation	sandy	volatile
• Electro reclamation	sandy	micro biodegradable
• Solidification/stabilization	all types	heavy metals
• Geohydrological isolation	all types	inorganic (organic)
• Mechanical/physical isolation	all types	all types
	all types	all types

treatment techniques have been investigated, including thermal treatment, biological treatment and water-based soil washing [11,12].

Table 2 lists the most important techniques along with field applications.

In situ treatment approaches have also been investigated, not only because of potential cost effectiveness but also because contaminated soils can not always be accessed easily due to the presence of surface structures, utility line, or other physical barriers. These bench and pilot scale studies have emphasized in situ flushing with aqueous solvent or surfactant solutions to enhance the rate and extent of tar solubilization [13] and in situ bioremediation for water-soluble tar contaminants [13]. Techniques aimed at volatilization of organic contaminants from soils (e.g., steam stripping or vapor extraction) are not expected to be effective for removal of the high molecular weight, low-volatility tars encountered at various sites.

Table 3 lists the most important in situ treatment techniques together with their field applications and possible modification. Research on in situ methods is largely focused on removal or destruction of tar at residual saturation. Although free tar may be directly pumped from selected locations in the subsurface [13], often the greatest mass of tar is that held at

residual saturation by capillary forces. Residual saturations of dense NAPLs (DNAPLs) are typically 5-25% of the pore volume [14]. Such tar is effectively immobile and fairly insoluble, but its dissolution is sufficient to contaminate large amounts of groundwater for decades and longer. Mobilization of the residual tar phase by interfacial tension lowering (e.g. through surfactant addition) or by viscosity reduction (e.g. through heating) is a possible remediation approach, but the difficulty in controlling the downward movement of a mobilized dense organic liquid may pose an unacceptable risk [15]. It is well recognized that microorganisms have the ability to degrade a wide variety of organic compounds.

In situ bioremediation appears to be a promising technology for cleaning up sites contaminated with hazardous waste. It has several advantages over thermal and other physicochemical methods. It neither alters the intrinsic soil properties nor does it involve the offsite transport of contaminants off.

In recent years, the treatment of hazardous waste has received considerable attention, thus the discovery of microorganism having the ability to degrade PAHs to less toxic products will greatly help in the development of processes for remediation of PAH contaminated sites.

Microorganisms capable of transforming

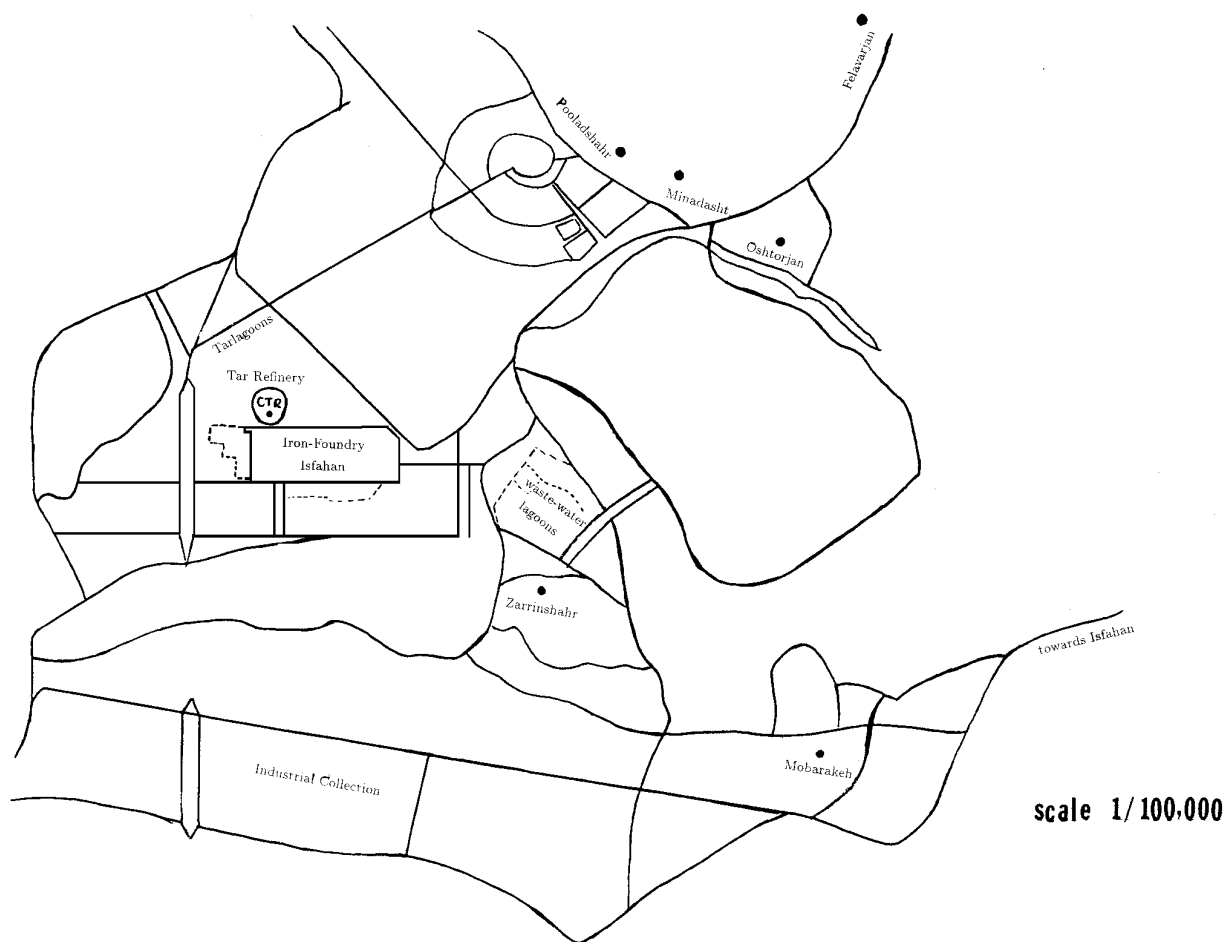


Figure 2. The map of site for collecting the samples (Sampling location*).

PAH compounds and remediating PAH contaminated soil are more abundant in PAH contaminated sites.

MATERIALS AND METHODS

A Case Study in Iran: Description of the Field

Site The site under study for this research is the Isfahan Iron Foundry Unit, located 45 km, south west of Isfahan, in a district neighboring Zarrinshahr, also called the Tabas Desert (Figure 2) The reason that this site was chosen for such a complex is the existence of high compacted soil layers that provide a good safety factor against the occurrence of earthquakes. Being near Zayandehrood River was for water supply, it's close distance to mines of

Yazd, and Kerman was for raw material.

In case to provide coke for purification and reduction of iron ore, coal is heat-treated in furnaces. In this way a valuable byproduct that is coal tar, with a capacity of 60,000 tons per year is obtained. Since the installation of the Iron Foundry Unit in 1971 and its operation up to 1997 when coal tar refinery was started, this by product was gathered in deposits located 5 km to the north of the refinery. Fortunately the operation of this refinery which is one of the largest in the Middle East in it's own type with a site of 5 hectares, to the north of Iron Foundry Unit, coal tar can be refined and valuable products can be obtained. The reason of choosing this place for tar refinery stand was its proximity to the center of tar production and storing, and also its closeness to

water sources and other facilities. Due to the accumulation of tar for so many years prior to the installation of a refinery, this area is a highly polluted zone and from this point of view is a special case of study in Iran.

Sampling Locations We initiated our studies by conducting a series of microbiological analyses of soil samples collected from six different locations.

The locations were designated respectively: (1) near the coal tar reservoirs, (2) around the coal tar reservoirs, (3) near the coke manufacturing unit, (4) around the coke manufacturing unit, (5) mix of coke and soil and (6) agricultural soil neighboring the site.

Materials Naphthalene, phenanthrene, anthracene (each used as PAH model) were purchased from Fluka AG, Buchs, Switzerland dichloromethane (proanalysis) was obtained from Merck Company. All of the solvent and chemicals were high purity grade reagents.

Culture Media Mineral medium contained, g/l: NaH_2PO_4 , 5.32; NH_4Cl , 2.67; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.06. mg/l: $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 0.6; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.09; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 2.4. The medium was adjusted to a pH of 7.1. To prepare mineral medium Agar, 6gr agar was added to 300 ml of mineral medium. It was used for screening after sterilization.

Microorganisms and Inoculums One gram of each soil sample was suspended in 10 ml of distilled water and then allowed to sit for sufficient time for the sediment of soil particles. The supernatant was assayed for the presence of PAHs - degrading microorganisms.

Microscope The microscope that was used in this study is a research photomicrograph microscope: Olympus, Vanox, AH BT3. The photomicrographs were taken on this microscope.

Screening Method The technique used for the isolation was based on that of Kiyohara [16]. The surface of the 18 agar plates (six soil samples for three chemicals) were coated lightly with

dichloromethane spray of dilute PAHs and dried for 24h at 35° C to volatilize the carrier solvent. Inoculate from each soil sample supernatant liquid was spread with a sterile rod on to agar surface of the petri dish and incubated for 3 weeks at 30°C.

Although many heterotrophic microorganisms grew on this medium, PAH-degrading microorganisms were distinguished as colonies surrounded by clear zones due to PAH utilization. These plating assays resulted in the isolation of some pure cultures, which were able to utilize PAHs during growth.

Therefore, after enough time at suitable temperature, colonies representative of each of different morphological types were removed and single colonies were repeatedly purified on mineral medium agar.

The isolates were subsequently maintained on N.A (Nutrient Agar) for bacteria and on P.D.A (Potato Dextrose Agar) for fungi and then stored as slant cultures under oil at 4°C.

RESULTS AND DISCUSSION

A few microorganisms utilizing PAHs had been isolated from Iran coal tar contaminated soil. The results of the microscopic and macroscopic observations are shown in Tables 4 and 6 for bacteria and fungi respectively. Colony morphology is described as macroscopic observations and cell morphology is described as microscopic observations. The results of bacteria's gram reactions, oxidase and catalase tests are shown in Table 5.

Soils and ground waters contain many kinds of microbes including fungi (molds and yeasts), protozoan and bacteria. Of these common native (indigenous) microorganisms, it is the bacteria and fungi that account for the degradation of practically all of the hydrocarbon and organic contamination entering the natural environment. It is the systematic enhancement of this natural biological degradation of contaminant soil by bacteria that comprises the art and science of environmental bioremediation.

The result of the aerobic degradation experiments for naphthalene, phenanthrene and anthracene show that in the fungal culture each PAH diminished from its water solubility limit to a detectable level.

TABLE 4. Macroscopic and Microscopic Observations of Bacterial Isolates.

Bacterial isolate	Sampling location	Macroscopic observation colonies on N. A:	Microscopic observation
B.Y : 22	5	Round, regular, smooth (entire), convex, mucoid translucent, yellow color	Polymorphic (coccid and shorted cells)
B.Y : 3	6	Mucoid, with yellow to orange color	Rod shaped
B.Y : 7	3	Round, regular, smooth, convex, mucoid, translucent	Club-shape (diplococcic and three or more bacteria from one center)
B.Y : 14	6	Round, regular, smooth, convex, mucoid, translucent	Coccid
B.Y : 9	1	Tiny white color	Coccid

TABLE 5. The Results of Bacteria's Gram Reaction, Oxidase and Catalase Tests.

Bacterial isolate	Gram reaction	Oxidase	Catalase
B.y : 22	+	-	-
B.y : 3	+	-	+
B.y : 7	+	-	+
B.y : 14	+	-	+
B.y : 9	+	+	+

TABLE 6. Macroscopic and Microscopic Observations of Fungal Isolates.

Fungal isolates	Sampling location	Macroscopic observation colonies on P.D.A:	Microscopic observation
F.y: 10	1	At initial time white color, powdery and then blue green color	Branching mycelium, septate, dome-shaped conidiophore at end of aerial hypha, spherical conidia in chain Result: <i>Penicillium. sp.</i>
F.y : 6	6	Powdery, at initial time yellow color and then black	Branching and septate hypha, hyaline, aerial hypha with spherical conidiophore Result: <i>Aspergillus.sp</i>
F.y : 1	3	Powdery, with light green color	Branching and septate hypha, hyaline, aerial hypha with spherical and green conidia. Result : <i>Aspergillus.sp</i>

Figure 3. Photograph of some microorganism isolated from the samples.

Figure 4. Photograph of some Fungi isolated from the samples.

As the control incubation showed that no significant loss of PAH occurred due to a biotic processes, all of this production is attributed to degradation by the fungi. However, the 45-65 hours required degrading naphthalene were substantially less than the 5 days for anthracene and the 7-8 days for phenanthrene. The mean concentration reduction rate for phenanthrene was

around 5.5 $\mu\text{g/L/hr}$, that for anthracene it was around 0.6 $\mu\text{g/L/hr}$.

CONCLUSION

It has been established that microbial metabolism of specific organic compounds is increased by

prior exposure to the compounds. Thus, the rates of pollutant degradation are more rapid in contaminated environments compared to pristine sites. This suggests that greater number of microorganisms capable of utilizing PAH, as a sole source of carbon and energy would be isolated from the site of highest PAHs contamination in the coal tar polluted soil.

This work presents strong evidence for the presence of indigenous microorganism. Stimulation of the native microbes for site remediation will be the next step of our research. Our goal is to gain satisfying results in future. Bioremediation can often be significantly less expensive (1/3 to 1/2 the cost) when compared to other site remediation alternatives. Therefore it can be useful technology for developing countries such as Iran.

NOMENCLATURE

PAHs	Polycyclic aromatic hydrocarbons
BTEX	Benzene, toluene, ethyl benzene and xylene
DNAPL	Dense nonaqueous – phase liquid
MGP	Manufactured gas plant
g/l	Gram per liter
Mg	Milligram
ml	Milliliter
H	Hour
C	Celsius degree
N.A	Nutrient agar
P.D.A	Potato dextrose agar
B	Bacterium
F	Fungus

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