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Full Length Research Paper

Remediation of anthracene in mycorrhizospheric soil using ryegrass

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Rhizosphere bioremediation has become an effective technique that uses green plants to enhance biodegradation of persistent organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), pesticides and radionuclides. Polycyclic aromatic hydrocarbons, due to their hydrophobic nature were found to be retained in the soil. Plants could be grown at the PAH contaminated sites to stimulate the biodegradation in the rhizosphere. In the present study, biodegradation of anthracene was studied using ryegrass in mycorrhizosphere soil by laboratory scale pot culture experiments. Ryegrass (*Lolium multiflorum*) was grown in pots containing soil contaminated with various levels of anthracene. Soil and plants from treated pots were sampled after 15, 30, 45 and 60 days and compared with uncontaminated planted pots. In the mycorrhizosphere, the concentrations of anthracene in the soil were found to be 5.2, 7.88, 15.43, 33.23 and 41.5 mg/kg at the 15 days harvest which further decreased to 0.31, 0.45, 0.89, 1.89 and 2.43 mg/kg over a period of 60 days when exposed to the initial concentrations of 10, 25, 50, 75 and 100 mg/kg amended in soil, respectively. Plant shoot and root dry biomass were observed to be significantly reduced at higher anthracene concentrations (75 and 100 mg/kg) whereas low concentrations had no distinct effect on plant biomass ($p<0.05$). The increase in the microbial counts was also monitored and quantified along the degradation of the anthracene in the soil. The findings of this research show that there is rapid degradation of anthracene under the influence of ryegrass mycorrhizosphere.

Key words: Rhizosphere bioremediation, *Lolium multiflorum*, arbuscular mycorrhizal fungi, PAHs.

INTRODUCTION

The industrial growth has brought the economic development in the fast developing countries like India. Along with industrial developments, environmental pro-blems are constantly increasing. The industrial effluent discharged in the environment has increased the level of contaminants in soil, sediments and aquatic systems causing environmental degradation and health problems. Polycyclic aromatic hydrocarbons (PAHs) are among the most concerned organic pollutants which persist in the environment. PAHs are hydrophobic organic contaminants composed of two or more fused aromatic rings and produced by combustion of organic matter (Corgie et al., 2004). They are found in particularly high levels at coal gasification and creosote- contaminated sites. Traditional clean-up techniques for PAH- contaminated soils involve expensive chemical or physical treatments (e.g., incineration and land disposal). Because these methods often require excavation, they increase potential transfer of contaminants to atmosphere (Lalande et al., 2003). Therefore there is an urgent need to develop eco-friendly and effective techniques for removal of polycyclic aromatic hydrocarbons from the contaminated environment. Out of the 16 major toxic polycyclic aromatic hydrocarbons, anthracene is a tricyclic aromatic hydrocarbon that is found in high concentrations in PAH contami-nated sediments, surface soils and waste sites. It has considered to be prototypic PAHs and serves as signature compound to detect PAH contamination, since its chemical structures are found in carcinogenic PAHs like benz(*a*)anthracene, dibenz(a,h) anthracene and benzo(a)pyrene (Moody et al., 2001).

Phytoremediation is an emerging green technology that uses plant to clean up by establishment of vegetation in soils contaminated with hazardous organic and inorganic compounds. The release of exudates and enzymes stimulate

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Table1. Physico-chemical characteristics of soil *

Parameters	Value
pH	7.4
Electrical Conductivity (mMohs)	0.46
Cation exchange capacity (meq/100gm)	7.2
Organic carbon (%)	1.3
Total N (%)	0.42
Total P (%)	0.039
Potassium (mg/kg)	25

* The values are the average of three replicates

microbial activity and biochemical transformations and enhancement of mineralization in the rhizosphere (the root-soil interface), which is attributable to mycorrhizal fungi and the microbial consortia (Schnoor, 1997). In phytoremediation, grasses are the most common plants evaluated. Kuiper et al. (2001) suggested that contaminant-resistant grasses with highly branched deep roots systems could be used to harbour PAH-degrading bacteria from contaminated soils. The large surface area of their fibrous roots and their intensive penetration of soil offer advantages for the phytoremediation of organic compound (Corgie et al., 2003). However, fine plant roots may be able to penetrate some of these pores, thereby increasing the contaminants available for degradation (Parrish et al., 2004). The rhizosphere bioremediation is a preferred mechanism by which hydrophobic organic contaminants like hydrocarbons are not just transferred to another medium, but transforms into simpler and less toxic compounds at a faster rate and to a greater extent than intrinsic bioremediation (Paquin et al., 2002; Pradhan et al., 1998). The cool seasoned ryegrass was therefore selected for anthracene phytoremediation which could be cost effective (Maila et al., 2005).

The aim of the present study was to investigate the phytoremediation of anthracene in mycorrhizosphere of ryegrass (*Lolium multiflorum*). The plant biomass, microbial analysis and dissipation of anthracene in soils were quantified at particular intervals to evaluate the degradation.

MATERIAL AND METHODS

Chemicals

The standard Anthracene (98%) was purchased from Merk (India). Total population of heterotrophic bacteria and fungi were estimated by plate count technique using Nutrient agar medium containing per 1 L; peptic digest of animal tissue 5 g, beef extract 1.5 g, yeast extract 1.5 g, sodium chloride 5 g and agar 15 g with final pH 7.4 ± 0.2 and Rose Bengal medium composed of (per 1 L) papic digest of soyabean meal 5 g, dextrose 10 g, monopotassium phosphate 1 g, magnesium sulphate 0.5 g Rose Bengal 0.05 g and agar 15 g (pH 7.2 ± 0.2 at 25°C) respectively (Kumar, 2004). These media were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India and prepared according to manufacturer's instructions. All the solvents used for analysis were of HPLC grade while other chemicals were of AR grade.

Soil collection

Soil was collected from a depth of about 0 - 15 cm along the banks of Surya River, Palghar (located 100 km away from Mumbai). The soil used in this study is referred as alluvial soil. Stones and plant tissues were carefully removed from the soil prior to drying process under laboratory condition. The soil was screened through 2 mm stainless steel sieve, and stored in a plastic bag at room temperature until use.

Soil characterization

The soil collected was characterized for its properties. The physicochemical parameters were measured by standard methods (Table 1). The pH and electrical conductivity (EC) were measured after 20 min of vigorous mixed samples at 1: 2.5 : : Solid : deionized water ratio using digital meters [Deluxe water and soil analysis kit, Model 191E] with a combination pH electrode (TL42) and a 1 cm platinum conductivity cell (Type CD10 electrode) respectively. Total nitrogen, total phosphorus and potassium were determined according to the standard methods of the American Public Health Association (1998). Cation exchange capacity was determined after extraction with ammonium acetate at pH 7.0 and the organic carbon was determined by using Walkley–Black method (Jackson, 1973).

Plant species

Plant selection criteria for phytoremediation studies were survival rate, growth of plant and PAH dissipation (Paquin et al., 2002). The aggressive growing cool-season grass - annual ryegrass was used in this study. Annual ryegrass (*L. multiflorum*) has found to grow in a wide range of soil textures, adapts well to poorly drained soils and has a high level of tolerance to climatic variations. This plant develops highly branched fibrous roots (large surface area and deep root penetration) and has quick seedling emergence (4-6 days) (Parrish et al., 2004). Ryegrass seeds (*Lolium multiflorum*, var. PRG-1) were procured from National Seeds Corporation limited (NSC), Beej Bhawan, New Delhi.

Fortification of soil

The dried and sieved soil used for fortification showed no background contamination of the anthracene. A stock solution of anthracene was prepared in methylene chloride and used for spiking soil according to the procedure described by Brinch et al. (2002). Anthracene was added with only 25% of the total quantity of dry soil and kept closed for few minutes to disperse. Then the solvent was made volatilized under fume-hood and the spiked soil was mixed with rest of non-polluted soil using metal spatula to obtain final anthracene concentrations of 10, 25, 50, 75 and 100 mg/ kg of potting mixture. The same protocol was used without anthracene as the control. The spiked soil was kept at room temperature until planting the next day. The advantages of this method are a minimization of the amount of solvent used during the spiking and preservation of the bacterial colonies in the soil. The presence of these bacteria is one of possible dissipation factors of the PAHs in soil (Campbell et al., 2002).

Experimental design

Experiments were conducted in controlled conditions in a green

house. There were six experimental conditions in this trial; contaminated soil spiked with five concentrations of anthracene and non-polluted soil served as control. All pots were planted with ryegrass. Ryegrass (*L. multiflorum*, var. PRG-1) was grown in plastic pots for conducting 'Pot Culture' experiments. For the experiments, the sieved soil spiked with the various concentrations was mixed with sand (< 2 mm) at a ratio of 3: l (w/w), which was standardized for the better porosity of the potting mixture to have good root growth of ryegrass. The air-dried soil- sand mixture was placed in each pot containing about 20% AM fungal inoculum applied to it. Thus, there were six experimental sets of anthracene contamination (including control); four harvest times (15, 30, 45 and 60 days) and three replicates giving a total of 72 pots. The pots were arranged in a greenhouse and were completely randomized.

Seeds of ryegrass were surface sterilized by soaking in 30% (v/v) H_2O_2 for 20 min and washed several times with distilled water. About fifteen pre-germinated seeds were then sown to one kg plastic pots containing 700gm artificially contaminated and control soil. Seedlings were thinned to ten after one week and the soil was covered with a layer of soil. Three replicates were done for this experiment and pots were randomly arranged. The pots were placed in green house at room temperature (26-27°C at day, 23 - 24°C at night) with natural light. Pots were watered on alternate days to maintain the moisture. Optimum conditions for plant growth were maintained in the greenhouse. NH_4NO_3-N (25 mg per pot) was provided in first and fourth week after seedling emergence. Any broadleaf weeds germinated in the treatments were removed periodically by hand before they reached 0.5 cm in size.

For the analysis, soil from planted pots was carefully collected, mixed and crushed by mortar and pestle. Pooled soil samples (approximately 30 to 40 g wet weight) were air dried overnight in a fume hood (in dark). Dried samples were then kept at 4°C to prevent microbial activity. The roots were removed from the soil with forceps and therefore, analyzed soil did not contain any root.

Chemical extraction

Anthracene was extracted using Soxhlet apparatus from 10 g dry soil with 200 ml methylene chloride (USEPA SW-846 Method 3540C). Soil extracts were filtered through Whatman No. 42 cellulose filter paper containing a bed (2 g) of anhydrous sodium sulphate to absorb any potential residual humidity. The soxhlet extracts were concentrated by rotary evaporator to 10 ml and each sample was stored in seal capped vials at 4°C until analysis. For the recovery study of freshly spiked soil; the soil was spiked, extracted and quantified after 24 h of incubation in dark using the same technique applied for samples. Mean anthracene recovery of recently spiked soil was calculated.

Chemical analysis

Anthracene was quantified by High Performance Liquid Chromatography (HPLC) [Jasco, Model UV-2075 Plus] (USEPA SW-846 Method 8310). Chromatographic separation was performed using HPLC system, equipped with the diode array detector (Varian) and with Borwin software. Twenty microlitres of soil extracts were injected into the isocratic mobile phase of acetonitrile and water (75:25 ratio), run at 1 ml min^{-1} with Varian C18 column (250 mm × 4.6 mm). Anthracene was further identified by compareng UV spectra and retention times with the standards. Detections were performed at 254 nm.

The extracts were also analyzed for identification of anthracene and its metabolites by Gas Chromatography-Mass Spectrometry and samples were quantified according to USEPA SW-846 Method 8270D (Gas Chromatography/Mass Spectrometry for Semivolatile Organic compounds). The Shimadzu gas chromatograph (Model QT 2010) equipped with electron ionization detector and mass selective detector was used. The injector temperature was programmed from ambient to 360°C where the oven temperature was fixed at 350°C.

Microbial analysis

One gram of each sample was immediately used for microbial enumerations. The enumeration of bacteria and fungi was done according to a standard procedure (Kumar, 2004). Briefly, 1 g of soil was mixed with 10 ml of sterile distilled water and serial dilutions were made using sterile distilled water. An aliquot of 0.1 ml of dilutions for each soil sample was spread plated on to agar from the appropriate dilution tubes and incubated at room temperature. The bacterial colonies were counted after 24 h. Only the plates showing between 25 to 300 colonies were tallied, and the results were averaged for each soil sample. To analyze the microflora of the ryegrass rhizosphere the bacterial and fungal counts were taken. Culturable heterotrophic bacteria were cultivated on Nutrient Agar. For fungi, Rose Bengal agar was used. The fungal colonies were counted after 48 - 72 h of incubation.

Plant biomass analysis

Plants were harvested and separated into above and below-ground fractions with scissors. From the rhizosphere system, roots were separated carefully by passing the soil through a sieve and washed to eliminate attached soil. However the criteria to select plants for phytoremediation include weight gain rate, shoot and root dry weights were determined upon reaching constant weight, after complete drying at 60°C.

Data analysis

These results of anthracene concentration remained in polluted soils at four harvesting periods (that is, 15, 30, 45 and 60 days) for all treatments were calculated and expressed in mg/ kg soil. The root and shoot biomass were estimated by measuring their dry weights at the end of the 60 days and the data means were compared to control by unpaired t- test for three replicates ($p < 0.05$) (Mahajan, 1991). The microbial count of bacteria and fungi were assessed for all the treatments for four harvesting periods.

RESULTS AND DICUSSION

Excessive combustion of organic matter for the industrial use has resulted into increase in polycyclic aromatic hydrocarbons contamination of soil-water environment. The remediation of these pollutants persisting in soil can be achieved by growing green plants at the contaminated area. Biological processes being solar-driven phytoremediation so on an average, could be ten fold cheaper than engineering-based remediation methods such as soil excavation, soil washing or burning or pump-and-treat systems. The fact that phytoremediation is usually carried out in situ contributes to its cost-effectiveness and may reduce exposure of the polluted substrate to humans, wildlife, and the environment (Smits, 2005). The plant-microbes symbiosis brings about favorable conditions in the rhizosphere which are responsible for rhizo

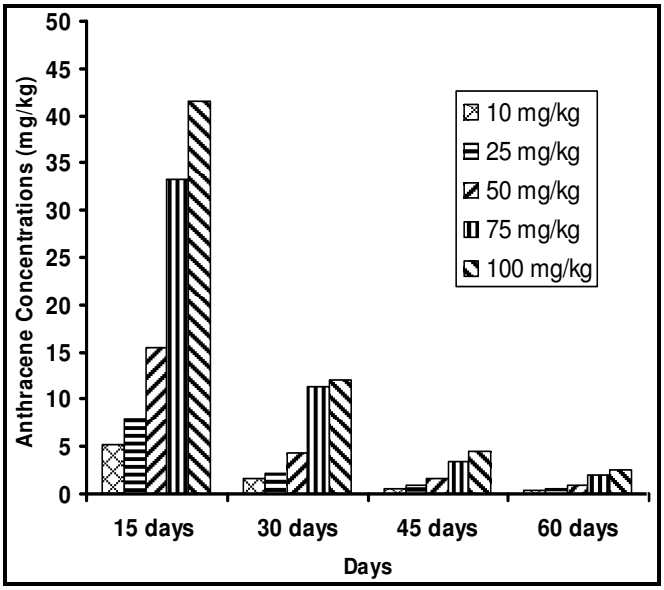


Figure 1. Concentration of anthracene found during the phytoremediation of anthracene contaminated soil using ryegrass.

sphere bioremediation of the contaminants. In the present research work, ryegrass was grown in the anthraxcene contaminated soil in the pot culture experiment. The soil was amended with various concentrations (10, 25, 50, 75 and 100 mg/kg) and sampled at four harvesting time i.e. at the 15, 30, 45 ad 60 days of plant growth period. Ryegrass was found to be tolerant at the highest concentration (100 mg/kg). The effect of anthracene on ryegrass, if any was also observed by measuring the changes in its dry biomass at the end of the experiment. The micro-flora present in the contaminated rhizospheric soil as well as non contaminated rhizospheric soil (control) were measured by taking the plate counts of heterotrophic bacteria and fungi.

Anthracene dissipation

The Pot culture experiments were conducted to study the degradation of anthracene in the soil. The concentrations of anthracene during the rhizosphere bioremediation of 10, 25, 50, 75 and 100 mg/kg anthracene amended soil are estimated and presented in Figure 1. The mean extraction recovery of anthracene from spiked potting soil was 96%. No anthracene or other PAHs were detected in the non contaminated soils over the course of this study. The extractable concentration of anthracene in soils gradually decreased with incubation as observed by Cam-bell et al. in 2002. The concentrations of anthraxcene reduced rapidly during the first 30 days and then the degradation rate slowed down. This degradation may be attributed to the microbial consortium present in the ryeryegrass rhizosphere.

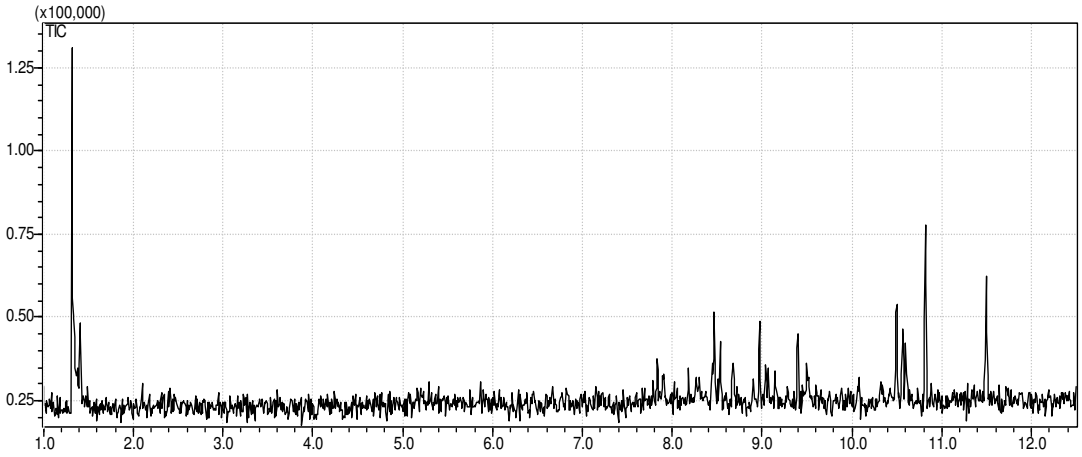
These results are in agreement with the findings of the previous experiments done by Schwab and Banks (1994); Günter et al. (1996) and Reilley et al. (1996) on PAHs such as anthracene and pyrene where a very rapid dissipation of these compounds in the rhizosphere of several plants in the early stages (40 days) was followed by slower rates. Joner et al. (2001) found that more than 98% of the low molecular weight (LMW) PAHs were degraded in ryegrass rhizosphere after 8 weeks. Binet et al. (2000b) studied the fate of eight PAHs (3 – 6 rings) in the mycorrhizosphere and showed that ryegrass was able to accelerate the dissipation of a range of PAHs, including 5 and 6 ring PAHs such as dibenzo(a,h) anthracene and benzo(g,h,l)perylene.

The disappearance of PAHs with three or more rings was stated to be mainly attributed to biotransformation or biodegradation (Lalande et al., 2003). The Mass spectrophotometry analysis of the soil samples showed anthraxquinone as an intermediate (Figure 2) analogous to other studies done on the anthracene contaminated soils (Binet et al., 2000b). Anthraquinone was found after 15 days in higher anthracene contaminated soil (50, 75, 100 mg/kg). The lower ones did not show the intermediate as the rapid degradation might have taken place under the influence of ryegrass mycorrhizosphere.

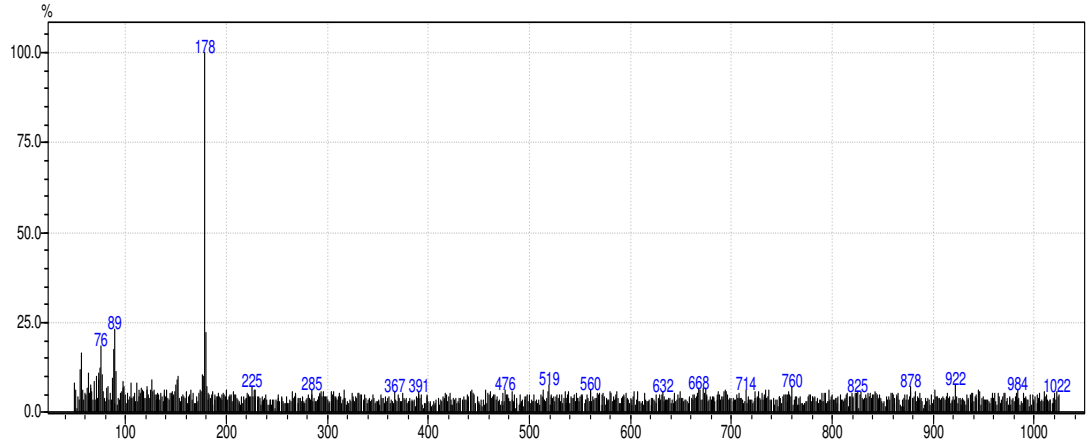
Microbial analysis

In the anthracene contaminated soil, the pattern of growth was characterized by an initial increase of microbial population in the soil (Figure 3). The viable counts of heterotrophs and fungi were determined at every 15 days for 2 months period. This increase of plate counts coincided with the rapid decrease of anthracene, apparently due to the consumption of the bioavailable part of the substrates. The plate counts of bacteria as well as fungi were observed to be increased along the concentrations which further became stable as the dissipation slower down. In the rhizosphere, the bacterial population reached higher values than the corresponding un- spiked planted soil, where as changes in the fungal count varied indistinctly. Thus bacterial population proved to be dominant in rhizosphere microbiota.

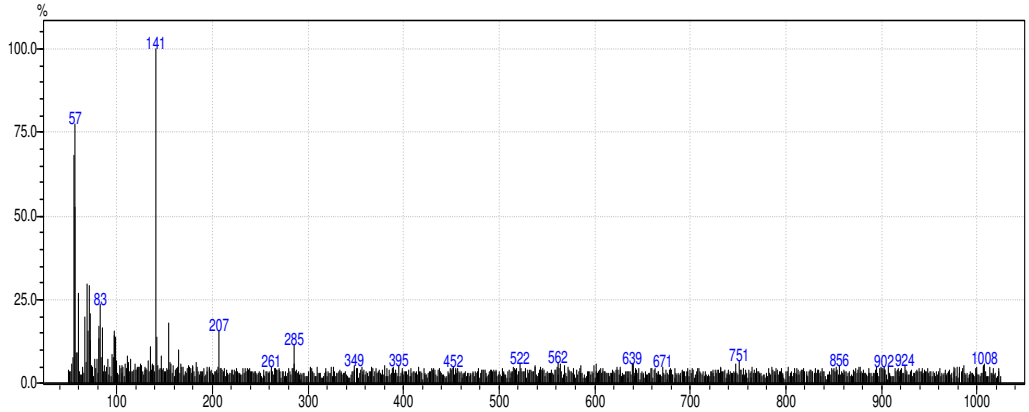
The results of the current study were found to be similar to the observations made by Kirk et al. (2005), where they investigate effect of ryegrass and alfalfa on microbial abundance and diversity in the petroleum contaminated soil. The findings of research work done by Corgie et al. (2006) and Günter et al. (1996) on phenanthrene and aliphatic hydrocarbons are also in accordance to the outcomes of the present study. In experimental systems that include living plants, it appears that degradation of contaminants is influenced not only by the rhizosphere microbiota, but also by unique properties of the host plant, soil, and environmental conditions (Walton et al., 1994). As a response to environmental conditions, plants



a. Gas Chromatogram

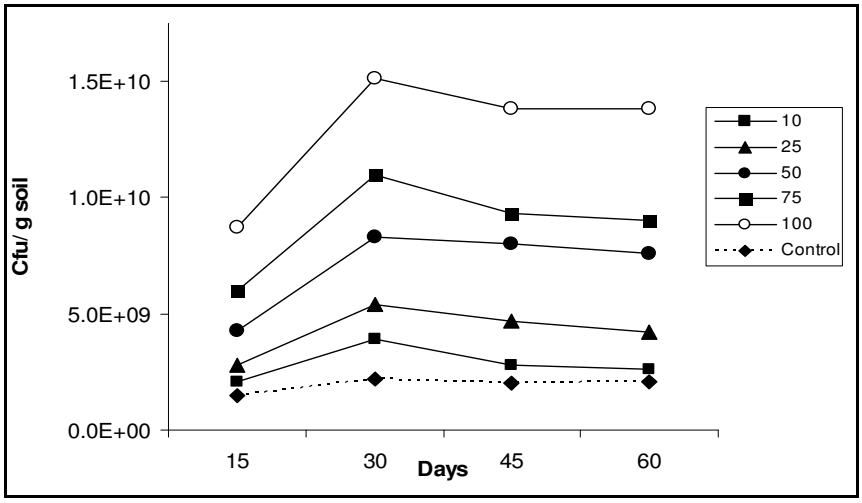


b. Anthracene [Retention time (RT) 9.670, *m/z* identification ion-178]

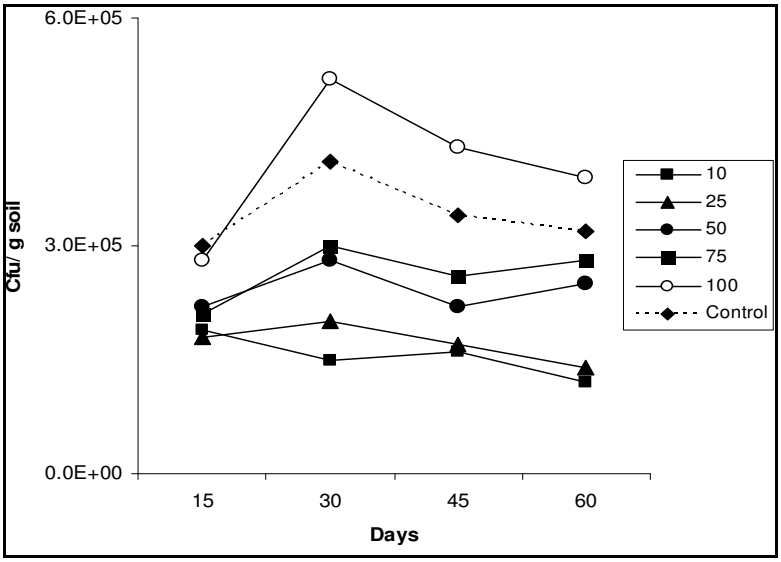


c. Anthraquinone [Retention time (RT) 11.764, *m/z* identification ion-207]

Figure 2. Mass chromatograms and spectra of anthracene and anthraquinone.



a. Bacterial count



b. Fungal count

Figure 3. Microbial counts of contaminated and uncontaminated (control) soil during the phytoremediation of anthracene using ryegrass.

produce allelopathic compounds, analogous to organic contaminants that stimulate microbial defense against toxic compounds (Siciliano et al., 2001). Kästner et al. (1994) reported 10^3 to 10^5 Cfu of bacteria per gram soil dry weight were able to grow on anthracene, phenanthrene, fluoranthene, or pyrene as a sole source of carbon at different contaminated sites.

Plant biomass analyses

The growth of the plants in soil contaminated with 10, 25, 50, 75 and 100 mg/kg of anthracene were determined by measuring the dry weight biomass of the plants at the end of the experiment (that is, 60 days of growth period) and are presented in Table 2. Results indicate that annual ryegrass can establish and survive in anthracene contaminated soil at a concentration of 100 mg/kg dry soiland showed no outward signs of phytotoxicity. With regard to plant (shoot and root) biomass, decrease was observed with rising pollutant content from 50 to 100 mg/kg of anthracene concentrations at the end of 60 days growth period. The plant growth responses to the lower (10 and 25 mg/kg) anthracene concentrations compared to control were not significant (p<0.05).

Table 2. Shoot and root dry biomass of the ryegrass plants after phytoremediation of anthracene contaminated soil and uncontaminated (control) soil.

Treatment of Soil amended with anthracene (mg/kg)	Shoot dry weight* (g)	Root dry weight* (g)
0(Control)	1.280±0.09 [a]	0.976±0.05[b]
10	1.270±0.08	1.100±0.07
25	1.107±0.08	0.995±0.06
50	0.980±0.07 [a]	0.955±0.05
75	0.871±0.05 [a]	0.689±0.04 [b]
100	0.850±0.05 [a]	0.612±0.04 [b]

*The values are the means and standard deviation of three replicates.
[a] indicates significant difference ($p < 0.05$, n = 3) between shoot biomass obtained from control and contaminated soil.
[b] indicates significant difference ($p < 0.05$, n = 3) between root biomass obtained from control and contaminated soil.

The plant biomass results obtained in the present study are similar to the study done by Günter et al. (1996) where shoot dry weight of ryegrass was reported to be lower (0.06 g/column) as compare to uncontaminated soil (0.13g/ column). Binet et al. (2000a) showed mixture of PAHs at the concentration of 1 g/kg reduced the ryegrass (root and shoot) biomass at the end of the 40 days experiments. There was no significant effect of phenanthrene (contaminated at 1000 mg/kg) was noticed on root-soot biomass of ryegrass compared to the non contaminated soil by Chiapusio et al. in 2007.

In this research work, results obtained show that natural biological processes occurring in the rhizosphere of ryegrass and its micro-flora could significantly degrade anthracene in the rhizosphere. Ryegrass was found tolerant even at the anthracene concentration of 100 mg/kg. It is efficient in remediation through the mechanism of increased aeration, deep rooted penetration and increased total number of indigenous micro-flora. The anthracene degradation found to be very fast over 30 days time and anthracene concentration reached very low at the end of the growing period. In the present case, bioremediation of anthracene has taken place by microbial consortium such as bacteria, fungi and actinomycetes present in the rhizosphere. Bacterial and fungal counts showed increase in number and activity along the concentrations and did correspond to the rate of degradation. The shoot and root biomass had slightly negative correlation with higher anthracene concentrations in the ryegrass planted soil. The plant assisted degradation of anthracene using ryegrass can be explored further as this technology may become a choice for organic contaminant remediation plans in developing countries because of its cost-efficiency and easy implementations.

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REFERENCES

APHA, AWWA WPCF (1998). Standard Methods for the Examination of Water and Wastewater. American Public Health Association / American WaterWorks Association/Water Environmental Federation, Washington DC.

Binet P, Portal JM, Leyval C (2000a). Dissipation of 3-6-ring polycyclic aromatic hydrocarbons in the rhizosphere of ryegrass. Soil Biol. Biochem. 32: 2011-2017

Binet P, Portal JM, Leyval C (2000b). Fate of polycyclic aromatic hydrocarbons (PAH) in the rhizosphere and mycorrhizoshere of ryegrass. Plant Soil. 227:207-213.

Brinch UC, Ekelund F, Jacobsen CS (2002). Method for spiking soil samples with organic compounds. Appl. Environ. Microbiol. 68 (4): 1808 – 1816.

Campbell S, Paquin D, Awaya JD, Li QX (2002). Remediation of Benzo[a]pyrene and Chrysene Contaminated Soil with Industrial Hemp (Cannabis sativa). Int. J. Phytoremed. 4:157-168

Chiapusio G, Pujol S, Toussaint ML, Badot PM, Binet P (2007). Phenanthrece toxicity and dissipation of grassland plants (Lolium perenne and Trifolium pretense) in three siked soils. Plant soil. 294: 103-112.

Corgie SC, Beguiristain T, Leyval C (2004). Spatial Distribution of Bacterial Communities and Phenanthrene Degradation in the Rhizosphere of Lolium perenne L. Appl. Environ. Microbiol. 70(6):3552-3557.

Corgie SC, Fons F, Beguiristain T, Leyval C (2006). Biodegradation of phenanthrene, spatial distribution of bacterial populations and dioxygenase expression in the mycorrhizosphere of Lolium perenne inoculated with Glomus mosseae. Mycorrhiza. 16:207-212

Corgie SC, Joner EJ, Leyval C (2003). Rhizosheric degradation of Phenanthrene is a function of proximity to roots. Plant Soil. 257:147-150.

Günter T, Dornberger U, Fritsche W (1996). Effects of ryegrass on biodegradation of hydrocarbons in soil. Chemosphere. 33(2): 203–215.

Jackson ML (1973). Soil Chemical Analysis. Prentice-Hall of India, New Delhi.

Joner EJ, Johansen A, Loibner AP, de la Cruz MA, Szolar OHJ, Portal JM, Leyval C (2001). Rhizosphere Effects on Microbial Community Structure and Dissipation and Toxicity of Polycyclic Aromatic Hydrocarbons (PAHs) in Spiked Soil. Environ. Sci. Technol. 35: 2773-2777.

Kästner M, Breuer-Jammali M, Mahro B (1994). Enumeration and characterization of the soil microflora from hydrocarbon-contaminated

soil sites able to mineralize polycyclic aromatic hydrocarbons (PAH). Appl. Microbiol. Biotech. 41: 267–273.
Kirk JL, Klironomos JN, Lee H, Trevors JT (2005). The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil. Environ. Pollut. 133: 455-465
Kuiper I, Bloemberg GV, Lugtenberg B (2001). Selection of a Plant-Bacterium Pair as a Novel Tool for Rhizostimulation of Polycyclic Aromatic Hydrocarbon-Degrading Bacteria. MPMI. 14: 1197-1205.
Kumar H (2004). Recent trends in biotechnology. Agrobios, India. pp. 55-58.
Lalande TL, Skipper HD, Wolf DC, Reynolds CM, Freedman DL, Pinerton BW, Hartel PG, Grimes LW (2003). Phytoremediation of Pyrene in a Cecil Soil under Field Conditions. Int. J. Phytoremed. 5:1-12.
Mahajan BK (1991). Methods in Biostatistics. Jaypee Brothers Medical publishers (P) Ltd., New Delhi, India. pp. 129-153.
Maila MP, Randima P. (2005). Multispecies and Monoculture Rhizoremediation of Polycyclic Aromatic Hydrocarbons (PAHs) from the Soil. Int. J. Phytoremed. 7:87-98
Moody JD, Freeman JP, Doerge DR, Cerniglia CE (2001). Degradation of Phenanthrene and Anthracene by Cell Suspensions of Mycobacterium sp. Strain PYR-1. Appl. Environ. Microbiol. 67:1476–1483.
Paquin D, Ogoshi R, Campbell S, Li QX (2002). Bench- Scale Phytoremediation of Polcyclic Aromatic Hydrocarbon-Contaminated Marine Sediment with Tropical Plants. Int. J. Phytoremed. 4: 297-313
Parrish ZD, Banks MK, Schwab AP (2004). Effectiveness of Phytoremediation as a Secondary Treatment for Polycyclic Aromatic Hydrocarbons (PAH's) in Composted Soil. Int. J. Phytoremed. 6:119-137
Pradhan SP, Conrad JR, Paterek JR, Srivastava VJ (1998) Potential of Phytoremediation for Treatment of PAHs in Soil at MGP Sites. Soil Sediment Contam. 7(4):467-480
Reilley KA, Banks MK, Schwab AP (1996). Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. J. Environ. Qual. 25:212–219.
Schoor JL (1997). Phytoremediation. Technical Evaluation Report for Ground-Water Remediation Technologies Analysis Center, Pittsburgh.
Schwab AP, Banks MK (1994). Biologically mediated dissipation of polyaromatic hydrocarbons in the root zone. In Anderson TA, Coats JR (eds.) Bioremediation through Rhizosphere Technology, ACS symposium series 563, American Chemical Society, Washington, DC, pp. 132–141.
Siciliano DS, Fortin N, Mihoc A, Wisse G, Labelle S, Beaumier D, Oullette D, Roy R, Whyte LG, Banks MK, Schwab P, Lee K, Greer CW (2001). Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. Appl. Environ. Microbiol. 67 (6): 2469–2475.
Smits E (2005). Phytoremediation. Annu. Rev. Plant Biol. 56:15–39.
US EPA (2007). SW-846, Soxhlet extraction method, HPLC for PNS and Gas Chromatography/Mass Spectrometry for Semivolatile Organic Compounds, Test methods for organic analytes. 4th revision. EPA methods 3540C, 8310 and 8270D Environmental Protection Agency, Washington, DC.
Walton BA, Hoylman AM, Perez MM, Anderson TA, Johnson TR, Guthrie EA, Christman RF (1994). Rhizosphere microbial communities as a plant defense against toxic substances in soils. In Anderson TA, Coats JR (eds.) Bioremediation through Rhizosphere Technology, ACS symposium series 563, American Chemical Society, Washington, DC, pp. 82–92.