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Bioremediation of Soils Polluted with Phenanthrene and Anthracene Using Ground Treatment Method and Chicken Manure as a Cosubstrate

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ABSTRACT

Introduction: Bioremediation convertssoils pollutants to safe and non-toxic substance through metabolic activities of microorganisms. This research was conducted to evaluate the effect of bacteria on removal of phenanthrene and anthracene from polluted soils using chicken manure as an auxiliary substrate.

Materials and Methods: First, uniformly-graded soils were transferred to the pilots, then activated sludge and chicken manure were added and mixed with the soilin specific ratios of 2:1 and 1: 1. Thereafter, phenanthrene and anthracene were manually added to the soil of each pilotat a rate of 12 mg/kg of soil. Anthracene and phenanthrene were measured using HPLC.

Results: The results showed that in control pilots (without chicken manure and sludge), the removal percentage of pollutants (phenanthrene + anthracene) was 15%. Nevertheless, when chicken manure and chicken manure + sludge were used, the removal percentageincreased to 80 and 84%, respectively. Control pilots showed the lowest percentage of COD removal and varied from approximately 7 to 10%. Although the percentage of COD removal was approximately 80%, with the addition of chicken manure + sludge, COD removal rate reached 90% (the highest removal percentage).

Conclusion: The use of chicken manure as a cosubstratecanbe considered as an appropriate alternative for increasing the efficiency of bioremediation of oil compounds in soil.

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Introduction

The release of oil compounds into the soil can cause toxicity to humans and other living organisms, and the pollution may also be transmitted to groundwater. Among pollutants entering the environment, polycyclic aromatic hydrocarbons (PAHs), such as phenanthrene and anthracene, are important in terms of damages imposed on humans and the environment, because the presence of phenanthrene and anthracene in the soil causes some biological effects. These effects include severe toxicity, mutagenesis, fetal defects, and impaired endocrine function [1]. Phenanthrene is one of the PAH compounds with three benzene rings. It is often found in soils around refineries

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and places using coal fuel [2]. It is widely distributed in natureand is placed in the list of most common pollutants by the EPA and can cause harmful biological effects [3]. Anthracene ($C_{14}H_{10}$) is a carcinogenic and toxic substance in the form of yellow crystals with blue fluorescence, soluble in alcohol and ether, and insoluble in water at a temperature of 27.4°C, specific gravity of 1.25°C, melting point of 217°C, a boiling point of 340°C and an ignition point of 250°C. Given that PAH compounds in the environment threaten the health of the community, it is necessary to remove them from the soil. There are many standard and common methods for cleaning up and decontaminating these oil products, which are not widely utilized due to their high cost and low efficiency. These methods also depend on the characteristics of the polluted site, the remediation targets, the cost and the time constraints. Bioremediation is one of the environmental cleaning methods where living organisms, especially fungi, bacteria and plants are used to decompose environmental pollutants and convert them into non-toxic compounds [2]. In comparison with other cleaning technologies such as burning and burying of oil sludge, the biological method is much cheaper and affordable. Bacteria are the most active microorganisms that decompose pollutants [3]. The results show that bacteria can remove different types of PAHs by producing various enzymes such as gelatinase enzyme.An experiment was carried out on the effect of nutrient concentrations on the biodegradation of crude oil via soil microbial population, and it was reported that the decomposition rate of these oil products was increased from 47 to 62% by adding nutrients [4]. Nevertheless, the use of reinforcing substance is effective in removing oil pollutants and optimization of the type and amount of additives that are economically feasible [5]. Investigating the bioremediation of PAHs in oil-polluted soils, the researchers concluded that with increasing incubation time, the rate of bioremediation of phenanthrene [6] and anthracene [7] increased. COD reduction is reported to reach over 97% by adding a cosubstrate [8]. A group of researchers reported that, COD removal rate by microorganisms increased as incubation time increased [9, 10]. The researchers reported that the microbial mass had an efficiency rate of 95 to 99.8% for mono, di-and tetrachlorophenol pollutants, and 85 to 97.8% for COD removal [11]. Therefore, considering the foregoing and the importance of removing harmful organic pollutants from the soil as well as lack of sufficient studies in this field in Iran, the aim of this study was to investigate the removal of phenanthrene and anthracene from polluted soils using biological solids and the effect of adding substrate (chicken manure) on this process. Bioremediation occurs in the decomposition of pollutants based on growth and cometabolism processes [12]. With regard to growth, pollutants are used as the only source of carbon and energy, which results in complete decomposition of pollutants; cometabolism is the metabolism of a compound in the presence of growth substrate, which is used as the primary source of carbon and energy [13].Cometabolism refers to the oxidation of substrates without the use of oxidation-derived energy, and according to this process, microbial growth is enhanced. Cometabolism is important especially for the decomposition of a mixture of polycyclic aromatic hydrocarbons [14]. This research was conducted to evaluate the effect of bacteria on removal of phenanthrene and anthracene contaminated soils using chicken manure as a cosubstrate.

Materials and Methods

Soil used in this research

Soil used in this study was an ordinary soil (free from explosive compounds) (Table 1) and chicken manure was obtained from a poultry farm. First, this soil sample was dried in open air and screened with a 2 mm pore size sieve so that debris and aggregates that could not be crushed and isolated from the soil are removed and a uniform mixture is obtained. The chicken manure was also dried and screened to remove excess waste, and later added to the pilot samples as a cosubstrate (Figure 1). The sludge used in this study was prepared from activatedsludge ponds in Southern Isfahan Refinery and added to the soil [14].

Table1: Characteristics of the soil studied in this experiment

Soil physical feature (%)			P (ppm)	N (%)	pH	EC (ds/m)	Text
S	Si	C					
52	27	21	260	0.30	8.05	15.1	Clay-Silt

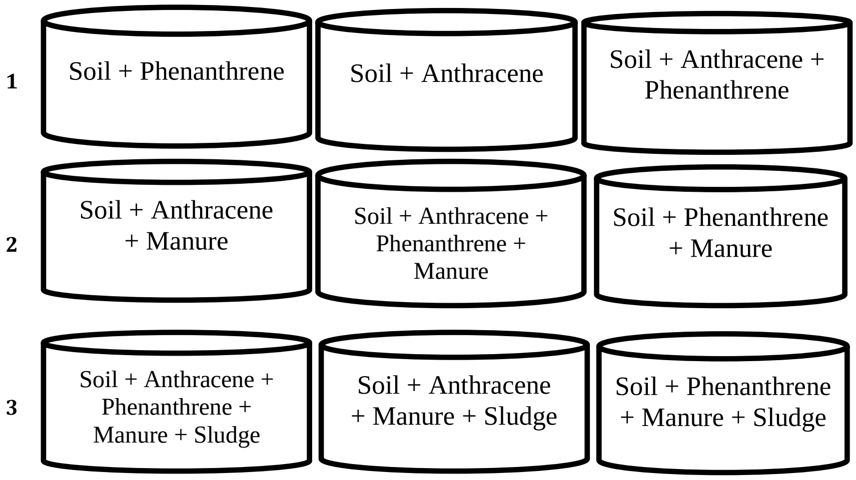

Figure 1: Pilotsstudy: 1. Control reactors; 2. Pilot containers containing chicken manure; 3. Pilot containers containing chicken manure + activated sludge

Chemicals

All chemicals used in this study were pure and purchased without any additional purification from the American Sigma Aldrich Company. Phenanthrene and anthracene were used by the American Sigma Aldrich (99% purity) as a soil polluting source. The intended pollutants were manually added to the pilot and turned so that the soil is uniformly distributed [15].

Preparation of pilots for bioremediation of soil polluted by oil compounds

Nine aluminum containers each with a volume of 21 L and dimensions of 25 * 25 * 35 cm were used for this study. Slots were designed at the bottom of the containers to drain the liquids as needed. When a relatively uniform mixture of soil and manure was obtained, soil and chicken manure samples were considered at a ratio of 1:1 for all containers and ratio 1:2 for activated sludge and soil. Finally, to manually add pollutants to the pilots (12 mg/kg soil), phenanthrene and anthracene were first dissolved in acetone as an intermediate solvent, and then double-distilled water was added to attain the required volume and was added to the soil in one step. In the end, the soil was mixed several times. In order to provide adequate aeration, 12 air stones were considered for each pilot, which was aerated daily through a pump and pilot containers were periodically watered in order to provide the required moisture. The studied oil compounds were measured every two weeks [16]. The EPA-3550B method was used to determine the concentration of phenanthrene and anthracene [16]. Before sampling, the soils were well turned and then 2 g of soil was removed and dried. It was then dissolved in 10 ml of acetonitrile and placed in an ultrasonic bath at 40-45°C for two minutes. The extracted solution was then placed in 200 rpm shaker for one hour. The supernatant was passed through Whatman filter paperand detected by HPLC (Model: HPLC Waters, Type and Specifications: C18 ultra sep ES PAH QC specia 60× 2mm ID, type of solvent for mobile phase: acetonitrile-water with ratio: 80%:20%, sample size: 20 µl, UV detector wavelength: 230 nm) [17].

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Results

The efficiency of the pilots in the removal of pollutants

Generally and as shown in figure 2, the average percentage of pollutant removal was 15% in control pilots (without substrate and without sludge), and the efficiency reached 80% when the chicken manure was added. Subsequently, the addition of sludge increased the percentage of pollutants removal to 84%. The highest efficiency in removing phenanthrene and anthracene was observed in chicken manure + sludge pilots withremoval rates of 89 and 87%, respectively (Figure 2). In control pilots, anthracene was removed to a greater extent than phenanthrene (15 and 14%, respectively), while the removal rate increased (16%) in pilot cases containing both pollutants. According to figure 2, in pilot containing chicken manure, unlike the control pilots, phenanthrene was removed at higher rate than anthracene (83 and 80% respectively). In fact, comparing the amount of pollutant removal after the addition of sludge indicated that increased anthracene removal rate was more prevalentcompared with phenanthrene. On the other hand, in pilots containing poultry + sludge, anthracene removal rate was highercompared to phenanthrene removal. The removal rate was more pronounced in control pilots containing both pollutants compared to control groups containing only one pollutant, although the opposite was achieved in non-control pilot cases (Figure 2).

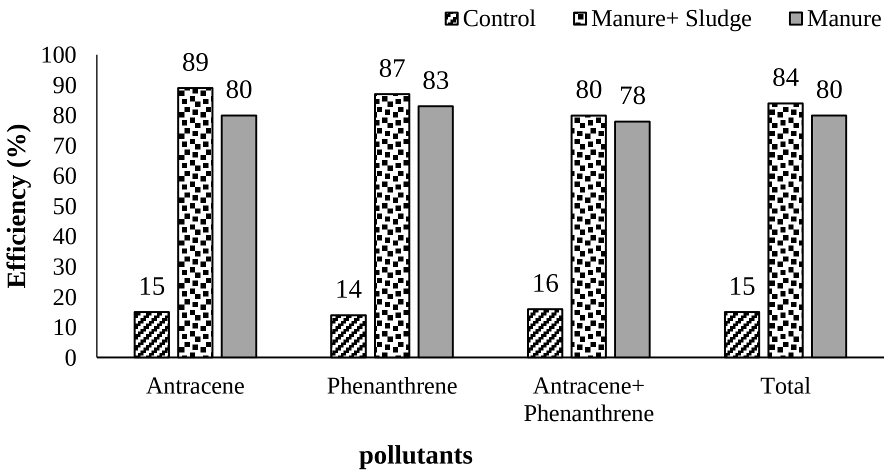

Figure 2: Comparison of the efficiency of the three main pilots in the removal of phenanthrene and anthracene pollutants

Investigating COD variations

In this experiment, chemical oxygen demand (COD) was measured as a bioremediation indicator during anthracene and phenanthrene bioremediation. According to figure 3, COD was reduced during bioremediation of pollutants in the studied pilots, and this decrease was lower in control pilots compared with pilots containing chicken manure + sludge (average efficiency of 9.12% in control pilots, 22.28%, in experimental pilots containing chicken manure, and 88.5% in experimental pilots containing chicken manure + sludge) (Figures 3, 4 and 5). COD variations revealed that this parameter had the lowest variation percentage in control pilot samples and varied from approximately 7 to 10% (Figure 6). The percentage of COD variations was approximately 80% in pilots containing soil + manure + pollutants. In other words, adding manure significantly increased the percentage of COD variations (Figure 6). While pilot soil containing + manure + sludge + pollutants showed the highest percentage of COD variations, COD variations were reducedby 90% in these pilots (Figure 6).

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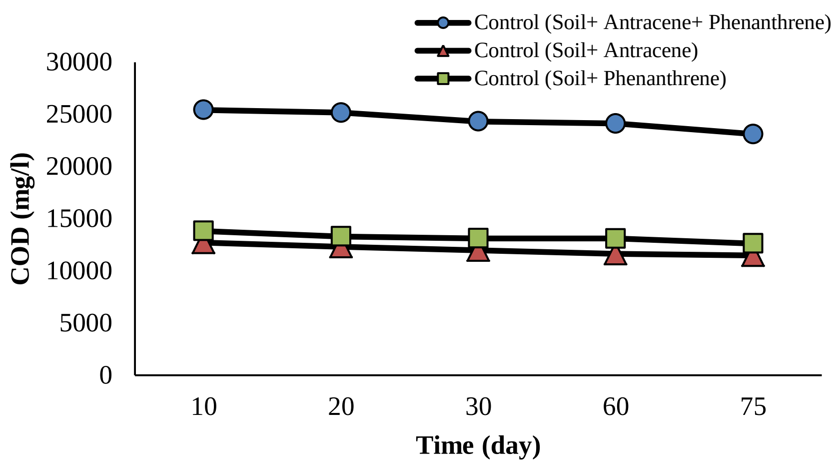

Figure 3: Variations in concentration of sample COD in control pilots during bioremediation

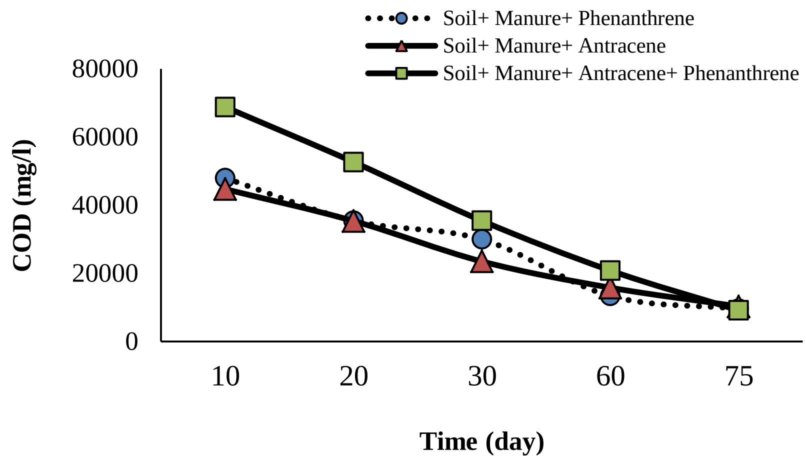

Figure 4: Concentration of sample COD in pilots-containing manure during bioremediation

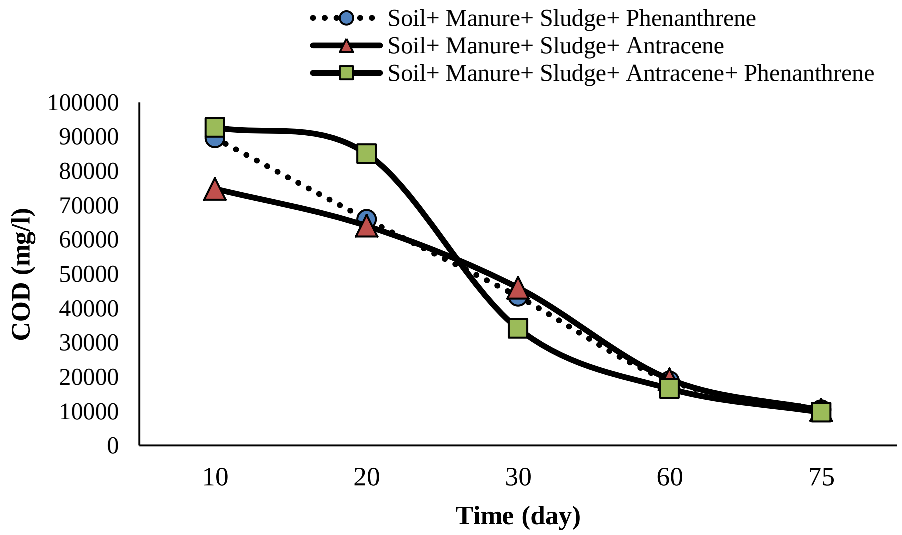

Figure 5: Concentration of sample COD in manure + sludge pilots during bioremediation

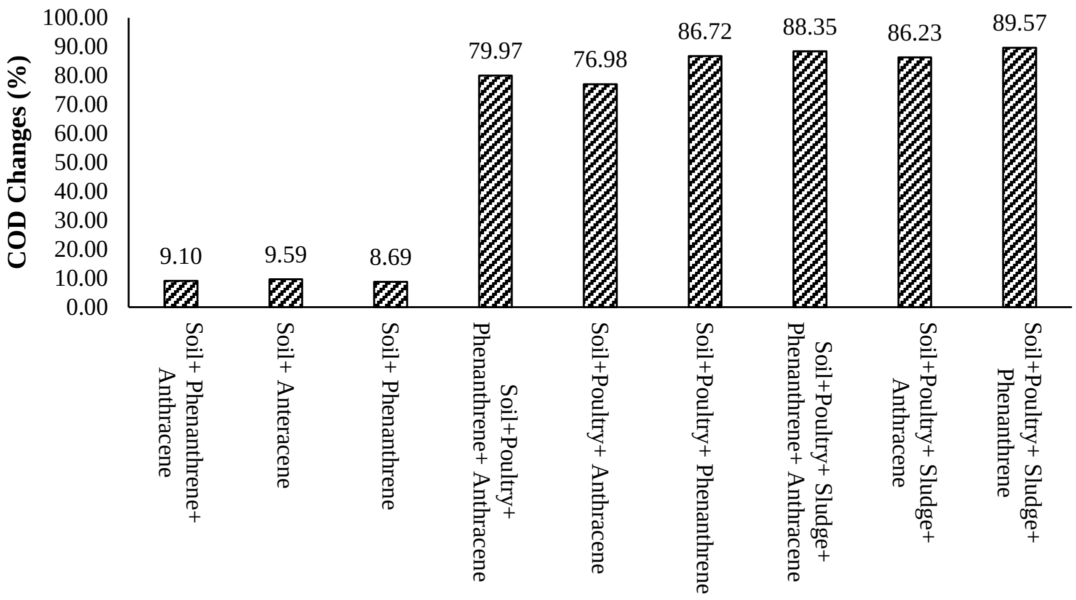

Figure 6: Percentage of COD changes during bioremediation

Discussion

The efficiency of the pilots in the removal of pollutants

The results showed that the anthracene removal rate was more than that of phenanthrene, which seems to be attributed to its molecular structure; anthracene has three linear benzene rings, while phenanthrene has three attached rings and anthracenemaybe used more by the bacterium due to its simpler molecular structure. The presence of active microbial populations in biological solids or cosubstrates may increase the removal of both anthracene and phenanthrene pollutants, and increased bioremediation may be due to high levels of carbon and nitrogen and other minerals in the chicken manure which support the growth of microorganisms. Necessary nutrients such as carbon, nitrogen, phosphorus, sulfur and other elements are essential for the growth and survival of bacteria to produce proteins, nucleic acids and other parts of the cell, and these requirements are met, regardless of whether bacteria are found in nature, or hazardous pollutants such as anthracene and phenanthrene are detoxicated [12]. Moreover, active microbial populations act in favor of bio-degradation of anthracene and phenanthrene [14]. Chicken manure has been reported to contain significant amounts of nitrogen due to its high levels of protein and amino acids, and as a bio-modifier, it contains almost all the factors required for bacterial growth [15]. The results of a study by Wong et al. showed that the increase in the amount of pig manure in the polluted soils increased the decomposition of three-ring PAHs [16]. The presence of these microorganisms increases the biological degradation of anthracene and phenanthrene in polluted soils. Some researchers,by investigating the bioremediation of soil polluted with PAHs through compost, concluded that the compost, as a cosubstrate, increased the removal of PAHs from soil polluted by microorganisms compared with the control group [17]. It has also been reported that chicken manure contains mineral salts, carbon and nitrogen, and while growing bacteria in the soil, it adds other microorganisms to decompose PAHs [13]. Chen et al. [18] stated that microorganisms could be used for bioremediation of polluted soils by adding the necessary nutrients to the soil and providing their energy requirement. To evaluate this process, the chicken manure was used as the feeding supply of bacteria in this experiment. In fact, the addition of chicken manure and sludge enhanced the removal of pollutants. On the other hand, considering the fact that the addition of substrate (chicken manure) significantly increased the removal of pollutants in comparison with control

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pilots, it seems that the cometabolism process has also contributed to the removal of pollutants in the experiment.

COD variations during bioremediation

In control trials, COD variations were very low and the lowest COD variations (compared to the 9 pilot cases studied) were observed in these trials. The probable cause seems to be due to the lack of nutrients required for the activity of microorganisms. Although the COD removal rate was significantly higher in the chicken manure pilots than that of control pilots, this removal rate was increased again with the addition of sludge. On the other hand, in pilots containing chicken manure + sludge, COD variations were much higher than the other 9 studied pilots. In addition, with increasing incubation time, COD levels weresignificantly decreased. This decrease in COD level indicates the activity of inoculated bacteria and microorganisms in the studied soil. The COD was used by these organisms and it decreased during the removal of anthracene and phenanthrene in this experiment and reached its lowest rate at the end of the experiment. Moreover, microorganisms used salts in the soil and the auxiliary substrates as a source of carbon and energy in the early stages of the experiment, and then, with the decrease in the amount of the salts and auxiliary substrate for a long time, the microorganisms used oxygen (for metabolic activities) and anthracene and phenanthrene (as a carbon source and energy for growth) and reduce them, especially COD. By investigating the bioremediation of PAHs in oil-polluted soils, some researchers concluded that decreasing COD was increased by increasing incubation time, but the bioremediation rate of phenanthrene significantly decreased [6]. It has been reported that the bacterium used glucose as a carbon source during low incubation, and then used anthracene as a carbon source and energy for growth during a long incubation (35 days) when glucose concentration is reduced, and then leads to a reduction in its level over a long time and COD level [7]. Also, investigating percentage of COD variations at different incubation times also showed that the 3 control pilots had the lowest percentage of COD variation and there was the slightest variation between their initial value and final value, and these very slight variations indicate that microorganisms were less active in control pilots. The percentage of COD variations was significantly increased by adding the chicken manure. In fact, it was observed that the addition of the chicken manure led to an increase in the activity of soil microorganisms and a significant decrease in the level of COD. Addition of sludge also increased the percentage of COD variations (such as the addition of chicken manure) in the pilots; it could be noted that microorganisms, in pilots containing chicken manure + sludge, use more oxygen because they were more active thus reducing COD to a greater extent. It has been reported that by adding auxiliary substrate (glucose), the removal of phenol and COD reaches more than 97% [18]. A group of researchers in their study reported that, as the incubation time increased, the COD removal rate by microorganisms increased [19]. In addition, during incubation (36 h), 99.8% of phenol and 92.5% of COD were removed [20]. It has been reported that the microbial mass had COD removal efficiency of 85 to 97.8% [21].

Conclusion

The results of bioremediation of anthracene and phenanthrene from polluted soils were discussed in this study. For this purpose, the effect of auxiliary substrate (chicken manure) and sludge on the amount of bioremediation of anthracene and phenanthrene was discussed. The results revealed that the decomposition of these compounds when sludge and chicken manure are used as a cosubstratecould be an effective or practical method to increase the bioremediation of polluted soils.

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Conflict of interest

The authors declare no competing interests.

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