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Phytoremediation of Contaminated Soil with Pyrene Using Sunflower (Helianthus annuus)

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Abstract

The study was conducted to evaluate the potential use of Sunflower (Helianthus annuus) to remediate contaminated soil with pyrene in phytoremediation experiments. Pyrene was used as an example of the PAHs at different concentrations. Representative soil samples were collected and contaminated by pyrene at three levels of concentrations: C1 (1.8 mg/kg), C2 (150 mg/kg), and C3 (300mg/kg). The experiments were conducted for 86 days. The pyrene removal from the contaminated soils was higher in most of the planted soil, compared with unplanted soil (controls) at different concentrations of the contaminants. In addition, the results of this study showed that sunflower (Helianthus annuus) were able to accumulate the tested pyrene in its tissues, and this accumulation was increased with the decrease in the contaminant concentrations in the soil. In addition, sunflower (Helianthus annuus) accumulated more amounts of pyrene in its aboveground parts (shoot and disc), compared with underground part (root). The results of this study imply that using sunflower (Helianthus annuus) in the phytoremediation process is an effective technique to remediate the contaminated soil with pyrene without causing any plant toxicity.

Keywords: *Pyrene, Phytoremediation,Sunflower, Contaminant, Helianthus annuus, Removal*

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds that have toxic effects on organisms. They can cause carcinogenic and mutagenic effects (e.g. Armstrong et al., 2004). The primary source of PAHs is the incomplete combustion of organic materials such as coal and oil as well as vehicular emissions and the indiscriminate disposal of refined petroleum products and used batteries (e.g., Imarhiagbe and Obayagbona, 2019).

PAHs are not synthesized chemically for industrial purposes. However, there are a few commercial uses for some chemicals of the PAHs such as pyrene. Pyrene is used in the manufacturing of pigments (Kaminski et al., 2008). Pyrene, $C_{16}H_{10}$ (four fused rings), is one of the PAHs that has recently gained attention due to its high toxicity, presence in all environmental ecosystems, high persistence, and resistance to biodegradation (Gabriele et al., 2021; Kim et al., 2019; Offiong et al., 2019; Sushkova et al., 2019; Zhang et al., 2018; Wang et al., 2012). Moreover, pyrene was selected among the 16 EPA-priority PAHs as a model compound based on its intermediate toxicity, hydrophobicity, and environmental persistence. Furthermore, pyrene represents the dominant PAH produced by the incomplete combustion of oil and oil byproducts (Kanaly and Harayama, 2000), and is frequently found in soil (Su et al., 2008) (Figure 1).

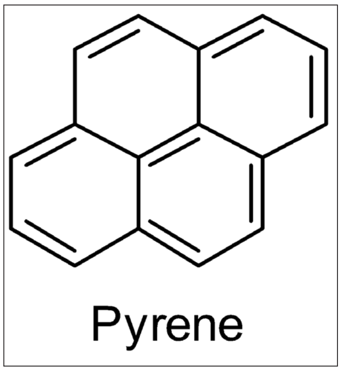

Figure 1. Structure of US-EPA Pyrene

Soil is considered similar to a sink reservoir for the presence of many atmospheric pollutants.Pyrenecontamination of soil occurs naturally because of the incomplete combustion of organic materials such as fossil fuels and vegetation. Moreover, pyrene is often monitored as an indicator of the amounts of PAHs in contaminated soils and sediments (Liu et al., 2020a, 2020b). Therefore, it is important to use technology that can remediate this pollutant from the soil. One of the important technologies is phytoremediation. Several studies showed that phytoremediation is an effective, sustainable, and reliable technique for the remediation of pyrene-contaminated soil and other toxic metals (Gabriele et al., 2021; (Omoregie and Ikhajiagbe, 2021). This technology utilizes plants to remove, stabilize, or reduce the toxicity

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of the pollutants or contaminants in the soil, groundwater, or other polluted media (USEPA, 2000; Ogeleka and Omoregie, 2022). However, phytoremediation is applied as a final step after other treatments in case of high levels of contamination and is an effective strategy used alone when the concentration of contaminants is low (Jones, 1991). One example of promising plants that could be used in this matter is sunflower (Helianthus annuus). Sunflowers are short-season plants that belong to the family Asteraceae, and there are more than 70 known species around the world. It originally comes from the temperate climate of North America (temperatures around 20- 25 °C) (Adeleke and Babalola 2020).

This plant has valuable benefits. Its growing season is relatively short. It has a large flower with a single stalk and differs from the wild sunflower, which is smaller and has multiple branches (Aboki et al., 2012). Many studies use sunflower (Helianthus annuus) to accumulate different contaminants because of its properties of rapid growth and production of large volumes of biomass (Martins et al., 2014).

A comparative greenhouse experiment was conducted between different plant families to remediate contaminated soil with aged polycyclic aromatic hydrocarbons (PAHs) by Olson et al. (2007). It showed that the largest populations of polycyclic aromatic hydrocarbons (PAHs) degraders were contained in the soil, planted with sunflower (Helianthus annus). Moreover, the results showed that sunflower (Helianthus annuus) was effective in stimulating PAH removal, and it may be a promising species in the remediation processes for the contaminated soil (Olson et al., 2007).

The accumulation of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) uptake by sunflower, willow, maize, and poplar in contaminated urban soil in a field experiment was studied (Kacálková and Tlustoš) (2011). They found that a higher concentration of polycyclic aromatic hydrocarbons was accumulated in sunflowers more than in maize. In addition, they observed that the highest accumulation levels of pyrene and phenanthrene were found in plants grown in soil with lower concentrations of pyrene and phenanthrene (Kacálková and Tlustoš, 2011). Another greenhouse experiment was conducted by Tejeda-Agredano and colleagues in 2013 to ascertain if the germination and development of sunflower (Helianthus annuus) plants can promote bio-accessibility and biodegradation of polycyclic aromatic hydrocarbons in the contaminated soil. The same study also explored the effects of sunflower (Helianthus annuus) planting on the removal of six polycyclic aromatic hydrocarbons (fluorene, phenanthrene, anthracene, fluoranthene, pyrene, and chrysene) in the contaminated soil. The results showed that planting sunflowers (Helianthus annuus) had a promoting effect on the removal of five contaminants (fluorene, anthracene, fluoranthene, pyrene, and chrysene) which decreased significantly from the soil in comparison with the unplanted controls. Nevertheless, according to phenanthrene, there was not any significant effect on the removal of this contaminant (Tejeda-Agredano et al., 2013).

Martins, Liduino, Oliveira, and Sérvulo (2014) studied the potential of three cultivars of sunflower (Helianthus annuus) plants to remediate multi-contaminated soil by hydrocarbons and heavy metals. They conducted another greenhouse experiment. Results indicated that the sunflower (Helianthus annuus) cultivars were able to remove Benzo] a[pyrene that is a high molecular weight polycyclic aromatic hydrocarbon from the contaminated soil (Martins et al., 2014).

In Jordan, there are many sources of polycyclic aromatic hydrocarbons (PAHs) that might affect directly or indirectly the health of plants as well as human beings, such as the Jordan petroleum refinery, Al-Hussein thermal power stations, traffic emissions, accidents (such as oil spills), and industrial areas (e.g. All these resources led to an increase in the probability of soil contamination by polycyclic aromatic hydrocarbons (PAHs). Therefore, the main objective of this study is to evaluate the potential of sunflower (Helianthus annuus) to remediate contaminated soil with three different concentrations of pyrene as an example of the PAHs. Other objectives are to study the accumulation of pyrene in the three parts of the sunflower plant (root, shoot, and disc) and to compare sunflower uptake of pyrene.

2. Materials and Methods

2.1. Soil physiochemical analysis

A representative uncontaminated topsoil samples (0-20 cm) were used in this study. This soil is usually cultivated with seasonal crops. The soil samples were air dried at room temperature, sieved with 2 mm mesh, and then subjected to several chemical and physical analyses for the basic soil parameters such as soil texture, past pH, paste electrical conductivity (EC), organic matter content (Black-Wakley method), and the concentration of the pyrene. The concentration of pyrene in soil and plant samples was determined according to Touraud et al., (1998) and Li et al. (2010).

2.2 Phytoremediation experiments

The experiment was performed by adding five kilograms in total of soil to each pot and mixing the contaminants with the upper layer of the soil. In detail, the used pots were bottom-sealed to prevent contaminants from leaching out of the soil. In each pot, 4.5 kg of the above-described soil was spiked with three concentrations of pyrene C1 (1.8 mg/kg), C2 (150 mg/kg), and C3 (300 mg/kg), mixed with the upper layer of the soil, three seeds of sunflower (Helianthus annuus) were sown directly in the contaminated soil and finally covered by 0.5 kg of soil. Locally cultivated sunflower (Helianthus annuus) in Jordan was used in this experiment, as it was adapted to the environmental conditions in Jordan. Furthermore, it doesn't need a lot of care. In addition, sunflower (Helianthus annuus) is considered a drought-tolerant plant that doesn't need a large amount of water.

The experiment was done in three replicates for each treatment. There was one control soil containing the original and the added contaminants, which will not be cultivated with any plant taking into consideration the independent variables like wind and temperature. The experiment was conducted for 86 days in two phases. The first phase was conducted in an open area because of the high temperature during the summer season, and the second phase was conducted in a plastic house because there was an expected drop in the temperature.

Seeds began to germinate, and seedlings emerged. During this period, the soil was kept moist to prevent hard crust formation, which might affect seed germination. About ten days after the end of the germination stage, the seedlings were thinned to keep just one seedling in each pot. The vegetative growth stage lasted much time in comparison with other stages; it lasted about forty days. During this stage, plants were irrigated carefully about one inch every three days. NPK fertilizer with micronutrients was added right at the beginning of this stage to maintain good health for the plants. After harvesting, plants were cut into the main parts (root, shoot, and disc). Fresh plant samples were washed through tap and distilled water to remove soil particles and dust completely from plant parts. Fresh weight was measured for each part of the plants, and the length of roots and shoots was measured. Air drying at room temperature in the laboratory of the faculty of Natural Resources and Environment was applied for all plants and soil samples until getting a constant weight. After drying, the dry weight for all plant samples was determined.

JMP version (8) statistical software was used to analyze the resulting data for all tested parameters. The statistical analyses included descriptive analysis using distribution command (e.g. measures of central tendency and dispersion), and comparison between means using Tukey Kramer Test (HSD) that is based on least significant difference testing at 95% confidence level.

3. Results and Discussion

3.1. Pyrene removal and uptake from the contaminated soil

The results of physiochemical properties of soil samples are presented in Table 1. The particle size distribution (42.37% sand, 37.45% silt, and 20.18% clay) indicated that the soil is loam. The pH of the soil is moderately alkaline (8.11), non-saline soil (EC< 4ds/m), moderately organic matter (6.14%), and 0.2 mg/kg of pyrene.

Table 1. Physiochemical properties of the studied soil samples

Parameter	Unit	value
Sand	%	42.37
Silt	%	37.45
Clay	%	20.18
Texture	-	Loam
pH	-	8.11
Electrical conductivity (EC)	dS/m	0.893
Organic matter content	%	6.14
Pyrene	mg/kg	0.2

After 86 days, the pyrene concentration in the non-cultivated, pyrene-spiked control soil decreased by 8.67%, 24.78%, and 39.71% for C1, C2, and C3 respectively as compared to the initial concentration (Table 2). This value was considered as the dissipation of pyrene in soil due to evaporation and degradation over the experimental period. There is pyrene removal from the unplanted control treatments, which likely refers to the microorganisms' activity in the soil (Olson et al., 2007). Furthermorepyrene is a volatile organic compound (VOC) and the area of evaporation is larger in bared soil (control) than in planted soil. Pyrene has a vapor pressure of 6.010-4 Pa (Mackay et al., 1992). These results are supported by several studies reported in literature. In a study by Liste and Alexander (2000), which investigated the capability of nine plant species to promote the degradation of pyrene in soil, it was found that higher pyrene was degraded in cultivated soil compared to non-cultivated soil. Moreover, Venkata et al., (2006) found a faster pyrene dissipation in the rhizosphere of several plants than in the bulk soil.

Table 2. Pyrene removal from the contaminated soil

Treatment	Initial (mg)	removal percent (%)
C 1	1.8	28.71[a]
Control C1	1.8	8.67[a]
LSD	-	25.78
C2	150	50.06[a]
Control C2	150	24.78[b]
LSD	-	19.03
C3	300	58.86[a]
Control C3	300	39.71[a]
LSD	-	26.8

*Values of C1, C2 and C3 are means of triplicate measurements, values followed by the same letter are not significantly different at ($p<0.05$).

The pyrene concentrations in soils cultivated with sunflower (Helianthus annuus) decreased respectively by 28.71, 50.07, and 58.86% with respect to the initial values. This decrease was not statistically significant except for C2. This result was agreed with Besalatpour et al. (2010) who found that sunflower (Helianthus annus) hadn't any significant effect on the reduction of the total petroleum hydrocarbons (TPHs) in the contaminated soil. Planting contaminated soil with sunflower (Helianthus annuus) stimulates pyrene degradation through root exudates, which have a clear impact on the development of PAHs degraders' populations (Ortega-Calvo et al., 2017; Olson et al., 2007).

Pyrene uptake by different plant parts (root, shoot, and disc) and by the whole plant are shown in Table 3. During the growth phase, plants can accumulate contaminants in roots, stems, and leaves, and pyrene is eventually removed by harvesting the plant when the treatment is terminated (Mahar et al., 2016).

Table 3. Pyrene uptake by plant parts (root, shoot and disc) and by the whole plant.

Treatment (mg/kg)	Root uptake (%)	Shoot uptake (%)	Disc uptake (%)	Plant uptake (%)
C 1	0.5[b]	12.1[b]	7.6[b]	20.27[a]
C 2	0.027[ab]	1.88[ab]	1.65[a]	3.56[a]
C 3	0.028[a]	0.86[a]	1.23[a]	2.72[a]
LSD	0.29	17.95	7.31	17.63

*Values of C1, C2 and C3 are means of triplicate measurements, values followed by the same letter are not significantly different at ($p<0.05$).

The results showed that roots, shoots, and discs significantly uptake more amount of pyrene at C1 treatment compared with other treatments (Table 3). There were no significant differences between the three treatments in pyrene uptake by the plant, taking into consideration the total amount of pyrene in the soil. However, the results showed that the increase in concentrations of pyrene in the soil leads to a significant decrease in the uptake percentage by different plant parts between treatments. The obtained results concur with Kacálková's and Tlustoš's (2011) study findings where the pyrene PAH uptake by the plant was significantly low when cultivated in soil contaminated with high PAH concentrations.

The extent to which an organic pollutant enters plant roots from contaminated soils depends on the physicochemical characteristics of the compounds including contaminant concentrations in the soil, octanol/water partition coefficient (log Kow), and other factors such as organic carbon content of the soil and plant species (Nwoko, 2010). Hydrophobic compounds with log Kow > 3.0 are bound so strongly to the surface of roots that cannot be easily translocated within the plant. Pyrene log Kow is 5.18, Mackay et al., 1992. This could explain the insignificance of pyrene accumulation in tested plant shoots and roots.

3.2 Plant characteristics at different concentrations of the contaminants

To observe the effect of pyrene uptake on plant growth, the fresh and dry weights of plant parts (root, shoot, and disc) and shoot and root heights were measured at the end of the growth cycle. Fresh and dry weights of plant parts (root, shoot, and disc), after the phytoremediation test, are presented in Figure 2. Statistical analysis of the data showed that pyrene, at low concentration applied (C1), was generally non-toxic to the three plant parts. The C1 concentrations of pyrene had no significant ($p<0.05$) effect on the fresh and dry weights of the different plant parts. No severe plant growth inhibition was apparent during the growth period. While C2 and C3 have a significant effect ($p<0.05$) among fresh and dry weights of plants (Figure 2). The maximum root, shoot, and disc for both fresh and dry weights were found at (C1) treatment. The minimum root, shoot, and disc fresh weights were found at pyrene (C2) treatment (Figure 2). As the pyrene concentration increases in soil, the sorption by soil particles increases resulting in the prevention of pyrene uptake by plants (Olu-Owolabi et al., 2014). Increasing soil pyrene concentration has increased the sorption by soil particles and hindered pyrene uptake by plants. This can be attributed to the fact that when the transport of pyrene at the external soil surface and within the internal pores is equal, less transboundary movement of pyrene is allowed. However, as the concentration increases, the transboundary movement of pyrene resumes, and the adsorption is concentration-dependent (Olu-Owolabi et al., 2014). This explains why the C3 treatment showed higher root, shoot, and disc weight compared with C2.

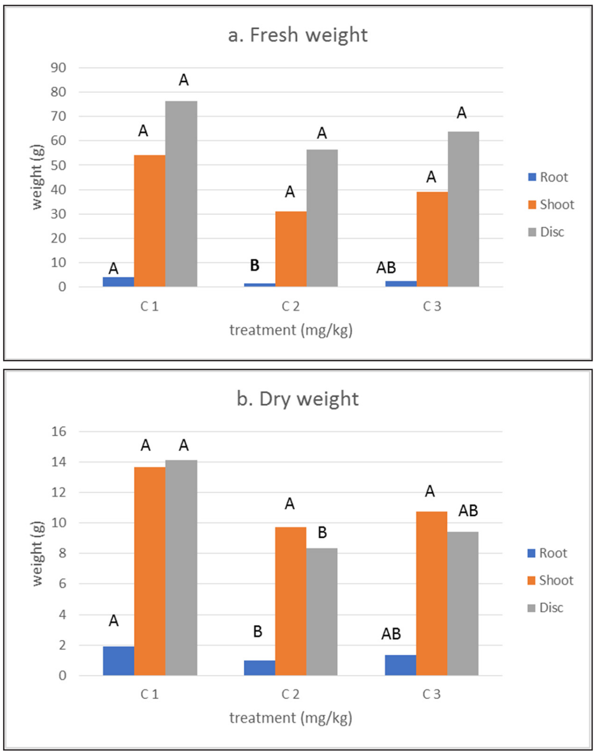

Figure 2. Weight (g) of plant parts (shoot, root and disc) after phytoremediation test fresh weight b. dry weight. (Values with the same letter are not significantly different at $p \leq 0.05$ (LSD test).

However, statistical analysis of the obtained data showed that there are no significant ($p < 0.05$) effects of the different concentrations of pyrene on the lengths of plant root and shoot (Figure 3). These results would imply that pyrene does not affect the development of sunflowers (Helianthus annuus) height. Same results were found in literature using L. perenne (D'Orazio et al., 2013), related this effect to the other factors associated with the plant itself and other soil factors.

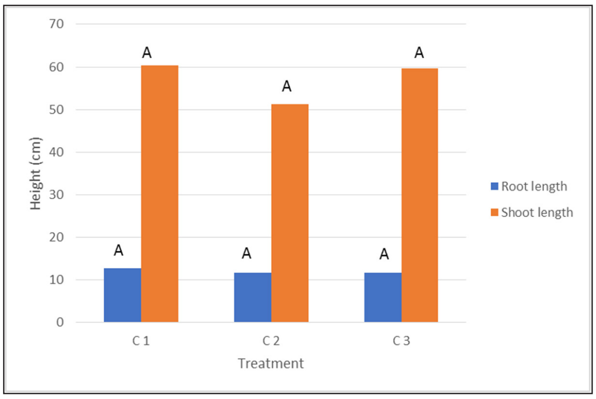

Figure 3. Shoot and root length (cm) after phytoremediation test of pyrene.
Values with the same letter are not significantly different at $p \leq 0.05$ (LSD test).

The results showed that phytoremediation mechanism for all treatments include extraction, degradation, volatilization, and stabilization by plants as indicated by more than 50% removal for higher concentration of pyrene. Moreover, the results indicate that the removal of pyrene does not result in any toxicity for the used plant.

Comparing pyrene removal from soil and plant uptake (Figure 4), each point is the average of triplicate. For the 86-day phytoremediation trial, the amount of pyrene in the uncultivated, pyrene-spiked control soil decreased by 8.67%, 24.78%, and 39.71% respectively. The distribution of pyrene in the soil and plants is shown by these findings. The soil used to cultivate sunflowers (Helianthus annuus) reduced pyrene by 28.71%, 50.10%, and 58.86% respectively, when measured in comparison to the initial levels. While the percentage of removed plants was 20.30%, 3.60%, and 2.70% respectively. These results demonstrated that plant removal decreased as soil pyrene content increased whereas the opposite was true for soil removal.

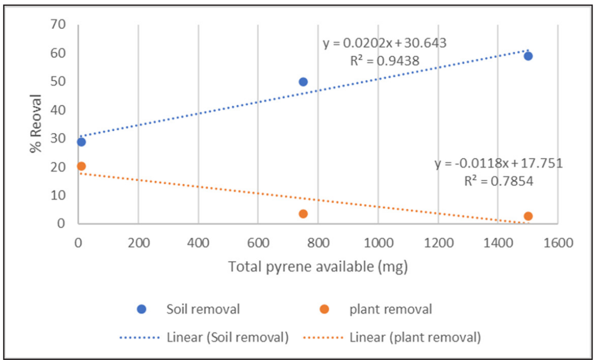

Figure 4. Total pyrene removal from spiked soil by soil and plant

These results (Figure 4) indicated that several factors play a major role in pyrene removal from soil. As the concentration of pyrene in soil increases, the effect of plant uptake is low, supporting that the pyrene removal is affected by other factors due to evaporation, degradation, and the presence of certain genera that are capable of using pyrene as energy and sole carbon source in the rhizosphere area and promote biodegradation of pyrene (Liu et al., 2013; Zeng et al., 2019; (Yang et al., 2021). Moreover, bioavailability plays a major role in plant uptake. Several factors affect the bioavailability such as aging or contamination. A study by Smith et al., 2011 showed that pyrene loss, through mechanisms not connected to plants, could more easily occur in freshly spiked soils. Presence of organic matter in high amounts (Table 1) more than 2% could play a role in limiting the bioavailability of pyrene for plants through adsorption. A study by Chekol et al., 2002 showed that under high soil organic matter (6.3%) conditions, adsorption, or covalent binding of pyrene to SOM are the dominant forces for pyrene retention in soil, limiting pyrene bioavailability for plants. Therefore, under the soil conditions of this study, sunflower (Helianthus annuus) with other soil factors showed a promising potential in promoting the phytoremediation of pyrene freshly added to the soil without any toxicity or any effect on plant growth. Moreover, the accumulation of pyrene in the aboveground parts helps remove pyrene by harvesting.

4. Conclusion

After 86 days of phytoremediation experiment, sunflower (Helianthus annuus) appears to be a promising process to remediate contaminated soil with the tested polycyclic aromatic hydrocarbon (pyrene). Pyrene removal from the contaminated soil was higher in the planted soil compared with unplanted soils at different concentrations of the contaminant. Sunflowers (Helianthus annuus) showed their ability to accumulate pyrene in their tissues, and this accumulation was increased with the decrease in the contaminant concentration in the soil without showing any toxicity. Therefore, plant cultivation on pyrene-contaminated soil appeared to play a significant role in ecosystem restoration by limiting the spread (erosion, leaching) of pyrene and restoring sites contaminated with pyrene. Thus, this practice represents an important tool for the control of pyrene migration in soil. Sunflower (Helianthus annuus) accumulated more amounts of pyrene in its aboveground parts (shoot and disc) compared with underground parts (roots).

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Conflicts of Interest:

The authors declare no conflicts of interest.

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