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THE USE OF ANNUAL BEARD GRASS IN PHYTOREMEDIATION OF PETROLEUM-CONTAMINATED SOILS

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Abstract: This greenhouse study investigated the ability of annual beard grass *Polypogon monspeliensis* to phytoremediation oil-contaminated soils. The research aimed to assess the plant's potential for removing oil from contaminated soils. Total petroleum hydrocarbon (TPH) levels were measured before and 30 days after planting in soils contaminated with 20, 30, 40, 50, and 60 grams of crude oil per kilogram of soil. The results showed that *P. monspeliensis* effectively reduced petroleum contamination, with the plant achieving a 64.85% reduction in treatment at 20 g/kg. The study also evaluated shoot height and root fresh and dry weight. Only treatment 60 g/kg resulted in a significant decrease in plant height compared to the control. Plant shoot height increased in the 20 g/kg and 30 g/kg petroleum-contaminated treatments. The fresh and dry weight of the root increased in the 20, 30, and 40 g/kg treatments but decreased in the 50 and 60 g/kg treatments. Shoot height increased in the 20 and 30 g/kg treatments, while root fresh and dry weight increased in the 20, 30, and 40 g/kg treatments. Results indicated that *P. monspeliensis* was significantly influenced by TPH concentration. In most treatments, the translocation factor was >1 except for treatment 60 g/kg. The plant investigated in this study demonstrated a good capacity for transferring hydrocarbons from the root.

Keywords: Sustainability, beard grass, hydrocarbons; oil, phytoremediation.

Introduction

Global oil consumption poses a risk to soils and water (Pichtel, 2016), and oil's presence affects the soil's nutrients and compounds. Studies have shown that phytoremediation is useful for reducing environmental risks by removing and degrading oil (Leewis *et al.,* 2013). The effectiveness of phytoremediation depends on the species' tolerance to the contaminant, making traditional flora imperative for minimizing ecological impact (Nouri *et al.,* 2011; Zaboon *et al.,* 2022).

Petroleum hydrocarbon (PHC) contamination of soil and water is a major environmental concern in oil production facilities. In recent decades, the impact of hydrocarbon pollution on the environment has been increasingly recognized (Perera, 2018). Petroleum-contaminated soil is crucial in pollution control efforts (Chen & Zhong, 2019). PHCs, a primary class of environmental pollutants (Srivastava *et al.,* 2019), are produced by industry and agriculture through distillation. These contaminants can be absorbed into soil particles and increasingly concentrated in surface water. However, they can penetrate the deep layer of soil and impact plants, animals, and humans (Hussain & Keçili, 2020). The presence of oil in the soil can harm the growth and development of animals and plants. A significant amount of PHCs in the atmosphere can also affect human health, ecological structure, and the quality of surface water and groundwater and cause soil degradation (Prematuri *et al.,* 2020).

The primary sources of petroleum soil contamination are extraction leaks from pipes and spills during transportation. Iraq is one of the world's top oil-producing countries, with a large annual output from its southern regions. Most of Iraq's refineries and petroleum facilities are in urban or agriculturally active regions.

The habitats in these areas are facing significant challenges due to oil emissions in some areas, highlighting the need for detoxifying soils contaminated with oil products to ensure environmental and agricultural product health (Wei & Pan, 2010).

Phytoremediation is an environmentally friendly and cost-effective method some aquatic plants use to treat water contaminated with pollutants (Azeez, 2021). It is also used for remediating soils contaminated with heavy metals and PHCs (Razmjoo & Adavi, 2012).

Rahbar *et al.* (2012) investigated the impact of hydrocarbons on growth and photosynthetic pigments in sunflower plants. Farzamisepher *et al.* (2013) and Farzamisepehr & Nourozi (2018) evaluated the ability of *Polypogon monspeliensis* to remediate petroleum-contaminated soils. They found that the plants used petroleum compounds as nutrients and had more rhizospheric microorganisms than the control treatment. Liu *et al.* (2018) reviewed phytoremediation using ornamental plants for contaminated soils, highlighting specific plants with strong accumulation ability and pollutant tolerance.

Tang & Angela (2019) investigated the impact of crude oil contamination on local plant species using phytoremediation. Zuzolo et al. (2021) evaluated the phytoremediation efficiency of two Poaceae species (*Festuca arundinacea* Schreb. and *Dactylis glomerata* L.) and two Fabaceae species (*Medicago sativa* L. and *Lotus corniculatus* L.) and found that Poaceae was more effective than Fabaceae. Azeez & Durgham (2021) discussed the potential of barley for remediating oil-contaminated soil in Iraq.

Beard grass, also known as annual grass, is a plant species native to Southern Europe but has been introduced to many other parts of the world. It can be considered a noxious weed in some areas. This annual grass typically grows to a height of between 5 cm and 1 m. The plant has a dense, soft, and fluffy inflorescence with green, plume-like panicles that can be divided into lobes. The inflorescence is characterized by long, thin, whitish awns on the spikelets, giving it a distinctive texture (Jara-Hermosilla *et al.,* 2017). This study investigates the ability of *P. monspeliensis* to grow in oil-polluted soil to determine its resistance to hydrocarbon pollutants and how this tolerance affects the plant's potential for the phytoremediation of oil-contaminated soils.

Materials and Methods

Study Area

Polypogon monspeliensis was obtained from plant seeds collected in the wild from the Eastern Hammar Marsh in Southern Iraq (Basra). A commercial mixture of silt and clay (4:1) was used as the soil for the experiments. The soil was manually mixed, homogenized, and sieved to a size of less than 2 mm. Its pH was 6.9, electrical conductivity was 1.79 dS m^{-1}, organic matter was 3.4%, total nitrogen was 0.14%, and phosphorus was 2.2 mg kg^{-1}. Crude oil was added to the soil to simulate various levels of oil contamination. The soil was then allowed to sit for three days to enable the evaporation of volatile hydrocarbons.

Before the initial concentrations were added, the physicochemical properties of the soil in the experiment were measured, including soil texture, electrical conductivity (dS m^{-1}), pH, total nitrogen (%), phosphorus (ppm), and organic matter (%).

Crude oil concentrations of 20, 30, 40, 50, and 60 g/kg of soil (w/w) were used to cover the low and high oil contamination range in the experimental soils, following the range suggested by Adam and Duncan (1999). A commercial soil mix was used as a control soil without oil.

Experiment Set-up

The seeds were germinated in Petri dishes, and the resulting seedlings were grown hydroponically in a diluted nutrient solution for two weeks. At four months old, the plants were transferred from hydroponic culture to control soils and acclimated to a nursery for six weeks.

Ten individual plants were selected randomly for each experimental group and were grown in soils containing 20, 30, 40, 50, and 60 g/kg (w/w) of crude oil, as well as a control group without crude oil. The length of each plant's leaves was measured on the day of soil transfer (day 0) and weekly until day 30. On day 0 and day 30, the length of each plant's primary root was also measured.

Measuring the Total Petroleum Hydrocarbons

Before using 1-month-old *P. monspeliensis* seedlings, different treatments of hydrocarbon concentration were tested using method 9071B (EPA) as described by Rauckyte *et al.* (2010). Additionally, PHCs were measured at the end of the plantation to assess the effects of different plant treatments on reducing total oils and grease in the soil. The plants were uprooted from their pots and stored in a glass container for 45 days after being planted in different soils. Each container was securely fastened with a lid and then placed in a refrigerator with a temperature of 4°C ± 2°C for extraction. The experimental soil samples were placed in clean mortars and washed with n-hexane. The samples were then placed in an oven at 50°C for 3 h to evaporate the water. Afterward, the leaves and mortar were washed in n-hexane, and the samples were crushed in a machine in three replications on Whatman No. 42 filter paper. Finally, we obtained 15 g of plant and sample-polluted water samples. After ensuring the papers were completely oil-free, they were transferred to the Soxhlet.

We washed the beakers of the Soxhlet in methanol to avoid leaving fingerprints, then removed them from the oven and immediately transferred them to plastic gloves. Anti-explosive weld powder was applied to each beaker, which was then measured. Next, n-hexane solvent was added to each beaker and then placed on a hot plate at 130°C. Filter containers with various treatments were placed into the beakers of n-hexane, and two processes of Soxhlet were conducted for immersion in three phases. After the solvent recovery stage, the beakers were picked up using pincers and shifted from side to side for brief moments to disperse the solvent vapours. Given the presence of sediment, we added 1 g of sodium sulfate to each beaker and allowed them to sit in a desiccator for 30 minutes before weighing them again.

To determine the percentage of total PHCs (TPHs) in the soil, we first subtracted the initial weight of the beakers and the sodium sulfate. This enabled us to calculate the percentage of PH within each treatment before and after the experiment. A UV-Vis spectrophotometer was used to quantify the crude oil concentration as TPH. Hydrocarbons in 1 g of soil were extracted with 10 ml of n-hexane using sonication and separated in a separatory funnel. The extract was then transferred to a volumetric flask and reconstituted with n-hexane to a final volume of 10 ml for examination with the UV-Vis spectrophotometer. The reconstitution made it easy to dilute crude oil extracts to fit the calibration curve. The UV-Vis spectrophotometer was tuned to a wavelength of 360 nm to detect absorbance and crude oil contents (Banda-Cruz *et al.,* 2016).

Plant Samples

Plant samples were analysed for total PHC using the Ultrasonic Extraction Method described in 3550 of USEPA (2007). The previously dried plant samples were mixed with anhydrous sodium sulfate until they resembled a free-flowing powder and were then mixed with dichloromethane as a solvent. The plant samples were sonicated in a specified pulse mode, and the extracted solvents were poured into a grade-A 100 mL volumetric flask through a glass funnel packed with anhydrous sodium sulfate. The extracts were then evaporated and concentrated, and the extracted solvent was injected into a GC-FID instrument for analysis.

Bioconcentration Factor

The bioconcentration factor (BCF) was calculated using the following formula:

$$BCF = C\ biota / C\ soil$$

where C biota is the concentration in the plant and C soil is the concentration in the soil.

Translocation Factor

The following formula was used to calculate the translocation factor:

$$TF = C\ leaf / C\ root$$

where C leaf is the concentration of TPH in the leaf sample and C root is the concentration of TPH in the root sample. The Translocation Factor (TF) was calculated to assess the potential mobility of PHC from the roots to the leaves.

Data Analysis

Statistical analyses were conducted using SPSS v21 at a probability level of 95%. The mean values and standard deviations were calculated for the treatments.

Results and Discussion

As presented in Table 1, the results showed that both the contamination-free and contaminated soils had silty-clay textures. The total nitrogen content in the two soils was 0.16% and 0.22%, respectively. These values suggest that the phosphorus level in both soil samples was within the optimal range for phosphorus in the soil, which is 15 ppm.

The measurements of the physicochemical properties of the contamination-free and oil-contaminated soils demonstrate significant similarities in terms of their properties, especially their texture, pH, and total nitrogen content.

Petroleum Hydrocarbons

Table 2 presents the concentrations of TPH in both soil and plant samples. The TPH concentration in the soil samples ranged from 2.6 g/kg to 31.2 g/kg. The results indicate that the concentration of TPH in the 60 g/kg treatment soil was higher than that in the other treatments, suggesting that the uptake and accumulation of TPH depend on the pollutant and its concentration in the environment. Different concentrations of TPH were observed in the roots and leaves of the tested plants.

Table 1: Physicochemical properties of soil in the experiment

Sample	Soil Texture	Electrical Conductivity (dS m^{-1})	pH	Total Nitrogen (%)	Phosphorus (ppm)	Organic Matter (%)
Non-contaminated soil	Silty-Clay	2.71	7.8	0.16	37	4.2
Contaminated soil	Silty-Clay	4.55	7.5	0.22	114	8.77

Table 2: Concentrations of total petroleum hydrocarbons in the soil and plant samples(g/kg) dry weight

Treatment	Soil	Root	Shoot(leaf)
20	2.6	8.03	9.37
30	5.8	11.56	12.64
40	9.2	16.2	14.6
50	18	19.53	12.47
60	31.2	21.38	7.42

Total Petroleum Hydrocarbon in Plant

The TPH concentration in the leaf samples ranged from 7.42 to 14.6 g/kg. These high concentrations of TPH in the leaf samples indicate that the plant leaves have a high capacity for PHC uptake. The TPH concentration in the root samples ranged from 8.03 to 21.38 g/kg, indicating a higher uptake of PHC by roots than by leaf samples. The comparison of TPH content in the plant samples showed that the plant had a good uptake of TPH through both the root and leaf samples, resulting in a higher concentration of TPH in the root than in the leaf samples.

This suggests that more PHC pollution in the soil was transferred toward the plant tissue via the roots. The roots of *P. monspeliensis* exhibited more uptake of PHC through phytostabilisation and rhizodegradation mechanisms. Phytostabilisation immobilizes contaminants in the soil through absorption and accumulation into the roots, adsorption onto the roots, or precipitation or immobilisation within the root zone, rendering the contaminants in a stable form. In rhizodegradation, contaminants are degraded in the soil through bioactivity produced and exuded by plants or soil organisms such as bacteria, yeast, and fungi. Kumari *et al*. (2019) demonstrated that *Zea mays* could grow in soil contaminated with polyaromatic hydrocarbons.

The results indicate that the samples collected from oil-contaminated soil contained PHC as dry weight. After 21 days, all treatments showed a significant reduction ($p \leq 0.05$) relative to the control treatments. The treatment of 20 g/kg resulted in the most significant reduction of up to 64.85% (Figure 1). The lowest reduction was 7.57%, which was observed in the 60 g/kg treatment without plants. The results revealed that the reduction rate in the plant rhizosphere decreases as the petroleum concentration increases. Moreover, the 60 g/kg treatment was associated with the minimum amount of petroleum reduction (22.42%) in the plant rhizosphere (Figure 1).

The control treatment differed significantly from the treatment of 60 g/kg in terms of plant height, which was higher. Plant height increased in the 30 g/kg contamination treatments compared to the non-contaminated treatment (Figure 2). This result is likely due to the plants using the oil compounds for growth or because the activity of most of the plant's rhizosphere bacteria in the treatments has improved plant height growth compared to the non-contaminated treatment.

Plant height decreased in the 40, 50, and 60 g/kg contamination treatments compared to the control treatment, indicating that the plant is responsive to higher oil concentrations. The maximum growth in plant height was associated with the 30 g/kg treatment at 32.6 cm, while the minimum growth was associated with the 60 g/kg treatment at 13.7 cm. A significant difference ($P \leq 0.05$) was observed in the fresh

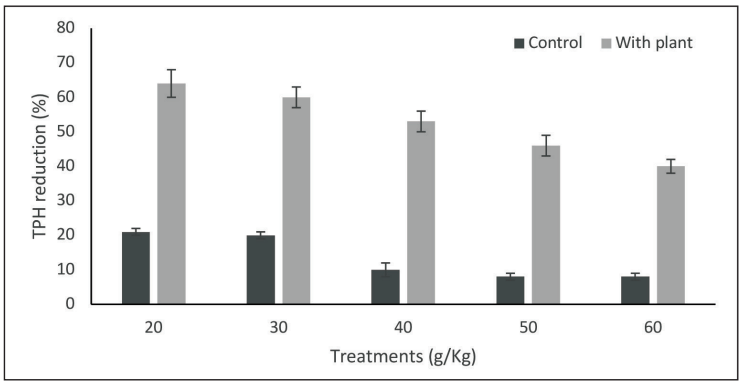

Figure 1: Reduction of petroleum hydrocarbons in different treatments with *Polypogon monspeliensis* and control treatment

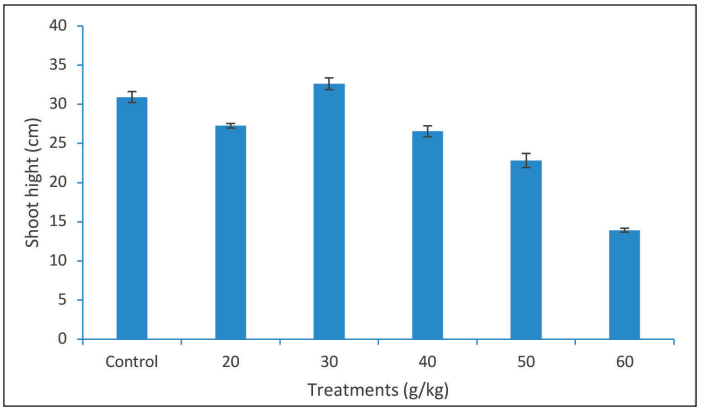

Figure 2: Shoot height of *Polypogon monspeliensis* planted in various contamination levels

weight of the control and all other treatments. Specifically, the 20, 30, and 40 g/kg treatments significantly increased fresh weight compared to the control treatment. The results indicated that plants exposed to 60 g/kg treatments or higher significantly decreased the fresh weight of their root tissues. Furthermore, no significant difference in the fresh weight of plants was observed between the 20 and 40 g/kg treatments.

As shown in Figure 3, the treatment at 30 g/kg was associated with the maximum fresh weight, reaching 0.058 g, while the treatment at 60 g/kg was associated with the minimum fresh weight, reaching 0.014 g. The difference in dry weight between the control treatment and the 30, 40, and 50 g/kg treatments was insignificant ($P \leq 0.05$) but significant compared to the other treatments. The 20 g/kg treatment resulted in a greater dry weight than the control treatment. Figure 4 shows that the maximum dry weight, 0.003 g, was associated with the 20 g/kg treatment, while the minimum dry weight was associated with the 60 g/kg treatment.

Bioconcentration and translocation factors

Figures 5 and 6 present the BCF and TF values of TPH in leaf and root samples, respectively. In all three types of plant samples, the BCF value of TPH was greater than 1, indicating PHC uptake via the roots. TPH BCF values increased in the order Root > Leaf, indicating that *P. monspeliensis* has phytoremediation potential through phytostabilisation in roots and phytodegradation in leaves.

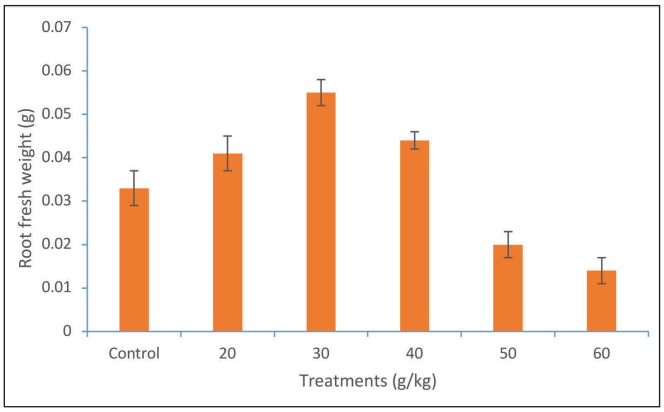

Figure 3: Root fresh weight (g) of *Polypogon monspeliensis* planted in various contamination levels

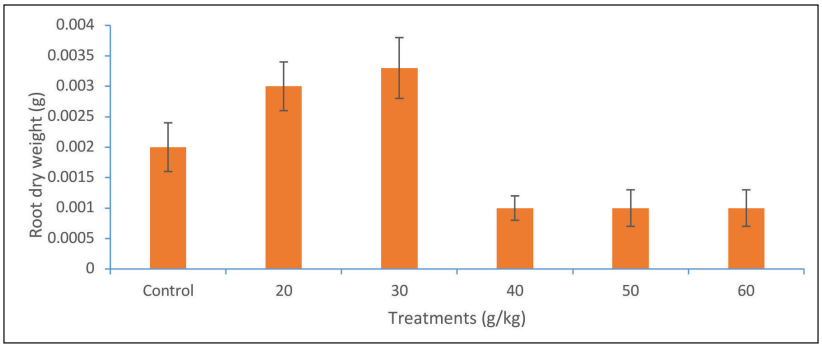

Figure 4: Root dry weight (g) of *Polypogon monspeliensis* planted in various contamination levels

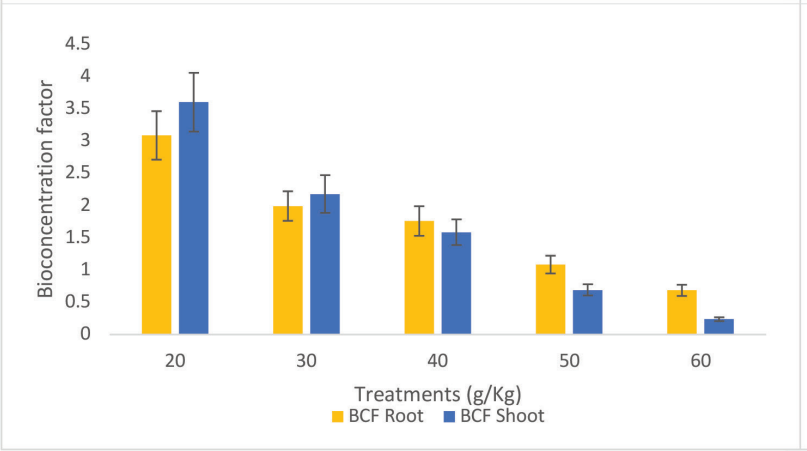

Figure 5: Bioconcentration factor in *Polypogon monspeliensis* planted in various levels of contamination± standard deviation

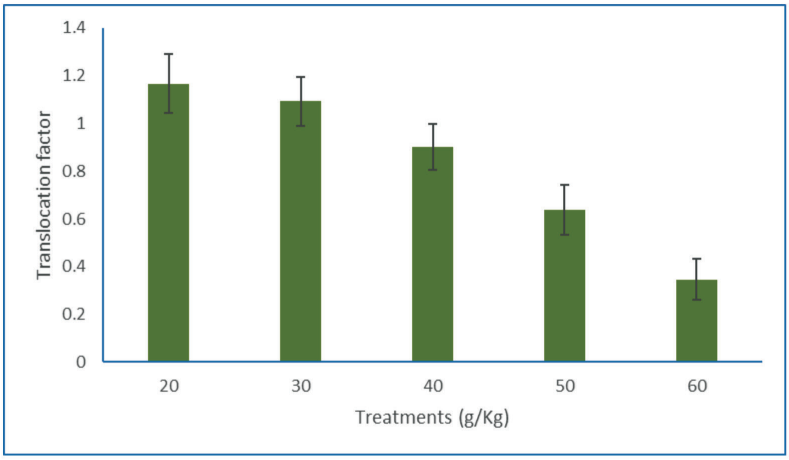

Figure 6: Translocation factor in *Polypogon monspeliensis* planted in various levels of contamination ± standard deviation

Phytostabilisation has played a significant role in the remediation of PHC contamination by *P. monspeliensis* in root samples, as indicated by the higher BCF value. The mean TF of PHC in plant samples was less than 1. The lower TF value of leaf samples suggests that PHCs are absorbed by roots and translocated through vascular systems to leaves. Highly water-soluble chemicals are generally not sufficiently absorbed into roots or actively transported through plant membranes. PHCs are typically not soluble in water or are firmly bound to the surface of roots due to the high proportion of lipids at the surface, making it difficult for them to be translocated into plants.

Polypogon monspeliensis has been recognized for its potential in the phytoremediation of soils polluted with crude oil hydrocarbons. This plant is known for its ability to tolerate soil contaminated with petroleum. This study successfully demonstrated its effectiveness as a phytoremediator due to its particular tolerance to petroleum contamination. The removal of PHCs showed its ability to thrive well in contaminated soil with crude oil. However, at higher oil treatment levels, the tested plants' biomass and height were significantly affected.

Another characteristic of this plant that makes it suitable for phytoremediation is its ability to bioaccumulate PH. Different types of plants thrive near emergent water in aquatic ecosystems like wetlands, making them suitable as phytoremediators for oil hydrocarbons. Since they exhibit rapid growth, they can be used to quickly repair the damage caused by oil pollution. However, their fast growth also makes them invasive species (Bhatia & Goyal, 2014). The fibrous roots of this plant provide large surfaces and dense rhizospheres for microbial colonisation (Nihorimbere *et al.,* 2011).

The efficiency of phytoremediation methods in industrial regions can be improved by selecting appropriate plant species and microorganisms. This approach promotes the sustainability of the local ecosystem and supports improving the local environment (Juwarkar & Jambhulkar, 2008). Low-molecular-weight hydrocarbons with a carbon number greater than 20 infiltrate the soil and disrupt its structure, while low-molecular-weight monocyclic aromatic hydrocarbons volatilize into the atmosphere during a crude oil spill (Yavari *et al*., 2015; Li *et al*., 2021).

Conclusion

This research has shown that petroleum contamination reduces the shoot and growth of annual beard grass. However, the study has also revealed that this species can remediate PHs in the soil, reducing TPHs contamination levels. The results suggest that *P. monspeliensis* is a useful and locally available plant with significant potential for phytoremediation of crude oil contamination in soils. Furthermore, the study highlights the plant's tolerance to varying crude oil concentrations. The BCF values confirm the suitability of using *P. monspeliensis* in phytoremediation efforts to control, prevent, and clean up PHC pollution. In phytoremediation experiments, it is important to consider the plants as waste and dispose of them in designated landfills. It is critical to examine the fate of PHCs in annual beard grass after the growth period and evaluate any potential risks associated with contaminants seeping into groundwater or surface water resources during disposal. All relevant regulatory requirements must be met for proper disposal practices to ensure these processes are carried out safely and effectively.

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