ISSN: 2287-688X

Annals of
Plant Sciences

Volume 10, Issue 11(2021) pp. 4294-4302

Research Article

Phytoremediation of Anthracene and Pyrene Contaminated Soil by *Lolium Perenne*

Muhammad Arbaz Madni[1], Syed Jahanzaib Rasool[2]*, Maira Naeem[3], Hafiz Usama Najeeb[4], Ahmad Ibrahim Maqbool[1], Azka Tariq[1], Syed Ijaz-ul-Haq[5], Safdar Ali[5], Muhammad Rizwan[5]

[1]*Institute of Environmental Engineering and Research, University of Engineering and Technology UET Lahore, 54890 Pakistan*

[2]*College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China,*

[3]*Department of Agricultural Engineering, Bahauddin Zakariya University, Multan 60800, Pakistan,*

[4]*Department of Environmental Science, International Islamic University, Islamabad, Pakistan*

[5]*Institute of Soil Science, PMAS Arid Agriculture University, Rawalpindi, Pakistan*

Abstract

Soil contamination can be addressed through phytoremediation technique which is one of the most important environmental problem. A pot study was conducted to examine the phytoremediation of anthracene and pyrene contaminated soil by Lolium perenne plant species for 45 days. The ability of Lolium perenne to remediate anthracene and pyrene spiked soil with initial concentration of 81.6 and 71.3 mg/kg was accessed. The anthracene and pyrene concentration in unplanted control soil on day 20 was 62.1 and 59.2 mg/kg respectively, whereas the respective concentration of unplanted control soil on day 45 was 21.7 and 22.4 mg/kg respectively. Lolium perenne was more effective in anthracene and pyrene removal from contaminated spiked soil during 45 days experiment. The results demonstrate the enhancement in disappearance of anthracene and pyrene by Lolium perenne on day 20 was 42.4 and 45.9 mg/kg, respectively, while there is complete removal of anthracene and pyrene by Lolium perenne was observed on day 45. The results shows that Lolium perenne could be more efficient in phytoremediation of PAHs-spiked soil

Keywords: Phytoremediation, anthracene, pyrene, Lolium perenne, PAHs

Introduction

In existing phase of development, soil contamination has increased massively due to industrialization and has become a global concern. Global advancement has created many problems in terms of pollution.

Environmental matrices (air, water, soil, biota) have highly effected due to anthropogenic activities such as vehicle emissions, shipwrecks, oil wells, accidental spillage and incomplete combustion of petroleum, wood, coal and open field burning of vegetation residues which are major source of PAH contamination in environment. Direct transfer of contaminants from other matrices lead to soil contamination (Cristaldi *et al*. 2017).

Phytoremediation is an ecological technological approach for remediation of soil contaminated with toxic metals. It is a technique in which pollutant accumulating plant is used to remove metals or organics from contaminated soil by concentrating them in harvestable parts of plants (Singh and Jain 2003), (Vamerali, Bandiera, and Mosca 2010). From this technique number of benefits can be obtained such as reclamation of soil, promoting local biodiversity, biomass from plant harvested in contaminated soil may be used as biofuel, plant continue to grow on site, atmospheric carbon dioxide fixation, regrowth of ecosystem and soil stability (Bell *et al*. 2014)

Polycyclic aromatic hydrocarbons (PAHs) are harmful organic pollutants due to their

***Corresponding Author:**
Syed Jahanzaib Rasool;

DOI: http://dx.doi.org/10.21746/aps.2021.10.11.1

oncogenic and mutagenic potential which pose serious risk to human health. Removal of these contaminants required high energy consumption and complex engineering processes. However, outcomes are not satisfactory which can cause changes in characteristics and texture of soil (Meng, Qiao, and Arp 2011). Many methods have been used for soil reclamation such as extraction of contaminant vapors, bioventing, slurry reactors and thermal reclamation. Whereas phytoremediation is best method for soil reclamation than other engineering techniques because it is cost effective and have least adverse effect on environment (Rostami and Azhdarpoor 2019).

PAHs in soil are commonly found in many regions in the world. Lower molecular weights PAHs such as anthracene and phenanthrene and higher molecular weights PAHs such as pyrene causes carcinogenicity and teratogenicity (Sarwar *et al*. 2017).

For soil reclamation by phytoremediation often plants with Poaceae, Cucurbitaceae, Leguminosae and Fabaceae families is used. In Thailand Butterfly Peas and Yam beans belongs to Leguminosae family shows stimulating anthracene and pyrene removal from soil. For example, the percentage of fluorene and anthracene degradation in 30 days of pot experiment with initial concentration of 138.9mg/kg and 95.9mg/kg were ranged from 7.8% and 24.2% respectively. Removal of fluoranthene concentration in soil planted with Long bean was 10.2% with initial concentration of 150mg/kg. In contrast amount of fluoranthene in soil without Long bean was 98.4% (Somtrakoon, Chouychai, and Lee 2018).

Biodegradation of polycyclic aromatic hydrocarbons (PAH) using endophytic bacteria is associated with rhizosphere effect of plants. Two infected and non-infected grass using endophytic fungi was investigated for degradation of petroleum hydrocarbons in contaminated soil. Results indicates that grass infected with endophytic fungi efficiently remove PAH and TPH in rhizosphere of plants 80-84% respectively (Soleimani *et al*. 2010). Arbuscular mycorrhizal fungi (AMF) have excessive potential for hydrocarbon degradation in contaminated soil. AMF assisted phytoremediation should have a suitable host plant and inoculation of plants with indigenous fungal stains is suggested for better effect of phytoremediation plans (Rajtor and Piotrowska-Seget 2016).

In soil microbial degradation of PAHs may be done by non-specific radical oxidation, mineralization and co-metabolic degradation (Smith *et al*. 2011). Microbial assisted phytoremediation is one of the most effective method in which plants and their assisted rhizosphere microbes degrade PAHs in soil (Sun *et al*. 2011). Alfalfa rhizobium symbiotic association will significantly degrade PAHs concentration in soil by 37.2% and 51.4% respectively which shows that pant-microbe partnership may be suitable for phytoremediation of PAHs contaminated soil (Teng *et al*. 2011). The inoculation of arbuscular mycorrhizal fungi and aromatic hydrocarbon degrading bacteria (ARDB) rapidly increase degradation rate of aged PAHs from soil which improves plant growth and reduce toxic effect to the plants (Zhang *et al*. 2010).

Plants of poaceae family has been reported for remediation of soil. For example, the removal of anthracene from contaminated soil is done by enhanced microbial activity in soil. Plant species belongs to poaceae family such as Lolium perenne was used for promoting growth of bacteria (bacillus aerophilus) for period of 20 and 45 days. This shows that disappearance of anthracene is due to increase in microbial activity of inoculated soil community as compared to uninoculated soils (Yarahmadi *et al*. 2017). In present study, Lolium perenne plant were selected to investigate the growth of plant species belongs to poaceae family and its potential for phytoremediation of anthracene and pyrene contaminated soils.

Materials and Methods

Soil collection and Analysis

Soil sample was collected from, Lahore district, Punjab province, Pakistan. The soil sample was collected very carefully to avoid roads, industries or any other activity which may harm or contaminate soil. It was also ensured that soil samples have no previous anthracene contaminated history. The soil was spread on large plastic sheet and dried at room temperature (29-30 °C) for 48 hours. After 48 hours weight is constant and then physiochemical analysis of soil was done for investigation of phytoremediation.

Table1. =Physiochemical properties of soil sample

Texture	Sand (%)	Silt (%)	Clay (%)	pH	OC (%)	EC (dS/m)	CEC (%)	$CaCO_3$ (%)	Ava-P(ppm)	Ava-K(ppm)
Silt Loam	49	29	22	8.25	0.94	1.5	11	3.21	6.80	140

Seedling Preparation

Perennial ryegrass (Lolium perenne L.) seeds were rinse and absorbed in sterile distilled water for at least 2 hr. Seeds were prepared as discussed by (Somtrakoon, Chouychai, and Lee 2015). Seeds were sprouted in moist spiked soil which containing anthracene in experimental posts maintaining room temperature (29-30 °C) and ensure that it will receive proper sunlight. The date of seedling at time of experiment was considered zero day. After ten days of seedling healthy plant seeds were picked up with comparable size and transplanted in pots.

Experimental Design

Experiment was performed in a plant nursery. The tested soil 2kg of dry weight spiked with anthracene was planted individually in 10 cm diameter pots with a ten-day old seedling. Almost 10 seeds of Lolium perenne were sowed in each pot which is covered within the thin layer of soil. At bottom of the pots a disk of filter paper was placed to avoid escaping of soil from small drainage holes. An aliquot of 20% of soil is mixed with anthracene (Fluka 98%) and pyrene (purity 98%) was dissolved in dichloromethane and poured into 500g of soil which will further mixed into 1.5kg of remaining soil. The soil was mixed completely at room temperature(29-30°C) until dichloromethane will be evaporated.

The initial concentration of anthracene spiked soil was 81.6 ± 41.7 and 71.3 ± 14.8 mg/kg. Then the spiked soil samples were transferred to pots. Three replicates of the soil samples were prepared in a completely randomized design. Following are the details of spiked and without spiked soil samples.

- Soil spiked with anthracene + pyrene and planted with perennial ryegrass (Lolium perenne L.) to investigate polluted soil and accumulation of pollutants in plant biomass.
- Soil spiked with anthracene + pyrene without plant to examine the ability of soil microorganism in removal of soil contaminants.
- Soil without anthracene and pyrene planted with Lolium perenne to inspect control for plant growth in non-contaminated soil.

The experiment was performed for 45 days by selecting two sampling periods for the investigation of anthracene and pyrene removal in soil. On first 20 days 1g of rhizospheric and bulk soil from planted pot soil were collected and then analyzed by gas chromatography-mass spectrometry (GC-MS) for investigation of contaminated PAH soil. It was predicted that anthracene and pyrene is not completely removed in first period. Whereas some amount of anthracene and pyrene was removed in planted soil than non-planted soil. Later on, after 45 days maximum amount of anthracene and pyrene removal in planted soil was expected than in unplanted soil.

PAH Analysis

The soil samples analysis was subjected to Soxhlet extraction as discussed by (Somtrakoon, Chouychai, and Lee 2014). Soil samples for determination of anthracene and pyrene concentration were analyzed through GC-MS. whereas the concentration of anthracene and pyrene in shoot and root of the plant were analyzed in similar way.

Statistical Analysis

All the values presented for soil analysis were expressed in term of ±SD. By using one way ANOVA means were compared using least significant differences accepted at P < 0.05. Multiple numbers of comparisons between treatments were performed using LSD test.

Results and Discussions

General morphology of plant in anthracene and pyrene contaminated soil

In unspiked soil the Lolium perenne grew up in 20 days of transplantation. Whereas the soil spiked with anthracene and pyrene the plant not grew up well in first period. Ability of plants varies with plant species either it will grow up in anthracene and pyrene (PAH) contaminated soil. In controlled unspiked soil perennial ryegrass did not shows any symptoms of stress during experimentation. In first period of experimentation the soil spiked with anthracene and pyrene the plant grew up but nodules formation was decreased. At 20 days perennial ryegrass (Lolium perenne) did not produce any nodules in spiked soil. However, on 45 day of experiment nodules were observed in plants spiked with anthracene and pyrene. It was observed that the soil spiked with anthracene and pyrene have toxic effect on plant growth and produce changes in the stem, root length, biomass and dry matter as well as course of proper leaf and nodule formation on first 45 days.

Effect of anthracene and pyrene on shoot and root growth of plant

The Lolium perenne with anthracene and pyrene spiked soil did not shows any adverse effect on shoots, compared to shoot length, shoot fresh and dry weight over day 45. In anthracene and pyrene spiked soil the shoot length, shoot fresh weight, shoot dry weight, of perennial ryegrass was similar to controlled plant which is grown in unspiked soil. The shoot length(cm), shoot fresh weight(g), and shoot dry weight(g) of both spiked and unspiked soils at day 20 was only 42.6-68.7 cm, 0.7-1.1g, 0.9-0.11g respectively.

In comparison, the shoot length of spiked soil at day 45 was 75.2 cm than that of controlled unspiked soil which was 85.1 cm. This shows that there is significantly decrease in shoot fresh weight and shoot dry weight of plant grown in.

Table 2. Shoot length, shoot fresh weight, shoot dry weight of Lolium perenne in anthracene and pyrene contaminated and non-contaminated soils

Perennial ryegrass (Lolium perenne L.)			
Soil	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Day 20			
Unspiked control	68.7 ± 12.2a	1.1 ± 0.1a	0.11 ± 0.01a
Anthracene + pyrene spiked	42.6 ± 21.8a	0.7 ± 0.2a	0.9 ± 0.02a
Day 45			
Unspiked control	85.1 ± 24.2a	2.5 ± 0.78a*	0.53 ± 0.21a*
Anthracene + pyrene spiked	75.2 ± 24.1a*	2.0 ± 1.11a*	0.39 ± 0.22a*

Note: Means followed by different letters show significant difference (P<0.05) according to LSD test. *Denotes significant difference between values for different days (P<0.05).

Table 3. Root length, Root fresh weight, Root dry weight of Lolium perenne in anthracene and pyrene contaminated and non-contaminated soils

Perennial ryegrass (Lolium perenne L.)			
Soil	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Day 20			

Unspiked control	8.1±5.3a	0.20 ± 0.10a	0.031 ± 0.005a
Anthracene + pyrene spiked	10.0 ± 3.7a	0.21 ± 0.11a	0.027 ± 0.016a
Day 45			
Unspiked control	26.0 ± 12.2a	1.02 ± 0.37a*	0.07 ± 0.03a*
Anthracene + pyrene spiked	20.1.2 ± 5.3a	1.21 ± 0.31a*	0.11 ± 0.03a*

Note: Means followed by different letters show significant difference (P<0.05) according to LSD test. *Denotes significant difference between values for different days (P<0.05).

The root of plant did not affect in presence of anthracene and pyrene on the root length, root fresh weight, root dry weight as compared to controlled plants grown in unspiked soil over 45 days as shown in Table 3. In contrast this is seen that the root length has significant effect in anthracene and pyrene contaminated soil on day 45 than that of controlled unspiked soil. Whereas root fresh weight and root dry weight of spiked and unspiked soil was around 1.21-1.02 g and 0.11-0.03g respectively, which shows that based on root growth characteristics perennial ryegrass (Lolium perenne L.) seemed to be tolerant in anthracene and pyrene contaminated soils.

Anthracene and pyrene in plant tissues

On the day 45 anthracene and pyrene were not detected in shoot and root of the plant. The detection limit was 2mg/kg. Probably anthracene and pyrene were removed from soil due to increase in microbial population. Sometimes plants and bacterial influence depending on soil environmental conditions may play major role on degradation of contaminants.

Therefore, soil indigenous microorganism is also important in decontamination of soils.

Anthracene and pyrene removal from soil by Lolium perenne

Planting of Lolium perenne in anthracene and pyrene spiked soil showed significant difference between studied treatments as shown in Table 4. Results shows that Lolium perenne have ability for significant removal of anthracene or pyrene from soil.

On day 20 amount of anthracene and pyrene in controlled unplanted soil which is spiked with anthracene and pyrene were 62.1 and 59.2 mg/kg, respectively. In contrast the amount of anthracene and pyrene on day 0 were 81.6 and 71.3 mg/kg which shows that there was rapidremoval rate of contaminants by indigenous soil microorganisms. Also, according to results there is more effective removal of both anthracene and pyrene from soil by Lolium perenne which is reduced to near half of initial concentration as 42.4 and 45.9 mg/kg on day 20. By day 45 amount of anthracene and pyrene remaining in unplanted soil were 21.7 and 22.4 mg/kg respectively. Lolium perenne could remove anthracene and pyrene completely from soil when the plant and its root system were fully developed on day 45. The rapid decrease in the concentrations of anthracene and pyrene from soil were observed which shows that Lolium perenne is more tolerant for decontamination of soils even with 81.6 mg/kg of anthracene and 71.3 mg/kg of pyrene in soil.

Table 4. Percentages of remaining anthracene and pyrene concentrations in soil planted with Lolium perenne

Amount of anthracene and pyrene (mg/kg)		
Treatments	**Anthracene**	**Pyrene**
Day 0	81.6 ± 41.7a	71.3 ± 14.8a
Day 20		
Control (Unplanted, Spiked with anthracene and pyrene)	62.1 ± 15.3a	59.2 ± 10.1a
Planted soil spiked with anthracene and pyrene	42.4 ± 26.2c	45.9 ± 28.6b
Day 45		
Control (Unplanted, Spiked with anthracene and pyrene)	21.7 ± 7.1c	22.4 ± 3.4b

Planted soil spiked with anthracene and pyrene	B.D.	B.D.

Note: Means followed by different letters show significant difference (P<0.05) between treatments according to LSD test. Abbreviation: B.D. represents below detection limit which is 2mg/kg.

shown in Tables 4 the enhanced removal of anthracene and pyrene from soil by Lolium perenne is observed.

In unspiked soil microorganism were active in removal of anthracene and pyrene with in suitable environment. Rhizosphere provide a suitable environment to soil microorganism and enhanced their ability to degrade anthracene and pyrene in planted soil. Lolium perenne is considered as an appropriate candidate for phytoremediation of the recalcitrant pollutants in soil. Increase in microbial community with the assistance of Bacillus aerophilus which is considered as growth-promoting bacteria shows enhanced dissipation for the Lolium perenne species which was 77.0-156.5 ppm at 180 ppm initial anthracene concentration (Yarahmadi *et al*. 2017).

Winged beans were considered as most effective plant for phytoremediation of soil because it enhanced removal of anthracene and fluorene concentrations in rhizospheric soil which were 7.8% and 24.2% respectively (Somtrakoon *et al*. 2014). Root exudates from alfalfa shows enhanced dissipation of anthracene by 25-80% as compared to unplanted soil controls (Kim *et al*. 2004). The soil planted with sorghum bicolor have higher removal efficiency of pyrene by Pseudomonas aeruginosa which was 52-92% than in unplanted soil treatments (Rostami *et al*. 2016),(Gałazka and Gałazka 2015). Fire Phoenix and Medicago sativa Linn were considered for the effective removal of PAH in contaminated soil (Liu, Dai, and Sun 2015). Fire Phoenix and Medicago sativa Linn also have ability to improve physiochemical properties of soil and removal rates for these two plants in case of pyrene was 93.27% and 79.53% respectively (Xiao *et al*. 2015).L. octovalvis and rhizobacteria have ability to degrade hydrocarbons in soil.

Living microorganism in soils have capability to degrade organic pollutants in gasoline contaminated soil (Al-Mansoory *et al*. 2017). The result of this study shows the potential of using Lolium perenne for phytoremediation of contaminated soil. In future studies this plant would be useful for the assessment of other pollutant tolerant plants in removal of PAHs from contaminated soil.

Conclusion

This study clearly demonstrates that Lolium perenne have ability to be used for phytoremediation of anthracene and pyrene in contaminated soil. Phytostimulation is most precise mechanism in anthracene and pyrene removal from contaminated soil. It remains to be studied that the ability of Lolium perenne in degradation of anthracene and pyrene from contaminated soil can be extended to other PAHs.

References

1. Mansoory, A., Asia. F., Mushrifah, I., Rozaimah, S.A.S. and Nurina, A. "Phytoremediation of Contaminated Soils Containing Gasoline Using Ludwigia Octovalvis (Jacq.) in Greenhouse Pots." Environmental Science and Pollution Research, 24.13 (2017): 11998–12008.
2. Bell, T.H., Simon, J., Frédéric, E.P. and Etienne, Y. "Increasing Phytoremediation Efficiency and Reliability Using Novel Omics Approaches." Trends in Biotechnology, 32. 5 (2014): 271–280.
3. Antonio, C., Gea, O.C., Eun, H.J., Pietro, Z., Alfina, G., Chiara, C. and Margherita, F. "Phytoremediation of Contaminated Soils by Heavy Metals and PAHs. A Brief Review." Environmental Technology and Innovation,8. (2017): 8:309–326.
4. Anna, G. and Rafał, G. "Phytoremediation of Polycyclic Aromatic Hydrocarbons in Soils Artificially Polluted Using Plant-Associated-Endophytic Bacteria and Dactylis Glomerata as the Bioremediation Plant." Polish Journal of Microbiology, 64.3 (2015):239–250.

5. Bum, K.Y., Young, P. K., Yong, C., Keun, C. O. and Bob, B. Bu. "Phytoremediation of Anthracene Contaminated Soils by Different Plant Species." Journal of Plant Biology, 47. 3 (2004):174–178.
6. Rui, L., Yuanyuan, D. and Libo, S. "Effect of Rhizosphere Enzymes on Phytoremediation in PAH-Contaminated Soil Using Five Plant Species." PLoS ONE, 10. 3 (2015): 1–14.
7. Liang, M., Min, Q. and Hans, P.H. "Phytoremediation Efficiency of a PAH-Contaminated Industrial Soil Using Ryegrass, White Clover, and Celery as Mono- and Mixed Cultures." Journal of Soils and Sediments, 11. 3 (2011): 482–490.
8. Rajtor, Monika, and Zofia Piotrowska-Seget. 2016. "Prospects for Arbuscular Mycorrhizal Fungi (AMF) to Assist in Phytoremediation of Soil Hydrocarbon Contaminants." Chemosphere 162:105–16. doi: 10.1016/j.chemosphere.2016.07.071.
9. Saeid, R. and Abooalfazl, A. "The Application of Plant Growth Regulators to Improve Phytoremediation of Contaminated Soils: A Review." *Chemosphere 220*, 2019: 818–827
10. saeid, R. and Abooalfazl, A., Majid, R. and Reza, S.M. "The Effects of Simultaneous Application of Plant Growth Regulators and Bioaugmentation on Improvement of Phytoremediation of Pyrene Contaminated Soils." *Chemosphere161*, 2016: 219–223.
11. Nadeem, S. Imran, M., Rashid, S. M., Wajid, I., Asif , K.M., Amar, M., Rehim, A. and Saddam, H. "Phytoremediation Strategies for Soils Contaminated with Heavy Metals: Modifications and Future Perspectives." *Chemosphere 171*, 2017:710–721.
12. Singh, O. V. and Jain, R. K. "Phytoremediation of Toxic Aromatic Pollutants from Soil." *Applied Microbiology and Biotechnology*, 63.2 (2003):128–135.
13. Smith, M. J., Flowers, T. H. Duncan, H. J. and Saito, H. "Study of PAH Dissipation and Phytoremediation in Soils: Comparing Freshly Spiked with Weathered Soil from a Former Coking Works." *Journal of Hazardous Materials*, 192.3 (2011): 1219–1225.
14. Mohsen, S., Majid, A., Afyuni, M., Hajabbasi, M. A., Nourbakhsh, F., Sabzalian, M. R. and Christensen, J. H. "Phytoremediation of an Aged Petroleum Contaminated Soil Using Endophyte Infected and Non-Infected Grasses." *Chemosphere*, 81.9 (2010):1084–1090.
15. Khanitta, S.,Waraporn, C. and Hung, L. "Comparing Anthracene and Fluorene Degradation in Anthracene and Fluorene-Contaminated Soil by Single and Mixed Plant Cultivation." International Journal of Phytoremediation, 16. 4 (2014):415–428.
16. Khanitta, S.,Waraporn, C. and Hung, L. "Removal of Anthracene and Fluoranthene by Waxy Corn, Long Bean and Okra in Lead-Contaminated Soil." Bulletin of Environmental Contamination and Toxicology, 95.3 (2015):407–413.
17. Khanitta, S.,Waraporn, C. and Hung, L. "Potential of Butterfly Pea (Clitoria Ternatea) and Yam Bean (Pachyrhizus Erosus) Plants for Phytoremediation of Anthracene- and Pyrene-Contaminated Soil." Songklanakarin Journal of Science and Technology, 40.3 (2018):725–731.
18. Mingming, S., Dengqiang, F., Ying, T., Yuanyuan, S., Yongming, L., Zhengao, L. and Peter, C. "In Situ Phytoremediation of PAH-Contaminated Soil by Intercropping Alfalfa (Medicago Sativa L.) With Tall Fescue (Festuca Arundinacea Schreb.) and Associated Soil Microbial Activity." Journal of Soils and Sediments, 11.6(2011):980–989.
19. Teng, Y., Yuanyuan, S., Yongming, L., Xianghui, S., Mingming, S., Dengqiang, F., Zhengao, L., and Peter, C. "Influence of Rhizobium Meliloti on Phytoremediation of Polycyclic Aromatic Hydrocarbons by Alfalfa in an Aged Contaminated Soil." *Journal of Hazardous Materials 186*, 2011, (2-3), 1271-1276.
20. Teofilo, V., Marianna, B. and Giuliano, M. "Field Crops for Phytoremediation of Metal-Contaminated Land. A Review." *Environmental Chemistry Letters*, 8.1 (2010):1–17.

21. Nan, X., Rui, L., Caixia, J. and Yuanyuan, D. “Efficiency of Five Ornamental Plant Species in the Phytoremediation of Polycyclic Aromatic Hydrocarbon (PAH)-Contaminated Soil.” *Ecological Engineering*, 75 (2015):384–391.
22. Zahra, Y., Jila, B., Reza, S., Yousef, M. A. and Nasrin, S.K. “The Efficiency of Lolium Perenne for Phytoremediation of Anthracene in Polluted Soils in the Presence of Bacillus Aerophilus.” *Petroleum Science and Technology*, 35.7 (2017):647–652.
23. Jing, Z., Rui, Y., Xiangui, L., Weiwei, L., Ruirui, C. and Xuanzhen, L. “Interactive Effect of Biosurfactant and Microorganism to Enhance Phytoremediation for Removal of Aged Polycyclic Aromatic Hydrocarbons from Contaminated Soils.” *Journal of Health Science*, 56 (2010):257–266.

Source of support: Nil; **Conflict of interest:** Nil.

Cite this article as:

Madni, A. M., Jahanzaib, S.R, Naeem, M., Usama, N. H., Ibrahim, M. A., Tariq. A., Ijaz-ul, H.S., Safdar, A. and Rizwan, M. “Phytoremediation of Anthracene and Pyrene Contaminated Soil by Lolium Perenne.” Annals of plant sciences, 10.11 (2021) pp. 4294-4302.

DOI: http://dx.doi.org/10.21746/aps.2021.10.11.1