Effect of single and mixed polycyclic aromatic hydrocarbon contamination on plant biomass yield and PAH dissipation during phytoremediation
Afegbua, Seniyat; Batty, Lesley

DOI:
10.1007/s11356-018-1987-1

License:
Creative Commons: Attribution (CC BY)

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):
Afegbua, S & Batty, L 2018, 'Effect of single and mixed polycyclic aromatic hydrocarbon contamination on plant biomass yield and PAH dissipation during phytoremediation', Environmental Science and Pollution Research. https://doi.org/10.1007/s11356-018-1987-1

Link to publication on Research at Birmingham portal

Publisher Rights Statement:
Checked for eligibility: 17/05/2018
https://doi.org/10.1007/s11356-018-1987-1

General rights
Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

• Users may freely distribute the URL that is used to identify this publication.
• Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
• Users may use extracts from the document in line with the concept of ‘fair dealing’ under the Copyright, Designs and Patents Act 1988 (?)
• Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy
While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Download date: 10. Mar. 2020
Effect of single and mixed polycyclic aromatic hydrocarbon contamination on plant biomass yield and PAH dissipation during phytoremediation

Seniyat Larai Afegbua\textsuperscript{1} \& Lesley Claire Batty\textsuperscript{1}

Received: 13 September 2017 / Accepted: 9 April 2018 © The Author(s) 2018

Abstract
Polycyclic aromatic hydrocarbon (PAH)-contaminated sites have a mixture of PAH of varying concentration which may affect PAH dissipation differently to contamination with a single PAH. In this study, pot experiments investigated the impact of PAH contamination on Medicago sativa, Lolium perenne, and Festuca arundinacea biomass and PAH dissipation from soils spiked with phenanthrene (Phe), fluoranthene (Flu), and benzo[\textalpha]pyrene (B[\textalpha]P) in single and mixed treatments. Stimulatory or inhibitory effects of PAH contamination on plant biomass yields were not different for the single and mixed PAH treatments. Results showed significant effect of PAH treatments on plant growth with an increased root biomass yield for F. arundinacea in the Phe (175\%) and Flu (86\%) treatments and a root biomass decrease in the mixed treatment (4\%). The mean residual PAHs in the planted treatments and unplanted control for the single treatments were not significantly different. B[\textalpha]P dissipation was enhanced for single and mixed treatments (71–72\%) with F. arundinacea compared to the unplanted control (24–50\%). On the other hand, B[\textalpha]P dissipation was inhibited with L. perenne (6\%) in the single treatment and M. sativa (11\%) and L. perenne (29\%) in the mixed treatment. Abiotic processes had greater contribution to PAH dissipation compared to rhizodegradation in both treatments. In most cases, a stimulatory effect of PAH contamination on plant biomass yield without an enhancement of PAH dissipation was observed. Plant species among other factors affect the relative contribution of PAH dissipation mechanisms during phytoremediation. These factors determine the effectiveness and suitability of phytoremediation as a remedial strategy for PAH-contaminated sites. Further studies on impact of PAH contamination, plant selection, and rhizosphere activities on soil microbial community structure and remediation outcome are required.

Keywords Phytoremediation \cdot Phenanthrene \cdot Fluoranthene \cdot Benzo[\textalpha]pyrene \cdot Medicago sativa \cdot Lolium perenne \cdot Festuca arundinacea \cdot Inhibition

Introduction
Recently, there has been a marked increase in research on phytoremediation as a promising eco-friendly remediation technology. This has been driven by reports of enhanced biodegradation of organic compounds including PAH in the presence of plants compared to unplanted soils (Siciliano et al. 2003; Xu et al. 2006; Olson et al. 2007; Vangronsveld et al. 2009; Wu et al. 2011). An enhanced dissipation in vegetated soils is attributed to rhizospheric effect through root exudation providing benefit such as improved soil condition, bioavailability, and stimulation of microbial activity (Kirk et al. 2005; Kaimi et al. 2006; Cheema et al. 2010; Hamdi et al. 2012). Apart from microbial degradation and rhizodegradation, abiotic processes such as volatilization, leaching, and adsorption to soil fractions may contribute to PAH loss (Kaimi et al. 2006).
PAH concentration in contaminated soils and plant tolerance level may influence plant biomass yield and PAH degradation as a result of the impact on seed germination, plant establishment, and growth (Smith et al. 2006; Lee et al. 2008; Gan et al. 2009). Interestingly, there are conflicting reports on the phytoremediation outcome (enhancement or inhibition) as a few studies have shown that presence of plants may not necessarily enhance PAH dissipation (Sun et al. 2010; Smith et al. 2011). Further, there are few studies on the contribution of different dissipation mechanisms during phytoremediation (Sun et al. 2010; Smith et al. 2011). Many phytoremediation studies have shifted towards mixed contamination remediation to reflect real site remediation scenarios as early studies were mainly on single contaminant remediation (Gan et al. 2009).

The aim of this study was to assess the impact of single and mixed PAH treatments on plant biomass and PAH dissipation and the contribution of abiotic processes and rhizodegradation to PAH dissipation during a greenhouse experiment. Soils were spiked with phenanthrene, fluoranthene, and benzo[a]pyrene in single and mixed treatments. Medicago sativa, Lolium perenne, and Festuca arundinacea were selected for this study based on their rhizodegradation potential attributed to their root structure and stress tolerance level in previous studies (Kaimi et al. 2006; Cheema et al. 2010; Lu et al. 2011). The following hypotheses were made; single PAH and mixed PAH treatments will affect biomass yields and PAH dissipation for selected plants but greater impacts would be observed in the mixed PAH treatment. Following the greenhouse experiments, mean residual PAH concentration of the different treatments will differ between vegetated soils and non-vegetated soils. PAH loss would be attributed to different dissipation pathways (abiotic processes and rhizodegradation).

Materials and methods

Chemicals

Phenanthrene (> 98% purity), fluoranthene (> 98% purity), and benzo[a]pyrene (> 96% purity) were obtained from VWR, UK. Internal standard mix (acenaphthene-d10, chrysene-d12, 1,4-dichlorobenzene-d4, naphthalene-d8, perylene-d12, and phenanthrene-d10), p-terphenyl-d14, and New Jersey Department of Environmental Protection (NJDEP) extractable petroleum hydrocarbon aromatics calibration standard 10/08 Rev.2 (2000 μg mL⁻¹ each ofacenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, 2-methyl-naphthalene, naphthalene, phenanthrene, pyrene, and 1,2,3-trimethylbenzene in methylene chloride) of chromatographic grade were purchased from Restek, UK. Other chemicals used were of analytical purity.

Soil preparation and experimental design

Sandy loam soil (pH 7.5, organic matter 6.19%, conductivity 1450 μS, moisture content 0.80%) sourced from a commercial supplier (Travis Perkins, UK) was air-dried and sieved (< 2 mm) prior to spiking with phenanthrene, fluoranthene, and benzo[a]pyrene for single and mixed PAH treatments in triplicate. The single PAH treatment was prepared by spiking 250 g of soil with phenanthrene (300 mg), fluoranthene (200 mg), and benzo[a]pyrene (5 mg) dissolved in 20 mL of acetone. Mixed PAH treatment used 250 g of soil spiked with all three compounds (phenanthrene, 300 mg; fluoranthene, 200 mg; benzo[a]pyrene, 5 mg) dissolved in 20 mL of acetone. The spiked soils were mixed and air-dried in a fume hood for 3 days before mixing with 750 g of unspiked soil and sieved through a 2-mm mesh and mixed thoroughly to achieve homogeneity (Cheema et al. 2010). The spiked soils were stored in the dark at room temperature for 4 weeks for equilibration before the pot experiment. The PAH spiked soils and control soils (without PAH) were dispensed into Desch plant plastic pot (diameter, 14 cm; depth, 12.4 cm; and capacity, 1.3 l) with 1 kg dry weight soil pot⁻¹. This was followed by the transplantation of 4-week old seedlings of Medicago sativa, Festuca arundinacea, and Lolium perenne in perlite. Plants were grown in a controlled environment growth chamber for 65 days (16 h, 25 °C day: 8 h, 20 °C night). Pots were watered as required and excess water collected in saucers. Fertilizer was not applied during the experiment. Abiotic controls were set up in triplicate using unplanted spiked soil with formalin (30 mL) added weekly to inhibit microbial growth (Sun et al. 2010). Abiotic controls assessed contribution made by abiotic processes while unplanted spiked controls without formalin were also set up in triplicate to assess contribution of abiotic processes as well as microbial degradation to PAH dissipation. The plant seedlings were thinned after 2 weeks to 20 seedlings per pot. Soil samples were collected before and after the 65-day greenhouse experiment for initial and final PAH concentration following thorough mixing to ensure homogeneity. For the initial PAH concentration, six soil samples were collected from the spiked soils of each treatment group. For the final soil PAH concentration, plants were harvested and shaken to remove loosely adhering soils. Rhizosphere soil samples were taken and stored at 4 °C prior to analyses.

Plant biomass

Following the harvest, plant roots were washed gently with water and then with deionized water to remove rhizosphere soil and the excess water blotted off roots with clean dry tissue paper. The
plant materials were oven-dried to a constant weight at 65 °C for 48 h and weighed using a weighing balance (Mettler, UK) for biomass calculation (Chigbo et al. 2013).

PAH analysis

Microwave extraction and solid phase extraction

Sodium sulfate (7 g) was added to soil samples (5 g) to remove any moisture and followed by addition of 15 mL of 2:1 hexane:acetone mixture, 5 mL of 1:4 triethylamine:acetone mixture, and p-terphenyl-d14 in a microwave extraction tube (Chigbo et al. 2013). The content of the tube was mixed using a vortex mixer (VWR, UK) and shaken by inversion to dislodge soil material from the base. Extraction was carried out with a microwave extraction unit (CEM MARS) with the following conditions: temperature ramp to 100 °C at 800 W for 12 min, hold at 100 °C at 800 W for 10 min then cool for 5 min in accordance with the USEPA method 3546 (USEPA 2007). Following the extraction, the clear extracts were transferred into glass tubes (20 mL). For the solid phase extraction, SPE HF Mega BE-SI 2 g 12 mL cartridges (Agilent, UK) were conditioned with 5 mL of hexane then, 1 mL of sample extract was added and eluted with 10 mL of 1:1 hexane:dichloromethane mixture. The eluant was collected in a clean 20 mL glass tube and concentrated to a final volume of 1 mL under a gentle stream of nitrogen gas. Samples were prepared in 2 mL vials (Agilent, UK) by adding a semi-volatile internal standard mix to the concentrated sample extracts from soil samples for GC-MS analysis.

GC-MS analysis

PAH analysis was performed with an Agilent gas chromatograph-mass selective detector (Agilent Technologies 6890N Network GC System) with HP 5MS fused silica capillary column of dimensions 30 m × 0.25 mm i.d. × 0.25 μm film thickness (Agilent, UK). The GC-MS was operated in selective ion mode using operating conditions for USEPA method 8270D with helium as a carrier gas at a constant flow rate of 30 cm s⁻¹ (USEPA 2014). PAH quantification was achieved in comparison with a standard curve for aromatics calibration standard and internal standard mixture (1, 4-dichlorobenzene-d4, naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12) while p-terphenyl-d14 was used as the surrogate standard. The calibration points were 50, 100, 500, 1000, 2000, 5000, and 10,000 pg μL⁻¹. Quality controls were set up with solvent blanks and matrix spikes. Percentage recovery for surrogate standard p-terphenyl was 46.04–93.3%.

Data analysis

Plant biomass and soil analyses data are presented as mean and standard error of replicate samples. Statistical analyses were carried out on the plant biomass and residual PAH concentration data using ANOVA followed by a Tukey Honest Significant Difference (HSD) post hoc test at a significance level of 0.05 on Statistical Package for Social Science (SPSS) (version 20.0 for Windows). The percentage PAH dissipation in each treatment equals total concentration of PAH dissipated divided by initial PAH concentration expressed in percentage. The proportion of overall dissipation attributable to plants and microbes (rhizodegradation) is calculated as difference between total dissipation from planted experiment and abiotic control expressed as a percentage of initial concentration.

Results

Plant biomass yield

*M. sativa* shoot biomass yield increase was 190, 180, 190, and 110% for Phe, Flu, B[a]P, and mixed PAH treatments respectively compared to the control. *M. sativa* root biomass yield increase relative to the control was 240, 160, 80, and 40% for the Phe, Flu, B[a]P, and mixed PAH treatments respectively. Root biomass yield decrease was observed for *L. perenne* with 5, 6, and 8% for Phe, Flu, and mixed PAH treatments respectively while a decrease (0.7%) was observed for the mixed PAH treatment. The effect of the single and mixed PAH treatments on the root and shoot biomass of *M. sativa* and *L. perenne* compared to the control plants was not significant (*p* > 0.05). One-way ANOVA showed that the effect of PAH treatments on *F. arundinacea* shoot biomass was not significant (*p* > 0.05) while that for *F. arundinacea*, root biomass (*p* < 0.01) was significant (Fig. 1). There was a decrease in *F. arundinacea* shoot biomass yield by 7 and 12% for Phe and PAH mixed treatments respectively while an increase in shoot biomass yield by 7 and 2% was observed for Phe and B[a]P treatments respectively. As for *F. arundinacea* root biomass, 170, 86, and 45% yield increase was observed in the Phe, Flu, and B[a]P treatments respectively compared to the control. A root biomass decrease of 4% was seen in *F. arundinacea* root after the mixed PAH treatment. Post hoc test revealed that the root biomass for the Phe and Flu treatments was not different from each other but different from those of the mixed PAH and control without PAH (Fig. 1). The shoot/root
Dry weight ratios for *M. sativa* were 1.57, 1.30, 1.68, 2.63, and 2.33 in the control, Phe, Flu, B[a]P, and mixed PAH treatments respectively. The shoot/root dry weight ratios in the control, Phe, Flu, B[a]P, and mixed PAH treatments were 0.63, 0.19, 0.47, 0.50, and 0.58 for *L. perenne* and 0.97, 0.33, 0.56, 0.68, and 0.89 for *F. arundinacea* respectively.

**PAH dissipation**

The initial PAH concentrations in the single PAH treatments were phenanthrene 222 ± 40.6 mg kg⁻¹, fluoranthene 104 ± 18.6 mg kg⁻¹, and benzo[a]pyrene 2.08 ± 0.208 mg kg⁻¹ and those for the mixed PAH treatment were phenanthrene 254 ± 42.2 mg kg⁻¹, fluoranthene 153 ± 17.7 mg kg⁻¹, and benzo[a]pyrene 2.65 ± 0.560 mg kg⁻¹ (mean ± SE, number of replicate = 6). At end of the greenhouse experiment, there was a decrease in PAH concentration in the single and mixed PAH treatments of the planted soils and unplanted controls. The PAH loss was greater in planted soils compared to unplanted controls for benzo[a]pyrene while phenanthrene and fluoranthene dissipation in planted soils was slightly greater or equal to those of the unplanted controls. PAH loss in the treatments varied between compounds for *M. sativa, L. perenne*, and *F. arundinacea* after the phytoremediation experiment.

**PAH dissipation in single PAH treatments**

Phenanthrene and fluoranthene dissipation was comparable in the single treatments with *F. arundinacea* and *L. perenne* and exceeded those of the controls (both abiotic and unplanted controls). It was observed that those of *M. sativa* were statistically different to the abiotic control but similar to the unplanted control (Table 1). The Tukey test revealed that the residual concentration of phenanthrene across the plants was different from that of the abiotic control (*p* = 0.05). Abiotic processes and rhizodegradation contributed to 68% and 30–31% of the total phenanthrene dissipation respectively (Table 1). For fluoranthene dissipation, abiotic processes and rhizodegradation accounted for 41% and 52–58% of the overall loss respectively (Table 1). For the benzo[a]pyrene treatment, the outcome was different, and the residual B[a]P concentrations for *L. perenne* (1.92 ± 0.434 mg kg⁻¹) and *F. arundinacea* (0.579 ± 0.123 mg kg⁻¹)
were significantly different from each other. Abiotic processes and rhizodegradation accounted 14% and −6–58% of the total benzo[a]pyrene dissipation respectively.

**Table 1** Phenanthrene, fluoranthene, and benzo[a]pyrene dissipation from single and mixed PAH treatments with *M. sativa*, *F. arundinacea*, and *L. perenne*. (Average values ± SE, n = 3). Different letters in each group indicate a significant difference at p < 0.05 according to Tukey’s HSD test. *Asterisked (negative) values represent percentage inhibition. Initial PAH concentration of single PAH treatments: phenanthrene (222 ± 40.6 mg kg⁻¹), fluoranthene (104 ± 18.6 mg kg⁻¹), benzo[a]pyrene (2.08 ± 0.208 mg kg⁻¹). Initial PAH concentration of mixed PAH treatment: phenanthrene (254 ± 42.2 mg kg⁻¹), fluoranthene (153 ± 17.7 mg kg⁻¹), benzo[a]pyrene (2.65 ± 0.560 mg kg⁻¹).

| PAH treatment | PAHs | Plant/control | Mean residual concentration (mg kg⁻¹) | Total PAH loss (%) | Rhizodegradation (% contribution by plants and microbes) |
|---------------|------|---------------|---------------------------------------|-------------------|--------------------------------------------------------|
| Single Phe    |      |               |                                       |                   |                                                        |
| M. sativa     | 3.62 ± 2.88a | 98            |                                       |                   | 30                                                     |
| L. perenne    | 1.53 ± 0.0918ab | 99          |                                       |                   | 31                                                     |
| F. arundinacea| 1.99 ± 0.0885ab | 99           |                                       |                   | 31                                                     |
| Abiotic control | 7.07 ± 0.740b | 68            |                                       |                   |                                                        |
| Unplanted control | 4.05 ± 2.01ab | 98           |                                       |                   |                                                        |
| Flu Flu       |      |               |                                       |                   |                                                        |
| M. sativa     | 7.51 ± 0.488a | 93            |                                       |                   | 52                                                     |
| L. perenne    | 1.06 ± 0.146a | 99            |                                       |                   | 58                                                     |
| F. arundinacea| 0.830 ± 0.294a | 99           |                                       |                   | 59                                                     |
| Abiotic control | 61.7 ± 3.91b  | 41            |                                       |                   |                                                        |
| Unplanted control | 6.92 ± 1.69a  | 93            |                                       |                   |                                                        |
| B[a]P B[a]P   |      |               |                                       |                   |                                                        |
| M. sativa     | 1.59 ± 0.132ab | 24           |                                       |                   | 10                                                     |
| L. perenne    | 1.92 ± 0.434a | 8             |                                       | −6*               |                                                        |
| F. arundinacea| 0.579 ± 0.123b | 72           |                                       |                   | 58                                                     |
| Abiotic control | 1.79 ± 0.190ab | 14           |                                       |                   |                                                        |
| Unplanted control | 1.58 ± 0.320ab | 24           |                                       |                   |                                                        |
| Mixed Phe     |      |               |                                       |                   |                                                        |
| M. sativa     | 36.2 ± 29.1a | 86            |                                       |                   | 19                                                     |
| L. perenne    | 2.10 ± 0.260b | 99            |                                       |                   | 33                                                     |
| F. arundinacea| 1.74 ± 0.400b | 99            |                                       |                   | 33                                                     |
| Abiotic control | 85.4 ± 4.03a  | 66            |                                       |                   |                                                        |
| Unplanted control | 1.78 ± 0.350b | 99            |                                       |                   |                                                        |
| Flu Flu       |      |               |                                       |                   |                                                        |
| M. sativa     | 22.4 ± 5.10a | 85            |                                       |                   | 24                                                     |
| L. perenne    | 1.87 ± 0.190b | 98            |                                       |                   | 38                                                     |
| F. arundinacea| 3.71 ± 1.54b | 98            |                                       |                   | 36                                                     |
| Abiotic control | 59.3 ± 3.18c  | 61            |                                       |                   |                                                        |
| Unplanted control | 8.54 ± 2.21b  | 94            |                                       |                   |                                                        |
| B[a]P B[a]P   |      |               |                                       |                   |                                                        |
| M. sativa     | 1.66 ± 0.130a | 37           |                                       | −11*              |                                                        |
| L. perenne    | 2.15 ± 0.0600b | 19           |                                       | −29.1*            |                                                        |
| F. arundinacea| 0.780 ± 0.0101c | 71          |                                       |                   | 22.6                                                   |
| Abiotic control | 1.39 ± 0.100a  | 48            |                                       |                   |                                                        |
| Unplanted control | 1.33 ± 0.120a  | 50            |                                       |                   |                                                        |

The mean residual concentration of phenanthrene and fluoranthene of the mixed PAH treatment with *M. sativa* differed significantly from those involving *F. arundinacea* and *L. perenne* (p < 0.01). B[a]P dissipation was greatest for *F. arundinacea* (1.87 mg kg⁻¹; 71%) and lowest for *L. perenne* (0.5 mg kg⁻¹; 19%) and was significantly different (p < 0.01) (Table 1).
Discussion

Phytoremediation is an eco-friendly and sustainable remediation technology for contaminants including PAHs. However, there are conflicting findings on the phytoremediation outcome and few studies on mixed PAH contamination that may reflect real site scenarios. The present study assessed the impact of single and mixed PAH treatments on plant biomass yield and PAH dissipation during a greenhouse experiment. The contribution of abiotic processes and rhizodegradation to the overall PAH dissipation was also assessed. Contrary to our hypothesis, single and mixed PAH treatments either had stimulatory or inhibitory effects on plant biomass yields. Also, the impact of single and mixed PAH treatments on plant biomass yields was not different. B[a]P dissipation was enhanced in treatments with *F. arundinacea* but inhibited in those with *L. perenne* and *M. sativa*. *L. perenne* inhibited B[a]P dissipation to a greater extent than *M. sativa* in both treatments. Phe and Flu dissipation was inhibited in vegetated PAH treatments. Abiotic processes such as volatilization and soil adsorption were more important as dissipation mechanisms compared to rhizodegradation in both treatments.

The inhibitory effect of PAH contamination on biomass yields of *L. perenne* and *F. arundinacea* is attributed to single or synergistic effect of PAHs in the single and mixed PAH treatments respectively. However, an increase in plant biomass yields and shoot to root ratio especially for *M. sativa* is contrary to most reports on phytotoxic effect of PAH contamination. Cheema et al. (2010) reported a 35% decrease in *M. sativa* biomass in soils spiked with phenanthrene (200 mg kg\(^{-1}\)) and pyrene (199 mg kg\(^{-1}\)). The biomass yield increase of *F. arundinacea* in the Phe and Flu treatments compared to the mixed PAH and control may be attributed to the PAH concentration. Jeelani et al. (2017) reported a significant increase in biomass yield of *Acorus calamus* grown on soils spiked with phenanthrene (50–100 mg kg\(^{-1}\)) and pyrene (25–50 mg kg\(^{-1}\)). Increase in plant biomass yields in the B[a]P treatment agrees with the findings of Sun et al. (2011) that B[a]P concentration ≤ 10 mg kg\(^{-1}\) of enhanced biomass yield of *Tagetes patula*. Chigbo and Batty (2013) also reported an enhanced germination and shoot elongation of *L. perenne* with B[a]P concentration of 1–4 mg L\(^{-1}\). With the exception of the phenanthrene treatment, the increase in shoot to root ratio for *M. sativa* in PAH treatments is supported by Salehi-Lisar and Deljoo (2015) who reported an increase of up to 1.29 times for *M. sativa* in fluorene treatments (0–100 mg/kg). The decrease in shoot to root ratio for *L. perenne* and *F. arundinacea* relative to their control agrees with the findings of Salehi-Lisar and Deljoo (2015) for *Triticum aestivum*. Abiotic processes and microbial degradation had a greater contribution than rhizodegradation in vegetated treatments that inhibited phenanthrene and fluoranthene dissipation. Sun et al. (2010) reported a significant loss of phenanthrene (83.4%) and pyrene (57.2%) from freshly spiked sterile soil as a result of abiotic process especially volatilization. Inhibition of PAH dissipation in the presence of vegetation is contrary to many reports of an enhanced PAH dissipation during phytoremediation (Olson et al. 2007; Hall et al. 2011). In a recent report, phenanthrene and pyrene dissipation was not significantly affected by the presence of the plant, *Acorus calamus* when compared to the unplanted control (Jeelani et al. 2017). We observed that *L. perenne* inhibited B[a]P dissipation to a greater extent than *M. sativa* in both treatments.

PAH contamination has adverse effect on water and nutrient uptake by plants with impact on biomass yield (Reiley et al. 1996; Cheema et al. 2010; Oguntimehin et al. 2010). Phytotoxicity is dependent on plant stress tolerance and degradation capability of indigenous soil microbes (Kechavarzi et al. 2007). Increase in *M. sativa* biomass yield and shoot to root ratio indicates tolerance to PAH contamination as well as a stimulatory effect on plant growth (Hall et al. 2011). On other hand, a decrease in the shoot to root ratio for *L. perenne* and *F. arundinacea* indicates phytotoxic effects of the PAH treatments with impacts on plant development and senescence (Kechavarzi et al. 2007; Cheema et al. 2010). Although there are several reports on tolerance of *M. sativa* to PAH contamination, conflicting findings may be related to differences in soil properties and soil microbial community. Abiotic processes and microbial degradation are principal dissipation mechanisms for phenanthrene due its low molecular weight (178.23 g mol\(^{-1}\)), vapor pressure (18 mPa), and solubility in water at 25 °C of 1.18 mg L\(^{-1}\) (Sun et al. 2010; Smith et al. 2011). However, volatilization is less likely to be a PAH dissipation mechanism for fluoranthene and benzo[a]pyrene with 3 or more rings and low vapor pressure. Also, high molecular weight PAHs adsorb to soil organic matter to facilitate formation of non-extractable bond residues thereby decreasing bioavailability (Kaimi et al. 2006; Hamdi et al. 2012). As such microbial degradation and rhizodegradation become relatively more important mechanisms for their dissipation in unplanted and planted soils respectively. Microbial degradation which involves mineralization, cometabolism, and non-specific radical oxidation depends on indigenous soil microorganisms, chemical properties of PAHs, soil properties, and environmental conditions (Smith et al. 2011; Toyama et al. 2011).

During phytoremediation, enhancement, or inhibition of PAH dissipation is determined by root exudate profile, catalolite repression, root morphology, and soil properties amongst others (Liste and Alexander 2000; Louvel et al. 2011; Jia et al. 2016). These factors also affect the extent of inhibition as observed with *L. perenne* compared to *M. sativa* for B[a]P dissipation. With respect to the effect of plants and root exudate on PAH dissipation, Guo et al. (2017) reported an enhanced PAH degradation at the early stage and then a
and remediation outcome. Ultimately, these factors amongst ture, microbial gene expression, plant-microbe interaction, and rhizosphere activities on soil microbial community struc-
stand critical controls of PAH contamination, plant selection, complex and variable. Further studies are required to under-
ment of specific abiotic processes such as volatilization 
We found that the effect of single and mixed PAH contamination on plant biomass yield and PAH dissipation was not different. Our study findings support few reports on the stimulatory effect of PAH contamination on plant growth while most studies report phytotoxic ef-
cultivation of abiotic control is difficult (Margesin et al. 2003; Kaimi et al. 2006). The contribu-
with nitrogen fixing ability. 
A stimulatory effect on B[a]P dissipation by L. perenne compared to M. sativa with nitrogen fixing ability. 
Conclusion
Mixed PAH contamination had a greater impact on plant biomass yield albeit non-significant and in most cases, there was a lower PAH dissipation in comparison to the single PAH treatment. Single or mixed PAH contamination can either inhibit or enhance plant growth. Enhancement of PAH dissipa-
tion in the presence of plants was only observed for single and mixed treatments with benzo[a]pyrene and F. arundinacea. Abiotic processes and microbial degradation were the most important PAH dissipation mechanisms. The PAH compound, presence of plants, and choice of plant species amongst other factors determine relative contribution of dissipation mecha-
nisms and remediation outcome, enhancement, or inhibition of PAH dissipation.
Acknowledgements I would like to acknowledge PTDF for funding my PhD programme and also Prof. Stuart Harrad at School of Public health, University of Birmingham, UK, for his professional guidance during the research.
Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References
Cheema SA, Imran Khan M, Shen C, Tang X, Farooq M, Chen L, Zhang C, Chen Y (2010) Degradation of phenanthrene and pyrene in spiked soils by single and combined plants cultivation. J Hazard Mater 177:384–389
Chigbo C, Batty L (2013) Effect of combined pollution of chromium and benzo[a]pyrene on seed growth of Lolium perenne. Chemosphere 90(2):164–169
Chigbo C, Batty L, Bartlett R (2013) Interactions of copper and pyrene on phytoremediation potential of Brassica juncea in copper–pyrene co-contaminated soil. Chemosphere 90(10):2542–2548
Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil 245:35–47
Fu D, Teng Y, Luo Y, Tu C, Li S, Li Z, Christie P (2012) Effects of alfalfa and organic fertilizer on benzo[a]pyrene dissipation in an aged con-
taminated soil. Environ Sci Pollut Res 19(5):1605–1611
Gan S, Lau EV, Ng HK (2009) Remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). J Hazard Mater 172:532–549

Guo M, Gong Z, Miao R, Su D, Li X, Jia C, Zhuang J (2017) The influence of root exudates of maize and soybean on polycyclic aromatic hydrocarbons degradation and soil bacterial community structure. Ecol Eng 99:22–30

Haichar F, Maro C, Berge O, Rangel-Castilo JR, Prosser JR, Balesdent J, Heulin T, Achoouak W (2008) Plant host habitat and root exudates shape soil bacterial community structure. Int Soc Microb Ecol 2:1221–1230

Hall J, Soole K, Bentham R (2011) Hydrocarbon phytoremediation in the family Fabaceae—a review. Int J Phytoremediation 13:317–332

Hamdi H, Benzarti S, Aymami L, Iezidi N (2012) Rehabilitation of degraded soils containing aged PAH based on phytoremediation with Alfalfa (Medicago sativa L.). Int Biodeterior Biodegrad 67:40–47

Jeelani N, Yang W, Xu L, Qiao Y, An S, Leng X (2017) Phytoremediation potential of Acorus calamus in soils co-contaminated with cadmium and polycyclic aromatic hydrocarbons. Sci Rep 7

Jia H, Lu H, Liu J, Li J, Dai M, Yan C (2016) Effects of root exudates on the leachability, distribution, and bioavailability of phenanthrene and pyrene from mangrove sediments. Environ Sci Pollut Res 23(6):5566–5576

Kaimi E, Mukaidani T, Miyoshi S, Tamaki M (2006) Loliolum perenne enhancement of biodegradation in diesel-contaminated soil. Environ Exp Bot 55:110–119

Kechavarzi C, Pettersson K, Leeds-Harrison P, Ritchie L, Ledin S (2007) Root establishment of perennial ryegrass (L. perenne) in diesel contaminated subsurface soil layers. Environ Pollut 145:68–74

Kirk JL, Klironomos JN, Lee H, Trevors JT (2005) The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil. Environ Pollut 133:455–465

Lee SH, Lee WS, Lee CH, Kim JG (2008) Degradation of phenanthrene and pyrene in rhizosphere of grasses and legumes. J Hazard Mater 153:892–898

Liste HH, Alexander M (2000) Accumulation of phenanthrene and pyrene in rhizosphere soil. Chemosphere 40:11–14

Louvel B, Cébron A, Leyval C (2011) Root exudates affect phenanthrene biodegradation, bacterial community and functional gene expression in sand microcosms. Int Biodeterior Biodegrad 65:947–953

Lu H, Zhang Y, Liu B, Liu J, Ye J, Yan C (2011) Rhizodegradation gradients of phenanthrene and pyrene in sediment of mangrove (Kandelia candel (L.) Druce). J Hazard Mater 196:263–269

Margesin R, Labbe D, Schinner F, Greer CW, Whyte LG (2003) Characterization of hydrocarbon-degrading microbial populations in contaminated and pristine alpine soils. Appl Environ Microbiol 69:3085–3092

Oguntimehin I, Issfa F, Sakugawa H (2010) Negative effects of fluoranthene on the ecophysiology of tomato plants (Lycopersicon esculentum Mill): Fluoranthene mists negatively affected tomato plants. Chemosphere 78:877–884

Olson PE, Castro A, Joern M, Duteau NM, Pilon-Smits EAH, Reardon KF (2007) Comparison of plant families in a greenhouse phytoremediation study on an aged polycyclic aromatic hydrocarbon–contaminated soil. J Environ Qual 36:1461–1469

Perelo LW (2010) Review: In situ and bioremediation of organic pollutants in aquatic sediments. J Hazard Mater 177:81–89

Reilley KA, Banks MK, Schwab AP (1996) Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. J Environ Qual 25:212–219

Salehi-Lisar SY, Deljoo S (2015) The physiological effect of fluorene on Triticum aestivum, Medicago sativa, and Helianthus annus. Cogent Food Agric 1:1020189

Siciliano SD, Germida JJ, Banks K, Greer CW (2003) Changes in microbial community composition and function during a polyaromatic hydrocarbon phytoremediation field trial. Appl Environ Microbiol 69:483–489

Smith MJ, Flowers TH, Duncan HJ, Alder J (2006) Effects of polycyclic aromatic hydrocarbons on germination and subsequent growth of grasses and legumes in freshly contaminated soil and soil with aged PAH residues. Environ Pollut 141:519–525

Smith MJ, Flowers TH, Duncan HJ, Saito H (2011) Study of PAH dissipation and phytoremediation in soils: comparing freshly spiked with weathered soil from a former coking works. J Hazard Mater 192:1219–1221

Sun TR, Cang L, Wang QY, Zhou DM, Cheng JM, Xu H (2010) Roles of abiotic losses, microbes, plant roots, and root exudates on phytoremediation of PAHs in a barren soil. J Hazard Mater 176:919–925

Sun Y, Zhou Q, Xu Y, Wang L, Liang X (2011) Phytoremediation for co-contaminated soils of benzo[a]pyrene (B[a]P) and heavy metals using ornamental plant Tagetes patula. J Hazard Mater 186(2):2075–2082

Toyama T, Furukawa T, Maeda N, Inoue D, Sei K, Kikuchi S, Ike M (2011) Accelerated biodegradation of pyrene and benzo[a]pyrene in the Phragmites australis rhizosphere by bacteria-root exudate interactions. Water Resour 45:1629–1638

USEPA (2007) USEPA Method 3546: Microwave extraction. https://www.epa.gov/sites/production/files/2015-12/documents/3546.pdf. Assessed 23 Aug 2017

USEPA (2014) Semivolatile organic compounds by gas chromatography/mass spectrometry. SW-846 Update V, Revision 5. https://www.epa.gov/sites/production/files/2015-12/documents/8270d.pdf. Assessed 23 Aug 2017

Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Theuwys T, Vlassiiev A, De Meers E, Nehevejova E, Van Der Lehe D, Mench M (2009) Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ Sci Pollut Res 16:765–794

Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321:385–408

Wu FY, Yu XZ, Wu SC, Lin XG, Wong MH (2011) Phenanthrene and pyrene uptake by arbuscular mycorrhizal maize and their dissipation in soil. J Hazard Mater 187:341–347

Xu SY, Chen YX, Wu WX, Wang KX, Lin Q, Liang XQ (2006) Enhanced dissipation of phenanthrene and pyrene in spiked soils by combined plants cultivation. Sci Total Environ 363:206–215