

Removal of soil polycyclic aromatic hydrocarbons derived from biomass fly ash by plants and organic amendments

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ABSTRACT

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Phytoremediation using maize (*Zea mays* L.) assisted by the compost or vermicompost amendments was the most appropriate strategy for bioremediation of soil contaminated by polycyclic aromatic hydrocarbons (PAHs) derived from biomass fly ash. Higher removal of low molecular weight PAHs than medium and high molecular weight PAHs within the same treatment were observed. The total PAH content in planted soil with compost or vermicompost was decreased in a range between 62.9–64.9%. There were no significant differences ($P < 0.05$) between the compost and vermicompost amendments on the total removal of ash-PAHs. The content of PAH derived by ash did not have adverse effect on maize cultivation and biomass yield. The contribution of PAH reduction by maize roots on the soil total PAH removal was negligible. Therefore, maize significantly boosted the PAH removal in soil. The harvested maize shoots did not represent any environmental risk.

Keywords: carcinogenic compound; combustion residues; contamination; degradation; soil amendments

Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic organic compounds with two or more benzene aromatic rings. Some PAHs are considered as potentially carcinogenic compounds to humans; they can be formed mainly during the incomplete combustion (Paris et al. 2018). The released PAHs tend to be persistent e.g. in soils, sediments and sewage sludge (Vácha et al. 2005, Dvořák et al. 2017, García-Sánchez et al. 2018). The unfavourable conditions of biomass combustion in power plants can also lead to the accumulation of PAHs in resulting ashes (Masto et al. 2015). The biomass ashes usually also contain high amounts of mineral nutrients such as Ca, K, P and Mg, and therefore there is an effort to recycle them as soil amendments and/or fertilizers (Ochecová

et al. 2017). The possible PAH content in ashes applied in soil have not received considerable attention. The increased content of PAHs in ashes can limit the soil ash application and elevate the agricultural soil contamination (Enell et al. 2008). Phytoremediation using maize (*Zea mays* L.) could be suitable strategy for clean-up of ash-PAHs contaminated soils because maize significantly enhanced the degradation of aged PAHs in soil from a wastewater-irrigated area (Guo et al. 2017). Moreover, the compost or vermicompost application into a soil amended by PAH-contaminated ashes could improve the removal similarly as in the case of bioremediation of artificially and aged PAHs contaminated soil described by Wang et al. (2012) and Feng et al. (2014).

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The main aims of this study were: (i) to determine the removal of PAHs derived from biomass fly ash in non-planted/planted ash-soil in comparison to non-planted/planted ash-soil amended with compost or vermicompost; (ii) to determine the contribution of maize on the PAH removal from soil.

MATERIAL AND METHODS

PAHs. In this study, 16 individual US EPA priority PAHs were investigated. The PAHs were sorted into four groups as follows: LMW PAHs – low molecular weight PAHs (the sum of naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene); MMW PAHs – medium molecular weight PAHs (the sum of fluoranthene, pyrene, benzo[*a*]anthracene, chrysene); HMW PAHs – high molecular weight PAHs (the sum of benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*c,d*]pyrene, dibenz[*a,h*]anthracene, benzo[*g,h,i*]perylene); total PAHs – the sum of all 16 individual PAHs.

Soil. The experimental soil originated from a long-term trial site close to the city of Humpolec in the Czech Republic (49°33'15"N, 15°21'00"E). The soil was obtained by mixing different subsamples collected from the field site at a depth of 0–20 cm. The non-sterilized soil was homogenized, air-dried at room temperature and passed through a 5 mm stainless steel sieve. The main physico-

chemical characteristics of the experimental soil (Cambisol – sandy loam according to the FAO soil classification) are listed in Table 1.

Compost and vermicompost. The compost was obtained from the composting of biowaste mixture following the methodology described by Habart et al. (2010). Briefly, the compost production was carried out in a 70 L plastic laboratory fermenter. The mixture was prepared from livestock manure, fresh grass, straw and waste paper in a ratio of 9:9:1:1 (w/w). The fermenter was placed in a laboratory at 25°C and the composting process was carried out with forced aeration. After 180 days, the compost was considered as mature and sufficiently stabilized to be used. The vermicompost was obtained from the vermicomposting of the same biowaste mixture mentioned above following the methodology described by Hanč et al. (2017). Briefly, vermicomposting was conducted in a plastic vermicompost reactors (Ekodomov, Prague, Czech Republic) placed in a laboratory. 5 kg of the biowaste mixture was inoculated with 0.5 kg of a substrate containing earthworms of the genus *Eisenia*. This mixture was placed into a 12 L plastic bowl of vermi-reactor and left 180 days for vermicomposting. The main physico-chemical characteristics of the 'ready to use' compost and vermicompost are shown in Table 1.

Ash. The experimental biomass fly ash was obtained from a commercial biomass power plant operated in the Czech Republic using a 20 MW grate boiler. The tested ash was derived from the

Table 1. Physico-chemical characteristics of soil, compost and vermicompost

Parameter	Soil	Compost	Vermicompost
pH _{CaCl₂}	5.2 ± 0.0	8.4 ± 0.0	8.7 ± 0.1
C _{tot} (g/kg)	18.3 ± 2.5	316 ± 2.0	317 ± 1.8
N _{tot} (g/kg)	1.35 ± 0.1	25.8 ± 0.3	29.9 ± 0.1
P _{avail} (mg/kg) ¹ /tot (g/kg) ²	80.2 ± 4.4	2.02 ± 0.3	3.12 ± 0.4
K _{avail} (mg/kg)/tot (g/kg)	190 ± 8.9	29.2 ± 0.6	19.2 ± 0.6
Ca _{avail} (mg/kg)/tot (g/kg)	1586 ± 72	6.95 ± 0.5	9.36 ± 0.7
Mg _{avail} (mg/kg)/tot (g/kg)	153 ± 10	1.99 ± 0.1	2.46 ± 0.2
Total PAHs (µg/kg)	nd	nd	nd

¹Avail. – available element contents (P, K, Ca and Mg) in soil (mg/kg) were determined using Mehlich 3 extraction;

²tot. – total element contents (P, K, Ca and Mg) in compost and vermicompost (g/kg) were determined according to the method used by Hanč et al. (2017). nd – not detected (individual polycyclic aromatic hydrocarbons (PAHs) were lower than the detection limit in the range between 1.8–5.6 µg/kg dry weight)

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combustion of wheat straw in the temperature range 600–700°C. The collected ash was a composite of four random sub-samples taken from a container with fly ash from electrostatic precipitator. The ash was air-dried at room temperature and thoroughly mixed in a laboratory. The main physico-chemical characteristics of the experimental ash determined according to Mercl et al. (2016) and Košnář et al. (2016) were: particle size – fraction < 0.25 mm, 67.8%; fraction 0.25–1.6 mm, 32.2%; $\text{pH}_{\text{H}_2\text{O}}$, 10.3; electrical conductivity, 9.9 mS/cm; loss on ignition, 52.6%; P_{tot} , 0.1%; K_{tot} , 9.5%; Ca_{tot} , 1.8%; Mg_{tot} , 0.57%; total PAHs, 160.2 mg/kg DW (dry weight).

Pot experiment. The experiment was conducted in an outdoor, atmospheric precipitation-controlled, vegetation hall with natural temperature and light using a series of 6 L polypropylene pots: open top, 21 cm; base, 18 cm; height, 20 cm. The pot experiment was carried out in 9 treatments each in four separated pots for replication as follows: S – soil (control); C – compost-soil; V – vermicompost-soil; A – ash-soil; CA – compost-ash-soil; VA – vermicompost-ash-soil; PS – planted soil (control for plants); PC – planted compost-soil; PV – planted vermicompost-soil; PA – planted ash-soil; PCA – planted compost-ash-soil; PVA – planted vermicompost-ash-soil. Each treatment (S and PS) contained 5 kg soil DW per pot and rest of the treatments contained 5 kg of amended soil per pot. The ash, compost and vermicompost in amended soil of the respective treatment represented 1% (w/w), 10% (w/w) and 10% (w/w), respectively. The initial PAH contents

of experimental treatments are shown in Table 2 excluding the treatments without the ash addition because the PAHs were not detected in them initially. As the experimental plant was tested maize (*Zea mays* L. var. Colisee). The maize seeds were gained from the KWS (Einbeck, Germany). Before sowing, each pot received 500 mg N in NH_4NO_3 water solution, 32 mg P and 80 mg K in K_2HPO_4 water solution. The maize seeds were sown directly in soil at a depth of 2–3 cm, at a rate of 8 seeds per pot. The plants were thinned to 3 per pot at the age of the third leaf emergence. The pots were manually watered with demineralized water regularly in order to keep soil moisture at 60–70% of the maximum water holding capacity. Soil samples were collected at the end of the 120-days experiment. Each soil sample was a composite of five sub-samples from different zones of each pot. The plant samples (roots and shoots separately) were obtained after the harvest. Roots were washed with distilled water to remove the attached soil particles. Before the PAH analysis the samples were separately air-dried at laboratory temperature, homogenized and plant samples were pulverized to a fine powder with a mill (Retsch, Haan, Germany).

PAH analysis. The extraction of PAHs from soil and plant samples was carried out according to the US EPA (2007) using an ultrasonic bath (Bandelin electronic, Berlin, Germany) with a continuous re-extraction cycles followed by the silica gel clean-up process in concordance to the US EPA (1996). The PAH identification followed by the PAH quantification was based on a gas

Table 2. Initial polycyclic aromatic hydrocarbon (PAH) contents ($\mu\text{g/kg}$ dry weight) in experimental treatments

Treatment	LMW PAHs	MMW PAHs	HMW PAHs	Total PAHs
A	745.4	371.5	484.8	1601.7
CA	750.1	376.7	477.3	1604.0
VA	739.2	387.4	493.8	1611.4
PA	730.8	417.8	526.6	1675.2
PCA	732.9	369.1	480.0	1582.0
PVA	725.8	401.0	483.3	1610.1

All values represent means ($n = 4$). There were no significant differences ($P < 0.05$) in initial PAH contents between the treatments: A – ash-soil; CA – compost-ash-soil; VA – vermicompost-ash-soil; PA – planted ash-soil; PCA – planted compost-ash-soil; PVA – planted vermicompost-ash-soil; LMW PAHs – low molecular weight PAHs; MMW PAHs – medium molecular weight PAHs; HMW PAHs – high molecular weight PAHs; total PAHs – the sum of all 16 individual PAHs

chromatography/mass spectrometry method described by US EPA (2014) using a 6890N-gas chromatograph with 5975-mass detector (Agilent Technologies, Santa Clara, USA). The separation of PAHs was carried out using a capillary column (20 m × 0.18 mm inner diameter, 0.14 µm film thickness) (Agilent J&W Scientific, Santa Clara, USA). The detailed chromatographic regime and analytical precision of the method were described elsewhere by Košnář et al. (2016).

Data processing and statistical analysis. The total soil PAH removal (%) in Figures 1 and 2 was calculated as follows:

$$\text{Total soil PAH removal} = 100 \times (\text{PAH}_{\text{residual}} - \text{PAH}_{\text{initial}}) / \text{PAH}_{\text{initial}}$$

Where: $\text{PAH}_{\text{residual}}$ – residual content of PAHs in soil (µg/kg DW) at the end of the 120-days experiment; $\text{PAH}_{\text{initial}}$ – initial content of PAHs in soil (µg/kg DW) at 0 days.

The plant PAH removal (%) in Table 3 was calculated as follows:

$$\text{Plant PAH removal} = 100 \times \left[\frac{\text{plant yield} \times \text{PAH}_{\text{concentration}}}{\text{PAH}_{\text{initial}}/\text{pot}} \right]$$

Where: plant yield – maize roots yield (kg roots DW/pot); $\text{PAH}_{\text{concentration}}$ – PAH concentration in maize roots (µg PAH/kg plant DW); $\text{PAH}_{\text{initial}}/\text{pot}$ – initial PAH content in soil at 0 days per each pot (µg PAH/pot).

The one-way ANOVA at $P < 0.05$ followed by the Tukey's test was performed to evaluate the statistical differences between the treatments. The data were evaluated using the Microsoft Excel 2010 (Microsoft Corporation, Redmond, USA) and Statistica 12.0 (StatSoft, Tulsa, USA).

RESULTS AND DISCUSSION

The influence of ash-PAHs in the development of bioremediation approaches was assessed by comparing of maize biomass yield in Table 3. The compost and vermicompost amendments in PC and PV treatments significantly increased ($P < 0.05$) the yield of maize roots in comparison to the planted control treatment (PS). The maize shoots yields were the same in all the treatments. The soil contaminated by ash-PAHs had no adverse effects on maize biomass (roots and shoots) yield as there were no significant differences ($P < 0.05$) between the respective treatments. Our results of biomass yield were comparable with 120 days experiment with maize grown in soil contaminated by pyrene and phenanthrene artificially (Liao et al. 2015). In our study, the PAHs were found only in maize roots cultivated on ash-amended soil (Table 3). The compost and vermicompost amendments could enhance

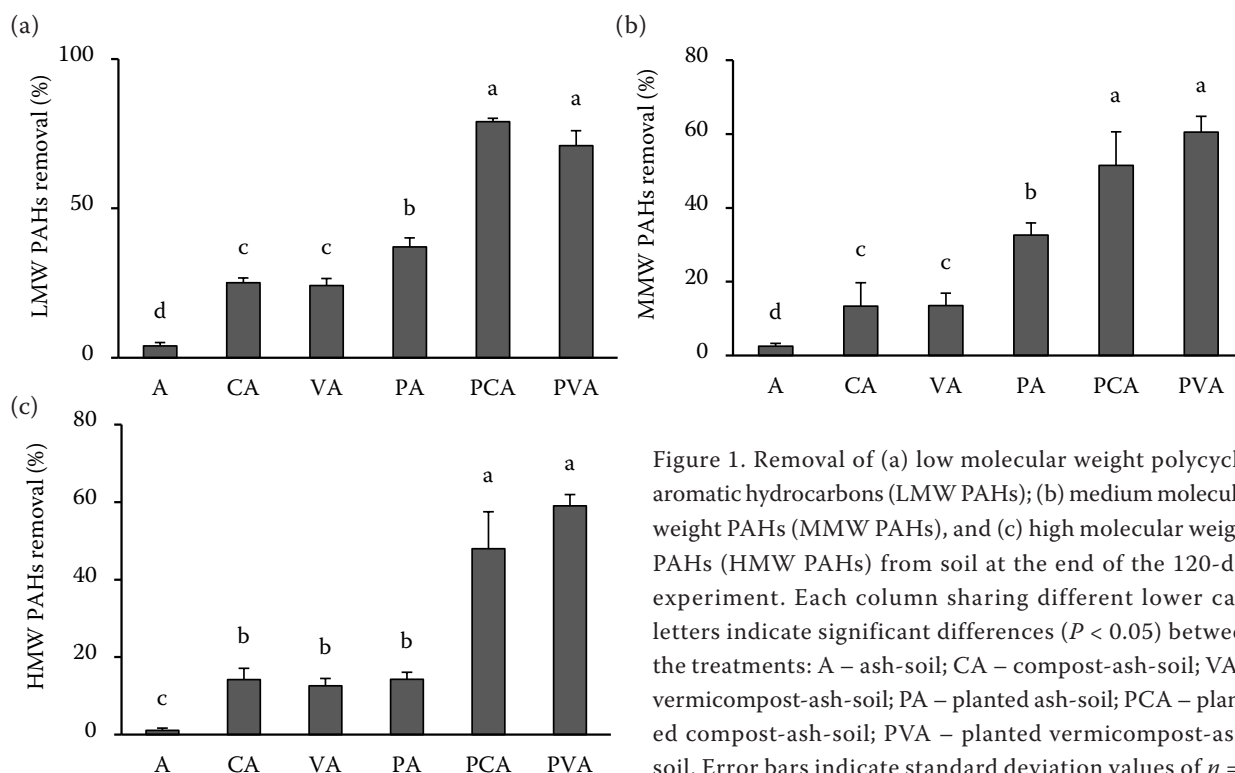


Figure 1. Removal of (a) low molecular weight polycyclic aromatic hydrocarbons (LMW PAHs); (b) medium molecular weight PAHs (MMW PAHs), and (c) high molecular weight PAHs (HMW PAHs) from soil at the end of the 120-day experiment. Each column sharing different lower case letters indicate significant differences ($P < 0.05$) between the treatments: A – ash-soil; CA – compost-ash-soil; VA – vermicompost-ash-soil; PA – planted ash-soil; PCA – planted compost-ash-soil; PVA – planted vermicompost-ash-soil. Error bars indicate standard deviation values of $n = 4$

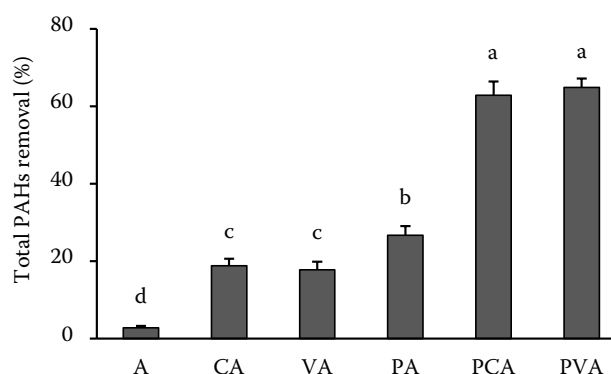


Figure 2. Removal of total polycyclic aromatic hydrocarbons (PAHs) from soil at the end of the 120-day experiment. Each column sharing different lower case letters indicate significant differences ($P < 0.05$) between the treatments: A – ash-soil; CA – compost-ash-soil; VA – vermicompost-ash-soil; PA – planted ash-soil; PCA – planted compost-ash-soil; PVA – planted vermicompost-ash-soil. Error bars indicate standard deviation values of $n = 4$

the bioavailability of soil ash-PAHs because significantly increased PAH content was observed in roots of PCA and PVA treatments than in PA treatment. The PAH content in maize shoots was not detected and translocation of ash-PAHs found in roots to the shoots remained unconfirmed. This was in line with the study by Gao et al. (2011) who indicated that the spiked PAHs are accumulated in roots rather than transported through the xylem flow to the shoots.

In this study, the PAH removal of ash origin from soil by maize roots was negligible in a range

between 0.02–0.04% (Table 3). This could indicate that the contribution of PAH accumulated in maize roots on the total PAH removal from soil of PCA and PVA treatments was minimal. This finding is consistent to the study by Kacáľková and Tlustoš (2011) who reported significantly lower than 1% accumulation of aged PAHs by maize from soil.

The removal of individual PAH groups is shown in Figures 1 and 2 excluding the treatments without the addition of ash because the PAHs were detected in them neither at the beginning nor in the end of the experiment. Different LMW, MMW and total PAH removal from soil of all investigated treatments was determined as follows: PCA ~ PVA > PS > CA ~ VA > A, and followed by PCA ~ PVA > PS ~ CA ~ VA > A for the HMW PAH removal. The phytoremediation of ash-soil assisted by compost or vermicompost (PCA and PVA) was the most appropriate strategy in the PAH removal. Cultivation of maize on ash-soil in combination with compost amendment in PCA treatment removed 79.0% of LMW PAHs, 51.5% of MMW PAHs and 48.1% of HMW PAHs from soil. These PAH removals were not statistically ($P < 0.05$) different to those reached in the PVA treatment using maize on vermicompost amended ash-soil. The higher LMW PAH removal than the MMW and HMW PAH removal in the same treatment indicated that the LMW PAHs of ash origin are susceptible to be biodegradable similarly to the aged PAHs as was described by Feng et al. (2014).

The results of total PAH removal in Figure 2 showed that the total PAHs derived from biomass

Table 3. Yield of maize roots and shoots, polycyclic aromatic hydrocarbon (PAH) concentration in roots and PAH removal by roots

Treatment	Root (g/pot DW)	Shoot (g/pot DW)	Total PAHs in roots (µg PAH/kg roots DW)	Plant PAH removal (%)
PS	15.8 ^b	106.9 ^a	nd	nd
PC	22.4 ^a	111.5 ^a	nd	nd
PV	22.1 ^a	105.5 ^a	nd	nd
PA	15.5 ^b	109.5 ^a	83.8 ^b	0.02 ^b
PCA	22.7 ^a	105.1 ^a	143.9 ^a	0.04 ^a
PVA	22.8 ^b	106.0 ^a	161.2 ^a	0.04 ^a

nd – not detected (individual PAHs were lower than the detection limit in the range between 1.8–5.6 µg/kg dry weight (DW)). All values represent means ($n = 4$). Different lower case letters within the same column indicate significant differences ($P < 0.05$) between the treatments: PS – planted soil (control for plants); PC – planted compost-soil; PV – planted vermicompost-soil; PA – planted ash-soil; PCA – planted compost-ash-soil; PVA – planted vermicompost-ash-soil

fly ash in non-amended bare soil (A treatment) were removed negligibly. The compost or vermicompost which were applied into the soil of non-planted treatments (CA and VA) separately decreased the total PAH content in soil significantly in comparison to the non-amended treatment (A) in the range between 15.1–17.8%. The phytoremediation of PAHs in ash-soil (PA treatment) showed that the maize has a significantly higher ability to remove ash-PAHs than the CA and VA treatments because the sum of total PAH content was removed by 26.7%. The total PAH content in the planted soil amended with compost and vermicompost (PCA and PVA treatments) decreased by 62.9% and 64.9%, respectively. However, there were no significant differences ($P < 0.05$) between the compost and vermicompost amendment on the removal of total ash-PAHs. The higher PAH removal in PCA and PVA treatments than in other treatments indicated that the maize plants could stimulate the growth and activity of soil autochthonous microorganism involved in PAH degradation due to the production of exudates released in maize rhizosphere as was reported by Nanekar et al. (2015). Moreover, the compost or vermicompost used together with maize could stimulate the activity of soil autochthonous PAH degraders which could be supported by PAH degraders augmented to soil from the amendments. Furthermore, the incorporation of these organic materials could support the PAH removal by the irreversible trapping processes of PAHs in soil as was described by Ouvrard et al. (2014).

This study showed that the maize cultivation on a PAH contaminated soil amended with the compost and vermicompost as separately applied amendments were the most efficient bioremediation approaches of soil contaminated by PAHs of ash origin. The resulted residual PAH contents in PCA (587.4 µg/kg DW) and PVA (565.6 µg/kg DW) were significantly lower than the limit of total PAHs (1000 µg/kg DW) for soils required by the Ministry of the Environment of the Czech Republic (2016). Moreover, the harvested above-ground biomass of maize did not represent any environmental risk.

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