



Polycyclic aromatic hydrocarbons in biochar amended soils: Long-term experiments in Brazilian tropical areas

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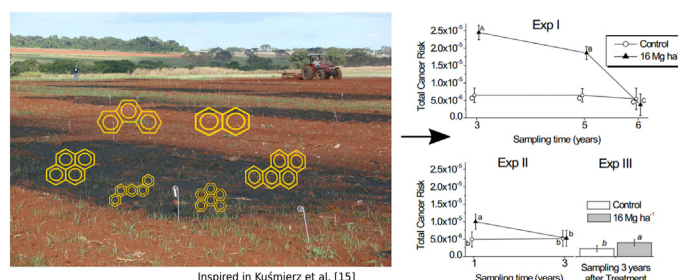
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HIGHLIGHTS

- Long-term field experiment dealing with PAH contamination after biochar application.
- Biochar application increases soil PAH concentrations.
- However the reached levels are far below the limits of prevention.
- The PAHs levels decrease with the time until equality with control after 3 or 6 years.
- This time depends of soil organic matter content (shortest for highest C content).

GRAPHICAL ABSTRACT



Inspired in Kuśmierz et al. [15]

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ABSTRACT

The interest in charcoal for agricultural use (biochar) has sharply increased in recent years. However, biochar can contain groups of compounds such as polycyclic aromatic hydrocarbons (PAHs) that are considered persistent pollutants, and are formed concomitantly with biochar during its production by pyrolysis. Soil samples were collected in three experimental areas at different intervals (1, 3, 5 or 6 years) after the application of 16 Mg ha⁻¹ of biochar. The total concentrations of PAHs; benzo[*a*]pyrene; and the estimated total cancer risk in biochar treated plots were larger than found in the control ones, but they decreased over time, equaling the control values after three years in the high C content soil (11.2 g C kg⁻¹ soil), or after six years in the low C content soil (6.8 g C kg⁻¹ soil). Nevertheless, the sum of PAH concentrations found in the biochar amended plots, in the range of 15.80–39.40 ng g⁻¹, were around two orders of magnitude below the limits of prevention established by Brazilian legislation (8100 ng g⁻¹) and some European regulations for soils (3000 ng g⁻¹) and also lower than the observed in previous studies about biochar amended soils. Our results indicate that, under the conditions evaluated, the application of biochar to soil in the studied proportion (16 Mg ha⁻¹, every six years) is safe concerning soil contamination by PAHs.

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1. Introduction

Biochar is the stable, carbon-rich material produced by biomass pyrolysis under low or no oxygen conditions. It has been used in agriculture to improve soil fertility, nutrient availability, soil water retention capacity and to provide a mechanism for long term carbon storage (Roberts et al., 2010; Chen et al., 2015; Khalid and Klarup, 2015). Due to these reasons, the interest in biochar use has sharply increased in recent years and thus, safe procedures and environmental protection are necessary for its application in soils (Fabbri et al., 2013; Novotny et al., 2015).

Polycyclic aromatic hydrocarbons (PAHs) are byproducts of pyrolytic or incomplete combustion reactions produced when materials containing carbon and hydrogen are heated at temperatures higher than 100–150 °C (Blumer, 1976). PAHs are introduced in the environment by natural sources, such as volcanic eruptions and wildfires, but mainly from anthropogenic sources such as incomplete combustion of fossil fuels and pyrolysis of organic matter (Zhang and Tao, 2009; Kamal et al., 2015). PAHs are widely distributed in the environment because of direct emission to certain environmental compartments, such as the atmosphere, transport into and/or between environmental compartments and accumulation in some of them, such as soils and sediments (Zhang and Tao, 2004; Kim et al., 2013; Kamal et al., 2015).

The United States Environmental Protection Agency (USEPA) has classified 16 PAHs as priority pollutants, since many PAHs exhibit mutagenic and/or carcinogenic properties. There are three different PAH exposure pathways for humans: inhalation, ingestion of contaminated food and/or water, and skin contact (Pereira Netto et al., 2004; IPCS, 1998). Therefore, workers involved in the production, transport and application of biochar may be exposed to a risk when in contact with biochar contaminated with PAHs and biochar amended soils (Fabbri et al., 2013; Buss and Masek, 2016; Kusmierz et al., 2016). In addition, PAHs are very persistent in soils, where they can exhibit toxic activity towards different organisms (plants, microorganisms and invertebrates) (Kusmierz and Oleszczuk, 2014; Oleszczuk et al., 2014). PAHs present in the soil can also be transported into water bodies, contaminating sediments and aquatic biota, and be re-suspended to the atmosphere, however this at low extent due to PAHs low water solubility and their high vapor pressures.

These facts justify the need for PAH monitoring in environmental compartments such as soils, atmosphere, sediments and water bodies, because of their potential effects on living organisms, including humans (Peng et al., 2011; Wang et al., 2011; Dimitriou and Kassomenos, 2017). The maximum permitted level of the 16 USEPA-PAH in soils is 3000 ng g⁻¹, according to some European countries' regulations (BBodSchV, 1999; Kusmierz and Oleszczuk, 2014). In Brazil, the National Environmental Council (CONAMA) establishes criteria for soil quality regarding several toxic compounds to protect human health and the environment, whereby the sum of the 16 USEPA-PAH concentrations cannot exceed 8100 ng g⁻¹ (CONAMA, 2009). The PAH classifications, according to USEPA and the International Agency for Research on Cancer (IARC), together with their CONAMA limits of molecular mass and vapor pressures, are shown in Table S1 (Supplementary Material).

PAHs and other organic contaminants can be formed during the thermo-chemical processing (pyrolysis) of biomass to produce biochar and their formation depends on the feedstock composition and pyrolysis conditions, such as temperature, residence time and carrier gas flow (Hale et al., 2012; Keiluweit et al., 2012; Oleszczuk et al., 2014; Buss et al., 2016). PAHs are formed through degradation of the lignin and cellulose contained in the biomass, through a mechanism reported in detail elsewhere (Hale et al., 2012; Keiluweit et al., 2012; Kusmierz et al., 2016).

Although higher PAHs concentrations were found in the liquid fraction compared to the solid fraction obtained from pyrolysis product (Schmidt and Noack, 2000; Hale et al., 2012; Wang et al., 2017; Weidemann et al., 2017), they must be monitored in soils after biochar application, due to environmental risk they represent. There are many reports about the PAH content of biochar (Freddo et al., 2012; Hale et al., 2012; Keiluweit et al., 2012; Kloss et al., 2012; Dai et al., 2014; Kusmierz and Oleszczuk, 2014; Chen et al., 2015; Khalid and Klarup, 2015; Zielinska and Oleszczuk, 2015; Stefaniuk et al., 2016) and maximum limits for the sum of 16 USEPA-PAHs have been established as 12 or 4 mg kg⁻¹ of dry mass for basic grade or premium grade biochar, respectively, according to the European Biochar Certificate (EBC, 2015) and with maximal of 6 mg kg⁻¹ for the International Biochar Initiative certificate guidelines (IBI, 2015).

Despite these values, very few studies have evaluated the PAH content of biochar amended soils and their decay over time (Fabbri et al., 2013; Khan et al., 2015; Kusmierz et al., 2016; Nicolini et al., 2015). Nicolini et al. (2015) observed that two years after soil treatment, PAH concentrations in biochar amended soils were lower than found before treatment. Rombola et al. (2015) also concluded that PAH concentrations in soil treated with biochar decreased with time (35 months after biochar application), but the increase of PAH concentrations after biochar treatment seemed to depend on the feedstock used for biochar production (De la Rosa et al., 2016). Kusmierz et al. (2016) found that the addition of biochar to soil led to an increase of PAH concentrations, but these sharply decreased 3.5 months after treatment. They also observed migration of PAHs from the surface soil to a deeper horizon.

In this context, the present study was carried out to evaluate the environmental safety of biochar application regarding soil contamination by PAHs and also to evaluate their persistence under field conditions in tropical areas, seeking to support the determination of safe biochar doses and treatment frequency regarding soil contamination. It is important to emphasize that this is the first study that evaluate the change of PAH concentration over time compared to the control soil under field and tropical conditions.

2. Materials and methods

2.1. Soil sampling and sampling sites

Surface soil samples were collected at 0–10 cm soil depth in three experimental areas (Exp. I, II and III) that had been treated once using biochar at a proportion of 16 Mg ha⁻¹. The soil samples were ground, sieved (<2 mm) and air-dried at room temperature.

The used biochar, that will be detailed below, was ground to a diameter <2 mm before application and incorporated into a 0–15 cm soil depth using a rotary hoe. The treatments were arranged in a randomized four-block design, corresponding to treated and non-treated blocks, to allow collecting control samples without biochar application in all experimental areas. Each experimental unit was an area of 40 m², of which 20 m² was considered for this study. Three subsamples were collected randomly within each experimental unit to build a composite sample. Routine chemical and texture analyses of the soils were performed for soil characterization (Supplementary Material).

Experiment I (Exp I) was conducted in Nova Xavantina, Mato Grosso state, Brazil (14° 35' 36" S and 52° 24' 04" W) in soil classified as dystrophic Ferralsol, sandy clay loam texture, with C content of 7 g kg⁻¹. The biochar application occurred in September, 2006. The area was used for soybean (*Glycine max*) production and soil samples were collected in 2009, 2011 and 2012 (3, 5 and 6 years after biochar application). The biochar used in this experiment was

a by-product (charcoal fines) of charcoal made from a mixture of savannah woods in a traditional circular bricks kiln at a temperature around 350 °C with a residence time of 120 h and showed an elemental composition of 490.6 g of total C kg⁻¹ and 6.6 g of total N kg⁻¹, more details about the biochar can be found in Madari et al. (2017).

Experiment II (Exp II) was conducted in Santo Antônio de Goiás, Goiás state, Brazil (49° 16' 54" S, 16° 29' 59" W) in a Dystric Ferralsol, clayey, with C content of 11.2 g kg⁻¹, in the area of the experimental farm of the Embrapa Rice & Beans research unit. The application of biochar occurred in June 2009. Before the experiment implantation, the area had been cultivated with corn (*Zea mays*) and bean (*Phaseolus vulgaris*) and after the biochar incorporation the area was cultivated with aerobic rice (*Oryza sativa*). The samples were collected one and three years after biochar application (2010 and 2012). The biochar used in this treatment was a eucalyptus (*Eucalyptus* sp) charcoal made in an industrial cylindrical metal kiln at 450 °C with a residence time of 12.3 h and showed an elemental composition of 774.0 of total C kg⁻¹ and 3.3 g of total N kg⁻¹. Further details about the biochars employed in this study can be found elsewhere (Madari et al., 2017).

Experiment III (Exp III) was also conducted in Nova Xavantina (14° 34' 50" S, 52° 24' 01" W) in a Dystric Plinthosol, sandy loam, with C content of 6.8 g kg⁻¹. Soil treatment occurred in December 2008. Before the experiment, the area was used for livestock with brachiaria (*Urochloa decumbens*) as the dominant species. Aerobic rice (*O. sativa*) was produced in this area after biochar application and soil samples were collected once in 2011, three years after biochar treatment. The biochar applied was the same used for Exp II.

2.2. Reagents and standards

Acetonitrile and methylene chloride, both HPLC grade, were purchased from Tedia (RJ, Brazil). A standard solution containing 16 USEPA-PAHs in concentrations between 0.1 and 2.0 mg L⁻¹ was purchased from Supelco (PA, USA). Solid standards of perylene and benzo[e]pyrene were obtained from Sigma-Aldrich (MO, USA), and the internal standards (naphthalene-d8, phenanthrene-d10, pyrene-d10, chrysene-d12, benzo[a]pyrene and perylene-d12) were purchased from Cambridge Isotope Lab., Inc. (PA, USA).

2.3. PAH extraction and analysis

PAHs can be extracted by Soxhlet, ultrasound bath and Accelerated Solvent Extraction (ASE). According to Wang et al. (2017), some studies reported that extractions by ultrasound could provide a comparable or even better extraction efficiency than Soxhlet extractions. The efficiency of the ASE for PAH extractions from biochar depends on the solvent used and it is not used as commonly as Soxhlet. Soil extraction was carried out as previously described for soil and street dust (Pereira Netto et al., 2004; Franco et al., 2017). Briefly, 10 g of each soil sample was ultrasonically extracted using four portions of 20 mL of dichloromethane for 20 min each. The combined extracts were spiked with a solution containing the internal standards and concentrated by rotary evaporation (40 °C), with solvent exchange to acetonitrile up to a final volume of 0.25 mL. The concentrated extracts were filtered through disposable syringe filters (PTFE; 25 mm; 0.45 µm, Millex, Millipore, USA). In order to evaluate the extraction procedure, aliquots of 2, 5 and 10 g of each soil sample were extracted and evaluated during implementation.

PAH determination was carried out using a gas chromatograph (Agilent 7890A) coupled to a mass spectrometer (Agilent 5975 C

inert XLEI/CIMSD). PAH separation was achieved using a DB-17 MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm, J&W Scientific, USA) and helium as carrier gas (1 mL min⁻¹). The oven temperature was kept at 60 °C for 1 min and then raised at 40 °C min⁻¹ to 230 °C, kept at this temperature for 1 min and increased again at 4 °C min⁻¹ to 310 °C, which was kept for 2 min. The temperatures of the injector and transfer liner were 350 °C and 310 °C, respectively. The volume injected was 1 µL and injection was performed in the splitless mode. The mass spectrometer was operated in electron impact mode (EI) at 70 eV. PAHs were detected in the selected ion monitoring (SIM) mode, and identified by retention times and elution order (Franco et al., 2017).

2.4. Quantitative analysis and quality control

PAH determination followed a previously published method (Franco et al., 2017). Briefly, analytical curves were obtained by the least-squares method after triplicate injections of standards containing the 18 PAHs studied in concentrations ranging between 0.5 and 500.0 µg L⁻¹ and constant concentrations of the internal standards (10 µg L⁻¹). The limits of detection (LOD) and of quantification (LOQ) were calculated according to the IUPAC criteria and obtained by dividing respectively 3 and 10 times the signal-to-noise ratios by the angular coefficients of the analytical curves of each PAH. Signal-to-noise ratios were estimated by the standard deviations of peak areas obtained after 7 subsequent injections of the least concentrated standard. LOD and LOQ were expressed as ratios to the mass of the soil samples, considering the extraction from 10 g of soil. The coefficients of determination (R²) of the calibration curves ranged between 0.997 and 1.000, indicating good adherence to linear models in the studied range. The LOQ varied between 0.28 ng kg⁻¹ (naphthalene) and 6.53 ng kg⁻¹ (benz[a]anthracene) (Table S2).

The accuracy of the extraction method evaluated through recovery assays using pooled soil samples spiked at two concentration levels (0.5 ng g⁻¹ and 2.5 ng g⁻¹) led to the results shown in Table S3. The recoveries varied between 52.7 ± 14.3% and 119.2 ± 4.5% and all of them showed relative standard deviation - RSD (%) < 30%. The recoveries found in level 1 (0.5 ng g⁻¹) varied between 64.3 and 110.3%, except for perylene (52.7%) and indene [1,2,3-*c,d*]pyrene (59.3%), with RSD (%) varying between 3.6 and 23.3%. With respect to the level 2 (2.5 ng g⁻¹), recoveries varied from 70.2 to 119.2%, except for indene[1,2,3-*c,d*]pyrene (56.0%), and all RSD (%) ranged from 4.5 to 15.1%. The results of recovery assays were considered satisfactory for all PAHs in both levels, considering sample complexity as well as previous considerations and acceptable values for the analysis of trace-level compounds in environmental samples (NOAA, 2006).

2.5. Data analysis

The data were previously treated with the acquisition program of the Chem Station (Agilent, USA) and with specific Microsoft Excel® spreadsheets.

Due to the multivariate characteristics of the data found in this study, factorial data analysis was employed, after mean-centering, seeking to detect data structure and eliminate the problem of redundancy and correlated variables, as well as to simplify the problem by reducing the number of original variables.

All the data (PAH concentrations and the obtained factor scores) were submitted to repeated measures analysis of variance (ANOVA) and when statistical significance was detected, the means were compared by Duncan's test at p = 0.05. The normality and homoscedasticity of the residuals were tested.

To simplify the analysis and overcome the problem of zero

concentrations, which degrade homoscedasticity, we decided to group the PAHs according to the IARC classification (Table S1). IARC group 2A was not considered here because the only PAH (dibenz[*a,h*]anthracene) classified in this group was not detected in any sample.

For the comparison among the different experiments, the Σ PAH values found in biochar amended plots in each sampling year and experiment minus their respective control plots (baseline) were considered. The variance (standard error) can be estimated considering that:

$$\text{Var}(\Sigma\text{PAH}_B - \Sigma\text{PAH}_C) = \text{Var}(\Sigma\text{PAH}_B) + \text{Var}(\Sigma\text{PAH}_C) + 2 \text{CoVar}(\Sigma\text{PAH}_B, \Sigma\text{PAH}_C)$$

where: Var = variance; CoVar = covariance; and ΣPAH_B and ΣPAH_C are the values of Σ PAH found in biochar amended and control soils, respectively.

2.6. Health risk assessment

The total toxic equivalent concentrations relative to benzo[*a*]pyrene (BaP_{eq}) were calculated according to Nisbet and Lagoy (1992). This allowed estimating the total cancer risk (TCR) (Liao and Chiang, 2006; Yu et al., 2014).

3. Results and discussion

3.1. Overall evaluation of PAH concentrations found in the soils studied

The individual and total concentrations of PAHs found in the experimental areas (I, II and III) are shown in Tables S4 and S5. The total concentrations of PAHs (Σ PAH) varied between $7.34 \pm 0.83 \text{ ng g}^{-1}$ and $26.3 \pm 6.64 \text{ ng g}^{-1}$ in the control soils, and between $19.2 \pm 0.49 \text{ ng g}^{-1}$ and $39.4 \pm 4.35 \text{ ng g}^{-1}$ in the biochar amended soils (Tables S4 and S5), indicating that in general the treated soils contained higher concentrations of PAHs than the control soils, however the obtained values were lower than the commonly reported in the literature (Fabbri et al., 2013; Nicolini et al., 2015; Rombola et al., 2015; De la Rosa et al., 2016; Kusmierz et al., 2016).

Naphthalene and phenanthrene were the predominant PAHs, followed by pyrene, fluoranthene, perylene and fluorene. These results corroborate those of previous studies of PAHs in biochar

obtained from different feedstocks (Freddo et al., 2012; Keiluweit et al., 2012; Oleszczuk et al., 2014). Acenaphthylene and dibenzo[*a,h*]anthracene were not detected in any of the samples studied (Tables S4 and S5).

None of the PAH concentrations, even in biochar amended plots, approached the maximum permitted levels established by CONAMA (2009) in Brazil or by some European countries (Table S3). Even the concentration of naphthalene, the predominant PAH, which showed a maximum of $23.08 \pm 2.36 \text{ ng g}^{-1}$ (Tables S4 and S5) considering all soil samples, was below the maximum concentration established by some European countries (1000 ng g^{-1}) and Brazilian regulations (8100 ng g^{-1}) (CONAMA, 2009; Kusmierz and Oleszczuk, 2014).

3.2. Evaluation of PAH concentration variations due the interaction time versus biochar application

Despite the very low concentrations of PAHs involved, there were statistically significant differences between treatments up to 5 years after application. In Exp I, which covered the longest period evaluated, with soil sampling at three, five and six years after a single application of 16 Mg of biochar per hectare, the interaction “biochar application” versus “time” was statistically significant for Σ PAH ($p = 8.49 \cdot 10^{-5}$), IARC Group 1 PAH, which contains only benzo[*a*]pyrene (BaP), ($p = 9.83 \cdot 10^{-5}$), and IARC Group 2B PAH (namely naphthalene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and indene[1,2,3-*c,d*]pyrene), ($p = 4.61 \cdot 10^{-3}$). The responses for Σ PAH and IARC Group 1 showed similar patterns (Fig. 1). The highest values were found three years after biochar application, followed by significant decrease five years after application. However, these last values were still larger than in control plots. Finally, there was a steady decrease of PAH concentrations in the biochar plots, falling to the level of the control plots six years after biochar application. On the other hand, for IARC Group 2B, similar Σ PAH values were found, for both treated and control soils, comparing three and five years after treatment, and a significant decrease of PAH concentrations with time in biochar amended plots occurred only six years after the treatment, again falling to the same concentrations as in the control plots (Fig. 1).

In Exp II, the interaction “biochar application” versus “time” was also statistically significant for Σ PAH ($p = 2.66 \cdot 10^{-3}$), IARC Group 1 ($p = 2.18 \cdot 10^{-3}$) and IARC Group 2B ($p = 0.012$). A similar trend, i.e., large PAH concentrations one year after biochar application with subsequent decrease was found (Fig. 2). Σ PAH decreased over time

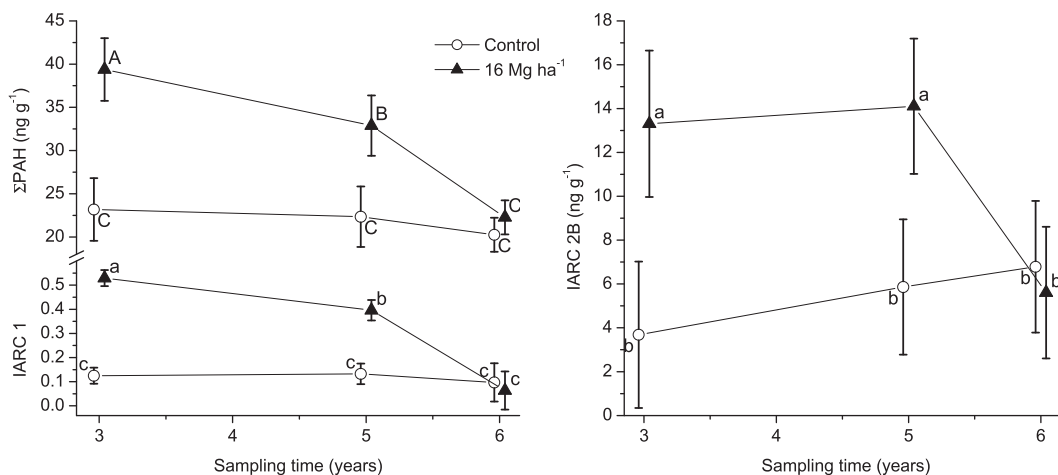


Fig. 1. Post-hoc comparisons of PAH data for Exp I. Left – Variation of total PAH (Σ PAH) and benzo[*a*]pyrene. Right – IARC Group 2B PAHs. Means marked with the same letters (uppercase for Σ PAH) do not differ statistically by Duncan's test ($p = 0.05$). Vertical bars denote the 0.95 confidence interval.

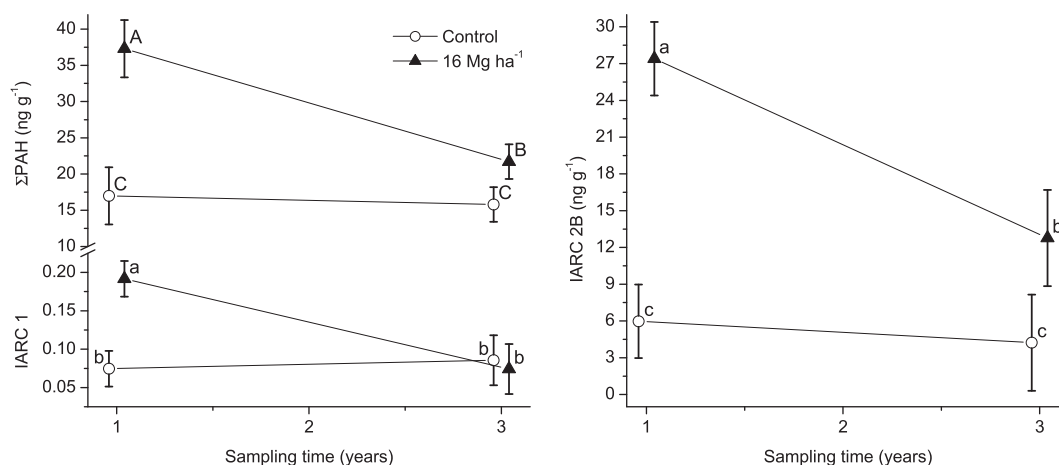


Fig. 2. Post-hoc comparisons of PAH data for the Exp II. Left – Variation of total PAH concentrations (Σ PAH) (ng g^{-1}) and (benzo[a]pyrene). Right - IARC Group 2B PAHs. Means marked with the same letters (uppercase for Σ PAH) do not differ statistically by Duncan's test ($p = 0.05$). Vertical bars denote 0.95 confidence interval.

after biochar application, becoming lower three years after the treatment than found after one year, but still larger than observed in control plots. However, unlike Exp I, in this case, three years was enough to for IARC Group 1 (BaP) concentrations in biochar amended and control plots to be the same. This possibly results of a lower input of benzo(a)pyrene of eucalyptus biochar produced under controlled industrial conditions than native biochar, produced under rustic traditional conditions (Table S4) (Wang et al., 2017). Thus, one year after biochar treatment, the increase of benzo(a)pyrene concentration in the treated soil was only 0.12 ng g^{-1} after industrial kiln biochar treatment, whereas it was 0.40 ng g^{-1} three years after traditional kiln biochar application. The higher soil organic matter content in Exp II than in Exp I, resulting in faster degradation of benzo(a)pyrene in Exp II than Exp I, is another hypothesis (Semple et al., 2003; Nam et al., 2008). On the other hand, the decrease of the concentrations of IARC Group 2B PAHs followed that of Σ PAH, remained larger for the treated plots than for the control plots.

In Exp III, the soils were studied once three years after the biochar application and larger Σ PAH values ($p = 1.59 \cdot 10^{-4}$); IARC Group 2B PAHs ($p = 5.35 \cdot 10^{-6}$); and IARC Group 3 PAHs ($p = 2.28 \cdot 10^{-3}$) were found in biochar amended plots than in the control ones (Fig. 3). However, for this site no statistical difference was observed for IARC Group 1 ($p > 0.19$).

Our results demonstrate that biochar is a source of PAHs in soils, although the dose (16 Mg of biochar per hectare of soil) and conditions employed were not sufficient to surpass national and international acceptable limits of PAHs in soils. Furthermore, our

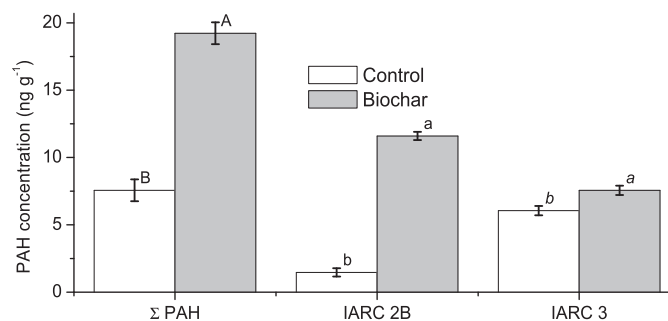


Fig. 3. Post-hoc comparisons of PAH means in Exp III. Means marked with the same letters (uppercase for Σ PAH and italics for IARC 3 PAHs) do not differ statistically by Duncan's test ($p = 0.05$). Vertical bars denote 0.95 confidence interval.

results show that, six years after soil treatment, Σ PAH was similar in both treated and untreated plots, indicating that successive applications of 16 Mg ha^{-1} , with at least a six-year interval, would not result in PAH accumulation in the soil.

The possible causes of Σ PAH decrease over time are microbiological degradation of the heaviest PAHs (five and six rings), volatilization of two-to four-ring PAHs and runoff (Nicolini et al., 2015), besides the adsorption of PAHs by soil organic matter may be another factor causing Σ PAH decrease. Some studies have also reported that biochar accelerates the degradation of PAHs in soils, and can favor the degradation of naturally occurring PAHs (Chen et al., 2012; Quilliam et al., 2013).

Another interpretation of our results can be obtained by comparing the values obtained subtracting the Σ PAH found in the control plots, which can be considered baseline values, from the values found in the biochar amended plots in each sampling year and experiment.

This approach showed that Σ PAH values were similar three years after biochar application in the three studied areas (Fig. 4).

The concentrations of benzo[a]pyrene, classified as carcinogenic to humans by IARC, decreased with time (Figs. 1 and 2) after biochar treatment. No statistical difference was detected between treated and control soils three years after biochar treatment in Exp II and III. On the other hand, in Exp I, the concentration profile of this PAH in biochar amended soils followed that of Σ PAH in these plots, decreasing with time and only becoming equal to that of the control plot six years after biochar application.

The profiles of the IARC Group 2B PAH were very similar of those of Σ PAH, except for Exp I, where the highest concentration was detected in the biochar amended soil five years after the treatment (Fig. 1). A similar increase was also observed in control soils (background levels), indicating possible soil contamination by other sources. However, six years after biochar application, all plots showed similar concentrations.

The IARC Group 3 PAH (PAH not classified as carcinogenic) (Table S1) showed completely different behavior than observed above. Except in Exp III, the application of biochar did not change the concentration of Group 3 PAHs in the studied soils, thus indicating that biochar was not the major source of these PAHs, which have a myriad of environmental sources such as fossil fuel and biomass combustion. Moreover, most of them have low octanol/water partition coefficient (K_{ow}) being more hydrophilic and so are more mobile in the environment than the high K_{ow} PAHs. The large content of some of these PAHs in the biochar treated plot of Exp III

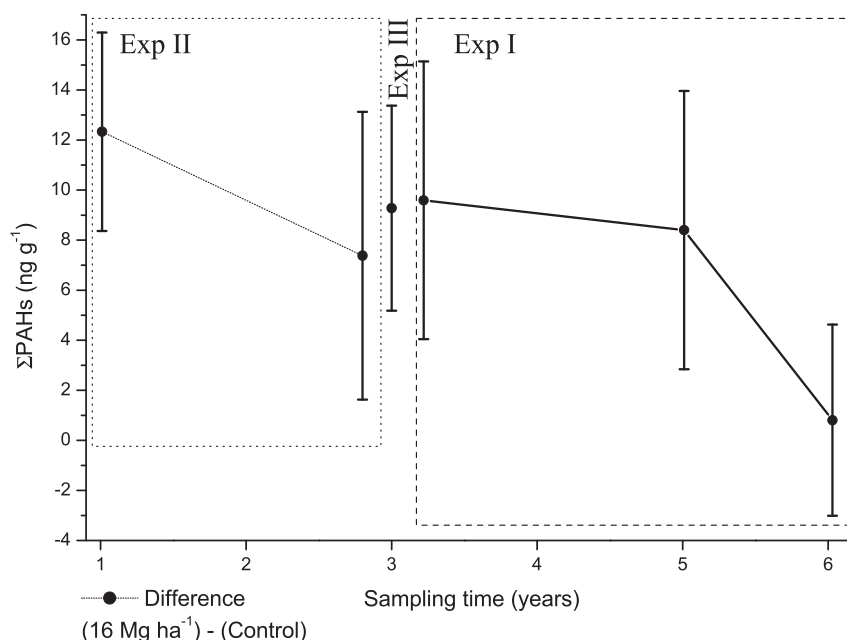


Fig. 4. Estimate of biochar contribution to total PAH concentrations (Σ PAH) (ng g^{-1}) over time. Values obtained subtracting Σ PAH found in control plots from that found in biochar treated plots. Vertical bars denote 0.95 confidence interval.

($p = 2.28 \cdot 10^{-3}$), compared to the control plot, can be due to the higher clay content in this experimental area than Exp I and II, which increased the persistence and immobilization of these PAHs there (Broholm et al., 1999; Carmo et al., 2000).

3.3. Factor analysis

After a varimax rotation of the principal components, the factor scores revealed a clear discrimination of the biochar feedstock (savannah woods or *Eucalyptus* sp.) or production system (traditional or industrial kilns). Factor 1, which accounted for 46% of total variance, is associated with traditional kiln biochar (Fig. 5) and is characterized by high loadings for two four-ringed isomeric PAHs: benz[a]anthracene and chrysene. On the other hand, Factor 2,

responsible for 12% of total variance, with high loadings for naphthalene, was associated with industrial kiln biochar. These factors were labeled as Traditional Kiln Factor and Industrial Kiln Factor. These two factors were correlated with two different PAH groups, respectively IARC Group 2B and IARC Group 3 PAH. This fact, together with the high BaP input from traditional kiln biochar compared to that of industrial kiln, already discussed, indicates that biochar produced in rustic traditional circular kilns might cause more soil contamination. Our results corroborate previous data of Buss et al. (2015), which stated that under uncontrolled pyrolysis conditions the PAH concentrations in biochar are typically increased, although in the conditions studied here, the achieved levels were far below the permitted ones. Anyway, these findings show that at present no generalization is possible concerning the security of biochar application and that the PAH evaluation is necessary for all biochar types.

Since the factor scores are linear combinations of the original random variables, weighted by the factor loadings, they are also random variables and therefore can be treated in this way. Thus, after this systematic grouping and mathematically insightful treatment, the obtained factor scores were analyzed using the same statistical model (repeated measures ANOVA).

The scores for Traditional Kiln Factor in Exp I showed statistical significance for the interaction of biochar versus time ($p = 2.16 \cdot 10^{-3}$) and the largest scores were found three years after biochar application. Although they decreased after five years, they remained larger than in control plots, and continued decreasing until the sixth year, when the scores were the same as those of the control plots (Fig. 6). Similar behavior was observed for Σ PAH and IARC Group 1 (BaP).

On the other hand, for the Industrial Kiln Factor in Exp II, the biochar versus time interaction was also significant ($p = 0.0203$), and only the biochar treatment after one year differed from other treatments and showed a large value (Fig. 6), regardless the PAH group. The fast decrease of this factor, which was equal to the control after three years, is probably due the large volatility of naphthalene, which has the highest vapor pressure among the PAHs (Table S1). However, for Exp III, which was only analyzed

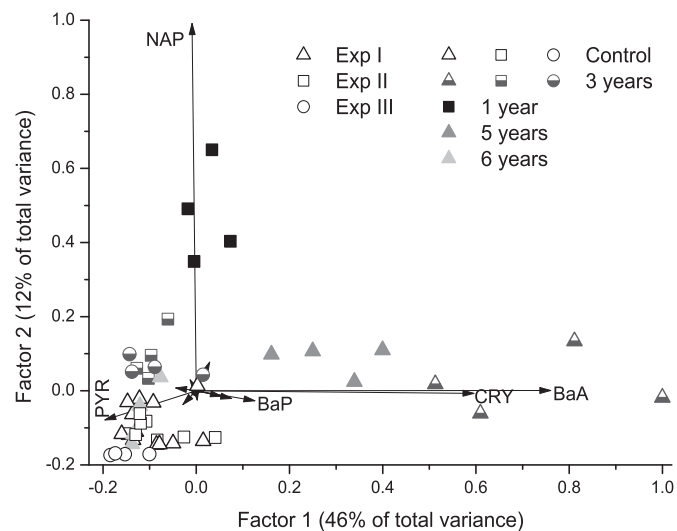


Fig. 5. Bi-plot of factor loadings (vectors) and scores (symbols). Selected PAHs vectors are represented by acronyms: naphthalene (NAP), pyrene (PYR), chrysene (CRY), benz[a]anthracene (BaA) and benzo[a]pyrene (BaP).

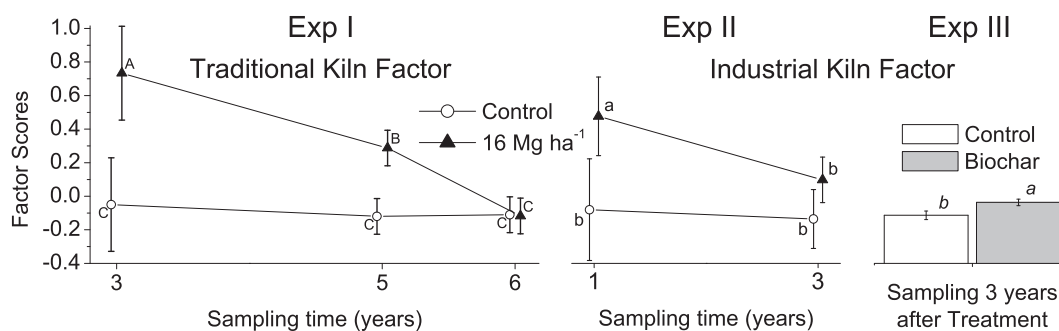


Fig. 6. Variation of factor scores obtained from PAH concentrations over time in Experiments I, II and III. Means marked with the same letters (uppercase for Traditional Kiln Factor in Exp I; lowercase for Exp II; and lowercase italics for Exp III, both Industrial Kiln Factor) do not differ statistically by Duncan's test ($p = 0.05$). Vertical bars denote 0.95 confidence interval.

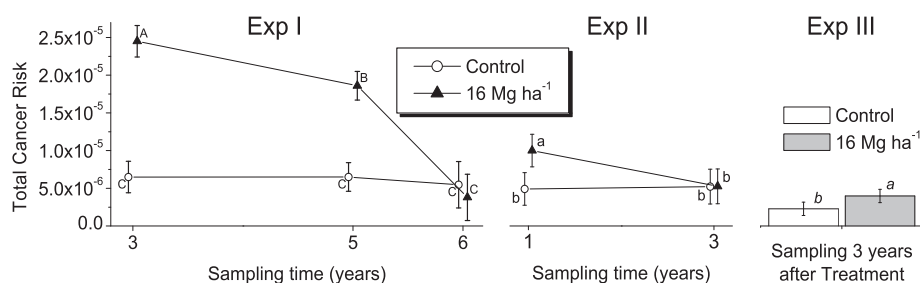


Fig. 7. Variation of total cancer risk (TCR) of PAH over time in Experiments I, II and III. Means marked with the same letters (uppercase for Exp I; lowercase for Exp II; and lowercase italics for Exp III) do not differ statistically by Duncan's test ($p = 0.05$). Vertical bars denote 0.95 confidence interval.

three years after biochar application, this treatment was significant ($p = 2.47 \cdot 10^{-4}$), and again, this result can be explained by the high clay content of this soil compared to that of Exp II (Broholm et al., 1999; Carmo et al., 2000).

3.4. Total cancer risk

Another way of grouping variables and comparing soils and treatments is by calculating the BaPeq and using these values to estimate the TCR. Although PAH levels were far below of the limits of prevention established by the Brazilian and European regulations, the highest TCR was observed in Exp I three years after biochar application ($2.45 \cdot 10^{-5}$ i.e., around two cases in 100,000 people), which is considered a moderate potential cancer risk. On the other hand, in the control plots, the TCR was $6.49 \cdot 10^{-6}$, close to the baseline value of acceptable potential cancer risk (one cancer case per million people) (Maertens et al., 2009).

In Exp I, the response profile of TCR (Fig. 7) was similar to those of \sum PAH and IARC Group 1, showing a statistically significant biochar versus time interaction ($p = 4.60 \cdot 10^{-4}$) with initial increase of TCR after biochar application followed by a decrease with time until reaching the baseline value represented by the control plots, six years after application. In turn, in Exp II, the biochar versus time interaction was also significant ($p = 1.03 \cdot 10^{-5}$), but after TCR increased due to biochar application, three years was sufficient to reduce the TCR value to the same value as the control plots (Fig. 7). In Exp III, the TCR in biochar amended plots was higher than in the control ones ($p = 0.02$) three years after biochar application.

4. Conclusions

The biochar treatment increased the PAH concentrations in the studied soils, however the obtained values were lower than the observed in previous studies and were still two orders of

magnitude below the maximum permissible levels established by the Brazilian and European guidelines. These results obtained under field conditions indicate there is a limited environmental risk of soil contamination after biochar application at a proportion of 16 Mg ha^{-1} under the studied conditions. The \sum PAH, benzo[a]pyrene concentrations and total cancer risk were higher in treated soils after a single biochar application than in control soil plots and they decreased with time, equaling the control plots after three years, in the soil with highest C content (11.2 g kg^{-1}), and after six years in the soil with lowest C content (7 g kg^{-1}). These results suggest that after six years there is no risk of PAH accumulation in the soil, result that contributed to the definition of safes biochar rate and application frequency.

The factor analysis showed some differences of soil contamination, and PAH predominance and elimination related to biochar origin, probably due to the production system (traditional circular bricks kiln or industrial cylindrical metal kiln). This fact points to the need of future investigations about soil resilience, concerning its functionality, to proof that the low concentrations of PAHs added with biochar don't pose any threat. And also adding some biochars with higher PAH concentrations to soil to see how the PAH concentration changes and how the soil reacts as a worst-case scenario. The possible interplay of biomass, pyrolysis conditions, PAH content, soil contamination and its resilience is yet to be established.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.chemosphere.2018.02.139>.

References

- BBodSchV, 1999. GmbH Ein Service des Bundesministeriums der Justiz in Zusammenarbeit mit der juris. Bundes-Bodenschutz- und Altlastenverordnung. <https://www.gesetze-im-internet.de/bbodschv/BBodSchV.pdf>.
- Blumer, M., 1976. Polycyclic aromatic-compounds in nature. *Sci. Am.* 234, 35–45.
- Broholm, M.M., Broholm, K., Arvin, E., 1999. Sorption of heterocyclic compounds on natural clayey till. *J. Contam. Hydrol.* 39, 183–200.
- Buss, W., Masek, O., 2016. High-VOC biochar—effectiveness of post-treatment measures and potential health risks related to handling and storage. *Environ. Sci. Pollut. Res.* 23, 19580–19589.
- Buss, W., Masek, O., Graham, M., Wust, D., 2015. Inherent organic compounds in biochar—Their content, composition and potential toxic effects. *J. Environ. Manag.* 156, 150–157.
- Buss, W., Grahamb, M.C., MacKinnon, G., Masek, O., 2016. Strategies for producing biochars with minimum PAH contamination. *J. Anal. Appl. Pyrolysis* 119, 24–30.
- Carmo, A.M., Hundal, L.S., Thompson, M.L., 2000. Sorption of hydrophobic organic compounds by soil materials: application of unit equivalent Freundlich coefficients. *Environ. Sci. Technol.* 34, 4363–4369.
- Chen, B.L., Yuan, M.X., Qian, L.B., 2012. Enhanced bioremediation of PAH-contaminated soil by immobilized bacteria with plant residue and biochar as carriers. *J. Soils Sediments* 12, 1350–1359.
- Chen, P., Sun, M.X., Zhu, Z.X., Zhang, J.D., Shen, G.Q., 2015. Optimization of ultrasonic-assisted extraction for determination of polycyclic aromatic hydrocarbons in biochar-based fertilizer by gas chromatography—mass spectrometry. *Anal. Bioanal. Chem.* 407, 6149–6157.
- National Environment Council (CONAMA), 2009. Resolução N° 420, de 28 de Dezembro de 2009. <http://www.mma.gov.br/port/Conama/res/res09/res42009.pdf>.
- Dai, Q., Jiang, X., Jiang, Y., Jin, Y., Wang, F., Chi, Y., 2014. Temperature influence and distribution in three phases of PAHs in wet sewage sludge pyrolysis using conventional and microwave heating. *Energy Fuels* 28, 3317–3325.
- De la Rosa, J.M., Paneque, M., Hilber, I., Blum, F., Knicker, H.E., Bucheli, T.D., 2016. Assessment of polycyclic aromatic hydrocarbons in biochar and biochar-amended agricultural soil from Southern Spain. *J. Soils Sediments* 16, 557–565.
- Dimitriou, K., Kassomenos, P., 2017. Aerosol contributions at an urban background site in Eastern Mediterranean – potential source regions of PAHs in PM10 mass. *Sci. Total Environ.* 598, 563–571.
- European Biochar Certificate (EBC), 2015. Guidelines for a Sustainable Production of Biochar. Version 6.3E. <http://www.european.biochar.org/biochar/media/doc/ebc-guidelines.pdf>.
- Fabbri, D., Rombola, A.G., Torri, C., Spokas, K.A., 2013. Determination of polycyclic aromatic hydrocarbons in biochar and biochar amended soil. *J. Anal. Appl. Pyrolysis* 103, 60–67.
- Franco, C.F.J., Resende, M.F., Furtado, L.A., Brasil, T.F., Eberlin, M.N., Pereira Netto, A.D., 2017. Polycyclic aromatic hydrocarbons (PAHs) in street dust of Rio de Janeiro and Niterói, Brazil: Particle size distribution, sources and cancer risk assessment. *Sci. Total Environ.* 599, 305–313.
- Freddo, A., Cai, C., Reid, B.J., 2012. Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar. *Environ. Pollut.* 171, 18–24.
- Hale, S.E., Lehmann, J., Rutherford, D., Zimmerman, A.R., Bachmann, R.T., Shitumbanuma, V., O'Toole, A., Sundqvist, K.L., Arp, H.P.H., Cornelissen, G., 2012. Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and dioxins in biochars. *Environ. Sci. Technol.* 46, 2830–2838.
- International Biochar Initiative (IBI), 2015. Standardized Product Definition and Product Testing Guidelines for Biochar that Is Used in Soil (Aka Ibi BiocharStandards). Version 2.1. http://www.biochat-international.org/sites/default/files/IBI_Biochar_Standards_V2.1_Final.pdf.
- International Programme on Chemical Safety (IPCS), 1998. Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons. World Health Organization. www.inchem.org/documents/ehc/ehc/ehc202.htm.
- Kamal, A., Cincinelli, A., Martellini, T., Malik, R.N., 2015. A review of PAH exposure from the combustion of biomass fuel and their less surveyed effect on the blood parameters. *Environ. Sci. Pollut. Res.* 22, 4076–4098.
- Keiluewei, M., Kleber, M., Sparrow, M.A., Simoneit, B.R.T., Prah, F.G., 2012. Solvent-extractable polycyclic aromatic hydrocarbons in biochar: influence of pyrolysis temperature and feedstock. *Environ. Sci. Technol.* 46, 9333–9341.
- Khalid, F.N.M., Klarup, D., 2015. The influence of sunlight and oxidative treatment on measured PAH concentrations in biochar. *Environ. Sci. Pollut. Res.* 22, 12875–12981.
- Kim, K.H., Jahan, S.A., Kabir, E., Brown, R.J.C., 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environ. Int.* 60, 71–80.
- Kloss, S., Zehetner, F., Dellantonio, A., Hamid, R., Ottner, F., Liedtke, V., 2012. Characterization of slow pyrolysis biochars: effects of feedstocks and pyrolysis temperature on biochar properties. *J. Environ. Qual.* 41, 990–1000.
- Kusmierz, M., Oleszczuk, P., 2014. Biochar production increases the polycyclic aromatic hydrocarbon content in surrounding soils and potential cancer risk. *Environ. Sci. Pollut. Res.* 21, 3646–3652.
- Kusmierz, M., Oleszczuk, P., Kraska, P., Palys, E., Andruszczak, S., 2016. Persistence of polycyclic aromatic hydrocarbons (PAHs) in biochar-amended soil. *Chemosphere* 146, 272–279.
- Liao, C.M., Chiang, K.C., 2006. Probabilistic risk assessment for personal exposure to carcinogenic polycyclic aromatic hydrocarbons in Taiwanese temples. *Chemosphere* 63, 1610–1619.
- Madari, B.E., Silva, M.A.S., Carvalho, M.T.M., Maia, A.H.N., Petter, F.A., Santos, J.L.S., Tsai, S.M., Leal, W.G.O., Zevianie, W.M., 2017. Properties of a sandy clay loam Haplic Ferralsol and soybean grain yield in a five-year field trial as affected by biochar amendment. *Geoderma* 305, 100–112.
- Maertens, R.M., Yang, X.F., Zhu, J.P., Gagne, R.W., Douglas, G.R., White, P.A., 2009. Mutagenic and carcinogenic hazards of settled house dust I: polycyclic aromatic hydrocarbon content and excess lifetime cancer risk from preschool exposure. *Environ. Sci. Technol.* 42, 1747–1753.
- Nam, J.J., Thomas, G.O., Jaward, F.M., Steinnes, E., Gustafsson, O., Jones, K.C., 2008. PAHs in background soils from Western Europe: influence of atmospheric deposition and soil organic matter. *Chemosphere* 70, 1596–1602.
- Nicolini, J., Khan, M.Y., Matsui, M., Cocco, L.C., Yamamoto, C.I., Lopes, W.A., de Andrade, J.B., Pillon, C.N., Arizaga, C.G.G., Mangrich, A.S., 2015. Evaluation of PAH contamination in soil treated with solid by products from shale pyrolysis. *Environ. Monit. Assess.* 187, 4123–4133.
- Nisbet, I.C., Lagoy, P.K., 1992. Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). *Regul. Toxicol. Pharmacol.* 16, 290–300.
- National Oceanic and Atmospheric Administration (NOAA), 2006. Organic Contaminant Analytical Methods of the National Status and Trends Program: Update 2000–2006, NOAA Technical Memorandum NOS NCCOS 30. <http://aquaticcommons.org/2116/1/organicsmethods.pdf>.
- Novotny, E.H., Maia, C.M.B.F., Carvalho, M.T.M., Madari, B.E., 2015. Biochar: pyrogenic carbon for agricultural use—a critical review. *Rev. Bras. Cir. Solo* 39, 321–344.
- Oleszczuk, P., Josko, I., Kusmierz, M., 2014. Biochar properties regarding to contaminants content and ecotoxicological assessment. *J. Hazard Mater.* 260, 375–382.
- Peng, C., Chen, W.P., Liao, X.L., Wang, M.E., Ouyang, Z.Y., Jiao, W.T., Bai, Y., 2011. Polycyclic aromatic hydrocarbons in urban soils of Beijing: status, sources, distribution and potential risk. *Environ. Pollut.* 159, 802–808.
- Pereira Netto, A.D., Cunha, I.F., Krauss, T.M., 2004. Persistence of polycyclic aromatic hydrocarbons in the soil of a burned area for agricultural purposes in Brazil. *Bull. Environ. Contam. Toxicol.* 73, 1072–1077.
- Quilliam, R.S., Rangelcroft, S., Emmett, B.A., Deluca, T.H., Jones, D.L., 2013. Is biochar a source or sink for polycyclic aromatic hydrocarbon (PAH) compounds in agricultural soils? *G.C.B. Bioenergy* 5, 96–103.
- Roberts, K.G., Gloy, B.A., Joseph, S., Scott, N.R., Lehmann, J., 2010. Life cycle assessment of biochar systems: estimating the energetic, economic, and climate change potential. *Environ. Sci. Technol.* 44, 827–833.
- Rombola, A.G., Meredith, W., Snape, C.E., Baronti, S., Genesio, L., Vaccari, F.P., Miglietta, F., Fabbri, D., 2015. Fate of soil organic carbon and polycyclic aromatic hydrocarbons in a Vineyard soil treated with biochar. *Environ. Sci. Technol.* 49, 11037–11044.
- Schmidt, M.W.I., Noack, A., 2000. Black carbon in soils and sediments: analysis, distribution, implications, and current challenges. *Global Biogeochem. Cycles* 14, 777–793.
- Semple, K.T., Morris, A.W.J., Paton, G.I., 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concept and techniques for analysis. *Eur. J. Soil Sci.* 54, 809–818.
- Stefaniuk, M., Oleszczuk, P., Bartmiski, P., 2016. Chemical and ecotoxicological evaluation of biochar produced from residues of biogas production. *J. Hazard. Mat.* 318, 417–424.
- Wang, W., Huang, M.J., Kang, Y., Wang, H.S., Leung, A.O.W., Cheung, K.C., Wong, M.H., 2011. Polycyclic aromatic hydrocarbons (PAHs) in urban surface dust of Guangzhou, China: status, sources and human health risk assessment. *Sci. Total Environ.* 409, 4519–4527.
- Wang, C., Wang, Y., Herath, H.M.S.K., 2017. Polycyclic aromatic hydrocarbons (PAHs) in biochar – their formation, occurrence and analysis: a review. *Org. Geochem.* 114, 1–11.
- Weidemann, E., Buss, W., Edo, M., Masek, O., 2017. Influence of pyrolysis temperature and production unit on formation of selected PAHs, oxy-PAHs, N-PACs, PCDDs, and PCDFs in biochar—a screening study. *Environ. Sci. Pollut. Res.* 1–8.
- Yu, B.B., Xie, X.J., Ma, L.Q., Kan, H., Zhou, Q.X., 2014. Source, distribution, and health risk assessment of polycyclic aromatic hydrocarbons in urban street dust from Tianjin, China. *Environ. Sci. Pollut. Res.* 21, 2817–2825.
- Zhang, Y.X., Tao, S., 2009. Global atmospheric emission inventory of polycyclic aromatic hydrocarbons (PAHs) for 2004. *Atmos. Environ.* 43, 812–819.
- Zielinska, A., Oleszczuk, P., 2015. The conversion of sewage sludge into biochar reduces polycyclic aromatic hydrocarbon content and ecotoxicity but increases trace metal content. *Biomass Bioenergy* 75, 235–244.