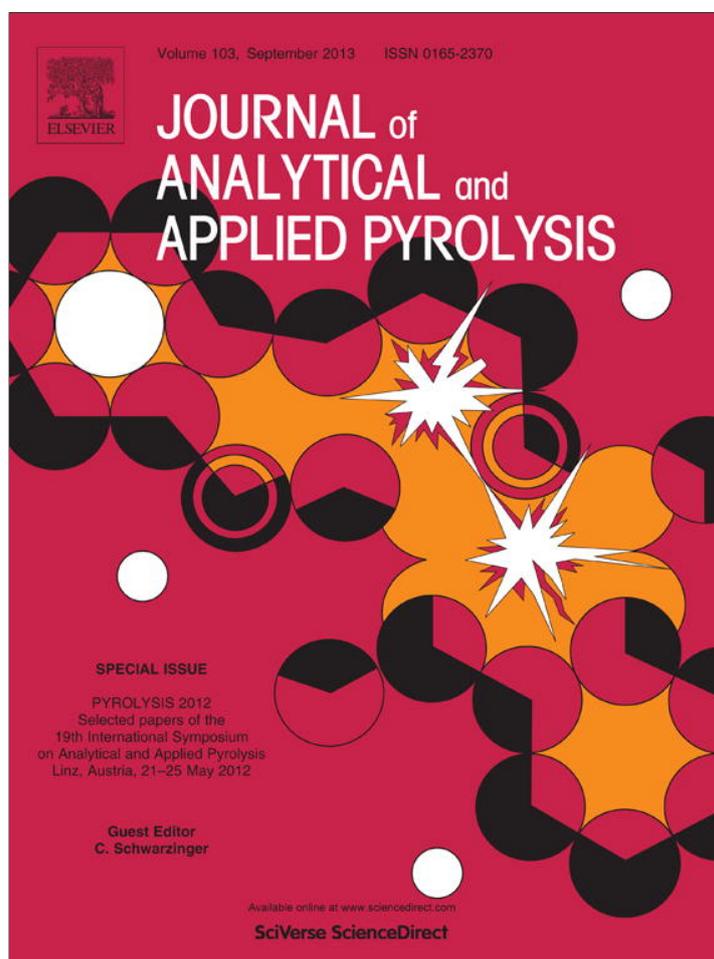


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Determination of polycyclic aromatic hydrocarbons in biochar and biochar amended soil

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ABSTRACT

A method for the determination of the 16 USEPA polycyclic aromatic hydrocarbons (PAHs) in biochar and biochar amended soil was developed. Samples were Soxhlet extracted with an acetone/cyclohexane (1:1) solvent mixture, and PAHs were analyzed by GC–MS after silica gel clean-up. In a comparative study based on reflux extraction, the Soxhlet solvent system acetone/cyclohexane exhibited a higher extraction efficiency of low molecular weight PAHs (e.g. naphthalene) than toluene or dichloromethane. Utilizing a reference biochar, this Soxhlet method possessed a 67–88% recovery of spiked deuterated PAHs (acenaphthene, phenanthrene, and chrysene), analytical precision (as assessed by relative standard deviations) between 5 and 18%, and a limit of detection in the 0.01–0.4 ng g⁻¹ range. The method was successfully validated through the analysis of a certified soil material, and was capable to quantify total PAHs following biochar addition at 1% (w/w). The concentration of the 16 USEPA-PAHs along with the 15 EU-PAHs (priority hazardous substances in food) was determined in a suite of currently available biochars for agricultural field applications, which were derived from a variety of parent materials and pyrolysis conditions. The total PAH levels ranged between 1.2–19 μg g⁻¹ and 0.2–5 μg g⁻¹ interval for USEPA and EU PAHs, respectively. Specifically, benzo[*a*]pyrene ranged between 0.01 and 0.67 μg g⁻¹ across these various biochars. Considering an application of 20–60 t biochar ha⁻¹, the degree of PAH contamination will be dependent on both the presence of background PAHs in soil and the sorbed concentrations of PAHs on the biochar. Our data, along with PAH levels determined in other studies, suggest that biochars produced by slow pyrolysis from woody biomass possess the lowest level of sorbed PAHs (<10 μg g⁻¹).

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1. Introduction

Biochar is a co-product from biomass pyrolysis that is targeted as a material with applications in environmental and agricultural management, as well as a vehicle for carbon sequestration [1,2]. As the interest toward biochar is steeply growing, safety procedures for ensuring human health and preservation of the environment are imperative. Polycyclic aromatic hydrocarbons (PAHs) are well known carcinogenic and persistent pollutants that are ubiquitous in the environment. PAHs are formed during the pyrolysis of biomass [3] and their occurrence in biochar [4–9] along with its possibly released into the environment need to be addressed. PAH production has also been confirmed during the production of charcoal by pyrolysis [10,11] and natural wildfires [12]. Human exposure of PAHs might occur through different pathways, such as inhalation

of particles generated during synthesis, handling and field applications of biochar or the ingestion of fruit/vegetables grown in biochar amended soil. Therefore, determining the content of PAHs in biochar is of utmost importance to establish risk assessment of biochar usage.

The worldwide distribution of PAHs in soils span over five orders of magnitude and is related to source (atmospheric input) and sorption ability of soil organic matter and black carbon [13]. The inclusion of carbonaceous residues in soil could increase PAHs sorption on humic matter [14–17] and biochar [18,19]. In this respect, soil application of biochar might represent a source and/or a sink of PAHs. All these aspects need to be considered when dealing with the origin of PAHs in soil amended with biochar.

A reliable methodology of PAH analysis is a first requisite toward risk assessment. Recent studies have examined the content of PAHs in biochar [4,5,7]. These results have provided a comprehensive picture on the levels and availability of PAHs in biochar [4], the influence of pyrolysis temperature [7], as well as critical aspects of validation [5]. Analytical methods described in these studies have utilized toluene as the extracting solvent. In fact, it was

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demonstrated that toluene is superior to other solvents for carbonaceous materials [20]. Nonetheless, extraction efficiencies are not always quantitative, especially in the case of low molecular weight (LMW) PAHs. In particular, naphthalene is problematic because of the high boiling point of toluene (111 °C) which causes the loss of semi-volatile PAHs during the preconcentration step [5,7]. Naphthalene is considered a possible carcinogenic to humans (IARC group 2B) and genotoxic to plants [21]. Incidentally, naphthalene is often the most abundant PAH in biochar [4–6,8,9,22]. Naphthalene and its isotopically labelled version are often employed in studies aimed at investigating the fate of PAHs in the environment [23–26]. In general, LMW PAHs are absorbed at higher rates than high molecular weight (HMW) PAHs [25,27], and naphthalene presence could affect the growth/response of the soil microbial community [28,29].

Although present at lower concentrations, HMW PAHs pose the highest health and environmental hazards due to the established carcinogenic potential of this class of compounds. Because of biochar's proposed use in crops and potential human exposure of biochar PAHs through bioaccumulation in agricultural products, biochar sorbed PAH concentrations could be a matter of concern [30–32]. On the basis of their occurrence and carcinogenicity, 15 PAHs have been identified as priority hazardous substances in food by the European Union (EU) [33] and 16 PAHs by US Environmental Protection Agency (USEPA) [34], 8 of them are shared across both lists. While studies have been reported on the occurrence of USEPA PAHs in biochar due to the widespread inclusion of these compounds in worldwide environmental legislation, very limited information is available on the occurrence of EU PAHs on biochar.

In addition, recent studies were focused on the analysis of PAHs in solely biochar, but the robustness of the solvent extraction method to extract PAHs when biochar is present in the soil was not fully investigated. It is important that a method developed for the analysis of solely biochar should be equally accurate for the biochar-soil matrix. In this context, the use of (cyclo)hexane/acetone mixtures as an extracting solvent in PAH determination in soil is rather common (e.g. [35–37]). In fact, a relatively polar solvents like acetone has been cited as beneficial for the extraction of hydrophobic PAHs from soil [38].

The present study is aimed at developing a well characterized method for the determination of PAH in biochars and soils amended with biochar by GC–MS. To this purpose, several solvent and extraction procedures were examined using the 16 EPA PAHs as targeted PAHs on a biochar utilized in agronomic field studies [39]. The method was then applied to a set of biochars investigated as soil amendments of different origin and from different process conditions [40]. Besides the EPA PAHs, the level of EU PAHs in these biochars was investigated as well.

2. Materials and methods

2.1. Reagents and standards

Cyclohexane, acetone, acetonitrile, dichloromethane, toluene, ethyl acetate (all ultra-purity), and surrogate standard mix (for USEPA 525) containing acenaphthene-*d*₁₀, phenanthrene-*d*₁₀ and chrysene-*d*₁₂ at concentrations of 500 mg l⁻¹ each in acetone were purchased from Sigma–Aldrich. PAH-Mix solution containing naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene certified at concentrations of 10 mg l⁻¹ for each species in acetonitrile was purchased from Sulpeco (Bellefonte, PA, USA). PAH-Mix standards in acetonitrile (10 mg l⁻¹) of EU PAHs

were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany): benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, cyclopenta[*c,d*]pyrene, dibenzo[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*c,d*]pyrene, 5-methylchrysene. Standard mix solutions containing the 15 PAHs at concentrations of 1 mg l⁻¹ were prepared in acetone/cyclohexane (1:1, v/v) and stored at room temperature in the dark.

A solution of 1,3,5-tri-tert-butylbenzene (TTB, 12.7 mg l⁻¹) in acetone:cyclohexane (1:1, v/v) was prepared by weighing the pure compound purchased from Sigma–Aldrich.

2.2. Soil and biochar samples

A natural matrix soil certified reference material ERM–CC013a (manufactured by Federal Institute for Materials Research and Testing; Berlin, Germany) containing 15 PAHs with concentrations ranging from 1.14 to 12.9 mg kg⁻¹ was used for the validation of the method for soil. An internal reference biochar sample (here named as reference biochar, or RB) was utilized for method optimization. This was a commercially available biochar created by the slow pyrolysis of orchard pruning, which was kindly provided by the Department of Agriculture and Environmental Sciences (DISA) University of Udine [39]. This reference biochar was homogenized and then mixed with an agricultural soil (dried and sieved 2 mm) at a 1.16% (w/w) amendment level. This concentration corresponded to an application of 36 t biochar ha⁻¹ (assuming a soil with 1.2 g cm⁻³ density and 0.3 m depth) [6,41], which is within the range currently investigated for biochar use in agriculture (20–60 t biochar ha⁻¹) [42].

Additional biochars evaluated were from an ongoing study on the impact of biochar additions on greenhouse gas production potentials conducted by the USDA–ARS Biochar and Pyrolysis Initiative. The full characterization of these biochars (i.e. ultimate and proximate analysis, Py–GC–MS, and microbial CO₂ production) was reported in a previous publication [40]. This group provides a cross-section of currently available biochars for agricultural field applications.

2.3. Sample treatment

2.3.1. Optimized sample pretreatment: soxhlet extraction and clean up

About 1 g of biochar (or 5 g soil sample) was placed into the extraction cellulose thimble, spiked with 0.1 ml of surrogate standard mix (Supelco for EPA 525 containing acenaphthene-*d*₁₀, phenanthrene-*d*₁₀ and chrysene-*d*₁₂ 5 μg ml⁻¹ each in acetonitrile). The thimble was covered with cotton wool, and inserted into the Soxhlet extractor. Soxhlet extraction thimbles (and the Soxhlet apparatus) were pre-cleaned by a 4 h Soxhlet extraction with acetone/cyclohexane (1:1, v/v). Extraction was carried out with 160 ml of extraction solvents (acetone/cyclohexane (1:1, v/v)) mixture for 36 h (4 cycles h⁻¹). The Soxhlet apparatus was covered with an aluminum foil to avoid exposure to daylight, which prevents PAH photodegradation. The extraction solvent was filtered, added with 1 ml of *n*-nonane, and then carefully evaporated by rotatory vacuum evaporation at 40 °C.

The concentrated extract was collected and loaded onto a silica gel cartridge (6 ml, 1 g DSC–Si Supelco washed with ethyl acetate, dried and conditioned with 4 ml cyclohexane). After purification with 1 ml of cyclohexane, PAHs were eluted with 4 ml of acetone/cyclohexane (1:1, v/v). The obtained solution was then blown down to 10–50 μl under nitrogen and spiked with 10 μl of the internal standard solution (TTB at 12.7 mg l⁻¹) prior to GC–MS analysis.

2.3.2. Reflux extraction

Four different solvent systems (toluene, dichloromethane, acetone:cyclohexane 1:1 (v/v) and acetone:cyclohexane 1:5 (v/v)) were compared by means of reflux extraction. To this purpose PAHs were extracted from the biochar (2 g reference biochar added with 0.1 ml of surrogate standard mix) by refluxing for 4 h with 80 ml solvent. The extract was filtered and concentrated to ~100 μl by using rotary evaporator and then under a nitrogen stream. The obtained solution was spiked with 10 μl of internal standard (12.7 mg l^{-1} TTB) and then analyzed by GC–MS.

2.3.3. Ultrasonication extraction

Each homogenized reference biochar sample (1 g) was transferred into a Pyrex tube, and 20 ml of acetone/cyclohexane (1:1, v/v) were added. The sample was ultrasonicated for 30 min with occasional swirling. The extraction solutions were then centrifuged and the supernatant filtered into a 50 ml beaker using a 9.0 cm GF/C glass microfibre filter (Whatman International, Maidstone, UK). The obtained solutions were reduced to 2 ml using a rotary evaporator and transferred into 4 ml vials. These solutions were further reduced using nitrogen gas, spiked with 10 μl of 12.7 mg l^{-1} TTB, and analyzed by GC–MS.

2.4. GC–MS

GC–MS analyses were performed using a 6850 Agilent HP gas chromatograph connected to a 5975 Agilent HP quadrupole mass spectrometer. Analytes were separated by a HP-5MS fused-silica capillary column (stationary phase poly[5% diphenyl/95% dimethyl]siloxane, 30 m \times 0.25 mm i.d., 0.25 mm film thickness), using helium as the carrier gas. Samples (1 μl) were injected under splitless conditions (1 min, then split ratio 1:50 to the end of analysis) with an injector temperature of 280 $^{\circ}\text{C}$. The following thermal program of the capillary column was used: 50 $^{\circ}\text{C}$ to 100 $^{\circ}\text{C}$ at 20 $^{\circ}\text{C min}^{-1}$, then from 100 $^{\circ}\text{C}$ to 300 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C min}^{-1}$, then a hold for 2.5 min at 300 $^{\circ}\text{C}$. The mass spectrometer operated under electron ionization (70 eV) and acquisition was performed on single ion monitoring (SIM) at the molecular ion of each PAH at the time windows corresponding to the elution region of the target PAH. Acenaphthene- d_{10} was utilized to quantify naphthalene, acenaphthylene, acenaphthene and fluorene; phenanthrene- d_{10} to quantify phenanthrene, anthracene, fluoranthene and pyrene; chrysene- d_{12} to quantify the remaining PAHs. Quantitation of EPA PAHs was based on the calibration curve (Section 2.5), while in the case of EU PAHs a single point calibration (1 mg l^{-1} , Section 2.1) was utilized.

2.5. Method validation

The figures of merit were reported for the EPA PAHs. Recovery of surrogated PAHs were determined with respect to the internal standard TTB. The procedural blank concentrations were determined as the average of five empty thimble runs. Procedural blanks were run periodically. Precision of the procedure was determined by four replicate analyses of reference biochar sample.

Calibration was performed in the 0.0025–1.25 mg l^{-1} interval by serial dilutions of the 10 $\mu\text{g ml}^{-1}$ EPA PAH calibration mix (Supelco). Three replicates were performed at each concentration level and the resulting instrumental response was homoscedastic for each PAH ($\alpha = 0.05$, Cochran test), therefore the least-squares regression line was utilized for quantification (R^2 values were 0.993–0.999).

Limit of detection (LOD) and limit of quantification (LOQ) were estimated for each analyte by using Eqs. (1) and (2)

$$\text{LOD} = \frac{3s_b}{a} \quad (1)$$

Table 1

Limits of detection (LOD), limits of quantification (LOQ), mean concentration of USEPA PAHs in reference biochar (RB) and relative standard deviations (RSD) from four replicates.

PAH	LOD (ng g^{-1})	LOQ (ng g^{-1})	RB ($\mu\text{g g}^{-1}$)	RSD (%)
Naphthalene	0.08	0.2	1.75	8
Acenaphthylene	0.01	0.03	0.026	13
Acenaphthene	0.03	0.1	0.034	5
Fluorene	0.03	0.1	0.071	10
Phenanthrene	0.4	1	0.71	12
Anthracene	0.03	0.1	0.13	13
Fluoranthene	0.08	0.3	0.30	11
Pyrene	0.06	0.2	0.35	11
Chrysene	0.1	0.4	0.095	9
Benzo[a]anthracene	0.08	0.3	0.095	9
Benzo[b]fluoranthene	0.2	0.5	0.13	6
Benzo[k]fluoranthene	0.09	0.3	0.10	18
Benzo[a]pyrene	0.2	0.8	0.19	14
Indeno[1,2,3- <i>cd</i>]pyrene	0.2	0.7	0.15	16
Dibenzo[a,h]anthracene	0.3	0.9	0.056	15
Benzo[ghi]perylene	0.1	0.4	0.15	8

$$\text{LOQ} = \frac{10s_b}{a} \quad (2)$$

where s_b stands for the mean standard deviation of peak areas integrated at the retention time of the PAH from procedural blanks and a for the slope of the calibration curve. Results of LOD, LOQ (calculated for biochar) and precision (%RSD) are listed in Table 1.

3. Results and discussion

3.1. Solvent selection

The choice of the extracting solvent is a crucial parameter in the analysis of PAHs in carbonized materials (soot, charcoal) because hydrophobic contaminants are tightly bound to the aromatic matrix [20]. In this study, the extraction ability of four different solvent systems was preliminary evaluated by means of reflux extraction under the same conditions. Toluene, solely [4,5] or mixed with methanol [7], was the solvent of choice in the determination of PAHs in biochar reported in recent literature and therefore included in this comparison. Dichloromethane is a rather common solvent in the extraction of PAHs in several matrices, including wood chars [43] and biochar [8]. Acetone/hexane mixtures were described in the analysis of PAHs in charcoal and soot samples [20].

The recovery of surrogate PAHs for each extraction system is reported in Table 2. Toluene is the best extracting solvent in the case of spiked *d*-phenanthrene and *d*-chrysene. This finding is in agreement with previous studies showing the strong extraction efficiency of toluene in comparison to other solvents and solvent/mixtures [5,20]. However, in the case of spiked *d*-acenaphthene, dichloromethane and acetone/cyclohexane 1:1 exhibited higher extraction efficiency than toluene (83 and 80% vs. 68%). The loss of LMW PAHs in the case of toluene was caused by the analytical procedure following the extraction step, as blank analysis with toluene (resulting from solvent evaporation) confirmed a recovery of 65 \pm 11% of *d*-acenaphthene. A similar result was reported by Hilber et al. [5], who suspected a cross-contamination by naphthalene possibly due to extended toluene removal. When examining the PAH concentrations as a function of solvent (Table A1 in supplementary materials), the detected concentrations of the LMW PAHs were the lowest with toluene (0.84 $\mu\text{g g}^{-1}$) and highest with acetone/cyclohexane 1/1 (1.37 $\mu\text{g g}^{-1}$). Therefore, the solvent mixture of acetone/cyclohexane was selected for the method optimization, because of its superior extraction efficiency for naphthalene (the most common PAH detected on biochar; see

Table 2
Recovery of surrogate PAHs using different extraction procedures of reference biochar (RB).

	Acenaphthene- <i>d</i> ₁₀ recovery (%)	Phenanthrene- <i>d</i> ₁₀ recovery (%)	Chrysene- <i>d</i> ₁₂ recovery (%)
Reflux extraction			
Acetone/cyclohexane 1/1	80	41	7
Acetone/cyclohexane 1/5	56	38	7
Dichloromethane	83	50	11
Toluene	68	68	58
Ultrasonication extraction			
Acetone/cyclohexane 1/1	9	4	0.4
Soxhlet extraction (18 h)			
Acetone/cyclohexane 1/1	75	66	29
Acetone/cyclohexane 5/1	76	37	10
Acetone	84	58	29
Soxhlet extraction (36 h)			
Acetone/cyclohexane 1/1	88	77	67

below), its widespread use in soil analysis of PAHs, and its reduced toxicity compared to toluene and dichloromethane.

3.2. Selection of the extraction procedure

The recovery of surrogate PAHs from reflux extraction with acetone:cyclohexane 1:1 were compared with Soxhlet extraction (18 h) and ultrasonic extraction (Table 2). Ultrasonic extraction had very low recoveries (<10%) and therefore was not investigated further. As expected, the recovery of *d*-chrysene by Soxhlet extraction increased with respect to reflux conditions. Increasing (100%, v/v) or decreasing (20%, v/v) the mixing ratio of acetone with respect to the 1:1 acetone:cyclohexane mixture (i.e. 50%, v/v) did not significantly improve the recovery of the surrogate PAHs. Therefore, the acetone:cyclohexane mixture 1:1 was selected to investigate the effect of the extraction time on the recovery. The results, depicted in Fig. 1, show that the higher recoveries were achieved with longer extraction times, which is in agreement with a previous study [5]. Interestingly, the same study showed that accelerated solvent extraction (ASE) was a less efficient than Soxhlet extraction [5]. However, prolonged extractions were problematic and did not guaranteed high recovery.

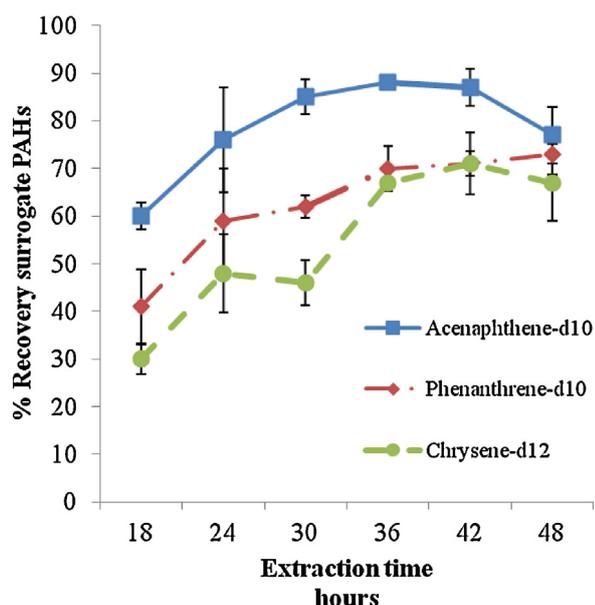


Fig. 1. Recovery of deuterated PAHs vs. soxhlet extraction times with acetone:cyclohexane 1:1 (v/v) of reference biochar (mean values and 1 s.d. from four replicates).

We decided to focus on the behavior of two HMW PAHs representative of five rings (benzo[*a*]pyrene) and six (indeno[1,2,3, *cd*]pyrene) rings as the target compounds for optimizing the extraction time (Table A2 in supplementary materials). Their concentrations increased significantly when the extraction time was increased from 18 to 36 h, after which time the concentration remained almost constant. Thus, 36 h of extraction were selected for the final procedure.

3.3. Final procedure applied to reference biochar and soil

The final procedure was described in detail in Section 2.3.1. The USEPA PAH concentrations of reference biochar are reported in Table 1 along with the relative standard deviations. A typical chromatogram is presented in Fig. 2. The precision (expressed as RSD from four replicates) was good, being within the 5–18% interval. The recoveries of surrogate PAHs were satisfactory (67, 77, and 88% for *d*-acenaphthene, *d*-phenanthrene, and *d*-chrysene, respectively, Table 2). This is also considered a good result considering that PAHs are strongly associated to the aromatic carbonaceous matrix of biochar. These results are on the higher end of PAH recoveries currently reported for biochar materials. Hilber et al. [5] reported 42–72% recovery range for several deuterated PAHs (from *d*-naphthalene to *d*₁₂-indeno[1,2,3-*cd*]pyrene), and similar values (56–79%) were reported by Hale et al. [4].

The accuracy of the method developed for biochar was tested on the soil matrix by the analysis of the certified soil (ERM-CC013a). The difference between the mean measured and certified values (Table 3) were lower than the expanded uncertainty of that difference for the majority of PAHs, attesting the validity of the method for the soil matrix [44]. Then, the ability of the method to analyze PAHs in the biochar amended soil was evaluated. The obtained concentrations of PAHs in the untreated soil and in the soil amended with biochar are presented in Table 4. The total PAH concentration in the amended soil is significantly higher than that in the untreated soil. In particular, the concentration of naphthalene is 0.0263 $\mu\text{g g}^{-1}$ against 0.0098 $\mu\text{g g}^{-1}$ in the untreated soil, a quite large difference due to naphthalene being the most abundant PAH in biochar at 1.75 $\mu\text{g g}^{-1}$. The excess naphthalene in the treated soil of 0.0263–0.0098 = 0.0165 $\mu\text{g g}^{-1}$ is slightly lower than that expected from the quantity of naphthalene added with biochar corresponding to 1.75 \times 1.16% = 0.0203 $\mu\text{g g}^{-1}$. Overall, the correspondence between the measured excess and expected is (0.0165–0.0203)/0.0203 = –0.19 (or –19%), which is an acceptable result and good demonstration of the accuracy of the method for LMW PAH compounds, which has been a shortcoming of some of the existing methods [i.e. 5]. A similar calculation was performed for the other PAHs, and the results are reported in last column of

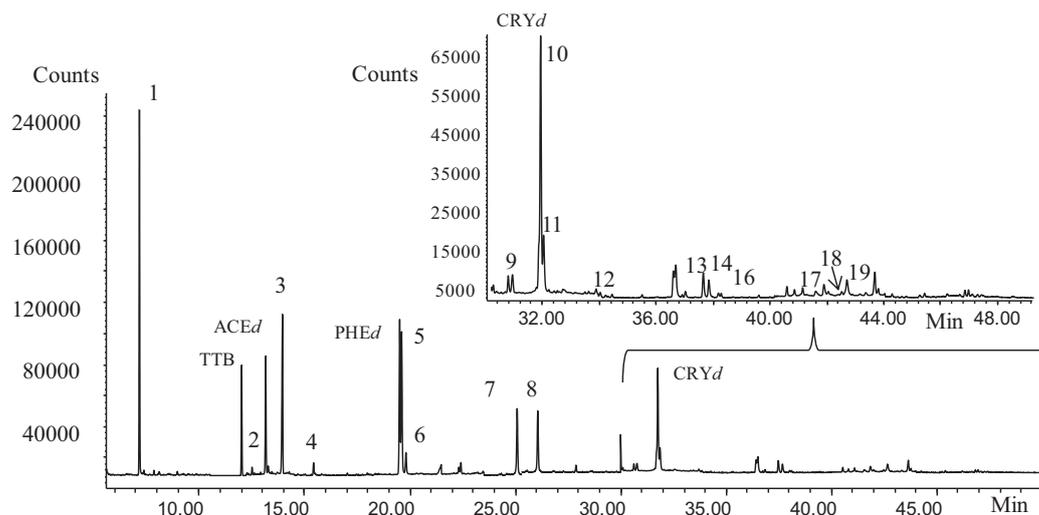


Fig. 2. GC-MS (SIM) chromatogram obtained from the analysis of the reference biochar (RB). Peak numbers refers to PAHs listed in Table 5.

Table 3

Method validation for the determination of PAHs in a soil matrix through the analysis of the certified soil material (ERM-CC013a).

PAH	Measured concentration ($\mu\text{g g}^{-1}$)	Certified value ($\mu\text{g g}^{-1}$)	Relative error (%)
Naphthalene ^a	2.2 ± 0.2	2.4 ± 0.5	-9
Fluorene ^a	1.30 ± 0.11	1.14 ± 0.11	+13
Phenanthrene ^a	12.4 ± 0.3	12.0 ± 0.6	+2
Anthracene	1.96 ± 0.09	1.41 ± 0.22	+32
Fluoranthene ^a	12.0 ± 0.5	12.9 ± 0.7	-9
Pyrene	8.4 ± 0.6	9.6 ± 0.3	-15
Benzo[a]anthracene ^a	5.1 ± 0.3	5.6 ± 0.5	-11
Chrysene	6.3 ± 0.3	5.3 ± 0.8	+15
Benzo[b]fluoranthene ^a	6.4 ± 0.4	7.1 ± 1.0	-12
Benzo[k]fluoranthene ^a	4.0 ± 0.4	3.4 ± 0.4	+14
Benzo[a]pyrene ^a	4.6 ± 0.4	4.9 ± 0.7	-8
Benzo[ghi]perylene ^a	4.3 ± 0.7	4.6 ± 0.5	-8
Indeno[1,2,3-cd]pyrene ^a	5.5 ± 0.9	5.2 ± 1.0	+3

^a There is no significant difference between the mean measured value ($n=4$ spread in two weeks) and the certified value [44].

Table 4. These differences between the calculated and measured values were satisfactory for the most abundant PAHs in biochar (Table 4; at the $\pm 20\%$ level). These data support that the proposed method was capable to extract PAHs from a biochar amended soil, a PAH contaminated soil, and the original biochar.

Obviously, the effect of biochar addition in soils on the level of PAHs will depend on the background level of PAHs in the soil before treatment [13,45], the concentration of PAHs in the original biochar, and the quantity of added biochar. Then, environmental processes (evaporation, biodegradation, or abiotic degradation)

Table 4

Observed concentration of PAHs in an agricultural soil and a corresponding biochar amended soil (1.16% (w/w) of reference biochar RB).

PAHs	Soil ($\mu\text{g g}^{-1}$)	Soil + biochar ($\mu\text{g g}^{-1}$)	Difference from expected (%)
Naphthalene	0.0098 ± 0.0002	0.0263 ± 0.0046	-19
Acenaphthylene	n.d.	n.d.	n.d.
Acenaphthene	n.d.	n.d.	n.d.
Fluorene	0.0023 ± 0.0008	0.0033 ± 0.0006	+13
Phenanthrene	0.0118 ± 0.0036	0.0212 ± 0.0063	+15
Anthracene	0.0003 ± 0.0002	0.0014 ± 0.0014	-24
Fluoranthene	0.0035 ± 0.0010	0.0075 ± 0.0030	+15
Pyrene	0.0031 ± 0.0007	0.0069 ± 0.0020	-6
Chrysene	0.0007 ± 0.0003	0.0014 ± 0.0010	-31
Benzo[a]anthracene	0.0039 ± 0.0007	0.0057 ± 0.0009	+60
Benzo[b]fluoranthene	0.0067 ± 0.0014	0.0091 ± 0.0029	+32
Benzo[k]fluoranthene	0.0005 ± 0.0001	0.0014 ± 0.0003	-51
Benzo[a]pyrene	0.0001 ± 0.0002	0.0019 ± 0.0009	-21
Indeno[1,2,3-cd]pyrene	0.0023 ± 0.0008	0.0040 ± 0.0022	-9
Dibenzo[a,h]anthracene	0.0009 ± 0.0002	0.0014 ± 0.0004	-18
Benzo[ghi]perylene	0.0046 ± 0.0011	0.0070 ± 0.0013	+36
Total	0.0506 ± 0.017	0.0986 ± 0.019	-2

Notes: Values in the tables are the mean value ± 1 standard deviation from four replicates. The last column reports the relative percent difference between the measured and expected value. The expected value is the concentration calculated from the PAH concentration obtained by summing the soil and biochar contributions (Table 1). This is expressed as a relative percentage of $(\text{measured} - \text{expected})/\text{expected} \times 100$.

Table 5

Concentrations of the 16 USEPA PAHs and (#) 15 EUPAHs ($\mu\text{g g}^{-1}$ mean of two duplicates). (RB reference biochar; characteristics of biochars from S-2 to S-20 were published elsewhere [40].)

Nr.	Sample Id. PAHs	RB	S-2	S-3	S-4	S-5	S-15	S-16	S-17	S-18	S-19	S-20
1	Naphthalene	1.75	1.57	1.71	2.39	0.44	0.47	0.93	2.58	0.78	0.49	3.36
2	Acenaphthylene	0.03	0.50	0.30	0.04	0.01	0.02	0.12	0.71	0.10	0.05	0.10
3	Acenaphthene	0.03	0.62	0.31	0.05	0.01	0.07	0.08	0.28	0.24	0.22	0.11
4	Fluorene	0.07	0.25	0.16	0.10	0.05	0.08	0.04	0.92	0.59	0.26	1.13
5	Phenanthrene	0.71	0.25	0.30	0.56	0.31	0.27	0.36	3.88	0.49	0.33	2.70
6	Anthracene	0.13	0.03	0.04	0.07	0.03	0.03	0.04	0.65	0.19	0.12	0.33
7	Fluoranthene	0.3	0.14	0.08	0.11	0.08	0.11	0.05	2.46	0.10	0.09	0.21
8	Pyrene	0.35	0.07	0.07	0.08	0.08	0.12	0.04	2.58	0.16	0.07	0.10
9	Cyclopenta[<i>c,d</i>]pyrene [#]	0.001	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.04	n.d.	0.03
10	Chrysene [#]	0.09	0.05	0.02	0.02	0.02	0.03	0.02	0.92	0.42	0.17	0.09
11	Benzo[<i>a</i>]anthracene [#]	0.09	0.04	0.02	0.05	0.04	0.04	0.02	0.83	0.46	0.08	0.17
12	5-methylchrysene [#]	0.01	0.11	0.04	0.02	0.02	0.02	0.09	0.27	0.21	n.d.	0.21
13	Benzo[<i>b</i>]fluoranthene [#]	0.13	0.02	0.05	0.04	0.04	0.05	0.02	0.70	0.29	0.05	0.07
14	Benzo[<i>k</i>]fluoranthene [#]	0.1	0.02	0.01	0.04	0.02	0.02	0.01	0.43	0.39	0.07	0.06
15	Benzo[<i>j</i>]fluoranthene [#]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
16	Benzo[<i>a</i>]pyrene [#]	0.19	0.02	0.02	0.10	0.01	0.05	0.02	0.67	0.32	0.06	0.22
17	Indeno[1,2,3- <i>cd</i>]pyrene [#]	0.15	0.02	0.01	0.13	n.d.	0.02	0.01	0.50	0.27	n.d.	0.03
18	Dibenzo[<i>a,h</i>]anthracene [#]	0.06	0.02	0.01	0.01	0.01	0.01	0.01	0.08	0.21	0.19	0.06
19	Benzo[<i>ghi</i>]perylene [#]	0.15	0.01	0.01	0.01	0.01	0.02	0.01	0.53	n.d.	n.d.	0.08
20	Dibenzo[<i>a,e</i>]pyrene [#]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
21	Dibenzo[<i>a,h</i>]pyrene [#]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22	Dibenzo[<i>a,i</i>]pyrene [#]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
23	Dibenzo[<i>a,l</i>]pyrene [#]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ 16 EPA PAHs		4.3	3.6	3.1	3.8	1.2	1.4	1.8	19	5.0	2.2	8.8
# Σ 15 EU PAHs		0.97	0.32	0.2	0.43	0.18	0.27	0.22	5.0	2.6	0.62	1.0

will affect the fate and levels of PAHs in amended soil. Due to the lipophilic nature of the PAHs, these compounds tend to bioaccumulate in plants [46,47]. Leafy vegetables typically accumulate higher levels of PAHs from the soil system than companion fruit or root crops [48]. The levels of PAH observed in some of the biochars (see below) do possess levels that could be of potential health and environmental concern, depending on the application rate, original soil concentrations, and end-use for the soil.

3.4. Determination of EPA and EU PAHs in different biochar samples

The method developed in this study was applied to the determination of USEPA and EU PAHs in a suite of ten biochar investigated in a previous study [40]. With the exception of biochar S-18 and S-19 (distillers grain) and S-17 (Macadamia nut shells), all the other biochars were derived from woody biomass (Table 5). Almost all 16 USEPA PAHs were detected and quantified in the biochars, as well as several EU PAHs. However, HMW EU PAHs were not detected (Table 5). The recovery of spiked deuterated PAHs ranged between 60 and 100% (Table A3 in supplementary materials) and for all the samples an average of 78%, 78 and 75% for *d*-acenaphthene, *d*-phenanthrene and *d*-chrysene, respectively, with ~10% RSD each.

Despite the difference in feedstock and process treatment the PAH levels were quite similar ($1\text{--}19 \mu\text{g g}^{-1}$). One sample (biochar S-17) was characterized by high levels of PAHs. However, the literature reports examples of biochar with much higher concentrations, some comparable to those observed on soot [5,6]. A large number of biochars investigated by Hale et al. [4] exhibited total PAHs in the $0.07\text{--}3.27 \mu\text{g g}^{-1}$ interval when produced from slow pyrolysis from different biomass at temperatures between 250 and 900 °C, and higher values ($45 \mu\text{g g}^{-1}$) from gasification. These examples underline the variety of PAH levels that could find in biochars.

With few exceptions (S-17), naphthalene was the most abundant PAH, in accordance to previous studies [4–6,8,9], followed by phenanthrene. However, it is interesting to note that benzo[*a*]pyrene was detected in all biochars analyzed here, with concentrations ranging from 0.01 to $0.67 \mu\text{g g}^{-1}$.

Sample S-2 was biochar obtained from the fast pyrolysis of hardwood sawdust at 500 °C, while S-3 the same biochar stored 1 year in an open drum subject to environmental conditions [40]. Table 5 shows that the levels of LMW PAHs did not change markedly, confirming the strong sorption of PAHs to biochar. However, Hale et al. [4] reported that artificial aging in aqueous solutions generally increased the concentration of PAHs on biochar, probably due to the leaching of hydrophilic components leaving the more hydrophobic biochar fraction.

Biochars S-18 and S-19 produced from the same feedstock (distiller grains) at similar pyrolysis temperatures (350 and 400 °C, respectively) exhibited significantly different PAH concentrations (total USEPA 5.0 and $2.2 \mu\text{g g}^{-1}$) suggesting the importance of pyrolysis conditions, as well as the role of temperature. A general trend has been observed of increasing PAH contents at shorter pyrolysis times and high pyrolysis temperatures [4]. A detailed study on the presence PAHs in biochar samples produced from woody and herbaceous biomass pyrolyzed at different temperatures showed that the concentration of pyrogenic PAHs peaked at 500 °C, a common temperature in slow pyrolysis [7]. Chagger et al. [49] demonstrated through modeling that PAHs are preferentially formed in a fluidized bed reactor versus a kiln style reactor, due to unstable combustion reactions present in a fluidized bed reactor. Schimmelpfennig and Glaser have underlined the importance of the particular technological process on the sorbed PAH concentrations, with wood gasifiers associated with the highest levels of PAHs on the solid residuals [6]. These authors proposed the naphthalene/phenanthrene ratio and the total PAHs concentrations as factors to differentiate pyrolysis processes between biochars. These hypotheses are also supported by our data, since biochars that are created by slow pyrolysis at longer residency times in kiln style reactors possess lower sorbed amounts of PAHs compounds.

Given the values of total PAHs reported in Table 5, as well as those reported in the literature [4,8] for the slow pyrolysis biochars and the level of biochar applications recommended in agriculture practices, the increased levels of elevated PAHs in biochar amended soil is not of universal concern. However, as also seen in our data and those from other studies [7,9], some biochars do have levels of

sorbed PAHs that do exceed existing and proposed guidelines for the usage of specific materials (e.g. sludge, wood ash) on land [7–9] including commercial biochar [5]. In addition, the bioaccumulation of PAH compounds in produce grown in biochar amended soils requires further investigation. Therefore, the development of valid analytical procedures for the determination of PAHs in biochar and biochar amended soils is critical.

4. Conclusions

A method for the determination of PAHs in biochar was developed making use of a solvent mixture (1:1 acetone:cyclohexane) in place of more toxic and/or hazardous solvents (e.g., dichloromethane, toluene) which was appropriate for the determination of LMW PAHs (including naphthalene) along with HMW PAHs. The method was validated with a certified reference soil and demonstrated its validity for the detection of PAHs deriving from biochar in a soil matrix amended with 1% biochar. Because of the strong affinity of PAHs toward biochar, solvent and duration time of the Soxhlet extraction were crucial parameters and at least 36 h was necessary to obtain a satisfactory recovery with 1:1 acetone:cyclohexane. Furthermore, this method provided satisfactory recovery when applied to a wide range of biochar samples obtained at different pyrolysis conditions from different biomass parent materials suggesting that this analytical procedure could be used successfully on different biochars. All the biochar analyzed contained the USEPA, as well as some of the EU PAHs at detectable levels ranging from 1.2 to 19 $\mu\text{g g}^{-1}$. In particular, the presence of EU PAHs on biochar could be of concern when biochars with elevated levels of PAHs are used in human food production due to the potential of contamination. However, this aspect requires further investigations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jaap.2012.10.003>.

References

- [1] S.P. Sohi, E. Krull, E. Lopez-Capel, R. Bol, A review of biochar and its use and function in soil, *Advances in Agronomy* 105 (2010) 47–82.
- [2] J.J. Manyà, Pyrolysis for biochar purposes: a review to establish current knowledge gaps and research needs, *Environmental Science and Technology* 46 (2012) 7939–7954.
- [3] D. Fabbri, A. Adamiano, C. Torri, GC–MS determination of polycyclic aromatic hydrocarbons evolved from pyrolysis of biomass, *Analytical and Bioanalytical Chemistry* 397 (2010) 309–317.
- [4] S.E. Hale, J. Lehmann, D. Rutherford, A.R. Zimmerman, R.T. Bachmann, V. Shitumbanuma, A. O’Toole, K.L. Sundqvist, H. Hans Peter, H.P.H. Arp, G. Cornelissen, Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and dioxins in biochars, *Environmental Science and Technology* 46 (2012) 2830–2838.
- [5] I. Hilber, F. Blum, J. Leifeld, H.-P. Schmidt, T.D. Bucheli, Quantitative determination of PAHs in biochar: a prerequisite to ensure its quality and safe application, *Journal of Agricultural and Food Chemistry* 60 (2012) 3042–3050.
- [6] S. Schimmelpfennig, B. Glaser, One step forward toward characterization: some important material properties to distinguish biochars, *Journal of Environment Quality* 41 (2012) 1–13.
- [7] M. Keilueit, M. Kleber, M.A. Sparrow, B.R.T. Simoneit, F.G. Prah, Solvent-extractable polycyclic aromatic hydrocarbons in biochar: influence of pyrolysis temperature and feedstock, *Environmental Science and Technology* 46 (2012) 9333–9341.
- [8] A. Freddo, C. Cai, B.J. Reid, Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar, *Environmental Pollution* 171 (2012) 18–24.
- [9] S. Kloss, F. Zehetner, A. Dellantonio, R. Hamid, F. Ottner, V. Liedtke, M. Schwanninger, M.H. Gerzabek, G. Soja, Characterization of slow pyrolysis biochars: effects of feedstocks and pyrolysis temperature on biochar properties, *Journal of Environment Quality* 41 (2012) 990–1000.
- [10] J. Mara Dos Santos Barbosa, N. Ré-Poppi, M. Santiago-Silva, Polycyclic aromatic hydrocarbons from wood pyrolysis in charcoal production furnaces, *Environmental Research* 101 (2006) 304–311.
- [11] N. Ré-Poppi, M. Santiago-Silva, Identification of polycyclic aromatic hydrocarbons and methoxylated phenols in wood smoke emitted during production of charcoal, *Chromatographia* 55 (2002) 475–481.
- [12] E.-J. Kim, J.-E. Oh, Y.-S. Chang, Effects of forest fire on the level and distribution of PCDD/Fs and PAHs in soil, *Science of the Total Environment* 311 (2003) 177–189.
- [13] J.J. Nam, A.J. Sweetman, K.C. Jones, Polynuclear aromatic hydrocarbons (PAHs) in global background soils, *Journal of Environmental Monitoring* 11 (2009) 45–48.
- [14] G. Cornelissen, O. Gustafsson, Importance of unburned coal carbon, black carbon, and amorphous organic carbon to phenanthrene sorption in sediments, *Environmental Science and Technology* 39 (2005) 764–769.
- [15] A.M.P. Oen, G. Cornelissen, G.D. Breedveld, Relation between PAH and black carbon contents in size fractions of Norwegian harbor sediments, *Environmental Pollution* 141 (2006) 370–380.
- [16] J. Poerschmann, D. Fabbri, T. Górecki, Investigation of the solvent extracts of humic organic matter (HOM) isolated from the Ravenna Lagoon to study environmental pollution and microbial communities, *Chemosphere* 70 (2007) 206–214.
- [17] R.C. Brandli, T. Hartnik, T. Henriksen, G. Cornelissen, Sorption of native polycyclic aromatic hydrocarbons (PAH) to black carbon and amended activated carbon in soil, *Chemosphere* 73 (2008) 1805–1810.
- [18] S. Hale, K. Hanley, J. Lehmann, A. Zimmerman, G. Cornelissen, Effects of chemical, biological, and physical aging as well as soil addition on the sorption of pyrene to activated carbon and biochar, *Environmental Science and Technology* 45 (2011) 10445–10453.
- [19] P. Oleszczuk, S.E. Hale, J. Lehmann, G. Cornelissen, Activated carbon and biochar amendments decrease pore-water concentrations of polycyclic aromatic hydrocarbons (PAHs) in sewage sludge, *Bioresource Technology* 111 (2012) 84–91.
- [20] M.T.O. Jonker, A.A. Koelmans, Extraction of polycyclic aromatic hydrocarbons from soot and sediment: solvent evaluation and implications for sorption, *Environmental Science and Technology* 36 (2002) 4107–4113.
- [21] R. Aina, L. Palin, S. Citterio, Molecular evidence for benzo[a]pyrene and naphthalene genotoxicity in *Trifolium repens* L., *Chemosphere* 65 (2006) 666–673.
- [22] K.A. Spokas, J.M. Novak, C.E. Stewart, K.B. Cantrell, M. Uchimiya, M.G. Dusaire, K.S. Ro, Qualitative analysis of volatile organic compounds on biochar, *Chemosphere* 85 (2011) 869–882.
- [23] S.R. Wild, K.C. Jones, The significance of polynuclear aromatic hydrocarbons applied to agricultural soils in sewage sludges in the UK, *Waste Management and Research* 2 (1994) 49–59.
- [24] M.P. Fraser, G.R. Cass, B.R.T. Simoneit, R.A. Rasmussen, Air quality model evaluation data for organics. 5. C6–C22 nonpolar and semipolar aromatic compounds, *Environmental Science and Technology* 32 (1998) 1760–1770.
- [25] A.M. Kippoulou, E. Manoli, C. Samara, Bioconcentration of polycyclic aromatic hydrocarbons in vegetables grown in an industrial area, *Environmental Pollution* 106 (1999) 369–380.
- [26] A. Motelay-Massei, B. Garban, K. Tiphagne-larcher, M. Chevreuil, D. Ollivon, Mass balance for polycyclic aromatic hydrocarbons in the urban watershed of Le Havre (France): transport and fate of PAHs from the atmosphere to the outlet, *Water Research* 40 (2006) 1995–2006.
- [27] S. Tao, Y.H. Cui, F.L. Xu, B.G. Li, J. Cao, W.X. Liu, G. Schmitt, X.J. Wang, W.R. Shen, B.P. Qing, R. Sun, Polycyclic aromatic hydrocarbons (PAHs) in agricultural soil and vegetables from Tianjin, *Science of the Total Environment* 320 (2004) 11–24.
- [28] A.P. Loibner, O.H.J. Szolar, R. Braun, D. Hirmann, Toxicity testing of 16 priority polycyclic aromatic hydrocarbons using Lumistox, *Environmental Toxicology and Chemistry* 23 (2004) 557–564.
- [29] A.S. Krang, Naphthalene disrupts pheromone induced mate search in the amphipod *Corophium volutator* (Pallas), *Aquatic Toxicology* 85 (2007) 9–18.
- [30] S. Ahn, D. Werner, R.G. Luthy, Modeling PAH mass transfer in a slurry of contaminated soil or sediment amended with organic sorbents, *Water Research* 42 (2008) 2931–2942.

- [31] A. Meudec, J. Dussauze, E. Deslandes, N. Poupart, Evidence for bioaccumulation of PAHs within internal shoot tissues by a halophytic plant artificially exposed to petroleum-polluted sediments, *Chemosphere* 65 (2006) 474–481.
- [32] L. Rey-Salgueiro, E. Martínez-Carballo, M.S. García-Falcón, C. González-Barreiro, J. Simal-Gándara, Occurrence of polycyclic aromatic hydrocarbons and their hydroxylated metabolites in infant foods, *Food Chemistry* 115 (2009) 814–819.
- [33] ECR, European Commission Regulation No 1881/2006 of 19th December; 2006. Setting maximum levels for certain contaminants in foodstuffs, available at: <http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF>, (accessed 12.06.11).
- [34] US EPA. Polycyclic organic matter, Washington, DC: Environmental Protection Agency, available at: <http://www.epa.gov/ttn/atw/hlthef/polycycl.html>, (2002).
- [35] M. Gfrerer, M. Serschen, E. Lankmayr, Optimized extraction of polycyclic aromatic hydrocarbons from contaminated soil samples, *Journal of Biochemical and Biophysical Methods* 53 (2002) 203–216.
- [36] Y.Y. Shu, T.L. Lai, H. Lin, T.C. Yang, C. Chang, Study of factors affecting on the extraction efficiency of polycyclic aromatic hydrocarbons from soils using open-vessel focused microwave-assisted extraction, *Chemosphere* 52 (2003) 1667–1676.
- [37] L. Beesley, E. Moreno-Jiménez, J.L. Gomez-Eyles, Effects of biochar and green-waste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil, *Environmental Pollution* 158 (2010) 2282–2287.
- [38] M.T. Pena, M.C. Casais, M.C. Mejuto, R. Cela, Optimization of the matrix solid-phase dispersion sample preparation procedure for analysis of polycyclic aromatic hydrocarbons in soils: comparison with microwave-assisted extraction, *Journal of Chromatography A* 1165 (2007) 32–38.
- [39] G. Fellet, L. Marchiol, G. Delle Vedove, A. Peressotti, Application of bio-char on mine tailings: effects and perspectives for land reclamation, *Chemosphere* 83 (2011) 1262–1267.
- [40] D. Fabbri, C. Torri, K.A. Spokas, Analytical pyrolysis of synthetic chars derived from biomass with potential agronomic application (biochar). Relationships with impacts on microbial carbon dioxide production, *Journal of Analytical and Applied Pyrolysis* 93 (2012) 77–84.
- [41] C. Zavalloni, G. Alberti, S. Biasiol, G. DelleVedove, F. Fornasier, J. Liu, A. Peressotti, Microbial mineralization of biochar and wheat straw mixture in soil: a short-term study, *Applied Soil Ecology* 50 (2011) 45–51.
- [42] S. Baronti, G. Alberti, G. DelleVedove, F. Di Gennaro, G. Fellet, L. Genesio, F. Miglietta, A. Peressotti, F.P. Vaccari, The biochar option to improve plant yields: first results from some field and pot experiments in Italy, *Italian Journal of Agronomy* 5 (2010) 3–11.
- [43] R.A. Brown, A.K. Kercher, T.H. Nguyen, D.C. Nagle, W.P. Ball, Production and characterization of synthetic wood chars for use as surrogates for natural sorbents, *Organic Geochemistry* 37 (2007) 321–333.
- [44] T. Linsinger, Comparison of a measurement result with the certified value, Application Note 1 EC-JRS IRMM (European Commission-Joint Research Center Institute for Reference Materials and Measurements), 2005 revision January 2010, Geel Belgium, 2 pp, available at: <http://irmm.jrc.ec.europa.eu/>
- [45] W. Wilcke, SYNOPSIS polycyclic aromatic hydrocarbons (PAHs) in soil – a review, *Journal of Plant Nutrition and Soil Science* 163 (2000) 229–248.
- [46] C. Duxbury, D.G. Dixon, B.M. Greenberg, Effects of simulated solar radiation on the bioaccumulation of polycyclic aromatic hydrocarbons by the duckweed Lemnagibba, *Environmental Toxicology and Chemistry* 16 (1997) 1739–1748.
- [47] Z.D. Parrish, J.C. White, M. Isleyen, M.P.N. Gent, W. Iannucci-Berger, B.D. Eitzer, J.W. Kelsey, M.I. Mattina, Accumulation of weathered polycyclic aromatic hydrocarbons (PAHs) by plant and earthworm species, *Chemosphere* 64 (2006) 609–618.
- [48] F.-F. Lei, J.-Y. Huang, X.-N. Zhang, X.-J. Liu, X.-J. Li, Determination of polycyclic aromatic hydrocarbons in vegetables by headspace SPME-GC, *Chromatographia* 74 (2011) 99–107.
- [49] H.K. Chagger, J.M. Jones, M. Pourkashanian, A. Williams, The formation of VOC, PAH and dioxins during incineration, *Process Safety and Environmental Protection* 78 (2000) 53–59.