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Bioavailability and bioaccessibility of polycyclic aromatic hydrocarbons from (post-pyrolytically treated) biochars

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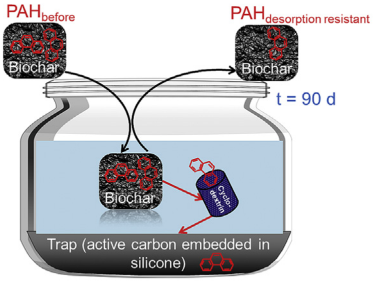
HIGHLIGHTS

- Desorption resistance of PAHs in biochar was determined with contaminant traps.
- PAHs in biochar were mostly desorption resistant.
- Where observed, bioaccessibility of PAHs in biochar was concentration independent.
- Exposure of PAHs in biochar becomes relevant at high concentrations (>10 mg/kg_{dw}).
- Biochars acted more as a sink than a source for PAHs.

GRAPHICAL ABSTRACT



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ABSTRACT

Bioaccessibility data of PAHs from biochar produced under real world conditions is scarce and the influence of feedstock and various post-pyrolysis treatments common in agriculture, such as co-composting or lacto-fermentation to produce silage fodder, on their bioavailability and bioaccessibility has hardly been studied. The total (C_{total}), and freely dissolved (i.e., bioavailable) concentrations (C_{free}) of the sum of 16 US EPA PAHs of 43 biochar samples produced and treated in such ways ranged from 0.4 to almost 2000 mg/kg, and from 12 to 81 ng/L, respectively, which resulted in very high biochar-water partition coefficients ($4.2 \leq \log K_D \leq 8.8$ L/kg) for individual PAHs. Thirty three samples were incubated in contaminant traps that combined a diffusive carrier and a sorptive sink. Incubations yielded samples only containing desorption-resistant PAHs (C_{res}). The desorption resistant PAH fraction was dominant, since only eight out of 33 biochar samples showed statistically significant bioaccessible fractions ($f_{bioaccessible} = 1 - C_{res}/C_{total}$). Bioavailability correlated positively with C_{total}/surface area. Other relationships of bioavailability and –accessibility with the investigated post-pyrolysis processes or elemental composition could not be found. PAH exposure was very limited (low C_{free}, high C_{res}) for all samples with low to moderate C_{total}, whereas higher exposure was determined in some biochars with $C_{total} > 10$ mg/kg.

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1. Introduction

Biochar can contain considerable amounts of polycyclic aromatic hydrocarbons (PAHs) produced during the pyrolysis process. Concentrations of PAHs range from a few to a few 100 mg/kg for the sum ($\sum$) of the 16 US environmental protection agency's (EPA) priority PAHs (Bucheli et al., 2015). In agriculture, biochar is often blended with mineral or organic fertilizers to increase its effect on soil fertility and crop yield (Atkinson et al., 2010; Sohi et al., 2010). In animal farming, the amendment of biochar into feed of ruminants is intended to reduce greenhouse gas emissions or improve the feed quality (Hansen et al., 2012; Silivong and Preston, 2015). To reduce nutrient losses during composting, biochars can be added to composting biomass (Khan et al., 2014). Biochar is also used to remediate contaminated soils and sediments by organic pollutants (Hale et al., 2015; Denyes et al., 2016).

While total concentrations (C_{total}) of pollutants such as PAHs are currently applied in legislation for the agricultural use of biochar (Bucheli et al., 2015), the C_{total} can significantly overestimate the actual risk of organic contaminants (Verstraete and Devliegher, 1996), particularly in cases where the pollutants themselves are strongly sorbed to the matrix (i.e., high partition coefficients) and where they are not readily desorbed from the matrix (i.e., high desorption resistant fraction). Actual exposure can be assessed by the chemical activity (i.e. expressed as freely dissolved concentration) and the bioaccessibility (i.e., readily desorbing fraction) (Reichenberg and Mayer, 2006). Freely dissolved (i.e., bioavailable) concentrations of native PAHs in soils or biochars (C_{free}) were earlier measured by, e.g., Hale et al. (2012), with non-depletive equilibrium sampling methods. Bioaccessibility can be measured with depletive sampling methods such as Tenax$^{®}$ (Hilber et al., 2009; Cui et al., 2013) or Sorptive Bioaccessibility Extraction (Gouliarmou and Mayer, 2012). These bioaccessibility techniques have recently been extended to samples with very high sorption capacities for PAHs, such as historically polluted soil or chimney soot (Gouliarmou and Mayer, 2012; Gouliarmou et al., 2013), but were unable to determine bioaccessible fractions ($f_{bioaccessible}$) for biochar (Mayer et al., 2016). As conceptually shown in Mayer et al. (2016), so far, $f_{bioaccessible}$ of PAHs from biochar can only be determined indirectly as one minus the ratio of desorption resistant PAH concentration (C_{res}) to C_{total}. The C_{res} can be quantified after incubation of biochars in contaminant traps, where cyclodextrin serves as diffusive carrier, and an active carbon (AC)/silicone composite as an infinite sink that maintains the desorption gradient (Mayer et al., 2011). This method is particularly suitable for matrices that have very high partition coefficients for organic contaminants (Mayer et al., 2016).

The aim of this study was to determine the bioavailability and −accessibility of native PAHs in biochars gathered from various producers in the field, both in pure form or after subjection to various post-pyrolysis treatments common in agriculture, or with biochar producers. The scientific hypothesis was that such treatments, potentially manifested in an altered biochar elemental composition and/or specific surface area, increase bioavailability and −accessibility of PAHs. For treatments involving microbial processes (e.g., composting, lacto-fermentation) in particular, this hypothesis is motivated by findings that microbes may promote desorption and degradation of PAHs sorbed to carbonaceous materials (Xia et al., 2010), and that degradation of PAHs by biofilm-producing bacteria was improved in presence of activated carbon (Leglize et al., 2008). To this end, physical-chemical properties, as well as PAH bioavailability and -accessibility were quantified in a set of diverse biochars, (some of) which were i) produced by different biochar providers from commonly used feedstock material, ii) pyrolysed under a 2% oxygen (O_2) atmosphere to simulate non-controlled pyrolysis conditions, iii) mixed and aged with compost to represent a common agricultural waste product with a drastically altered microenvironment, iv) subjected to lacto-fermentation because biochar is used as a feed additive and usually applied in combination with lacto-fermented feed-products (Hansen et al., 2012; Silivong and Preston, 2015), and v) treated post-pyrolytically with ozone, a process which may increase PAH bioaccessibility (Bernal-Martinez et al., 2007). Please note that this is a field, and common practice orientated experiment where bioavailability and −accessibility including physical-chemical biochar properties were surveyed, rather than a mechanistic lab study under controlled conditions. Still, this study sheds new and relevant light on the exposure assessment of biochar produced, and post-pyrolytically treated in the field. Due to the width and breadth of the investigated samples, the here presented results should be equally relevant for scientists, legislators, and biochar practitioners.

2. Material and methods

2.1. Samples

Table 1 provides details about the 43 biochar and four compost samples. The majority of the feedstocks of the biochar samples (24) consisted of “wood”, with the remainder being divided as follows: two “sugar beet” samples, six “*Miscanthus*” (elephant grass), five “lop” (green waste) and six “miscellaneous” materials, e.g., grape pomace, coffee grounds, sewage sludge. The names in the quotation marks refer to the feedstock groups listed in Table 1, which also includes the feedstock of the composts specified in the supporting information (SI). All biochars were produced by slow pyrolysis by different professional and amateur biochar producers under field scale real world conditions at temperatures between 400 and 750 °C.

Table 1 also lists the treatment in the “sample description” and the group it belongs to in the “**treatment group**” (bold in the text). Biochars that were not treated or blended with other additives after pyrolysis are labeled “**pure**”. Some of the biochars were blended with microorganisms such as lactobacilli and photosynthetic bacteria, yeasts, actinomycetes, and fungi, all commercially available at the agro-market (the product is described in detail in the SI). These samples are referred to as treatment group “**microorganisms**” and were fermented for 2 weeks at 35 °C following microorganism addition. Some biochars were mixed with biomass at different ratios and co-composted at the facilities of a commercial compost producer. These are labeled “**blend**”. Corresponding pure composts are named “**compost**”. The feedstock and composting process is described in detail in the SI. Furthermore, aliquots of the sieved coniferous wood residues, sugar beet, and elephant grass (*Miscanthus*) feedstocks were slowly pyrolysed under a 2% O_2 atmosphere and allocated to the $\mathbf{O_2}$ treatment group. The *Miscanthus* biochar was also treated with ozone after pyrolysis at 700 °C and was labeled $\mathbf{O_3}$. Pure biochars in Table 1 were numbered consecutively and their respective treatments received a corresponding letter in alphabetic order. The last column in Table 1 defines the samples that were subjected to contaminant traps. Thirty three samples were selected for the trap experiments (see section 2.5) and these samples represented all six treatment groups as well as samples with high and low C_{total} from the **pure** treatment group.

The samples were stored and treated according to Hilber et al. (2012). Briefly, the samples were dried at 40 °C overnight, ground to ≤0.75 mm with a cutting mill SM1 Retsch GmbH (Haan, Germany) to reduce constitutional heterogeneity and allow for representative subsampling (Hilber et al., 2017), and mixed thoroughly

Table 1
Overview of investigated biochar and compost samples: The samples are first grouped according to their feedstock. The composting is described in the supporting information (SI). All samples and their treatment during/after pyrolysis are specified in the "sample description". Pure biochars are numbered consecutively and mixtures thereof or alternatively produced or treated analogues labeled with an additional letter. The samples are further grouped according to their treatments ("treatment group"). The samples that were subjected to contaminant traps (+trap) are indicated in the last column.

No.	Feedstock group	Sample description	Treatment group	+trap
1	Wood	Biochar from 20% hard- and 80% pinewood (different batches)	Pure	Yes
2	Wood		Pure	Yes
2a	Wood	Biochar from 20% hard-, 80% pinewood mixed with microorganisms (lacto ferments and photosynthetic bacteria, yeasts, actinomycetes, fungi)	Micro-organism	No
3	Wood	Biochar from 20% hard- and 80% pinewood (different batches)	Pure	Yes
3a	Wood	Biochar from 20% hard-, 80% pinewood mixed with microorganisms and fodder bokashi (silage animal fodder)	Micro-organism	Yes
3b	Wood	Biochar from 20% hard-, 80% pinewood mixed with microorganisms in molasses water (by-product of sugar production)	Micro-organism	Yes
4	Wood	Biochar from 20% hard- and 80% pinewood (different batches)	Pure	Yes
5	Wood	Biochar from 20% hard- and 80% pinewood (different batches)	Pure	No
C1	See SI	Pure compost C1	Compost	Yes
5a	Wood	10% biochar from 20% hard-, 80% pinewood mixed with compost C1	Blend	Yes
C2	See SI	Pure compost C2	Compost	No
5b	Wood	20% biochar from 20% hard-, 80% pinewood mixed with compost C2	Blend	No
C3	See SI	Pure compost C3	Compost	Yes
5c	Wood	20% biochar from 20% hard-, 80% pinewood mixed with compost C3	Blend	Yes
6	Wood	Biochar from 1 year old grape wine wood	Pure	Yes
C4	See SI	Pure compost C4	Compost	Yes
6a	Wood	30% biochar from grape wine wood mixed with compost C4	Blend	Yes
7	Wood	Biochar from sieved coniferous wood residues (different batches)	Pure	Yes
8	Wood		Pure	No
9	Wood		Pure	Yes
10	Wood		Pure	Yes
10a	Wood	Sieved coniferous wood residues pyrolysed with max. 2% O_2	O_2	Yes
11	Wood	Spruce wood	Pure	No
12	Wood	Not specified wood (different batches)	Pure	Yes
13	Wood		Pure	No
14	Wood	Wood chips pyrolysed in a gasifier	Pure	Yes
15	Wood	Biochar from beech wood	Pure	No
15a	Wood	10% biochar from beech wood mixed with unpyrolysed beech wood pellets, fodder bokashi and microorganisms	Blend	No
16	Sugar beet	Biochar from sugar beet	Pure	Yes
16a	Sugar beet	Sugar beet pyrolysed with max. 2% O_2	O_2	Yes
17	*Miscanthus*	Biochar from *Miscanthus* (elephant grass, different batches)	Pure	No
18	*Miscanthus*		Pure	Yes
19	*Miscanthus*		Pure	Yes
19a	*Miscanthus*	Biochar from *Miscanthus* was exposed to ozone after pyrolysis	O_3	Yes
20	*Miscanthus*	Biochar from *Miscanthus* (elephant grass, different batches)	Pure	Yes
20a	*Miscanthus*	*Miscanthus* pyrolysed with max. 2% O_2	O_2	Yes
21	Lop	Biochar from lop (green waste from cut garden trees, different batches)	Pure	No
22	Lop		Pure	Yes
23	Lop		Pure	No
23a	Lop	Biochar from lop mixed with compost	Blend	No
23b	Lop	Biochar from lop mixed with compost and soil where barley was grown	Blend	No
24	Miscellaneous	Biochar from marc of grapes	Pure	Yes
25	Miscellaneous	Biochar from coffee ground	Pure	Yes
26	Miscellaneous	Biochar from brown coal	Pure	Yes
27	Miscellaneous	Biochar from mixed wood chips	Pure	Yes
28	Miscellaneous	Biochar from paper sludge and wheat husks	Pure	Yes
29	Miscellaneous	Biochar from sewage sludge	Pure	Yes

with a Turbula shaker-mixer Bachofen AG (Muttenz, Switzerland) prior to beginning experimental work. While we cannot exclude any effect of biochar sample preparation on bioavailability analysis of PAHs, we consider this rather unlikely in our case, as PAHs overwhelmingly reside on particles <0.63 mm (Hilber et al., 2017), i.e., such that were smaller than the cutting mill cutoff and therefore not physically altered.

2.2. *Elemental analysis*

Elemental analysis was described in Bucheli et al. (2014). Briefly, carbon (C) and hydrogen (H) were quantified following dry combustion. Oxygen (O) was determined after pyrolysis of the sample at 1000 °C and reduction to carbon oxide. The specific surface area (SSA) was determined by nitrogen adsorption (Bucheli et al., 2014).

2.3. *Total PAH concentrations (C_{total})*

The 16 US EPA PAHs (naphthalene (NAP), acenaphthylene (ANY), acenaphthene (ANA), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benzo[*a*]anthracene (BaA), chrysene (CHR), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), indeno[1,2,3-*cd*]pyrene (IPY), dibenz[*a,h*]anthracene (DBA), and benzo[*ghi*]perylene (BPE)), as well as their deuterated PAH homologues, and indeno[1,2,3-*cd*]fluoranthene (IFL, internal standard) were determined by gas chromatography-mass spectroscopy (Agilent GC 6890N, MS 5973 *inert*). Briefly, biochars were extracted for 36 h with toluene using a Soxhlet extraction. Deuterated recovery standards were spiked onto the biochars immediately before the Soxhlet extraction. Following extraction, the extract solvent was concentrated to 1 mL with a Syncore Analyst (Büchi, Flawil, Switzerland). Total concentrations (mg/kg$_{\text{dry weight (dw)}}$) of the $\sum$16 US EPA PAHs were determined according to Hilber et al. (2012) using GC-MS (for details see SI). Quantification was carried out by means of the internal standard method and the calibration standards containing different amount of analytes (10–2500 ng/mL).

2.4. Freely dissolved PAH concentration (C_{free})

The freely dissolved (i.e., bioavailable) concentrations (C_{free}) of the PAHs were determined for the biochars and biochar mixtures using an equilibrium passive sampling method according to Hale et al. (2012). Briefly, 0.2 g of the samples (in triplicates) were mixed with water and a 0.4 g polyoxymethylene (POM) passive sampler for 30 days. Following this, POM samplers were extracted using acetone:heptane (20:80 vol:vol) and the concentration of PAHs in the POM determined. The POM-water partition coefficients for the PAHs were taken from Hawthorne et al. (2011). Ten blanks in total containing only POM strips in water were run with the sample batches and treated as all of the other samples. The maximum PAH concentration in the blank samples were as follows: 8.5, 0.8, 2.3, 0.4, 0.3, and 12.0 ng/L for NAP (Fig. S1A), FLU (Fig. S1D), PHE (Fig. S1E), FLT (Fig. S1G), PYR (Fig. S1H), and $\sum$16 US EPA PAHs (Fig. 1B), respectively. The blank concentrations were up to an order of magnitude lower than those determined in the samples. None of the other PAHs were detected in the blanks. Consequently, the results were not blank corrected, but only concentrations of PAHs above the blank values were used for calculations of partition coefficients (K_D, defined as C_{total} (μg/kg)/C_{free} (μg/L)).

2.5. Desorption resistant PAHs (C_{res}) determined with contaminant traps

Samples (0.1 g, in duplicates) were incubated in contaminant trap glass jars containing a composite of silicone and 5 g of AC (referred to as + trap), which served as infinite sink sorbent. The sample was suspended in 20 mL hydroxylpropyl-β-cyclodextrin (HPCD)-solution (140 $g_{HPCD}/L_{\text{Milli-Q-water}}$) to enhance the mass transfer of the PAHs from the biochar to the AC/silicone trap (Mayer et al., 2016). After 90 days (Mayer et al., 2016) of exposure (constant temperature of 19 ± 1 °C, without shaking) the suspension was filtered (Mayer et al., 2011). The filter, including the sample, was dried at 40 °C (losses of PAH at this temperature were found to be negligible (Desaules et al., 2008)) for one day and subsequently subjected to Soxhlet extraction (Hilber et al., 2012). As a control, a HPCD-solution with 0.1 g of sample (n = 2) but without the silicone/AC layer (referred to as -trap) was used and treated exactly the same way as the +trap. The concentration of the samples exposed to −trap and +trap are described as C_{-trap} and C_{+trap} (mg/kg$_{dw}$). The experimental design does not allow to back-extract PAHs from the infinite sink sorbent (Mayer et al., 2016). The bioaccessible fractions (see introduction) were thus translated from the conceptual to an operational approach as follows:

$$f_{bioaccessible}[\%] = (1 - C_{res}/C_{total})*100\% = \left(1 - C_{+trap}/C_{-trap}\right)*100\% \qquad (1)$$

2.6. Statistics

All statistical analysis was carried out with R version 3.1.1 (2014-

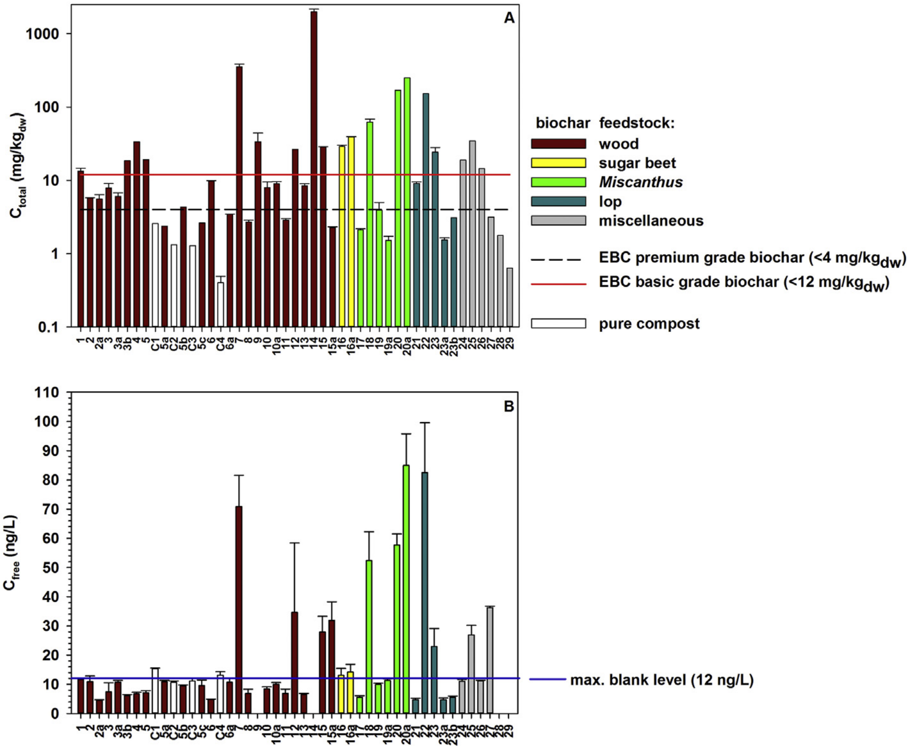

Fig. 1. Total concentrations (C_{total}, A) and the freely dissolved concentrations (C_{free}, B) of the sum ($\sum$) of 16 US EPA PAHs of 47 different samples (biochars, biochar blends, composts). Bar colours, feedstock groups, and the numbers on the x-axis correspond to the sample description in Table 1. Where indicated, error bars reflect the standard deviation of triplicates (B) and duplicates (A). The maximum blank level of 10 samples with polyoxymethylene and water but without biochars is indicated at 12 ng/L as blue line (B). Absent columns (B) indicate that the specific sample was not analysed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

07-10). An analysis of variance (anova, two-sided) was carried out to test the influence of the biochar feedstock on the C_{total}.

To assess the influence of the biochar properties on C_{free} and $f_{bioaccessible}$, multiple linear regressions with C_{free} and $f_{bioaccessible}$, respectively, as dependent variable, and C_{total}/SSA, H/C and O/C as independent variables were carried out. The mechanistic hypotheses tested by these models were that biochars i) which have more PAHs adsorbed per surface area exhibit a higher PAH bioavailability, ii) with a higher aromaticity (lower H/C numbers) exhibit stronger PAH affinity, and iii) biochars with a higher fraction of functional groups (higher O/C numbers) exhibit lower PAH affinity.

To evaluate the difference of PAH concentrations in the traps, unpaired, 1-sided t-tests were carried out based on the assumption that PAH concentrations in samples exposed to +trap were lower than in -trap. A statistically significant difference (trap evaluation and anova) was assigned if the p-value was ≤ 0.05. Descriptive parameters such as mean and standard deviations were calculated using Microsoft Excel 2010.

3. Results and discussion

3.1. Elemental analysis

The C-, H-, and O-contents of the biochars are given in Table S1. Most of the **pure, O_2** and **O_3** samples had a C-content $\geq$60%, which is Class 1 biochar according to the International Biochar Initiative ((IBI, 2015), Class 2: C-content $\geq$30% and <60%). With C-contents >50%, all of these samples except sample 13 (Table S1) can be classified as biochars according to the European Biochar Certificate (EBC, 2012). **Blend** and **compost** samples mostly had C-contents below 20% and reflected compost C enriched by biochar C. For instance Jindo et al. (2012) amended 10% biochar to composts and observed significant increased C contents and C/N ratios after 12 weeks of co-composted biochars in comparison to the unamended control. The O- and H contents of the **pure, O_2** and **O_3** samples averaged at 8.9 and 1.5%, respectively, and the H/C and O/C ratios 0.24 and 0.10, respectively. The H/C ratio was higher, and the O/C ratio lower than what Hale et al. (2016) reported (H/C: 0.03, O/C: 0.25) in a compilation of 28 studies about biochar.

Both EBC and IBI define a H/C_{org} ratio of 0.7 as upper threshold for biochars (EBC, 2012; IBI, 2015). All of the **pure, O_2** and **O_3** samples met this criterion. For the O/C_{org} ratio, the (EBC, 2012) uses a value of <0.4 to define a biochar, this value being met by the **pure** and **O_2** samples. However, the IBI (2015) has not defined such a guideline value. Hence and in summary, most of the **pure, O_2** and **O_3** samples exhibited elemental composition typical and indicative for biochar, indicating their representativeness for this kind of carbonaceous material. The SSA for half of the biochar samples (**pure, O_2** and **O_3**) was >150 m^2/g (Table S1), as recommended by the EBC. The measurements with N_2 are in the line with the SSA results from other studies (Kloss et al., 2012; Bachmann et al., 2016; Kupryianchyk et al., 2016).

3.2. Total PAH concentrations (C_{total})

The C_{total} (Fig. 1A, Table S1) of the samples for the Σ16 US EPA PAHs ranged from 0.4 to 1987 mg/kg_{dw}. The highest C_{total} measured was for a biochar produced by gasification (sample 14). This observation is in agreement with Hale et al. (2012), who reported the highest total PAH concentration in biochars produced from gasifiers. Elevated PAH concentrations in gasification relative to pyrolysis-produced biochars may be explained by more pronounced recondensation from the gas phase to less residual solid material (Bucheli et al., 2015). According to the EBC, a biochar can be defined as premium grade when the Σ16 US EPA PAHs content is < 4 mg/kg_{dw} and as basic grade when it is < 12 mg/kg_{dw} (EBC, 2012). Out of the **pure, O_2** and **O_3** samples, 24% qualified as premium grade. The IBI guidelines (IBI, 2015) indicate a maximally allowed threshold of PAHs between 6 and 300 mg/kg_{dw}; two of the **pure, O_2** and **O_3** biochars exceeded the upper concentration limit.

The feedstock type had no significant influence on the C_{total} of the Σ16 US EPA PAHs, neither for the biochars of the **pure** and **O_2** treatments, nor for the biochars treated after pyrolysis (**microorganisms**, **O_3**). This finding is in the line with results from other studies which found no clear influence of the biochar feedstock biochar's PAH content (Buss et al., 2016; Madej et al., 2016).

Sieved coniferous wood residues, sugar beet, and *Miscanthus* were pyrolysed under normal (samples 10, 16, 20) and slightly increased O_2 atmospheres (samples 10a, 16a, 20a). This process had no consistent influence across the three feedstock types on the C_{total} of the Σ16 US EPA PAHs. For biochar produced from *Miscanthus* and sieved coniferous wood residues, no significant differences in PAH concentrations between the biochars pyrolysed under normal (i.e., marginal) and elevated O_2 levels were observed. In contrast, the sugar beet biochar (sample 16, 16a) had a significantly higher PAH concentration in the biochar produced under the enhanced O_2 environment. The indistinct influence of O_2 is in the line with the study of Madej et al. (2016). The **O_3** treatment (19a) reduced the C_{total} in comparison to the control (sample 19) and the difference was almost significant (p-value = 0.09). On average 48% (max. 82%) of the C_{total} in biochars (**pure, O_2** and **O_3**) consisted of NAP and 15% (max. 35%) of PHE. These fingerprints were similar to the ones summarized from the literature in Bucheli et al. (2015).

Generally, the samples from the groups **blend**, **compost**, and **microorganisms** showed low C_{total} in comparison to the **pure** or **O_2** treated biochars. The **blend** (samples 5a, 5b, 5c, 6a) group in this study mainly reflected the "diluted" concentrations of PAHs found in the original biochar (samples 5, 6) because the **composts** (C1 - 4) contained less (<2.6 mg/kg_{dw}) native PAHs.

3.3. Freely dissolved PAH concentration (C_{free})

The C_{free} (Fig. 1B, Table S1) ranged from <12 ng/L to 85 ± 11 ng/L for the Σ16 US EPA PAHs in all samples. The highest C_{free} was found in the *Miscanthus* biochar pyrolysed under increased **O_2** content (sample 20a). For comparison, Hale et al. (2012) reported C_{free} in a lower range from 0.2 to 10 ng/L, with the highest concentration of 162 ng/L listed for a biochar produced by gasification. Only one third of all biochars had C_{free} concentrations above the maximum blank level of 12 ng/L for the Σ16 US EPA PAHs (Fig. 1B, blue line). There was not a clear correlation between C_{total} and C_{free} (Fig. S1, panels A–Q) for C_{total} in the lower concentration range, neither for individual PAHs, nor for their sum. A linear relationship could be observed only in case of high C_{total} for individual light PAHs such as NAP and ACY (>10 mg/kg_{dw} NAP, Fig. S1A and >1 mg/kg_{dw} ACY, Fig. S1B).

The multiple linear regression model that relates C_{free} (Table S2) with the C_{total}, SSA, C, H, and O was significant (R^2 = 0.40, p-value = 1.7e-04), and correlated positively with C_{total}/SSA, and negatively with H/C. No correlation was found with O/C. Accordingly, we can confirm the first, but must reject the second and third of the mechanistic hypotheses formulated in Section 2.6. This finding indicates that the bioavailabilities of our real world biochars were not easily predictable based on their basic physical-chemical properties.

Similar to the C_{total}, the C_{free} was dominated by the low molecular weight PAHs, particularly NAP (median: 12.4 ng/L, maximum 77.2 ng/L). Up to 90% of the quantified PAHs in the C_{free} consisted of NAP, which also dominated in the 10 experimental blanks (74%). Cross-contamination of samples with NAP in the laboratory can occur and was previously reported (Hilber et al., 2012) as a consequence of the ubiquitous presence of NAP, its volatility, and its dominance in biochars that have partly very high C_{total}. Still, NAP was not removed from the data analysis because (i) of its general dominance among the 16 US EPA PAHs in biochar, and (ii) it contributed to the highest C_{free} values. Freely dissolved concentrations of the other PAHs in biochars were near or below ambient environmental levels (Lang et al., 2015). Benzo[*a*]pyrene, the most carcinogenic PAH compound, ranged from 0.002 to 0.01 (Fig. S1M) with a median of 0.008 ng/L. For comparison, the BaP C_{free} (0.07 ± 0.05 ng/L) of the surface sediments in the Baltic Sea (Lang et al., 2015) were about ten times higher than the ones measured in some wood and *Miscanthus* derived biochars in this study. As NAP is prevalent in C_{free}, it was compared with BaP in terms of its toxicity equivalent factor (TEF), which is 0.001 (Andersson and Achten, 2015). On this basis, the C_{free} of NAP were similar to those of BaP in the surface sediments of the Baltic Sea (Lang et al., 2015), which is still considered as ambient environmental concentration.

The K_D values were determined for 39% of all individual PAH results (16 US EPA PAHs in each of the 43 biochars). The log K_D (L/kg) minima - maxima of some representative individual PAHs for the different biochars ranged for NAP from 4.2 to 6.4, for ANT from 5.2 to 7.6, for PYR from 5.3 to 7.6, for BaP from 6.5 to 8.5, and for BPE from 6.2 to 8.0, respectively (Table S3). These K_Ds are well within the range reported by Hale et al. (2015) and they are also similar to values for other black carbon materials such as AC (log K_{AC} were 5.6 for 2-rings, 6.1–6.6 for 3-rings, 7.1–7.6 for 4-rings, 7.6–7.8 for 5-rings, and 8.1–8.5 for 6-rings, calculated from QSAR (Thorsen et al., 2004)) or soot (log K_{soot} were 4.6 for NAP, 6.0 for FLU, 6.6 for PHE, and 7.0 for PYR, compounds spiked into the water phase (Bucheli and Gustafsson, 2000)). Generally, the K_D of condensed geosorbents like biochars is one to two orders of magnitude higher than the non-pyrogenic organic carbon-water partition coefficients (Cornelissen et al., 2005; Koelmans et al., 2006; Hale et al., 2015).

From these elevated K_Ds, it appears that the biochars acted more as a sink than a source of PAHs. This result confirms initial findings of Mayer et al. (2016), where the C_{free} of PAHs from two highly contaminated biochars were below environmental levels as well. In that study, the sink capacity of the biochar was confirmed by an experiment with labeled PAHs spiked into the water where silicone rod and biochar were present as sinks. The recoveries of the labeled PAHs in the silicone rod were <5% and the biochar acted as the dominant sorptive sink (Mayer et al., 2016).

3.4. *Desorption resistant PAHs (C_{res}) determined with contaminant traps*

The desorption resistant PAH concentrations (C_{res}, Fig. 2, low concentrations depicted in Fig. S2) were high in all samples. This is illustrated by the close agreement between the concentrations in the +traps (C_{+trap}) and the -trap (C_{-trap}, control) of the ∑16 US EPA PAHs to the 1:1 line (Fig. 2). Only eight (out of 33) samples showed significantly lower C_{+trap} than C_{-traps} (black symbols, Figs. 2 and S2) for the ∑16 US EPA PAHs and for these, the $f_{bioaccessible}$ was concentration independent at 30% on average (Fig. 3).

In seven samples, NAP and PHE had significantly reduced C_{+trap} in comparison to C_{-trap}. This was also the case for PYR in six samples and for BaP in three samples. In these cases, the mean $f_{bioaccessible}$ was 18% for NAP, 29% for PHE, 26% for PYR, and 51% for BaP. About two thirds of the individual PAHs (except for ANA, ANT, BaA, BaP, IPY, and DBA) showed data near the 1:1 line thereby confirming the PAHs were desorption resistant (Fig. S3, panels A, B, D, F, G, H, J, K, L, P). This result is in line with Mayer et al. (2016) who assessed highly desorption resistant concentrations of individual PAHs in two model biochars and the $f_{bioaccessible}$ were between 0 and 10%. The same multiple linear regression model as for C_{free} was run for $f_{bioaccessible}$. All independent variables (i.e., C_{total}/SSA, H/C, O/C) had no significant influence on the $f_{bioaccessible}$ (Table S4). Hence, for the set of biochars investigated here, PAH bioaccessibility was even less “predictable” than bioavailability.

The **O_2** treatment did not increase the bioaccessibility in the samples 10a and 16a but in the *Miscanthus* (20a) biochar. For this sample, 15 of the 16 PAHs exhibited significantly lower C_{+trap} than C_{-trap} (Fig. S3, dots labeled with 20a). The corresponding sample 20 only had two PAHs (NAP, PHE) that were significantly reduced in the C_{+trap}. The K_Ds of sample 20a and 20 hardly differed (Table S3). However, the influence of increased O_2 during pyrolysis was not consistent, neither in the bioaccessibility nor in the C_{total} assessment (see Chapter 3.2). For the latter, it was the sugar beet biochar (sample 16a) which was increased in comparison to the control (sample 16).

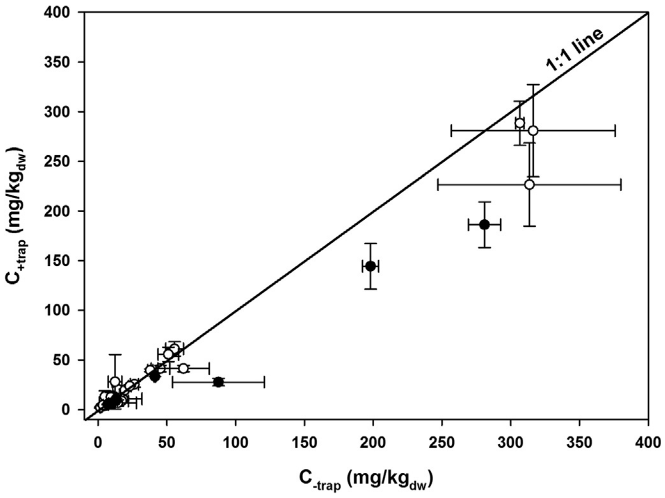

Fig. 2. The ∑16 US EPA PAH concentrations (C) in the samples subjected to contaminant traps (+traps) versus those in HPCD suspensions only (-traps). Error bars on x- and y-axis show the standard deviation of duplicates. The straight line indicates the 1:1 unity. Full symbols, eight in total, indicate significantly lower concentrations in the +trap than in the −traps. For identical plots of individual compounds, see Fig. S3 and for a zoom in on the low concentration range, see Fig. S2.

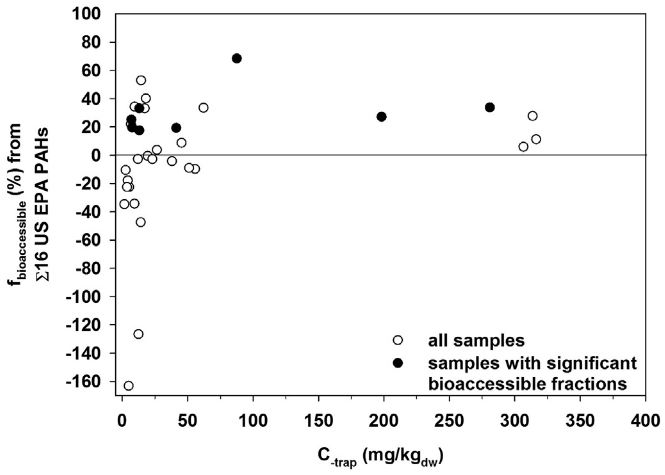

Fig. 3. Bioaccessible fractions ($f_{bioaccessibility;}$ eq. (1)) of the ∑16 US EPA PAH concentrations in various biochars, biochar mixtures and compost. Bioaccessible fractions below zero reflect the uncertainties caused by error propagation at low concentrations in the +trap and −trap samples.

The biochar that was lacto-fermented from the group **microorganisms** (sample 3b) was the sample with the second most PAH compounds (except NAP, ANA, PYR, BkF, Fig. S3, dots labeled with 3b, Fig. S4) that exhibited significantly less $C_{+\text{trap}}$ than $C_{-\text{trap}}$. The corresponding trapped sample 3 only showed four significantly reduced PAHs. Thus, it is possible that the microorganisms might have rendered the native PAHs more amenable for desorption in sample 3b than sample 3. In the real world, biochar in fermented material e.g., corn silage and biochar (Hansen et al., 2012) is fed to ruminants to decrease greenhouse gases and odor emissions. Zhang et al. (2015) investigated the bioaccessibility of PAHs in soot in a human digestive model and found the apparent bioaccessibility was not influenced in the gastric section but rather in the small intestine where bile acids join the food. It is possible that the mobilized PAHs in the **microorganism** treated biochars in this study would easily, and maybe even more efficiently, be absorbed by the digestive tract of ruminants. PAHs in co-composted (**blend**) biochars, as well as those in the corresponding pure composts (**compost**) were fully desorption resistant (Fig. S4). Thus, co-composting biochar did not alter the bioaccessibility of the PAHs. Finally, opposite to urban sludge treatment by Bernal-Martinez et al. (2007), **O_3** did not facilitate bioaccessibility of the PAHs (sample 19a in comparison to the control, sample 19).

Bioaccessibility can also be depicted as trap concentration differences (i.e., $C_{-\text{trap}}$ - $C_{+\text{trap}}$; Fig. 4) vs. $C_{-\text{trap}}$. While there is some scatter in the data because of error propagation particularly at low $C_{-\text{trap}}$ (also Fig. 3), it is apparent that most differences are marginal (and some even negative), confirming dominating C_{res}. Low molecular weight PAHs such as NAP and FLU displayed generally higher bioaccessibility (Fig. 4), which is in the line with the fingerprint of the C_{total} of biochar (Bucheli et al., 2015) and the lower K_D of light PAHs than of heavy ones. Thus, also the trap experiment confirmed that the biochars generally should be seen as PAH sink rather than as source. However, at highest $C_{-\text{trap}}$ (>10 mg/kg$_{\text{dw}}$, individual PAHs), bioaccessibility can be considerable, with up to several tens of mg/kg for individual PAHs (Fig. 4). Consequently, highly contaminated biochars can release PAHs that are relevant in terms of mass. Therefore, biochars shall meet the EBC guidelines for C_{total} to minimize the exposure risk.

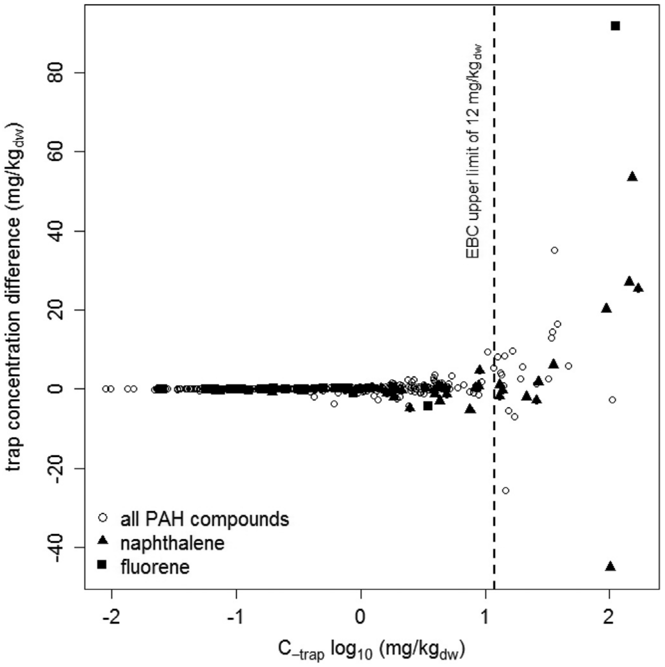

Fig. 4. Trap concentration difference ($C_{-\text{trap}} - C_{+\text{trap}}$) versus $C_{-\text{trap}}$ of naphthalene and fluorene, and of all remaining individual PAHs in various biochars, biochar mixtures and compost samples. The dashed line indicates the upper PAH concentration of the European Biochar Certificate (EBC) of 12 mg/kg$_{\text{dw}}$ for the $\sum$16 US EPA PAHs.

4. Conclusions

The majority of the biochar samples showed marginal bioavailable and −accessible, i.e., highly desorption resistant, PAHs. Potential effects of post-pyrolytical treatments of the biochars on C_{total} and $f_{\text{bioaccessibility}}$ were inconsistent and hardly significant, and the corresponding scientific hypotheses must therefore be rejected. Nevertheless, high C_{total} must be avoided (which is generally the case when biochar is properly produced; Bucheli et al. (2015)) according to the precautionary principle because of the risk of correspondingly high bioaccessible mass of pollutants. Note that these conclusions are solely based on data obtained with chemical proxy methods for bioavailability in the pure aqueous phase. In our view, the risk of humans and animals by oral exposure of native PAHs in biochar still needs to be addressed, to assess whether the desorption-resistant fraction is also largely non-accessible when the biochars are ingested or exposed *in vitro* to artificial human and animal gut fluids (Zhang et al., 2015). Finally, PAHs data in this paper were largely presented as Σ16 US EPA PAHs. We did this because current legislations and biochar quality assurance and control guidelines are based on this parameter. In light of the dominance of light and less toxic PAHs in biochar, TEF as proposed, e.g., by Andersson and Achten (2015) may be more adequate for an ecotoxicological risk assessment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.02.014.

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