Application of biochar to soils may result in plant contamination and human cancer risk due to exposure of polycyclic aromatic hydrocarbons

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ABSTRACT

Biochars are added to soil to improve agronomic yield. This greenhouse- and field-scale study evaluated polycyclic aromatic hydrocarbon (PAH) contamination in 35 commercial and laboratory-produced biochars, and assessed the effects of biochar amendment of soils on PAH accumulation in vegetables and the risk for cancer. The total and bioavailable PAH concentrations in biochars varied from 638 to 12,347 µg/kg and from below the detection limit (BDL) to 2792 µg/kg, respectively. PAH formation in biochars decreased with increasing production temperature (350–650 °C). Root exudates enhanced PAH release from biochars. The total PAH concentrations in eight edible vegetables growing in biochar-amended soil varied according to biochar and vegetables type from BDL to 565 µg/kg. A health risk assessment framework was integrated with the benzo[a]pyrene toxic equivalency quotient and the incremental lifetime cancer risk (ILCR) to estimate the exposure risk for human beings via ingestion of PAH-contaminated vegetables. The total ILCR for adults was above 10^{-6}, which suggests a risk to human health from direct exposure to PAHs in vegetables grown in biochar-amended soil. These results demonstrate that biochar application may lead to contamination of plants with PAHs, which represents a risk to human health. The PAH levels in biochars produced using different conditions and/or feedstocks need to be evaluated and biochars should be pretreated to remove PAHs before their large-scale agronomic application.

1. Introduction

Approximately 140 billion metric tons of agricultural residues are produced per year worldwide, with 800 million tons in China (United Nations Environmental Program, 2010; Fu et al., 2017). How to reuse this agricultural waste is an important issue. The application of biochar derived from crop residues via thermal pyrolysis to land facilitates nutrient cycling and is increasingly important for agricultural sustainability for a growing population. According to the State of the Biochar Industry Report of the International Biochar Initiative (IBI, 2017), up to 800,000 tons of crop residues were converted into biochar from 2016 to 2017 in China, which is expected to increase to 3 million tons within five years.

The application of biochar to soil is a new approach to reusing crop straw. Biochar is a low-cost and environmentally friendly agent that increases crop productivity and reduces soil pollution. A 3-year field trial found a significant increase (3.0 t/ha) in the above-ground biomass of *Dactylis glomerata* growing in a field amended with biochars derived from mechanically chipped trunks and large branches pyrolyzed at 450 °C for 48 h (Jones et al., 2012). Similarly, amendment with biochar pyrolyzed from wheat straw at 10 and 40 t/ha enhanced rice yields by 12% and 14% on the Tai Lake Plain, China (Zhang et al., 2010). Moreover, biochar may decrease the bioavailability of toxic elements and organic compounds in contaminated soils and thus reduce their accumulation in crops (Khan et al., 2014; Lehmann, 2007; Herath et al., 2013). Khan et al. (2014) observed that adding biochar (10%) to soil markedly reduced the accumulation of As(III) (72%), dimethylarsinic acid (DMA) (74%), and As(V) (62%) in rice. In another study, adding peanut shell-based biochar (5%) to soil significantly decreased (84%) PAH accumulation in turnip (Khan et al., 2015a). However, most studies did not assess the risk of contamination due to biochar application.

Biochar may be produced from different biomass such as manure, crop residue, sludge, wood, etc. by thermal pyrolysis (Lehmann, 2007). However, PAHs are produced during pyrolysis (carbonization) in the

absence of air. Biochars produced by pyrolysis at different temperatures and from different feedstocks may contain different levels of PAHs. Hilber et al. (2012) reported that the total concentrations of 16 United States Environmental Protection Agency (US-EPA)-priority PAHs in four biochars ranged from 9113 μg/kg (coniferous residues) to 355,295 μg/kg (wood residues). In a previous study, the total concentrations of 11 three- to five-ring PAHs in grass (tall fescue straw)- and wood (ponderosa pine)-derived biochars produced at 400 °C and 500 °C were 130–26,000 and 50–30,200 μg/kg, respectively (Keiluweit et al., 2012). In another study, the total PAH level in biochar produced by thermal pyrolysis of birchwood was10,000 μg/kg (Fagernäs et al., 2012). Hence, biochar used as a soil amendment agent may act as a source of PAHs (Mukherjee and Lal, 2014; Qadeer et al., 2017). Biochar amendment of soils reportedly also has adverse effects (Mukherjee and Lal, 2014; Kuppusamy et al., 2016). Rombolà et al. (2012) reported that the total PAH concentrations in soil amended with orchard pruning-derived biochars were 34,500–42,900 μg/kg. Biochar amendment led to a marked increase in the total extractable PAH concentrations, which subsequently decreased from 153 to 78 μg/kg after 35 months. These PAH concentrations were significantly higher than that of unamended control soil (24 μg/kg). Similarly, Quilliam et al. (2013) found that the concentrations of 16 US-EPA-priority PAHs in soil amended with wood-based biochar (50 t/ha) for 3 years was 1953 μg/kg, significantly higher than that of control soil (1131 μg/kg).

PAHs are carcinogenic and mutagenic, and thus are classified as persistent organic health-threatening pollutants (Perera, 1997). The application of PAH-containing biochar to agricultural soil may represent a risk to human health, as vegetables may accumulate PAHs at high concentrations from such soils (Gao and Collins, 2009). Hence, an understanding of the negative effects of PAH-containing biochars on crop security and human health is critical for their large-scale application. To date, research has focused on the effects of biochar on crop productivity; there is little information on the risk of vegetable contamination and risk to human health. Although soil PAH levels are reportedly increased by biochars (Quilliam et al., 2013), to the best of our knowledge, few studies have investigated PAH accumulation in vegetables and the risk for human cancer due to biochar amendment of agricultural soil.

To address this crucial knowledge gap, we conducted greenhouse and field experiments to: (1) elucidate the PAH accumulation in vegetables grown in biochar-amended soil; and (2) estimate the incremental lifetime cancer risk (ILCR) via dietary intake of vegetables contaminated with biochar-associated PAHs in soil.

2. Materials and methods

2.1. Chemicals, soil, and biochar

A standard solution of the 16 EPA-priority PAHs (98% purity) was purchased from O2si Smart Solutions Co. (Charleston, SC). The properties of those PAHs are listed in Table S1.

The Typic Paleudalf soil used for the greenhouse experiments was collected from Nanjing, China, and has the following physicochemical characteristics: pH 6.14, 15.5 g/kg total organic carbon content, 1.1 g/kg total nitrogen content, 24.7% clay, 13.4% sand, and 61.9% silt. Soil pH was determined by agitating the mixture of soil: distilled water (1:2.5) for 30 min and then standing quietly for 1 h. The pH of supernatant was measured by a pH meter. The total organic carbon content was measured using the potassium bichromate titrimetric method according to National Standards of China (GB 9834-88). The total nitrogen content was determined using an automatic Kjeldahl nitrogen meter (SKD-1000, Shanghai, China). After collection, the soil was air-dried and sieved (< 4 mm). The field experiment was performed in suburban agricultural fields in Nanjing, and the soil was classified as a typic Paleudalf. The characteristics of the field soil are as follows: pH 6.84, 8.5 g/kg total organic carbon content, 2.3 g/kg total nitrogen

content, 24.4% clay, 12.1% sand, and 63.5% silt. The initial concentrations of PAHs in test soils for greenhouse and field experiments were under the detection limits.

Twenty biochars were purchased at a market and had been produced by pyrolysis at 250–900 °C from apple wood (B1), elm wood (B2), lychee tree (B3), oak tree (B4), walnut shell powder (B5), coconut husk (B6), pine wood (B7), jujube wood (B8), corn stover (B9), wheat straw (B10), pear tree (B11), rice straw (B12 and B13 in Nanjing in 2016 and 2017; B14 in Liyang), rice husk (B15, B16, and B17 in Nanjing, Shanghai, and Nantong, respectively), and bamboo (B18, B19, and B20 from Zhejiang, Guangxi, and Jiangxi, respectively).

To assess the effects of feedstock and pyrolysis temperature on PAH concentrations in biochar, 15 biochars were produced in our laboratory. The feedstocks and pyrolysis temperatures used were as follows: reed (B21, B22, and B23 produced at 350 °C, 500 °C, and 650 °C, respectively); sesame stalks (B24, B25, and B26 produced at 350 °C, 500 °C, and 650 °C, respectively), pine needles (B27, B28, and B29 produced at 350 °C, 500 °C, and 650 °C, respectively), soybean straw (B30, B31, and B32 produced at 350 °C, 500 °C, and 650 °C, respectively), and cypress wood (B33, B34, and B35 produced at 350 °C, 500 °C, and 650 °C, respectively). All of these biochars were produced by pyrolysis in muffle furnace under an oxygen-free condition. The heating rate was employed at 5 °C/min, and kept for 8 h when reaching the set temperature (350 °C, 500 °C, and 650 °C).

The general physicochemical properties of the commercial and laboratory-produced biochars are listed in Table S2 and Figs. S1 and S2 in Supporting information (SI).

2.2. Greenhouse and field experiments

Each commercial biochar was manually mixed with the test soil at a 3% ratio, corresponding to 48 t/ha (assuming a soil depth of 30 cm and density of 1.2 g/cm^3). Each cell of eight-cell potted containers (Fig. S3 in SI) was filled with 1 kg biochar-mixed soil, irrigated with deionized water, allowed to stand for 1 day, and planted with seedlings of carrot (*Daucus carota*), Chinese cabbage (*Brassica chinensis*), water spinach (*Ipomoea aquatica Forsk*), pterocladia tenuis (*Brassica rapa*), spinach (*Spinacia oleracea*), pakchoi (*Brassica campestris*), lettuce (*Cichorium endivia*), or cherry radish (*Raphanus sativus*). To produce seedlings, plant seeds were soaked in warm water (55–60 °C) for 15 min and transferred to Petri dishes in a 28 °C incubator for 3 days to germinate. The containers were placed in random locations in the greenhouse at 25 ± 3 °C (day) and 20 ± 3 °C (night), with 12 h of natural light, until reaching maturity. Each cell contained 8 seedlings. Half-strength Hoagland's nutrient solution (2 mL) was added to each cell once for the first 2 weeks; subsequently, full-strength Hoagland's nutrient solution (50 mL) was added to each cell once weekly until harvest. Each experiment was conducted in triplicate. Chinese cabbage, water spinach, pterocladia tenuis, spinach, pakchoi, and lettuce were harvested after 45 days, and carrot and cherry radish were harvested after 70 days. The photos of test plants in greenhouse experiments were shown in Fig. S4 in SI. The harvested plants were separated into shoot and root parts, and thoroughly washed with deionized water, freeze-dried, and pulverized. Soil samples were collected from each cell, freeze-dried, and passed through 2 mm sieves. Plant and soil samples were stored at −20 °C until analyses.

The field experiment was performed in suburban agricultural fields in Nanjing, China using the B2, B5, B7, B9, B16, B18, B31, and B34 biochars. The field experiment was of randomized design, involving eleven 2 × 2 m^2 plots. The biochars were manually mixed with the field soil at a 3% ratio, corresponding to 48 t/ha (assuming a soil depth of 30 cm and density of 1.2 g/cm^3). Base fertilizer comprising 176 g N, 39 g P, and 61 g K was added to each plot. Two vegetables, namely, Chinese cabbage (*Brassica chinensis*) and pakchoi (*Brassica campestris*), were grown in each plot. During the growth period, the plots were irrigated with water every 2 days, and plant samples were collected after

45 days. Each experiment was conducted in triplicate. The photos of plants in field study were shown in Fig. S5 in SI. The harvested plants were thoroughly washed with deionized water, freeze-dried, separated into shoot and root parts, and pulverized. The soil samples were collected, freeze-dried, and passed through 2 mm sieves. Plant and soil samples were stored at $-20\,°C$ until analyses.

2.3. Root exudate-mediated release of PAHs from biochars

Chinese cabbage and water spinach were used for production of natural root exudates (NRE). Plant culture and NRE collection were performed according to Mench and Martin (1991). The characteristics of the NREs are listed in Table S3. The total organic carbon contents of NREs from Chinese cabbage and water spinach were 9.8 and 11.6 mg/L, respectively. An artificial root exudate (ARE) stock solution was prepared by mixing 26.7 mmol/L lactic acid, 26.7 mmol/L fructose, 26.7 mmol/L glucose, 13.3 mmol/L sucrose, 13.3 mmol/L citric, 13.3 mmol/L serine, 13.3 mmol/L alanine, 19.8 mmol/L succinic acid and 7.8 mmol/L glutamic acid (Baudoin et al., 2003). The total organic carbon content of ARE stock solution was 10,000 mg/L, and the total organic carbon content of ARE working solution used for the release of PAHs from biochars was 100 mg/L.

Next, we evaluated the release of PAHs from biochars B2, B8, B16, B18, and B33. Biochar (10 g) was packed into Erlenmeyer flasks, to which 150 mL NRE, ARE, or deionized water (control) was added, followed by 50 mmol/L NaN_3 to inhibit microbial activity. The flasks were capped, wrapped in tinfoil, and shaken (25 °C, 120 r/min) in the dark for 14 days. After centrifugation at 2500 r/min for 6 min, the aqueous fraction (100 mL) from each flask was enriched on a solid-phase extraction column. PAHs were eluted from the column with 10 mL hexane, and the eluate was dried under a nitrogen stream. The residue was reconstituted in 1 mL chromatographic methanol, filtered, and the PAH concentrations were quantified by high-performance liquid chromatography (HPLC) using an ultraviolet detector and a fluorescence detector The HPLC detection parameters were described in the following section. Each experiment was conducted in triplicate.

2.4. PAH analyses in plants and biochars

The total amounts of 16 US-EPA PAHs were extracted from plant and biochar samples as described previously (Gao and Collins, 2009). Plant and biochar extracts were generated by ultrasonication three times for 30 or 80 min each in 10 mL n-hexane and dichloromethane (DCM) (1:1 v:v). The extract was resolved by centrifugation, combined, and passed through a silica gel column (top layer, 2 g anhydrous Na_2SO_4; sub-layer, 2 g silica gel). The column was eluted with 11 mL 1:1 (v/v) DCM. The samples were evaporated and exchanged with methanol to a final volume of 1 mL. After passage through 0.22 μm filter units, the PAH solutions were analyzed by HPLCwith ultraviolet and fluorescence detectors. The HPLC was ftted with a $\Phi 4.6 \times 250$ mm reverse-phase ^{18}C column using methyl alcohol and ultrapure water as the mobile phase at a flow rate of 1 mL/min. Chromatography was performed at 40 °C, and injection volume was 20 μL.External standard method was used to ensure the accuracy of the PAH detection. The detection procedure of HPLC, detection limits and recoveries of PAHs were shown in Tables S4–S6 in SI.

Bioavailable PAHs in biochars were analyzed as described by Stokes et al. (2005). Briefly, 1 g biochar sample was placed in a 40 mL glass centrifuge tube with 20 mL 60 mmol/L cyclodextrin (HPCD, Sigma Aldrich, Poole, UK). The samples were agitated at 250 r/min for 20 h on an orbital shaker, and centrifuged at 2500 r/min for 15 min. The supernatant was collected, passed through 0.22 μm filter units, mixed with methanol, and the PAH concentrations were determined.

2.5. Toxic equivalency quotient of PAHs in biochar and edible vegetables

Benzo[a]pyrene (BaP) is the most toxic and carcinogenic PAH (US-EPA, 2017). Therefore, it is used as an indicator in mixed-PAH toxicity analyses (Nakata et al., 2003). The BaP toxic equivalency quotient (TEQ) of biochar and vegetable samples was calculated by summing the BaP-equivalent toxicity of each PAH, as follows:

$$TEQ = \sum_{i=1}^{16} (PAH_i \times TEF_i)$$

where PAH_i is the concentration of PAH congener i, and TEF_i is the published toxic equivalent factor (TEF) of PAH congener i (Table S1).

2.6. Incremental lifetime cancer risk for PAHs

The ILCR values of PAHs in edible portion of vegetables were calculated referred to Xia et al. (2010). First, we calculated the BaP-equivalent concentration (BEC_j; μg/kg) of 16 USEPA PAHs in a certain vegetable j according to the equationas follows:

$$BEC_j = \sum_{i=1}^{n} C_i \times TEF_i$$

where C_i (μg/kg) is the concentration of PAH congener i in edible part of vegetable j, and TEF_i is the TEF of PAH congener i (Table S1). Then the total daily dietary PAH exposure level (E_D; ng/d) of a local adult due to intake of the eight vegetables was calculated:

$$E_{Dj} = BEC_j \times IR_j \qquad E_D = \sum_{j=1}^{8} BEC_j \times IR_j$$

where E_{Dj} (ng/d) is the daily dietary PAH exposure level due to intake of vegetable j. IR represents the total amount of vegetables ingested per day by an adult (236 g/d) (Zhai and Yang, 2006), and IR comprises the eight test vegetables: carrot (10%), pterocladia tenuis (10%), lettuce (10%), charry radish (10%), spinach (15%), Chinese cabbage (15%), pakchoi (15%), and water spinach (15%). IR_j is the amount of vegetable j ingested per day by an adult. Then the total Incremental lifetime cancer risk (Total ILCR) of PAHs in vegetables was determined using the following equations:

$$ILCR_j = \frac{(E_{Dj} \times EF \times ED \times SF \times CF)}{BW \times AT}$$
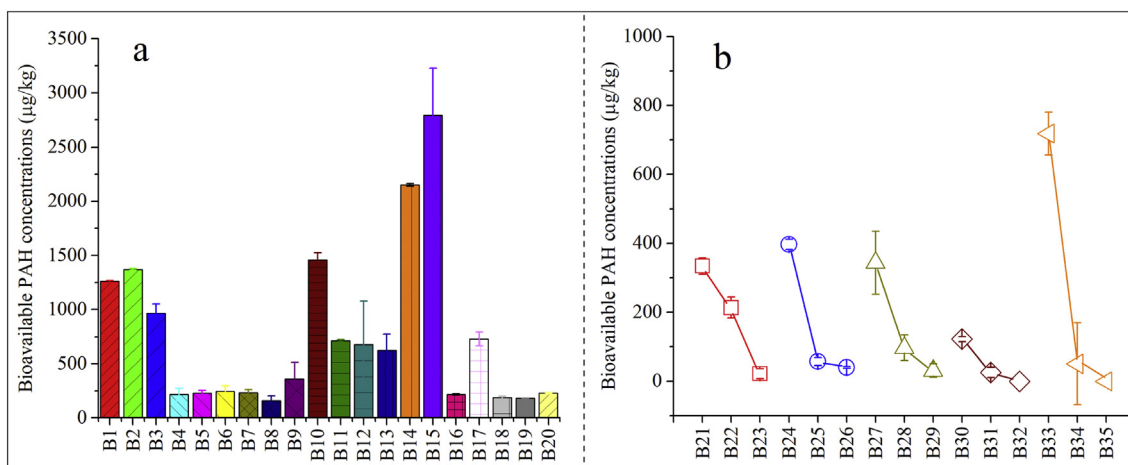
$$Total\ ILCR = \sum_{j=1}^{8} ILCR_j$$

where $ILCR_j$ is the Incremental lifetime cancer risk of PAHs imposed by vegetable j. SF is the slope factor of oral cancer for BaP. Generally, the slope factor is a plausible upper-bound estimate of the probability of a response per unit intake of a chemical over a lifetime, and the slope factors is 4.5, 5.9, 9.0, and 11.7 with a geometric mean of 7.3 (mg/kg)/d (US-EPA, 1993). ED is the exposure duration (43 years for adults from age 18 to 60 years) (Xia et al., 2010) and BW is the body weight of an adult (62.82 kg). AT is the average lifespan of carcinogens (25,550 days). EF is the exposure frequency (365 days/year). CF is a conversion factor (10^{-6} mg/ng).

2.7. Statistical analyses

All data were processed with Microsoft Excel 2013 (Microsoft, Redmond, WA). Data were analyzed using SPSS v. 19.0 (SPSS, Inc., Chicago, IL). Figures were generated by Origin (v. 9.2, MA). The differences between treatments were tested using ANOVA, while LSD's test ($P < 0.05$) was used for mean significance. Each experiment was conducted in triplicate. Data are means, and error bars indicate standard deviations (n = 3).
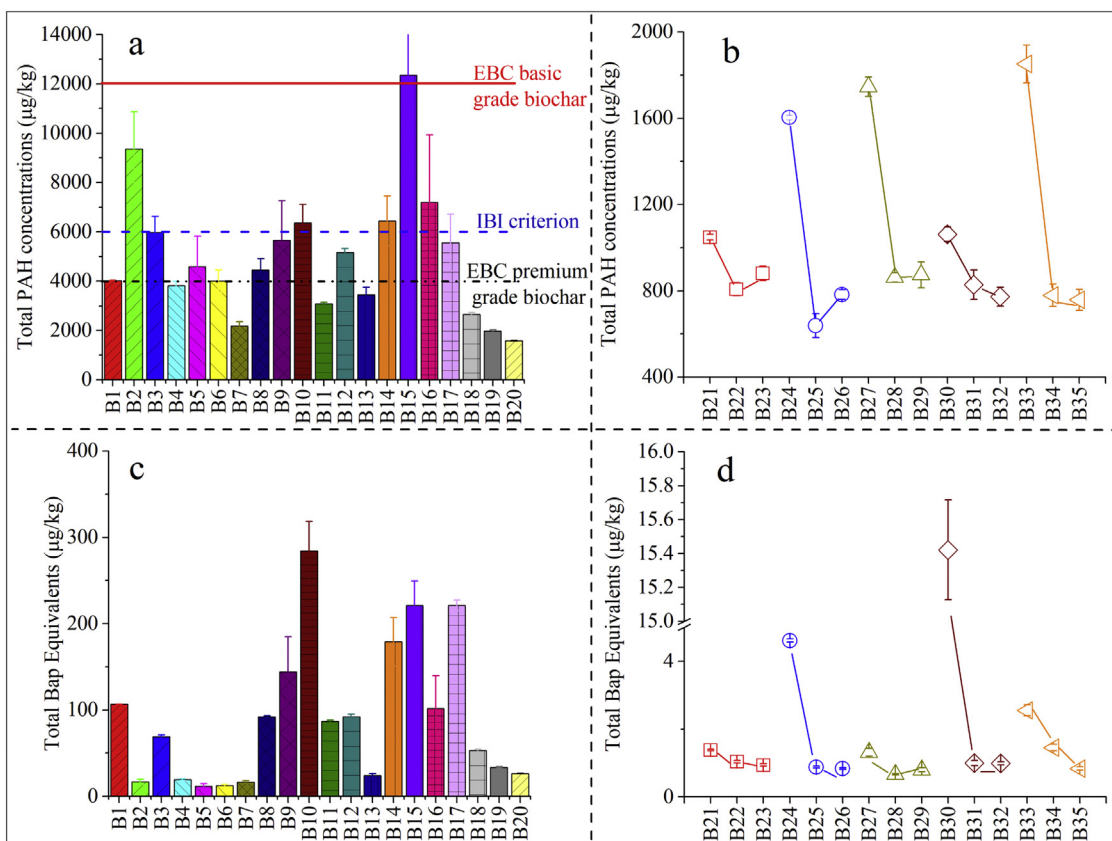
Fig. 1. Total PAH concentrations in commercial (a) and laboratory-produced (b) biochars, and TEQ values of commercial (c) and laboratory-produced (d) biochars. B1–B20, commercial biochars produced at 250–900 °C from apple wood (B1), elm wood (B2), lychee tree (B3), oak tree (B4), walnut shell power (B5), coconut husk (B6), pine wood (B7), jujube wood (B8), corn stover (B9), wheat straw (B10), pear tree (B11), rice straw (B12 and B13 from Nanjing in 2016 and 2017, B14 from Liyang), rice husk (B15, B16, and B17 from Nanjing, Shanghai, and Nantong, respectively), and bamboo (B18, B19, and B20 from Zhejiang, Guangxi, and Jiangxi, respectively). B21–B35 were produced by pyrolysis for 8 h in the laboratory from reed (B21, B22, and B23 at 350 °C, 500 °C, and 650 °C, respectively), sesame stalks (B24, B25, and B26 at 350 °C, 500 °C, and 650 °C, respectively), pine needles (B27, B28 and B29 at 350 °C, 500 °C, and 650 °C, respectively), soybean straw (B30, B31, and B32 at 350 °C, 500 °C, and 650 °C, respectively), and cypress wood (B33, B34, and B35 at 350 °C, 500 °C, and 650 °C, respectively). Error bars represent standard errors.

Fig. 2. Bioavailable concentrations of 16 EPA-priority PAHs in the commercial (a) and laboratory-produced (b) biochars. B1–B20, commercial biochars produced at 250–900 °C from apple wood (B1), elm wood (B2), lychee tree (B3), oak tree (B4), walnut shell power (B5), coconut husk (B6), pine wood (B7), jujube wood (B8), corn stover (B9), wheat straw (B10), pear tree (B11), rice straw (B12 and B13 from Nanjing in 2016 and 2017, B14 from Liyang), rice husk (B15, B16, and B17 from Nanjing, Shanghai, and Nantong, respectively), and bamboo (B18, B19, and B20 from Zhejiang, Guangxi, and Jiangxi, respectively). B21–B35 were produced by pyrolysis for 8 h in the laboratory from reed (B21, B22, and B23 at 350 °C, 500 °C, and 650 °C, respectively), sesame stalks (B24, B25, and B26 at 350 °C, 500 °C, and 650 °C, respectively), pine needles (B27, B28 and B29 at 350 °C, 500 °C, and 650 °C, respectively), soybean straw (B30, B31, and B32 at 350 °C, 500 °C, and 650 °C, respectively), and cypress wood (B33, B34, and B35 at 350 °C, 500 °C, and 650 °C, respectively). All the laboratory-made biochars were produced with the pyrolysis residence time of 8 h. Error bar represent standard error.

3. Results

3.1. Concentrations of PAHs in biochars

The total concentrations of PAHs ($C_{\Sigma PAH}$) in the commercial and laboratory-produced biochars were 608–12,347 µg/kg (Fig. 1, Table S7). Commercial biochars generally have higher $C_{\Sigma PAH}$ values than those produced in the laboratory. The $C_{\Sigma PAH}$ of the laboratory biochars produced from reeds, sesame stalks, pine needles, soybean straw, and cypress wood generally decreased as pyrolysis temperature increased from 350 °C to 650 °C; the $C_{\Sigma PAH}$ of biochars produced at 350 °C was 22–69% higher than those produced at other temperatures. The PAH composition of biochars differed markedly according to the pyrolysis temperatures used (Fig. S6, Table S9). The laboratory-produced biochars predominantly contained three and four benzene-ring PAHs (> 50%); five-ring PAHs were detected at negligible levels. Two- to four-ring PAHs (> 90%) predominated in the commercial biochars; five- and six-ring PAHs were scarce.

The mean BaP toxic equivalency quotient values of the 16 EPA-priority PAHs in the commercial and laboratory-produced biochars were 11.69–284.44 and 0.66–15.42 µg/kg, respectively (Fig. 1). The wheat straw and pine needle biochars (500 °C) had the highest and lowest TEQ values, respectively. The commercial biochars had much higher BaP toxic equivalency quotient values than the laboratory-produced biochars. The BaP toxic equivalency quotient values of PAHs in biochars generally decreased as the pyrolysis temperature increased.

The bioavailable concentrations of PAHs in the commercial and laboratory-produced biochars were 159–2792 µg/kg and BDL to 718 µg/kg, respectively (Fig. 2), which represented 3–33.3% and 0–31.9% of the total PAHs. The bioavailable PAH concentrations in commercial biochars were generally much higher than those of laboratory-produced biochars. The bioavailable concentrations of PAHs in laboratory-produced biochars were dependent on the feedstock used (Fig. 2) and decreased with increasing pyrolysis temperature; the bioavailable PAH concentrations of biochars produced at 350 °C were 36–100% higher than those produced at 500 °C and 650 °C.

3.2. PAH accumulation in vegetables

The PAH concentrations in vegetables varied according to the biochar applied and the vegetable species (Fig. 3). Because the eight vegetables died in soils amended with B15 (commercial biochar from rice husk; highest PAH concentration), PAH data are not available for B15-amended soils. The PAH concentrations ($C_{\Sigma PAH}$) in carrot, Chinese cabbage, water spinach, and lettuce grown in soil amended with the 19 commercial biochars were 10.31–347.39, 5.63–523.87, 0.27–565.04, and 0.50–445.08 µg/kg, respectively. PAHs were detectable in 84% of pterocladia tenuis, 84% of packchoi, 89% of cherry radish, and 58% of spinach samples with the maximum $C_{\Sigma PAH}$ of 392.69, 425.43, 469.10, and 406.77 µg/kg, respectively.

The BaP toxic equivalency quotient (TEQ) values of vegetables grown in biochar-amended soil in the greenhouse ranged from 0.37 to 5.12 µg/kg for carrot, 0.03 to 14.80 µg/kg for Chinese cabbage, BDL to 15.67 µg/kg for water spinach, BDL to 10.60 µg/kg for pterocladia tenuis, BDL to 13.69 µg/kg for spinach, BDL to 10.21 µg/kg for pakchoi, BDL to 6.84 µg/kg for lettuce, and BDL to 2.29 µg/kg for cherry radish. According to the China Food and Drug Administration (CFDA), the BaP TEQ maximum contaminant level (MCL) in cereal is 5 µg/kg. But to date, there is still no MCL of BaP toxic equivalency quotient in vegetables. If based on the MCL in cereal, 5%, 60%, 45%, 25%, 10%, 15%, and 10% of the carrot, Chinese cabbage, water spinach, pterocladia tenuis, spinach, pakchoi, and lettuce samples, but none of the cherry radish samples, had PAH concentrations greater than the MCL. One notes that the daily dietary PAH exposure level of cereal and vegetable should actually be different. Results basing on the MCL of cereal may over- or under-estimate the actual BaP toxic equivalency quotient of

test vegetables, and MCL for BaP toxic equivalency quotient in vegetables needs to be built up in future.

Six commercial biochars and two laboratory-produced biochars were used in the field study. The $C_{\Sigma PAH}$ values of Chinese cabbage and pakchoi harvested from the field were 98–1494 and 93–1277 µg/kg, with averages of 534 and 449 µg/kg, respectively. The corresponding BaP toxic equivalency quotient values of Chinese cabbage and pakchoi were 4.02–13.30 and 4.89–11.86 µg/kg, and 75.0% of Chinese cabbage and 87.5% of pakchoi samples had BaP toxic equivalency quotient values higher than the MCL of 5 µg/kg (Table 1).

3.3. Intake of PAHs in vegetables and associated health risks

The ILCR values for an adult induced by exposure to PAHs due to vegetable consumption are shown in Fig. 4. The total ILCR values of vegetables grown in biochar-amended soil in the greenhouse were around 10^{-6}; the highest value was 6.57×10^{-6}. ILCR values $< 10^{-6}$ are indicative of no risk to human health, values of 10^{-6} to 10^{-4} indicate a low risk, and ILCR values $> 10^{-4}$ are indicative of a high risk (US-EPA, 1996). The ILCR values for 10% of carrot, 40% of Chinese cabbage, 40% of water spinach, and 5% of spinach samples were 10^{-6} to 10^{-4}, which suggests a low risk to health. The ILCR values of pakchoi, pterocladia tenuis, lettuce, and cherry radish were lower than 10^{-6}. The total ILCR values of the eight vegetables grown in 80% biochar-amended soils were 10^{-6} to 10^{-4}, which indicates a low risk to health.

The field experiments also suggested a risk to human health (Table 1). The ILCR values of PAHs for intake by an adult of Chinese cabbage and pakchoi grown in biochar-amended fields were 6.00×10^{-7}–1.44×10^{-6} and 3.90×10^{-7}–1.30×10^{-6}, respectively. The total ILCRs were 1.49×10^{-6}–2.16×10^{-6}, which indicates a risk to human health.

3.4. Root exudates-mediated release of PAHs from biochar

The high PAH levels in, and risk to human health of, vegetables grown in biochar-amended soils may be due to the enhancement of PAH release by root exudates (Table 2). The artificial root exudates markedly increased PAH release (to 28.61–70.32 µg/kg) from commercial and laboratory-produced biochars after 14 days compared to the control. PAH release was also significantly increased by natural root exudates for some studies biochars (like B16 and B18), despite their lower total organic carbon content compared to the artificial root exudates. For instance, 62.24 µg/kg PAHs were released from B16 in the presence of Chinese cabbage NRE, which was 88% higher than the control. The amounts of PAHs released from test biochars by water (control treatment), NRE-1 (Chinese cabbage), and NRE-2 (water spinach) were 8.69–29.90, 7.31–62.24, and 9.24–37.46 µg/kg, respectively. PAHs with two to four benzene rings represented the majority of the PAHs released from biochars by the natural root exudates (Fig. S7).

4. Discussion

Our findings demonstrate that PAHs are produced during biochar production by thermal pyrolysis of agricultural residues. High concentrations of the 16 USEPA-priority PAHs were detected in commercial (1580–12,350 mg/kg) and laboratory-produced (505–1745 µg/kg) biochars, as has been reported by others. Hale et al. (2012) reported that biochars derived from dairy manure, food waste, paper-mill waste, corn stover, wheat straw, rubberwood sawdust, lodgepole pine, pine wood, switch grass, laurel oak, loblolly pine, eastern gamma grass, hardwood, heartland pine, secondary mixed wood, corn cob, rice husk, maize residues, corn stover, sawdust, empty fruit bunches, and coconut shell pyrolyzed at 250–900 °C contained 70–3270 µg/kg PAHs. PAH concentrations of 27,000 and 17,000 µg/kg were detected in biochars from wood (400 °C) and paper sludge (500 °C), respectively (Keiluweit et al.,
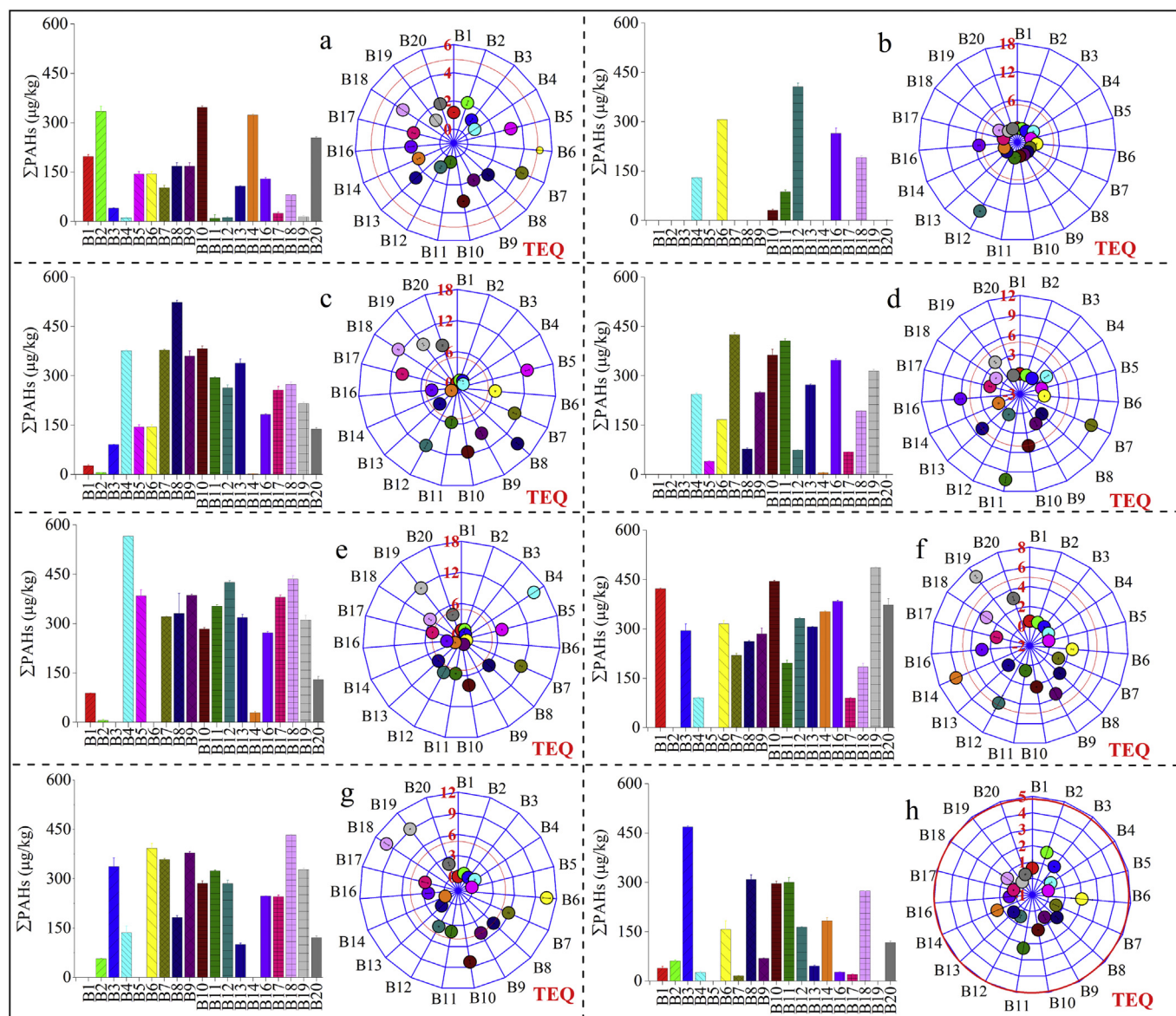
Fig. 3. Total PAH concentrations ($C_{\Sigma PAH}$) and TEQ values in carrot (a), spinach (b), Chinese cabbage (c), pakchoi (d), water spinach (e), lettuce (f), *Pterocladia tenuis* (g), and cherry radish (h) grown in biochar-amended soil in the greenhouse. Red circle, MCL (5 μg/kg) of BaP in cereal according to the CFDA. Because the eight vegetables died in soils amended with B15 (commercial biochar from rice husk; highest PAH concentration), PAH data are not available for those soils. B1–B20, commercial biochars produced at 250–900 °C from apple wood (B1), elm wood (B2), lychee tree (B3), oak tree (B4), walnut shell power (B5), coconut husk (B6), pine wood (B7), jujube wood (B8), corn stover (B9), wheat straw (B10), pear tree (B11), rice straw (B12 and B13 from Nanjing in 2016 and 2017, B14 from Liyang), rice husk (B15, B16, and B17 from Nanjing, Shanghai, and Nantong, respectively), and bamboo (B18, B19, and B20 from Zhejiang, Guangxi, and Jiangxi, respectively). All the commercial biochars bought from the market, and produced at 250–900 °C. Error bars represent standard error.

2012; Devi and Saroha, 2014). The concentrations of PAH generated depended on the biochar production conditions such as pyrolysis temperature. With respect to the pyrolysis temperature in present study, the higher temperature (650 °C) resulted in the lowest total PAH concentrations in biochars. PAHs concentration in biochars produced at 650 °C were 16%–59% lower than those at 350 °C irrespective of the different feedstocks. Wang et al. (2013) reported that giant reed-derived biochars (pyrolysis at 200–600 °C for 2 h) contained 119.27–2125.56 μg/kg PAHs, and PAHs in biochar obtained at 600 °C was 92% lower than those pyrolyzed at 350 °C. In a comprehensive study, the PAH content of biochars from thermomechanical pulp sludge was 400–236,000 μg/g, depending on the temperature (450 °C, 500 °C, and 550 °C) and residence time (30, 60, 120 min), and biochars pyrolyzed at 500 °C for 60 min held higher PAH concentrations than others (Khan et al., 2015b).

The bioavailable PAH contents of the biochars comprised 33.3% and 31.9% of the total PAHs for the commercial and the lab-made biochars, respectively, and the bioavailable PAHs in biochars decreased with increasing the production temperature (350 °C, 500 °C, and 650 °C). Hilber et al. (2017) reported that wood, sugar beet, Miscanthus (elephant grass), lop (green waste), grape pomace, coffee grounds, sewage sludge, pine, switchgrass, and hardwood-based biochars produced by slow pyrolysis contained 12–80 μg/kg bioavailable PAHs.

Regarding toxicity, Lyu et al. (2016) found that the BaP toxic equivalency quotient values of PAHs in pine wood biochar produced at 250–700 °C were 3.43–15.48 μg/kg. However, in a different study, the BaP toxic equivalency quotient values of PAHs in biochars generated at 450 °C, 500 °C, or 650 °C for 30, 60, or 120 min were 0.16 to 938.5 μg/kg (Khan et al., 2015b), with which our findings are in agreement.

The PAH concentrations in biochar are affected by the pyrolysis

Table 1
Total PAH concentrations and TEQs of PAHs in vegetables grown in biochar-amended soils at a field-scale, the associated ILCR value of each vegetable, and the total ILCR of the eight vegetables for an adult.

Biochar	Vegetables	Total PAH concentrations in vegetable (μg/kg)	TEQ of PAHs in vegetable (μg/kg)	ILCR of individual vegetable ($\times 10^{-6}$)	TILCR of vegetables ($\times 10^{-6}$)
B2	Chinese cabbage	774 (37.0)	8.33 (0.28)	1.01 (0.03)	1.52 (0.04)
	Pakchoi	573 (0.2)	5.28 (0.18)	0.51 (0.02)	
B5	Chinese cabbage	98 (8.3)	11.86 (3.37)	1.44 (0.33)	1.83 (0.37)
	Pakchoi	179 (17.0)	4.02 (0.43)	0.39 (0.04)	
B7	Chinese cabbage	569 (4.8)	7.11 (0.04)	0.87 (0.00)	2.17 (0.04)
	Pakchoi	637 (5.6)	13.30 (0.34)	1.30 (0.03)	
B9	Chinese cabbage	1495 (49.7)	8.71 (3.57)	1.06 (0.35)	1.96 (0.42)
	Pakchoi	1277 (47.2)	9.24 (0.78)	0.90 (0.08)	
B16	Chinese cabbage	266 (26.0)	8.99 (1.56)	1.09 (0.15)	1.52 (0.26)
	Pakchoi	93 (20.5)	4.44 (1.14)	0.43 (0.11)	
B18	Chinese cabbage	468 (26.6)	4.89 (1.52)	0.60 (0.15)	1.50 (0.22)
	Pakchoi	217 (23.0)	9.21 (0.71)	0.90 (0.07)	
B31	Chinese cabbage	164 (19.3)	7.52 (0.20)	0.92 (0.02)	1.58 (0.29)
	Pakchoi	263 (0.6)	6.82 (2.78)	0.66 (0.27)	
B34	Chinese cabbage	437 (26.3)	10.87 (0.24)	1.32 (0.02)	1.90 (0.31)
	Pakchoi	350 (0.4)	5.96 (2.96)	0.58 (0.29)	

Numbers B2, B5, B7, B9, B16, B18 are commercial biochars derived from elm wood, walnut shell powder, pine wood, corn stover, rice husk (Shanghai), and bamboo (Zhejiang), respectively. B31 and B34 are biochars derived from soybean straw (500 °C) and cypress wood (500 °C) produced in laboratory. Date in bracket represent standard error.
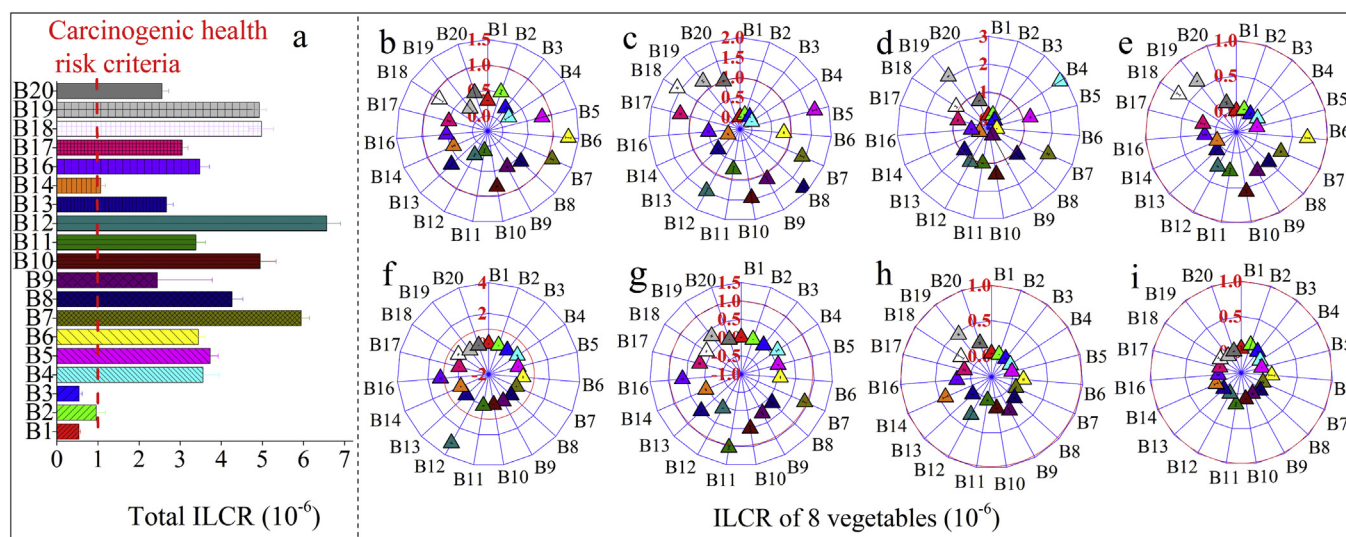
Fig. 4. ILCR values for an adult due to consumption of vegetables grown in soils amended with the commercial biochars. Total ILCR for an adult due to consumption of the eight vegetables (a), and the ILCR of each of the eight vegetables (b–h, carrot, spinach, Chinese cabbage, pakchoi, water spinach, lettuce, pterocladia tenuis, and cherry radish, respectively). Because the eight vegetables died in soils amended with B15 (commercial biochar from rice husk; highest PAH concentration), PAH data are not available for those soils. B1–B20, commercial biochars produced at 250–900 °C from apple wood (B1), elm wood (B2), lychee tree (B3), oak tree (B4), walnut shell power (B5), coconut husk (B6), pine wood (B7), jujube wood (B8), corn stover (B9), wheat straw (B10), pear tree (B11), rice straw (B12 and B13 from Nanjing in 2016 and 2017, B14 from Liyang), rice husk (B15, B16, and B17 from Nanjing, Shanghai, and Nantong, respectively), and bamboo (B18, B19, and B20 from Zhejiang, Guangxi, and Jiangxi, respectively). Error bars represent standard error.

temperature and the feedstock used (Bucheli et al., 2015). In present study, the concentrations of PAHs with low molecular weight (2- and 3-rings) decreased, while concentrations of high molecular weight PAHs increased with the incease of pyrolysis temperature. During pyrolysis at < 500 °C, plant biopolymers undergo unimolecular cyclization and aromatization, leading to the formation of aromatic structures, and more 2–3 ring PAHs may be produced. However, during pyrolysis at > 500 °C, PAHs are produced by pyro-synthesis of free radicals into larger aromatic structures, generating low-molecular-weight PAHs, which undergo zig-zag-addition to produce high-molecular-weight PAHs as the pyrolysis temperature increases (Keiluweit et al., 2012), which was in agreement with our observations. A portion of PAHs are locked up within the biochar structure, leading to low bioavailability. PAH concentrations generally decrease with increasing pyrolysis time and temperature (Hale et al., 2012). The total PAH concentrations and

their related BaP toxic equivalency quotient in laboratory-produced biochars were negatively correlated with pyrolysis temperature (350–650 °C). Thus, based on our results as well as those in documents, biochars produced at higher temperatures (500 °C–650 °C) relatively contain less PAHs.

According to the International Biochar Initiative (IBI) guidelines, PAHs in biochar used in soil must be below 20 mg/kg (IBI, 2013); this was the case for 25% of the commercial biochars in this study. According to the guidelines of the European Biochar Certificate (EBC) (EBC, 2017), the biochar PAH content (sum of the 16 EPA-priority PAHs) must be under 12 mg/kg for basic-grade biochar and under 4 mg/kg for premium-grade biochar. All of the laboratory-produced biochars met the EBC and IBI criteria, but 2.6% and 31.43% of the commercial biochars exceeded the basic- and premium-grade EBC criteria, respectively. However, application rates were unrealistic in some

Table 2
Amounts (µg/kg) of PAHs released from biochars by natural and artificial root exudates.

Treatment	Biochars				
	B2	B8	B16	B18	B33
Water control	9.04 (0.03)	8.69 (0.03)	29.90 (0.18)	10.02 (0.04)	11.19 (0.14)
NRE-1	7.31 (0.05)	10.88 (0.09)	62.24 (1.30)	11.21 (0.09)	9.36 (0.13)
NRE-2	9.24 (0.03)	8.50 (0.03)	34.41 (0.09)	37.46 (0.07)	12.82 (0.03)
ARE	32.80 (0.29)	93.95 (0.02)	70.33 (2.67)	70.22 (0.12)	28.61 (0.43)

NRE-1 and NRE-2 are natural root exudates collected from Chinese cabbage and water spinach, respectively. ARE means artificial root exudates. B2, B8, B16, B18 and B33 represent biochars derived from elm wood, jujube wood, rice husk (Shanghai), bamboo (Zhejiang), and cypress wood (350 °C). Date in bracket represent standard error.

cases when biochars used as the soil conditioner or fertilizer (Mukherjee and Lal, 2014), and therefore biochar-associated PAHs were introduced into soil and serve as a potential PAH source. On the other hand, the environmental fates of biochar-associated PAHs after addition into soil are still poorly understood. What's more, biochemical processes in soil may change biochar physicochemical properties, which may result in the release of occluded PAHs from biochar. On a whole, it seems possible that even PAH concentrations in biochars meet the IBI and EBC criteria, addition of these biochars into soil will still have potential PAH risk.

Little information is available on PAH accumulation by vegetables grown in soils with biochar amendment, although biochar application reportedly increases the soil PAH concentration (José et al., 2016). Active or passive uptake of PAHs by plant roots results in them entering the food chain (Gao and Collins, 2009). Khan et al. (2015a, b) reported that the PAH concentrations in lettuce grown in pulp-sludge biochar produced at 450 °C, 500 °C, and 550 °C in a growth chamber were 10.3–87.9 µg/kg. Our results suggest that biochar amendment of soil may lead to PAH accumulation in edible plants, possibly at levels exceeding the maximum defined by the China Food and Drug Administration.

PAH accumulation by edible vegetables is not solely dependent on the soil PAH content, as indicated by the lack of a correlation of vegetable and biochar PAH levels; this has previously been reported (Chiou et al., 2001). Uptake of biochar-derived PAHs by vegetables requires first release of the PAHs from biochar, their sorption/desorption in soil, absorption by roots, and translocation from roots to shoots. These processes are affected by soil conditions and by the type of biochar and plant species, which may explain the lack of a correlation between the PAH concentrations in the vegetables and the biochars. In addition, biochars from different feedstocks cause different effects on the soil biota (Weyers and Spokas, 2011; Lehmann et al., 2011). Biochar application can reduce microbial abundance (Birk et al., 2009; Warnock et al., 2007), decreasing PAH biodegradation in soil and increasing its accumulation in plants.

The release of PAHs from biochar is the first step in their uptake by vegetables. During growth, plant roots actively or passively release a range of organic compounds known as root exudates; indeed, up to 20–30% of the carbon fixed by photosynthesis in plant shoots is released into soil (Kuzyakov and Domanski, 2000). Root exudates accelerate nutrient-cycling, plant growth, and rhizosphere microbial activity (Phillips et al., 2011). Moreover, root exudates can enhance desorption of organic pollutants from environmental matrices (Kozdrój and van Elsas, 2000; Gao et al., 2010; LeFevre et al., 2013). LeFevre et al. (2013) reported that the presence of surface-active compounds (glycerolipids and glycoproteins) in root exudates enhance naphthalene desorption in

soil. Here, we found that an artificial root exudates and several natural root exudates enhanced the release of PAHs from biochars, possibly by altering their surface structures and/or dissolving the solid organic matter to which PAHs are bound (Joseph et al., 2010). The PAHs released from biochar into soil are subsequently absorbed by plants.

Consumption of contaminated food is a major route of human exposure to PAHs. This is the first study of the risk to human health of PAH contamination of edible vegetables in biochar-amended soils. Although the PAH concentrations in the 35 biochars did not exceed the International Biochar Initiative PAH criterion of 20 mg/kg, and the PAH concentrations in a large proportion of the biochars were lower than the European Biochar Certificate PAH criterion, the ILCR values indicated a significant health risk. The ILCR values (most were ~10^{-6}) of the vegetables were increased by growth in biochar-amended soil. According to the EPA human health assessment standards (US-EPA, 2017), consumption of vegetables grown in soils amended with PAH-containing biochars represents a risk to human health.

Therefore, soils contaminated by PAHs derived from biochars may require remediation. The cost of soil PAH remediation varies according to the technique used, but even using the lowest figure of $10/t (Sehnoor, 1997), could cost $24,012/ha, which negates the economic benefit of an enhanced vegetable yield/ha. Therefore, biochar application not only increases the cost of crop production but also poses a potential risk to human health. Large-scale utilization of biochar in agriculture should take these disadvantages into consideration.

5. Conclusions

To the best of our knowledge, this is the first study of PAH contamination and the associated health risks in edible vegetables grown in biochar-amended soils at the greenhouse and field scale. PAHs are produced during biochar production, and their concentrations and benzo[a]pyrene toxic equivalency quotient values decrease as the pyrolysis temperature increases. Vegetable uptake and accumulation of PAHs may result in human toxicity and increased the incremental lifetime cancer risk. Thus, not all biochars are safe for use in agricultural soil. The results provide insight into the negative effects of biochar application in soil management and vegetation production. Reslults indicate that large-scale field application of biochar should take into consideration of biochar-induced vegetation PAH contamination and human health risks, and biochars should be cleaned before their application in agriculture. Further studies involving other crops and biochars are needed to evaluate the risk to human health of soil amendment with biochar.

Conflict of interests

The authors declare no competing financial interest.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.09.010.

References

Baudoin, E., Benizri, E., Guckert, A., 2003. Impact of artificial root exudates on the

bacterial community structure in bulk soil and maize rhizosphere. Soil Biol. Biochem. 35, 1183–1192. https://doi.org/10.1016/S0038-0717(03)00179-2.

Birk, J.J., Steiner, C., Teixeira, W.C., Zech, W., Glaser, B., 2009. Microbial Response to Charcoal Amendments and Fertilization of a Highly Weathered Tropical Soil. Springer, Berlin, pp. 309–324. https://doi.org/10.1007/978-1-4020-9031-8_16.

Bucheli, T.D., Hilber, I., Schmidt, H.P., 2015. Polycyclic Aromatic Hydrocarbons and Polychlorinated Aromatic Compounds in Biochar. Routledge earthscan, London, pp. 593–624. https://www.researchgate.net/publication/280052910.

Chiou, C.T., Sheng, G., Manes, M., 2001. A partition-limited model for the plant uptake of organic contaminants from soil and water. Environ. Sci. Technol. 35, 1437–1444. https://doi.org/10.1021/es0017561.

Devi, P., Saroha, A.K., 2014. Effect of pyrolysis temperature on polycyclic aromatic hydrocarbons toxicity and sorption behaviour of biochars prepared by pyrolysis of paper mill effluent treatment plant sludge. Bioresour. Technol. 192, 312–320. https://doi.org/10.1016/j.biortech.2015.05.084.

European Biochar Certificate (EBC), 2017. Guidelines of the European Biochar Certificate. 14/08/2017. http://www.european-biochar.org/en/download%20the%20certificate.

Fagernäs, L., Kuoppala, E., Simell, P., 2012. Polycyclic aromatic hydrocarbons in birch wood slow pyrolysis products. Energy Fuel 26, 6960–6970. https://doi.org/10.1021/ef3010515.

Fu, P., Yi, W., Li, Z., Bai, X., Wang, L., 2017. Evolution of char structural features during fast pyrolysis of corn straw with solid heat carriers in a novel V-shaped down tube reactor. Energy Convers. Manag. 149, 570–578. https://doi.org/10.1016/j.enconman.2017.07.068.

Gao, Y., Collins, C.D., 2009. Uptake pathways of polycyclic aromatic hydrocarbons in white clover. Environ. Sci. Technol. 43, 6190–6195. https://doi.org/10.1021/es900662d.

Gao, Y., Cheng, Z., Ling, W., Huang, J., 2010. Arbuscular mycorrhizal fungal hyphae contribute to the uptake of polycyclic aromatic hydrocarbons by plant roots. Bioresour. Technol. 101, 6895–6901. https://doi.org/10.1016/j.biortech.2010.03.122.

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. https://doi.org/10.1021/es203984k.

Herath, H.M.S.K., Camparbestain, M., Hedley, M., 2013. Effect of biochar on soil physical properties in two contrasting soils: an Alfisol and an Andisol. Geoderma 209, 188–197. https://doi.org/10.1016/j.geoderma.2013.06.016.

Hilber, I., Blum, F., Leifeld, J., Schmidt, H.P., Bucheli, T.D., 2012. Quantitative determination of PAHs in biochar: a prerequisite to ensure its quality and safe application. J. Agric. Food Chem. 60, 3042–3050. https://doi.org/10.1021/jf205278v.

Hilber, I., Mayer, P., Gouliarmou, V., Hale, S.E., Cornelissen, G., Schmidt, H.P., Bucheli, T.D., 2017. Bioavailability and bioaccessibility of polycyclic aromatic hydrocarbons from (post-pyrolytically treated) biochars. Chemosphere 174, 700–707. https://doi.org/10.1016/j.chemosphere.2017.02.014.

International Biochar Initiative (IBI), 2013. Standardized Product Definition and Product Testing Guidelines for Biochar That Is Used in Soil, Version 1.1. 11 November, 2013. https://www.biochar-international.org/wp-content/uploads/2018/04/IBI_Biochar_Standards_V1.1.pdf.

International Biochar Initiative (IBI), 2017. IBI Newsletter. 27/12/2017. http://www.biochar-international.org/sites/default/files/2017-12-IBI-Newsletter-1.pdf.

Jones, D.L., Rousk, J., Edwards-Jones, G., Deluca, T.H., Murphy, D.V., 2012. Biochar-mediated changes in soil quality and plant growth in a three year field trial. Soil Biol. Biochem. 45, 113–124. https://doi.org/10.1016/j.soilbio.2011.10.012.

José, 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. https://doi.org/10.1007/s11368-015-1250-z.

Joseph, S.D., Campsarbestain, M., Lin, Y., Munroe, P., Chia, C.H., Hook, J., van Zwieten, L., Kimber, S., Cowie, A., Singh, B.P., Lehmann, J., Foidl, N., Smernik, R.J., Amonette, J.E., 2010. An investigation into the reactions of biochar in soil. Aust. J. Soil Res. 48, 501–515. https://doi.org/10.1071/SR10009.

Keiluweit, M., Kleber, M., Sparrow, M.A., Simoneit, B.R., Prahl, F.G., 2012. Solvent-extractable polycyclic aromatic hydrocarbons in biochar: influence of pyrolysis temperature and feedstock. Environ. Sci. Technol. 46, 9333–9341. https://doi.org/10.1021/es302125k.

Khan, S., Reid, B.J., Li, G., Zhu, Y.G., 2014. Application of biochar to soil reduces cancer risk via rice consumption: a case study in Miaoqian village, Longyan, China. Environ. Int. 68, 154–161. https://doi.org/10.1016/j.envint.2014.03.017.

Khan, S., Waqas, M., Ding, F., Shamshad, I., Arp, H.P.H., Li, G., 2015a. The influence of various biochars on the bioaccessibility and bioaccumulation of PAHs and potentially toxic elements to turnips (*Brassica rapa* L.). J. Hazard. Mater. 300, 243–253. https://doi.org/10.1016/j.jhazmat.2015.06.050.

Khan, A., Mirza, M., Fahlman, B., Rybchuk, R., Yang, J., Harfield, D., Anyia, A.O., 2015b. Mapping thermomechanical pulp sludge (TMPS) biochar characteristics for greenhouse produce safety. J. Agric. Food Chem. 63, 1648–1657. https://doi.org/10.1021/jf502556t.

Kozdrój, J., van Elsas, J.D., 2000. Response of the bacterial community to root exudates in soil polluted with heavy metals assessed by molecular and cultural approaches. Soil Biol. Biochem. 32, 1405–1417. https://doi.org/10.1016/S0038-0717 (00) 00058-4.

Kuppusamy, S., Thavamani, P., Megharaj, M., Venkateswarlu, K., Naidu, R., 2016. Agronomic and remedial benefits and risks of applying biochar to soil: current knowledge and future research directions. Environ. Int. 87, 1–12. https://doi.org/10.1016/j.envint.2015.10.018.

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. J. Plant Nutr. Soil Sci. 163, 421–431. https://doi.org/10.1002/1522-2624 (200008)163.

Lefevre, G.H., Hozalski, R.M., Novak, P.J., 2013. Root exudate enhanced contaminant desorption: an abiotic contribution to the rhizosphere effect. Environ. Sci. Technol. 47, 11545–11553. https://doi.org/10.1021/es402446v.

Lehmann, J., 2007. A handful of carbon. Nature 447, 143–144. https://doi.org/10.1038/447143a.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota—a review. Soil Biol. Biochem. 43, 1812–1836. https://doi.org/10.1016/j.soilbio.2011.04.022.

Lyu, H., He, Y., Tang, J., Hecker, M., Liu, Q., Jones, P.D., Codling, G., Giesy, J.P., 2016. Effect of pyrolysis temperature on potential toxicity of biochar if applied to the environment. Environ. Pollut. 218, 1–7. https://doi.org/10.1016/j.envpol.2016.08.014.

Mench, M., Martin, E., 1991. Mobilization of cadmium and other metals from two soils by root exudates of *Zea mays* L. *Nicotiana tabacum* L. and *Nicotiana rustica* L. Plant Soil 132, 187–196. https://doi.org/10.1007/BF00010399.

Mukherjee, A., Lal, R., 2014. The biochar dilemma. Aust. J. Soil Res. 52, 217–230. https://doi.org/10.1071/SR13359.

Nakata, H., Sakai, Y., Miyawaki, T., Takemura, A., 2003. Bioaccumulation and toxic potencies of polychlorinated biphenyls and polycyclic aromatic hydrocarbons in tidal flat and coastal ecosystems of the Ariake Sea, Japan. Environ. Sci. Technol. 37, 3513–3521. https://doi.org/10.1021/es021083h.

Perera, F.P., 1997. Environment and cancer: who are susceptible? Science 278, 1068–1073. https://doi.org/10.1126/science.278.5340.1068.

Phillips, R.P., Finzi, A.C., Bernhardt, E.S., 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecol. Lett. 14, 187–194. https://doi.org/10.1111/j.1461-0248.2010.01570.x.

Qadeer, S., Anjum, M., Khalid, A., Waqas, M., Batool, A., Mahmood, T., 2017. A dialogue on perspectives of biochar applications and its environmental risks. Water Air Soil Pollut. 228, 281–307. https://doi.org/10.1007/s11270-017-3428-z.

Quilliam, R.S., Rangecroft, 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? GCB Bioenergy 5, 96–103. https://doi.org/10.1111/gcbb.12007.

Rombolà, A.G., Meredith, W., Snape, C.E., Baronti, S., Genesio, L., Vaccari, F.P., Miglietta, F., Fabbri, D., 2012. Fate of soil organic carbon and polycyclic aromatic hydrocarbons in a vineyard soil treated with biochar. Environ. Sci. Technol. 49, 11037–11044. https://doi.org/10.1021/acs.est.5b02562.

Sehnoor, J.L., 1997. Phytoremediation. Phytoremediation. Technology Evaluation Report, Prepared for Ground-water Remediation Technologies Analysis Center. http://gwatac.org.

Stokes, J.D., Wilkinson, A., Reid, B.J., Jones, K.C., Semple, K.T., 2005. Prediction of polycyclic aromatic hydrocarbon biodegradation in contaminated soils using an aqueous hydroxypropyl-β-cyclodextrin extraction technique. Environ. Toxicol. Chem. 24, 1325–1330. https://doi.org/10.1897/04-336R.1.

United Nations Environmental Program (UNEP), 2010. Converting Waste Agricultural Biomass Into a Resource - Compendium of Technologies. February 2010. https://www.unenvironment.org/resources/report/convertingwasteagriculturalbiomassresourcecompendiumtechnologies.

United States Environmental Protection Agency (USE-PA), 1993. Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons. (EPA 600/R-93/089). https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=49732.

United States Environmental Protection Agency (US-EPA), 1996. Soil Screening Guidance: User's Guide. Office of Solid Waste and Emergency Response (EPA/540/R-96/018). https://rais.ornl.gov/documents/SSG_nonrad_user.pdf.

United States Environmental Protection Agency (US-EPA), 2017. Integrated Risk Information System (IRIS): Toxicological Review of Benzo[*a*]pyrene (EPA/635/R-17/003Fa). https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0136tr.pdf.

Wang, Z., Zheng, H., Luo, Y., Deng, X., Herbert, S., Xing, B., 2013. Characterization and influence of biochars on nitrous oxide emission from agricultural soil. Environ. Pollut. 174, 289–296. https://doi.org/10.1016/j.envpol.2012.12.003.

Warnock, D.D., Lehmann, J., Kuyper, T.W., Rillig, M.C., 2007. Mycorrhizal responses to biochar in soil—concepts and mechanisms. Plant Soil 300, 9–20. https://doi.org/10.1007/s11104-007-9391-5.

Weyers, S.L., Spokas, K.A., 2011. Impact of biochar on earthworm populations: a review. Appl. Environ. Soil Sci. 1–12. https://doi.org/10.1155/2011/541592.

Xia, Z., Duan, X., Qiu, W., Liu, D., Wang, B., Tao, S., Jiang, Q., Lu, B., Song, Y., Hu, X., 2010. Health risk assessment on dietary exposure to polycyclic aromatic hydrocarbons (PAHs) in Taiyuan, China. Sci. Total Environ. 408, 5331–5337. https://doi.org/10.1016/j.scitotenv.2010.08.008.

Zhai, F., Yang, X., 2006. Chinese National Health and Nutrition Survey Report Two: Diet and Nutrient Intake Status (in Chinese). People's Medical Publishing House, Beijing, pp. 145–184.

Zhang, A., Cui, L., Pan, G., Li, L., Hussain, Q., Zhang, X., Crowley, D., 2010. Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China. Agric. Ecosyst. Environ. 139, 469–475. https://doi.org/10.1016/j.agee.2010.09.003.