Polycyclic Aromatic Hydrocarbons in Biochar and Their Bioavailabilty

by

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Abstract

Biochar may be utilized as a soil-amendment to sequester carbon and enhance soil fertility through improved nutrient retention, increased plant-available water, and improved drainage for excess water. Before biochar can be applied on a widespread scale, potential threats to the soil environment must be evaluated. In this study, total and bioavailable polycyclic aromatic hydrocarbons (PAHs) were evaluated for nine slow pyrolysis biochars and three gasification biochars. Slow pyrolysis biochars were produced from loblolly pine (*Pinus taeda*), sorghum (*Sorghum bicolor*), and switchgrass (*Panicum virgatum*) feedstocks at temperatures of 400, 550, and 700°C. Gasification biochars were produced from hardwood and switchgrass feedstocks at a temperature of 1,100°C and pine feedstocks at 1,000°C. Total PAH concentrations ranged from 1.7 μg/g of pine char produced at 550°C to 31,957 μg/g of pine char at 1,000°C. A significantly higher concentration of PAHs was found in slow pyrolysis chars produced from sorghum stalks and gasification chars produced from pine. Concentrations of bioavailable PAHs in gasification biochars were further evaluated using polyoxymethylene (POM) passive samplers and PAH uptake from lettuce and soybean plants grown in two soil types. Bioavailable PAH concentrations ranged from 0.04 μg/g in blank POM assays to 27.89 μg/g in pine biochar POM assays. Bioavailable PAH concentrations ranged from 0.51 to 5.11 μg/g for lettuce plants grown in a Compass soil and 0.60 to 0.80 μg/g for lettuce plants grown in a Waynesboro soil. Bioavailable PAH concentrations ranged from 0.29 to 2.49 μg/g for soybean plants grown in a Compass soil and 0.31 to 0.34 μg/g for soybean plants grown in a Waynesboro soil. Although soybean and lettuce plants were only able to assimilate small amounts of the total solvent-extractable PAHs, plant uptake due to biochar amendment may still pose a hazard to humans.

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List of Abbreviations

GC-MS	Gas Chromatography-Mass Spectroscopy
HMW	High molecular weight
HSD	Honest Significant Difference
LMW	Low molecular weight
PAH	Polycyclic Aromatic Hydrocarbon
IBI	International Biochar Initiative
EBC	European Biochar Certificate
POM-SPE	Polyoxymethylene Solid Phase Extraction
PDMS-SPME	Polydimethylsiloxane-Coated Solid Phase Micro Extraction

Chapter 1. Literature Review

A 110% increase in global food production is necessary to accommodate the 9.6 billion people that the United Nations projects to inhabit the world by 2050 (Hanjra and Qureshi, 2010). To accommodate the industrial, transportation, and domestic needs that correspond to an increase in food production, increased energy production will also be essential. Burning additional fossil fuels for energy production will deplete an already finite resource in addition to increasing anthropogenic carbon emissions, altering the carbon cycle and intensifying climate change. Enhanced crop production, carbon sequestration, and the production of sustainable, carbon neutral or carbon negative energy will be critical for future generations.

Although the utilization of biomass for energy production is not a new concept, geopolitical tensions and rising oil prices in the 1980s helped reestablish the necessity and viability of biofuel production (Ma and Hanna, 1999). Biofuels provide a sustainable source of energy by utilizing biomass such as pine wood, sorghum, switchgrass, or any number of agricultural wastes. Biofuel production has the potential to become a carbon negative process with the utilization of biofuel's co-product, biochar (IBI, 2017; Sohi et al., 2010).

Biochar is a carbonaceous material formed through the thermal decomposition of organic material under anoxic or low-oxic conditions (Hale et al., 2012; Kloss et al., 2012). Biochar can be formed as a co-product of the pyrolysis process or produced specifically for soil amendment. Conventionally, biochar was pyrolyzed to initiate the primary bioenergy processes or dry biomass prior to pyrolization (Gaunt and Lehmann, 2008; Sohi et al., 2010). An interest in the use of biochar for soil amelioration and carbon sequestration arose after studying fertile black soils in the Brazilian Amazon (Schimmelpfennig and Glaser, 2012; Sohi et al., 2009). These soils, referred to as 'terra preta de Indio' or 'terra preta', consist of 1 to 100 ha tracts of land known for their 40-80 cm thick soil horizons (Garcia-Perez and Metcalf, 2008). These thick black soil horizons are believed to originate from the amendment of charred organic material produced from cooking fires dating back 500-2500 years ago (Garcia-Perez and Metcalf, 2008; Jeffery et al., 2011). With high levels of humus, charcoal, and black carbon particulates, terra preta soils show enhanced levels of fertility in comparison to the surrounding infertile tropical soils (Garcia-Perez and Metcalf, 2008; Schimmelpfennig and Glaser, 2012). Terra preta soils retain more water and provide an increased availability of nutrients such as phosphorous, calcium, manganese, and zinc, allowing crops to grow three times faster than in the surrounding soils (Garcia-Perez and Metcalf, 2008; Sohi et al., 2009). The aromatic carbon structures within the charred material also appear relatively recalcitrant to degradation, suggesting that the char provides a carbon sink in addition to enhanced soil fertility (Jeffery et al., 2011). The numerous long-term benefits associated with the terra preta soils helped popularize the concept of biochar as a soil amendment.

Along with being a co-product in the production of renewable bioenergy, biochar has potential applications for waste management, carbon sequestration, greenhouse gas mitigation, and soil improvement (Hale et al., 2012; Woolf et al., 2010). Formerly considered a waste product, crop residue can be repurposed through thermochemical processes to produce syngas, bio-oil, and biochar. Lignocellulosic food crops produce about 4000 MT/yr of crop residue worldwide (Singh et al., 2015). In the past, a common practice for disposing of crop residue was open burning. This practice led to the brown cloud phenomenon and the emission of pollutants such as non-methane hydrocarbon compounds and particulate matter (Singh et al., 2015). Utilizing crop residue for biofuel production and the coproduction of biochar can prevent harmful emissions, offset carbon emissions, and reduce the dependence on fossil fuels (Woolf et al., 2010). In the United States, nearly half of the annual consumption of oil could be met by the pyrolysis of 1.3×10^9 metric tons dry biomass (Garcia-Perez and Metcalf, 2008).

Higher carbon contents and the low likelihood of soil CO_2 emissions make biochar ideal for carbon sequestration and the mitigation of climate change (Lehmann, 2007; Sohi et al., 2010). With a residence time 10-1,000 times longer than soil organic matter, the carbon compounds composing biochar can remain in the soil for hundreds to thousands of years (Schimmelpfennig and Glaser, 2012; Sohi et al., 2010). Biochar amendment also increases the retention of nitrogen in the soil, reducing the emission of nitrous oxide by up to 50% (Gaunt and Lehmann, 2008). By removing crop residue, plant decomposition is eliminated and emissions of methane and nitrous oxide are prevented (IBI, 2017; Woolf et al., 2010). Enhanced soil fertility also stimulates crop growth and reduces chemical fertilizer requirements, reducing emissions from fertilizer production and leading to a higher CO_2 consumption from enhanced crop growth (IBI, 2017).

Without accounting for the utilization of carbon sequestration methods, slow pyrolysis emits 91-360 kg of CO_2 per MWh of electricity produced (Gaunt and Lehmann, 2008). Comparatively, fossil fuels generate 600-900 kg of CO_2 per MWh of electricity produced. By using biochar as a soil amendment, the biofuels industry can reduce emissions by an additional 12-84% (Sohi et al., 2009). The reduction in emissions attained through the soil amelioration of biochar could lead to net-negative carbon emissions and provide incentives for carbon trading (Gaunt and Lehmann, 2008). Over the period of a century, large-scale biochar amendment has the potential to reduce net emissions by up to 130 Pg CO_2-C without jeopardizing soil conservation, habitat or food security (Gaunt and Lehmann, 2008).

In addition to benefits associated with climate mitigation and crop residue management, soil fertility may be enhanced due to positive impacts on soil biota and physicochemical properties resulting from biochar amendment (Sohi et al., 2009). Possible mechanisms behind the increase in soil fertility include: the availability of nutrients in biochar, improved nutrient retention, increased cation exchange capacity (CEC), increased plant-available water, and improved drainage for excess water (Hansen et al., 2015; Jeffery et al., 2011). Soil CEC refers to the availability of cationic nutrients in the soil and their ability to resist leaching (Verheijen et al., 2010). In neutral or basic soil conditions, anionic nutrients (such as nitrates) are retained poorly in the soil while cations are held on soil colloids. At lower pH, the soil CEC tends to be low and retains less nutrients. The high pH of biochar can produce a liming effect in the soil and increase soil CEC. Additional benefits resulting from biochar amendment include increased microbial biomass and microbial activity, increased mycorrhizal abundance, increased earthworm abundance and activity, and reduced nutrient and agricultural chemical runoff (Hilber et al., 2012; Verheijen et al., 2010; Woolf et al., 2010).

The extent of the benefits associated with biochar amendment are dependent on native soil characteristics. The most significant benefits to crop productivity and soil fertility occur in acidic, free-draining soils and highly weathered, poorly buffered soils, such as Ultisols (Purakayastha et al., 2016; Verheijen et al., 2010; Woolf et al., 2010). The liming effect of biochar increases crop productivity and fertility considerably in acidic soils. Although the pH of biochar may vary from slightly acidic to alkaline, most biochars have a pH ranging from 8 to 10 (Singh et al., 2015). The capacity of biochar to neutralize soil pH is highest in wood biochars, which generally provide a higher CEC to amended soils (Singh et al., 2015). However, by aging the biochar, the alkalinity associated with biochar may decrease or neutralize entirely (Singh et al., 2015). Biochars with high ash contents have produced the most significant increases in soil pH and soil electrical conductivity (EC) (Purakayastha et al., 2016). Increases in EC and pH are attributed to the amounts of metals, phosphates, organic and inorganic nitrogen, and carbonates of alkali and alkaline earth metals held within the ash residue of biochar (Raison, 1979). Soil pH and EC may also be affected by the high surface area and porosity of biochar (Purakayastha et al., 2016).

In fine-grained clay soils, increased porosity and permeability often result from biochar amendment (Barnes et al., 2014). Higher porosity and permeability lead to higher infiltration rates and enhanced crop productivity. In coarse-textured soils, biochar amendment decreases crop water stress by increasing the water holding capacity of the soil and improving plant water availability. Degraded, sandy soils may benefit from sorption increases resulting from biochar amendment (Kloss et al., 2012).

Antecedent organic matter composition and fertility will also affect the benefits associated with biochar amendment (Singh et al., 2015). Crop yield has been observed to increase in fields previously experiencing low contents of soil organic matter (SOM). Comparatively, in fields with high levels of SOM, no increase in yield is generally observed (Singh et al., 2015). Furthermore, benefits to crop growth, crop nutrient, and soil quality are not as evident following biochar application to fertile soils. Overall, any potential benefits experienced as a result of biochar amendment will depend on soil type and conditions, biochar amendment rate, and biochar properties (Barnes et al., 2014).

Although the benefits of soil amelioration with biochar are numerous, there are several potential complications that need to be addressed prior to widespread production and application. First, the effects of biomass source and pyrolysis conditions on biochar properties must be assessed. The effectiveness of soil amendment is likely to vary considerably between various types of biochars based on biochar properties and soil characteristics (Singh et al., 2010). Additionally, research must address the interaction between biochar and the soil environment (Verheijen et al., 2010). The effect on SOM dynamics, soil compaction, priming effects, soil water retention and availability, and biochar's sorption affinity to organic compounds, such as herbicides, must be determined (Hale et al., 2012; Verheijen et al., 2010). Another essential item to consider is biochar's loading capacity. Maximizing the positive effects of biochar amendment and storage within soils while minimizing negative impacts on other soil functions is imperative due to the longevity of biochar within the soil. Research must also identify the relationship between biochar production and human health (Jeffery et al., 2015). Although smallholder farms in developing countries may see the largest benefit from biochar amendment, they may also be susceptible to health-related issues. The emission of particles, methane, and carbon monoxide from traditional kilns may lead to problems such as pneumoconiosis. The quantity of crop residue in developing nations may also be scarce due to the utilization of residual biomass for animal feed, mulch, and roofing materials. These problems may be addressed through the adoption of pyrolysis stoves for cooking as an alternative to burning firewood (Jeffery et al., 2015).

The effects of various trade-offs must also be addressed and evaluated prior to widespread biochar amendment. The removal of crop residue for the production of biochar is of primary concern (Verheijen et al., 2010). Crop residue removal may lead to the erosion of topsoil and a depletion of SOM, thereby reducing crop yields. Additionally, residue removal may negatively affect soil biota that rely on the breakdown of plant residues for their carbon and energy source. Replacing crop residue with a recalcitrant form of carbon that cannot be utilized may alter ecosystem function. Furthermore, biochar must be incorporated into the topsoil through tillage in order to reduce the erosive effects of wind and water (Jeffery et al., 2015). Tillage may reduce native SOM and produce negative effects on lands previously accustomed to no-till farming. Finally, the utilization of heavy machinery for biochar amendment may lead to soil compaction (Verheijen et al., 2010).

The trade-off between bioenergy and biochar production must also be addressed. Biochar can be produced as a co-product of biofuel production or for the sole purpose of soil amendment. Biochar produced for soil amelioration will generate char with optimal characteristics for amendment but decrease the quantities of biomass available for biofuel production (Jeffery et al., 2015). For biochars produced as a co-product, the slow pyrolysis of biomass produces the most char and would provide the maximum potential for carbon sequestration and crop productivity. However, producing biochar through slow pyrolysis produces 30% less energy than optimal bioenergy methods (Gaunt and Lehmann, 2008).

The presence of toxic organic chemicals in biochar should also be considered prior to widespread utilization. High temperatures break down the complex chemical structures of biomass during pyrolysis (Schimmelpfennig and Glaser, 2012). During the process of resynthesis, carcinogenic and mutagenic compounds such as polycyclic aromatic hydrocarbons (PAHs) may be generated. The general permanency and irretrievability of biochar from the soil necessitates a thorough contamination assessment prior to biochar amendment (Sohi et al., 2010).

1.1 Polycyclic aromatic hydrocarbons (PAHs)

Although there are clear benefits to biochar amendment, risks must be evaluated before utilization can begin at a large scale. Based on the longevity of char within the Amazonian terra preta soils, any pollution or unforeseen negative effects may persist for hundreds to thousands of years (Singh et al., 2010). To ensure the sustainability and safety of biochar amendment, the assessment of contaminants, such as PAHs, are essential (Keiluweit et al., 2012).

Well-known carcinogenic and persistent pollutants, PAHs extend over five orders of magnitude in soils worldwide, making them ubiquitous pollutants in the soil environment (Chen and Yuan, 2011; Fabbri et al., 2013). When the chemical structure of raw organic material is broken down during incomplete combustion, or pyrolysis, PAHs are generated (Hale et al., 2012; Schimmelpfennig and Glaser, 2012). Unstable radicals formed during pyrolysis then undergo a secondary process, called pyrosynthesis. During pyrosynthesis, the radicals fuse to form more thermodynamically stable parent PAHs (Keiluweit et al., 2012). Low-molecular weight (LMW) PAHs, such as naphthalene, are first to form. High-molecular weight (HMW) PAHs are formed when temperatures and residence times are increased and additional rings are added to positions containing the highest π-electron density. HMW PAHs become significant at temperatures greater than 650°C (Keiluweit et al., 2012).

Although natural processes, such as grassland/forest fires or volcanic eruptions, can produce PAHs within the environment, the greatest source of pollution results from anthropogenic processes (Quilliam et al., 2013; US EPA, 2008). Large amounts of atmospheric pollution are generated from the burning of fossil fuels and smoke from industrial, municipal or domestic origins (US EPA, 2014). Other anthropogenic sources include exhaust from motor vehicles or engines, cigarette smoke, asphalt processing and use, and stoves or furnaces. Foods that are broiled or cooked with charcoal may also become contaminated with PAHs.

Considered one of the most difficult organic contaminants to treat, many PAHs are regulated due to their teratogenic, mutagenic, and carcinogenic properties (Keiluweit et al., 2012; Quilliam et al., 2013). In the United States, the EPA has included 16 priority PAHs on the Priority Pollutant List created under the Clean Water Act (Wick et al., 2011). These PAHs were chosen due to their frequency of occurrence in hazardous waste as well as their potential toxicity. Seven of these PAHs are considered possible or probable carcinogens and are also listed on the US EPA's Priority Chemical List (US EPA, 2008). Biochar associations such as the International Biochar Initiative (IBI) and the European Biochar Certificate (EBC) have also been developing guidelines to assess and monitor biochar quality and production (Bucheli et al., 2014). The IBI recommends that the sum of the 16 EPA regulated PAHs not exceed 6-20 mg/kg dry weight. The EBC recommends that the sum of the 16 PAHs remain below 4 mg/kg dry weight for premium biochar and below 12 mg/kg dry weight for basic grade biochars (Gustafsson, 2013).

PAHs can be found in the atmosphere, water, sediment, soils and plants (Wick et al., 2011). PAHs are predominant in the atmosphere of urban areas due to the close proximity to anthropogenic combustion activities (Nam et al., 2009). The concentration of atmospheric PAHs is highest in the winter due to the prevalence of heating and a reduction in thermal and photo-decomposition (Wick et al., 2011). In North America, atmospheric concentrations of PAHs generally range from 3.7 to 450 ng/m^3 and consist primarily of phenanthrene, fluoranthene and pyrene. Atmospheric PAHs can reside in either a gas or particulate phase. Most HMW PAHs reside in the particulate phase, while LMW PAHs reside in a gaseous phase (Wick et al., 2011).

PAH concentrations in water are limited due to the hydrophobicity resulting from their lipophilic structure (Quilliam et al., 2013). With low solubility, the presence of PAHs in water is often the result of surface runoff following PAH adsorption to particulate matter (Wick et al., 2011). Other potential causes of PAH contamination in waterbodies include: the deposition of atmospheric particulate matter, pollution by industrial effluents, oil spills, leachate from coal tar, or municipal wastewater discharge (US EPA, 2014).

The accumulation of PAHs within plants occurs mainly due to atmospheric deposition and uptake by aboveground plant matter (Wick et al., 2011). PAHs can also be adsorbed onto plant roots. Although translocation to aboveground plant matter is unlikely due to the hydrophobicity of the compounds, the lipophilic structure of PAHs can lead to bioaccumulation within plants (Fabbri et al., 2013). Leafy vegetables are known to accumulate higher levels of PAHs than fruit, root crops, and subterranean stem vegetables (Lei et al., 2011).

The rapid sorption of PAHs to soil particles can result in an environmental sink (Quilliam et al., 2013). In North America, the concentrations of PAHs in marine sediments range from 2.17 to 170,000 $ng/g_{sediment}$ and generally consist of HMW compounds (Wick et al., 2011). Soil contamination by PAHs can occur through processes such as wet and dry atmospheric deposition, forest fires, and road runoff (Chen and Yuan, 2011). Urban industrial soils contain higher PAH concentrations than remote soils and industrial activities have resulted in increased soil PAH concentrations within the past 100-150 years (Wick et al., 2011).

Physiochemical properties, such as SOM and CEC, affect the ability of biochar or biochar-amended soils to adsorb and retain PAHs (Novotny, 2002). Soil CEC is regulated by the surface area and nature of particles. Small particles, such as clay, have the highest adsorption capacity. The proclivity of PAHs to sorb to black carbon and SOM is also of concern when applying biochar (Fabbri et al., 2013; Keiluweit et al., 2012). High SOM contents and small particle sizes may enhance the sorption of PAHs and other organic pollutants to biochar and cause the contaminants to resist microbial decomposition (Novotny, 2002; Quilliam et al., 2013). The strong sorption capacity of biochar and resistance to microbial degradation may lead to an accumulation and persistence of PAHs within the soil. Alternatively, PAHs generated during the pyrolysis of biomass may be a source of contamination to soil amended with biochar (Quilliam et al., 2013). Risks associated with the addition of biochar must be assessed before biochar is incorporated into the soil (Sohi et al., 2009).

The widespread distribution of atmospheric PAHs and the consumption of PAH-laden foods, such as leafy greens and cooked meats, generate varying levels of PAH exposure to humans on a daily basis (Menzie et al., 1992a; US EPA, 2008, 2014; Wick et al., 2011). For nonsmokers, the consumption of foods contaminated with PAHs accounts for more than 70% of the total exposure (Lei et al., 2011; Wild et al., 2005). The average American's rate of dietary exposure is estimated at 1-5 μg PAH/day and has been associated with an increased risk of some cancers (Lei et al., 2011; Menzie et al., 1992a). Upon entering the body, PAHs can spread and target fat tissues such as the kidney and liver (US EPA, 2008). Acute exposure of PAHs may lead to a suppression of the immune system and red blood cell damage. Chronic exposure may result in developmental or reproductive issues (US EPA, 2014).

Chemical structure affects the carcinogenic and mutagenic properties of PAHs. LMW PAHs, which contain 2-3 fused aromatic rings, are directly toxic to humans. HMW PAHs, containing four or more fused aromatic rings, are genotoxic and may cause damage to DNA (Wick et al., 2011). The rate of absorption of LMW PAHs is higher than HMW PAHs; however, HMW PAHs may have the potential for greater health and environmental hazards (Fabbri et al., 2013).

As the molecular weight of the PAH compound increases, hydrophobicity, recalcitrance and lipophilicity increase while water solubility and bioavailability decrease. In the soil, LMW PAHs are degraded more quickly due to their higher solubility and the ability of microorganisms to use them as a carbon source. HMW PAHs are more recalcitrant in the soil due to their fused aromatic ring structure and water solubility (Wick et al., 2011). The half-life of PAHs within the soil can range from 2 days to 1.9 years (US EPA, 2014). Rates of degradation depend on the molecular weight and solubility of the PAH in question (Wick et al., 2011). The high sorptive capacity of biochar may limit microbial degradation and facilitate the persistence of PAHs in the soil (Quilliam et al., 2013). Additional information regarding PAH sorption to biochar and the potential for contamination must be determined before large scale application of biochar can occur (Keiluweit et al., 2012; Sohi et al., 2009).

1.2 PAHs in Biochars

Large-scale application of biochar cannot be advocated until potential risks are accurately evaluated and quantified (Jeffery et al., 2015). A consistent extraction method is crucial to accurately quantify PAHs. Highly divergent methods have resulted in substantial variation, making it difficult to optimize biochar production for quality, control, registration, and legislation (Hilber et al., 2012). In an effort to optimize techniques that assess total and bioavailable PAHs, various studies have been conducted.

Soxhlet extraction and accelerated solvent extraction (ASE) are the most common methods for extracting PAHs from biochar. Although ASE is less time-consuming, the equipment is expensive and does not quantify PAHs as effectively as soxhlet extraction (Fabbri et al., 2013; Hilber et al., 2012). Hilber et al. (2012) determined that ASE was only able to detect 60-80% of the 16 US EPA PAHs quantified by soxhlet extraction. Additionally, ASE was unable to extract PAHs heavier than chrysene (Hilber et al., 2012).

Soxhlet extraction produced the highest extraction recoveries (Fabbri et al., 2013). To optimize extractions, Fabbri et al. (2012) focused on the behavior of HMW PAHs. PAH concentration was significantly enhanced when extraction times were increased from 18 to 36 hours, however, increasing the extraction time did not guarantee a high recovery of PAHs (Fabbri et al., 2013). With this in mind, Hilber et al. (2012) sought to identify the most efficient extraction solvent. Extractions were performed using a variety of solvents, ranging from 100% toluene, heptane, and hexane to an assortment of solvent mixtures. Soxhlet extractions performed with 100% toluene provided the best recoveries of PAHs. The difference in PAH quantification between the 18 and 36 hour extraction times only equated to about a 4% increase.

Additional research is necessary to establish the relationship between feedstock source, production methods, and the concentration of PAHs within biochar. In 2012, Hale et al. compared slow pyrolysis methods with local and industrial methods of biochar production for temperatures varying between 250 and 900 ˚C. Biochars produced through slow pyrolysis varied in PAH concentration as a result of biomass source, temperature, and residence time (Figure 1-1) (Hale et al., 2012). For slow pyrolysis conditions, PAH concentrations ranged from 0.17 ng/L to 10.0 ng/L. These values are lower than the concentrations of PAHs found in clean urban sediments and remain less than the environmental quality standards. Hale et al. (2012) concluded that temperatures of 350 to 550 ˚C produced the greatest PAH concentrations.

The effects of slow pyrolysis temperature and feedstock source on the concentration and composition of PAHs was also analyzed by Keiluweit et al. (2012). Biochars were produced from two types of wood and grass under slow pyrolysis conditions. Temperatures ranged from 100 to 700 ˚C. When pyrolyzed at temperatures in the 400 to 500 ˚C range, wood and grass biochars contained the highest concentration of PAHs. Grass biochars contained higher concentrations of PAHs than wood biochars. To minimize the formation of PAHs, Keiluweit et al. recommended that biochar be produced from a woody feedstock using slow pyrolysis temperatures between 500 and 600 ˚C.

1.3 Bioavailability of PAHs

Particulate matter, soil, and sediments tightly bind PAHs and limit their bioavailability (US EPA, 2014, 2012). Although solvent extraction methods are useful in determining total PAH concentration, these methods are unable to quantify the concentrations of dissolved contaminants that are bioavailable to organisms (US EPA, 2012). The bioavailable concentration is the portion that is freely dissolved and available to interact with an organism (Bao et al., 2013; US EPA, 2012). Bioavailable contaminants are mobile and may be accumulated in the lipids of fish, benthic organisms, plants, soil organisms, or other flora and fauna exposed to the contaminant (Novotny, 2002). Although total PAH concentrations in biochar and biochar-amended soils are important, an accurate determination of the roughly 1% stored as bioavailable contaminants is critical when assessing ecological risk (Bao and Zeng, 2011; Novotny, 2002). Two common methods for assessing risks posed by PAHs within the soil environment are plant uptake and passive sampler analysis.

1.3.1 Plants and Bioavailability

In nonsmoking adults, the consumption of cooked meats, leafy vegetables, and grains is one of the primary sources of PAH exposure (Menzie et al., 1992b; Wild et al., 2005). Evaluating PAH bioaccumulation in plants is essential when assessing the risks associated with biochar amendment.

PAHs may bioaccumulate in plant roots and shoots despite the strong sorption of PAHs and SOM (Wild et al., 2005). The main pathway that transports PAHs within the root system is apoplastic transport (Wild et al., 2005). Once the PAHs have entered the cortex cells, lateral movement occurs as the PAH compounds travel towards the plant shoot (Wild et al., 2005). Since the transport of PAHs is closely associated with the flow of water, compound solubility is an important parameter in evaluating plant uptake. Consequently, the process of plant uptake is often slow due to the repulsion of the hydrophobic PAHs from water molecules (Jakob et al., 2012; Wild et al., 2005). Due to the mechanism of transport, the highest PAH concentrations are found within the roots of plants, followed by stems and leaves (Parrish et al., 2006).

Lei et al. (2011) found that 2 and 3-ring PAHs were predominately taken up in samples of white gourd, cucumber, towel gourd, lettuce, Indian lettuce, bok-choy, radish, potato, and lotus root produced in soils previously contaminated with PAHs. Of the PAHs found, fluorene was found in all vegetable samples while naphthalene and acenaphthene were found in most (Lei et al., 2011). Attributes such as greater water solubility, volatility, and bioavailability may explain the predominance of LMW PAHs within the vegetable samples. Of the nine vegetable types studied, the highest level of total PAHs was found in Indian lettuce (96.98 ng/g). Other leafy vegetables also showed high concentrations of total PAHs. Root and subterranean stem vegetables showed the lowest levels of PAHs. Although cooking did reduce total PAH concentrations in vegetable samples, leafy vegetables such as lettuce are often eaten as uncooked green leaves (Khan et al., 2008; Lei et al., 2011). As a result, the uptake of total PAHs within leafy vegetables may be of particular interest in future studies.

Several studies have considered whether biochar application provides a source or sink for bioavailable PAHs. Brennan et al. (2014) compared the use of an activated carbon with two sources of biochar as part of a phytomanagement practice to remediate degraded soil and eliminate soil contaminants. This study found that the two slow pyrolysis biochars (derived from pine woodchip and maize stubble) produced no detrimental effects on the soil environment

(Brennan et al., 2014). Results also indicated that the utilization of biochar may be beneficial as part of an integrated phytomanagement approach to dealing with contaminants. Similarly, biochar application has reduced bioaccumulation rates for PAHs in lettuce and cucumbers following soil amendment (Khan et al., 2013; Waqas et al., 2014). Waqas et al. (2014) studied the application effects from sewage sludge and sewage sludge biochar on PAH bioavailability in plants. PAH bioavailability was significantly greater for biochar than sewage sludge and the application of biochar increased the biomass yield of the cucumber fruit.

1.3.2 POM and bioavailability

While the use of plants is essential in determining the effect of biochar amendment on crop uptake, organismal biodiversity produces variations in the uptake of freely dissolved organic contaminants (Bao and Zeng, 2011). To fill this technological gap, passive samplers are utilized. Passive samplers offer a fast, inexpensive and reliable technique for the assessment of bioaccessible organic contaminants in sediment porewater. Simple to use and capable of being placed in situ for days to months, passive samplers can provide a time-averaged representation of contaminants (Bao and Zeng, 2011; US EPA, 2012).

Passive samplers provide a more biologically relevant measure of bioaccessible hydrophobic contaminants than studies in crop uptake (US EPA, 2012). Incorporating the fundamental biochemical knowledge of “like dissolves like”, passive samplers are composed of a hydrophobic, organic polymer with a composition similar to the contaminant of concern. When the concentration of contaminant within the sampler reaches sorption equilibrium with the environment, the samplers are recovered and wiped clean to remove any biological growth, soil, or site water (Endo et al., 2011; US EPA, 2012). The sampler is then solvent extracted. Passive sampler analyses predict the dissolved contaminant concentration and the potential for bioaccumulation within organisms. Analyses may also be used in comparison to water quality standards and criteria, background levels, and risk-based values.

Various passive samplers and extraction methods are available to assess bioaccumulation. Two common passive sampling methods include polyoxymethylene solid phase extraction (POM-SPE) and polydimethylsiloxane (PDMS)-coated solid phase micro extraction (SPME) (Gomez-Eyles et al., 2012). The POM-SPE method is capable of detecting low concentrations of contaminants and may be up to 400 times as sensitive as the PDMS-SPME method (Jonker and Koelmans, 2001). POM-SPE also has a high level of reproducibility and employs a cleanup step to remove interfering chemicals and simplify complex chromatograms.

Appropriate for both water columns and sediment deployment, POM provides a simple and inexpensive partitioning method (Jonker and Koelmans, 2001; US EPA, 2012). The rigidity, cleaning ability, and easy application of POM strips make them essential in both the scientific and regulatory communities (US EPA, 2012). Although POM strips take longer to reach equilibrium than SPME, most studies have shown an equilibrium time of 10 to 28 days to be sufficient (Cornelissen et al., 2008; Endo et al., 2011; Gomez-Eyles et al., 2012; Jonker and Koelmans, 2001).

Although POM can be beneficial when quantifying bioavailable contaminants, discrepancies may occur between passive sampler analyses and other methods for quantifying bioaccumulation. Gomez-Eyles (2011) found that passive samplers were within a factor of 10 when predicting PAH accumulation in earthworms. In comparison to PAH extractions of plant roots, POM-SPE was found to underpredict bioaccumulation. Soil particle adherence to plant roots or chemicals bound to root exudates are possible explanations for this underprediction, although more research is necessary to be conclusive.

1.4 Further Research

Further research regarding potential risks and benefits of biochar amendment are necessary before large-scale application can commence. Although benefits of biochar amendment are evident, it is not clear whether the mechanisms behind these impacts can be attributed to unique properties expressed by biochar or the short term consequences attributed to biochar's liming effect (Jeffery et al., 2015).

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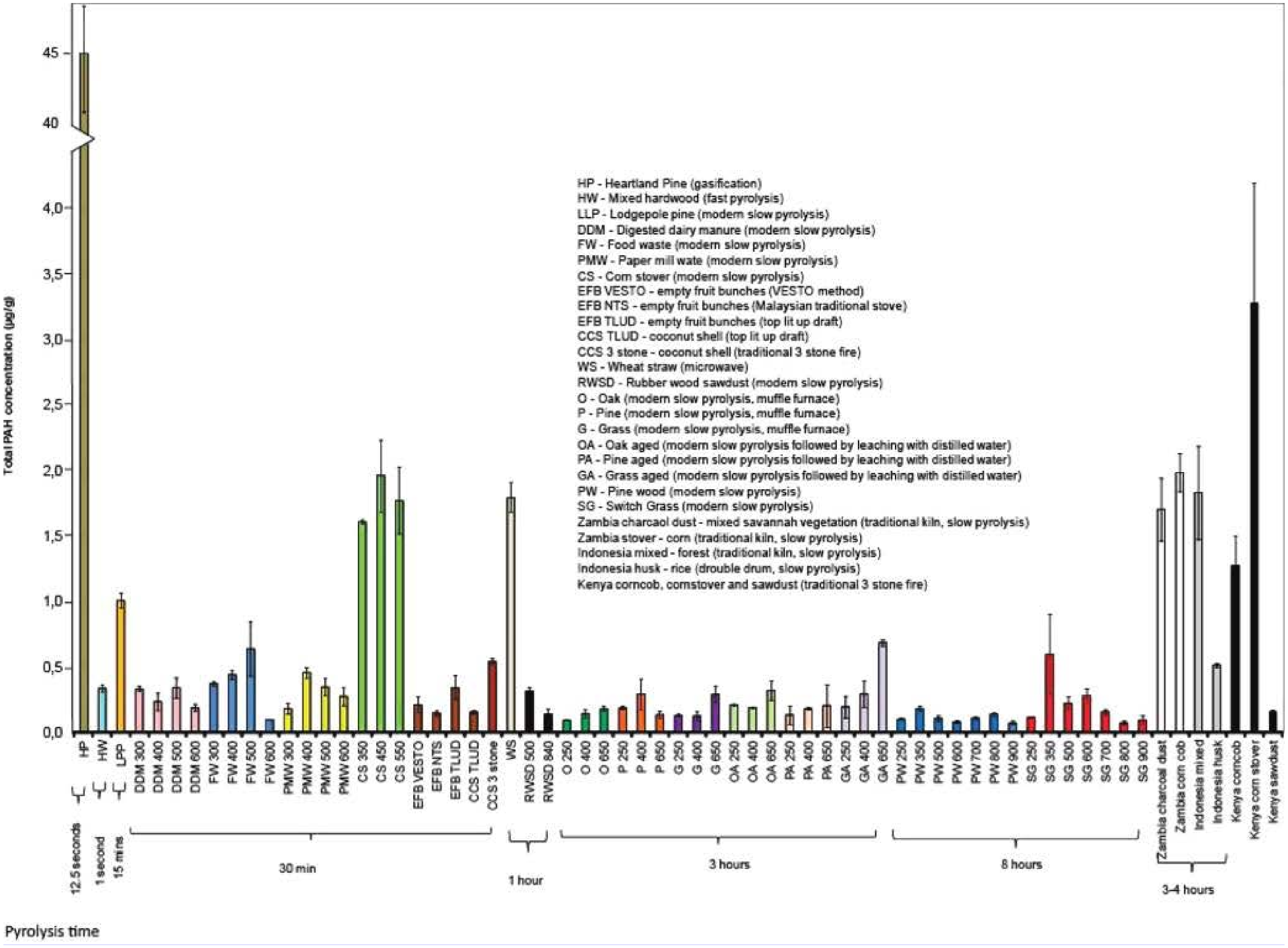

Figure 1-1: Total PAH concentration (µg/g) quantified in biochars by Hale et al. (2012).

Chapter 2. Quantification of Total and Bioavailable PAHs in Biochar

2.1. Introduction

With global populations on the rise and an increased demand on natural resources, soil health and productivity are becoming increasingly vital. Increased crop productivity, enhanced water retention and acquisition, and carbon sequestration are just a few of the potential benefits of biochar amendment. Although biochar amendment has the potential to help farmers grow more food with fewer resources, a thorough risk analysis is necessary before widespread application can commence.

Biochar is a carbonaceous material formed through the thermal decomposition of organic matter under anoxic or low-oxic conditions (Hale et al., 2012; Kloss et al., 2012). Higher crop yield, increased soil fertility, and a long-term sequestration of carbon (C) are potential benefits associated with soil amendment of biochar (IBI, 2017; Lehmann, 2007; Sohi et al., 2009). Benefits may be attributed in part to the highly porous structure of biochar, the resulting liming effect, an increased cation exchange capacity, and increased nutrient use efficiency (Hansen et al., 2015; Jeffery et al., 2011).

Biochar can be formed as a byproduct of biofuel production or for the purpose of soil amendment (Hale et al., 2012; Kloss et al., 2012). Biofuel production processes vary in heating temperatures, oxygen availability, and heat treatment residence time. Slow pyrolysis occurs under low to moderate heat for long residence times in the absence of oxygen (Sohi et al., 2010). Products of slow pyrolysis are approximately 35% biochar, 35% syngas, and 30% bio-oil. Gasification is a process which occurs at high temperatures under low-oxic conditions. Products of gasification are approximately 10% biochar, 85% syngas, and 5% tar (Sohi et al., 2010).

Production temperature and process influence biochar characteristics and require further study. Additionally, the source of feedstock utilized in production is essential in biochar assessment.

Feedstock, production temperature, and process affect both beneficial and detrimental biochar characteristics. During pyrolysis, fragmentation and recombination reactions occur due to the combustion of the biomass particles and may produce hazardous compounds such as polycyclic aromatic hydrocarbons (PAHs) (Hale et al., 2012). PAHs are potential mutagens, carcinogens, and teratogens and any potential exposure to humans must be assessed and mitigated prior to biochar use as soil amendment. Health problems attributed to PAH exposure include decreased immune function, cataracts, kidney and liver damage, asthma-like symptoms, and lung function abnormalities (Abdel-Shafy and Mansour, 2016). In an effort to monitor human exposure to PAHs, the U.S. Environmental Protection Agency (EPA) and the U.S. Agency for Toxic Substances and Disease Registry (ATSDR) compiled a list of 16 PAHs that pose the most significant potential threat to human health (Zelinkova and Wenzl, 2015). The 16 PAHs identified were frequently found in environmental monitoring samples and were prioritized based on a combination of their toxicity, occurrence, and potential for human exposure. Although regulatory standards and guidelines regarding U.S. EPA PAHs are available for environmental monitoring programs, no maximum limits for PAHs in food products currently exists in U.S. legislation (Zelinkova and Wenzl, 2015).

In an effort to identify risks associated with biochar, total and bioavailable PAH concentrations were evaluated in this study. Biochars produced from three feedstock sources and at three production temperatures were evaluated for the slow pyrolysis process. Three feedstock sources were evaluated for the gasification process. After total PAH concentration was quantified, passive sampler and plant uptake analyses were used to estimate bioavailable and bioaccessible PAHs in biochar.

2.2. Materials and Methods

2.2.1. Biochar Preparation

Biochars utilized in the study were generated by two thermochemical processes: slow pyrolysis and gasification. Slow pyrolysis biochars were prepared in accordance with the methodology presented in Keiluweit et al. (2012). The biomass feedstock used in the slow pyrolysis process included loblolly pine (*Pinus taeda*) sawdust, switchgrass (*Panicum virgatum*), and sorghum (*Sorghum bicolor*) stover. Plant biomass was placed in an Inconel crucible (Metal Technology, Inc., Albany, OR) and dried overnight at 60˚C. The crucible filled with biomass was covered with a tight fitting Inconel lid to create oxygen-limited conditions during biochar production. The biomass-filled crucible was placed in a Fisher Scientific muffle furnace and allowed to reach a heat treatment temperature of 400, 550, or 700 °C. Once the specified temperature was reached, temperature was maintained for one hour. The muffle furnace was then turned off and the biochar was allowed to cool. Once cool, the biochar was ground with a mortar and pestle and passed a 60-mesh sieve. Representative samples of each type of biomass were pyrolized at 400, 550, and 700 °C, resulting in nine slow pyrolysis biochars total. The ground samples were placed in glass jars with polypropylene caps and stored at room temperature.

The three gasification biochars were obtained from other sources. Proton Power, Inc. (PPI) produced the hardwood and switchgrass gasification biochars. PPI uses biomass to produce hydrogen, which can then be converted to synthetic fuels, electricity, and heat. Cellulose to Hydrogen Power (CHyP) at a production temperature of 1,100 °C was used to produce the biochars and about four percent of the total biomass placed into the CHyP was converted to biochar. The biochar produced from this process was sold under the product name “Pro-C Carbon Soil enhancer.” In addition to PPI biochars, loblolly pine biochar was obtained from Auburn University’s mobile gasification and power generation laboratory. Biochar was produced from pine sawdust at a production temperature of 1,000 °C. Gasification biochars were ground and stored in the same manner as slow pyrolysis biochars.

2.2.2. Chemical Characterization of Biochar

Total C and N contents of biochars were determined by dry combustion (Elementar Vario Macro CNS Analyzer, Hanau, Germany). Inorganic carbon (IC) contents were measured following the method of Rodriguez et al. (2016) using 100 mg of biochar sample. Total organic C contents were calculated based on the difference between total C and IC. To determine pH and electrical conductivity, 0.5 grams of biochar and 10 ml of Millipore water were mixed in a 20-ml glass vial. Following gentle hand mixing, samples were mixed on a platform shaker at 120 rpm for an hour and a half. Electrical conductivity and pH were determined using a Fisher Scientific accumet AB30 conductivity meter and a model 25 pH meter, respectively. The ash and volatile matter content of biochar samples were evaluated with a Perkin Elmer Thermogravimetric Analyzer (PerkinElmer, Inc., Waltham, MA).

Cation exchange capacity (CEC) of biochar was determined using the modified barium chloride compulsive exchange method (Hendershot and Duquette, 1986; Kloss et al., 2012). Water soluble ions were removed prior to CEC determination. Briefly, distilled water (20 ml) was added to 0.5 g of biochar sample and the mixture was shaken on a platform shaker at 180 rpm overnight. After shaking, the sample was filtered through 0.22-μm nylon membrane filter under vacuum followed by four washes with distilled water (4 x 20 ml, 5 min per wash). The water-extracted ions in the filtrate were analyzed by ICP-AES (Spectro Analytical Instruments, Cleves, Germany). Biochar was scraped off the membrane filter and transferred to a 50-ml centrifuge tube using 10 ml distilled water, and then 10 ml of 0.2 M $BaCl_2$ was added. The mixture was shaken for 2 hours and filtered under vacuum. The filtrate was collected for analysis of exchangeable cations (Ca, Mg, K, Na, Al, Mn, Fe and Zn).

2.2.3. Total PAH Concentrations in Biochars

2.2.4.1 Soxhlet Extractions

PAHs in biochar were extracted using triplicate Soxhlet extraction prior to quantification. To perform the extractions, a 250 mL round bottomed boiling flask was filled with 90 mL of toluene. Three boiling stones were added to the boiling flask. The flask was then placed in a mantle and the extraction chamber was inserted into the boiling flask. A cellulose thimble (80 mm long x 25 mm i.d.), holding 0.5 grams of biochar, was placed into the extraction chamber. The condenser was then placed on top of the extraction chamber and a hose for cooling water was attached to connect all three condensers. The water hose connecting to the final condenser was left to drain into an adjoining sink.

When setup was complete, the mantle was used to heat toluene to boil. The toluene then evaporated, condensed, and dripped into the extraction chamber and onto the 0.5 grams of biochar that was held within the thimble. Once the solvent surpassed the bubble in the siphon arm, the solvent emptied back into the boiling flask and the cycle was repeated. The cycle repeated every 4 ½ minutes for 6 hours. At the completion of the extraction run, the mantles were turned off and allowed to cool. Once cool, the condenser and base were rinsed with solvent into the round-bottomed flask. Thimbles were discarded after use.

Following Soxhlet extraction, each sample was evaporated using a rotary evaporator. A vacuum pump and regulator were used to keep the pressure within the rotary evaporator constant. The temperature of the water bath (50 ˚C) and the vacuum pressure (680 mm Hg) were set in order to minimize sample loss through bumping. A cold trap was used to protect the vacuum pump from any bumping that may occur. Once the sample was reduced to 1 mL, the sample was transferred to a test tube using a Pasteur pipette. Each flask was rinsed three times with toluene to ensure the efficiency of sample transfer. Solvent then underwent further reduction using N-Evap (Organomation Associates, Inc., Berlin, MA). Once a final sample volume of 1 ml was reached, the solvent mixture was passed through a silica gel column for a cleanup procedure.

2.2.4.2 Silica Gel Cleanup

Silica gel cleanup was performed in accordance with U.S. EPA method 3630C and Herzfelder and Golledge (2004). The cleanup was performed in order to separate compounds of a different chemical polarity that may interfere with the gas chromatography-mass spectroscopy (GC-MS) analysis (US EPA, 1996). To prepare for the cleanup procedure, 2 g of silicic acid (Unisil 100–200 mesh, Clarkson Chromatography Products, Inc., South Williamsport, PA) was weighed out and activated by heating at 105°C overnight, per manufacturer recommendation. Anhydrous sodium sulfate was purified by heating at 400°C for four hours. All materials were cooled and stored in a desiccator following purification and activation.

A glass column (15 cm long x 1 cm i.d.) with a PTFE stopcock was used for the cleanup procedure. Glass wool was placed in the bottom of the column in order to retain the silica gel adsorbent. After the addition of the glass wool, the column was packed with 2 g of activated silicic acid. The column was then tapped gently to settle the silicic acid before the addition of sodium sulfate. About 1 centimeter of sodium sulfate was added to the top of the silica column, after which the column was tapped again to ensure uniform packing. Pre-elution of the column was accomplished with 10 ml of hexane. Once the hexane reached the top of the sodium sulfate, the stopcock was closed and the sample was added. Once the sample reached the sodium sulfate surface, a rinse of the sample tube was added. Once again, the column was drained to the level of the sodium sulfate. From there, hexane was added in 1-2 ml increments in order to achieve plug-flow (Herzfelder and Golledge, 2004). Sample was collected once the toluene completed its pass through the column and the cloudy or opaque color had returned. About 15 ml of sample was collected, after which the sample was evaporated to 1 ml under nitrogen. Prior to GC-MS analysis, a 15 µl spike of PCB 77 at 100 µg/ml was added as an internal standard. The sample was then brought up to 2 ml and transferred to a GC vial and stored at -20˚C prior to analysis.

2.2.4. Bioavailable PAH Concentrations

Bioavailable PAH concentrations were determined through the use of two types of bioavailability assays. Polyoxymethylene passive samplers were utilized in order to quickly achieve equilibrium and provide comparable results to bioavailability assays in living organisms. Uptake of PAHs by soybean and lettuce was also evaluated in greenhouse experiments.

2.2.4.1 Polyoxymethylene Passive Samplers

Polyoxymethylene (POM) sheets were used to determine the concentration of bioavailable PAHs within three gasification biochars following a procedure modified after (Hale et al., 2012). The POM sheets (CS Hyde Company, Lake Villa, IL) were cut into 4x9 cm strips. Each strip weighed 0.4 g and was 76 µm thick. To eliminate background contaminants, POM strips were precleaned by soaking in methanol, hexane, and Millipore water for one day each. Strips were then air-dried and placed in 40 ml tubes containing 0.2 grams of biochar and 40 ml of an aqueous solution containing 200 mg/L of sodium azide and HPLC grade Millipore water.

Samples were equilibrated on a platform shaker at 150 rpm in the dark for a period of 30 or 60 days.

Following an equilibration period of 30 or 60 days, the POM strips were removed from the aqueous solution. Excess biochar was removed and strips were blotted dry. POM strips were then placed in a 40 ml tube and extracted with 40 ml of hexane:acetone mixture (80:20) for two days. The tubes were shaken for 30 seconds each day. Following extraction, POM strips were removed and solvents were reduced to 1 ml under nitrogen. Silica gel cleanup was then performed as described above. The hexane/acetone extracts were reduced to 1 ml under nitrogen. A 7.5 µl spike of PCB 77 at 100 µg/ml was added to each sample before transference to a GC vial. Vials were stored at –20 ˚C prior to GC-MS analysis.

2.2.4.2 Greenhouse Experiments

To evaluate bioaccumulation of PAHs in food crops, two types of soil were utilized. The first soil was collected from the EV Smith Research Center (Shorter, Alabama) and classified as a Compass loamy sand (coarse-loamy, siliceous, subactive, thermic Plinthic Paleudults). The second soil came from Lenoir City, Tennessee and was classified as a Waynesboro loam (fine, kaolinitic, thermic Typic Paleudults). The Waynesboro loam soil was amended with switchgrass gasification biochar at a rate of 10 tons per acre (22,417 kg/ha) and 40 tons per acre (89,668 kg/ha) in the field 18 months prior to soil sample collection for greenhouse experiments. The Compass loamy sand was amended with pine, hardwood, and switchgrass gasification biochars at rates of 10 and 40 tons per acre prior to potting. All biochars were ground to pass a 60-mesh sieve before mixed with the Compass loamy sand. Soil samples were mixed homogenously in a container prior to planting and analysis of soil treatments. For the 10 ton per acre treatments, 30 g of char was added to 3000 g of soil. For the 40 ton per acre treatment, 120 g of char was mixed into 3000 g of soil. Soil samples from all treatments were analyzed at the Auburn University Soil Testing Laboratory, where the N and C contents, pH, and fertilizer recommendations were determined (Table 2-1).

In order to evaluate the level of background PAHs in the Compass loamy sand and Waynesboro loam soils, three soxhlet extractions were performed using 5 grams of soil and 2.5 grams of anhydrous sodium sulfate (US EPA, 1996). The setup, extractions, and cleanup of the samples followed the same procedures as the biochar extractions. A 7.5 µl spike of 100 µg/ml PCB 77 was added to each sample as an internal standard.

Buttercrunch lettuce (*Lactuca sativa* L. cv. Buttercrunch) and Roundup Ready® Prichard soybean (*Glycine max* L. Merr.) were used in the greenhouse experiments. Treatments were arranged in a randomized complete block design with four blocks. Within the greenhouse, an average temperature of 24 °C was maintained. Temperatures within the greenhouse fluctuated between a minimum of 12 and maximum of 34°C during the growing period. Water was applied manually and as needed in an effort to reduce nutrient leaching. Fertilizer was applied to lettuce before planting and every two weeks after planting in accordance with recommendations from the Auburn University Soil Testing Lab. For soybean, only the control for Waynesboro soil received fertilizer at the recommended rate (Table 2-1).

Prior to planting, lettuce seeds were surface sterilized in a 0.8% NaClO (10% bleach) solution for 10 minutes, rinsed with autoclaved distilled water three times, and then allowed to soak for 10 minutes. Three lettuce seeds were placed on the soil surface in each Ray Leach cell (14 cm deep x 3.8 cm i.d., 107 mL). The seeds were covered with either control or amended soil in accordance with their respective treatments. After two weeks, germination rates were determined and plants were thinned to two seedlings per cell. Eight weeks after planting, lettuce plants were harvested. Immediately following harvest, lettuce plants were separated into roots and shoots, rinsed, dried with a paper towel, freeze dried, and stored at –20 ˚C prior to use.

For soybeans, seeds were soaked in a 0.8% NaClO solution for 5 minutes. Afterwards, the seeds were rinsed with autoclaved distilled water three times and allowed to stand for 10 minutes. Rhizobial inoculant was added to the seeds prior to planting. Two seeds were planted at a depth of 1.5 cm in each Ray Leach super cell (21 cm deep x 3.8 cm i.d., 164 mL). The seeds were covered with either control or amended soil in accordance with their respective treatments. After two weeks, germination rates were determined and plants were thinned to one seedling per cell. Six weeks after planting, aboveground plant material was harvested by cutting at the first node. All root material was extracted from the soil. Immediately following harvest, soybean plants were rinsed and dried with a paper towel. Root nodules were counted. Shoots and nodulated roots were then freeze-dried and stored at –20 ˚C prior to use.

2.2.4.3 Extraction of PAHs from Plants

The freeze-dried plant samples were ground in a handheld electric coffee grinder prior to PAH extraction. To facilitate grinding, roots were cut and leaves crushed before being placed in the grinder. To overcome the problem caused by the small sample volume, root and shoot samples were combined. Dry ice was added to the grinder and ground before adding plant sample. Additional dry ice was added to the grinder as necessary and the samples were ground until they reached a fine powder. Samples were weighed and stored in 20 mL glass vials.

Lettuce and soybean samples were extracted in accordance with U.S. EPA Method 3550C. To prepare for extraction, 0.5 grams of lettuce or 0.8 grams of soybean sample were transferred to clean 20 ml glass vials. Anhydrous sodium sulfate was mixed with plant sample at a ratio of 1:1 by weight.

To determine the recovery, a 5 µl spike of a 200 µg/ml deuterated standard was added to the mixture. The deuterated spike comprised of 1,4-dichlorobenzene-D4, naphthalene-D8, acenaphthene-D10, phenanthrene-D10, chrysene-D12, and perylene-D12. After adding the deuterated standard, 10 ml of 1:1 acetone:hexane mixture was added to each of the samples. Samples were then extracted for two minutes using a Misonix S-4000 Sonicator (Misonix, Inc., Farmingdale, NY) fitted with a 1/8 inch (3.175 mm) tapered microtip probe. The sonicator was set at an amplitude of 5 with a pulse-on time of 30 seconds and a pulse-off time of 15 seconds.

The cleanup process began with filtration through glass funnels loosely packed with 2-3 cm of glass wool and the filtered samples were collected in a 20 ml glass vial. Immediately before silica gel cleanup, samples were transferred to 15 ml graduated tubes and the volumes were reduced to 0.8 ml under nitrogen. Silica cleanup proceeded in accordance with U.S. EPA method 3630C and Herzfelder and Golledge (2004). Due to the differing characteristics of the plant samples, the above methodology for the silica gel cleanup of the char samples was modified. Sample collection began once 8 ml of hexane had passed through the column following sample application. A total of 40 ml of sample was collected. Samples were then reduced to 1 ml under nitrogen. Prior to GC-MS analysis, a 7.5 µl spike of 100 µg/ml PCB 77 was added as an internal standard. The sample was then transferred to a GC vial and stored at –20 ˚C prior to analysis.

2.2.5. GC-MS Analysis

The GC-MS procedure was performed in accordance with ISO method 18287 and U.S. EPA method 8270D (US EPA, 1996). An Agilent Technologies 7890A gas chromatograph coupled with an Agilent 5975C mass spectrometer (GC-MS) was used to determine the total PAH concentrations of the biochar samples. The extraction efficiency of this method was determined by spiking a mixture of deuterated PAHs prior to Soxhlet extraction of biochars. The deuterated standards mixture included 1,4-Dichlorobenzene-D4, Naphthalene-D8, Acenaphthene-d10, Phenanthrene-D10, Chrysene-D12, and Perylene-D12. 1,4-Dichlorobezene-D4 was not used in the calculations of extraction efficiency because it is not a PAH. Two µl of PAH extract was injected; the injector temperature was set at 40˚C. PAH separations were accomplished using an Agilent DB-EUPAH column (length 20 m; inner diameter 0.18 mm; film thickness 0.14 µm) with helium as the carrier gas. Temperature programming began at 40˚C and ramped up to 300˚C at a rate of 8˚C/min. The temperature was held at 300˚C for 10 minutes.

2.2.6. Data Analysis

Data analysis was conducted in R Studio version 1.0.136 (RStudio Team, 2016) using the car (Fox and Weisberg, 2011), nlme (Pinheiro et al., 2017), and phia (De Rosario-Martinez, 2015) packages. Analysis of variance and linear model analysis were conducted in order to determine the effects of treatments. Biochar production batches were divided into three pseudoreplicates. Analysis of covariance and a mixed effects model were utilized to determine the effect imposed by pseudoreplication. All models were evaluated for independence, linearity, homoscedasticity, and normality. Log base 10 transformations of the data from POM assays were performed to correct for discrepancies in the model. Tukey's Honest Significance Difference (confidence level = 95%) was also conducted.

2.3. Results and Discussion

2.3.1. Characteristics of Biochar

Table 2-2 summarizes key characteristics of biochars produced via slow pyrolysis and gasification. For the three biochars produced via slow pyrolysis, total C, TOC, and IC contents increased with increasing temperatures. Although total N contents for pine biochar increased with an increase in production temperature, a decreasing trend was observed for switchgrass and sorghum biochars. The C:N ratio decreased with increasing production temperature with the ratios in pine char up to seven times higher than those in switchgrass or sorghum. In sorghum and switchgrass biochars, the C:N ratio increased with an increase in production temperatures. Overall, pine biochar had the highest and sorghum had the lowest C:N ratios at all pyrolysis temperatures.

For slow pyrolysis biochars, trends resulting from the analysis of total C, TOC, IC, and total N were consistent with analyses found previously in the literature (Purakayastha et al., 2016; Singh et al., 2010). By increasing the production temperature for pyrolysis, total C, TOC, and IC contents increased. With the exception of pine biochar, increased production temperature decreased N content and increased the C:N ratio. The higher total C content and lower N contents of pine biochar were consistent with results found by Singh et al. (2010). Trends in biochar total C, TOC and IC contents appear to correspond to the ash content of biochar samples. Higher percentages of C remained within biochar samples which contained high ash contents (Singh et al., 2010). Switchgrass and sorghum chars, however, showed the opposite trend in our study.

For gasification biochars, pine contained the lowest percentages of total C, TOC, and total N and the highest percentage of IC. Hardwood biochar had the lowest percentage of IC and highest percentages of total C and TOC. Switchgrass contained the highest percentage of N, with total C and TOC values slightly lower than that of hardwood biochar.

In comparison with the pine char produced using slow pyrolysis, pine char produced using gasification resulted in 40-55% less total C and 42-58% less TOC. The pine gasification biochar had the highest IC content, whereas IC in pine biochar produced under slow pyrolysis was barely detectable. Pine biochar produced from gasification had a slightly higher percentage of N than those from slow pyrolysis, continuing the trend of increasing N content with higher production temperatures. The low total C content in the pine gasification biochar resulted in a much lower C:N ratio compared with pine biochars produced using slow pyrolysis. Results for switchgrass biochar produced through gasification were similar to that of biochar produced from slow pyrolysis. Compared with the biochars produced at lower temperatures using slow pyrolysis, the percentage of IC increased for the switchgrass gasification biochar.

Increasing production temperatures during slow pyrolysis resulted in biochars with a higher pH. As production temperature increased, increases in EC and CEC were also observed in switchgrass char. For pine char, pH and EC increased but CEC varied. Sorghum char did not show a clear trend for EC or CEC. The EC and CEC concentrations of sorghum biochars were the highest for their respective production temperatures.

Pine char produced from gasification had a higher pH, EC, and CEC compared with slow pyrolysis pine char. Compared with slow pyrolysis switchgrass char, switchgrass gasification char exhibited increased EC; CEC did not show a clear trend. The pine char produced from gasification resulted in the highest pH, EC, and CEC among the gasification and slow pyrolysis chars evaluated. The pine char produced from slow pyrolysis resulted in the lowest observed pH, EC, and CEC at a given temperature. Most chars had a basic pH ranging from 8.1 to 11.4. Acidic to neutral pHs (5.6 and 6.6, respectively) were found for pine at 400°C and switchgrass at 400°C.

High concentrations of common base-forming cations, such as Ca, Mg, K, Na, enhanced biochar alkalinity (McCauley et al., 2017). All biochar samples contained elevated concentrations of alkali matter contents and macro- and micronutrients. Although concentrations varied for processes and temperatures, increasing pH levels correlated with an increase in total alkali matter and ash content (Table 2-2). These results are similar to findings reported in the literature (Kloss et al., 2012; Singh et al., 2015). The high pH, high CEC, and abundance of nutrients within biochar may produce benefits when applied to acidic and nutrient-poor soil (Singh et al., 2015).

2.3.2. PAHs within Biochar

Total PAH concentration was determined for biochars produced from slow pyrolysis and gasification at various temperatures. Analysis of variance revealed a significantly higher concentration of total PAHs for the gasification biochars in comparison to the slow pyrolysis biochars ($p < 0.001$). Biochars produced from the slow pyrolysis and gasification production processes were evaluated separately.

Figure 2-1 shows the average concentration of solvent-extractable PAHs from biochars produced from slow pyrolysis at three temperatures. Total PAH concentrations for slow pyrolysis biochars ranged from 1.7 μg/g for pine at 550°C to 17.9 μg/g for sorghum at 400°C. Sorghum biochars contained a significantly higher concentration of PAHs than pine or switchgrass biochars at 400°C ($p < 0.001$).

Compared with biochars produced from the same feedstock at higher temperatures, switchgrass and sorghum biochars produced at 400°C contained a significantly higher concentration of PAHs. Total PAH concentrations decreased as pyrolysis temperature increased for sorghum and switchgrass chars. The trend of decreasing PAH concentration with increasing temperature is analogous to previous findings reported by Hale et al. (2012). No trend was evident for pine biochar, which had the lowest concentration of PAHs at 550°C.

For biochar treatments derived from the gasification process, PAH concentrations ranged from 2779 μg/g for Hardwood at 1,100°C to 31,957 μg/g for Pine at 1,000°C (Table 2-3).

Analysis of variance showed a significantly higher total PAH concentration in pine biochar ($p < 0.001$). Tukey's Honest Significance Test (confidence level = 95%) also revealed a significant difference between pine and hardwood ($p < 0.001$) and pine and switchgrass ($p < 0.001$). No significant difference was detected between switchgrass and hardwood chars. After accounting for pseudoreplication, PAH concentrations in switchgrass and hardwood chars were still significantly lower than the concentrations in pine biochar.

Analysis of variance for LMW and HMW PAHs in gasification biochars showed a significantly higher concentration for pine biochar compared with switchgrass and hardwood biochars ($p < 0.001$) (Table 2-3). After combining factors and accounting for pseudoreplication, the concentration of HMW PAHs in pine biochar ($p = 0.04$) remained significantly higher than the combined switchgrass and hardwood factor ($p = 0.01$). Similarly, the concentration of LMW PAHs within pine biochar ($p = 0.03$) was significantly higher.

On average, gasification biochars contained between 1630 and 1785 times more solvent-extractable PAHs than slow pyrolysis biochars. These trends are consistent with the literature (Hale et al., 2012; Ledesma et al., 2002). Results indicate that heat treatment temperature and residence time are influential factors in determining the concentration of solvent-extractable PAHs. For temperatures below 700°C, increasing the slow pyrolysis production temperature led to a decrease in PAH concentration. At temperatures above 700°C, this trend is no longer applicable and high concentrations of PAHs have been identified (Garcia-Perez and Metcalf, 2008; Ledesma et al., 2002). High PAH concentrations resulting from increased production temperatures are believed to be the result of secondary chemical reactions that occur as the material in organic feedstock is thermally degraded (Garcia-Perez and Metcalf, 2008; Ledesma et al., 2002; Verheijen et al., 2010). As temperatures rise, the aromatic rings that compose the feedstock lose functional groups and coalesce to form stable PAH compounds. Gasification biochars, which are produced at temperatures greater than 700°C, are processed with shorter residence times and higher production temperatures than slow pyrolysis biochars. Shorter residence times increase the likelihood of PAH condensation onto the surface of biochar rather than emission to the atmosphere (Quilliam et al., 2013). Greater access to oxygen may also be a factor contributing to the high PAH concentrations within gasification biochars (Hale et al., 2012). Although no standards currently exist for biochar application to cropland within the United States, the Finnish Ministry of Agriculture and Forestry requires ash products that are anticipated for dispersal on forested land to contain less than 2 $\mu g_{PAH}/g_{soil}$ (Hale et al., 2012). In this study, pine biochar produced at 550°C would be the only biochar that would comply with this standard.

Slow pyrolysis biochars contained two types of PAHs, naphthalene and phenanthrene (Table 2-4). Concentrations of naphthalene ranged from 1.2 to 8.4 µg/g and comprised 47 to 100% of the total PAH concentration contained in slow pyrolysis biochars. The predominance of naphthalene and phenanthrene in biochar are consistent with the literature (Fabbri et al., 2013; Hilber et al., 2012; Quilliam et al., 2013; Verheijen et al., 2010). Due to the low concentrations of these PAHs, biochar produced from slow pyrolysis would present low toxicological and environmental implications if applied to the soil.

Gasification processes produced an assortment of low and high molecular weight PAHs (Table 2-3). Of the 16 PAHs regulated by the U.S. EPA, hardwood contained 13, switchgrass contained 15, and pine biochar contained all 16 PAHs. Naphthalene was the most abundant individual PAH in all three biochars. Naphthalene concentrations ranged from 537 $\mu g/g_{char}$ to 5,375 $\mu g/ g_{char}$. High naphthalene concentrations were consistent with previous studies of gasification biochars (Fabbri et al., 2013; Hilber et al., 2012; Quilliam et al., 2013; Schimmelpfennig and Glaser, 2012). Naphthalene's genotoxicity to plants and possible carcinogenic nature make soil amendment of gasification biochars high in naphthalene and other PAHs disadvantageous (Fabbri et al., 2013).

Hardwood and pine biochars contained a higher concentration of LMW PAHs than HMW PAHs (Table 2-3). On average, LMW PAHs composed 60% of the total solvent-extractable PAHs within hardwood biochar and 54% of the total solvent-extractable PAHs in pine biochar. HMW PAHs composed 58% of the total solvent-extractable PAHs within switchgrass biochar. Although HMW PAHs pose the greatest health and environmental concerns, their low solubility makes them less bioavailable (Fabbri et al., 2013).

Differences were observed in the properties of biochars originating from wood and grass feedstocks. Consistent with previous studies, gasification biochars produced from woody feedstocks resulted in a lower concentration of HMW PAHs compared with LMW PAHs (Hale et al., 2012). In slow pyrolysis biochars, the total PAH concentrations of biochars derived from grass feedstocks were higher than those derived from wood feedstocks at temperatures of 400 and 550°C (Figure 2-1). Further investigation is necessary in order to ascertain the full extent of these differences.

Slow pyrolysis biochars contain trace concentrations of PAHs and would not constitute a significant source of contamination in the soil environment. On the other hand, contamination introduced by gasification biochars could be considerable. Table 2-5 compares the calculated concentrations of PAHs for soil amended with 10 and 40 tons per acre of gasification biochars with the U.S. EPA standard for individual PAHs in the soil. At an application rate of 10 and 40 tons per acre (89,668 kg/ha), many of the PAHs have the potential to exceed the standard.

Although all three gasification biochars exhibited the potential for contamination, considerable contamination may be induced following pine biochar amendment. For pine biochar amended at a rate of 10 tons per acre (22,417 kg/ha), 10 of the 16 EPA regulated PAHs may be present in concentrations that exceed the standard. The potential for violations increased to 13 at a 40 ton per acre pine (89,668 kg/ha) biochar amendment rate. Hardwood biochar posed the least potential threat to the soil environment. Potential PAH concentrations after soil amendment with hardwood biochar produced three potential violations of the standard at 10 tons per acre (22,417 kg/ha) and six potential violations at 40 tons per acre (89,668 kg/ha). For all biochars, the majority of individual PAHs that showed a potential for violation were the more persistent and highly regulated HMW PAHs. The potential for PAH concentration to exceed the U.S. EPA standard increased as application rate increased. At both amendment levels, all three biochars contained a high enough concentration of PAHs to potentially exceed U.S. EPA standards for acceptable PAH levels within soil. Utilizing a soil amendment that may pose a threat to the soil environment is unacceptable from both a regulatory and environmental standpoint.

2.3.3. Bioavailable PAHs within Biochar

2.3.3.1. Polyoxymethylene Passive Sampler Assay

Bioavailable PAHs extracted from polyoxymethylene passive samplers were determined for three gasification biochars at 30 or 60-day equilibration periods (Figure 2-2). Analysis of variance was performed to assess the interaction effects of biochar source and equilibration period on total bioavailable PAH concentration. No significant difference resulted from either the 30 or 60 day period. Tukey’s Honest Significance Difference (confidence level = 95%) was conducted and revealed a significantly higher concentration of bioavailable PAHs for pine char compared with the blank control, hardwood, and switchgrass chars ($p<0.001$). There was no significant difference between the two equilibrium periods.

Significantly higher concentrations of bioavailable PAHs were found in the pine biochar POM assay compared with the control, switchgrass, and hardwood POM assays. For the 30 and 60 day equilibration periods, POM film exposed to pine biochar absorbed 24.45 and 27.89 µg of PAHs per gram of biochar (Table 2-7). The second highest PAH concentration was contained within POM assays exposed to switchgrass biochar. Switchgrass POM assays contained 0.14 and 0.25 µg of PAHs per gram of biochar for the 30 and 60 day equilibrium periods. These concentrations were over 100 times lower than concentrations contained within pine POM assays.

Compared with the solvent-extractable PAH concentrations within biochar, less than 0.01% of the LMW, HMW, and total PAHs were bioavailable in switchgrass and hardwood biochars. A significantly higher concentration of bioavailable PAHs was observed in pine biochar for both equilibration periods ($p < 0.001$). On average, 0.14% of the LMW PAHs found within biochar sample were bioavailable and assimilated into the POM passive samplers. On average, POM passive samplers were able to assimilate 0.08% of the solvent-extractable PAHs contained in pine biochar. Bioavailable PAH concentration increased numerically with equilibration period for pine and switchgrass POM assays but decreased for hardwood assays. Increasing the equilibration time allows additional opportunities for the PAHs to reach equilibrium between the dissolved phase and the assimilated concentration in the membrane of the POM. Although increased concentrations of bioavailable PAHs uptake with increasing equilibrium periods can be expected, further analysis is necessary in order to determine why a decrease in concentration occurred for hardwood POM assays.

Pine POM assays contained a greater variety of individual PAHs than hardwood or switchgrass assays (Table 2-6). Pine assays contained seven types of PAHs for the 30 day equilibrium period and five types of PAHs for the 60 day equilibration period. The decrease in types of individual PAHs may be a result of inadequate mixing during the duration of the 60 day period. Hardwood POM assays produced the second highest variety of PAHs for the 30 day equilibration period, with four types of PAHs present. Switchgrass assays contained the second highest variety of PAHs for the 60 day equilibration period, with three types of individual PAHs present. For the 60 day equilibration period, switchgrass assays were the only treatments in which the number of individual PAHs increased. Fewer types of individual PAHs were found in pine and hardwood assays for the 60 day equilibrium period.

Naphthalene had the highest individual PAH concentration for all POM assays and both equilibration periods (Table 2-6). Compared with switchgrass and hardwood biochars, significantly more of the total solvent-extracted naphthalene was bioavailable in the pine biochar assays. The percentage of bioavailable naphthalene in the total amount of naphthalene solvent-extracted from pine biochar was 0.41 and 0.45% for the 30 and 60 day equilibrium periods. By comparison, percentages of bioavailable naphthalene in hardwood and switchgrass POM assays averaged 0.01% and 0.02%, respectively. Overall, bioavailable naphthalene concentrations comprised 68 to 100% of the total bioavailable PAHs detected within biochar POM assays.

LMW PAHs comprised 92 to 100% of the total bioavailable PAHs within the POM assays (Table 2-7). Fluoranthene and pyrene were the only HMW PAHs detected (Table 2-6). Both compounds were present in the pine biochar samples for the 30 and 60 day equilibration periods. For the 30 day period, fluoranthene was also extracted from the hardwood sample.

Neither the 30 nor 60 day period for switchgrass nor the 60 day period for hardwood resulted in any high molecular weight PAHs.

Table 2-8 shows biochar-water partitioning coefficients for PAHs in the three gasification biochars as well as their octanol-water partitioning coefficients. Biochar-water partitioning coefficients were calculated by dividing solvent-extractable PAH concentrations (μg/g) by the passive-sampler extracted PAH concentrations (μg/ml) for each biochar. Coefficients were high for each of the biochars, indicating a strong sorption that is further evidenced by the low bioavailable concentration detected by the passive samplers. Compared with the octanol-water partitioning coefficient and the biochar-water partitioning coefficients for switchgrass and hardwood assays, biochar-water partitioning coefficients for napthalene and phenanthrene were lower in the pine biochar assays. These individual PAHs are more likely to be bioavailable, a trend that is consistent with previously published studies (Fabbri et al., 2013; Hilber et al., 2012; Quilliam et al., 2013). Despite the high concentrations of solvent-extractable PAHs within gasification biochars, high biochar-water partitioning coefficients indicate that PAHs are bound tightly to the particulate matter and may not be readily available in the soil environment.

2.3.3.2. Greenhouse Experiment: Plant Uptake of PAHs

PAH uptake was evaluated for lettuce and soybean plants grown in Compass and Waynesboro soils amended with gasification biochars. For the Compass soil, three types of gasification biochars were applied at two application rates prior to planting. For the Waynesboro soil, switchgrass biochar was applied in the field at two application rates 18 months prior to soil sample collection. Plants grown in the Compass and Waynesboro soil series were evaluated separately.

2.3.3.3. *Effect of Biochar Amendment on Soil Characteristics*

Background PAH concentrations were evaluated for Waynesboro and Compass soils. Both soils contained naphthalene, phenanthrene, fluoranthene, and pyrene in varying concentrations (Table 2-9). Naphthalene was found in the highest concentrations, composing 50% of the total concentration for the Compass soil and 44% of the total concentration for the Waynesboro soil. LMW PAHs consisted of 82% and 56% of the total concentration of PAHs extracted from Compass and Waynesboro soils, respectively. The Compass soil contained a lower total PAH concentration than the Waynesboro soil. Background PAH concentrations may originate from natural or anthropogenic sources (US EPA, 2007). Possible natural sources contributing to background concentrations include forest fires and atmospheric deposition.

Control and biochar-amended soils were analyzed by the Auburn University Soil Testing Laboratory prior to planting. A summary of the results and recommendations are provided in Table 2-1. For Compass soil, biochar amendment increased the soil pH, total N, and total C. Biochar amendment eliminated additional P requirements for lettuce plants within all Compass soil treatments except the 10 ton per acre hardwood and switchgrass char amendments. Lettuce recommendations for N remained unchanged following the biochar amendment.

Although a liming effect was detected following switchgrass amendment in Waynesboro soil, the effect was not as substantial as in the Compass soil due to the Waynesboro soil’s higher CEC. Biochar amendment eliminated the need for additional nutrients for soybeans and reduced P and K requirements for lettuce. Similar to the Compass soil, there was no decrease in N requirements for lettuce after application of biochar to Waynesboro soil, suggesting that biochars were not a significant source of N.

2.3.3.4. Effects of Biochar Amendment on Plant Growth

Plant growth was evaluated throughout the duration of the greenhouse experiments. Interveinal chlorosis was observed on the lower trifoliates of soybean plants, eventually resulting in lemon yellow leaves. Additionally, soybean plants grown in Compass soil amended with the switchgrass 40 ton per acre and the pine 10 and 40 ton per acre were small in stature with yellow to very light green leaves. Chlorophyll readings, root nodule counts, and soil characteristics for these treatments revealed a probable metal deficiency. The switchgrass 40 ton per acre and pine 10 and 40 ton per acre amendments contained chlorophyll readings of 24.9 27.4 and 12.6 SPAD units, respectively (Table 2-10). Chlorophyll readings for these treatments were low in comparison to other Compass soil treatments and substantially low in comparison to the Waynesboro soil treatments.

Tukey’s HSD showed that in the Compass soil, the pine 40 ton/acre application produced a root nodule count that was significantly lower than the control ($p<0.001$), hardwood 10 and 40 ton/acre, and switchgrass 10 and 40 ton/acre treatment applications (Table 2-10). Soybeans grown in Compass soil amended with pine biochar at 10 tons per acre produced significantly fewer root nodules in comparison to the hardwood switchgrass 10 ton per acre applications ($p<0.001$). For chlorophyll, the pine 40 ton per acre treatment resulted in the lowest readings in comparison to all other treatments. No significant difference in chlorophyll readings or root nodule count was observed for soybeans grown in the Waynesboro soil.

Soybeans grown in the Compass soil amended with pine 10 and 40 ton per acre had the lowest root nodule counts out of all Compass and Waynesboro soil treatments. There were ten root nodules for soybeans grown in the 10 ton per acre pine biochar-amended soil and two root nodules for soybeans grown in the 40 ton per acre treatment. The biochar-amended soils for these two treatments had a high pH (9.3 and 10). These pH values are significantly higher than the recommended soil pH for soybean growth, which ranges between 6 and 7. As soil pH increases, the availability of nutrients such as Mg, Fe, Mn, Cu, and Zn are limited and plant health is adversely affected. Trace element deficiency and unfavorable conditions for biological nitrogen fixation stunted plant growth, leading to low root nodule counts and yellow to pale green leaves.

The amendment of biochar to Compass soils resulted in a liming effect for all soil treatments. Soil pH, total C, and total N increased with an increase in application rate. Chlorophyll readings decreased with an increasing application rate in soybean plants. For lettuce plants, the high soil pH in the 40 ton per acre char-amended Compass soil inhibited growth. Additioanlly, many of the lettuce plants grown within the switchgrass and pine 40 ton per acre treatments did not emerge or were unable to grow normally. Lettuce plants grown in the Compass control soil, switchgrass 10 ton per acre soil, and hardwood 10 ton per acre soil produced healthy plants.

Brown spotting was evident on the lower trifoliates of soybeans grown in the Waynesboro fertilized and unfertilized control soils. Spotting eventually resulted in the yellowing, necrosis, and drop-off of the leaves. For these treatments the pH of the soil was 6.5, which was within the optimal range for soybean plants. The upper trifoliates maintained a healthy green color that was inconsistent with the problems seen in the older leaves. The symptoms seem to have been consistent with that of Septoria brown spot, a soybean disease that results in brown spots on unifoliate and lower trifoliate leaves, progresses to a yellow coloration of the leaves, and leaf necrosis. Waynesboro soils amended with switchgrass biochar maintained an overall healthy appearance. Amendment resulted in a slight pH increase and an increase in total C and total N. Although some discoloration was evident, char-amended Waynesboro soil treatments maintained an overall healthy spread of dark green leaves. The higher buffering capacity of the Waynesboro soil may have prevented soil pH from reaching levels that were unsuitable for plant growth. Plants grown within Waynesboro soils were larger and healthier than those grown within the biochar-amended Compass soil treatments.

Biomass data were collected and evaluated for lettuce and soybean plants after harvest. No significant difference in plant weight was found for lettuce plants grown in the char-amended Compass or Waynesboro soils (Table 2-10). In the Waynesboro soil, lettuce plants grown in the control without fertilizer produced a significantly lower ($p<0.001$) total and shoot weight than what?. Root weight for the control without fertilizer was not significantly different from the other Waynesboro soil treatments.

Compared to other Compass soil treatments, soybean root, shoot, and total weight were significantly lower for the pine 40 ton per acre char application ($p<0.001$). A significantly higher total plant weight was observed for the switchgrass 10 and 40 ton per acre Waynesboro soil treatments. For shoot weight, the switchgrass 10 and 40 ton per acre treatments were significantly lower for the control with fertilizer but not the control without fertilizer. No significant difference was seen for root weights.

2.3.3.5. PAH Uptake from Compass Soil

With total PAH concentrations ranging from 1.64 to 5.11 $\mu g/g_{biomass}$, lettuce plants grown in pine char-amended soils contained significantly higher concentrations of total PAHs than plants grown in other soil treatments (Figure 2-3 and Table 2-11). Total PAH concentrations for soybean plants grown in pine char-amended soils ranged from 2.34 to 2.49 $\mu g/g_{biomass}$ (Figure 2-4 and Table 2-11). Overall, soybean plants grown in pine char-amended soils contained significantly higher total PAH concentrations than other treatments, with the exception of the hardwood 40 ton/acre treatment. Although plants grown in soil amended with hardwood at 40 ton/acre contained the third highest concentration of total PAHs, the concentration was on average 1 μg/g less than the total PAHs assimilated in the pine 10 ton/acre treatments.

With the exception of plants grown in pine-amended soils, lettuce plants grown in Compass soils assimilated predominantly LMW PAHs, with roughly two thirds of the total PAH concentration originating from LMW PAHs (Table 2-11). An overall trend was not evident for PAH assimilation within soybean plants. Soybean plants grown in the control and switchgrass char-amended soils primarily assimilated LMW PAHs; however, results varied for other char treatments. Lettuce plants grown in soil amended with pine at 10 tons/acre contained a significantly higher concentration of HMW and total PAHs than other lettuce treatments ($p < 0.05$). Lettuce plants grown in pine at 40 ton/acre contained a significantly higher concentration of LMW, HMW and total PAHs than other lettuce treatments ($p < 0.001$).

Although soybeans grown in pine char-amended soils contained the highest concentrations of HMW PAHs, levels of significance differed between treatments. Soybean plants grown in soil amended with pine char at 10 tons/acre contained significantly higher concentrations of LMW PAHs compared to all other treatments except the pine 40 ton/acre treatment ($p < 0.001$) (Table 2-11). Soybean plants grown in soil amended with pine char at 40 ton per acre contained significantly higher concentrations of LMW PAHs for all treatments except for the control, the pine 10 ton/acre, and hardwood 40 ton/acre treatments ($p < 0.05$). Additionally, soybeans grown in the 40 ton/acre pine treatments contained significantly higher concentrations of HMW PAHs compared to all other treatments except the pine 10 ton/acre treatments.

With a wide range of total PAH concentrations across Compass soil treatments, no significant difference was discernible between the concentrations of PAHs assimilated by lettuce and soybean plants. Less than 1% of the total solvent-extractable PAHs available within the soil treatments were assimilated into the tissues of lettuce and soybean plants. Increasing the rate of biochar application reduced the percentage of PAHs assimilated by lettuce and soybean plants. The decrease in uptake was indicative of an enhanced sorptive capacity of the soil following biochar amendment. Increased sorption may be the result of an increase in soil organic matter (Dutta et al., 2017).

2.3.3.5. PAH Uptake from Waynesboro Soil

Unlike plants grown in the Compass soil, lettuce and soybean plants grown in biochar-amended Waynesboro soils contained a much narrower range of bioavailable PAHs. Concentrations of bioavailable PAHs within lettuce plants ranged from 0.60 to 0.80 $\mu g/g_{biomass}$ and soybean concentrations ranged from 0.31 to 0.34 $\mu g/g_{biomass}$ (Table 2-12). The concentration of PAHs in lettuce plants was significantly higher than the concentration in soybeans; however, treatment levels did not produce a significant difference. Since treatment levels were not significant, clear trends were not identified for LMW, HMW, and total PAH concentrations in lettuce or soybean plants.

The uptake of solvent-extractable PAHs was highest for lettuce plants grown in the Waynesboro control soils. Lettuce plants grown in the fertilized and unfertilized control soils assimilated 0.36% and 0.20% of the soil’s total solvent-extractable PAHs. Comparatively, the percentage of the soil’s total solvent-extractable PAHs assimilated by soybean plants grown in the fertilized and unfertilized control soils were 0.20% and 0.21%. For all lettuce and soybean plants grown in Waynesboro soil treatments, less than one percent of the total solvent-extractable PAH concentration was assimilated. Soybean plants had a significantly lower uptake of PAHs than lettuce plants, confirming previous studies which indicate that leafy vegetables accumulate higher PAH concentrations than fruit or root crops (Fabbri et al., 2013; Menzie et al., 1992a).

Individual PAH concentrations varied for soybean and lettuce plants grown in the biochar-amended Compass and Waynesboro soils. For both soils, lettuce plants contained naphthalene, phenanthrene, and pyrene (Table 2-14). Fluoranthene was also found in Compass soils amended with pine char. Additional individual PAHs were identified in soybean plants (Table 2-15). More soybean biomass was available for extraction in comparison to lettuce plants. The difference in available biomass may have affected the individual PAH distribution that was detected. Additional research is necessary to more accurately determine individual PAH concentrations in lettuce and soybean plants.

The uptake of PAHs from plants and passive samplers were low in comparison to the high concentrations of total solvent-extractable PAHs in biochar. The strong affinity of PAHs towards biochar and soil particulates limited the bioavailability and bioaccessibility of PAH compounds (Fabbri et al., 2013; Hale et al., 2012). The bioaccessibility of PAHs in food crops was affected by individual PAH characteristics such as ring structure, lipophilicity, and compound solubility (Abdel-Shafy and Mansour, 2016). As the number of ring structures increased, lipophilicity increased and solubility decreased (Alexander et al., 2008; Sverdrup et al., 2002). As a result, HMW PAHs were bound tightly to soil organic matter and were highly insoluble, leading to their persistence within the soil environment (Parrish et al., 2006). Table 2-

13 illustrates the relationship between compound molecular weight, ring size, lipophilicity, and water solubility.

The higher solubility and lower lipophilicity of LMW PAHs enhanced assimilation within the plant tissues. Both plant species were able to assimilate individual PAHs with four or less aromatic rings and molecular weights less than 202. The two HMW PAHs assimilated, fluoranthene and pyrene, had a higher solubility and lower lipophilicity than all other HMW PAHs regulated by the US EPA. More individual PAHs were identified for pine char treatments in both lettuce and soybean plants (Table 2-14 and Table 2-15). Since pine char contained the highest concentration of solvent-extractable PAHs, higher concentrations of a variety of PAHs were available in the soil for plant uptake.

Although soybean and lettuce plants were only able to assimilate small concentrations of PAHs, plant uptake due to biochar amendment may still pose a hazard to humans. For non-smokers, food is believed to be the primary mechanism for PAH uptake in humans (Menzie et al., 1992a). The typical American ingests 1 to 5 μg_{PAH}/day. Total daily exposure for nonsmokers ranges from 3 to 15 $\mu g_{PAH/}$day (Menzie et al., 1992a). Although there are no standards for the concentrations of PAHs within foods, it is recommended that exposure be limited due to the potential carcinogenic, teratogenic, and mutagenic effects of PAHs. Higher PAH concentrations on broad, leafy vegetables such as lettuce can occur due to atmospheric deposition. The consumption of these leafy vegetables may pose a greater threat to humans due to their tendency to be consumed uncooked (Menzie et al., 1992a). Table 2-16 shows the PAH content that would be ingested if the lettuce treatments grown in this study were to be consumed in a main-dish salad. If 150 g of lettuce were consumed, total PAH contents would range from 3 (control) to 31 µg (pine 40 ton/acre) for the Compass soil and 4 µg (switchgrass 10 ton/acre) to 5 µg (control with fertilizer) for the Waynesboro soil. Concentrations of PAHs in uncooked foods generally range between 0.01 to 1 ppb, or 0.0015 to 0.15 μg in 150 g of lettuce (Abdel-Shafy and Mansour, 2016). Based on the calculated concentration of PAHs within lettuce treatments grown in the Waynesboro and Compass soils, the projected total PAH content is likely to be higher than the expected range for PAHs in uncooked foods. Additionally, all of the values in Table 2-16 are significantly higher than the average daily intake of PAHs found to be ingested by Americans in Menzie et al. (1992). Coinciding with significance findings discussed previously, ingestion of lettuce plants grown in the pine 10 and pine 40 ton/acre biochar applications would be significantly higher than other treatments. The highest percentage of PAHs assimilated by lettuce plants was only 0.36% of the total solvent-extractable concentration present within the soils, indicating that the overall potential for exposure could be significantly higher.

2.4. Conclusions and Future Research

In conclusion, gasification biochars are not recommended as a soil amendment due to their high potential for contamination. Based on results from the chars tested, slow pyrolysis biochars produced at longer residence times with temperatures of 550°C or higher could be safe for soil amendment. Although bioavailable PAH concentrations in POM passive sampler assays and soybean and lettuce uptake experiments were low compared to the solvent-extractable concentrations, site contamination and plant and organism uptake may still pose a hazard to humans and the environment.

Further research is necessary before the land application of biochar can become a widespread practice. Biochar feedstock source, production process and production temperature affect characteristics of biochar that influence potential toxicity and interaction within the soil environment. A pervasive assay of common feedstocks and processes must be performed before a standard characterization of common biochars can be compiled. Once compiled, research should be conducted to assess the interaction effects of biochar upon soil amendment. Soil type and characteristics are key parameters that must be thoroughly assessed. Optimal application rates for biochar may vary depending on soil type, biochar type, and crops planted. Effects stemming from the aging of biochar prior to application as well as aging within the soil must be considered. The need for re-application of biochar must be assessed and the lifespan of potential benefits must be quantified.

In regards to the PAH composition within biochar, sorption characteristics must be analyzed. An understanding of how biochar interacts with the soil microbial community will aid in understanding the fate and persistence of biochar-induced PAH deposition in the environment. To be effective, these processes must be observed under a variety of soil and climatic conditions.

Evidence suggests that risk can be substantially reduced depending on the processes, temperatures, and feedstock sources by which biochar is produced. To fully ascertain the risks and benefits of biochar amendment, additional research must be performed. Based upon these findings and the current body of knowledge, gasification biochars are not recommended for use as a soil amendment. Slow pyrolysis biochars produced at longer residence times with temperatures of 550 °C or higher will produce negligible concentrations of PAHs and would be safe for soil amendment. Additional research is necessary to ensure optimal production conditions and feedstock sources.

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Table 2-1: Selected characteristics and fertilizer recommendations for the control and biochar-amended Compass and Waynesboro soil treatments.

Soil Series	Ton/Acre	Biochar	pH	N (%)	C (%)	Soybean $N\text{-}P_2O_5\text{-}K_2O$ (lb/Acre)	Lettuce $N\text{-}P_2O_5\text{-}K_2O$ (lb/Acre)
Compass	0	None	7.1	0.07	0.64	-	120-60-0
Compass	10	Switchgrass	7.9	0.12	1.65	-	120-60-0
Compass	40	Switchgrass	8.8	0.10	3.24	-	120-0-0
Compass	10	Pine	9.3	0.08	0.97	-	120-0-0
Compass	40	Pine	10	0.09	2.08	-	120-0-0
Compass	10	Hardwood	7.7	0.12	1.67	-	120-60-0
Compass	40	Hardwood	8.9	0.09	3.64	-	120-0-0
Waynesboro	0	None	6.5	0.19	1.41	0-40-0	120-120-120
Waynesboro	10	Switchgrass	6.8	0.21	3.76	-	120-0-60
Waynesboro	40	Switchgrass	6.6	0.22	5.89	-	120-0-60

Table 2-2: Pyrolysis temperature, pH, electrical conductivity (EC), cation exchange capacity (CEC), ash and volatile matter content, total carbon, total organic carbon (TOC), inorganic carbon (IC), total nitrogen, and overall nutrient contents of slow pyrolysis and gasification biochars.

Biochar	**Temp °C**	**pH**	**EC (µS/cm)**	**CEC mmol(+)/kg**	**Ash %**	**Volatile Matter %**	**Total C %**	**TOC %**	**IC %**	**Total N %**	**C:N**	**Ca mg/kg**	**Mg mg/kg**	**K mg/kg**	**Na mg/kg**	**P mg/kg**	**S mg/kg**	**Fe mg/kg**
Slow Pyrolysis:																		
Pine	400	5.6	43.6	13.3	1.2	34.4	71.6	71.6	BD[†]	0.11	651	244.4	131.8	390.2	1.2	40.7	17.4	9.0
	550	8.1	56.6	26.3	1.7	17.7	81.8	81.8	BD	0.15	545	169.8	34.8	691.9	0.3	41.9	30.9	8.6
	700	9.4	91.2	19.9	1.9	8.8	87.1	87.1	0.04	0.17	512	434.0	95.2	1178.9	5.2	41.5	7.0	8.4
Switchgrass	400	6.6	185.8	74.9	6.5	30.5	68.5	68.4	0.11	0.83	82	730.9	745.7	1387.2	21.4	415.0	131.0	9.3
	550	9.7	229.9	78.7	8.1	17.0	75.2	75.0	0.15	0.83	90	215.7	613.6	2323.0	19.9	500.4	225.2	8.5
	700	10.4	313.9	87.3	8.0	15.2	76.3	76.1	0.18	0.65	117	232.9	966.5	3238.3	24.1	428.4	47.3	8.6
Sorghum	400	8.1	651.6	224.1	15.7	32.0	60.5	60.3	0.15	1.58	38	251.1	726.8	7521.1	12.4	1122.4	117.4	11.5
	550	9.7	614.0	121.8	21.0	16.4	64.9	64.7	0.26	1.06	61	138.6	541.0	11644.9	0.8	975.1	292.1	8.8
	700	10.8	999.3	159.4	20.7	16.2	66.5	66.1	0.36	0.96	69	195.7	1055.7	16426.1	5.8	804.8	52.3	8.5
Gassification:																		
Pine	1000	11.4	6041.5	452.8	66.3	29.6	32.3	29.0	3.22	0.18	161	3573.5	11.5	60266.8	869.1	1.9	5256.4	10.1
Switchgrass	1100	10.5	1099.5	82.8	16.1	14.2	70.8	70.5	0.39	0.64	110	351.4	613.1	12461.1	242.5	275.4	114.9	8.6
Hardwood	1100	10.8	623.4	71.0	14.8	11.0	73.1	73.0	0.16	0.62	118	664.7	726.0	9712.8	169.2	122.8	114.7	9.1

[†] BD indicates the value is below the limit of detection

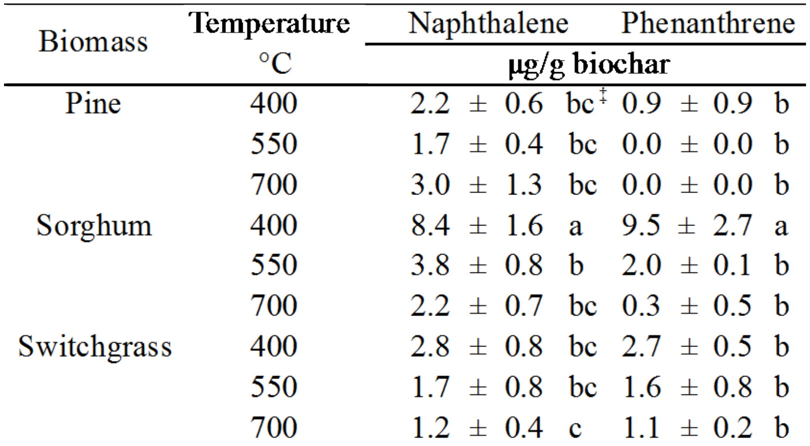
Table 2 - 3: Concentrations of the 16 US EPA-regulated polycyclic aromatic hydrocarbons (PAHs) within gasification biochars[†].

PAH	Hardwood 1100°C		Pine 1000°C		Switchgrass 1100°C	
			μg/g biochar			
Naphthalene	784.0 ± 19.0	b[‡]	5375.2 ± 619.9	a	537.1 ± 229.9	b
Acenaphthylene	234.3 ± 11.6	b	3661.4 ± 437.2	a	306.4 ± 170.1	b
Acenaphthene	15.3 ± 1.2	b	642.4 ± 95.5	a	13.1 ± 6.1	b
Fluorene	21.4 ± 2.3	b	1658.9 ± 277.6	a	6.8 ± 5.0	b
Phenanthrene	510.7 ± 120.3	b	135.5 ± 12.6	b	620.8 ± 454.0	b
Anthracene	87.5 ± 22.6	b	5915.1 ± 601.6	a	125.3 ± 91.4	b
Fluoranthene	351.2 ± 97.4	b	5162.7 ± 318.7	a	503.2 ± 407.2	b
Pyrene	410.5 ± 105.8	b	3248.1 ± 230.7	a	819.2 ± 510.6	b
Benzo(a)anthracene	130.6 ± 45.6	b	1880.3 ± 81.6	a	70.9 ± 51.5	b
Chrysene	48.5 ± 16.4	b	1412.3 ± 56.6	a	57.8 ± 50.2	b
Benzo[B]fluoranthene	38.4 ± 16.3	b	1125.2 ± 44.6	a	106.8 ± 96.7	b
Benzo[k]fluoranthene	25.3 ± 8.4	b	160.7 ± 1.2	a	29.6 ± 27.1	b
Benzo(a)pyrene	112.3 ± 35.6	b	1414.3 ± 21.6	a	229.6 ± 194.6	b
Dibenzo(A,H)Anthracene	ND	b	18.4 ± 8.4	a	ND	b
Benzo(G,H,I)Perylene	ND	b	69.2 ± 1.7	b	87.2 ± 91.6	b
Indeno(1,2,3-CD)Pyrene	8.9 ± 14.4	b	77.5 ± 3.2	b	161.9 ± 141.3	a
LMW PAHs	1653.3 ± 168.5	b	17388.5 ± 2029.2	a	1609.6 ± 953.0	b
HMW PAHs	1124.9 ± 339.5	b	14568.8 ± 639.7	a	2064.9 ± 1310.1	b
Total	**2778.8 ± 507.9**	b	**31957.3 ± 2620.2**	a	**3674.5 ± 2186.8**	b

[†] Values are presented as the mean ± standard deviation (n=3). Values that were not detected are identified with ND.

[‡] Means within each row followed by different letters are significant different at $P < 0.001$.

Table 2-4: Concentrations of naphthalene and phenanthrene in slow pyrolysis biochars[†].



Biomass	Temperature °C	Naphthalene	Phenanthrene
		µg/g biochar	
Pine	400	2.2 ± 0.6 bc[‡]	0.9 ± 0.9 b
	550	1.7 ± 0.4 bc	0.0 ± 0.0 b
	700	3.0 ± 1.3 bc	0.0 ± 0.0 b
Sorghum	400	8.4 ± 1.6 a	9.5 ± 2.7 a
	550	3.8 ± 0.8 b	2.0 ± 0.1 b
	700	2.2 ± 0.7 bc	0.3 ± 0.5 b
Switchgrass	400	2.8 ± 0.8 bc	2.7 ± 0.5 b
	550	1.7 ± 0.8 bc	1.6 ± 0.8 b
	700	1.2 ± 0.4 c	1.1 ± 0.2 b

[†] Values are presented as the mean ± standard deviation (n=3).

[‡] Means within each column followed by different letters are significant different at $P < 0.001$.

Table 2-5: US EPA criteria for polycyclic aromatic hydrocarbons (PAHs) in soil compared with the potential concentrations of individual PAHs introduced to the soil at an application rate of 10 and 40 ton/acre. Bolded values exceed US EPA criteria (US EPA, 2007).

PAH	**EPA Criteria**	**10 ton/acre**			**40 ton/acre**		
		Hardwood	**Pine**	**Switchgrass**	**Hardwood**	**Pine**	**Switchgrass**
	µg/g soil	**µg/g soil**			**µg/g soil**		
Naphthalene	29-100	8	**54**	5	**31**	**215**	22
Acenaphthylene	29-100	2	**37**	3	9	**146**	12
Acenaphthene	29-100	0	6	0	1	26	1
Fluorene	29-100	0	17	0	1	**66**	0
Phenanthrene	29-100	5	1	6	20	5	25
Anthracene	29-100	1	**59**	1	4	**237**	5
Fluoranthene	29-100	4	**52**	5	14	**207**	20
Pyrene	1.1-18	**4**	**32**	**8**	**16**	**130**	**33**
Benzo(a)anthracene	1.1-18	**1**	**19**	1	**5**	**75**	**3**
Chrysene	1.1-18	0	**14**	1	**2**	**56**	**2**
Benzo[B]fluoranthene	1.1-18	0	**11**	**2**	**2**	**45**	**6**
Benzo[k]fluoranthene	1.1-18	0	**2**	0	1	**6**	**2**
Benzo(a)pyrene	1.1-18	**1**	**14**	**2**	**4**	**57**	**9**
Dibenzo(A,H)Anthracene	1.1-18	0	0	0	0	1	0
Benzo(G,H,I)Perylene	1.1-18	0	1	**1**	0	**3**	**5**
Indeno(1,2,3-CD)Pyrene	1.1-18	0	1	**2**	0	**3**	**10**

Table 2 - 6: Concentrations of individual polycyclic aromatic hydrocarbons (PAHs) in polyoxymethylene (POM) passive sampler assays equilibrated for a period of 30 or 60 days[†].

PAH	Blank		Hardwood		Pine		Switchgrass	
	30 Days	60 Days	30 Days	60 Days	30 Days	60 Days	30 Days	60 Days
	µg/g biochar		µg/g biochar		µg/g biochar		µg/g biochar	
Naphthalene	0.02 ± 0.02 b[‡]	ND	0.08 ± 0.03 b	0.04 ± 0.07 b	22.06 ± 2.49 a	24.19 ± 4.38 a	**0.10** ± 0.04 b	**0.17** ± 0.05 **b**
Acenaphthylene	ND	ND	ND	ND	1.03 ± 0.09 b	1.87 ± 0.14 b	ND	ND
Acenaphthene	ND	ND	ND	ND	0.07 ± 0.06 b	ND	ND	ND
Fluorene	ND	ND	ND	ND	ND	ND	ND	ND
Phenanthrene	0.03 ± 0.03 b	ND	0.01 ± 0.02 b	ND	0.35 ± 0.61 b	1.44 ± 0.07 a	0.04 ± 0.04 b	0.0 ± 0.04 b
Anthracene	ND	ND	0.02 ± 0.03 b	ND	0.67 ± 0.59 b	ND	ND	0.1 ± 0.10 b
Fluoranthene	ND	ND	0.01 ± 0.01 b	ND	0.06 ± 0.00 a	0.16 ± 0.02 b	ND	ND
Pyrene	ND	ND	ND	ND	0.20 ± 0.04 b	0.23 ± 0.01 b	ND	ND
Benzo(a)anthracene	ND	ND	ND	ND	ND	ND	ND	ND
Chrysene	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[B]fluoranthene	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[k]fluoranthene	ND	ND	ND	ND	ND	ND	ND	ND
Benzo(a)pyrene	ND	ND	ND	ND	ND	ND	ND	ND
Dibenzo(A,H)Anthracene	ND	ND	ND	ND	ND	ND	ND	ND
Benzo(G,H,I)Perylene	ND	ND	ND	ND	ND	ND	ND	ND
Indeno(1,2,3-CD)Pyrene	ND	ND	ND	ND	ND	ND	ND	ND

[†] Values are presented as the mean ± standard deviation (n=3). Values that were not detected are identified with ND.

[‡] Means within each row followed by different letters are significant different at $P < 0.001$.

Table 2-7: Low molecular weight (LMW), high molecular weight (HMW), and total polycyclic aromatic hydrocarbon (PAH) concentrations (µg/g biochar) extracted from polyoxymethylene passive samplers equilibrated for 30 and 60 day periods[†].

Period	Treatment	LMW	HMW	Total
		µg/g biochar		
30 Days	Blank	0.05 ± 0.05 b[‡]	ND b	0.05 ± 0.05 b
	Hardwood	0.11 ± 0.04 b	0.01 ± 0.01 b	0.12 ± 0.04 b
	Pine	24.19 ± 2.72 a	0.26 ± 0.04 a	24.45 ± 2.72 a
	Switchgrass	0.14 ± 0.06 b	ND b	0.14 ± 0.06 b
60 Days	Blank	ND b	ND b	ND b
	Hardwood	0.04 ± 0.07 b	ND b	0.04 ± 0.07 b
	Pine	27.50 ± 4.45 a	0.38 ± 0.02 a	27.89 ± 4.44 a
	Switchgrass	0.25 ± 0.13 b	ND b	0.25 ± 0.13 b

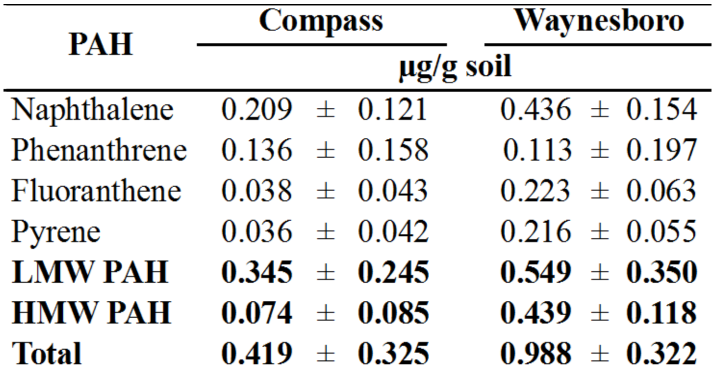
[†] Values are presented as the mean ± standard deviation (n=3). Values that were not detected are identified with ND.

[‡] Means within each column followed by different letters are significant different at $P < 0.001$.

Table 2-8: Comparison of biochar-water partitioning coefficients ($\log K_{\text{biochar-water}}$ in ml/g) for individual polycyclic aromatic hydrocarbons (PAHs) extracted from polyoxymethylene passive samplers equilibrated for periods of 30 and 60 days (Sverdrup et al., 2002).

PAH	logKow	30 Day POM			60 Day POM		
		Pine	**Switchgrass**	**Hardwood**	**Pine**	**Switchgrass**	**Hardwood**
Naphthalene	3.32	3.09	4.43	4.68	3.05	4.21	5.01
Acenaphthylene	4.07	4.25	-	-	3.99	-	-
Acenaphthene	3.94	4.65	-	-	-	-	-
Fluorene	4.23	-	-	-	-	-	-
Phenanthrene	4.5	3.29	4.86	5.25	2.67	5.17	-
Anthracene	4.6	4.64	-	4.40	-	4.03	-
Fluoranthene	5.2	5.63	-	5.52	5.22	-	-
Pyrene	5.2	4.92	-	-	4.85	-	-
Benzo(a)anthracene	5.66	-	-	-	-	-	-
Chrysene	5.8	-	-	-	-	-	-
Benzo[B]fluoranthene	6.4	-	-	-	-	-	-
Benzo[k]fluoranthene	6.4	-	-	-	-	-	-
Benzo(a)pyrene	6.4	-	-	-	-	-	-
Dibenzo(A,H)Anthracene	6.5	-	-	-	-	-	-
Benzo(G,H,I)Perylene	6.9	-	-	-	-	-	-
Indeno(1,2,3-CD)Pyrene	6.7	-	-	-	-	-	-

Table 2-9: Individual, low molecular weight (LMW), high molecular weight (HMW) and total background concentrations of polycyclic aromatic hydrocarbons (PAHs) extracted from unamended Compass and Waynesboro soils†.



PAH	Compass	Waynesboro
	µg/g soil	
Naphthalene	0.209 ± 0.121	0.436 ± 0.154
Phenanthrene	0.136 ± 0.158	0.113 ± 0.197
Fluoranthene	0.038 ± 0.043	0.223 ± 0.063
Pyrene	0.036 ± 0.042	0.216 ± 0.055
LMW PAH	**0.345 ± 0.245**	**0.549 ± 0.350**
HMW PAH	**0.074 ± 0.085**	**0.439 ± 0.118**
Total	**0.419 ± 0.325**	**0.988 ± 0.322**

†Values are presented as the mean ± standard deviation (n=3).

Table 2-10: Average mass of root and shoot biomass for lettuce and soybean plants grown in Compass and Waynesboro soil treatments. Average root nodule count and chlorophyll content is also included for soybean treatments[†].

	Lettuce		**Soybean**			
	Root g	**Shoot g**	**Root g**	**Shoot g**	**Chlorophyll SPAD unit**	**Nodule No.**
Compass						
Control w/ Fert	0.10 ± 0.02 a[‡]	0.29 ± 0.07 a	0.33 ± 0.02 a	0.80 ± 0.06 a	28.4 ± 1.7 a	23 ± 5 a
Hardwood						
10 tons/acre	0.09 ± 0.03 a	0.28 ± 0.06 abc	0.33 ± 0.04 a	0.71 ± 0.07 a	32.6 ± 2.0 a	18 ± 6 ab
40 tons/acre	0.09 ± 0.05 a	0.23 ± 0.08 bcd	0.31 ± 0.04 ab	0.69 ± 0.09 ab	26.2 ± 4.4 a	16 ± 7 ab
Pine						
10 tons/acre	0.10 ± 0.05 a	0.25 ± 0.12 cd	0.31 ± 0.04 ab	0.67 ± 0.07 ab	27.4 ± 3.7 a	10 ± 1 bc
40 tons/acre	0.09 a	0.17 d	0.21 ± 0.09 b	0.48 ± 0.17 b	12.6 ± 5.5 b	2 ± 4 c
Switchgrass						
10 tons/acre	0.12 ± 0.04 a	0.39 ± 0.14 ab	0.31 ± 0.02 a	0.81 ± 0.07 a	29.3 ± 3.6 a	20 ± 3 ab
40 tons/acre	0.15 ± 0.00 a	0.38 ± 0.10 bcd	0.30 ± 0.02 ab	0.74 ± 0.07 a	24.9 ± 2.3 a	16 ± 7 ab
Waynesboro						
Control w/ Fert	0.10 ± 0.02 a	0.32 ± 0.09 a	0.33 ± 0.04 a	0.58 ± 0.10 b	46.2 ± 4.6 a	12 ± 3 a
Control w/o Fert	0.07 ± 0.01 a	0.21 ± 0.03 b	0.32 ± 0.05 a	0.64 ± 0.01 ab	46.1 ± 5.6 a	14 ± 2 a
Switchgrass						
10 tons/acre	0.08 ± 0.01 a	0.34 ± 0.04 a	0.35 ± 0.07 a	0.81 ± 0.11 a	45.0 ± 1.6 a	15 ± 6 a
40 tons/acre	0.09 ± 0.02 a	0.32 ± 0.03 a	0.33 ± 0.01 a	0.79 ± 0.05 a	47.8 ± 2.6 a	15 ± 4 a

[†] Values are presented as the mean ± standard deviation (n=4). Two lettuce treatments had missing data. Lettuce plants grown in the pine 40 ton/acre biochar application had one replicate. Lettuce plants grown in the switchgrass 40 ton/acre application had two replicates.

[‡] Means within each column followed by different letters are significant different at $P < 0.001$.

Table 2-11: Concentrations of low molecular weight (LMW), high molecular weight (HMW), and total polycyclic aromatic hydrocarbons (PAHs) extracted from lettuce and soybean plants grown in Compass soil treatments[†].

Plant	Treatment	Rate (tons/acre)	LMW		HMW		Total	
			µg/g biomass					
Lettuce	Control	0	0.35 ± 0.25	b[‡]	0.16 ± 0.12	c	0.51 ± 0.34	c
	Hardwood	10	0.37 ± 0.31	b	0.20 ± 0.14	c	0.57 ± 0.43	c
	Hardwood	40	0.40 ± 0.31	b	0.23 ± 0.16	c	0.64 ± 0.45	c
	Pine	10	0.81 ± 0.18	b	0.83 ± 0.36	b	1.64 ± 0.48	b
	Pine	40	1.83	a	3.28	a	5.11	a
	Switchgrass	10	0.39 ± 0.27	b	0.14 ± 0.09	c	0.52 ± 0.36	c
	Switchgrass	40	0.40 ± 0.10	b	0.22 ± 0.04	c	0.62 ± 0.13	c
Soybean	Control	0	0.29 ± 0.08	bc	0.14 ± 0.04	c	0.43 ± 0.11	c
	Hardwood	10	0.23 ± 0.07	c	0.45 ± 0.16	bc	0.68 ± 0.21	c
	Hardwood	40	0.35 ± 0.08	bc	0.84 ± 0.43	bc	1.19 ± 0.50	bc
	Pine	10	1.34 ± 0.52	a	1.00 ± 0.27	ab	2.34 ± 0.77	ab
	Pine	40	0.82 ± 0.30	ab	1.67 ± 0.70	a	2.49 ± 0.98	a
	Switchgrass	10	0.25 ± 0.13	c	0.16 ± 0.04	c	0.42 ± 0.15	c
	Switchgrass	40	0.17 ± 0.10	c	0.12 ± 0.03	c	0.29 ± 0.11	c

[†] Values are presented as the mean ± standard deviation (n=4). Two lettuce treatments had missing data due to plant death. Lettuce plants grown in the pine 40 ton/acre biochar application had one replicate. Lettuce plants grown in the switchgrass 40 ton/acre application had two replicates.

[‡] Means within each column followed by different letters are significant different at $P < 0.001$.

Table 2-12: Concentrations of low molecular weight (LMW), high molecular weight (HMW), and total polycyclic aromatic hydrocarbons (PAHs) extracted from lettuce and soybean plants grown in Waynesboro soil treatments[†].

Plant	Treatment	Rate (tons/acre)	LMW	HMW	Total
			µg/g biomass		
Lettuce	Control w/ Fert	0	0.44 ± 0.29	0.35 ± 0.08	0.80 ± 0.31
	Control w/o Fert	0	0.43 ± 0.30	0.26 ± 0.07	0.69 ± 0.36
	Switchgrass	10	0.36 ± 0.31	0.24 ± 0.12	0.60 ± 0.43
	Switchgrass	40	0.45 ± 0.38	0.27 ± 0.17	0.72 ± 0.55
Soybean	Control w/ Fert	0	0.15 ± 0.05	0.19 ± 0.07	0.33 ± 0.06
	Control w/o Fert	0	0.17 ± 0.09	0.16 ± 0.04	0.34 ± 0.13
	Switchgrass	10	0.16 ± 0.03	0.17 ± 0.14	0.33 ± 0.14
	Switchgrass	40	0.14 ± 0.06	0.17 ± 0.11	0.31 ± 0.11

[†] Values are presented as the mean ± standard deviation (n=4).

Table 2-13: Molecular weight (MW), ring size, n-Octanol:water partition coefficient (logKow), and water solubility (WS) for the 16 US EPA-regulated polycyclic aromatic hydrocarbons (PAHs) (Sverdrup et al., 2002).

PAH	MW	Ring Size	logKow	WS (µg/L)
Naphthalene	128	2	3.32	31700
Acenaphthylene	152	3	4.07	3930
Acenaphthene	154	3	3.94	16100
Fluorene	166	3	4.23	1980
Phenanthrene	178	3	4.5	73
Anthracene	178	3	4.6	1290
Fluoranthene	202	4	5.2	135
Pyrene	202	4	5.2	260
Benzo(a)anthracene	228	4	5.66	14
Chrysene	228	4	5.8	2
Benzo[B]fluoranthene	252	5	6.4	1.5
Benzo[k]fluoranthene	252	5	6.4	0.8
Benzo(a)pyrene	252	5	6.4	3.8
Dibenzo(A,H)Anthracene	278	5	6.5	0.6
Benzo(G,H,I)Perylene	278	6	6.9	0.26
Indeno(1,2,3-CD)Pyrene	276	6	6.7	0.19

Table 2 - 14: Average concentrations of individual polycyclic aromatic hydrocarbons (PAHs) extracted from lettuce samples grown in Compass and Waynesboro soil treatments[†].

Soil	Treatment	Rate tons/acre	Naphthalene	Phenanthrene	Fluoranthene	Pyrene
			µg/g biomass			
Compass	Control	0	0.14 ± 0.12	0.21 ± 0.15	ND	0.16 ± 0.12
	Hardwood	10	0.16 ± 0.15	0.21 ± 0.17	ND	0.20 ± 0.14
	Hardwood	40	0.13 ± 0.09	0.27 ± 0.22	ND	0.23 ± 0.16
	Pine	10	0.27 ± 0.08	0.54 ± 0.12	0.30 ± 0.17	0.53 ± 0.20
	Pine	40	0.33	1.50	1.52	1.76
	Switchgrass	10	0.16 ± 0.11	0.23 ± 0.18	ND	0.14 ± 0.09
	Switchgrass	40	0.21 ± 0.02	0.19 ± 0.08	ND	0.22 ± 0.04
Waynesboro	Control w/ Fert	0	0.22 ± 0.15	0.22 ± 0.14	ND	0.35 ± 0.08
	Control w/o Fert	0	0.20 ± 0.13	0.23 ± 0.18	ND	0.26 ± 0.07
	Switchgrass	10	0.19 ± 0.14	0.17 ± 0.17	ND	0.24 ± 0.12
	Switchgrass	40	0.22 ± 0.18	0.23 ± 0.20	ND	0.27 ± 0.17

[†] Values are presented as the mean ± standard deviation (n=4). Two lettuce treatments had missing data due to plant death. Lettuce plants grown in the pine 40 ton/acre biochar application had one replicate. Lettuce plants grown in the switchgrass 40 ton/acre application had two replicates. Values that were not detected are identified with ND.

Table 2 - 15: Average concentrations of individual polycyclic aromatic hydrocarbons (PAHs) extracted from soybean samples grown in Compass and Waynesboro soil treatments[†].

Soil	Treatment	Rate tons/acre	Naphthalene	Acenaphthylene	Acenaphthene	Phenanthrene	Fluoranthene	Pyrene
			µg/g biomass					
Compass	Control	0	0.11 ± 0.06	ND	ND	0.18 ± 0.04	ND	0.14 ± 0.04
	Hardwood	10	0.05 ± 0.02	ND	ND	0.18 ± 0.06	0.16 ± 0.07	0.29 ± 0.09
	Hardwood	40	0.07 ± 0.03	ND	ND	0.27 ± 0.09	0.30 ± 0.22	0.54 ± 0.22
	Pine	10	0.38 ± 0.19	0.05 ± 0.09	ND	0.92 ± 0.38	0.39 ± 0.17	0.61 ± 0.11
	Pine	40	0.16 ± 0.04	ND	0.04 ± 0.07	0.63 ± 0.21	0.72 ± 0.35	0.94 ± 0.35
	Switchgrass	10	0.09 ± 0.07	ND	ND	0.16 ± 0.06	0.01 ± 0.02	0.15 ± 0.04
	Switchgrass	40	0.05 ± 0.04	ND	ND	0.12 ± 0.06	0.01 ± 0.02	0.11 ± 0.04
Waynesboro	Control w/ Fert	0	0.04 ± 0.02	ND	ND	0.11 ± 0.03	ND	0.19 ± 0.07
	Control w/o Fert	0	0.04 ± 0.02	ND	ND	0.13 ± 0.08	ND	0.16 ± 0.04
	Switchgrass	10	0.04 ± 0.01	ND	ND	0.12 ± 0.02	0.03 ± 0.06	0.14 ± 0.08
	Switchgrass	40	0.04 ± 0.02	ND	ND	0.10 ± 0.04	0.02 ± 0.04	0.15 ± 0.07

[†] Values are presented as the mean ± standard deviation (n=4). Values that were not detected are identified with ND.

Table 2-16: Expected amount of polycyclic aromatic hydrocarbons (PAHs) in 150 g (2 cups) of fresh lettuce.

Soil	**Treatment**	**Rate tons/acre**	**LMW**	**HMW**	**Total**
				μg_{PAH}	
Compass	Control	0	2	1	3
	Hardwood	10	2	1	3
	Hardwood	40	2	1	4
	Pine	10	5	5	10
	Pine	40	11	20	31
	Switchgrass	10	2	1	3
	Switchgrass	40	2	1	4
Waynesboro	Control w/ Fert	0	3	2	5
	Control w/o Fert	0	3	2	4
	Switchgrass	10	2	1	4
	Switchgrass	40	3	2	4

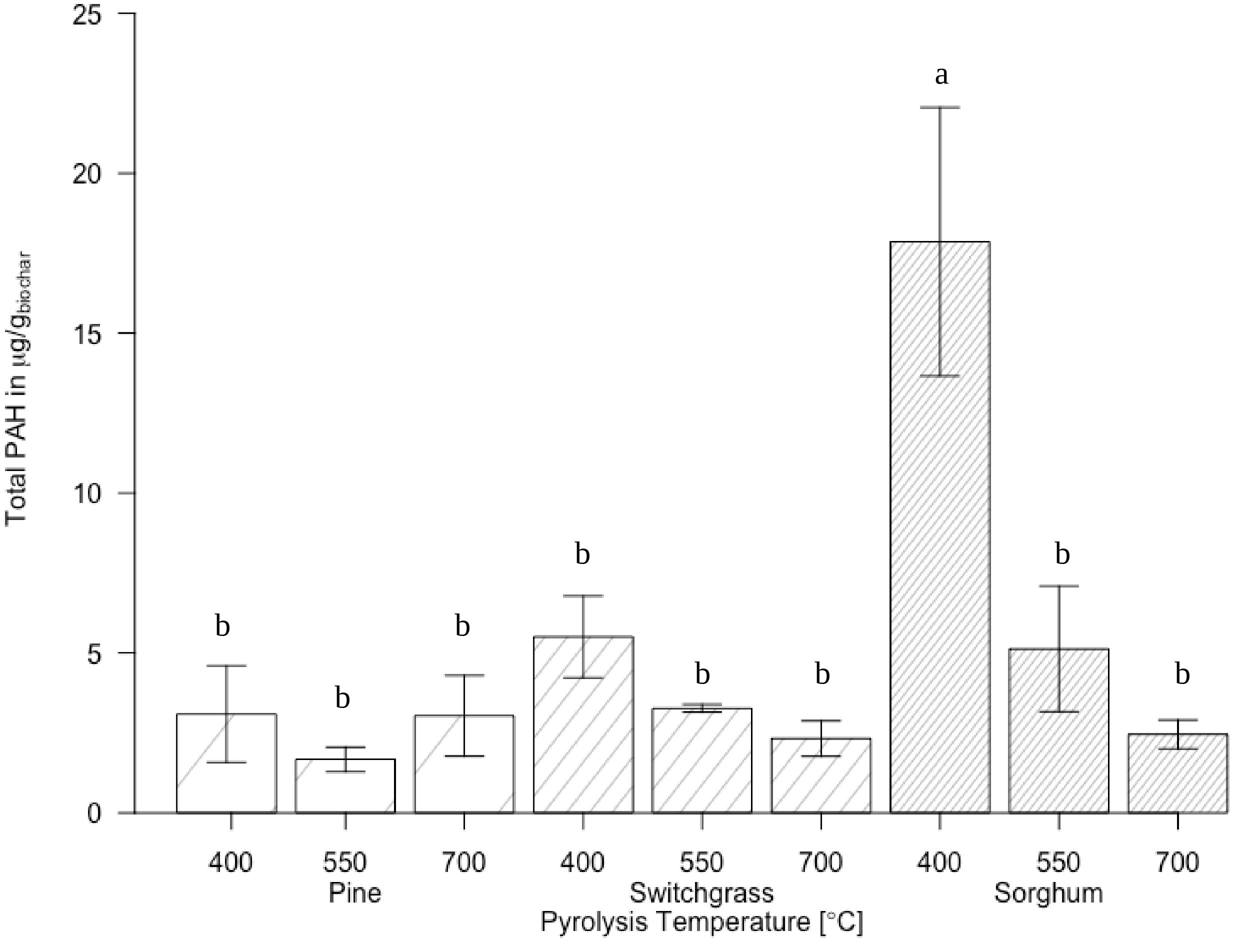


Figure 2-1: Effect of feedstock source and production temperature on PAH concentration in slow pyrolysis biochars. Error bars indicate ± standard deviation (n=3).

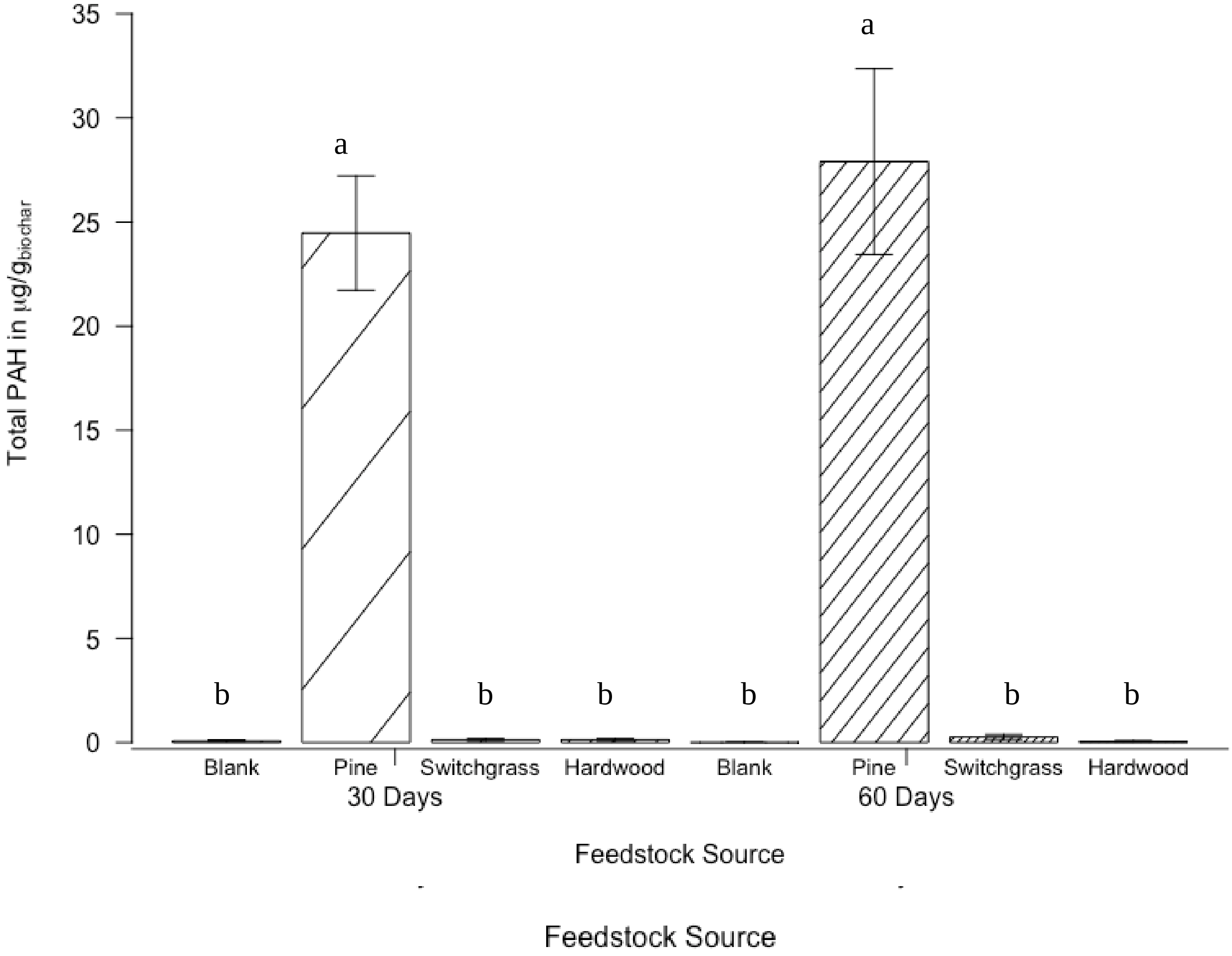


Figure 2-2: Effect of feedstock source, equilibration time, and total uptake of PAHs in polyoxymethylene (POM) passive sampler. Error bars indicate ± standard deviation (n=3).

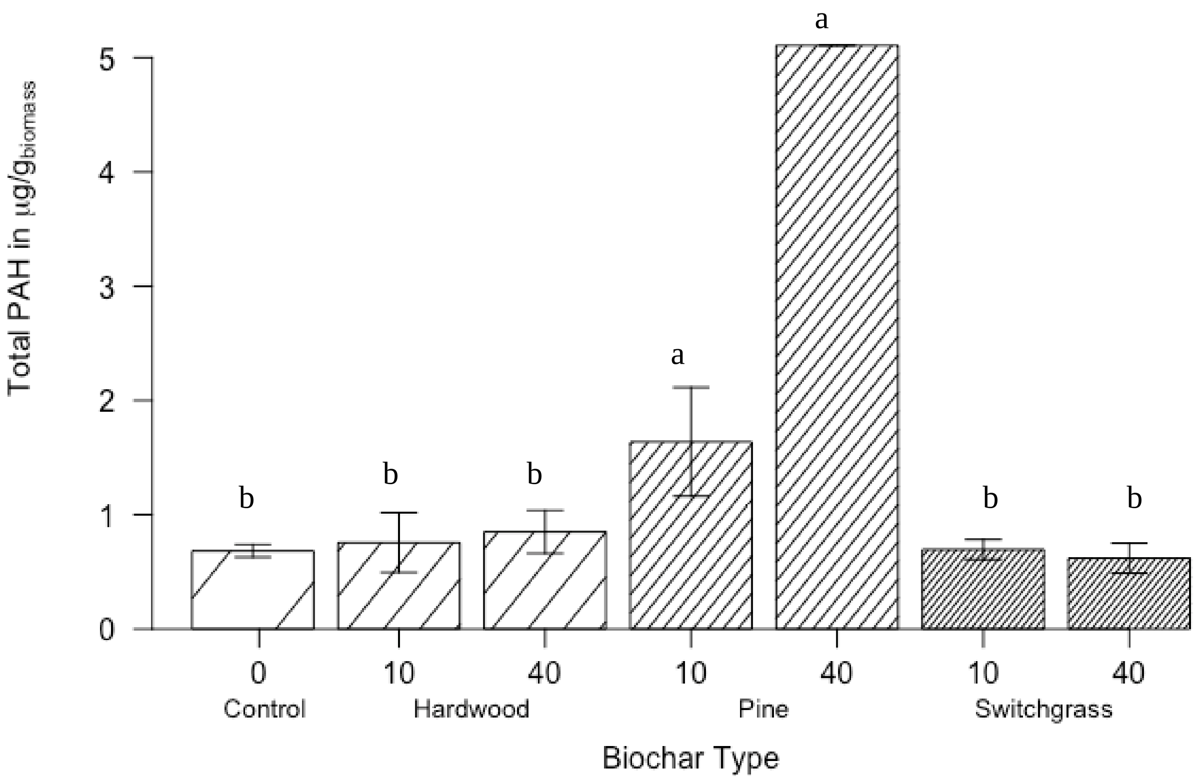


Figure 2-3: Total PAH concentrations extracted from lettuce plants grown in biochar-amended Compass soil. Error bars indicate ± standard deviation (n=4). Two lettuce treatments had missing data. Lettuce plants grown in the pine 40 ton/acre biochar application had one replicate. Lettuce plants grown in the switchgrass 40 ton/acre application had two replicates.

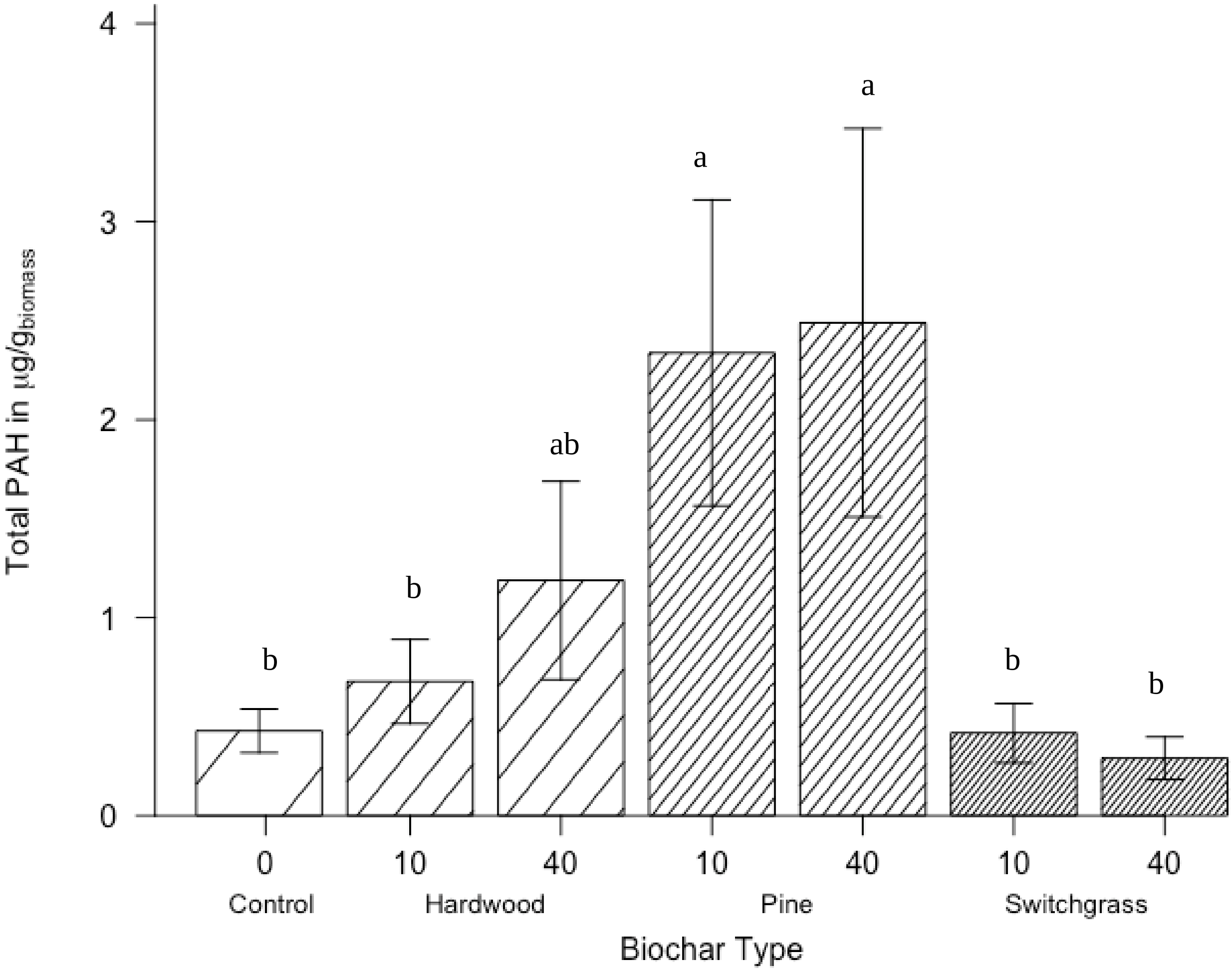


Figure 2-4: Total PAH concentrations extracted from soybean plants grown in biochar-amended Compass soil. Error bars indicate ± standard deviation (n=4).